NUTRITIONAL AND BIOLOGICAL ACTIVITY EVALUATION OF DEBITTERED *CHENOPODIUM QUINOA* SEEDS FOR DEVELOPMENT OF FUNCTIONAL FOOD PRODUCTS

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by

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DECLARATION

I, Intelli Kaur, student of Ph.D. Nutrition and Dietetics (regular), Lovely Professional University, hereby declare that the thesis entitled "Nutritional and Biological Activity Evaluation of Debittered *Chenopodium quinoa* for Development of Functional Food Products" has been prepared by me under the esteemed guidance of Dr. Beenu Tanwar (guide) and Dr. Manju Reddy (co-guide). No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

We certify that Intelli Kaur has prepared her thesis titled "Nutritional and Biological Activity Evaluation of Debittered *Chenopodium quinoa* Seeds for Development of Functional Food Products" for the award of PhD degree of Lovely Professional University**,** under our guidance. She has carried out the research work at the School of Agriculture, Lovely Professional University.

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ABSTRACT

The present study, was carried to explore Indian *Chenopodium quinoa* seeds, grown under the project "Anantha", in Anantapur district of Andhra Pradesh, India. The study titled "Nutritional and biological activity evaluation of debittered *Chenopodium quinoa* seeds for development of functional food products," was carried at Lovely Professional University. Towards the accomplishment of first objective, the *Chenopodium quinoa* seeds were evaluated for their nutritional quality. The seeds were subjected to proximate, nutritional, phytochemical and *in vitro* analysis. Indian *Chenopodium quinoa* seeds were processed by the domestic processes, namely, soaking and germination and compared with respect to raw and industrially processed seeds on the basis of proximate, nutritional, and phytochemical composition. Soaking resulted in increase in 8.5% of moisture content while germination resulted in decrease in moisture content by 17.5% and industrial processing led to 8.5% decrease in moisture content. Soaking and germination resulted in decrease of carbohydrate content by 1.6% and 2% respectively. The carbohydrate content of industrially processed and domestically processed seeds was significantly ($P<0.05$) different. Soaking and germination of raw seeds caused significant decrease ($P<0.05$) in fat content by 20.5% and 32.5%. Crude protein content of raw, domestically processed and industrially processed quinoa seeds was significantly ($P<0.05$) different. Soaking resulted in 0.6% increase in ash content which was statistically non-significant ($P<0.05$) with respect to ash content of raw quinoa seeds while significant increase ($P<0.05$) in ash content, by 22.5%, was observed after germination. Industrially processed seeds were reported with 4.7% reduced ash content. The fiber content of all quinoa seeds were significantly ($P<0.05$) different. Domestic and industrial processing of seeds led to non-significant change ($P<0.05$) in contents of β- carotene. Soaking and germination led to 0.1% and 0.8% increase in β- carotene. Domestic processing of seeds i.e. soaking and germination, led to 10% and 31% significant increase ($P<0.05$) in TDF (Total Dietary Fiber) content of quinoa seeds. Vitamin C content increased by 15% in soaked quinoa seeds and by 46% in germinated quinoa seeds. A significant difference in Vitamin C content of raw and industrially processed seeds was also observed. Industrial processing decreased the vitamin C

content by 30%. Soaking and germination led to 50% and 66.6% decrease in tannin content. Soaking and germination led to 1% and 15%, significant decrease ($P<0.05$) in alkaloid content of quinoa. Soaking and germination resulted in 30% and 55% decrease in oxalate content. Industrially processed seeds were also reported with 10% reduction in oxalate content. Germination led to significant decrease ($P<0.05$) in phytic acid content by 68%. Industrial processing led to non-significant decrease ($P<0.05$) in phytic acid content by 3.2%. Industrially processed seeds exhibited 4.6% increase in total phosphorous content, which was non-significant ($P<0.05$) as compared to the total phosphorous content of raw seeds. Soaking resulted in significant decrease ($P<0.05$) of 24% in saponin content. Germination led to 98% decrease in saponin content. Industrially processed seeds exhibited 97% reduced saponin content as compared to the raw seeds. Soaking resulted in 4.7% decrease in trypsin inhibitor activity. Industrially processed quinoa seeds exhibited 20.7% decrease in trypsin inhibitor activity. Germination resulted in 9.1%, significant increase ($P<0.05$) in protein digestibility. Soaking of quinoa seeds resulted in 1.52%, non-significant increase in starch digestibility while germination led to 1.80%, significant increase with respect to the raw seeds. Soaked quinoa seeds exhibited 7% decrease in antioxidant activity as compared to raw seeds. FRAP values of germinated quinoa seeds increased by 89%. However industrial processing of the seeds lead to decline in antioxidant activity.

Industrially processed Indian *Chenopodium quinoa* seeds were also compared with industrially processed American *Chenopodium quinoa* seeds. It was noticeable that the protein content of Indian quinoa (13.11±0.08 g/100g) was 7.02% more than the protein content of American quinoa (12.25±0.92 g/100g). Indian quinoa seeds exhibited significantly higher ($P<0.05$) β-Carotene content than American seeds. Vitamin C content of American quinoa was, 14.4%, significantly higher ($P<0.05$) than the vitamin C content of Indian quinoa seeds. Calcium, iron and zinc contents of American quinoa seeds were 15.6, 43.2 and 26.1% higher than Indian quinoa, respectively. American quinoa seeds exhibited significantly lower ($P<0.05$) protein digestibility (75.15%) than Indian quinoa seeds.

The objective two of the study was accomplished by a biological trial using animal model. Male wistar rats (42; divided in 7 groups with 6 rats in each group) for a period of 45 days and testing any cholosterolemic effect of supplementing

germinated quinoa (debittered) and raw quinoa (unwashed) seeds. The rats fed with raw and germinated quinoa along with basal diet showed 28.2 and 31.9% reduction in total cholesterol level. Triglyceride level of rats fed with bitter and debitterd quinoa along with basal diet reduced by 10.4 and 14.5%, respectively. The HDL levels were observed to decrease significantly ($P<0.05$) in rats fed with diets supplemented with raw (5.5%) and germinated (3.3%) quinoa along with hypercholesterolemic diet as compared to serum HDL levels of rats fed with hypercholesterolemic diet (positive control) which reveals negative effect of quinoa supplementation along with hyper cholesterolemic diet on serum HDL levels. Lipid lowering effect of quinoa (raw and germinated) was higher than the beneficial effect of statin, which was reported to cause 17.7% decline in (VLDL+HDL) values. The reduction was almost almost twice in germinated quinoa. The drug statin administered to rats along with hypercholesterolemic diet, caused 23.7% reduction in atherosclerotic index, while supplementation of raw and germinated quinoa along with hypercholeterolemic diet resulted in 18.4 and 37.2% reduction in atherosclerotic index. The results infer beneficial effect of quinoa, mainly the germinated one, in hypercholesterolemia over the statin drug.

Towards the accomplishment of third objective, functional foods, namely, quinoa bar, quinoa cracker and quinoa beverages were prepared from quinoa grains. Bars, control bar and quinoa bar, were prepared using chickpea flour and germinated quinoa flour, respectively. They were evaluated for different parameters at an interval of 15 and 30 days. Ash, fat, protein and fiber content of quinoa bar was significantly higher ($P<0.05$) than control bar. Antioxidant activity of quinoa bars, as evaluated by DPPH and FRAP method, was reported significantly higher ($P<0.05$) than the control bars. A significant decrease ($P<0.05$) in over acceptability of snack bars was observed after storage period of 15 and 30 days. In general, although the overall acceptability was observed to decrease with storage period in both snack bars but overall acceptability of quinoa bar was more than the control bar after different storage intervals. Crackers, control and quinoa cracker, were prepared from wheat flour and quinoa flour, respectively. Ash, fat and protein was observed to increase with increase in the ratio of quinoa incorporation to the crackers. Protein content of quinoa crackers was higher than that of wheat crackers. Antioxidant activity was observed to increase significantly ($P<0.05$) with

incorporation of quinoa flour. Cracker incorporated with 40% quinoa flour received highest score for texture, mouthfeel, flavor, and taste on nine point hedonic scale. Storage period of 15 days had no significant effect ($P<0.05$) on overall acceptability of wheat (control, T_0) cracker while overall acceptability of crackers incorporated with 20 and 40% quinoa decreased significantly ($P<0.05$). Beverages, namely raw quinoa beverage (RQB), soaked quinoa beverage (SQB) and germinated quinoa beverage (GQB) were prepared from raw, soaked and germinated quinoa seeds, respectively. Addition of 0.5% (*w/v*) xanthan gum increased the viscosity and helped in textural stabilization of the beverages by reducing serum separation. All quinoa beverages had total phenolic content well correlated to its anti oxidant activity. Upon sensory evaluation, it was observed that GQB qualified all positive aspects and was rated similarly to the commercial soya milk used as control.

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Dated: **Intelli Kaur**

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REVIEW OF LITERATURE

Review of literature provides a sound base for any scientific investigation. Any research study, demands an acquaintance with the studies done already on a particular subject, in order to develop a clear picture of the problem in hand and to comprehend the study correctly. It also helps us to elucidate the correct methodology for the study.

The existing literature on C*henopodium quinoa*, has been extensively referred in order to identify the research gap and formalize the objectives and methodologies for this research. In this chapter, the literatures related to this study are presented under the following heads.

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2.1 Proximate analysis

2.1.1 Moisture content

Moisture content refers to the water content of any food. Isengard, 2011, stated importance of quantification of moisture content of food for understanding its thermodyanamic properties and shelf life. Miranda et al., 2013 reported 7.7 to 15.1g/100g moisture content in quinoa seeds grown in northern, central, and southern Chile. Lower moisture content is indicator of longer product shelf life (Sanni et al., 2006). Quinoa moisture content is highly influenced by variation in seed variety. Miranda et al., 2013 reported difference (6.5 to 12.5 g/100gm) in 'Regalona Baer' and 'Vicuna' varieties of quinoa grown in Temuco and Villarrica regions in Chile.

Effect of processing on moisture content

Moisture diffusivity of quinoa is dependent on moisture content of surrounding media (Vilche et al., 2009). As influenced by processing methods like soaking and germination, Desalegn, 2016 reported increase in moisture content after soaking and decrease in moisture content after germination of chickpea. The increase in moisture content after soaking may be due to uptake of water by dry seed resulting in cell hydration and cell multiplication within the seed (Nonogaki et al., 2010) while decrease in moisture content on germination may be attributed to utilization of water for the synthesis of metabolites (Wang et al., 2009).

2.1.2 Crude Ash

Ash content is the inorganic part of any food sample left as residue after charring. Ashing method used mainly for the estimation of proximate composition is the dry ashing (Azeke et al., 2011). Ash content of a foodstuff is indicator of its mineral content. While dry ashing method measures the total ash content, the wet ashing method is used to measure individual mineral content constituting total ash content (Azeke et al., 2011). Nascimento et al., 2014 reported 2g/100g ash content of quinoa seeds. According to USDA, 2015 (Table 2.1) ash content of quinoa is more than rice, wheat, barley, corn, rye, and sorghum.

Ash content of quinoa varies with variation geographical area of growth. Miranda et al., 2013 reported 3.45g/100g ash content in 'Regalona baer' quinoa variety

grown in Temuco region while 4.2g/100g ash content was exhibited by same quinoa variety grown in Vicuna region in Chile. Ash content also varies with difference in variety of quinoa seed, despite of similar geographical areas of growth. Miranda et al., 2013 reported 4.2g/100g ash content in 'Regalona baer' quinoa variety and 5g/100g ash content in 'Villarrica' quinoa variety grown in same Temuco region in Chile.

Effect of processing on ash content

Various processing methods like soaking, germination, toasting etc have influential effect on ash content of cereals. Inyang and Zakari, 2008 stated activation of phytase on germination, and soaking, resulting in hydrolysis of protein-enzyme bond which leads to release of minerals, due to lixiviation of anti nutrients, as possible cause for increase in ash content on germination. Blessing ang Gregory, 2010 reported increase in ash content of mung bean after toasting. Kavitha and Primalavalli, 2014 reported decrease in ash content of germinated groundnut, which might be due to lixiviation of some minerals in water, while Chikwendu and Ndirika, 2015, reported increase in ash content of germinated ground bean. Afify et al., 2012 also reported decrease in ash content of different varieties of sorghum after soaking and germination.

Table 2.1: Proximate composition of other grains and quinoa

Content /100g	Quinoa	Rice	Barley	Wheat	Corn	Rye	Sorghum
Crude Ash	2.5	0.19	0.62	1.13	0.67	0.98	0.84
Crude protein	14.12	6.81	9.91	13.68	9.42	10.34	10.62
Crude Fat	6.07	0.55	1.3	2.47	4.74	1.63	3.46
Crude Fiber	7	2.8	15.6	10.7	7.3	15.1	6.7
Carbohydrate	64.16	81.68	77.72	71.13	74.26	75.86	72.09

*(USDA, 2015)

2.1.3 Crude protein

According to USDA, 2015, protein content of quinoa is higher than the commonly used grains (Table 2.1). Quinoa's protein content ranges from 11.32 to 16.10 g/100gm (Miranda et al., 2012). Unlike wheat, barley, maize and corn, quinoa

contains albumin (cystine, arginine, histidine) and globulin (chenopodin) as major proteins constituting 35 and 37% of protein content, respectively (James, 2009).

In addition, the presence of non-quantifiable amounts of glutamic acid and prolamine (<7%), an irritant protein for celiac patients, make quinoa fit for consumption by patients suffering from celiac disease (Zevallos et al., 2012). This also makes quinoa a suitable candidate for formulation of gluten free functional food products (Manikandan et al., 2013). Protein content of food is also known to be inversely related to its glycemic index (Shin et al., 2013), Thus, quinoa is also fit for inclusion in list of low glycemic index foods as higher protein content leads to fullness, delayed gastric emptying and decelerated digestion rate (Pineli et al., 2015). The protein quality of quinoa protein has been found to be almost alike the milk protein, casein (Vega-Galvez et al., 2010). The low sulfur amino acid content of globulins is well balanced by the sulfur rich amino acids in albumins (Mardini Filho et al., 2015). Quinoa is known to be an exceptional grain having both methionine (40 to 100mg/100g) and lysine (510 to 640mg/100g), which are limiting amino acids in legumes and cereals, respectively (Gorenstein et al., 2004, Bhargava et al., 2007, Gesinski and Nowak, 2011). Quinoa contains all essential amino acids except tryptophan (Elsohaimy et al., 2016). Hence, the presence of well-balanced amino acid composition renders quinoa protein as a "complete protein" with high nutritional and biological value (Comai et al., 2007).

Besides bestowing unique nutritional properties, protein content of quinoa is also responsible for its diverse functional properties. It imparts properties like foaming, structural, and thermal stabilization to functional food products developed from quinoa. Addition of quinoa protein to film derived from chitin improved the tensile property and thermal stability of quinoa-chitosan film (Araujo-Farro et al., 2010).

Effect of processing on protein content

Domestic processing methods like soaking, germination, cooking, etc are known to greatly influence the protein content of food items. Increase in protein content after soaking of soyabeans was reported by Kayembe and Rensburg, 2013. Nutritive value of cereals is also known to enhance after germination (Hubner and Arendt, 2013). Moongngarm and Sateung, 2010 reported 29% increase in protein content of rice after germination. Inyang and Zakari, 2008 reported increased protein content in germinated peal millet. The increase in protein content may be due to increased activity of protease leading to degradation of peptides to amino acids and further synthesis of new protein (Laetitia et al., 2014). On the contrary, Martinez et al., 2013 reported decreased protein content in germinated legumes, which may be due to excessive hydrolysis of protein on prolonged germination period, leading to its mobilization from the seeds. Industrial processing methods like dehulling and pearling are also known to influence protein content. Ghavidel and Prakash, 2007 reported increase in protein content of dehulled cow pea, green

gram, chick pea and lentils with respect to the raw ones which may be due to the removal of hulls and concentration of protein in embryo while Tosi et al., 2011 reported decrease in protein content of pearled wheat grains due to removal of aleurone layer during the process of pearling.

2.1.4 Crude fat

Quinoa fat content ranges from 4.6 to 5.7 g/100g (Miranda et al., 2013) and is reported to be similar to the Andean grain, kaniwa (Repo-Carrasco-Valencia et al., 2011). The qualitative and quantitative characteristics of quinoa fat make it suitable to be used as an oil crop. The fat content, (Table 2.1) is higher than the common cereals (USDA, 2015), is uniquely rich in essential fatty acids (James, 2009).

In addition it contains less saturated fatty acids and abundant unsaturated fatty comprised of mono and poly unsaturated fatty acid (Marmouzi et al., 2015). According to Marmouzi et al., 2015, quinoa fat is 25 to 29% oleic acid (MUFA), 59% linoleic acid (PUFA) and 12.3% palmitic acid (SFA). The fatty acid profile of quinoa is also reported to be similar to soyabean and maize (Borges et al., 2013). Higher amounts of PUFA have also been known to be beneficial in cardiovascular diseases and insulin sensitivity (Oliver et al., 2012). In addition, the ratio of ω-6/ ω-3 fatty acid in quinoa (10:1/9:1) is higher than western diets (Marmouzi et al., 2015), which also proves quinoa as a beneficial grain for cardio vascular disorders. The fatty acids in quinoa are accompanied by antioxidants, which serve as savior against oxidative rancity (Ng et al., 2007). Among antioxidants, tocopherols have great contribution in imparting antioxidant activity to quinoa (Tang et al., 2014). Presence of γ-tocopherlos (797 ppm) and α-tocopherols (721 pm) braces the use of quinoa in food applications and enhance the shelf life of quinoa oil (Repo-Carrasco-Valencia et al., 2011). The saponification index of quinoa oil (192%) is lower than butter (242%) and coconut oil (250%) but similar to cottonseed (193%) and soyabean (190%) oil (Sundarrajan, 2014). Besides tocopherols, the unsaponifiable oil fraction of quinoa contains squalene and phytosterols. Presence of squalene (54 to 89 mg/100g), an organic compound also present in shark liver oil, is known to impart cardio vascular protective potential to quinoa seeds (Ryan et al., 2007). Graf et al., 2014 concluded phytoecdysteroid content of quinoa to be directly correlated to its oil content. Phytosterols in quinoa have although not been given much importance, but quinoa grains are known to contain about 118mg/100g of phytosterols (Varli et al., 2016). Presence of phytosterols, mainly campesterol (16 mg/100g), stigmasterol (3.4 mg/100g) and β-sitosterol (64 mg/100g) impart anti-inflammatory and anti carcinogenic and hypo cholestrolemic potential to quinoa (Villacres et al., 2013). Phytosterol content of quinoa is higher than corn and millets (Ryan et al., 2007). Phytosterols are also known to have great impact in lowering cholesterol levels by competitive inhibition of cholesterol absorption due to structural similarity with cholesterol (Graf et al., 2015).

Effect of processing on fat content

As influenced by processing methods like soaking and germination, Kayembe et al., 2013 reported increase in fat content after germination of soyabean while Kajihausa et al., 2014 reported decrease in fat content after germination of sesame seeds. The reported decrease may be due to cell growth during the process. Seed growth because of water imbibition by cells on soaking, consumes required energy from fat, a major carbon source in seeds, which may lead to decrease in fat content after soaking (Rumiyati et al., 2012). Germination of seeds leads to metabolite synthesis. This metabolic change requires energy, which is liberated by oxidation of fatty acid resulting in reduced fat content in germinated seeds (Hahm et al., 2009).

2.1.5 Crude fiber

Crude fiber content is measure of mainly the resistant or insoluble fiber (roughage), which is resistant to digestion in human body (Slavin and Lloyd, 2012). Estimation crude fiber content in food does not measure the soluble fibres (pectins, gums, certain poly- and oligo-saccharides) which have been reported to be associated with various health benefits (Chawla and Patil, 2010). Quinoa fiber content of quinoa seeds (Table 2.1) is greater than rice, corn and sorghum (USDA, 2015), which makes it a potential fiber rich food source and can be included in planning dietary strategy to increase fiber intake through diet.

Effect of processing on crude fiber content

Soaking, germination, cooking, toasting, etc are various processing methods employed to enhance nutritional value of foods (Pandey and Awasthi, 2015). Kanensi et al., 2011, reported increase in crude fiber content of Amaranth seeds with increase in steeping period. Increase in fiber content of chickpea, cowpea and mung bean on germination was also reported by Uppal and bains, 2012. Synthesis of insoluble fibers, which are constituents of cell wall, namely cellulose and hemicelluloses may be the cause for increase in fiber content after germination (Pandey and Awasthi, 2015). Kavitha and Parimalavalli, 2014 reported increase in crude fiber content of roasted and germinated wheat flour. Decrease in fiber content after industrial processing has been reported in mung bean by Blessing and Gregory, 2010 which may be due to removal of hulls during industrial processing, which largely account for the fiber content of seed.

2.1.6 Carbohydrates

Starch, the major macro constituent of carbohydrate content in quinoa ranges from approximately 54 to 70% of total carbohydrate content (Steffolani et al., 2013). Quinoa starch granules, as a unit or aggregate, are found mainly in perisperm and are polygonal in shape having 0.6 to 2.3µm diameter (Li et al., 2016). The starch grains are small in size with respect to the size of starch grains of wheat, rice and

maize (Vega-Galvez et al., 2010). Rich content of quinoa starch makes it fit to be used as rice replacement pseudocereal. According to USDA, 2015, (Table 2.1) quinoa contains lower carbohydrate content as compared to some common cereals.

Starch, in general, comprises of 2 types of molecules, namely, amylose and amylopectin. With difference in quinoa varieties, quinoa starch differs with respect to content, structure, granular size and physiochemical characteristics. The amylose content of quinoa starch ranges from 7.7 to 25.7% (Li et al., 2015) and the amylopectin content comprising approximately 78% (Tari et al., 2003) is composed largely of short chains and some long chains which gives it unique structural and functional characteristics (Araujo-Farro et al., 2010). Lindeboom et al., 2005 demonstarted extensive variation in amylase content of quinoa starches, which was directly related to its functional characteristics. The crystalline polymorphic form of quinoa starch shows diffraction pattern of type A, which corresponds to polymorphic form of the cereal starch (Lopez-Rubio et al., 2008). Quinoa starch is known to be the main player behind the quality attributes of resultant food products (Perez et al., 2009). The excellent functional properties of quinoa starch like small granular size, low pasting temperature, high water absoption capacity, swelling power, low gelatinization temperature, freeze-thaw stability and storage stability make it apt to be used as thickener and textural stabilizer for dressings, sauces and other creamy food stuffs (Wu et al., 2012). Rayner et al., 2012 revealed that on basis of weight, ten times less amount of quinoa starch (maximum coverage 1590mg/m^2) is required to stabilize a pickering emulsion, with respect to the barley starch (maximum coverage 16400mg/m^2). The emulsions stabilized using quinoa starch also showed excellent stability for upto 2 years (Rayner et al., 2012). Pagno et al., 2015 revealed antimicrobial characteristics of an edible biofilm prepared from quinoa starch and gold nanoparticles. Hydrophobic alteration in quinoa starch granules make it apt to be used as a stabilization agent in double Pickering where they were efficient in encapsulation hydrophilic samples and showed stability upto 1 month (Matos et al., 2013). Araujo-Farro et al., 2010 prepared a colorless edible film with quinoa starch and glycerol. The homogenous film produced, exhibited best mechanical properties and a smooth surface. Matos et al., 2013 also demonstrated effective use of quinoa starch for encapsulation of cosmetics and pharmaceutical products.

Besides imparting nutritional and functional properties, quinoa carbohydrates have also proven their potential health benefits. Tang et al., 2014 revealed antioxidative and immunolegulatory characterisctic of quinoa polysachharides in an *in vitro* study using RAW 264.7 cells.

Effect of processing on carbohydrate content

Carbohydrate content of food is influenced by various processing methods. Rosa et al 2009 reported decrease in carbohydrate content after soaking and

germination, which may be due to activation of α-amylase in quinoa seeds and breakdown of starch to simple sugars on hydration during both processes. As influenced by industrial processing, Makinde and Akinoso, 2013, reported decrease in carbohydrate content after dehulling of Nigerian sesame. Miranda et al., 2012 and Miranda et al., 2013 also demonstrated different carbohydrate content of quinoa seeds grown in different environmental conditions, revealing influence of varied environmental constraints on nutritional and quality attributes of grain.

2.2 Nutritional analysis of *Chenopodium quinoa*

2.2.1 Dietary fiber

Dietary fiber is the indigestible component of carbohydrate, other than the starch, which is easily digestible. Dietary fiber is composed of non starch polysaccharides and oligosaccharides. Non-starch polysaccharides may be classified as "soluble dietary fiber" or "insoluble dietary fiber" (AACCI, 2011). On the basis of water solubility, dietary fiber can be classified as, Insoluble dietary fiber (cellulose, hemicelluloses and lignin) and soluble dietary fiber (Pectins, gums and mucilages). Among various methods to determine the dietary fiber content of food, detergent methid i.e., ADF (acid detergent fiber) and NDF (neutral detergent fiber) method measure cell components insoluble in acid and neutral detergent, respectively. ADF includes cellulose and lignin while NDF includes hemicelluloses and ADF, which involves measurement of hemicelluloses by difference method (Caprita and Adrian., 2011). Lamothe et al., 2015 reported 10g/100g dietary fiber content in quinoa seeds. Cereals are one of the important sources of dietary fiber (Vega-Galvez et al., 2010) Marmouzi et al., 2015 reported 72.03% NDF (Neutral detergent fiber) and 27.06% ADF (Acid detergent fiber) in Moroccan quinoa seeds. Miranda et al., 2013 reported 11.5 to 15.07 g/100g total dietary fiber (9.9 to 12.1g/100g insoluble dietary fiber and 0.36 to 2.8 g/100g soluble dietary fiber) content in two genotypes of quinoa. Further, the dietary fiber content of food is known to be associated with various physiological benefits like, hypertension, hypercholesterolemia, cardio vascular diseases, diabetes etc (Dhingra et al., 2012). Lamothe et al., 2015 revealed that dietary fiber content of quinoa is similar to amaranth but greater than maize. In addition, dietary fiber is also known to possess antioxidant properties. Zhu et al., 2009 reported 96.3% antioxidant activity of rice bran polysaccharides concluding its effectiveness similar to ascorbic acid.

Effect of processing on dietary fiber

Different processing methods have different effect on dietary fiber content of foods (Dhingra et al., 2012). Cetrain processing methods involving enzymes, chemicals, heat treatments etc., can also cause structural modification of dietary fibres. Increase in dietary fiber contents upon soaking and germination has been previously reported in cereals and legumes (Vasishtha and Srivastava, 2013, and

Megat et al., 2016).The increase content may be due to enlargement of cell body and growth initiation upon water imbibition during soaking and germination (Martin-Cabrejas et al., 2003). Similarly, extrusion cooking has been also reported to increase dietary fiber content due to chemical interactions that take place under heat and pressure. Pushparaj and Urooj, 2011, have reported detrimental effect of *industrial* processing on dietary fiber in pearl millet.

2.2.2 Vitamin C

Vitamin C content in *Chenopodium quinoa*, as reported by Miranda *et al.,* (2010) ranges from 12 to 23 mg/100g. Miranda et al., 2013, reported even higher vitamin C content (22 to 31 mg/100g) in two quinoa genotypes from Temuco and Vacuna localities in Chile. Vitamin C content may be vary according to different environmental and storage conditions, as factors like light intensity, amount of nitrogen fertilizers, frequency of irrigation and temperature of the region strongly affect the vitamin C content in crops (Lee and Kader, 2000).

Effect of processing on Vitamin C content

Ascorbic acid which is practically absent in dry grain legumes (Xu et al., 2005) increased in significant amount after sprouting (Khattak et al. 2007). The metabolic changes during sprouting affect the bioavailability, palatability and digestibility of essential nutrients. However, the effect of sprouting depends on the types of legume and conditions and duration of sprouting process (Savelkoul et al., 1992). Several enzyme systems become active during sprouting that brings about profound changes in the nutritional quality of cereals and pulses. Masood et al., 2014 revealed significant ($p< 0.01$) effect of sprouting time on ascorbic acid level of mung bean and chickpea seeds. Dry seeds had no ascorbic acid but phenomenal linear increase was observed in mung bean and chickpea with the progress in sprouting.

2.2.3 β-carotene

β- Carotene belong to a group of pigments, carotenoids, commonly found in whole grains (Borneo and Leon, 2011). Total carotenoid index (TCI) of quinoa (leaves), as measured by Tang et al., 2014 is 49.6 to 73.8 g/100 and is more than TCI of amaranth leaves. α- carotene, β- Carotene, cryptoxanthin, leutin and zeaxanthin are the commonly found carotenoids in dietary sources. Quinoa leaves contain β- Carotene, as primary carotenoids with concentration higher than β-Carotenecontant of amaranth leaves (Tang et al., 2014). Lutein, second most dominant and zeaxanthin, in traces, were the two other lipophylic carotenoids isolated by Tang et al., 2015 in quinoa.Carotenoids have been known to be the influencial factors that impart antioxidant activity to quinoa (Tang et al., 2015). α-carotene, β- Carotene lead to synthesis of vitamin A and xanthophylls. Commonly found xanthophylls in quinoa, leutin and zeaxanthin act as antioxidants and

protect the cell membranes. One of the most important contribution of carotenoids in quinoa is protection against light-induced damages by acting as photosensitizers (Asensi-Fabado et al., 2010).

Effect of processing on β- Carotene content

Increase in β- Carotene upon soaking and germination has been reported by Luthariya and Singh, 2014 and Suryanti, 2016. Lee et al., 2013 also repored increased β- Carotene contents in soyabean sprouts as compared to the seeds. This may be attributable to the fact that β- Carotene content in cereals and pulses is directly proportional to the growth progression in the seed (Ahn et al., 2012).

2.2.4 Mineral content

Although, the mineral composition of a grain acquires quantitatively insignificant portion, but its function and chemistry plays a quite significant role in nutritive value of a grain (Singh et al., 2012).

Table 2.2: Mineral composition (mg/100g) of quinoa with respect to oat and barley

Minerals	Quinoa seed[a]	Oat [b]	Barley [b]	Wheat[c]
Iron	5.5	5.4	2.5	3.3
Magnesium	206	235	79	96.4
Calcium	32	58	29	34.8
Zinc	1.8	3.11	2.1	1.2

* [a]Konishi et al., 2004; [b]USDA, 2015; [c]Jubete et al., 2010

Inadequate intake and less bioavailability of minerals can trigger various health related complications. Quinoa is considered as a pseudo cereal rich in minerals (Jubete et al., 2010). It's mineral concentration, shown in Table 2.2 is higher than the mineral content in commonly found grains (USDA, 2015). Vega-Galvez et al., 2010 reported higher magnesium content (0.26%) in quinoa grain with respect to wheat (0.16%) and corn (0.14%).

Moreover, the main minerals in quinoa, i.e. iron, calcium, magnesium and zinc, etc. are found in readily bio-available form, which aids in mineral absorption by human body and are almost adequate to meet the requirements of a balanced diet (Vega-Galvez, 2010). Besides, the mineral content in quinoa genotypes is highly dependent on environmental conditions (Miranda et al., 2013). Miranda et al., 2013 and Nascimento et al., 2014 reported 44 to 110 mg/100g calcium content in quinoa seeds. Nascimento et al., 2014 reported 5.4 g/100g iron content in quinoa seeds. Miranda et al., 2013 and Nascimento et al., 2014 reported 2.9 to 9.5 mg/100g zinc content in quinoa seeds. Miranda et al., 2010, and Marmouzi et al., 2015 reported 176 to 192 mg/100g magnesium content in quinoa seeds.

Effect of processing on mineral content

Various processing methods influence the content of mineral retention and mineral loss in grains. Mota et al., 2016 reported up to 20% decrease in mineral content of cereals and pseudo cereals caused by cooking. The study revealed better retention of minerals under steaming as compare to boiling. Domestic processing of seeds i.e. soaking and germination increase calcium content Chaparro et al., 2011. Hahm et al., 2009 also reported 0.7% increase in calcium content of sesame seeds after germination. Chapparo et al., 2011 reported post germination increase of 11.4% in iron content of quinoa seeds. The apparent reason, for increase in calcium and iron content, may be the decrease in phytic acid content post domestic processing. Phytic acid is known to bind with minerals to form insoluble mineral-phytate complexes and thus, making them less bio-available for proper utilization in body (Coulibaly et al., 2012)

Afiffy et al., 2012 reported 14% and 20% reduction in zinc content of sorghum seeds post soaking and germination. Decrease in magnesium content after germination has been reported in black beans by Sangronis and Machado, 2007. The apparent reason behind decrease in zinc and magnesium contents may be lixiviation of minerals into soaking media during domestic processing of seed.

Konishi et al., 2004, in their study related to depiction of mineral distribution in quinoa stated an industrial processing technique, abrasion, as a potent cause of calcium loss from quinoa seeds, as the latter is located in pericarp, which is usually removed during industrial processing. Reduction in zinc and iron contents of industrially processed seeds has been reported by Pal et al., 2016 who reported losses in Zn and Fe content of dehulled horsegram.

2.3 Phytochemical analysis of *Chenopodium quinoa*

2.3.1 Phytic acid

Phytic acid is an anti nutritional component quinoa seeds (Konishi et al., 2003). Quinoa contains 1.18g/100g phytic acid content (Valencia-Chamorro, 2004). Unlike, wheat and rye, which contain phytic acid only in the outer layers, phytic acid in quinoa is present in embryo and seed coat of the seeds (Bastidas et al., 2016) The negatively charged phytic acid molecules bind with the minerals resulting in formation of mineral-phytate chelates and hence interfers with the mineral bioavailability (Frontela and Martinez, 2011). Phillippy et al., 2014 reported low phyate content in foods to be directly related to their potential health benefit in atherosclerosis, dyslipidemia and hypercholestrolemia.

Effect of processing on content of phytic acid

Domestic processing methods like soaking and germination, tend to decrease the phytic acid content and the reason behind the decrease may be leaching of phytic acid in the soaking media (Vadivel et al., 2011). Liang et al., 2008, also reported

reduction in contents of phytic acid in brown rice upon soaking. Ibrahim et al., 2005, reported reduction in phytic acid content of cereals after germination. The decrease may be due to the increased activity of enzyme, phytase, upon germination, which hydrolyzes phytic acid to release phosphorous (Kumar, 2013). Pal et al., 2016 have also reported decrease in phytic acid content of horsegram after germination.

2.3.2 Total phosphorous

Total phosphorous content has recently been much focused on to create sustainable global phosphorous reserves, which are non-renewable in nature (Rose et al., 2013). Dairy based foods and cereal grains, which are rich in protein have also been known to be rich in dietary phosphorous content (Welch et al., 2009). Phytate, inorganic phosphates, DNA, ATP, RNA are various forms in which phosphorous is found in cereal grains (Raboy, 2009). Phosphorous content in dietary sources is of great importance as both excess and deficiency lead to detoriation of bone health (Takeda et al., 2012). Rosero et al., 2013 reported 0.44-0.5g/100g total phosphorous content in four different varieties of quinoa seeds. Ando et al., 2002 and Konishi et al., 2004 reported that about 60% of phytic acidis localized in embryo of quinoa seeds, and is a major indicative of phosphorous.

Effect of processing on total phosphorous

Various domestic processing methods like soaking and germination lead to increase in total phosphorous content. Hydration of seeds leads to activation of enzyme phytase and thus the release of inorganic phosphorous as consequence of phytic acid degradation (Kumar, 2013). Increase in total phosphorous content of cereals upon germination has also been reported by Azeke et al., 2011. As phytic acid disintegrates upon soaking and germination release of in organic phosphorous results in increase of total phosphorous (Baruah et al., 2007). Mota et al., 2016 reported 100% retention of phosphorous in quinoa after steaming.

2.3.3 Oxalates

Quinoa leaves and stems contain high amounts of total oxalate content (875 to 1960 mg/100g) as compared to the seeds (144 to 234 mg/100g) (Jancurova et al., 2009). Oxalate content of food corresponds to soluble oxalate content, that binds sodium, potassium and ammonium ions while soluble oxalte content binds to calcium, magnesium and iron (Savage et al., 2009). Quinoa seeds have been reported to have 1.8g/100g total oxalate content and soluble oxalate content comprises of 71% (131mgmg/100g) of total oxalate content (Siener et al., 2006). Oxalate content in quinoa is less than spinach, beet, rhubarb, etc., which belong to the same family “Chenopodieceae”. Lopes et al 2009, demonstrated 381 mg/100g oxalic acid content in wholemeal from quinoa variety ‘BRS Paibiru”, which was found to be higher than the oxalic acid content of spinach (823 mg/100) and similar to the oxalic acid content of beet (330 mg/100g). Oxalic acid present in

foods is known to form chelates by binding with mineral content of food and making it less bioavailable during digestion (Nile and Park, 2014). Oxalic acid, upon chelation with divalent calcium, forms calcium oxalate, commonly known as root cause of kidney and gall bladder stone. Hyperoxaluria, is caused due to excessive oxalate excretion in urine (Hang et al., 2014). Hence, low oxalate content foods are beneficial for mineral bioavailability from mineral rich foodstuffs.

Effect of processing on oxalate content

Domestic processing methods like soaking and germination tend to decrease the oxalate content of food due to lixiviation of soluble oxalate content into soaking media (Makinde and Akinoso, 2013). Hang et al., 2014 demonstrated detrimental effect of boiling and soaking on oxalate content of taro leaves and petioles by 84.5 and 69.2%, respectively. Boiling causes disruption of cell wall due to excessive heat, and hence the oxalate leaches out in to boiling media (McEwan et al., 2014). Juajun et al., 2012 reported cooking as an effective method to reduce oxalate content of vegetables.

2.3.4 Tannins

Tannins are biomolecules comprising of carboxyl and hydroxyl groups and are polyphenolic in nature. They form complex linkages with carbohydrates and proteins present in plants (Santos, 2006). Because of their interference with absorption of proteins and certain minerals like iron, tannins are considered as antinutrients (Kumar and Udhayaya, 2012). Tannins are also responsible for astringency and nasty color of food product leading to its decreased palatability. In addition they contribute to undesirable biological effect like inhibition of carbohydrate, mineral and vitamin absoption (Santos, 2006). Quinoa seeds contain 0.53g/100g tannin content (Valencia-Chamorro, 2003).

Effect of processing on tannin content

Khandelwal et al., 2010 stated soaking and germination as effective domestic processing methods to reduce tannin contents in legumes. The decrease might be attributed to leaching out of tannins in soaking media during soaking. Megat and Azrina, 2016 also reported decrease in tannin content of peanuts post germination. Germination is known to trigger the disintegration of tannin-protein-enzyme-mineral complex (Echendu et al., 2009) which might cause decrease in tannin content. Akin-Idowu et al., 2009 reported decrease in tannin content of tubers after boiling. Decrease in tannin content has also been known to increase iron bioavailability (Enes et al., 2014).

2.3.5 Alkaloids

Alkaloids are nitrogen-containing compounds, present mainly in plants. First alkaloid discovered was, Morphine (Laux, 2013). Genus *Chenopodium* has been

reported to contain tropane, piperidine and pyridine alkaloids (Kokanova-Nedialkova et al., 2009). Dini et al., 2006 reported presence of five betaines in *Chenopodium quinoa. Chenopodium murale* was reported to contain alkaloid piperine . Chenoalbacine is an alkaloid isolated from roots of *Chenopodium album* (Dini et al., 2005).

Effect of processing on alkaloid content

Soaking and germination lead to decrease in alkaloid content due to solubility of betaine (an alkaloid present in quinoa) in polar solvents such as water (Wang and Zhu, 2012). Sanchez et al., 2009 reported decrease in alkaloid content upon soaking and germination of pigeon pea and lupin seeds, respectively.

2.3.6 Saponins

Quinoa saponins, the natural detergents, are the major negative factor responsible for its bitter taste, lower palatability, and lower consumer acceptability. Majorly, the triterpene saponins derived from oleonic acid, hederagenin and serjanic acid with galactose, arabinos and glucose as sugar moieties have been located in all parts of quinoa plant (Zhu et al., 2002). Kuljanabhagwad and Wink, 2009 have concluded presence of monodesmosidic (with single carbohydrate chain), didesmosidic (with two carbohydrate chains) and tridesmosidic (with three carbohydrate chains) triterpene saponins in quinoa. In quinoa seeds, saponins are located in the outer layer, i.e. the papillose cells of hulls (Raamsdonk et al., 2010). Saponin content in bitter varieties of quinoa is 0.1 to 5 g/100g while sweet varieties are known to contain 0.02 to 0.05 g/100g of saponin content (Valencia-Chamorro, 2003 and Mastebroek et al., 2000). Saponin content in quinoa is less than the saponin content of legumes (James, 2009). In addition to cross hybridization as possible solution to production of sweet quinoa varieties for increasing its consumer acceptability, regulation of soil salinity and irrigation level is also known to greatly influence the saponin level in quinoa seed (Gomez-Caravacca et al., 2014). Soliz-Guerrero et al., 2002 revealed that high soil water deficit may also help in yielding quinoa crops with lower saponin content.

Saponins are known to engage in hemolytic activity of red blood cells and result in toxicity (Araujo-Farro et al., 2010).They also interfere in absorption of minerals by forming tight saponin-mineral linkages (Jancurova et al., 2009). Although saponins adversely affect quinoa acceptability in market but they are to have positive influence on crop protection against pests (Chaeib, 2010). In addition, saponins are reported to have anti microbial and cholesterol lowering potential (Afrose et al., 2010). Yao et al., 2014 also demonstrated anti-inflammatory potential of quinoa saponins. Saponins are also known as “cell permeability modifiers” and used as precursors which intensifies the uptake of certain drugs by small intestine, which represents its pharmacological potential (Vega-Galvez et al., 2010).

Effect of processing on saponin content

Domestic processing methods like soaking and germination are known to be effective in removal of saponins. Raamsdonk et al., 2010 demonstrated destruction of papillose cells, the major location site of saponins in quinoa, during washing process. Nwosu, 2010 reported 25% decrease in saponin content of bean after soaking for 24 hours. Adekanmi et al., 2009 and Mittal et al., 2012 have also reported decrease in saponin content after soaking tiger nut and chickpea, respectively.

2.3.7 Trypsin inhibitor activity

Trypsin Inhibitor is a protease inhibitor, which inhibits the action of pancreatic enzyme, trypsin, and hence interfers with the intestinal digestion of proteins. Pesoti et al., 2015 isolated novel trypsin inhibitor "CqTI" from C*henopodium quinoa* seeds. Quinoa trypsin inhibitor activity is not of much concern as the content of trypsin inhibitors in quinoa (1.38 to 5.1 TIU/mg) is quite less and lower than trypsin inhibitor activity of lentils (18 TIU/mg), beans (20 TIU/mg) and soyabean (40 TIU/mg) (Jancurova et al., 2009). Presence of trypsin inhibitors interferes with the action of trypsin on proteins and may lead to gastric and pancreatic distress (Horton et al., 2006)

Effect of processing on Trypsin inhibitor activity

Mubarak, 2005, reported decrease in trypsin inhibitor activity, by 5.2%, after soaking mung bean seeds. El-Adawy, 2002 reported 33.9% decrease in trypsin inhibitor activity of chickpeas after soaking. McEwan et al., 2014 reported reduction in trypsin inhibitor activity of tubers after cooking due to heat sensitivity of trypsin inhibitors.

2.3.8 Total phenolic content

Quinoa total phenolic content depends on grain variety and environmental conditions (Miranda et al., 2011). Nasimba et al., 2008 reported higher phenolic content of Japanese quinoa (150 mg/g tannic acid equivalent) as compared to the Bolivian quinoa, which exhibited 94mg/g tannic acid equivalent of total phenolic content. Hirose et al., 2010 suggested the presence of quercitin glycosides as major responsible factors for higher antioxidant activity of Japanese quinoa as compared to the South American quinoa. Quinoa seeds and sprouts have been known to be rich in total phenolics and antioxidant activity (Pasko et al., 2010a). The study also revealed higher total phenolic content of quinoa as compared to the amaranth grain and positive correlation between total phenlic content and antioxidant activity, suggesting antioxidative potential of quinoa phenolics.

Effect of processing on total phenolic content

Xu and Chang, 2008 reported 26-56% loss in total phenolic content of black beans (*Phaseolus vulgaris* L.). Lower phenolic content was also reported in soaked faba beans by Siah et al., 2015. Hydrolysis and leaching of some condensed polyphenols during prolonged soaking period into water used for soaking may be the reason for reduction in TPC in soaked seeds (Segev, 2011). Germination leads to increase in phenolic content of seeds (Duenas et al., 2009) as synthesis of phenolic acid is enhanced by seed growth during germination (Cevallos-Casals and Cisneros-Zevallos, 2010). Increase in total phenolic content in germinated quinoa has also been reported by Carciochi et al., 2014a (56 % after 48 hours and 101.2% after 72 hours of germination) and Jubete et al., 2010 (107% after 82 hours of germination). Difference in total phenolic increase can be explained on the basis of varying germination time and techniques (Khattak et al., 2007). Among common processes involved in industrial seed processing is decortication, also known as pearling, which removes its saponins present mainly in outer layer of quinoa (Bastidas et al., 2016). Decrease in phenolic compounds of pearled quinoa (abrasion degree of 30%) was reported by Gomez-Caravaca et al., 2014 with 21.5% and 35.2% decrease in free and bound phenolic compounds, respectively. Decrease in TPC after decortication (industrial abrasive dehulling) has also been reported in soyabean (11%), chickpeas (37%), and yellow peas (34%) by Xu and Chang, 2010. Han, Baik, 2008 (in wheat), Madhujith and Shahidi, 2006 (in barley), and Cardador-Martinez et al., 2002 (in common beans) have also reported decrease in TPC after decortication of seeds.

2.4 *In vitro* analysis of *Chenopodium quinoa*

2.4.1 *In vitro* starch digestibility

Starch, is the major constituent of carbohydrate content in quinoa and corresponds to 54 to 70% of total carbohydrate content (Steffolani et al., 2013). Repo-Carrasco-Valencia and Serna, 2011 reported 65.1 to 68.6% starch digestibility in 4 different varieties of quinoa seeds. High starch digestibility of quinoa can also be attributed to the small size (0.3 to 2µm) of quinoa starch granules (Kong and Bertoft, 2010). Li and Zhu, 2016 reported significant amount of rapidly digestible starch (RDS) content in quinoa, which is readily susceptible to enzyme action.

Effect of processing on in vitro starch digestibility

Soaking and germination are known to increase the in vitro starch digestibility due to better subjection of seed starch matrix to degradation upon hydration and action of α-amylase on soaking and germination (Chung et al., 2012). It also results in starch gelatinization and increased activity of α-amylase, an enzyme responsible for disintegration of starch into sugars, hence, reducing starch into readily digestible form (Preet and Punia, 2000). Kaur et al., 2016 reported increased glycemic index and starch hydrolysis of germinated (glycemic index: 26.8, starch hydrolysis: 54.4) and soaked (glycemic index: 19.1, starch hydrolysis: 50.2%) mungbean as compared to the raw (glycemic index: 17, starch hydrolysis: 49.15)

mugbean . Capriles et al., 2014 also reported 7.02% increase in starch digestibility of industrially processed (extruded) amaranth seeds. Raghuvanshi et al., 2011 and Oghbaei and Prakash, 2016 reported improvement in starch digestibility of dehulled and milled cereals. Thapliyal et al., 2014 also reported 9.8 to 14% increase in starch digestibility of industrially processed (dehulled) chickpeas of different varieties. Removal of bran and outer fibrous tissue of cereals during dehulling and milling leads to concentration of starch rich fraction during separation, hence whole and refined flours have higher *in vitro* starch digestibility (Oghbaei and Prakash, 2016).

2.4.2 *In vitro* protein digestibility

Interactions between proteinic and non proteinic components like starch, polyphenol, lipids, phytates affect the digestibility of dietary protein (Wong et al., 2009). Repo-Carassco-Valencia and Serna, 2011 reported 76.32 to 80.54% protein digestibility in different quinoa varieties. Presence of anti nutrients likes tannin, trypsin inhibitors, etc. impede the digestibility and solubility of protein (Pushparaj, 2011). Phytic acid, anti nutrient present in quinoa seeds is known to interfere by binding with protein and suppressing proteolysis hence, lowering protein digestibility (Cowieson et al., 2006). Protein digestibility of cereals and legumes varies with the variation in cultivar (Torres et al., 2016).

Effect of processing on in vitro protein digestibility

El-Sayed Embaby, 2010, reported 0.9 to 1.4 % increase in protein digestibility of lupin seeds after soaking. Ghavidel and Prakash, 2007 also reported 14 to 15% increase in protein digestibility of various legumes after germination. Increase in protein digestibility after soaking, germination and industrial processing may be due to decrease in anti nutrients. Nergiz and Gokgoz, 2007 reported pressure cooking as an effective method to increase protein digestibility of foods. Decrease in contents of antinutrients like tannins and phytate lead to increase in protein digestibility as otherwise they bind with endogenous proteins and make them unavailable for digestion (Blessing and Gregory, 2010). Madhu et al., 2012reported an increase in *in vitro* protein digestibility of rice to increase with increase in degree of flaking in rice. Khattab et al., 2009 reported increase in in vitro protein digestibility of soaked and fermented legumes. Rasane et al., 2015 reported improvement in protein quality of oats after germination.Raghuvanshi et al., 2011 reported 69 to 75% improved protein digestibility of dehulled and fried mung beans. Ghavidel and Prakash, 2007 reported 2.2 to 13.2% increase in protein digestibility of dehulled cow pea, green gram and chickpea. Removal of hulls and depletion of protein binding anti nutrients during dehulling leads to concentration of proteins into endosperm (Ghavidel and Prakash, 2007).

2.5 Health benefits of *Chenopodium quinoa*

Quinoa, due to its impeccable nutritional characteristics holds a great health potential (Arneja et al., 2015). Presence of high protein content and richness of bioactive compounds make quinoa an interesting dietary source with health benefits (Nowak et al., 2015).

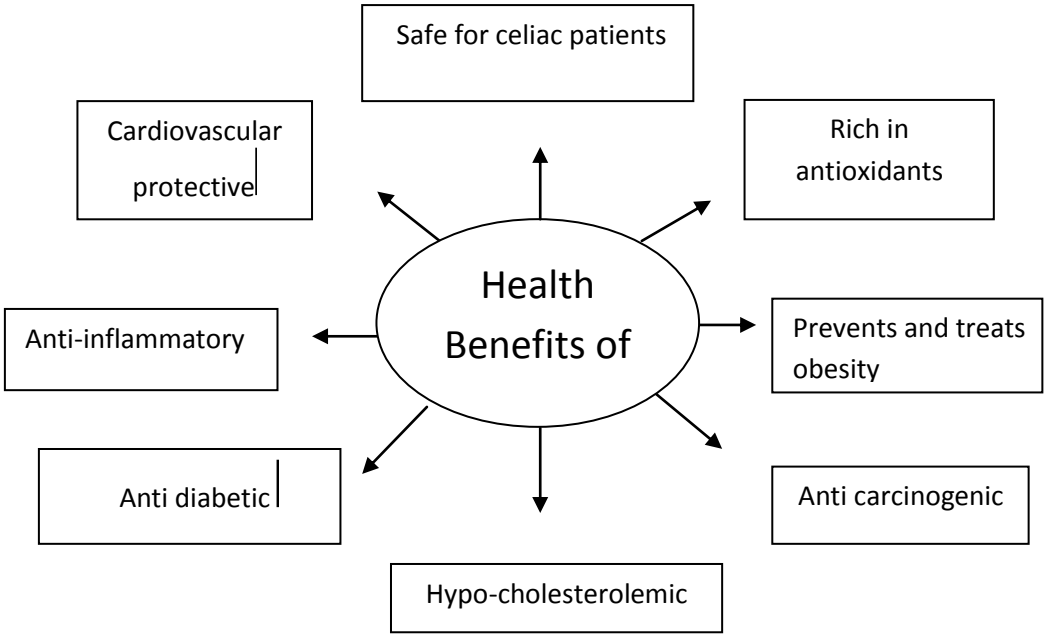

Figure 2.1: Various health benefits of *Chenopodium quinoa*

Non-detectable amount of prolamines in quinoa seed is beneficial for consumption by celiac patients as the grain consumption doesnot cause any intestinal or gastric irritation to individuals allergic to gluten (Zevallos et al., 2014). Querctin, kaempferol and rutin are most abundant phenolic compounds in quinoa (Carciochi et al., 2014b). Being rich in antioxidants quinoa is known to be helpful in various degenerative metabolic disorders like cancer, obesity, diabetes, cardio vascular diseases, etc. (Hejazi, 2016). Researches show both *in vitro* and *in vivo* evidences of health potential of quinoa. Kaur and Tanwar, 2016 revealed anti diabetic and anti hypertensive potential of quinoa beverage prepared from soaked, germinated, and malted quinoa seeds. Some of the work done to explore health benefits of quinoa in *in vitro* and *in vivo* models has been tabulated in Table 2.3 below.

Table 2.3: Health benefits of quinoa in *in vitro* and *in vivo* models.

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
1	***In vitro studies***				
	Quinoa seeds	α-amylase, α-glucosidase inhibition and angiotensin converting enzyme inhibition	Dose dependant	• Antioxidative potential • Treatement of typeII diabetes • Anti hypertensive	Ranilla et al., 2009
	Quinoa milled fractions	α-amylase, α-glucosidase inhibition	Dose dependant	• Dehulled and milled quinoa seed showed increased metal chelating activity • Bran and hull showed anti diabetic potential	Hemalatha et al., 2016
	Soaked and germinated quinoa seed (in form of beverage)	α-amylase, α-glucosidase inhibition and angiotensin converting enzyme inhibition	Dose dependant	• Anti diabetic and antihypertensive potential of malted quinoa beverages.	Kaur and Tanwar, 2016
	Quinoa flour	angiotensin converting enzyme inhibition		• Antihypertensive potential • Antioxidative potential	Asao and Watanabe, 2010

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
	Quinoa saponin fractions	Cell line (RAW 264.7)	50, 100, 200µg/ml of saponins	• Anti inflammatory potential • Inhibition of inflammatory mediators and cytokines	Yao et al., 2014 a
	Quinoa polysachharide	Cell line (RAW 264.7 cells)	10, 50, 150 200µg/ml of polysachharide	• Antioxidant • Immunoregulator	Yao et al., 2014 b
	Quinoa leaf extract	Dunning rat model (Rat prostate cancer cells)	NK	• Chemopreventie • Anticarcinogenic	Gwalik-Dziki et al., 2013
	Quinoa leaf extract	ABTS decolorization And degree of linoleic acid peroxide inhibition	NK	• Antioxidative potential	Gwalik-Dziki et al., 2013
2	***In vivo studies***				
a	Animal model				
	Milled Quinoa flour	Diet induced obese 30 male albino rats	30,40,50,60% quinoa flour incorporated in a formulation	• Increase in hemoglobin and platelets • Decrease in cholesterol, LDL • Decreased liver function parameters in rats fed with 60% quinoa	Hejazi, 2016

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
	Saponin	Mice	300µg	• Safe to consume • Non lethal	Verza et al., 2012
	Quinoa seed extract	Diet induced obese mice	250 and 500 mg/kg quinoa leachate (0.86% 20-hydroxyecdysone, 1% phytoecdysterois, 2.59%flavonoid glycoside, 11.9% oil, 20.4% protein)	• Lower fasting glucose level	Graf et al., 2014
	Quinoa seeds	Fructose fed 24 male wistar rats	310g quinoa seeds/kg fodder for 5 weeks	• Decrease in serum cholesterol • Decrease in LDL • Reduction in blood glucose levels • Inhibition of HDL decrease which was initially induced by fructose	Pasko et al., 2010 a
	Quinoa seeds	Male wistar rats	310g quinoa seeds/kg fodder for 5 weeks	• Decreased MDA (malondialdehyde) in plasma • Reduced lipid peroxidation • Enhanced antioxidant activity of body organs	Pasko et al., 2010b

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
	Quinoa seed extract	Obese male mice	Quinoa seed extract + 20 hydroxyecdysterone (6mg/day/kg body weight) for 3 weeks	• Reduction in adipose tissue development • Inhibition of lipid storage linked gene expression • Reduction in fatty acid uptake	Foucault et al., 2012
	Saponin rich quinoa seed coat	Adult male Sprague-dawley rats	25, 50,75,100µl/10^7RBC	• RBC hemoglobin release at lower concentration • Surfactant at low concentration • Disulfide reducing agent • Antioxidant potential due to presence of thiol compounds	Letelier et al., 2011
	Hydrolyzed quinoa extract	64 adult wistar rats	2000mg/kg body weight for 30 days	• Nill hepatic/renal toxicity • Decreased body weigh • Low fat accumulation and triglygeride level	Meneguetti et al., 2011
b	**Clinical trial**				
	Quinoa seeds	19 celiac patients	50g quinoa/day for 6 weeks	• Well tolerated by celiac patients • Better histology and serology relate parameters • Hypocholestrolemic	Zevallos et al., 2014

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
	Quinoa flakes	35 overweight women	25g quinoa flakes for 4 weeks	• Decrease in triglycerides and LDL • Higher level of glutathione (antioxidant)	De Carvalho et al., 2013
	Quinoa cereal bar	9 males;13 females	19.5g quinoa/day for 30 days	• Lower cholesterol, triglyceride and LDL-c • Normal urea, aspartate transaminase (AST) and alanine transaminase (ALT) levels	Farinazzi-Machado et al., 2012
	Quinoa seeds (in milk form)	12 volunteers	312.5ml	• Low glycemic index • Beneficial for diabetics	Pineli et al., 2015
	Quinoa seeds	Boys (50-65 months)	100g quinoa + baby food, twice a day, for 15 days	• Increase in plasma inulin growth factor • Potential to fight malnutrition	Ruales and Nair, 2002
	Low glycemic index diet including quinoa	Diabetic patients	210 diabetic participants	• Decreased HbA1c parameters • Increased HDL	Jenkins et al., 2008

*NK: not known

2.5.1 Hypocholesterolemic effect of *Chenopodium quinoa*

Cholesterol level in human body is mainly dependant on *de novo* synthesis (by liver), and diet intake. Cholesterol elimination process includes conversion of cholesterol to bile salts, steroids, excretion through stools etc. Liver is mainly associated with cholesterol homeostasis in body. Disturbance or interference in cholesterol homeostasis may lead to increased serum cholesterol levels, which have been known to be directly linked to cardio vascular diseases (Gunness and Gidley, 2010). Researches so far have shown positive effect of presence of dietary fibres and bioactive compounds on cholesterol (Gunness and Gidley, 2010; Thilakarathna and Rupasinghe, 2013). Being rich in dietary fiber, magnesium content, phenols, flavanoids and antioxidants quinoa is known to exhibit cholesterol lowering health potential (Pasko et al., 2010; Hejazi, 2016). Dietary fibres, mainly indigestible soluble dietary fibres, are fermented by the colon and bacteria, which results in production of short chain fatty acids (acetate, butyrate and propionate). These short chain fatty acids, mainly propionate, interefere with cholesterol metabolism in liver and result in depletion of cholesterol levels (Gunness and Gidley, 2010). In addition, high magnesium content of quinoa (250mg/100g) as compared to wheat (170mg/100g) and barley (75mg/100g) impart hypocholestrolemic effect to quinoa (Jubete et al., 2010 and Valencia-Chammaro, 2003). Magnesium influences cholesterol level by dilating the blood vessels and leading to lowering of serum cholesterol level (Nasseri and Hakemi, 2013). Quinoa has also been reported to contain low phytate (1 to 1.8g/ 100 g) content (Valencia-Chamarro, 2003). Phillippy et al., 2014 reported low phytate content of foods to be directly related to their potential health benefit in hypercholesterolemia. Flavanol glycosides, main polyphenols in quinoa have been reported to possess hypeocholestrolemic health potential (Gomez-Caravaca et al., 2011). Diadzein and genistein, the bioactive compounds in quinoa, isolated by Lutz et al., 2013, have been reported to greatly influence the lipid metabolism and have cholesterol lowering properties (Takahashi et al., 2009). Diadzein and genistin enhance translocation of fatty acid and deecreases the synthesis of Low density lipoprotein (LDL) in body (Takahashi et al., 2009). Pasko et al. 2009 reported presence of anthocyanins in coloured quinoa seeds. Anthocyanin supplementation have been reported to be beneficial in increasing serum HDL level and help in proper regulation of cholesterol homeostasis (Zhu et al., 2014).

Recently, Hejazi, 2016 reported beneficial effect of quinoa incorporation at 60% level in diet fed to 30 obese male albino rats. The study concluded hypocholestrolemic effect of quinoa incorporation. Konishi et al. 2004 reported that quinoa pericarp incorporation in diet at 3% level led to significant decrease in serum cholesterol level in mice. Takao et al., 2005 reported hypocholesterolemic effect of incorporation of quinoa protein (at 0, 2.5 and 5% level) to mice fed with 0.5 % cholesterol diet 4 weeks. Pasko et al., 2010a reported 26% lower serum cholesterol, 57% lower LDL, 11% lower triglycerides, 10% lower glucose level in high fructose fed male wistar rats after incorporation of 310g/kg quinoa seeds in rat fodder for 5 weeks. De Carvalho, 2013 reported decrease in cholesterol (191 ± 35 to 181 ± 28 mg/dl) and LDL (129 ±

35 to 121 ± 26 mg/dl) level of 35 obese women after consumption of 25g quinoa flakes in 4 week clinical trial on 35 women with weight excess who consumed 25 grams of quinoa flakes (QF) or corn flakes (CF) daily during a period of four consecutive weeks. Jenkins et al., 2008 reported increased HDL level of diabetic patients who were fed with quinoa incorporated low glycemic index diet. Farinazzi-Machado et al., 2012 reported lower cholesterol, triglyceride and LDL levels of subjects who consumed 19.5g quinoa per day in form of a quinoa cereal bar for 30 days.

2.6 Functional foods developed from *Chenopodium quinoa*

Owing to varied functional properties and an additional advantage of being a gluten free pseudocereal, quinoa has been extensively used in formulation of functional food products. Breads (fermented/steamed), biscuits, snacks, pasta, edible films, beverages, etc., are some of the recently developed food products using quinoa as an ingredient (Wang and Zhu, 2016). Quinoa being rich in dietary fiber and a good source of anti oxidants, holds a great potential in today's food industry (James, 2009). It is also an ideal candidate for inclusion in "composite flour technology" which is based on incorporation of cereal/legume/millet flour to wheat flour for extended bioavailability of nutrients (Valcarel-Yamani and Caetano, 2012). In corporation of quinoa has known to affect both, functional and nutritional properties of the resultant food product (Wang and Zhu, 2016).

Jubete et al., 2010, incorporated 50% quinoa flour (QF) in wheat flour bread formulation and revealed better nutritional and textural properties of the resultant bread. Rodriguez-Sandoval et al., 2012 demonstrated decreased volume and bulkiness of bread incorporated with 10 and 20% QF. The quality characteristics of quinoa food products largely depend on the physiochemical attributes of its protein and starch components (Kong and Bertoft, 2010). Addition of quinoa protein to film derived from chitin improved the tensile property and thermal stability of quinoa-chitosan film (Araujo-Farro et al., 2010). Modification in quinoa starch granules make it apt to be used as a stabilization agent in Pickering emulsions as well as production of edible films (Matos et al., 2013). Incorporation of quinoa to the wheat dough can modify its thermo mechanical properties (Hager et al., 2012). This so because addition of quinoa flour to gluten containing flour causes weakening of cohesive bonds in gluten matrix leading to lower springiness and cooking stability of dough (Rodriguez-Sandoval et al., 2012).

Quinoa incorporation also extends the longevity and reduces microbial spoilage of resultant food product. Hager et al., 2012 and Wolter et al., 2014 demonstrated 95 and 400% reduced staleness in quinoa flour bread as compared to the wheat flour bread, respectively. Pronged longevity of quinoa food products is due to lower degradation rate of its starch molecule (Lindeboom et al., 2005) while presence of phytochemicals like phenols inhibit mould growth in quinoa based food products (Wang et al., 2015).

Quinoa is known to add on to nutritional aspects of food products due to its markable nutritional profile (Arneja et al., 2015). Stikic et al., 2012 revealed 16% improvement in protein quality of bread incorporated with 20% quinoa seeds as compared to wheat flour bread. Chlopika et al., 2012 found increased antioxidant activity, phenolic and flavonoid content by 11, 11 and 36%, respectively upon addition of 15% quinoa flour to wheat bread.

Some quinoa functional food products have also been known to hold therapeutic potential. Pineli et al., 2015 developed protein rich quinoa milk from quinoa seeds with low glycemic index. Kaur and Tanwar, 2016 dveloped quinoa beverages from soaked, germinated, and malted quinoa seeds, which exhibited anti diabetic and anti hypertensive potential. Farinazzi-Machado et al., 2012 developed a quinoa cereal bar which exhibited hypocholesterolemic potential. De Carvalho et al., 2015 demonstrated antioxidative and cardiovascular protective potential of quinoa flakes. Some of the recently developed functional food products from quinoa and their quality characteristics have been summarized below in Table 2.4

Table 2.4: Functional foods developed from *Chenopodium quinoa*

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
1	**BREADS**				
	Non-sourdough breads				
	Wheat and quinoa bread	Wheat flour + 20% and 50% quinoa flour	100% wheat flour bread	• Decrease in loaf volume • Increase in nutritional value • Acceptable by consumers	Iglesias-Puig et al., 2014
	Wheat and quinoa bread	Wheat flour + 10 and 20% quinoa incorporation	100% wheat flour bread	• Decreased water absorption of dough • increased stability of dough • Lower weight of bread • Increased fluffiness of bread	Rodriguez-Sandoval et al., 2012
	Wheat and quinoa bread	Wheat flour + 15 and 30% quinoa incorporation	100% wheat flour bread	• Inrease in total phenolic content, total favonoid content and antioxidant activity • Better sensory characteristics	Chlopika et al., 2012
	Wheat and quinoa bread	100% quinoa flour	100% wheat flour bread	• Decrease in dough development height • Decrease in springiness • Increase in chewiness • Decrease in slice area	Hager et al.,2012
	Wheat and quinoa bread	80, 85, 90, 100% Wheat flour + 10,15 and 20% quinoa flour	100% wheat flour bread	• Increase in proximate and mineral content • Better sensory attributes (sensory score: 5 to 4.98 on nine point hedonic scale)	Stikic et al., 2012
	Wheat and quinoa bread	Wheat flour + 50 and 100% quinoa seeds	100% wheat flour bread	• Increase in antioxidant activity	Jubete et al., 2010

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
	Wheat and quinoa bread	Wheat flour + 6% quinoa seed	Wheat flour+6% flaxseed	• Reduction in levels of saturated fatty acids • Reduction in levels of trans fatty acids.	Calderilli et al., 2010
	Quinoa malt incorporated bread	1,2.5,5% quinoa malt + 50% rice flour + 50 % potato flour	Oat malt bread	• No significant effect on baking properties • No effect on bread density	Manikandan et al., 2013
	Quinoa and wheat bread	40% wheat flour + 15 % quinoa + 15% buckwheat + 5% pumpkin seed kernel	Wheat bread	• Increased protein, fat and fiber content • Higher energy value • Better sensory value	Milovanivic et al., 2014
	Sourdough breads				
	Wheat and quinoa bread	50% quinoa flour + 50% wheat flour	Wheat flour bread	• Increased elasticity • Decreased bake loss • Increase moisture content • Decreased slice area	Wolter et al., 2014
	Quinoa	quinoa flour + buckwheat + amaranth + chickpea flour	Wheat flour bread	• Decrease in specific volume • Increased hardness • Decreased springiness	Coda et al., 2010
2	**Snacks**				
	Quinoa and corn snack	20% quinoa flour+80% corn flour	100% corn flour snack	• More dietary fiber • Increased hardness • Lower moisture content	Martin and Diaz , 2013
	Quinoa and cashew snack	100% quinoa flour+ 12.5% whey protein concentrate +12.5% cashew pulp	Quinoa fried snack	• decreased water absorption index on adding whey protein concentrate • decreased moisture content increased volume expansion	Onwulata et al., 2010

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
	Quinoa cookie	100% quinoa flour	100% Wheat flour cookies	• increased chewiness • increased overall acceptability	Harra et al., 2011
	Quinoa cake	25, 50, 75, 100% quinoa flor	100% wheat cake	• increased water absorption and dough weakening • decreased stability • good quality cake with equal substitution of wheat and quinoa flour	Atef et al., 2012
3	**Pasta/Spaghetti**				
	Quinoa flour Pasta (taglaitell)	69.7,69.8, 69.9% quinoa flour + 5,10, 15% quinoa starch)	semolina	• no difference in rheological properties of quinoa dough	Chillo et al., 2009
	Pre-gelatinized quinoa flour spaghetti	53.3% quinoa flour and 20% quinoa starck	100% quinoa flour spaghetti	• increased dough gelatinization • improved colour • no difference in sensory attributes	Chillo et al., 2010
	Quinoa pasta	20% amaranth flour + 20% quinoa flour + 60% buckwheat flour	20% amaranth flour + 20% quinoa flour + 60% buckwheat flour + egg white	• better agglutination • increased cooking stability • decreased cooking loss	Schoelencher et al., 2010

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
4	**Breakfast cereal**				
	Quinoa flakes	Wheat flour + rice flour + quinoa flour	Wheat flour + rice flour flakes	• No significant difference in gamma-amino butyric acid level • Increased glutamic acid level after roating	De Calvalho et al., 2015
5	**Quinoa edible films**				
	Quinoa starch film	100% Quinoa starch + glycerol	-	• Peelable • Colourless • Transparent and Smooth surface • Best mechanical properties • Homogenous	Araujo-Farro et al., 2010
	Quinoa protein film	Quinoa protein + chitosan	Chitosan film	• Increased thichkness • Good tensile strength • Thermal stability	James, 2009
	Antimicrobial biofilm	Quinoa starch + gold nanoparticles		• Perfect for food packaging • Protective potential against *E.coli* and *S. aureus*	Pagno et al., 2015

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
6.	**Emulsion stabilizer**				
	Stabilizer in Pickering emulsion	88.2% octenyl succinic anhydride + 1.8% Quinoa starch	-	• Efficient for encapsulation • Potential to be used used in formulation of food products with reduced fat content	Matos et al., 2013
7	**Beverages**				
	Quinoa milk	White quinoa:water (1:7)	Rice milk	• Increased protein content • Low glycemic index • Sensory acceptance at par with rice milk	Pineli et al., 2015
	Quinoa liquid suspension	White/ red quinoa: water (1:10)	Oat milk	• White quinoa was best variety for quinoa beverage preparation • Smooth beverage • Good in taste • Perfectly gelatinized	Thuresson, 2015
	Fermented quinoa beverage	100,70,50,30 % quinoa extract	Soy extract	• 70% soy and 30% quinoa extract was most acceptable. • Quinoa fermented beverages with >30% quinoa extract were less preferred as compared to the soy extracts	Bianchi et al., 2015

CHAPTER 3

RESEARCH METHODOLOGY

This chapter refers to the research design of this study. It explains in detail the methods used to accomplish the specified research objectives.

3.1 MATERIALS

3.1.1 Sample collection

To conduct this study Indian white C*henopodium quinoa* grains "Royal White", grown under project "Anantha," were procured from TSIPARD (Telangana State Institute of Panchayat Raj and Rural Development), Hyderabad, Telangana, India. The American white *Chenopodium quinoa* grains "Royal White", imported from Bolivia, South America, were procured from Devshree grains and pulses, New Delhi, India.

3.1.2 Sample processing

To debitter the raw white Indian *Chenopodium quinoa*, the grains were subjected to domestic processing methods viz. soaking and germination to obtain debittered *Chenopodium quinoa* seeds. The industrially processed grains refer to the product as available in the market for consumer consumption after undergoing industrial processes for debittering of quinoa.

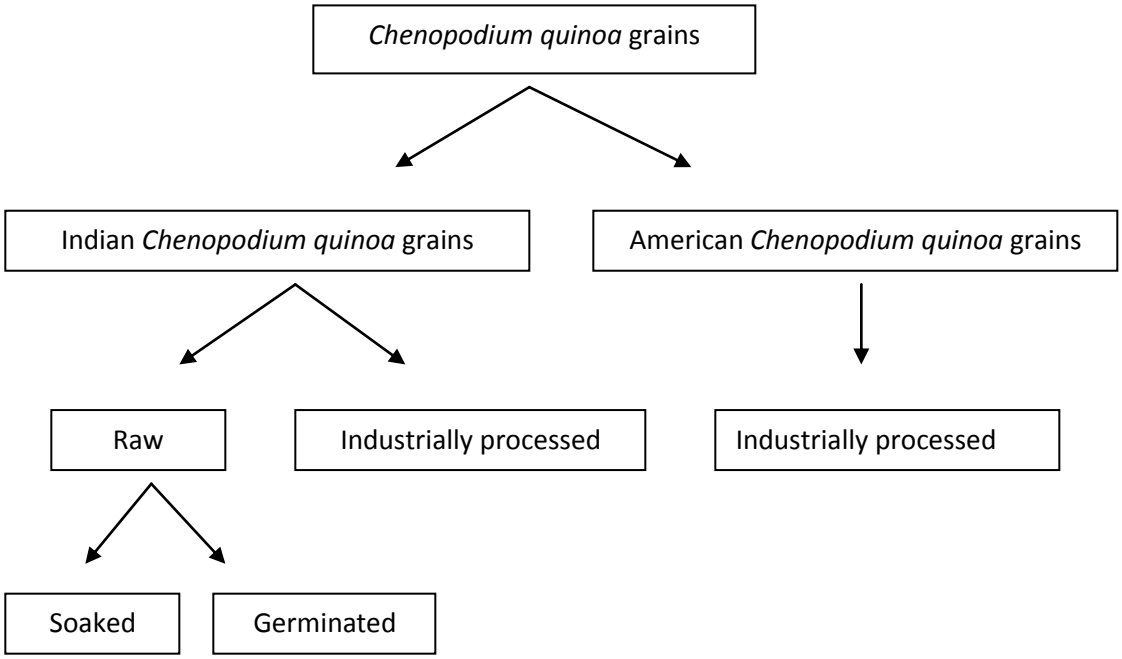

Figure 3.1: Processing of *Chenopodium quinoa* grains

a) Soaking

Raw Indian *Chenopodium quinoa* seeds were soaked for 24 hours in deionized water (1:5 *w/v*) obtained through Millipore (Merck-Milli-Q® Direct 8 Water Purification System, USA). Water used for soaking was changed thrice at regular interval of 8 hours. After 24 hours, the water was discarded and seeds were washed once again with the de ionized water. Soaked seeds were further dried in vacuum drying oven at 40±5°C. Dried seeds were ground to finely powdered flour with a laboratory grinder (Philips HL1606/03 500 W Mixer Grinder). Soaked quinoa seed flour was stored at 4°C for further analysis.

b) Germination

Raw Indian *Chenopdium quinoa* seeds were thoroughly washed with and soaked in de ionized water (1:5 *w/v*) obtained through Millipore (Merck-Milli-Q® Direct 8 Water Purification System, USA) for 24 hours. Seeds were then spread on to petri dishes covered with autoclaved filter paper and incubated at 20°C in an incubator (Biotechnics, India) for 72 hours (Carciochi et al., 2014a) for development of sprouts. Water was changed every 8 hours. Germinated seeds were then dried in vacuum drying oven at 40±5°C. Dried germinated seeds were ground to flour with a laboratory grinder (Philips HL1606/03 500 W Mixer Grinder). Finely powdered germinated quinoa seed flour was stored at 4 °C for further analysis.

3.2 METHODS

Objective 1: To evaluate the nutritional quality of *Chenopodium quinoa.*

Under this objective, nutritional quality of Indian and American *Chenopodium quinoa* was assessed and compared. The sub parts of this objective were:

A) Nutritional evaluation of bittered and debitterd (domestically and industrially processed) Indian *Chenopodium quinoa* seeds

B) Comparison of nutritional quality of industrially processed Indian and American *Chenopodium quinoa* seeds

To accomplish this objective of the study, the samples were analyzed for their proximate, nutritional, and phytochemical composition. Also the *invitro* analysis was carried out to determine antioxidant activity, starch and protein digestibility of samples.

3.2.1 Proximate analysis of *Chenopodium quinoa*

Moisture, crude protein, crude fat, crude fiber and crude ash content were determined according to standard methods given by Association of Official Analytical Chemists AOAC, 2000. The samples were analyzed in triplicates.

3.2.1.1 Moisture content

Moisture content of samples was determined by drying the sample at 105°C until a constant weight was observed. 2g of sample was taken in a previously weighed crucible (W_c). The crucible containing the sample (W_{cs}) was then subjected to drying in oven at 105°C. At an interval of every 4 hours, the sample was taken out of the oven with the help of a pair of tongs. It was then immediately put in a dessicator to avoid moisture (present in air) absorption by the sample and was allowed to cool. The crucible containing the sample after oven drying was reweighed (W_{csd}). The readings were noted and the process was repeated until a constant weight of crucible containing the sample was obtained. Moisture content in 100g of sample was calculated using following formula

$$\text{Moisture content} = \frac{(W_{cs} - W_c) - (W_{csd} - W_c)}{(W_{cs} - W_c)} \times 100$$

Where:

W_c : Weight of emplty crucible

W_{cs} : Weight of crucible + sample prior to drying

W_{csd} : Weight of crucible + sample after drying

3.2.1.2 Crude ash

Crude ash content of samples was determined by placing 2 g of sample in muffle furnace at 550°C for 6 hours. Ash content in 100 g of sample was calculated using following formula

$$\text{Crude ash content} = \frac{W_{ca} - W_c}{W_{cs} - W_c} \times 100$$

Where

W_{ca} : Weight of crucible with ash

W_c : weight of empty crucible

W_{cs} : weight of crucible with sample

3.2.1.3 Crude protein

Kjeldahl method was employed to determine the crude protein content of samples. The analysis involves 3 processes viz. digestion, distillation, and titration. 5g of sample was digested in 10 ml sulphuric acid for about 3 hours to obtain a clear solution. Kjeldahl distillation unit was set up and clear liquid obtained was distilled by adding 40% concentrated sodium hydroxide solution in 50 ml water. The process results in liberation of ammonia which was then collected over 25 ml boric acid solution containing indicator (bromo cresol green and methyl red). It was then titrated against 0.05N sodium hydroxide solution. Blank was prepared similarly without using the sample. Quantification of crude protein content was done by converting nitrogen to protein using conversion factor of 6.25. Crude protein content in 100g of sample was calculated using following formula

$$\text{Crude protein content} = \frac{V_s - V_b}{W_s} \times 14 \times 6.25 \times 100$$

Where,

V_s: Titration volume of sample

$V_{b:}$ Titration volume of blank

14: Molecular weight of nitrogen

6.25: Nitrogen to protein conversion factor

3.2.1.4 Crude fat

Soxhlet method was employed for determination of crude fat content of samples. About 2g of moisture free sample put in a thimble. Petroleum ether (50ml), used as fat extraction solvent was taken in round bottom flask. Soxhlet apparatus was set up. Crude fat content in 100g of sample was calculated using following formula

$$\text{Crude fat content} = \frac{W_{fr} - W_{ir}}{W_{ts} - W_t} \times 100$$

Where

Wt = weight of empty thimble

Wts = weight of thimble + sample

Wir = initial weight of round bottom flask

Wfr = final weight of round bottom flask

3.2.1.5 Crude fiber

Acid base digestion method was employed for determination of cruder fiber content of samples. A 2g of sample was boiled in 0.1M HCl and then treated with 0.3M sodium hydroxide. The samples were then put in muffle furnace at 550°C for 5 hours. Crude fiber content in 100g sample was calculated using following formula

$$\text{Crude fiber content} = \frac{W_{cr} - W_{cs}}{W_s} \times 100$$

Where

W_s = weight of sample

W_{cs} = weight of crucible with ash

W_{cr} = weight of crucible with residue after acid base digestion

3.2.1.6 Carbohydrate

Carbohydrate content in samples was measured using anthrone method. A 100 mg of sample was hydrolyzed with 5ml 0g 2.5N HCl and placed in a boiling water bath for 2 hours. The solution was then neutralized with sodium carbonated till the effervescence ceased. Further, the volume make was done upto 100 ml using distilled water. The resultant solution was centrifuged and standards were prepared using 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard, where 0 served as blank. The volume was further made upto 1 ml in all tubes and 4 ml of ice cold anthrone reagent was added. The solution was further heated for eight minutes in a boiling water bath, cooled rapidly and green colored solution was read at 630 nm. Glucose solution was used as a standard.

3.2.2 Nutritional analysis of *Chenopodium quinoa*

3.2.2.1 Dietary Fiber

Dietary fiber constituents include Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Lignins, Cellulose and Hemicellulose.

a) Neutral detergent Fiber (NDF)

Neutral Detergent Fibre in samples was estimated by the method as suggested by Van Soest and Wine (1967)

Reagents

Neutral Detergent Solution

Sodium borate decahydrate	: 6.18 g
Sodium Lauryl Sulphate	: 30.0 g
2- ethoxy ethanol	: 10.0 ml
Disodium ethylene diamino tetra acetate (EDTA)	: 18.16 g
Water	: 1.0 litre
Disodium Hydrogen Phosphate	: 5.0 g

EDTA and sodium borate decahydrate was weighed together in a large beaker and water was added. The solution was heated till the contents dissolved. Then sodium lauryl sulphate was added to this solution. In a separate beaker disodium hydrogen phosphate was weighed and dissolved in remaining water by heating. The solution was then added to the beaker containing other ingredients and volume was made to 1ltr in a volumetric flask.

Procedure

Air-dried sample (in triplicate) was weighed 500 mg and transferred in to beakers of the refluxing apparatus. To this 100 ml neutral detergent solution was added and heated to boiling. As it will start boiling, the heat was reduced to avoid foaming and allowed to reflux for 60 minutes. The solution was filtered through a weighed gooch crucible with minimum of hot water. Then it was washed with acetone in the same manner. The crucible was dried in hot air oven at 100^{o} C for 8 hours and weighed after cooling.

Calculation

$$\text{NDF (\%)} = \frac{(\text{Wt. of crucible + fiber content}) - \text{Wt. of crucible}}{\text{Weight of sample (g)}} \times 100$$

b) Acid Detergent Fiber (ADF)

Acid Detergent Fibre in samples was estimated on the basis of the method as suggested by Van Soest and Wine (1967).

Reagents

Acid Detergent Solution

Cetyl trimethyl ammonium bromide (CTAB) : 20.0 g 1 N H_2SO_4

Add 20.0 g of CTAB to 1N H_2SO_4 to make total volume of one liter and stir.

Procedure

Air-dried sample was weighed 500mg and transfered in to a beaker of the refluxing apparatus and 100 ml of acid detergent solution was added to it. Mixture was heated to boiling ,refluxed for 60 minutes and was filtered through weighed Gooch crucibles. The samples was rinsed in to crucibles with minimum of hot water (90 to 100^o C). Liquid was filtered and washed repeated. Finally the residue was washed twice with acetone in the same manner. All the lumps was broken, so that the solvent can come in to contact with all particles of the fiber. Acid detergent fiber was dried at 100^o C for 8 hours in hot air oven and weighed.

Calculation

$$\text{ADF (\%)} = \frac{(\text{Wt. of crucible + Fiber content}) - \text{Wt. of crucible}}{\text{Weight of sample (g)}} \times 100$$

Hemicellulose

Hemicellulose was calculated using following formula

Hemicellulose = NDF- ADF

3.2.2.2 Vitamin C

N- bromosuccinimide (NBS) method for determination of vitamin C as given by Barakat et al.1955 and Miranda et al. (2010) was used for determination of vitamin C in quinoa samples. Slight modifications were made in analysis accordingly. The method includes preparation of standard ascorbic acid solution, standardization of NBS with ascorbic acid and estimation of ascorbic acid in sample extract. a) Preparation of standard ascorbic acid: Standard ascorbic acid of concentration0.4mg ml^{-1} was prepared by dissolving 200mg ascorbic acid in 500 ml distilled water. b) Standardization of NBS Solution: Standard ascorbic acid solution (20ml) was added to a flask containing 4 ml of 4% potassium iodide solution (KI), 1.6 ml of 10% acetic acid (CH_3COONa), 4 drops of 1% starch (used as an indicator) and 25 ml distilled water. It was then titrated with NBS (0.2mg ml^{-1}). Appearance of permanent blue colour was considered as end point of titration. c) Estimation of ascorbic acid in sample: Quinoa extracts, acidified with 0.4 g oxalic acid was added to a flask containing 10 ml of 4% potassium iodide solution (KI), 4 ml of 10% acetic acid (CH_3COONa), 4 drops of 1% starch (used as an indicator) and 40 ml distilled water. Final vitamin C content was expressed as mg 100^{-1} using following equation:

$$\text{Vit C content} = \frac{\text{concentration of standard ascorbic acid solution} \times \text{volume of NBS corresponding to quinoa extract (ml)}}{\text{volume of NBS corresponding to std ascorbic acid solution(ml)} \times \text{Sample mass (g)}}$$

3.2.2.3 β-carotene

β carotene in the samples was estimated by the method of Ranganna, (1995).

Reagents

Petroleum ether B.P. (60-80^{o}C)

Acetone

Procedure

One gram sample was extracted with petroleum ether (60-80^{o}C) and acetone (3:2) by grinding with sand in 50 ml silica dish with a glass mortar. Extract was decanted in to a 50 ml volumetric flask and extraction was continued 4-5 times till all fat-soluble pigments was completely dissolved. Volume was adjusted to 50 ml and absorbance was read at 450 nm against a suitable blank. The results was expressed in terms of beta- carotene.

Calculation

$$\beta \text{ Carotene } (\mu g/ 100g) = \frac{\text{O.D.} \times 13.9 \times 10^4 \times 100}{\text{Wt of sample} \times 560}$$

3. 2.2.4 Mineral content

Mineral content of samples was estimated by digestion in Diacid (Piper, 1966)

Reagents

Diacid mixture (25ml) of nitric acid and perchloric acid in the ratio of 3:1 was used for digesting the samples, taking care to prepare the reagent fresh before use.

Procedure

1 g of sample was digested with 25 ml of diacid mixture in a conical flask (100-250 ml). The contents were kept overnight for cold digestion and then heated at low temperature on a hot plate till about clear, colourless liquid with precipitate was left. The contents were allowed to cool and then transferred with deionized water into a 50

ml volumetric flask after repeated washing and then volume was made up to the mark. The digested samples were filtered through Whatman paper No.42 filter paper and stored in the decontaminated dried labeled air tight plastic bottles for the mineral determination. For blank, 25 ml of diacid mixture was digested as in case of sample and volume was made to 50 ml with deionized water.

The digested samples were analysed for iron by using atomic absorption spectrophotometer while calcium, magnesium and zinc were analyzed by using flame photometer

3.2.3 Phytochemical analysis of *Chenopodium quinoa*

3.2.3.1 Phytic Acid

Phytic acid was estimated in the samples by the method of Haugh and Lantzch 1983.

Reagents

Phytate reference solution: Sodium phytate (30.54 mg) was dissolved in 100 ml of 0.2 N HCl, which will give a solution containing 200 ml phytic acid per ml.

Ferric ammonium sulphate solution: Ferric ammonium sulphate (0.2 g) was dissolved in 100 ml 2N HCl and made to 1000 ml with water.

Bipyridine solution: 2', 2' bi-pyridine (10 g) was and 10 ml thioglycollic acid was dissolved in water and made to 1 litre. The solutions are stable for several months at room temperature.

Extraction

Finely ground sample (1g) was extracted with 25 ml of 0.2 N HCl for three hours with continuous shaking in a shaker. After proper shaking, it was filtered through Whatman No.1 filter paper and volume was made 25 ml with 0.2 N HCl.

Estimation

Above mentioned extract (0.5ml) was pipetted into a test tube fitted with a glass stopper and 1 ml of ferric ammonium sulphate solution was added. Tube was heated and centrifuged for 30 minutes at 3,000 rpm. One ml supernatant was transferred to another test tube and 1.5 ml bipyridine solution was added. The absorbance was read at 519 nm against distilled water. For plotting a standard curve different concentration i.e. 0.2 to 1.0 ml of standard sodium phytate solution containing 40-200 µg phytic acid was taken and made to 1.4 ml with water. 0.5 OD will correspond to 120 µg phytic acid.

Calculation

$$\text{Phytic acid (mg/100g)} = \frac{\text{Reading of Graph X} \quad \text{ml of volume made}}{\text{Weight of sample taken X} \quad \text{ml of aliquot taken}} \text{ X } 100$$

3.2.3.2 Total phosphorous

Total phosphorus concentration was determined spectrophotometrically by the molybdo-vanadate reagent after ashing of the sample with HCL according to AOAC, 1995.

Phytate phosphorus was derived by using the following formula:

Phytate phosphorus, mg = A X 28.18/ 100

Where A = the phytate content (mg)

Non-phytate Phosphorus

Non-phytate Phosphorus was calculated as the difference between Total Phosphorus – Phytate Phosphorus.

3.2.3.3 Oxalates

Oxalate was estimated by the method of Day and Underwood 1986.

To 1 g of the ground powder, 75 ml of 15 N H_2SO_4 was added. The solution was carefully stirred intermittently with a magnetic stirrer for 1 hr and filter using Whatman No. 1 filter paper. 25 ml of the filtrate was then collected and titrated against 0.1 N $KMnO_4$ solution, till a faint pink color appears that persists for 30 seconds.

3.2.3.4. Tannins

Sample (100 mg) was mixed with 40 ml distilled water. The suspension was then boiled for 30 min cooled and subsequently centrifuged at 2000 rpm for 10 min and used as a source for tannin estimation. Tannins were estimated using Folin-Denis reagent. After extraction, 1 ml of the clear supernatant was used as a source of tannins and to this 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solution were added followed by dilution to 100 ml with water. The tubes were incubated at room temperature for 30 min and the color thus developed was read at 700nm using a spectrophotometer.

3.2.3.5 Alkaloids

Five grams of the dried powder of each sample was weighed into a 250 ml beaker and 200 ml of 10 per cent acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath until it reaches to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated.

3.2.3.6 Saponins

Twenty grams of sample was placed into a conical flask and 100 ml of 20 per cent aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml 20 per cent ethanol. The combined extracts was reduced to 40 ml over water bath at 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10 ml of 5 per cent aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples was dried in the oven to a constant weight and saponin content was calculated as percentage.

3.2.3.7 Trypsin Inhibitor Activity

Trypsin inhibitor activity in the samples was determined by the method given by Roy and Rao 1971.

Reagents

0.1 M phosphate buffer (pH 7.6): Sodium dihydrogen phosphate (16 ml) (0.2 M) and 84 ml Disodium hydrogen phosphate (0.2 M) was diluted to 200 ml with distilled water and pH was adjusted to 7.6.

0.05 M phosphate buffer (pH 7.0): 50 ml of 0.1 M phosphate buffer was diluted to 100 ml with distilled water and the pH adjusted to 7.0.

2 % casein: A suspension of 2 g casein was prepared with phosphate buffer (0.1 M, pH 7.6) and dissolved by warming on steam bath for 10 minutes. The cooled solution was made to 100 ml with phosphate buffer and stored in the refrigerator.

Trypsin solution (5 mg/ml): 125 mg Trypsin (20,000 F gross units/g) was dissolved in 20 ml phosphate buffer (0.1M, pH 7.6)

Trichloroacetic acid solution (5 %): 5 g TCA was dissolved in water to make 100 ml.

Procedure

Extraction

To 5 g sample taken in conical flask add 25 ml of 0.05 M phosphate buffer (pH 7.0). The contents was shaken for 3 hrs and centrifuged for 10,000 rpm for 20 minutes. The following sets of incubation mixture was prepared.

Phosphate	Test	Control	Blank
Buffer (0.1M pH 7.6)	0.1 ml	1.1 ml	1.0 ml
Trypsin (5 mg/ml)	0.5 ml	0.5 ml	0.5 ml
HCl (0.001N)	0.4 ml	0.4 ml	0.4 ml
TCA (5 per cent)	-	-	6.0 ml
Casein (2 per cent)	2.0 ml	2.0 ml	2.0 ml
Extract incubated at 37 ^{o}C for 20 minutes	1.0 ml	-	0.1 ml
TCA (5 per cent)	6.0 ml	6.0 ml	-

Protein Determination

After incubation the contents was centrifuged at 10,000 rpm for 10 minutes. The TCA soluble proteins was determined by the method of Lowry et al. (1951).

Reagents

A: 2 % Na_2CO_3 in 0.1 N NaOH

B: 0.5 % copper sulphate in 1% sodium citrate solution

Alkaline copper sulphate solution: 50 parts of solution A and 1 part of solution B was mixed just before use.

Folin phenol reagents: as indicator

Determination: To 0.5 ml of supernatant 5 ml alkaline copper sulphate solution was added, mixed thoroughly and allowed to stand for 10 minutes at room temperature. After this, 0.5 ml Folin phenol reagent (double diluted the original reagent) was added

and immediately mixed. Water blank was also run side by side. The color intensity was read after 30 minutes at 520 nm.

For preparing standard curve 0.1 ml of 0.5 ml casein solution (400 µg/ml) was taken.

Trypsin inhibitor units: One unit of trypsin was defined as amount of enzyme which converted 1mg casein to TCA soluble component at 37^0 C for 20 minutes at 7.6 pH. One unit of inhibitory activity is that which reduces the activity of trypsin by one unit under assay condition.

3.2.3.8 Total phenolic content

Total phenolic content (TPC) was assessed according to method described by Ainsworth and Gillespie, (2007) using Folin-ciocalteau reagent. Sample extract (0.5ml) was diluted and volume was made upto 1ml. After 2 minutes 2ml 0f 10% folin-ciocalteau reagent and vortex thoroughly. At sixth minute, add 8 ml of 700mM Na_2CO_3 and incubate the mixture for 2 hours. Transfer 2 ml of mixture to quartz cuvette and read the absorbance at 765nm using spectrophotometer (Shimadzu, Japan).The readings were compared to gallic acid standard curve (linearity range 50-250 mg/ml and R^2=0.991). Final total phenolic content expressed as mg of gallic acid equivalent (GAE) per 100 g i.e. mg GAE/100g

3.2.3.9 Total flavonoid

Total flavonoid was determined according to procedure followed by Carciochi et al., 2014a. Quinoa extract (0.5ml) was taken in a test tube. To the test tube 4 ml of distilled water and 0.5 ml of 20% NaNO2 (Sodium nitrite) was added. Mixture was allowed to stand for about 5 minutes and then 0.3ml of 10% $AlCl_3$ (Aluminium Chloride) was added. After 1 minute 0.5 ml 0f 2M NaOH (Sodium hydroxide) was added to the reaction mixture. Absorbance was read at 510nm using spectrophotometer (Shimadzu, Japan). Quercitin was used as standard. Results were expressed as mg of quercitin equivalent/100g i.e. mg QE/100g.

3.2.4 *In vitro* tests

3.2.4.1 Protein *in vitro* digestibility

Digestibility of proteins was determined by an *in vitro* method according to Hsu et al., 1977. The multi-enzymatic method is based on the decrease of pH during 10 minutes. The percentage of digestibility was calculated using the Equation:

$$Y = 210.464 - 18.103\,X \quad (3)$$

Where: X = pH of the protein suspension after 10 minutes of digestion and Y = percentage of protein hydrolysis.

3.2.4.2 *Starch in vitro digestibility*

The digestibility of starch was determined by an *in vitro* method according to Holm et al. (1985). Starch (500 mg) was mixed with phosphate buffer (pH 6.9) and incubated with α-amylase at 37 °C for 1 hour. The sugars released was determined by spectrophotometry.

3.2.4.3 *In vitro* antioxidant activity

In vitro antioxidant activity of samples was determined by following 2 methods:

a) 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) assay

DPPH i.e. 2,2,-diphenyl-2-picryl-hydrazyl assay was proceeded according to method followed by Jubete et al. (2010b) and Sun and Ho, (2005) with some modification. Aliquots of quinoa extract, in increasing trend, (i.e. 100, 200, 400, 800, 1000 µl) were taken for serial dilution. Diluted quinoa extracts (1 ml) from each serial dilution was added to cuvettes respectively. DPPH solution of concentration 200µM (absorbance 1.4) was freshly prepared and 1 ml of this solution was added to each cuvette containing quinoa extract. The mixture was vortexed and incubated in dark for 30 minutes. Absorbance was measured at 517 nm using spectrophotometer (Shimadzu, Japan). DPPH was expressed as mgTE/100g. Trolox (0.02M) was used as standard.

b) Ferric reducing ability of plasma (FRAP) assay

FRAP i.e. Ferric reducing ability of plasma assay was proceeded according to procedure followed by Jubete et al., 2010b and Benzie and Strain, 1996 with some modification. FRAP reagent was prepared by mixing 2.5 ml of 0.01M TPTZ in 0.04M hydrochloric acid, 2.5ml of 0.02M ferric chloride and 25 ml of 0.3M sodium acetate buffer (pH 3.6). Quinoa extracts (100µl-300µl) and 2ml of FRAP reagent was taken in a 5ml volumetric flask. Distilled water was used to make up the volume. Solutions were kept in dark at 37°C for 60 minutes. Absorbance was read at 595nm using spectrophotometer (Shimadzu, Japan). Trolox stock solution of 0.02M was used as standard for the assay FRAP reagent (2ml), made up to 5 ml in a volumetric flask was used as blank.

3.2.4.4 Statistical analysis

The experiments were performed in triplicates and the data was expressed as mean±standard deviation. The data was analyzed on Microsoft office excel, 2007 and Graphpad prism 5 software (La jolla, CA, USA). Means were compared using one way analysis of variance (ANOVA) followed by Tukeys multiple comparison test for comparison between means. The values were considered significant at $p \leq 0.05$.

Objective 2: To assess cholesterolemic effect of *Chenopodium quinoa*

To accomplish objective two of the study was accomplished by a biological trial using animal model

3.2.5 Biological trial

Forty two (42) adult male albino rats, wistar strains, weighing 252± 5 g, aged 28 days, were housed individually in stainless steel mesh cages. They were fed on standard diet, AIN 93G for 10 days before experiments begun (Adaptation period). The Institutional Animal Ethics Committee at Lovely Professional University approved the protocols for animal experiments.

The diets were prepared every week in the laboratory. The water and diets was given *ad libitum*. Induction of hypercholesterolemia was carried out on 4 groups (G2-5; 24). The animals (42) were divided into 7 groups (6 rat/ group) as follows:

Table 3.1: Experimental grouping for biological trial

Group 1	Rats fed on basal diets (Control group, negative control)
Group 2	Hypercholesterolemic rats (positive control)
Group 3	Hypercholesterolemic rats fed on basal diet supplemented with hypocholesterolemic drugs (Statin)
Group 4	Hypercholesterolemic rats fed on basal diet supplemented with Chenopodium (Unwashed)10%
Group 5	Hypercholesterolemic rats fed on basal diet supplemented with Chenopodium (debittered) 10 %
Group 6	Normal rats fed on basal diet supplemented with Chenopodium (Unwashed) 10 %
Group 7	Normal rats fed on basal diet supplemented with Chenopodium (debittered) 10 %

The standard atrovastatin tablets were procured from Lupin Pharmaceuticals Limited, Tarapur, India. At the end of the experimental period (6 weeks after treatment), rats were fasted over night before the blood was collected. The blood was collected in

tubes coated with EDTA through retro orbital puncture. It was then centrifuged; serum was separated and stored at -80°C until analysis.

3.2.5.1 Blood Lipid Profile

The serum total cholesterol (TC), serum high density lipoprotein cholesterol (HDL-C) level, and serum triacylglycerol (TG) level were determined using colorimetric enzymatic kits.

The VLDL+LDL were calculated by an equation reported previously by Ibrahim et al., 2005:

VLDL+LDL cholesterol = total cholesterol-HDL cholesterol.

The atherosclerotic index was calculated by an equation reported previously by Ibrahim et al., 2005:

Atherosclerotic index = (VLDL+LDL-cholesterol)/ (HDL-cholesterol)

3.2.5.2 Statistical analysis

The experiments were performed in triplicates and the data was expressed as mean±standard deviation. The data was analyzed on Microsoft office excel, 2007 and Graphpad prism 5 software (La jolla, CA, USA). Means were compared using one way analysis of variance (ANOVA) followed by Tukeys multiple comparison test for comparison between means. The values were considered significant at $p \leq 0.05$.

Table 3.2 Composition of Experimental Diets (g/100 g)

Ingredients	Control diet	Control diet with cholesterol	Control diet with cholesterol and statin	Control diet with cholesterol and raw *Chenopodium quinoa*	Control diet with cholesterol and debittered *Chenopodium quinoa*	Control diet with raw *Chenopodium quinoa*	Control diet with debittered *Chenopodium quinoa*
Casein	10	10	10	9.38	9.28	9.38	9.28
Ground nut oil	10	10	10	9.78	9.82	9.78	9.82
Sucrose	10	10	10	10	10	10	10
Cellulose	5	5	5	4.64	4.68	4.64	4.68
Mineral mixture	4	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1	1
Cholesterol	0	1	1	1	1	0	0
Starch	60	59	59	55.2	55.2	56.2	56.2
Quinoa	0	0	0	5	5	5	5

* All diets contained 10% protein including the crude protein from C*henopodium quinoa*
** All diets contained 10% fat including the crude fat from C*henopodium quinoa*
*** All diets contained 5% fibre including the crude fibre from *Chenopodium quinoa*

Table 3.3 Composition of Mineral Mixture (AIN 93G)*

Ingredients	g/kg mineral mixture
Calcium Carbonate	357
Sodium chloride	74.00
Potassium citrate monohydrate	70.78
Potassium sulphate	46.6
Magnesium oxide	24.00
Ferric citrate	6.06
Magnesium carbonate	0.63
Zinc carbonate	1.65
Cupric carbonate	0.30
Potassium iodate	0.01
Sodium selenite pentahydrate	1.45
Chromium Potassium sulphate dodecahydrate	0.275
Sucrose	221.02
Boric acid	0.08
Sodium fluoride	0.06
Nickel carbonate	0.03
Ammonium vandate	0.006

*Based on the National Academy of Science recommended levels for rats (BARR committee on Animal Nutrition, 1972)

Table 3.4 Composition of Vitamin Mixture (AIN 93G)*

Ingredients	g/kg vitamin mixture
Thiamine hydrochloride	0.6
Riboflavin	0.6
Pyridoxine hydrochloride	0.7
Nicotinic acid	3.0
Calcium pantothenate	1.6
Folic acid	0.2
Biotin	2
Retinol acetate	0.8
Cholecalciferol	1
D-alpha tocopherol	To provide 5000 IU of Vitamin E activity
Cyanocobalamine	2.5
Sucrose	To make 1000 g

*Based on the National Academy of Science recommended levels for rats (BARR committee on Animal Nutrition, 1972)

Objective 3: To develop and analyze value added gluten free products from *Chenopodium quinoa*

To accomplish objective three functional foods, namely, quinoa bar, quinoa cracker and quinoa beverages were prepared from quinoa grains and evaluated for proximate composition, total phenolic content and antioxidant activity. Sensory evaluation and storage studies of functional food products developed in this study was also done. Beverages prepared from quinoa were analyzed, additionally, for pH, viscosity, total soluble solids, and serum separation.

3.2.6 Development of functional food products

3.2.6.1 Development of snack Bar

Quinoa snack bar was prepared according to method followed by Sharma and Mridula, 2015, with slight modifications.

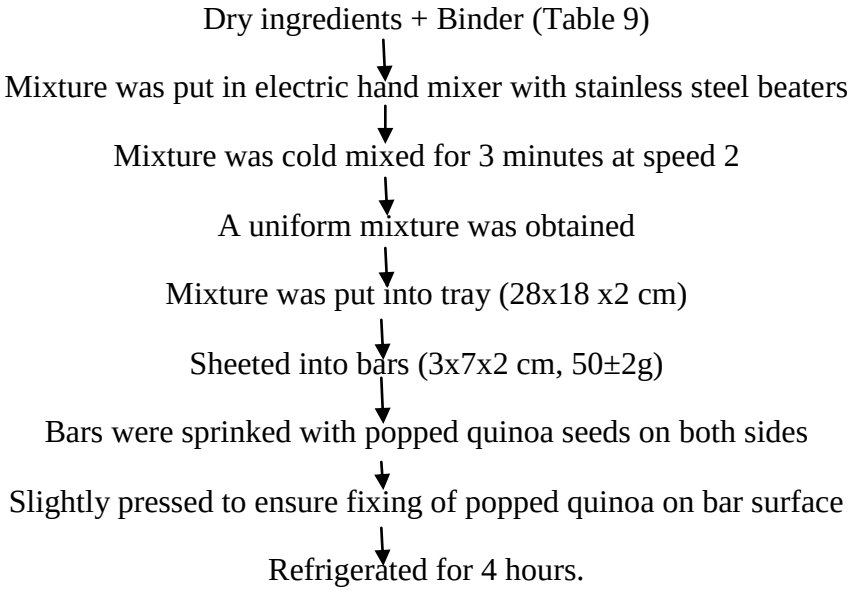

Figure 3.2: Flowchart for method of preparation of quinoa cereal bar

The prepared snack bars were analysed for

a) **Proximate compostion** as described in 3.2.1

b) **Total phenolic content** as described in 3.2.3.8

c) **Antioxidant activity** as described in 3.2.4.3

d) **Sensory evaluation**:

Sensory evaluation was carried out by serving bars to 20 untrained panel of judges for evaluating different sensory attributes like appearance, color, texture, flavor, mouth feel, taste and overall acceptability on a 9-point Hedonic scale grading 9 for extremely like and 1 for extremely disliked samples.

e) Storage study: Formulated snack bars were carefully packed in zip lock bags and stored in refrigerator for 30 days. Bars were evaluated for different parameters (a to e) at an interval of 15 and 30 days

Table 3.5: Proportion of dry ingredients and the binders used for preparation of snack bars

Ingredients	Quantity (%)	
	Control Bar	Quinoa Bar (QB)
Dry Ingredients		
Oat flakes	15	15
Roasted chickpea flour	50	25
Germinated quinoa flour	-	20
Coco powder	5	5
Sugar	5	5
Popped quinoa seeds	-	5
Binders		
Honey	10	10
Vegetable oil	10	10
Water	5	5

3.2.6.2 Development of crackers

The crackers were prepared according to the method described in table below

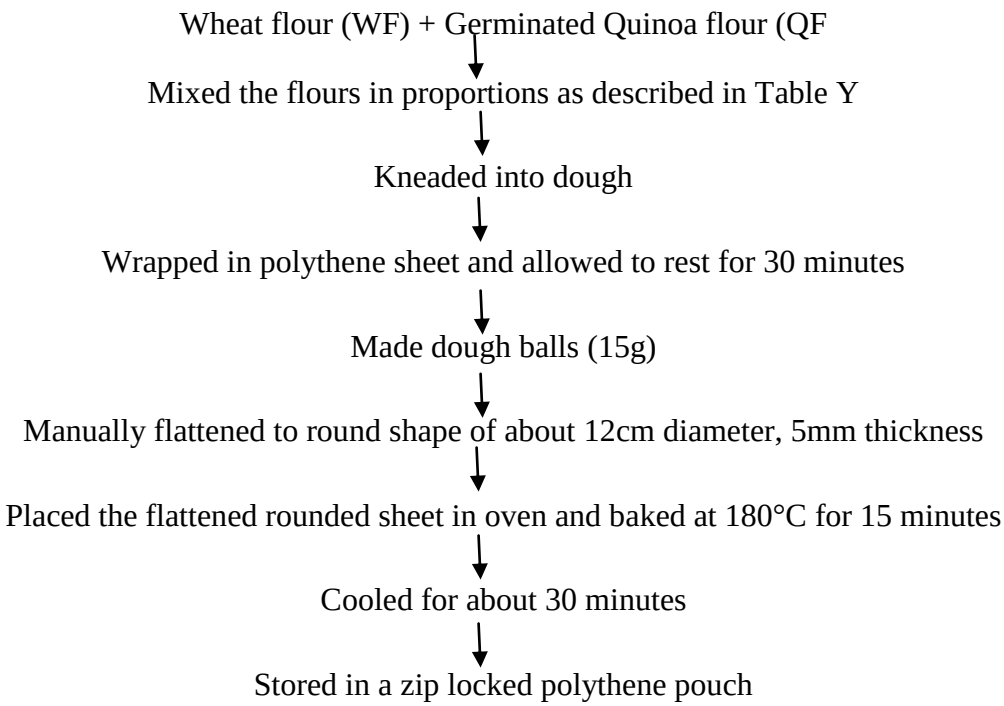

Figure 3.3: Flowchart for method of preparation of cracker (*Khakhra*) incorporated with 20 and 40% quinoa flour

Table 3.6: Basic formulation of quinoa cracker using all ingredients

Ingredients (g)	T_0 (Control)	T_1 (80:20)	T_2 (60:40)
WF	150	120	90
QF	0	30	60
Cumin seeds	5	5	5
Salt	5	5	5
Oil	3	3	3
Turmeric Powder	1.5	1.5	1.5
Baking Powder	2	2	2
Water (ml)	40	40	40

Table 3.7: Substitution ratio of wheat flour and quinoa flour for preparation of quinoa cracker (*Khakhra*)

Sample	Wheat flour (WF) (%)	Quinoa flour (QF) (%)
Control (T_0)	100	0
Quinoa cracker (T_1)	80	20
Quinoa cracker (T_2)	60	40

The prepared crackers were analysed for

a) **Proximate compostion** as described in in 3.2.1

b) **Total phenolic content** as described in 3.2.3.8

c) **Antioxidant activity** as described in 3.2.4.3

d) **Sensory evaluation**:

Sensory evaluation was carried out by serving crackers to 20 untrained panel of judges for evaluating different sensory attributes like appearance, color, texture, flavor,

mouth feel, taste and overall acceptability on a 9-point Hedonic scale grading 9 for like extremely and 1 for disliked extremely samples.

e) Storage study:

Formulated snack bars were carefully packed in zip lock bags and stored in refrigerator for 30 days. Bars were evaluated for different parameters (a to e) at an interval of 15 and 30 days

3.2.6.3 Development of Beverages

Basic grain treatment prior to beverage preparation

Clean *Chenopodium quinoa* seeds were soaked for 10 minutes in 10% sodium hypochlorite solution (NaOCl) for surface sterilization and the washed with distilled water obtained from milli pore (Merck-Milli-Q® Direct 8 Water Purification System). The seeds were then steeped in 0.03 mol/L sodium chloride and maintaining pH 5.0 for better protein yield as described by Pineli et al., 2015. Further raw were used as such, soaked, and germinated for production of their respective quinoa beverage.

Soaked quinoa seeds: Quinoa seeds were soaked in milli Q water for 24 hours at room temperature. Water was changed every 8 hours. Soaked seeds were further processed on the same day for beverage preparation.

Germinated quinoa seeds: Quinoa seeds soaked in milli Q water for 24 hours, were spread on to petri dishes layered with filter paper dipped in 3ml distilled water and incubated at 20°C in an incubator (Biotechnics, India) for 72 hours (Carciochi, 2014b). Water was changed and checked for dryness every 6 hours. Germinated seeds were further processed on the same day for beverage preparation.

Preparation method for raw, soaked and germinated quinoa beverages

Basic process for preparation of raw, soaked, and germinated beverages was followed according to Ma et al., 2015 with some modifications. Flowchart representation of the preparation process has been described in Figure 1. All quinoa beverages were stored at 4°C for further analysis.

The prepared beverages were analysed for

a) **Physical analysis**

pH was measured using a digital pH meter at 20°C.Total soluble solids were measured according to method followed by Kim et al., 2012. Viscosity was measure using a rotational viscometer (Cole-Parmer Basic Viscometer, Cole-Parmer India Pvt. Ltd,

India). To determine the effectiveness of xanthum gum, serum separation was assessed using a graduated cylinder (of volume 50 ml) according to method used by Koksoy and Kilic, (2004).

b) **Proximate compostion** as described in in 3.2.1

c) **Total phenolic content** as described in 3.2.3.8

d) **Antioxidant activity** as described in 3.2.4.3

e) **Sensory evaluation**

Beverages were assessed for organoleptic acceptance using a nine point hedonic scale, from extremely dislike to extremely like. The samples were randomly marked and served at room temperature in white paper cups to a semi-trained panel of 20 members. Commonly available commercial soya milk (Sofit natural unflavored soya milk, Hershey India Pvt. Ltd.) was used as a reference beverage to valuate acceptance. Panellists were asked about their favourite and least favourite beverage and also about positive and negative sensory aspects of each beverage.

CHAPTER 4

RESULT AND DISCUSSION

The results of the data collected for the study on nutritional evaluation of quinoa, its cholesterolemic effect in wistar rats and development of functional food products, were statistically analyzed, critically discussed, and presented in this chapter.

Objective 1: To evaluate the nutritional quality of *Chenopodium quinoa*

A) Nutritional evaluation of bittered and debittered (domestically and industrially processed) Indian *Chenopodium quinoa* seeds

4.1 Proximate Analysis of Indian *Chenopodium quinoa*

4.1.1 Moisture content

The moisture content of raw and processed quinoa seeds is shown in Table 4.1. The raw seeds were reported to have 11.30±0.08 g/100g of moisture content. The result was in agreement with the values reported by Nascimento et al., 2014. A significant ($P<0.05$) difference in moisture content of raw, domestically processed and industrially processed seeds was observed. Soaking resulted in increase in 8.5% of moisture content while germination resulted in decrease in moisture content by 17.5%. Similar trend of variation in moisture content after soaking and germination of chickpea has been reported by Desalegn, 2016. The increase in moisture content after soaking may be due to uptake of water by dry seed resulting in cell hydration and cell multiplication within the seed (Nonogaki et al., 2010) while decrease in moisture content on germination may be attributed to utilization of water in synthesis of metabolites (Chung et al., 2014). Industrial processing of quinoa led to 8.5% decrease in moisture content. This may be due to removal of hulls, during the process. Chauhan, 1992 reported 11.3% moisture content in quinoa hulls which account for about 8% of total seed weight. Lower moisture content is indicator of longer product shelf life (Sanni et al., 2006). Thus, the results suggest better shelf life of germinated quinoa seeds.

Table 4.1: Proximate composition of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		**Moisture (g/100g)**	**Carbohydrate (g/100g)**	**Crude fat (g/100g)**	**Crude protein (g/100g)**	**Ash (g/100g)**	**Crude fibre (g/100g)**
Raw		11.30±0.08[a]	65.11±0.12[a]	5.17±0.18[a]	12.54±0.03[a]	3.19±0.03[a]	2.62±0.01[a]
Domestically Processed	Soaked	12.26±0.34[b]	64.01±0.36[b,d]	4.29±0.28[b]	13.18±0.03[b, e]	3.21±0.16[a, c]	2.9±0.05[b]
	Germinated	9.29±0.15[c]	63.78±0.32[c, d]	3.9±0.04[c,b]	14.96±0.04[c]	3.92±0.05[b, d]	3.3±0.03[c]
Industrially processed		10.35±0.20[d]	64.90±0.09[a]	5.11±0.05[a]	13.11±0.08[d,e]	3.04±0.03[a]	2.49±0.04[d]

4.1.2 Crude Ash

The ash content of raw and processed quinoa seeds is shown in Table 4.1. The raw seeds were reported to have ash content of 3.19±0.03 g/100g which is similar to the ash content reported by Miranda et al., 2012 in different varieties of *Chenopodium quinoa* seeds. Soaking resulted in 0.6% increase in ash content which was statistically non significant ($P<0.05$) with respect to ash content of raw quinoa seeds while significant ($P<0.05$) increase in ash content, by 22.5%, was observed after germination. This may be due to decrease in carbohydrate (0.3 to 2%) and crude fat (15.7 to 32.5%) content of processed seeds which can be accounted for increase in ash content (Chaudhary and Vyas, 2014). Inayang and Zakari, 2008 stated activation of fitase on germination, resulting in hydrolysis of protein-enzyme bond and hence release of minerals as possible cause for increase in ash content on germination. Industrial processed seeds were reported to have 4.7% reduced ash content. The apparent reason behind decreased ash content can be removal of hulls which accounts for 8.5% of total ash content in quinoa seeds (Chauhan et al., 1992).

4.1.3 Crude protein

Crude Crude protein content of quinoa seeds is shown in Table 4.1. Raw quinoa seed was reported to have 12.54±0.03g/100g crude protein content. The result is similar to the protein content of unprocessed quinoa (12.06 g/100g) reported by Coehlo et al., 2007 and protein content of Moroccon quinoa (12.5g/100 gm) reported by Marmouzi et al., 2015. Crude protein content of raw, domestically processed and industrially processed quinoa seeds was significantly ($P<0.05$) different. Soaking and germination led to increase in protein content by 4.8% and 19.2%. Increase in protein content after soaking was also reported in soyabeans by Kayembe and Rensburg, 2013. Nutritive value of cereals is known to enhance after germination (Hubner and Arendt, 2013). Moongngarm and Sateung, 2010 reported 29% increase in protein content of germinated rice. The result is also supported by findings of Inyang and Zakari (2008) in germinated peal millet. This increase in protein content may be due to increased activity of protease leading to degradation of peptides to amino acids and further synthesis of new protein (Laetitia et al., 2014). Increase in protein content of industrially processed quinoa seeds was similar to the increase reported after soaking. As most protein content

of quinoa is located in embryo (Prego et al., 1998), increased protein content of industrially processed seeds as compared to raw seeds may be due to removal of hulls and concentration of protein in embryo (Ghavidel and Prakash, 2007).

4.1.4 Crude fat

Crude fat content of Indian quinoa is shown in Table 4.1. Raw Indian quinoa had 5.17±0.18 g/100g crude fat content which falls within the range of fat content for various quinoa varieties reported by Miranda et al., 2013. Soaking and germination of raw seeds caused significant ($P<0.05$) decrease in fat content by 20.5% and 32.5%. Similar decrease in fat content has been reported in germinated sesame seeds by Kajihausa et al., 2014. Seed growth, because of water imbibition by cells on soaking, consumes required energy from fat, a major carbon source in seeds, which may lead to decrease in fat content after soaking (Rumiyati et al., 2012). Germination of seeds leads to metabolite synthesis. This metabolic change requires energy which is liberated by oxidation of fatty acid resulting in reduced fat content in germinated seeds (Hahm et al., 2009). Industrially processed seeds had 1.6% reduced fat content as compared to the raw seeds. This may be due to removal of hulls which contain 5.7% of crude fat. No significant ($P<0.05$) difference was observed in change in fat content of soaked, germinated and industrially processed quinoa seeds. The results reveal chances of good oil yield from raw quinoa seeds as compared to soaked, germinated and industrially processed seeds.

4.1.5 Crude fiber

The crude fiber content of raw and processed quinoa seeds is shown in Table 4.1. Raw quinoa seeds had crude fiber content of 2.22±0.01 g/100g. The fiber content of all quinoa seeds were significantly ($P<0.05$) different. Soaking led to 31.8% increase in fiber content and germination caused further increase by 13.7%. The findings are supported by increase in fiber content on germination of chickpea, cowpea and mungbean as reported by Uppal and bains, 2012. Synthesis of insoluble fibers, which are constituents of cell wall, namely cellulose and hemicelluloses may be the cause for increase in fiber content after germination (Pandey and Awasthi, 2013). Industrial processing led to 7.6% decrease in fiber content. Chauhan et al., 1992 reported hulls to account for 5.6% fiber content in

quinoa seeds. Decrease in ash content may be attributed to the fact that industrial processing leads to removal of hulls, resulting in decreased fiber content of seeds. Decrease in fiber content after germination has also been reported by Blessing and Gregory, 2010.

4.1.6 Carbohydrate

The carbohydrate content of quinoa seeds is shown in Table 4.1. Carbohydrate content of raw quinoa seeds was 65.11±0.12 g/100g, which is in accordance with the value reported reported by Marmouzi et al., 2015. The results were significantly ($P<0.05$) different between raw and domestically processed seeds. However no significant difference ($P<0.05$) in carbohydrate content of seeds subjected to soaking and germination was observed. Soaking and germination resulted in decrease of carbohydrate content by 1.6% and 2% respectively. This decrease may be due to activation of α-amylase in quinoa seeds and breakdown of starch to simple sugars on hydration during soaking and germination (Rosa et al., 2009). Although non significant ($P<0.05$) but 0.3% decrease in carbohydrate content of industrialy processed seeds was observed. Also, the carbohydrate content of industrially processed and domestically processed seeds was significantly ($P<0.05$) different. Industrial processing of grains leads to removal of hulls (Slavin, 2003). Chauhan, 1992 reported that quinoa hulls account for 55% carbohydrate content. Hence lower carbohydrate content of industrially processed seeds may be because of removal of hulls. Decrease in carbohydrate content after dehulling was also reported by Makinde and Akinoso, 2013.

4.2 Nutritional analysis of Indian *Chenopodium quinoa*

4.2.1 Dietary fiber

Total dietary fiber content of quinoa seeds is shown in Table 4.2 . Raw quinoa seeds were reported with 10.26±0.17 g/100g of dietary fiber content. The results are similar to the dietary fiber content in quinoa seeds as reported by Lamothe et al., 2015. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) constituted about 71.9 to 73.3% and 26.6 to 28% of total dietary fiber, respectively. The results correspond well to the findings of Marmouzi et al., 2015 who reported 72.03% NDF and 27.06% ADF in Moroccan quinoa seeds. Different processing methods have different effect on dietary fiber content of foods

(Dhingra et al., 2012). Domestic processing of seeds i.e. soaking and germination, led to 10% and 31% significant increase ($P<0.05$) in TDF content of quinoa seeds, respectively. Both the constituents of dietary fiber i.e. NDF (Lignin, cellulose and hemicellulose) and ADF increased upon soaking and germination. Increase in dietary fiber contents upon soaking and germination has also been previously reported in cereals and legumes (Vasishtha and Srivastava, 2013, Benitez et al., 2013 and Megat et al., 2016). The increased content may be due to enlargement of cell body and growth initiation upon water imbibition during soaking and germination (Martin-Cabrejas et al., 2003). Industrially processed seeds exhibited 9.9%, significant decrease ($P<0.05$) in TDF content. Pushparaj and Urooj, 2011, have also reported detrimental effect of industrial processing on dietary fiber in pearl millet.

4.2.2 Vitamin C

The vitamin C content of Indian quinoa seeds is shown in Table 4.2. Vitamin C content of raw quinoa seeds was found to be 13mg/100g, which is within the range of vitamin c content as reported by Miranda et al., 2010 (12-23mg/100g) and is close to the vitamin C content of Cahuil variety (13.8 mg/100g) among six chilean quinoa ecotypes studied in his study. The value reported in this study is greater than the values reported by Koziol, 1992 (4mg/100g) and less than as reported by Ruales and Nair, 2002 (16.4 mg/100g), Miranda et al., 2013 (22-31 mg/100g) in two quinoa genotypes from Temuco and Vacuna localities in Chile. This difference may be due to different environmental and storage conditions, as factors like light intensity, amount of nitrogen fertilizers, frequency of irrigation and temperature of the region strongly affect the vitamin C content in crops (Lee and Kader, 2000).

Also there is significant difference in vitamin C content of raw and domestically processed Indian quinoa seeds ($P<0.05$). As depicted in this study, vitamin C content increased by 15% in soaked quinoa seeds and by 46% in germinated quinoa seeds. Higher increase in germinated seeds observed might be due to synthesis of vitamin C during the process of germination (Sattar et al., 1995 and Fernandez-Orozco et al., 2006). Tang et al., 2015 reported vitamin C content in sprouted mungbean where as nil vitamin C content was reported in raw seeds, which confirms vitamin C synthesis during germination process.

Table 4.2 Nutritional composition of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		**Dietary fiber (g/100g)**						**β- Carotene (µg/100g)**	**Vitamin C (mg /100gm)**
		NDF	**ADF**	**Lignin**	**Cellulose**	**Hemi-cellulose**	**Total dietary fibre**		
Raw		7.38±0.07	2.88±0.02	2.4±0.04	3.6±0.03	1.38±0.01	10.26±0.17[a]	535.9±3.6[a]	13.43±0.4[a]
Domestically Processed	**Soaked**	8.24±0.11	3.05±0.02	2.6±0.04	3.9±0.03	1.74±0.03	11.29±0.23[b]	536.4±2.07[a]	15.09±0.17[b]
	Germinated	9.87±0.06	3.59±0.03	2.8±0.02	4.2±0.06	2.87±0.02	13.46±0.19[c]	540.6±1.5[a]	19.38±0.28[c]
Industrially processed		6.69±0.03	2.50±0.07	2.2±0.02	3.3±0.04	1.19±0.05	9.24±0.21[d]	535.6±2.8[a]	9.45±0.35[d]

NDF: Neutral Dtergent Fiber; ADF: Acid Detergent Fiber

Increased vitamin C content in germinated Indian quinoa seeds is also supported by the findings of Khattak et al., 2007 where linear relationship was observed between germination time and content of vitamin C in chickpea seeds. In addition, a significant difference in Vitamin C content of raw and industrially processed seeds was also observed. Industrial processing decreased the vitamin C content by 30%. De hulling, pearling, shelling etc are post harvest industrial treatments applied to cereal grains, which lead to loss of their nutritional content (Singh and Jambunathan, 1990).

4.2.3 β- Carotene

The β- Carotene content of quinoa seeds is shown in Table 4.2. Raw quinoa seeds were reported to have 535.9±3.6 µg/100g of β- Carotene content. The results are in accordance with the β- Carotene content in *Chenopodium quinoa* as reported by Sharma et al., 2012. Domestic and industrial processing of seeds led to non-significant change ($P<0.05$) in contents of β- Carotene. Soaking and germination led to 0.1% and 0.8% increase in β- Carotene, respectively. Increase in β- Carotene upon soaking and germination has also been reported by Luthriya and Singh, 2014 and Suryanti, 2016. Lee et al., 2013 also repored increased β- Carotene contents in soyabean sprouts as compared to the seeds. This may be attributable to the fact that β- Carotene content in cereals and pulses is directly proportional to the growth progression in the seed (Ahn et al., 2012). Industrially processed seeds exhibited almost similar content of β- Carotene as compared to the raw seeds.

4.2.4 Mineral Content

Mineral content of Indian quinoa seeds is reported in Table 4.3 . Minerals like calcium, iron, zinc and magnesium were assessed in raw, domestically processed and industrially processed Indian quinoa seeds. Raw quinoa seeds were reported with 85.3±0.25 mg/100g calcium content. The results are in accordance with the values of calcium content (44 to 110 mg/100g) in quinoa seeds reported by Miranda et al., 2013 and Nascimento et al., 2014. Domestic processing of seeds i.e. soaking and germination led to 0.59% and 0.94%, non-significant increase ($P<0.05$) in calcium content, respectively, which is supported by findings of Chaparro et al., 2011. Hahm et al., 2009 also reported 0.7% increase in calcium

content of sesame seeds after germination. The iron content in raw Indian quinoa seeds was 5.2±0.01 mg/ 100g. The results are in agreement with the findings of Nascimento et al., 2014 who reported 5.4 g/100g iron content in quinoa seeds. A significant increase (P<0.05) of 1.9% and 13.4% was observed in quinoa seeds subjected to domestic processing methods i.e. soaking and germination, respectively. Chaparro et al., 2011 also reported a similar post germination increase of 11.4% in iron content of quinoa seeds. The apparent reason, for increase in calcium and iron content, may be the decrease in phytic acid content post domestic processing as reported in this study. Phytic acid is known to bind with minerals to form insoluble mineral-phytate complexes and thus, making them less bio-available for proper utilization in body (Coulibaly et al., 2010).

Table 4.3: Mineral content of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		**Calcium (mg/100g)**	**Iron (mg/100g)**	**Zinc (mg/100g)**	**Magnesium (mg/100g)**
Raw		85.3±0.25 a	5.2±0.01 a	6.6±0.04 a	182.4±0.11 a
Domestically Processed	**Soaked**	85.8±0.70 a	5.3±0.02 b	5.9±0.12 b	180.2±0.14 b
	Germinated	86.1±0.05 a, b	5.9±0.01 c	5.7±0.06 c	179±0.16 c
Industrially processed		85.06±0.15 a, c	5.1±0.02 d	6.5±0.13 a	182.2±0.15 a

The zinc content of raw quinoa seeds was 6.6±0.04 mg/100g. The results lie within the range of zinc content in quinoa seeds (2.9 to 9.5 mg/100g) reported by Miranda et al., 2013 and Nascimento et al., 2014. Zinc content was significantly reduced (P<0.05) by 10.6% and 13.5% after soaking and germination, respectively. Afiffy et al., 2012 also reported 14% and 20% reduction in zinc content of sorghum seeds post soaking and germination. Raw quinoa seeds were reported to have magnesium content of 182.4±0.11 mg/100g. The values are consistent with the magnesium content in quinoa seeds (176 to 192 mg/100g) reported by Miranda et al., 2010, Gonzales Martin et al., 2014 and Marmouzi et al., 2015. Domestic processing methods like soaking and germination led to 1.21% and 1.8% significant decrease (P<0.05) in magnesium content,

respectively. Decrease in magnesium content after germination has also been reported in black beans by Sangronis and Machado, 2007. The apparent reason behind decrease in zinc and magnesium contents may be lixiviation of minerals into soaking media during domestic processing of seed.

Overall, industrially processed seeds exhibited lower mineral content as compared to the raw seeds. A non-significant reduction ($P<0.05$) was observed in calcium (0.3%), zinc (1.5%) and magnesium (0.1%) content of industrially processed seeds while a significant depreciation ($P<0.05$) was observed in iron (2%) content. Konishi et al., 2004, in their study related to depiction of mineral distribution in quinoa stated an industrial processing technique, abrasion, as a potent cause of calcium loss from quinoa seeds, as the latter is located in pericarp, which is usually removed during the process. The study also supports non-significant reduction in magnesium content as the latter is located in the embryo of the seeds and hence, least affected by the industrial processing methods. The observed reduction in zinc and iron contents of industrially processed seeds are supported by the findings of Pal et al., 2016 who reported losses in Zn and Fe content of dehulled horsegram.

4.3 Phytochemical analysis of Indian *Chenopodium quinoa*

4.3.1 Phytic acid

The phytic acid content of raw and processed quinoa seeds is shown in Table 4.4. Raw quinoa seeds were reported to have 1.25±0.22 g/100g phytic acid. The results were similar to the phytic acid content reported by Ruales and Nair, 2002 (1g/100g) and Valencia-Chamorro, 2003 (1.18 g/100g). Soaking resulted in reduction of phytic acid content by 2.5%, which was statistically non-significant ($P<0.05$) as compared to raw seeds. In quinoa seeds, phytic acid is present in seed coat as well as the embryo (Konishi et al., 2004). The reason behind the decrease in phytic acid content after soaking may be leaching of the same in the soaking media (Vadivel et al., 2011). Liang et al., 2008, also reported reduction in contents of phytic acid in brown rice upon soaking. Germination led to significant decrease ($P<0.05$) in phytic acid content by 68%. The present results are supported by findings of Ibrahim et al., 2002, who reported reduction in phytic acid content of cereals after germination. The decrease may be due to increased activity of enzyme, phytase, upon germination, which hydrolyzes phytic acid to release

phosphorous (Kumar et al., 2013). Pal et al., 2016 have also reported decrease in phytic acid content of horsegram after germination. Industrial processing led to non- significant decrease ($P<0.05$) in phytic acid content by 3.2% which might be due to removal of seed coat during the process. Decrease in phytic acid content after industrial processing has also been reported by Ghavidel and Prakash, 2007.

4.3.2 Total phosphorous

Total phosphorous content of quinoa seeds is indicated in Table 4.4. Total phosphorous content of raw Indian quinoa seeds was 0.43±0.22 g/100g. The result is in accordance with Rosero et al., 2013 who reported 0.44-0.5g/100g total phosphorous content in four different varieties of quinoa seeds. Soaking resulted in a non-significant increase ($P<0.05$) in total phosphorous content by 7%. The result coincides with the non-significant reduction ($P<0.05$), in phytic acid content of quinoa seeds as reported earlier. Hydration of seeds leads to activation of enzyme phytase and thus the release of inorganic phosphorous as consequence of phytic acid degradation (Kumar et al., 2013). The results are in accordance with the findings of El-Hady and Habiba, 2003. Germination of quinoa seeds was observed to cause a significant increase ($P<0.05$) in total phosphorous content. The results correlate well with the significant decrease ($P<0.05$) in phytic acid content as reported earlier, based on the fact that increased activity of phytase during germination, breaks down phytic acid to release phosphorous (Sung et al., 2005). Increase in total phosphorous content of cereals upon germination has also been reported by Azeke et al., 2011. Konishi et al., 2004 reported that about 60% of phytic acid is localized in embryo of quinoa seeds, and is a major indicative of phosphorous. As phytic acid disintegrates upon soaking and germination release of in organic phosphorous results in increase of total phosphorous. Industrially processed seeds exhibited 4.6% increase in total phosphorous content, which was non-significant ($P<0.05$) as compared to the total phosphorous content of raw seeds. This may be due to decrease in phytic acid content as reported earlier. The results infer better phosphorous bioavailability of germinated quinoa seeds because of increased phytase activity and phytic acid breakdown (Baruah et al., 2007).

Table 4.4: Phytochemical composition of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		Total phosphorous (g/100 g)	Phytic acid/ Phytate (g/100g)	Phytate phosph-orous	Non phytate phosphorous	Saponin (g/100g)	Trypsin Inhibitor Activity TIU/100g	Oxalates (g/100g)	Alkaloids (g/100gm)	Tannins (g/100g)
Raw		0.43±0.22[a]	1.25±0.22[a]	0.35	0.8	2.01±0.15[a]	6633±7.5[a]	0.2±0.02 [a]	2.11±0.01[a]	0.6±0.08[a]
Domestically Processed	**Soaked**	0.46±0.06[a]	1.22±0.14[a]	0.34	1.94	1.52±0.19[b]	6321±10[b]	0.14±0.03[b]	2.09±0.04 [a,c]	0.33±0.03[b]
	Germinated	0.68±0.07[b]	0.40±1.21[b]	0.11	3.41	0.03±0.01[c]	5116.3±5.6[c]	0.09±0.02 [c]	1.78±0.00 [b,c]	0.23±0.03[b]
Industrially processed		0.45±0.01[a]	1.21±0.31[a]	0.26	1.62	0.06±0.02[d, c]	5254.6±11.5[d]	0.18±0.04 [d]	1.9±0.02 [b,c]	0.35±0.0[b]

significant decrease ($P<0.05$) in phytic acid content by 3.2% which might be due to removal of seed coat during the process. Decrease in phytic acid content after industrial processing has also been reported by Ghavidel and Prakash, 2007.

4.3.3 Oxalates

Total oxalate content of quinoa seeds is shown in Table 4.4. Total oxalate content of raw Indian quinoa seeds was 2.11±0.01 g/100g. Results are in agreement with Siener et al., 2006, who reported 1.8g/100g oxalate content in quinoa seeds. All the processing methods led to reduction in oxalate content. Soaking and germination resulted in 30% and 55% decrease in oxalate content. Savage and Dubois, 2006 also reported 25% decrease in oxalate content of Taro leaves after soaking. This may be due to presence of 71% (131mg/100g) of soluble oxalates quinoa with respect to total oxalate content (182 mg/100g) in quinoa seeds (Siener et al., 2006), which may leach out upon soaking and germination. Industrially processed seeds were also reported with 10% reduction in oxalate content. The results are in agreement with findings of Makinde and Akinoso, 2013 who reported decrease in oxalate content of dehulled sesame seeds.

4.3.4 Tannin

Tannin content of quinoa seeds is depicted in Table 4.4. Raw quinoa seeds were reported to have 0.6±0.08g/100g of tannin content. The findings are close to the results reported by Valencia-Chamorro, 2003 (0.53g/100g). Soaking and germination led to 50% and 66.6% decrease in tannin content. Khandelwal et al., 2010 stated soaking and germination as effective domestic processing methods to reduce tannin contents in legumes. The decrease might be attributed to leaching out of tannins in soaking media during soaking. Megat and Azrina 2012 also reported 58% decrease in tannin content of germinated peanuts. Germination triggers disintegration of tannin-protein-enzyme-mineral complex (Echendu et al., 2009) which might cause decrease in tannin content. Industrially processed seeds were reported to have reduction in tannin content similar to the reduction observed after soaking. This might be due to removal of hulls during industrial processing which also account for tannin content in quinoa seeds (Valencia-Chammaro, 2003).

4.3.5 Alkaloids

Total alkaloid content of quinoa seeds is shown in Table 4.4. Total alkaloid content of raw Indian quinoa seeds was 2.11±0.01 g/100g. Sanchez et al., 2004, have also reported similar results for total alkaloid content in lupin seeds. Genus *Chenopodium* has been reported to contain tropane, piperidine and pyridine alkaloids (Kokanova-Nedialkova et al., 2009). Dini et al., 2005 reported presence of five betaines in *Chenopodium quinoa.* Soaking and germination led to 1% and 15%, significant decrease ($P<0.05$) in alkaloid content of quinoa. This might be due to solubility of betaine (an alkaloid present in quinoa) in polar solvents such as water (Wang et al., 2012). The result is in agreement with reported decrease in alkaloid content upon soaking and germination in pigeon pea and lupin seeds, respectively (Sanchez et al., 2002). Industrialy processed seeds exhibited 10% decrease in alkaloid content.

4.3.6 Saponins

Saponin content of quinoa seeds is shown in Table 4.4. Raw quinoa seeds were reported to contain 2.01±0.15 g/100g saponin content. The results are supported by findings of Valencia-Chamorro, 2003 who reported 0.1 to 5 g/100g saponin content in bitter varieties of quinoa. Soaking resulted in significant decrease ($P<0.05$) of 24% in saponin content. The present result is supported by the findings of Nwosu, 2010 who reported 25% decrease in saponin content of bean after soaking for 24 hours. Adekanmi et al., 2009 and Mittal et al., 2012 have also reported decrease in saponin content after soaking in tigernut and chickpea, respectively. As saponin is located in outer covering of quinoa seeds (Chauhan et al., 1999), the decrease may be due to leaching of saponin in water during soaking period. Germination led to 98% decrease in saponin content. The saponin content reported after germination (0.03±0.01 g/100g) was within the range of saponin content of sweet quinoa varieties (0.02 to 0.05 g/100g) as reported by Mastebroek et al., 2000. Thus, germination can be a preferred method to debitter raw quinoa seeds. Lorenz and Nayanzi, 1989 also stated saponin lowering effect of wet processing methods in quinoa seeds. Industrially processed seeds exhibited 97% reduced saponin content as compared to the raw seeds. Riechert et al., 1986 also reported decrease in saponin content after industrial processing of quinoa. Ridout et al., 1991 also reported similar effect of wet processing and industrial processing

on saponin content in quinoa seeds. The reason behind reduction in saponin content after industrial processing may be removal of outer coat, containing 34% saponins (Chauhan et al., 1992), during the process. With respect to the germinated seeds, although the reduction was statistically non-significant ($P<0.05$) but germinated seeds were reported to have 50% lower saponin content than industrially processed seeds. Thus, the results demonstrate the efficacy of domestic processing, mainly germination, over industrial processing in removal, saponin, the major anti nutrient present in quinoa seeds.

4.3.7 Trypsin Inhibitor Activity

Trypsin Inhibitor activity of quinoa seeds is shown in Table 4.4. Raw seeds were reported to have 6633±7.5 TIU/100g trypsin inhibitor activity. The results are almost similar to and supported by values of trypsin inhibitor activity (6890 TIU/ 100g) reported by Ando et al., 2002 in quinoa seeds. All the processing methods led to significant decrease ($P<0.05$) in trypsin inhibitor activity. Soaking resulted in 4.7% decrease in trypsin inhibitor activity, which may be due to leaching of trypsin inhibitors from seed coat to soaking media (Sharma and Sehgal, 1992). Mubarak, 2005, reported almost similar decrease in trypsin inhibitor activity, by 5.2%, after soaking mung bean seeds. Decrease in trypsin inhibitor activity, by 22.8%, was also observed in germinated seeds. The result is in agreement with findings of El-Adawy, 2002 who reported 33.9% decrease in trypsin inhibitor activity of chickpeas after soaking. Industrially processed quinoa seeds exhibited 20.7% decrease in trypsin inhibitor activity. This may be due to removal of hulls from seeds during industrial processing, which accounts for 10 to 12% of whole seed weight (Hemlatha et al., 2016) and have major participation (16%) in trypsin inhibition (Chauhan et al., 1992) as compared to the whole seed. Highest decrease in trypsin inhibitor activity in germinated seeds also infers better protein digestibility of the same as compared to raw, soaked, and industrially processed seeds.

4.3.8 Total phenolic content

Total phenolic content (TPC) of Indian *Chenopodium quinoa* is shown in Table 4.5. TPC of raw Indian quinoa seeds, was reported to be 43.2±0.28 mgGAE/ 100g which corresponds well to TPC content reported by Repo-Carrasco-Valencia et

al., 2010 (42 mg GAE 100^{-1}) and Vollamannova et al., 2013 (45mgGAE /100g) in Carmen variety of quinoa.

Also the value of TPC content found in this study lies close to reported the values of TPC in raw quinoa seeds by Carciochi et al., 2014 (39 mgGAE /100g), Pasko et al., 2009 (38mgGAE /100g), higher than as reported by Miranda et al., 2010 (28mg GAE/100g). Higher reported values in this study can be explained as raw seeds (direct from the field) used were with the seed coat while the quinoa seeds procured by these authors were as available in the local market, which might be industrially processed for removal of seed coat which leads to decrease in phenolic content . Significant difference in phenolic contents reported by various other authors may be due to different environment conditions for growth, extraction solvents (Marmouzi et al., 2015), quinoa varieties with coloured testa (Tang et al., 2015). Total phenolic content reported in soaked quinoa seeds was significantly less (28%, $P \leq 0.05$) as compared to raw seeds. The result corresponds well to 26-56% loss in total phenolic content of soaked black beans (*Phaseolus vulgaris* L.) reported by Xu and Chang 2008. Germinated seeds were found to exhibit 134% increase in total phenolic content as compared to the raw quinoa seeds. This is because germination leads to increase in phenolic content of seeds as synthesis of phenolic acid is enhanced by seed growth during germination (Cevallos-Casals and Cisneros-Zevallos, 2010). Increase in total phenolic content in germinated quinoa has also been reported by Carciochi et al., 2014 (56 % after 48 hours and 101.2% after 72 hours of germination) and Jubete et al., 2010 (107% after 82 hours of germination). Industrial processed quinoa seeds exhibited 20% decrease in total phenolic content (34 mgGAE /100g). Similar decrease in phenolic compounds of pearled quinoa (abrasion degree of 30%) was reported by Gomez-Caravaca et al., 2014 with 21.5% and 35.2% decrease in free and bound phenolic compounds respectively.

4.3.9 Total flavonoids

Total flavonoid content (TFC) of quinoa seeds is shown in Table 4.5. Total flavonoid content of raw Indian quinoa was reported as 11.40 mg QE /100g. Results agree with the findings of Carciochi et al., 2014 (11.06 mg QE /100g) and Chirinos et al., 2013 (11 mg QE /100g). The total flavonoid content reported in our study is significantly different to the values reported by Marmouzi et al.,

2015; Carciochi et al., 2014 and Chlopika et al., 2012. This might be due to different solvents used for extraction, difference in temperature during extraction process and different methods of flavonoid analysis used (HPLC or spectrophotometry). Total flavonoid content of soaked quinoa seeds decreased by 36% (7.2 mg QE /100g). Similar decrease in flavonoid content after soaking has been found in white sorgum (Afiffy et al., 2012). Germination of quinoa seeds lead to significant increase (56%) in flavoniod content (18 mg QE /100g). Similar increase in flavonoid content (59%) has been reported by Carciochi et al., 2014 in germinated quinoa seeds. The increase in flavonoid content on germination of seeds is due to synthesis of metabolites like flavonoids by phenylproponoid pathway, common to all plants, during process of seed germination (Wu et al. 2011). Industrial processing of quinoa seeds led to reduction in flavonoid content by 47%. The findings may be attributed to the fact that most of the flavonoids are contained in the seed coat and industrial processing involves removal of outer layer of seed thus causing decrease in the flavonoid content (Xu and Chang, 2008).

Table 4.5: Total phenolic content and total flavonoid content (TFC) of Raw, domestically and industrially processed Indian *Chenopodium quinoa* seeds

Indian *Chenopodium quinoa*		**Total Phenolic Content (mg GAE/100g)**	**Total Flavonoids (mg QE/100g)**
Raw		43.2±0.28[a]	11.4±0.08[a]
Domestically Processed	**Soaked**	7.2±0.08[a]	31.1±0.35[b]
	Germinated	18.02±0.2[a]	101.2±0.29[c]
Industrially processed		34.6±0.33[a]	5.8±0.10[a]

4.4 *In-Vitro* analysis

3.4.1 In vitro starch digestibility

The starch digestibility of quinoa seeds is shown in Table 4.6. Raw quinoa seeds exhibited 65.7±0.15% starch digestibility. The results are in agreement with the findings of Repo-Carassco-Valencia, 2011 who reported 65.1 to 68.6% starch

digestibility in 4 different varieties of quinoa seeds. Soaking of quinoa seeds resulted in 1.52%, non-significant increase in starch digestibility while germination led to 1.80%, significant increase with respect to the raw seeds. This can attributed to the fact that soaking and germination result in starch gelatinization and increased activity of α-amylase, an enzyme responsible for disintegration of starch into sugars, hence, reducing starch into readily digestible form (Preet and Punia, 2000). High starch digestibility can also be attributed to small size (0.3 to 2µm) of quinoa starch granules (Kong and Bertoft, 2010). Li and Zhu 2016 also reported significant amount of rapidly digestible starch (RDS) content in quinoa, which is readily susceptible to enzyme action. Industrialy processed seeds exhibited 1.05%, non-significant increase ($P<0.05$), in starch digestibility. Capriles et al., 2014 also reported 7.02% increase in starch digestibility of industrially processed (extruded) amaranth seeds. Thapliyal et al., 2014 also reported 9.8 to 14% increase in starch digestibility of industrially processed (dehulled) chickpeas of different varieties. Starch digestibility was higher after soaking and germination as compared to industrial processing, this may be due to better subjection of seed starch matrix to degradation upon hydration and action of α-amylase on soaking and germination (Chung et al., 2012).

4.4.2 *In vitro* Protein digestibility

The protein digestibility of raw and processed Indian quinoa seeds is shown in Table 4.6. Raw quinoa seeds exhibited 75.3±0.33% protein digestibility. Repo-Carassco-Valencia and Serna, 2011 also reported 76.32 to 80.54% protein digestibility in different quinoa varieties. Among all quinoa seeds (raw and processed), raw quinoa seeds had lower protein digestibility value. This may be attributed to presence of anti nutrients like tannin, trypsin inhibitors, etc. which impede the digestibility and solubility of protein (Pushparaj and Urooj, 2011). Soaking resulted in 1.5% increase in protein digestibility. The results are supported by findings of El-Sayed Embaby, 2010, who reported 0.9 to 1.4 % increase in protein digestibility of lupin seeds after soaking. Germination resulted in 9.1%, significant increase ($P<0.05$) in protein digestibility. Ghavidel and Prakash, 2007 also reported 14 to 15% increase in protein digestibility of various legumes after germination. Industrially processed seeds exhibited 1.2% increase in

protein digestibility value. Increase in protein digestibility after soaking, germination and industrial processing may be due to decrease in anti nutrients. Phytic acid, anti nutrient present in quinoa seeds is known to interfere by binding with protein and suppressing proteolysis hence, lowering protein digestibility (Cowieson et al., 2006).

Table 4.5: In vitro analysis of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		**Invitro protein digestibility (%)**	**In vitro Starch Digestibility (%)**	**Antioxidant activity**	
				FRAP mg TE/ 100g	**DPPH mg TE/ 100g**
Raw		75.3±0.33[a]	65.7±0.15[a]	84.46±5.9[a]	59.61±0.39[a]
Domestically Processed	**Soaked**	76.5±0.32[b]	66.7±0.05[a]	96.46±1.5[a]	53.51±0.56[b]
	Germinated	82.2±0.36[c]	66.9±0.2[a,b]	159.23±0[.b]	61.41±1.89[c]
Industrially processed		76.2±0.1[d, b]	66.4±0.6[b]	72.35±1.82[a]	49.69±1.5[a]

4.4.3 *In vitro* antioxidant activity

Antioxidant activity of Indian *Chenopodium quinoa* seeds is shown in Table 4.6. Raw quinoa seeds were reported to have 59.6 mgTE /100g as calculated by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) method and 84.4 mg TE /100g as calculated by FRAP (Ferric reducing antioxidant activity) method. The results were close to antioxidant activity according to Jubete et al., 2010 (57.7 mg TE/100g and 34.8 TEAC by DPPH method and 84.1 mg TE /100g by FRAP method). Antioxidant activity of quinoa reported by Ranilla et al., 2009 (by DPPH) was much higher (86 mg TE /100g) than as reported in this study (59.6 mgTE /100g). This is because red quinoa were used by Ranilla et al., 2009 and difference in color of seeds strongly effects antioxidant activity with dark colored seed coats exhibiting highest antioxidants activities (Tang et al., 2015). Quinoa seeds exhibit higher antioxidant activity (evaluated by FRAP and DPPH) as compared to grain Amaranth (Nsimba et al., 2008 and Pasko et al., 2009, Vollmannova et al., 2013)

and some Peruvian Andean fruit like Tuna and grain Kwicha (Chirinos et al. 2015). It is found to exhibit lower antioxidant activity as compared to buckwheat (Jubete et al., 2010 and Vollmannova et al., 2013), oat and rice (Halvorsen et al., 2002), higher than amaranth (Jubete et al., 2010) and almost similar to wheat (Jubete et al., 2010).

Antioxidant activity of quinoa also depends on intensity of colour of seed coat (Tang et al. 2015). Soaked quinoa seeds exhibited 7% decrease in antioxidant activity as compared to raw seeds. The results is supported by findings of Afiffy et al., 2012, who reported decrease in antioxidant activity in soaked white sorghum. Xu and Chang, 2008 also reported decrease in antioxidant activity of soaked green pea (9%), yellow pea (8%), and lentil (7%). As phenols and flavonoids contribute significantly to antioxidant activity (Thaipong, 2006), the decrease may be due to leaching of phenols and flavonoids in water used for soaking the seeds (Afiffy et al., 2012). Quinoa seeds germinated in day light for 7 days exhibit significantly high antioxidant activity than raw seeds (Pasko et al., 2010a). In our study antioxidant activity of germinated seeds (after 4 days or 48 hours) was found to increase by 90% (calculated by DPPH method). The result is supported by findings of Carciochi et al., 2014a which showed 100% increase in antioxidant activity of germinated quinoa seeds as evaluated by DPPH method. Similarly, increase in antioxidant activity of germinated quinoa seeds has also been reported by Pasko et al., 2008. FRAP values of germinated quinoa seeds increased by 89%. The result is supported by increase in FRAP values of quinoa sprouts (79%) as reported by Jubete et al., 2010b. Quinoa (*Chenopodium quinoa*) sprouts have lower antioxidant activity (evaluated by FRAP) as compared to Amaranth (*Chenopodium album*) sprouts (Pasko et al., 2009). However industrial processing of the seeds lead to decline in antioxidant activity. Processed quinoa seeds showed decline of 14% and 19% antioxidant activity as evaluated by FRAP and DPPH, respectively. The result is supported by decrease in antioxidant activity of wheat (Hung et al., 2009), after undergoing industrial processing like decortications and pearling. Decline in antioxidant activity after processing can be due to removal of hulls, which are majorly responsible for antioxidant activity (Cardador-Martinez et al., 2002).

B) Comparison of nutritional quality of industrially processed Indian and American *Chenopodium quinoa* seeds

4.5 Proximate Composition of industrially processed Indian and American *Chenopodium quinoa* seeds

Proximate composition of quinoa seeds grown in India and South America is shown in Table 4.7. Moisture, carbohydrate, protein and crude fiber content of quinoa seeds grown at two different countries were significantly different ($P<0.05$) while ash and crude fat contents were statistically non-significant ($P<0.05$). The results of proximate composition of American quinoa reported in this study are in agreement with proximate composition of Peruvian quinoa (grown in Peru, South America) and Argentinean quinoa (grown in Argentina, South America) reported by Nascimento et al., 2013 and Villa et al., 2014, respectively. Significant difference ($P<0.05$), observed in moisture contents of grains might be due to varying climatic conditions and soil water holding capacities, which highly affect constitutional aspects of the crops (Kang et al., 2009).

Table 4.7: Proximate Composition of industrially processed Indian and American

Chenopodium quinoa	Moisture (g/100g)	Carbohydrate (g/100g)	Crude fat (g/100g)	Crude protein (g/100g)	Ash (g/100g)	Crude fibre (g/100g)
Indian	10.35±0.20 [a]	64.90±0.09 [a]	5.11±0.05 [a]	13.11±0.08 [a]	3.04±0.03 [a]	2.49±0.04 [a]
American	11.23±0.32 [b]	67.93±0.84 [b]	4.63±0.15 [a]	12.25±0.92 [b]	3.18±0.08 [a]	2.14±0.31 [b]

It was noticeable that the protein content of Indian quinoa (13.11±0.08 g/100g) was 7.02% more than the protein content of American quinoa (12.25±0.92 g/100g). Foste et al., 2015 reported similar protein content of Bolivian quinoa (grown in Bolivia, South America). Gonzalez et al., 2014 also reported different protein content of quinoa seeds grown at different sites. Although, protein content of grain varies with the variation in genotype but environmental elements are also

known to exert a pronounced effect on the protein content (Kumar et al., 2006). Marmouzi et al., 2015 reported lower protein content of Moroccan quinoa as compared to American quinoa, which shows effect of environmental conditions and their interactions on protein content of grain.

A significant difference ($P<0.05$) observed in crude fiber contents of grain might be attributed to the different processing methods and the extent of processing, applied to grains for saponin removal, which involves removal of outer fibrous covering of seeds (Ghavidel and Prakash , 2007).

4.6 Nutritional Composition of industrially processed Indian and American *Chenopodium quinoa* seeds

Dietary fiber and β-Carotene contents of American and Indian quinoa seeds is given in Table 4.8. Dietary fiber content of American and Indian quinoa seeds is given in Table . The results are in accordance with the values reported by Marmouzi et al., 2015. A non-significant difference ($P<0.05$) was observed in dietary fiber content of American and Indian quinoa seeds. Miranda et al., 2013 also reported non-significant difference ($P<0.05$) in quinoa seeds grown at different places.

Indian quinoa seeds exhibited significantly higher ($P<0.05$) β-Carotene content than American seeds. The results are in accordance with the β-Carotene content of American chenopodium species reported by Sharma et al., 2012. According to Gastol et al., 2012, plant macro and micronutrients are highly dependent on soil composition, method of farming and management practices. Hence, the difference in β-Carotene contents of quinoa seeds observed in this study might be due to variation in these factors.

Vitamin C content of Indian and American quinoa seeds is given in Table 4.8. Vitamin c content of American quinoa was, 14.4%, significantly higher ($P<0.05$) than the vitamin C content of Indian quinoa seeds. The results are in agreement with the vitamin c content of South American (Chilean) quinoa varieties reported by Miranda et al., 2010. The difference might be due to certain influential factors like fertilizer quality, regional temperature, soil water management etc., which highly affect the vitamin c content of crop (Lee and Kader, 2000).

Table 4.8: Nutritional Composition of industrially processed Indian and American

Indian *Chenopodium quinoa*	Vitamin C (mg/100g)	β-Carotene (µg/100g)	Dietary fiber (g/100g)					
			NDF	ADF	Lignin	Cellulose	Hemi-cellulose	Total dietary fibre
Indian	13.43±0.4 [a]	535.6±2.8 [a]	6.69±0.03	2.50±0.07	2.2±0.02	3.3±0.04	1.19±0.05	9.24±0.21 [a]
American	15.38±0.16 [b]	165.24±1.2 [b]	6.93±0.31	2.45±0.46	2.13±0.18	3.52±0.10	1.22±0.01	9.41±0.73 [a]

Mineral composition of Indian and American quinoa is shown in Table 4.9. Mineral content of American quinoa was found to be significantly higher ($P<0.05$) than the Indian quinoa. Calcium, iron and zinc contents of American quinoa seeds were 15.6, 43.2 and 26.1% higher than Indian quinoa, respectively. The results are in agreement with the findings of Miranda et al, 2013 who reported similar calcium, iron and zinc content in South American quinoa grown in Argentina. Magnesium content of American quinoa was 6.1% higher than Indian quinoa. The resultsa re in agreement with the findings of Coelho et al., 2011 who reported similar results for magnesium content in Argentinean quinoa. Miranda et al., 2013 also demonstrated different soil characteristics of different regions where quinoa was cultivated. The difference in mineral content of quinoa grains grown at two different place may be due to difference in soil composition, as soil type is known to have great influence on crop characteristics (Baratasevec et al., 2013).

4.7 Phytochemical analysis of industrially processed Indian and American *Chenopodium quinoa* seeds

The content of phytochemical in Indian and American quinoa grain is shown in Table 4.10. A significant difference ($P<0.05$) was observed in antinutritional content of both grains grown at different places. The difference observed in phytic acid

Table 4.9: Mineral content of industrially processed Indian and American C*henopodium quinoa* seeds

Indian *Chenopodium quinoa*	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Magnesium (mg/100g)
Indian Quinoa	85.06±0.15 [a]	5.1±0.02 [a]	6.5±0.13 [a]	182.2±0.15 [a]
American quinoa	98.38±0.22 [b]	7.32±0.15 [b]	8.24±0.76 [b]	193.34±0.65 [b]

Table 4.10: P**hytochemical Composition of industrially processed Indian and American *Chenopodium quinoa* seeds**

Indian *Chenopodium quinoa*	Total phosphorous (g/100 g)	Phytic acid/ Phytate (g/100g)	Phytate phosphorous	Non phytate phosphorous	Saponin (g/100g)	Trypsin Inhibitor Activity TIU/100g	Oxalates (g/100g)	Alkaloids (g/100gm)	Tannins (g/100g)
Indian	0.45±0.01 [a]	1.21±0.31[a]	0.26	1.62	0.06±0.02 [a]	5254.6±11.5 [a]	0.18±0.04 [a]	1.9±0.02 [a]	0.35±0.0 [a]
American	0.62±0.16 [a]	1.29±0.15[b]	0.33	0.96	0.02±0.15 [b]	5249.3±9.32 [b]	0.24±0.16 [b]	1.2±0.41 [b]	0.41±0.18 [b]

contents may be due to environmental conditions and difference in crop variety that are known to have influential effect on crop's phytic acid content (Mahmood et al., 2010). In addition, the crop harvesting time is also known to influence phytic acid content, which further influences its content of total phosphorous (Kim et al., 2002). Difference in saponin, trypsin inhibitor activity, oxalate, alkaloid and tannin contents may be due to difference in extent and type of processing technique applied to the grain for increasing its palatability and acceptability by consumers (Preedy, 2014). The antinutrients are largely located and congregated in outer layers of grains, which get removed during various industrial processes applied to them post harvesting (Mao et al., 2011), so extent of processing applied largely affects the quantity of anti nutrient removed and retained.

Total phenolic content and total flavonoid content of Indian and American quinoa (Table 4.11) was significantly ($P<0.05$) different. Significant difference in phenolic contents due to different environment conditions for growth has also been rep orted by Miranda et al., 2013.

4.11 Total phenolic content and total flavonoid content of industrially processed Indian and American *Chenopodium quinoa* seeds

Indian *Chenopodium quinoa*	Total phenolic content	Total flavonoid content
Indian quinoa	34.6 ± 0.33^{a}	5.8 ± 0.10^{a}
American quinoa	30.16 ± 0.49^{b}	4.6 ± 0.18^{b}

4.8 *In vitro* analysis of industrially processed Indian and American *Chenopodium quinoa* seeds

***3.8.1 In vitro* starch digestibility**

Invitro starch digestibility of quinoa seeds is shown in Table 4.12. American quinoa seeds exhibited significantly higher ($P<0.05$) starch digestibility (67.14%) than Indian quinoa seeds. The results lie within the range of values of in vitro starch digestibility of four different South American quinoa varieties reported by

Repo Carassco-Valencia and Serna, 2011. The difference in *invitro* protein and starch digestibility of Indian and American quinoa seeds may be due to varietal difference of the grain because of different environmental conditions of cultivation and also different processing methods used for saponin removal from raw seed. Shimelis and Rakshit, 2007 demonstrated effect of processing on *invitro* protein digestibility of different varities of kidney beans. Souilah et al., 2015 reported different *invitro* starch digestibility in different varieties of sorghum.

4.8.2 *In vitro* protein digestibility

Invitro protein digestibility of quinoa seeds is shown in Table 4.12. American quinoa seeds exhibited significantly lower ($P<0.05$) protein digestibility (75.15%) than Indian quinoa seeds. The results are in agreement with findings of Rehman and Shah, 2001 and Repo Carassco-Valencia and Serna, 2011 who reported 75.3 to 80.4% protein digestibility of different variety of quinoa seeds procured from Peru, South America.

Table 4.12: *In vitro* analysis of industrially processed Indian and American *Chenopodium quinoa* seeds

Indian *Chenopodium quinoa*	Invitro protein digestibility (%)	In vitro Starch Digestibility (%)
Indian quinoa	76.22±0.13 [a]	66.48±0.62 [a]
American quinoa	75.15±0.35 [b]	67.14±0.38 [b]

4.8.3 *In vitro* antioxidant activity

The total antioxidant activity of Indian *Chenopodium quinoa* (Table: 4.13) was 72.35±1.82 mgTE/100g (by FRAP) and 49.69±1.50 (by DPPH) while American seeds were reported with antioxidant activity of 70.53±0.15 (by FRAP) and 46.38±0.41 (by DPPH). Miranda et al., 2013 also reported 41 to 73% difference in antioxidant activities of quinoa seeds grown in contrasting environmental regions. Nsimba et al., 2008, revealed antioxidant activity of two quinoa genotypes with

mean values of 72.1% of radical scavenging activities for Bolivian genotype and 59.2% for Japan sea-level type. The results suggest in that case that non phenolic compounds might also play an important role in the free radicals scavenging activity.

Table 4.13: Antioxidant activity of industrially processed Indian and American *Chenopodium quinoa* seeds

Indian *Chenopodium quinoa*	***In vitro* antioxidant activity**	
	FRAP mgTE/100g	**DPPH mgTE/100g**
Indian quinoa	72.35±1.82[a]	49.69±1.5[a]
American quinoa	70.53±0.15[b]	46.38±0.41[b]

Objective 2: To assess cholesterolemic effect of *Chenopodium quinoa* seeds.

Plasma lipid levels of rats fed with experimental diet for 45 days is shown in Table 4.14.

4.9.1 Effect on serum chlosterol levels

The total serum cholesterol level of rats fed with basal diet (Group1) was reported as 160.16±16 mg/dl. The total concentration of plasma cholesterol was observed to reduce significantly ($P<0.05$) upon addition of quinoa to the experimental diet. It was observed that, as compared to the Group 2, the positive control (rats fed on hypercholesterolemic diet), concentration of cholesterol reduced by 8.2% in diet fed with statin, while 15.5 and 24.4% reduction in serum cholesterol was observed in rats fed with raw (Group 4) and germinated quinoa (Group 5) along with hypercholesterolemic diet, respectively. The rats fed with raw (Group 6) and germinated quinoa (Group 7) along with basal diet showed 28.2 and 31.9% reduction in total cholesterol level. The results are in agreement with the findings

of Foucault et al., 2012 who reported reduction in serum cholesterol levels of mice fed with quinoa extract for 3 weeks. The results reveal higher hypercholesterolemic effect of germinated quinoa seeds as compared to the raw quinoa seeds

4.9.2 Effect on plasma triglycerides level

The levels of plasma triglycerides showed a declining trend with supplementation of quinoa to the experimental diet. As compared to the positive control, which showed 96.6±2.9mg/dl triglyceride content, the levels were reported to decrease significantly ($P<0.05$) in rats fed with quinoa diet. However, reduction in triglyceride content by quinoa supplemented along with hypercholestrolemic diet was observed to be lower than the reduction induced by statin administered along with hypercholestrolemic diet. El-gawad et al., 2005, have also reported similar trend. In rats fed with quinoa along with hypercholersterolemic diet, the triglyceride levels reduced by 12.5 and 4.5% in Group 4 and Group 5, respectively. Triglyceride level of rats fed with quinoa along with basal diet i.e. in Group 6 and Group 7, reduced by 10.4 and 14.5%, respectively. Takao et al., 2005 also reported similar reduction in triglyceride level of rats fed with quinoa 2.5 and 5% quinoa protein. Mithila et al., 2015 also reported reduction of triglycerides in rats fed with quinoa supplemented diet. This could be due to the wide variety of phytoconstituents present in quinoa which offer a synergistic effect in exerting hypolipidimic effects (González and Rodriguez 2011).

4.9.3 Effect on HDL level

The serum HDL level of rats fed on basal diet were reported as 91.15±4.8 mg/dl while of rats fed on hypocholesterolemic diet was 90.1±2.2 mg/dl . The HDL levels were observed to decrease significantly ($P<0.05$) in rats fed with diets supplemented with raw (5.5%) and germinated (3.3%) quinoa along with hypercholesterolemic diet as compared to serum HDL levels of rats fed with hypercholesterolemic diet (positive control) which reveals negative effect of quinoa supplementation along with hyper cholesterolemic diet on serum HDL levels. Mithila and Khanum, 2015, have reported similar results. Supplementation of raw and quinoa seeds with basal diet showed an increase in HDL level by 6.5 and 9.8%, respectively effect as compared to the supplementation of quinoa with basal diet.

The results are in accordance with the results reported by Pasko et al., 2010 and Hejazi, 2016.

4.9.4 Effect on serum (VLDL+HDL) level

The serum (VLDL+HDL) values of rats fed on basal diet were reported as 68.82±7.2 mg/dl while of rats fed on hypercholesterolemic diet was 128.9±3.5 mg/dl. The results were observed to decrease significantly ($P<0.05$), by 21.3 and 39.4% with supplementation of raw quinoa and germinated quinoa to

Table 4.14: Effect of quinoa supplementation (raw and germinated) on blood lipid profile

	Cholesterol (mg/dl)	**Triglyceride (mg/dl)**	**HDL (mg/dl)**	**(LDL+VLDL) (mg/dl)**	**Atherosc-lerotic Index (mg/dl)**
Group 1	160.16±4.8 [a]	86.3±1.6 [a]	91.15±4.8 [a]	68.82±7.2 [a]	0.75±0.2 [a]
Group 2	219.10±11.3 [b]	96.6±2.9 [b]	90.1±2.2 [b]	128.9±3.5 [b]	1.43±0.3 [b]
Group 3	201.13±2.9 [c]	84±2.5 [c]	96±1.7 [c]	105.13±5.4 [c]	1.09±0.4 [c]
Group 4	185.5±2.8 [d]	92.3±1.6 [d]	85.1±2.2 [d]	100.4±1.9 [d]	1.17±0.16 [d]
Group 5	165.18±2.7 [e]	88±3.5 [e]	87.02±1.4 [e]	78.16±1.3 [e]	0.89±0.1 [e]
Group 6	157.14±3.1 [f]	86.6±2.8 [f]	97.33±1.6 [f]	59.81±1.1 [f]	0.61±0.1 [f]
Group 7	149.5±1.0 [g]	82.16±1.4 [g]	100±1.5 [g]	49.5±1.2 [g]	0.49±0.1 [g]

VLDL: Very low-density lipoprotein-cholesterol; LDL: low-density lipoprotein-cholesterol; VLDL+LDL-cholesterol=Total cholesterol- HDL.
Atherosclerotic index= (VLDL+LDL-cholesterol)/(HDL-cholesterol)

hypercholesterolemic diet, respectively. The results also infer beneficial effect of germinated quinoa over raw quinoa. This may be due to higher phenolic content of germinated quinoa which might be responsible for lipid lowering beneficial effects (Carciochi et al., 2013). Also, it is noteworthy that lipid lowering effect of quinoa (raw and germinated) was higher than the beneficial effect of statin, which was reported to cause17.7% decline in (VLDL+HDL) values. The reduction was almost almost twice in germinated quinoa. The rats fed on diet containing raw and germinated quinoa supplemented with basal diet also reported 13.2 and 27.3% reduction in (VLDL+HDL) values. The results infer lipid lowering beneficial effect of quinoa, mainly germinated quinoa.

4.9.5 Effect on Atherosclerotic index

Atherosclerotic index of rats fed with basal diet was reported as 0.75±0.2 mg/dl while of rats fed on hypercholestrolemic diet was reported as 1.43±0.3 mg/dl. The drug statin administered to rats along with hypercholesterolemic diet, caused 23.7% reduction in atherosclerotic index, while supplementation of raw and germinated quinoa along with hypercholeterolemic diet resulted in 18.4 and 37.2% reduction in atherosclerotic index. The results infer beneficial effect of quinoa, mainly the germinated one, in hypercholesterolemia over the statin drug. The rats fed on raw and germinated quinoa supplemented with basal diet also showed 18.6 and 34.5% reduction in atherosclerotic index. This may be due high dietary fiber and phenolic content of germinated quinoa which may be responsible for lipid lowering effect (Carciochi et al., 2013 and Marmouzi et al., 2016). The results also are in line with the lipid lowering and beneficial effects of quinoa supplementation, mainly the germinated quinoa, reported in this study. To our knowledge, no study till now has reported effect of quinoa supplementation on atherosclerotic index.

Objective 3: To develop and analyze value added products from *Chenopodium quinoa*

4.10 Development of Snack bar

4.10.1 Proximate composition of snack bars

Proximate composition of snack bars is shown in Table 4.15. Moisture content of formulated bars ranged from 15.21±0.18 to 15.19±0.12 g/100gm. The results are in

agreement with the moisture content of snack bars (15.56 to 18.52 g/100gm) formulated by Nadeem et al., 2012. Moisture content of control bar (CB) and quinoa bar (QB) were almost similar and statistically non-significant to each other ($P<0.05$). Moisture content of snack bar formulated in this study was found to be lower than flaxseed incorporated cereal bars prepared by Khouryeih and Aramouni, 2013 which also indicates better shelf life of CB and QB. Ash, fat, protein and fiber content of quinoa bar was significantly higher ($P<0.05$) than control bar. The results are supported by findings of Slinkard, 2014 who reported better nutritional value of pasta formulated using combination of quinoa and chickpea flour with respect to the pasta formulated with quinoa flour alone.

Table 4.15: Proximate composition of snack bars

Proximate composition	**Quantity (g/100g)**	
	Control Bar (CB)	**Quinoa Bar (QB)**
Moisture	15.21±0.18 [a]	15.19±0.12 [a]
Ash	2.03±0.25[a]	3.16±0.92[b]
Fat	6.19±0.57[a]	7.31±0.13[b]
Protein	8.14±0.43[a]	13.41±0.53 [b]
Carbohydrate	67.14±0.13 [a]	60.87±0.73 [b]
Fiber	2.16±0.93 [a]	3.03±0.16 [b]

4.10.2 Total phenolic content and antioxidant activity

The total phenolic content and antioxidant activity of quinoa bar is shown in Table 4.16. Total phenolic content of quinoa bar was significantly higher ($P<0.05$) than the control bar. The reported results reveal higher total phenolic content of quinoa bar in comparison to the total phenolic content (30.7mgGAE/ 100g) of quinoa breads as reported by Jubete et al., 2010. Carciochi et ., 2014 reported rich polyphenolic content of quinoa. Total phenolic content of quinoa bars reported in this study is also higher than the total phenolic content of crackers (84 to 148mg GAE/100g) reported by Sedej et al., 2011.

Antioxidant activity of quinoa bars, as evaluated by DPPH and FRAP method, was reported significantly higher ($P<0.05$) than the control bars. The results are supported by the findings of Bhaduri and Navder, 2014 who reported 137% in antioxidant activity of muffins incorporated with quinoa. Carciochi et al., 2016 reported significantly higher antioxidant activity of germinated quinoa seeds, which might be the probable reason behind higher antioxidant activity of quinoa bars because of incorporation of germinated quinoa flour in formulated bar. Antioxidant activity of quinoa snack bars formulated in this study is higher than the snack bars formulated by Bailek et al., 2016 using popped amaranth seeds and oat flakes.

Table 4.16: Total phenolic content and antioxidant activity

Bar	**Antioxidant Activity**		**Total phenolic Content (mg GAE/100g)**
	DPPH (%)	**FRAP (mg Te/100g)**	
CB	59.34±0.31 [a]	101.22±0.66 [a]	89.43±0.34 [a]
QB	62.12±0.12 [b]	103.05±0.21 [b]	96.59±0.65 [b]

4.10.3 Sensory evaluation of bars

The data analysis of sensory evaluation (Table 4.17), revealed significant

Table 4.17: Sensory evaluation of snack bars

Bars	**Appearance**	**Colour**	**Texture**	**Mouth feel**	**Flavour**	**Taste**	**Overall Acceptability**
CB	7.1±0.70 [a]	6.2±0.70 [a]	6.7±0.18 [a]	6.9±0.14 [a]	6.5±0.2 [a]	7.7±0.13 [a]	6.4±0.03 [a]
QB	7.9±0.17 [b]	6.9±0.14 [b]	6.9±0.98 [a]	7.2±0.21 [b]	7.1±0.6 [b]	8.4±0.09 [b]	7.5±0.13 [b]

difference (P<0.05) in appearance, colour, mouthfeel, flavor and taste of control bars and quinoa bars, while difference in texture was revealed statistically non-significant (P<0.05).

Similarly in texture of both snack bars may be due to similar processing methods used for formulation of bars. The results infer better consumer acceptability of quinoa bars rather than control bars formulated with chickpea four and are parallel to the findings of Slinkard, 2014 who reported better consumer acceptability of quinoa-chickpea composite pasta. Revelations by Bahaduri and Navder, 2014 who demonstrated positive effect of incorporation of quinoa flour to food products for better sensory characteristics and higher consumer acceptability, also support the results in this study. According to scoring on nine point hedonic scale, overall acceptability of quinoa snack bars indicated "like very much" while control bars which indicated "like slightly". Highest score was scored by taste aspect (8.4 ± 0.09^{j}) of quinoa bar, which indicated "like extremely" on a nine point hedonic scale while lowest score was scored by colour aspect of control bar which indicated "like moderaltely" on a nine point hedonic scale.

4.10.4 Storage study

4.10.4.1 Effect on moisture content

Figure 4.1 represents moisture content of snack bars as affected during storage period of 30 days. The figure depicts increase in moisture content of snack bars upon storage. A significant increase (P<0.05), in moisture content was observed in both control bar and quinoa bar at an interval of 15 and 30 days. However, moisture content at the end of 30 days was highest as compared to the initial moisture content of snack bars. Increased moisture content of quinoa bars over the period may be due to the changes in water holding capacity of quinoa during the storage as already reported by Abugoch et al., 2009. Inglett et al., 2015 also reported increased water holding capacity of quinoa-oat composites. Increase in moisture content might also be due to water vapour transmission through the polythene packaging material used to store the bars (Bertrand et al., 2013). As, the moisture content of food is inversely related to its shelf life (Genkawa et al., 2008), the results depict degradation in shelf life of formulated bars with time.

4.10.4.2 Effect on crude ash

Figure 4.2 represents ash content of formulated snack bars as affected during storage period of 30 days. Ash content of snack bars was observed to decrease non-significantly ($P<0.05$) upon storage for 15 days while a significant decrease in ash content was observed after storage period of 30 days. Decrease in ash content during storage period has also been reported by Nadarajah et al., 2015 in coconut cookies. Decrease in ash content may also be due to increase in moisture content, which favours microbiological growth. During their growth period, the microbes utilize minerals and other nutrients, which result in decrease in ash content (Adams and Moss, 2005).

4.10.4.3 Effect on crude fat

Figure 4.3 represents changes in fat content of snack bars during the storage period of 30 days. A non significant change ($P<0.05$) in fat content was observed after storage period of 15 days while a significant decrease ($P<0.05$) was observed after storage period of 30 days. Decrease in fat content of cereals over a period during storage has also been reported by Sharma et al., 2014. This might be attributed to the lipid oxidation due to their larger surface area of snack bar which is in contact with air and moisture (Maisuthisakul et al., 2007). Decrease in fat content can also be attributed to increased activity of lipase which is highly influenced by moisture content of food (Agrahar-Murgkar and Jha, 2011). Lipase is responsible for oxidative rancidity leading to hydrolysis of fat present in food matrix and formation of free fatty acids which also imparts off flavor to the food product (Adawiyah et al., 2012).

4.10.4.4 Effect on crude protein

Figure 4.4 represents effect of storage period on protein content of snack bars. A non-significant decrease ($P<0.05$) was observed in protein content of snack bars after storage period of 15 days while significant decrease ($P<0.05$) was observed after storage period of 30 days.. Decrease in protein content may be attributed to increase in proteolyic activity due to increase in moisture content (Butt et al., 2010).

4.10.4.5 Effect on carbohydrate content

Figure 4.5 represents effect of storage period on carbohydrate content of snack bars. The carbohydrate content of control snack bars was observed to decrease significantly ($P<0.05$) after storage period 15 and 30 days as compared to the initial carbohydrate content while quinoa snack bars exhibited non significant

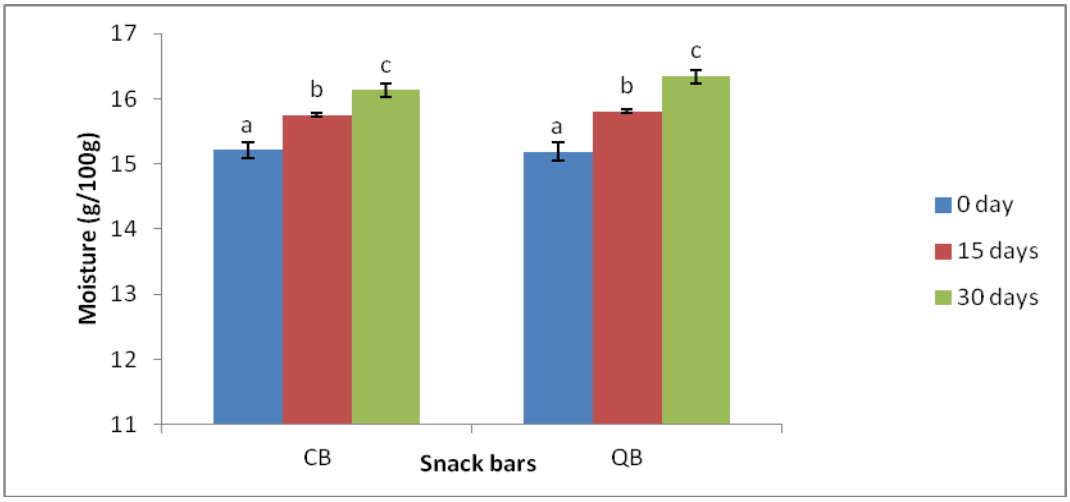

Figure 4.1: Effect of storage period on moisture content of snack bars.

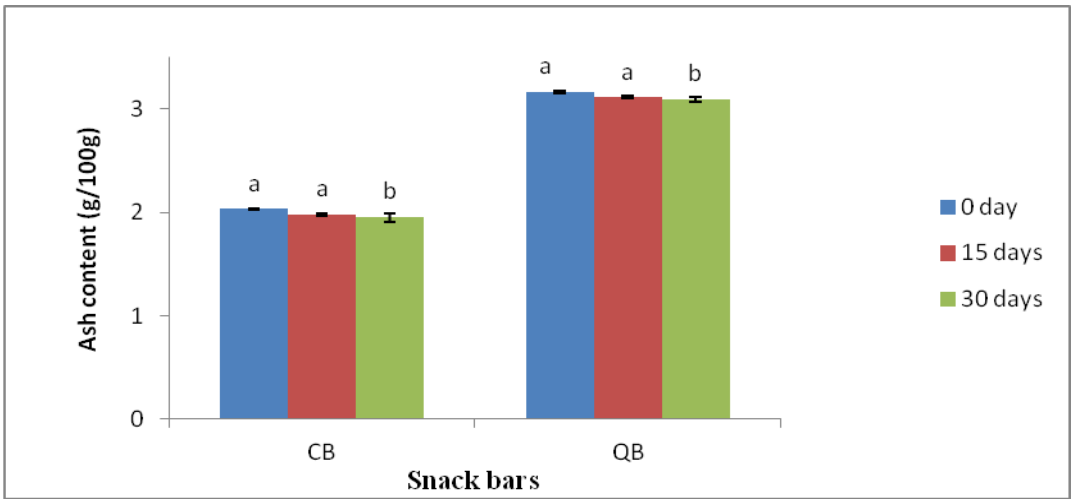

Figure 4.2: Effect of storage period on ash content of snack bars.

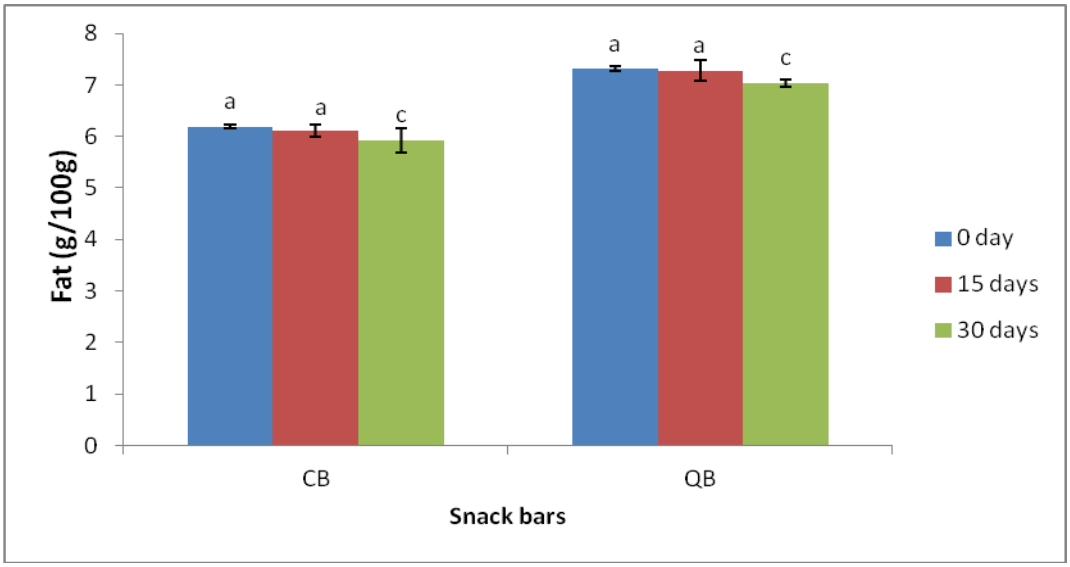

Figure 4.3: Effect of storage period on fat content of snack bars.

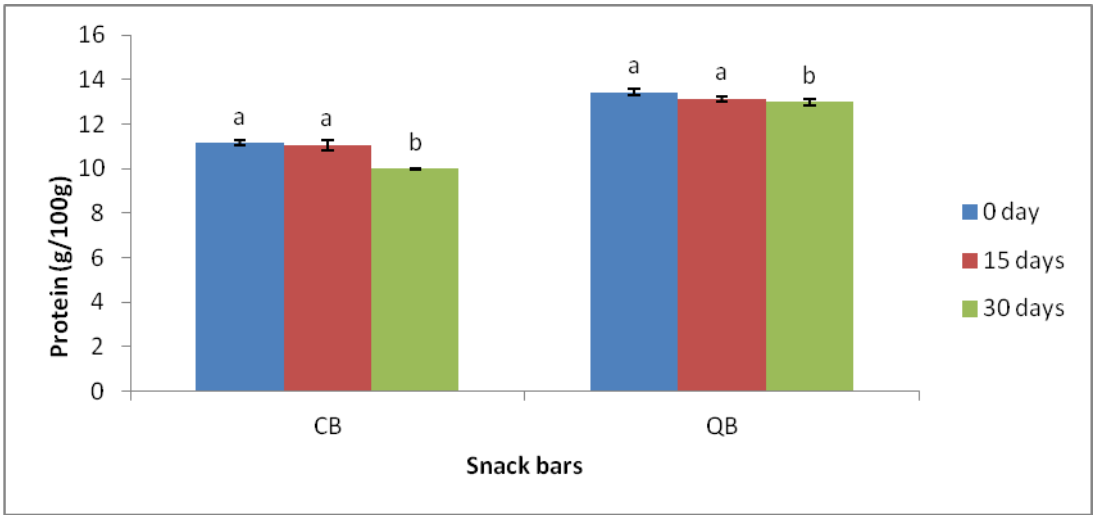

Figure 4.4: Effect of storage period on protein content of snack bars.

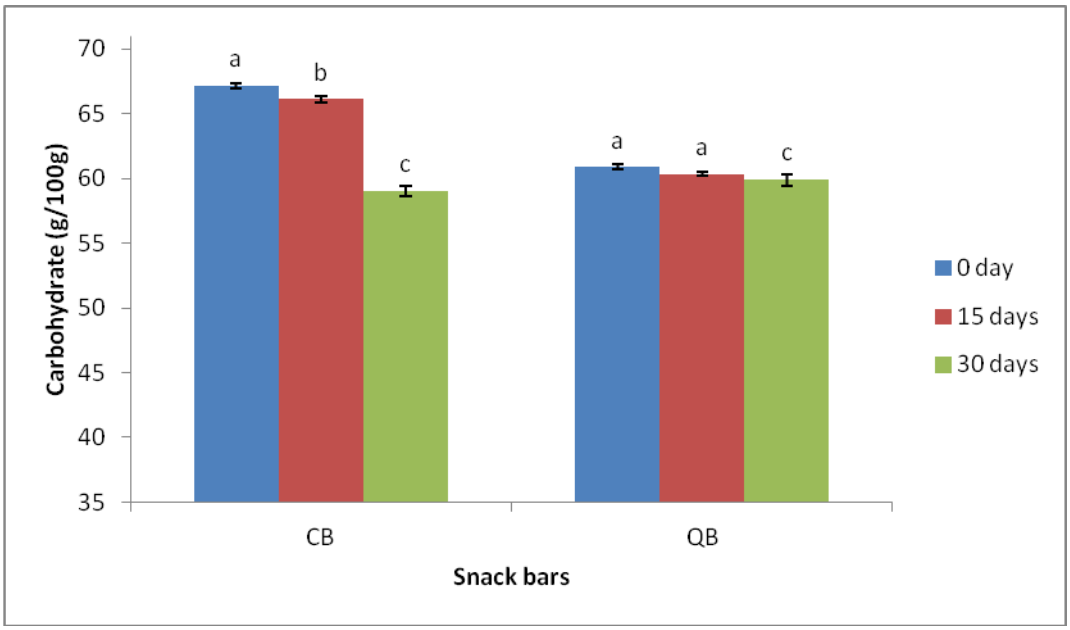

Figure 4.5: Effect of storage period on carbohydrate content of snack bars.

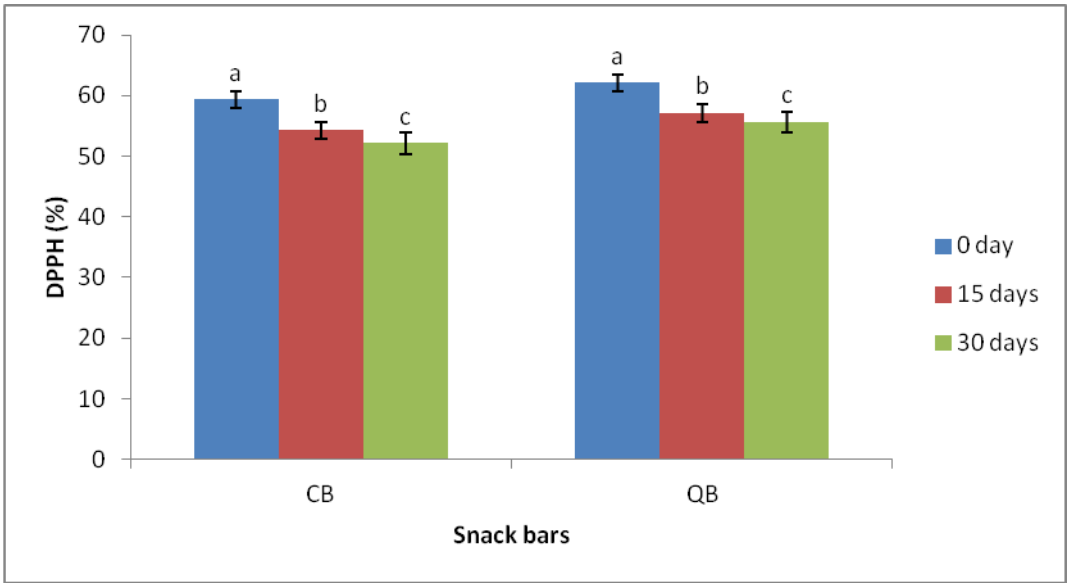

Figure 4.6: Effect of storage period on antioxidant activity (DPPH) of snack bars.

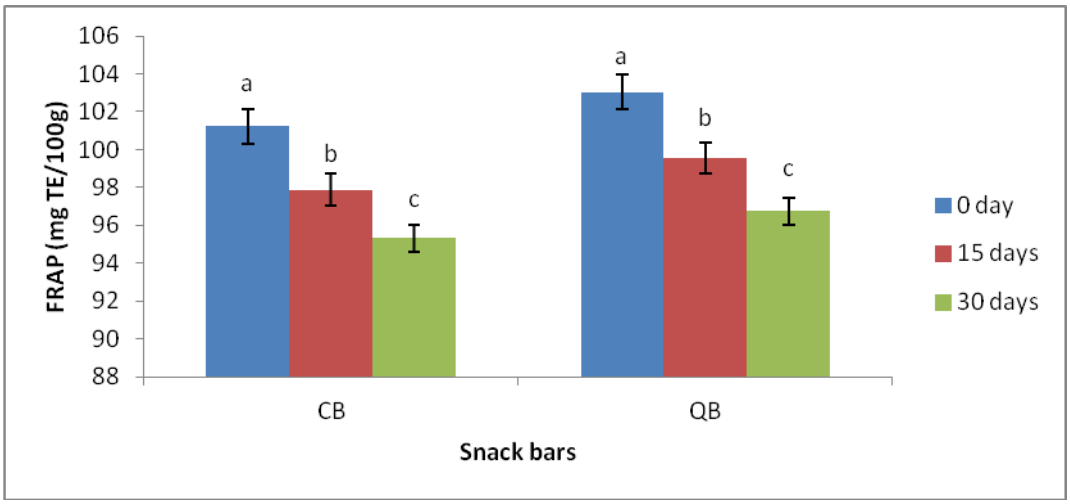

Figure 4.7: Effect of storage period on antioxidant activity (FRAP) of snack bars.

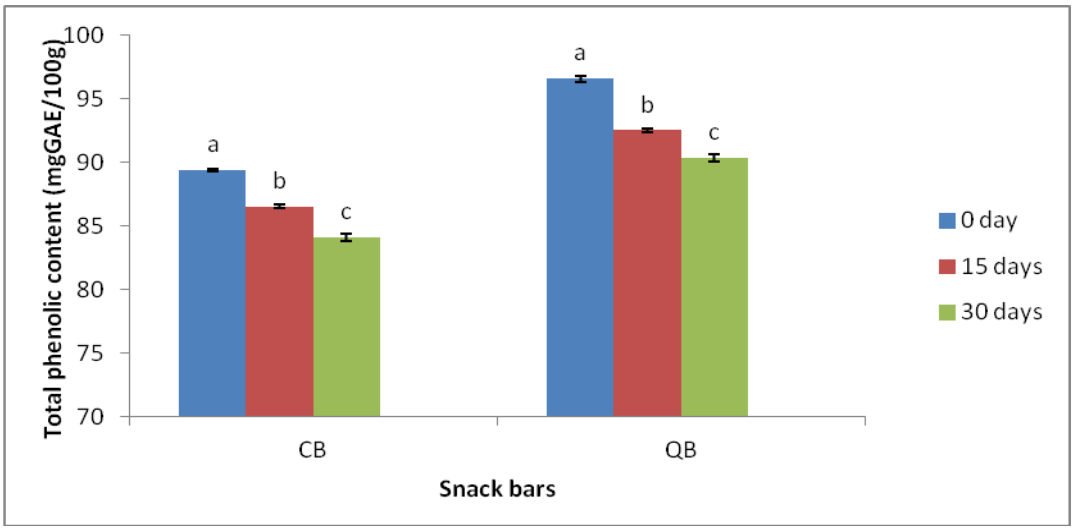

Figure 4.8: Effect of storage period on total phenolic content of snack bars.

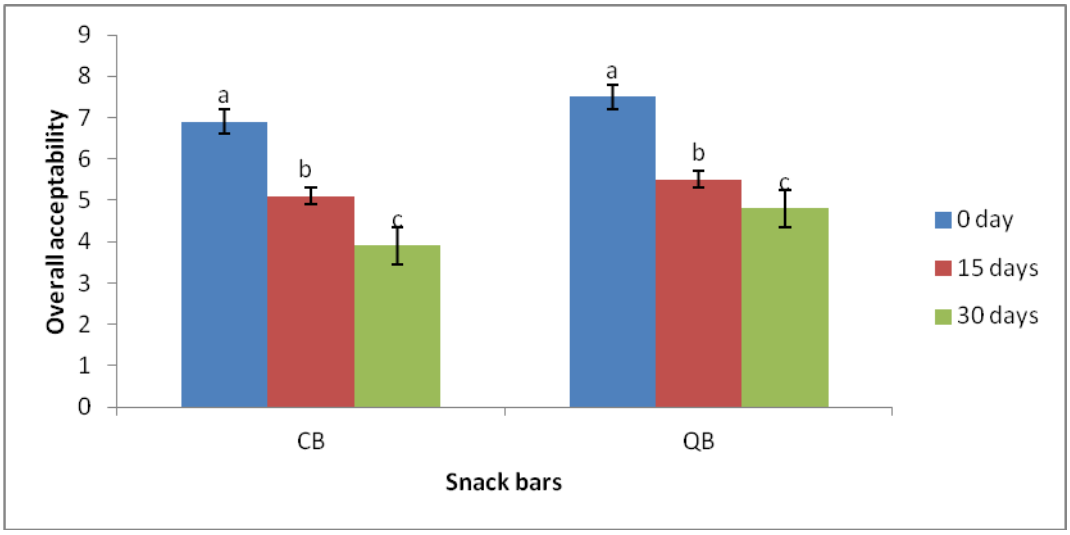

Figure 4.9: Effect of storage period on overall acceptability of snack bars.

decrease (P<0.05) in carbohydrate content after 15 days and a significant decrease after 30 days. This may be due to activation of enzyme, α-amylase, upon increase in moisture content, which results in degradation of starch (Rosa et al., 2004).

4.10.4.6 Effect on antioxidant activity

Figure 4.5 and Figure 4.6 represent effect of storage on antioxidant activity measured by DPPH and FRAP method, respectively. A significant decrease (P<0.05) in antioxidant activity of snack bars was observed after storage period of 15 and 30days. Antioxidant activity of a product depends on storage temperature, moisture content and surface area of food product in contact with air (Sharma et al., 2015). The observed decrease in antioxidant activity may be due to oxidation of lipids due to large surface area of snack bars (Maisuthisakul et al., 2007). The results correspond well with the decrease in fat content of snack bars due to increased lipid oxidation, which is mainly due to retarded antioxidant activity.

4.10.4.7 Effect on total phenolic content

Figure 4.7 depicts effect of storage period on total phenolic content of snack bars. A significant decrease (P<0.05) in total phenolic content of snack bars was observed after storage period of 15 and 30days. The decrease in total phenolic content upon storage may be due to hydrolysis of phenolic acids present in snack bars (Wong et al., 2009).

4.10.4.8 Effect on overall acceptability

Figure 4.8 represents overall acceptability of snack bars. A significant decrease ($P<0.05$) in over acceptability of snack bars was observed after storage period of 15 and 30 days. The apparent reason behind decrease in overall acceptability is unpleasant mouthfeel caused by oxidative rancidity of the food product. The free radicals released after fat oxidation also result in colour detoriation of the product (Zamora and Hidalgo, 2005). Another reason behind decreased overall acceptability might be cross linkaging of oxidized lipids with protein present in food resulting in modification of product texture (Estevez et al., 2005). In general, although the overall acceptability was observed to decrease with storage period in both snack bars but overall acceptability of quinoa bar was more than the control bar after different storage intervals. Bars exhibited least overall acceptability after storage period of 30 days. According to the scoring on nine point hedonic scale, overall acceptability of quinoa bars indicated "like very much" initially and decreased after 15 and 30 days indicating "neither like nor dislike" and "dislike slightly", respectively, while the overall acceptability of control bar decreased from "like slightly" initially to "neither like nor dislike" to "dislike moderately" after 15 and 30 days, respectively.

4.11 Development of crackers

4.11.1 Proximate composition of crackers

Proximate composition of wheat and quinoa incorporated crackers are shown in Table 4.18. Moisture content of crackers ranged from 3.51±0.07 g/100g to 3.55±0.10g/100g. The results are in agreement with the moisture content of various types of crackers reported by Owusu et al., 2011. Although the moisture content of quinoa incorporated crackers was less than the wheat (control, T_0) crackers, but the difference observed was statistically non-significant ($P<0.05$). This may be due to similar cooking and temperature conditions while preparation of all the crackers. Ash, fat and protein was observed to increase with increase in the ratio of quinoa incorporation to the crackers. Ash content of wheat cracker was significantly lower ($P<0.05$) than the 20 and 40% quinoa incorporated crackers but was statistically similar in crackers incorporated with 20 and 40% quinoa flour. Increase in ash content of wheat-quinoa blend with increase in quantity of quinoa incorporation has also been reported by Enriquez et al., 2003.

Protein content of quinoa crackers was higher than that of wheat crackers. The results are supported by the reported higher protein content in quinoa seeds as compared to the other common cereals like wheat (Arneja et al., 2015). Enriquez et al., 2003 also reported better protein quality of food products after incorporation of quinoa to wheat flour.

Table 4.18: Proximate composition of crackers.

Crackers	Moisture (g/100g)	Ash (g/100g)	Fat (g/100g)	Protein (g/100g)	Carbohydrate (g/100g)	Fiber (g/100g)
T_0	3.55±0.10 [a]	1.93±0.31 [a]	4.28±0.03 [a]	10.6±0.08 [a]	80.10±1.03 [a]	2.10±0.21 [a]
T_1 (80:20)	3.51±0.07 [a]	3.01±0.08 [b]	4.31±0.10 [a]	11.9±0.05 [b]	77.13±0.01 [b]	2.17±0.26 [a]
T_2(60:40)	3.53±0.01 [a]	3.22±0.08 [c,b]	4.46±0.35 [a]	12.21±0.08 [c]	77.5±0.35 [c,b]	2.29±0.04 [a]

Highest carbohydrate content, 80.10±1.03 was reported in wheat crackers. Carbohydrate content was found to decrease with the increase in the ratio of quinoa incorporation to the crackers. This may be due to observed increase in fat and protein content. The result correspond well to the findings of Ibrahium, 2015 who reported higher carbohydrate content in wheat biscuits as compared to biscuits supplemented with 20 and 40% quinoa flour.

Fiber content was lowest, 2.10±0.21g/100g in wheat (control, T_0) cracker and was found to increase with incorporation of quinoa but was statistically similar ($P<0.05$) in all the crackers. The results are in line with the findings of Jancurova et al., 2009 who reported higher crude fiber content in quinoa as compared to the wheat.

4.11.2 Total phenolic content (TPC) and antioxidant activity

The total phenolic content (TPC) and antioxidant activity of crackers is shown in Table 4.19. The total phenolic content of wheat cracker (control, T_0) was observed

as 88.16±0.02 mg GAE/ 100g. The results are similar to the findings of Sedej, 2011 who reported 84 to 148 mg GAE/ 100gm total phenolic content in wheat crackers used as experimental control in their study. The TPC was found to increase significantly ($P<0.05$) with incorporation of quinoa flour to the crackers and was directly related to ratio of quinoa flour added. Crackers incorporated with quinoa flour showed 3.5 to 7% higher TPC than the wheat cracker (control).The results correspond well to the findings of Jubete et al., 2010 who reported higher total phenolic content of quinoa as compared to the wheat. Brend et al., 2012 also reported increase in total phenolic content of quinoa as influenced by baking. Highest TPC (94.25±0.14 mg GAE/100g) was observed in cracker incorporated with 40% quinoa flour (T_2).

Table 4.19: Total phenolic content (TPC) and antioxidant activity of crackers

Cracker	**Antioxidant Activity**		**Total phenolic Content (mg GAE/100g)**
	DPPH (%)	**FRAP (mg Te/100g)**	
T_0	41.68±0.46 [a]	96.29±0.30 [a]	88.16±0.02 [a]
T_1	45.93±0.14 [b]	109.12±0.04 [b]	91.32±0.51 [b]
T_2	50.13±0.90 [c]	118.08±0.15 [c]	94.25±0.14 [c]

The antioxidant activity of wheat cracker (control, T_0) was observed as 41.68±0.46 % (by DPPH method) and 96.29±0.30 mgTE/100g (by FRAP method). Antioxidant activity was observed to increase significantly ($P<0.05$) with incorporation of quinoa flour. The results are supported by the findings of Sedej et al., 2011 who reported increased antioxidant activity of buckwheat crackers as compared to the wheat crackers. Highest antioxidant activity was observed in crackers incorporated with 40% quinoa flour (50.13±0.90 % by DPPH method and 118.08±0.15 mg TE/ 100g by FRAP method). Increase in antioxidant activity upon incorporation of cereal and pseudocereal flours like sorghum and buckwheat has also been reported earlier by Chiremba et al., 2009 and Sedej et al., 2011.

4.11.3 Sensory evaluation

The data analysis of sensory evaluation, Table 4.20, revealed non-significant difference ($P<0.05$) in appearance and colour of all crackers. As compared to the wheat cracker ($T_{0,}$ control), texture of quinoa incorporated crackers was more acceptable. This may be due to better water holding capacity of quinoa as compared to wheat, which enhances evenness in consistency and hence imparts better texture to resultant product (Inglett et al., 2015).

Table 4.20: Sensory evaluation of crackers

	Appearance	**Colour**	**Texture**	**Mouthfeel**	**Flavour**	**Taste**	**Overall Acceptability**
T_0	7.3±0.70 ^{a}	6.5±0.8 ^{a}	6.9±0.5 ^{a}	7.1±0.78 ^{a}	6.2±0.9 ^{a}	6.1±1.05 ^{a}	6.5±0.94 ^{a}
T_1	7.7±0.40 ^{a}	7.2±0.6 ^{a}	7.2±0.4 ^{b}	7.5±0.5 ^{b}	7.7±0.4 ^{b}	8.0±0.5 ^{b}	7.6±0.58 ^{b}
T_2	7.6±0.50 ^{a}	7.3±0.7 ^{a}	7.3±0.52 ^{c,b}	7.4±0.72 ^{c,b}	8.2±0.6 ^{c,b}	8.44±0.52 ^{c,b}	7.94±0.71^{c,b}

Similarly, mouthfeel, flavor, taste of quinoa incorporated crackers (20 and 40%, respectively) was significantly highly ($P<0.05$) acceptable than the wheat crackers. The results are supported by findings of Elsohiamy et al., 2015 who reported water and oil absorption characteristics of quinoa better than the wheat and similar to soy, which is responsible for better mouthfeel and taste of the product. Cracker incorporated with 40% quinoa flour received highest score for texture, mouthfeel, flavor and taste on nine point hedonic scale which was although non-significant to the scores received by crackers incorporated with 20% quinoa flour. The results also indicated that the increase in ratio of quinoa incorporation resulted in increase in panelist's rating for texture, mouthfeel, flavor and taste of the resultant quinoa cracker. Overall acceptability was lowest for the wheat ($T_{0,}$ control) crackers and highest for crackers incorporated with 40% quinoa flour (T_2). The findings are in

line with the results reported by Bhathal et al., 2015 who revealed higher overall acceptability of food products prepared with quinoa than the control products. Statistically, the results indicated significantly lower ($P<0.05$) overall acceptability of wheat cracker ($T_{0,}$ control) as compared to quinoa incorporated crackers (20 and 40%), while overall acceptability of cracker incorporated with 40% quinoa flour was although higher but statistically non-significant to the cracker incorporated with 20% quinoa flour. The results are supported by Bahaduri and Navder, 2014 who demonstrated positive effect of use of quinoa flour in baked food products for better sensory characteristics and higher consumer acceptability.

4.11.4 Storage study

4.11.4.1 Effect proximate composition of crackers

Figure 4.10 represents moisture content of crackers as affected during storage period of 15 days. The figure depicts increase in moisture content of all the crackers upon storage. A non-significant increase ($P<0.05$), in moisture content was observed in wheat cracker (control, T_0) while the moisture content of quinoa incorporated crackers increased significantly ($P<0.05$) after 15 days. Highest increase in moisture content was observed in T_2 (3.6%) followed by T_1(3.3%) and T_0 (0.6%). Increased moisture content of quinoa crackers over the period may be due to the changes in water holding capacity of quinoa during the storage as already reported by James et al., 2009. Increase in moisture content might also be due to water vapour transmission through the polythene packaging material used to store the crackers (Bertrand et al., 2013). As, the moisture content of food is inversely related to its shelf life (Genkawa et al., 2008), the results depict lower shelf life of formulated wheat and quinoa crackers.

4.4.11.2 Effect on crude ash

Figure 4.11 represents ash content of crackers as affected during storage period of 15 days. Ash content of all the crackers was observed to decrease non-significantly ($P<0.05$) upon storage for 15 days. Decrease in ash content during storage period has also been rported by Nadarajah et al., 2015 in coconut cookies. Highest decrease in ash content was observed in T_1 (1.99%) followed by T_2 (1.24%) and T_0 (1.04%). This decrease in ash content may be due to mineral binding properties of by products formed during millard reaction (Nadarajah et al., 2015). Decrease in

ash content may also be due to increase in moisture content, which favours microbiological growth. During their growth period, the microbes utilize minerals and other nutrients, which result in decrease in ash content (Adams and Moss, 2005).

4.11.3 Effect on crude fat

Figure 4.12 represents changes in fat content of crackers during the storage period of 15 days. Fat content of wheat cracker (control, T_0) and cracker incorporated with 20% quinoa (T_1) decreased non-significantly by 0.9 and 0.4%, respectively, while a significant decrease of 2.6% was observed in cracker incorporated with 40% quinoa (T_2). Decrease in fat content of cereals over a period of time during storage has also been reported by Sharma et al., 2015. Fat in presence of moisture causes tenderness in baked food products. Crackers are highly susceptible to lipid oxidation due to their larger surface area which is in contact with air and moisture (Maisuthisakul et al., 2007). Decrease in fat content can be attributed to increased activity of lipase which is highly influenced by moisture content of food (Agrahar-Murgkar and Jha, 2011). Lipase is responsible for oxidative rancidity leading to hydrolysis of fat present in food matrix and formation of free fatty acids which also imparts off flavor to the food product (Adawiyah et al., 2012).

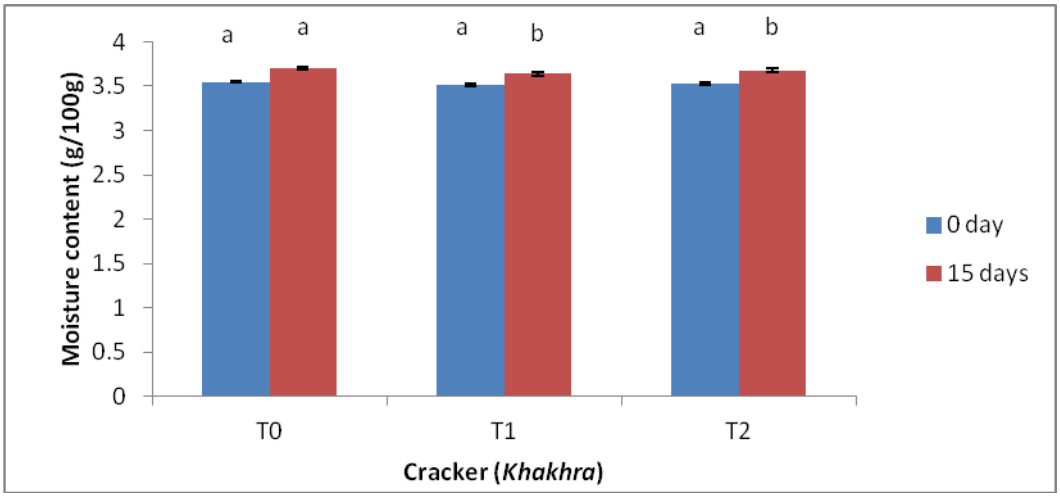

Figure 4.10: Effect of storage period on moisture content of crackers

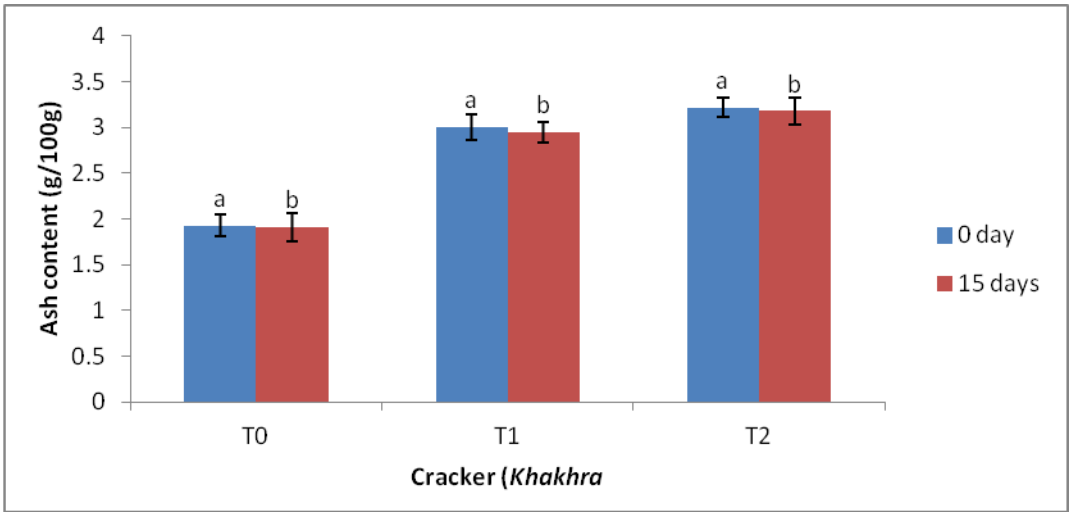

Figure 4.11: Effect of storage period on ash content of crackers.

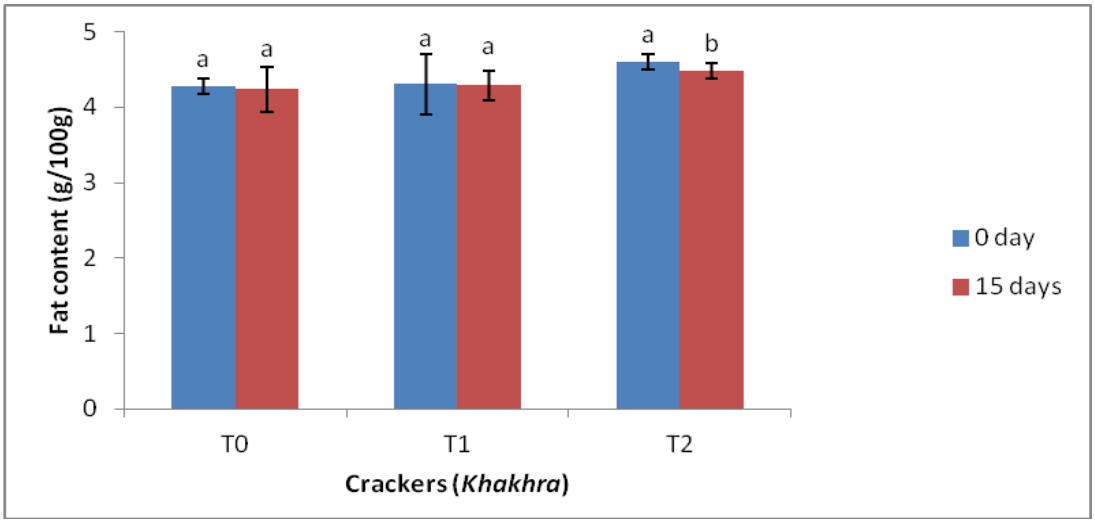

Figure 4.12: Effect of storage period on fat content of crackers.

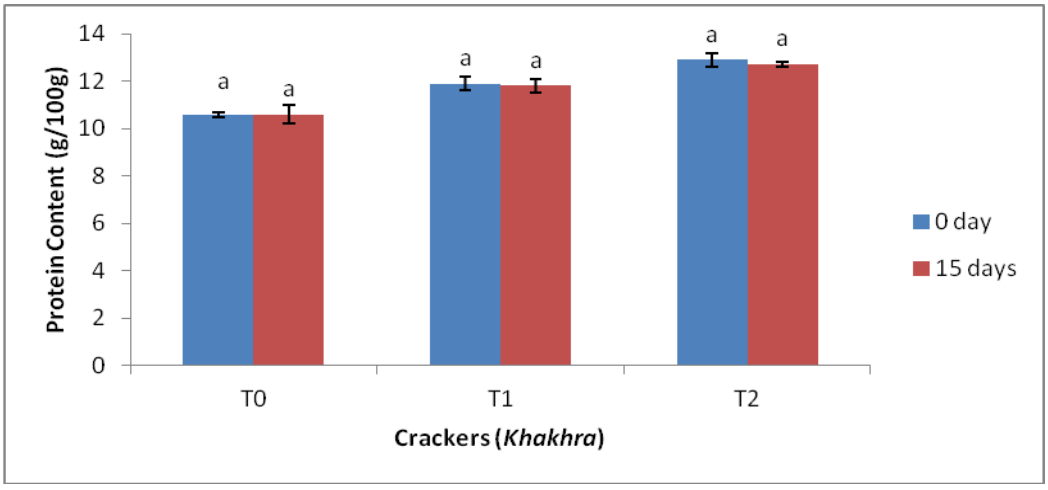

Figure 4.13: Effect of storage period on protein content of crackers.

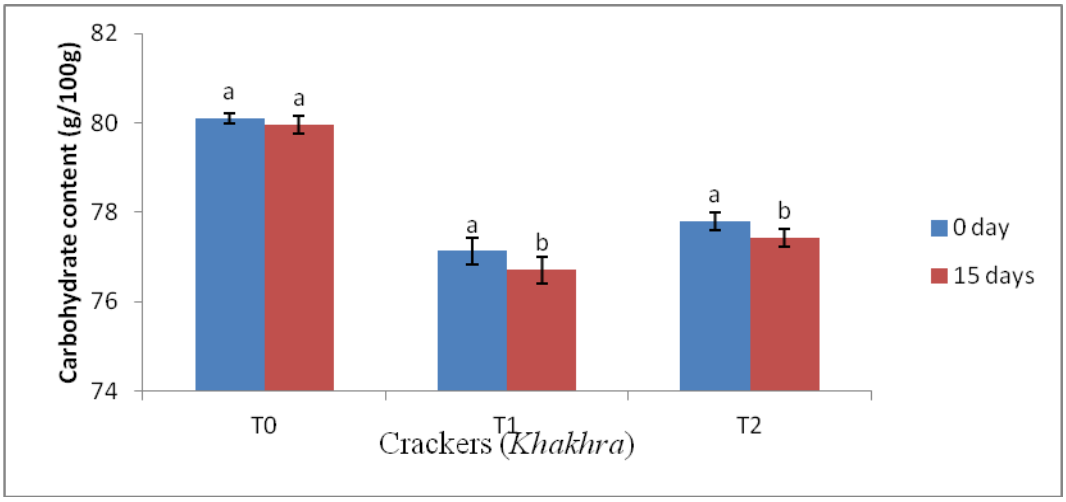

Figure 4.14: Effect of storage period on carbohydrate content of crackers

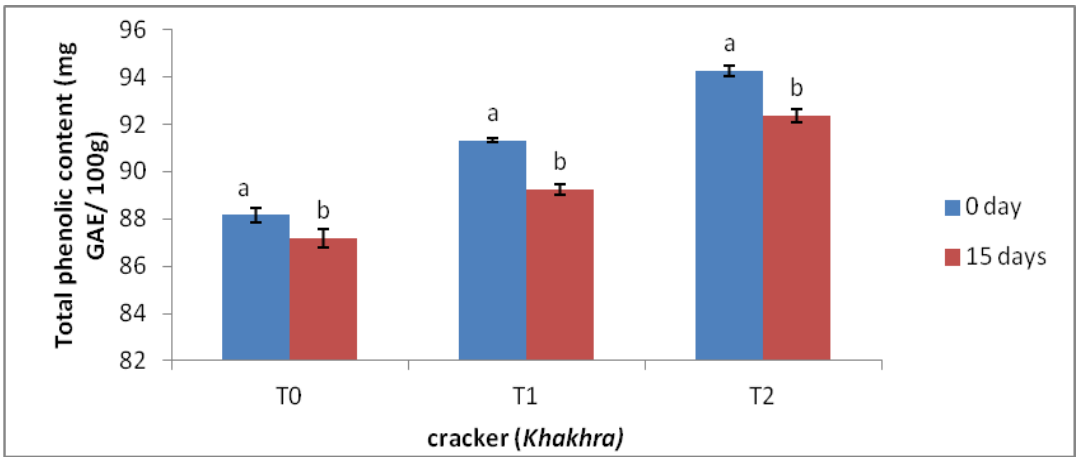

Figure 4.15: Effect of storage period on total phenolic content of crackers.

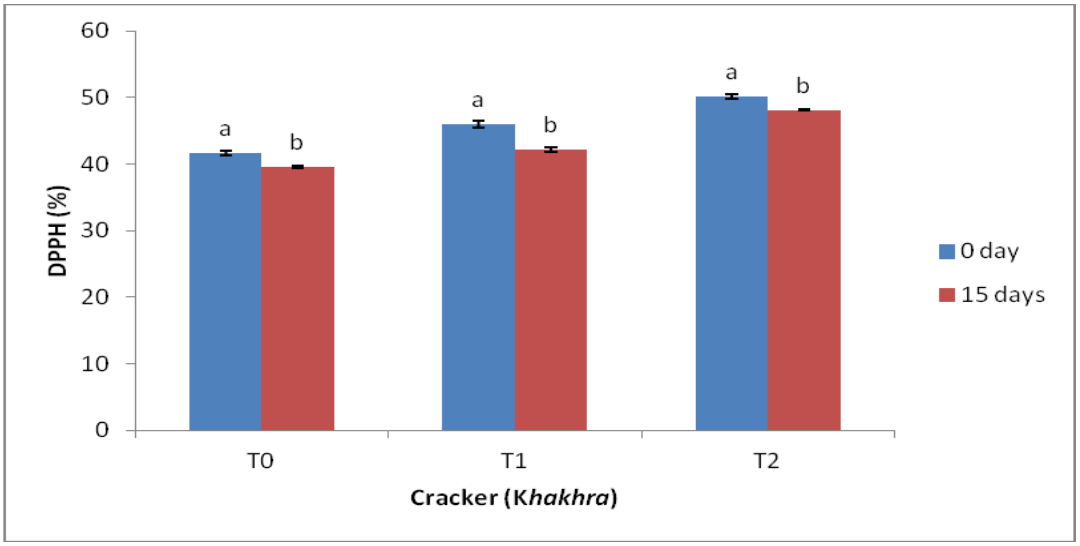

Figure 4.16: Effect of storage period on antioxidant activity (DPPH) of crackers.

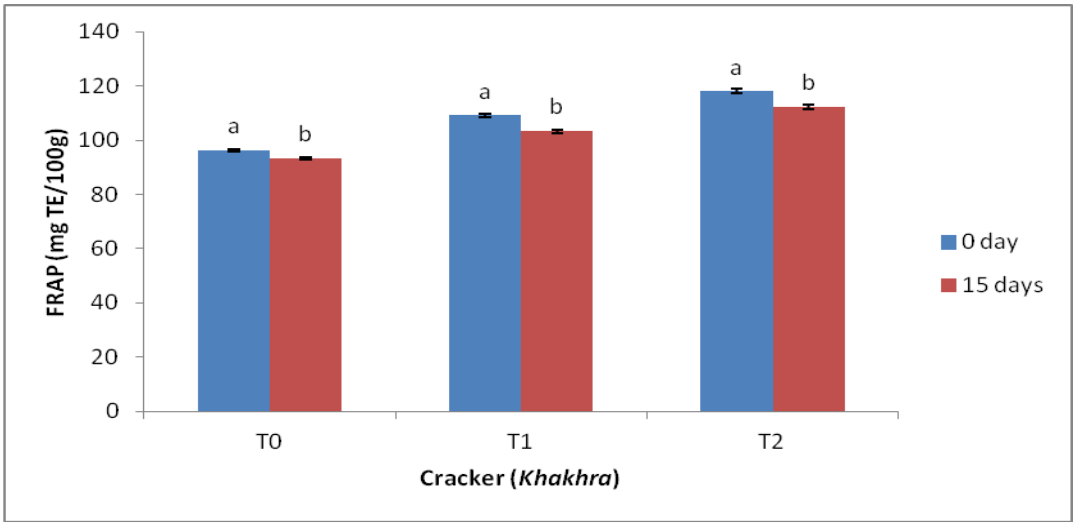

Figure 4.17: Effect of storage period on antioxidant activity (FRAP) of crackers.

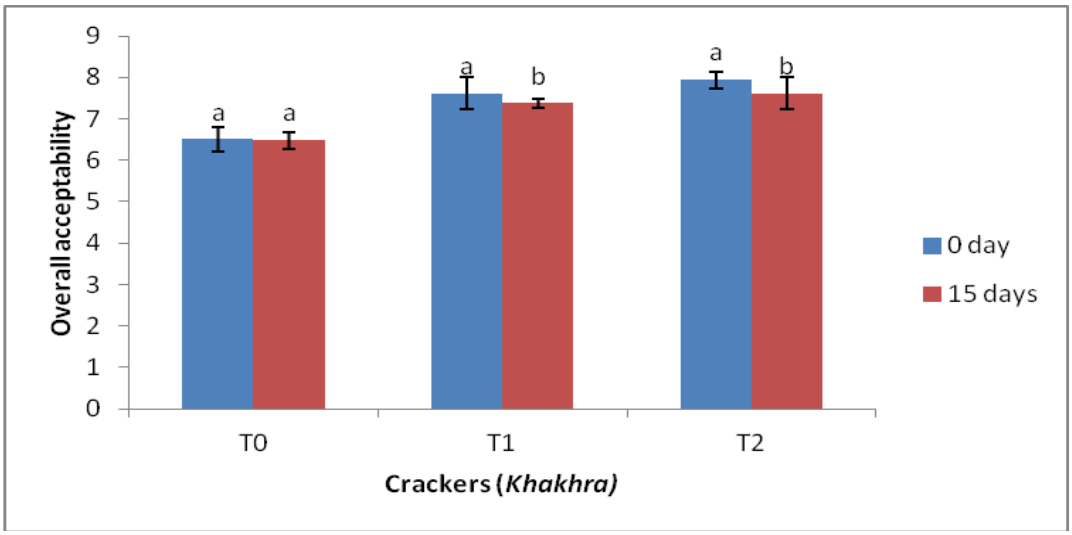

Figure 4.18: Effect of storage period on overall acceptability of crackers.

4.11.4 Effect on protein

Figure 4.13 represents effect of storage period on protein content of crackers. In general, 1 to 1.5% non-significant decrease ($P<0.05$) was observed in protein content of crackers after storage period of 15 days. Decrease in protein content during storage of pumpkin incorporated cake has also been reported by Bhat and Bhat, 2013. Decrease in protein content may be attributed to increase in proteolyic activity due to increase in moisture content (Butt et al., 2004). Another cause of decrease in protein content can also be ascribed to susceptibility of baked food products to millard reaction, which results in protein detoriation (Nadarajah et al., 2015).

4.11.5 Effect on carbohydrate

Figure 4.14 represents effect of storage period on carbohydrate content of crackers. Overall, carbohydrate content of crackers was observed to decrease after storage period of 15 days. A non-significant decrease ($P<0.05$), in carbohydrate content was observed in wheat cracker (control, T_0) while the carbohydrate content of quinoa incorporated crackers decreased significantly ($P<0.05$) after 15 days. This may be due to activation of enzyme, α-amylase, upon increase in moisture content, which results in degradation of starch (Rosa et al., 2004). Furthermore, decrease in carbohydrate of crackers was observed to follow statistically similar trend to increase in moisture content after storage period of 15 days.

4.11.6 Effect of total phenolic content (TPC)

Figure 4.15 depicts effect of storage period on total phenolic content of crackers. A significant decrease ($P<0.05$) in total phenolic content of crackers was observed during storage period of 15 days. The highest decrease in total phenolic content was reported in crackers incorporated with 20% quinoa (2.3%) followed by 40% quinoa incorporated cracker (2.1) and wheat (control, T_0) cracker (1.5%). The decrease in total phenolic content upon storage may be due to hydrolysis of phenolic acids present in cracker (Wong et al., 2006).

4.11.7 Effect on antioxidant activity

Figure 4.16 and Figure 4.17 represent effect of storage on antioxidant activity measured by DPPH and FRAP method, respectively. A significant decrease ($P<0.05$) in antioxidant activity of crackers was observed during storage period of 15 days. Antioxidant activity of a product depends on storage temperature, moisture content and surface area of food product in contact with air (Decker et al., 2010 and Sharma et al., 2015). The observed decrease in antioxidant activity may be due to oxidation of lipids due to large surface area of cracker (Maisuthisakul et al., 2007). The results correspond well with the decrease in fat content of cracker, reported in this study, which may be probably due to increased lipid oxidation, generally caused by decreased antioxidant activity. Thus, reduction in total phenolic content might be the apparent reason behind decrease in antioxidant activity of crackers during storage.

4.11.8 Effect on overall acceptability

Figure 4.18 represents overall acceptability of crackers. Overall acceptability of wheat (control, T_0) had no significant effect ($P<0.05$) after storage period period of 15 days while overall acceptability of crackers incorporated with 20 and 40% quinoa decreased significantly ($P<0.05$). The apparent reason behind decrease in overall acceptability unpleasant mouthfeel caused by oxidative rancidity of the food produt. The free radicals released after fat oxidation also result in colour detoriation of the product (Zamora and Hidalgo, 2005). Another reason behind decreased overall acceptability might be cross linkaging of oxidized lipids with protein present in food resulting in modification of product texture (Estevez et al., 2005). In general, highest decrease in overall acceptability, 4.1% was observed in overall acceptability of 20% quinoa incorporated cracker followed by 3.9% decrease in 40% quinoa incorporated cracker and 1.5% decrease in wheat (control, T_0).

4.12 Beverages

4.12.1 Physical analysis

Physical analysis Parameters are tabulated in Table 4.21. pH ranged from 6.2± 0.01 to 6.5 ± 0.10. Statistically, no significant difference ($P<0.05$) was observed in pH content of all quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Thuresson, 2015 also reported 5.4 to 6.4 pH value of quinoa beverage formulated from white *Chenopodium quinoa* seeds.

Total soluble solid content ranged from 9.08± 0.02 to 9.45 ± 0.01%. No significant difference ($P<0.05$) was observed in total solid of quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Results reported in our study are quite similar to total solid content (%) in soymilk as reported by Kim et al., 2012.

Viscosity of quinoa beverages ranged from 15.02 ± 0.01 to 15.31 ± 0.04 cp. No significant difference ($P<0.05$) was observed in viscosity of quinoa beverages prepared from raw, soaked and germinated quinoa seeds. The results are similar to the viscosity (13 cp) of quinoa beverage reported by Thuresson, 2015. The slight difference noted, on higher side, in viscocity of quinoa beverage prepared in this

study may be due to addition of hydrocolloid, xanthan gum, which tends to thicken and increase the viscosity of the resultant product (Saha and Bhattacharya 2010).

4.12.2 Proximate analysis of beverages

Moisture content of raw, soaked and germinated quinoa beverage (Table 4.21) ranged from 84.01± 0.01 to 84.07±0.13 g/100ml. The results are similar to the moisture content of fermented quinoa bevereges reportd by Bianchi et al., 2014. The results also correspond well to the 88.9 g/100ml moisture content of soy beverage reported by Jackson et al., 2002. Moisture content of all the formulated quinoa beverages was found to be statistically similar ($P<0.05$). Moisture content of quinoa beverage reported in this study is less than the moisture content of quinoa beverage reported by Thuresson, 2015, which may be due addition of hydrocolloid, xanthan gum, which serves as a thickening agent (Panovska et al., 2012).

Protein content of quinoa beverages ranged from 0.68 to 1.5 g/100 ml. The values of protein content in quinoa beverages reported in this study are similar to the protein content in quinoa milk (0.49- 1.72 g/100g, *wb*) reported by Pineli et al., 2015. The results are also supported by findings of Thuresson, 2015 who reported 1.43g/100ml protein content in quinoa beverage. Significant increase ($P<0.05$) in protein content was reported in GQB as compared to RQB. This may be due to increased activity of enzyme protease, during germination of seed, leading to degradation of peptides to amino acids and further synthesis of new protein (Laetitia et al., 2005). Protein content of quinoa beverage was observed to be less than the protein content of soy beverages (1.68 to 2.36 g/100ml) as reported by Terhaag et al., 2013. This may be due to higher protein content of soyabean as compared to quinoa (Jancurova et al., 2009).

Carbohydrate content of quinoa beverages ranged from 14.9±0.1 to 16.2±0.02g/ 100 ml with trend in increasing order being GQB<SQB<RQB. Statistically, the difference between carbohydrate content of all quinoa beverages was signinificant ($P<0.05$). The results were close to the the carbohydrate content in quinoa milk (14.7 g/100g) reported by Pineli et al., 2015. Carbohydrate content of SQB and GQB was observed to be 4 and 8% to be lower with respect to RQB, respectively. Similar decrease in carbohydrate content upon soaking and germination has been

reported by Uppal and Bains, 2012. This decrease may be accounted to hydrolysis of starch during process of soaking and germination (Tang et al., 2015).

Fat content of beverages ranged from 0.23 to 0.93 g/ 100ml. GQB was reported with least fat content which may be due to use of fat as energy during grain sprouting (Kayembe and Rensburg, 2013). RQB and SQB were reported with significantly higher ($P<0.05$) fat content as compared to GQB. The results were similar to the lipid content in quinoa milk reported by Pineli et al., 2015.

Thuresson, 2015 also reported 1.43g/100ml of fat content in quinoa beverage. The difference may be due to varietal difference in *Chenopodium quinoa* seeds used for the formulation of beverage.

Ash content of quinoa beverages ranged from 0.11±0.01 to 0.28 gm/100ml. The results are similar to the ash content of beverages prepared with 100% quinoa extracts, reported by Bianchi et al., 2015. A significant increase in ash content ($P<0.05$) was reported in GQB as compared to RQB. This may be due to increase in ash content upon germination as reported by Echendu et al., 2009.

4.12.3 Total phenolic content and Antioxidant activity

Predominantly, all quinoa beverages, as indicated in Figure 4.19, had total phenolic content well correlated to its anti oxidant activity. Total phenolic content of quinoa beverages ranged from 1.9 to 2.4 mg GAE/ g. The results are close to total phenolic content in quinoa beverage (1.52 mg GAE/ g) reported by Thuresson, 2015. Germinated quinoa beverage (GQB) showed highest phenolic content (2.4 ± 0.2 mg GAE/g), followed by SQB (2.1 ± 0.2 mg GAE/g) and RQB (1.0 ± 0.3 mg GAE/ g). Megat et al., 2016, also reported an increment in total phenolic content upon germination. Higher phenolic content in germinated quinoa beverage might be attributed to better liberation of bound phenolic contents from the cereal matrix during the process of germination (Sharma et al., 2015).

Antioxidant activity of quinoa beverages (Figure 4.19), as determined by DPPH method ranged from 52 to 92%. Potential to inhibit DPPH free radical i.e. the anti oxidant activity of quinoa beverages followed the same trend as their total phenolic content i.e. GQB>SQB>RQB. Also, the antioxidant activity was found to have good correlation with the total phenolic content ($r = 0.95$, $P<0.05$), which suggests

potential contribution of phenolic compounds in quinoa to its antioxidant activity. Good pearson coefficient correlation between antioxidant activity and total phenolic content has also been reported in soy beverages by Durazzo et al., 2015. The findings are also supported by linear correlation between antioxidant activity and phenolic compounds in germinated quinoa reported by Carciochi et al., 2014b.

Table 4.21: Physical and proximate analysis of beverages

Quinoa bevera-ges	pH	Total soluble Solid (% Brix)	Viscocity (cp)	Serum Separation		Moisture (g/100ml)	Protein (g/100ml)	Carbohydrate (g/100ml)	Fat (g/100ml)	Ash (g/100 ml)
				Water phasewithout xanthan Gum (cm)	Water phase with Xanthan gum					
RQB	6.2± 0.01[a]	9.08± 0.02 [a]	15.31 ± 0.04[a]	15.3±0.02	5.4±0.04	84.01±0.02 [a]	0.68± 0.01[a]	16.2 ± 0.02[a]	0.93 ± 0.02[a]	0.13± 0.04[a]
SQB	6.5 ± 0.10[b]	9.45 ± 0.01[b]	15.12± 0.01[b]	14.8±0.13	4.2±0.11	84.05±0.11 [a]	1.2 ± 0.10[b]	15.5± 0.01[b]	0.81 ± 0.01[b]	0.11± 0.01[b]
GQB	6.3 ± 0.03[a,c]	9.39 ± 0.04[c]	15.02 ± 0.01[c]	14.5±0.11	4.3±0.16	84.07±0.13 [a]	1.5 ± 0.01[c]	14.90± 0.1[c]	0.23 ± 0.04[c]	0.28 ± 0.01[c]

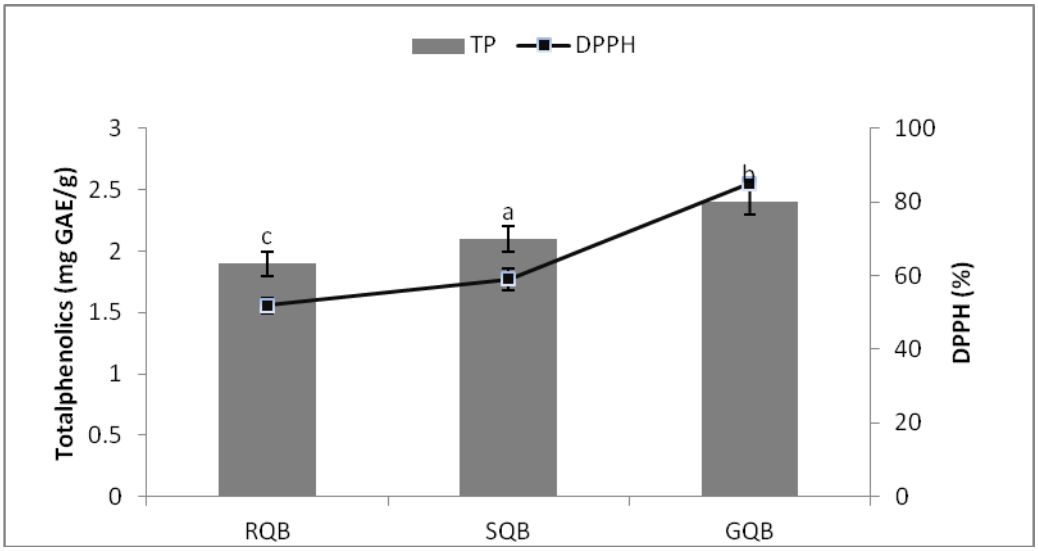

Figure 4.19: DPPH and total phenolic content of quinoa beverages.

4.12.4 Effect of addition of xanthan gum in quinoa beverages:

Xanthan gum has been used extensively in gluten free formulations (Lazaridou et al., 2007). Addition of 0.5% (*w/v*) xanthan gum increased the viscosity and helped in textural stabilization of the beverages by reducing serum separation. Serum separation reduction ranged from 64.7 to 71.6%. The results are supported by findings of Panovska et al., 2010 who reported reduced rate of phase separation on addition of 0.1-0.8% (*w/w*) xanthan gum in beverage. Xanthum gum has been reported as successful hydrocolloid for textural stabilization of beverages due to its peculiar structural characteristics (Desplanques et al., 2012).

4.12.5 Sensory Evaluation

Panelists opinion on positive (Color, flavor, texture, mouthfeel, taste, consistency) and negative aspect (after taste), of formulated quinoa beverages is indicated in spider diagram in Figure 4.20. Color aspect of all quinoa beverages ranged from 5.1±0.02 to 5.3±0.11 indicating "neither like nor dislike". Flavor of quinoa beverages ranged from 5.3±0.03 to 7.1±0.12. Flavor of RQB was rated as "dislike very much" as compared to SQB ("like slightly") and GQB ("like moderately"). Low consumer acceptance of quinoa beverage prepared from raw quinoa seeds due to the presence of strong beany flavor of quinoa has also been reported by Thuresson, 2015. Flavor aspect of GQB was rated similar to the soya beverage used as control. Texture and consistency of all quinoa beverages was rated as "like slightly." This may be due to addition of xanthan gum which is known to

Table 4.22: Sensory evaluation of quinoa beverages

Quinoa Beverage	Favourite (%)	Least favourite (%)	Overall acceptability
RQB	0	88	2.2 ± 0.1^{a}
SQB	24	4	3.9 ± 1.4^{b}
GQB	28	4	6.8 ± 2.5^{c}
Commercial Soya milk	32	4	6.9 ± 1.4^{c}

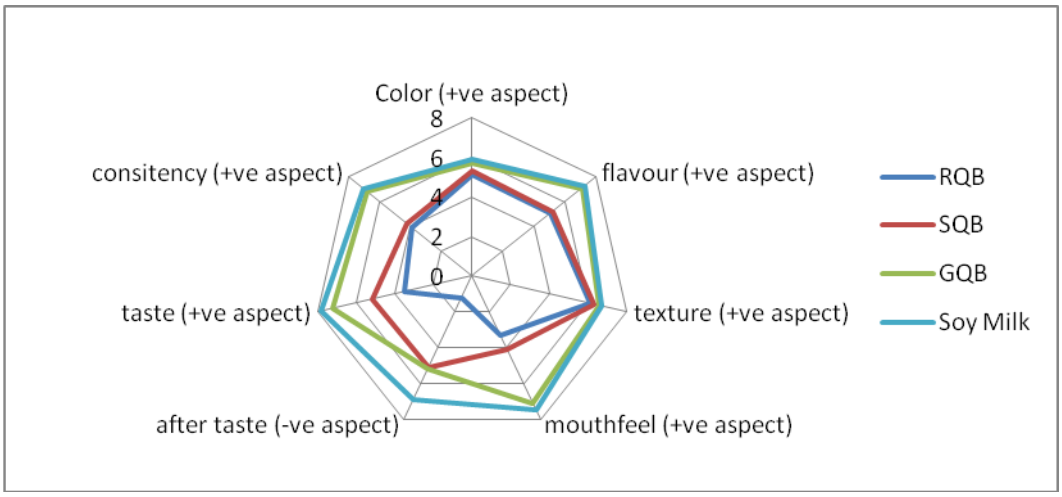

Figure 4.20: Panelists opinion on positive and negative aspects of quinoa beverages with respect to commercial soy milk.

improve texture and stabilize consistency of food products (Desplanques et al., 2012). Among all the beverages, RQB indicated lowest score on nine point hedonic scale for after taste (1.2±0.1), indicating "dislike extremely" and least good mouth feel indicating "dislike very much" (2.3±0.4). The after taste of raw quinoa beverage, as stated by panelists was "bitter". This may be due to presence of saponins in raw quinoa, which imparts bitter taste to quinoa (Miranda et al., 2014) and their lixiviation into water used for formulation of beverage during preparation. Similar negative comments about quinoa beverages from raw quinoa seeds have also been stated by Thuresson, 2015. After taste aspect SQB and GQB was better than RQB and marked as "neither liked nor disliked" and "like slightly," respectively. This is due to reduction in content of bitter taste imparting

antinutrients like saponins post processing methods like soaking and germination (Kayembe and Rensburg, 2011). In general, it was observed that GQB qualified all positive aspects and was rated similarly to the commercial soya milk used as control.

On evaluation of panelists choice of favorite and least favorite beverage (Table 4.22), it was observed that among quinoa beverages, RQB was rated as least favorite beverage by 88% panelists (22 out of 25) and GQB was most favorite beverage of 28% (7 out of 25) panelists. Overall acceptability of quinoa beverages, as indicated in Table 2, ranged from dislike very much to like moderately with acceptability trend in increasing order being RQB<SQB<GQB. RQB was disliked because of its bitter after taste and beany flavor while GQB was most liked among all quinoa beverages with overall acceptability score significantly similar ($P<0.05$) to that of commercial soya milk.

CHAPTER 5

SUMMARY AND CONCLUSION

1. Towards the accomplishment of objective one, the *Chenopodium quinoa* seeds were evaluated for their nutritional quality. The seeds were subjected to proximate, nutritional, phytochemical and *in vitro* analysis.

a) Indian *Chenopodium quinoa* seeds were processed by the domestic processes, namely, soaking and germination and compared with respect to raw and industrially processed seeds and evaluated for proximate, nutritional, and phytochemical composition.

i. **Proximate composition**:

The raw seeds were reported to have 11.03±0.08 g/100g of moisture content. A significant ($P<0.05$) difference in moisture content of raw, domestically processed and industrially processed seeds was observed. Soaking resulted in increase in 8.5% of moisture content while germination resulted in decrease in moisture content by 17.5%. Industrial processing of quinoa led to 8.5% decrease in moisture content.

The Carbohydrate content of raw quinoa seeds was 65.11±0.12 g/100g. The results were significantly ($P<0.05$) different between raw and domestically processed seeds. However no significant difference ($P<0.05$) in carbohydrate content of seeds subjected to soaking and germination was observed. Soaking and germination resulted in decrease of carbohydrate content by 1.6% and 2% respectively. The carbohydrate content of industrially processed and domestically processed seeds was significantly ($P<0.05$) different.

The raw Indian quinoa had 5.17±0.18 g/100g crude fat content. Soaking and germination of raw seeds caused significant decrease ($P<0.05$) in fat content by 20.5% and 32.5%. Industrially processed seeds had 1.6% reduced fat content as compared to the raw seeds. No significant ($P<0.05$) difference was observed in change in fat content of soaked, germinated and industrially processed quinoa seeds.

The raw quinoa seed was reported to have 12.54±0.03g/100g crude protein content. Crude protein content of raw, domestically processed and industrially processed quinoa seeds was significantly ($P<0.05$) different. Soaking and germination led to increase in protein content by 4.8% and 19.2%. Increase in protein content of industrially processed quinoa seeds was similar to the increase reported after soaking.

The raw seeds were reported to have ash content of 3.19±0.03 g/100g. Soaking resulted in 0.6% increase in ash content which was statistically non-significant ($P<0.05$) with respect to ash content of raw quinoa seeds while significant increase ($P<0.05$) in ash content, by 22.5%, was observed after germination. Industrially processed seeds were reported with 4.7% reduced ash content.

The raw quinoa seeds had crude fiber content of 2.22±0.01 g/100g. The fiber content of all quinoa seeds were significantly ($P<0.05$) different. Soaking led to 31.8% increase in fiber content and germination caused increase by 4%. Industrial processing led to 7.6% decrease in fiber content.

ii. Nutritional composition:

Raw quinoa seeds were reported to have 535.9±3.6 μg/100g of β- Carotene content. Domestic and industrial processing of seeds led to non-significant change ($P<0.05$) in contents of β- carotene. Soaking and germination led to 0.1% and 0.8% increase in β- carotene.

Raw quinoa seeds were reported with 10.26±0.17 g/100g of dietary fiber content. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) constituted about 71.9 to 73.3% and 26.6 to 28% of total dietary fiber, respectively. Domestic processing of seeds i.e. soaking and germination, led to 10% and 31% significant increase ($P<0.05$) in TDF content of quinoa seeds. Both the constituents of dietary fiber i.e. NDF (Lignin, cellulose, and hemicellulose) and ADF increased upon soaking and germination. Industrially processed seeds exhibited 9.9%, significant decrease ($P<0.05$) in TDF content.

Vitamin C content of raw quinoa seeds was found to be 13mg/100g. There was significant difference in vitamin C content of raw and domestically processed Indian quinoa seeds ($P<0.05$). Vitamin C content increased by 15% in soaked

quinoa seeds and by 46% in germinated quinoa seeds. A significant difference in Vitamin C content of raw and industrially processed seeds was also observed. Industrial processing decreased the vitamin C content by 30%.

Raw quinoa seeds were reported with 85.3±0.25 mg/100g calcium content. Domestic processing of seeds i.e. soaking and germination led to 0.59% and 0.94%, non-significant increase ($P<0.05$) in calcium content. The iron content in raw Indian quinoa seeds was 5.2±0.01 mg/ 100g. A significant increase ($P<0.05$) of 1.9% and 13.4% was observed in quinoa seeds subjected to domestic processing methods i.e. soaking and germination, respectively. The zinc content of raw quinoa seeds was 6.6±0.04 mg/100g. Zinc content was significantly reduced ($P<0.05$) by 10.6% and 13.5% after soaking and germination, respectively. Raw quinoa seeds were reported to have magnesium content of 182.4±0.11 mg/100g. Domestic processing methods like soaking and germination led to 1.21% and 1.8% significant decrease ($P<0.05$) in magnesium content, respectively. Overall, industrially processed seeds exhibited lower mineral content as compared to the raw seeds. A non-significant reduction ($P<0.05$) was observed in calcium (0.3%), zinc (1.5%) and magnesium (0.1%) content of industrially processed seeds while a significant depreciation ($P<0.05$) was observed in iron (2%) content.

iii. Phytochemical composition

Raw quinoa seeds were reported to have 0.6±0.08g/100g of tannin content. Soaking and germination led to 50% and 66.6% decrease in tannin content. Industrially processed seeds were reported to have reduction in tannin content similar to the reduction observed after soaking.

Total alkaloid content of raw Indian quinoa seeds was 2.11±0.01 g/100g. Soaking and germination led to 1% and 15%, significant decrease ($P<0.05$) in alkaloid content of quinoa. Industrialy processed seeds exhibited 10% decrease in alkaloid content

Total oxalate content of raw Indian quinoa seeds was 2.11±0.01 g/100g. All the processing methods led to reduction in oxalate content. Soaking and germination resulted in 30% and 55% decrease in oxalate content. Industrially processed seeds were also reported with 10% reduction in oxalate content.

Raw quinoa seeds were reported to have 1.25±0.22 g/100g phytic acid. Soaking resulted in reduction of phytic acid content by 2.5%, which was statistically non-significant ($P<0.05$) as compared to raw seeds. Germination led to significant decrease ($P<0.05$) in phytic acid content by 68%. Industrial processing led to non-significant decrease ($P<0.05$) in phytic acid content by 3.2%

Total phosphorous content of raw Indian quinoa seeds was 0.43±0.22 g/100g. Soaking resulted in a non-significant increase ($P<0.05$) in total phosphorous content by 7%. Germination of quinoa seeds was observed to cause a significant increase ($P<0.05$) in total phosphorous content. Industrially processed seeds exhibited 4.6% increase in total phosphorous content, which was non-significant ($P<0.05$) as compared to the total phosphorous content of raw seeds.

Raw quinoa seeds were reported to contain 2.01±0.15 g/100g saponin content. Soaking resulted in significant decrease ($P<0.05$) of 24% in saponin content. Germination led to 98% decrease in saponin content. Industrially processed seeds exhibited 97% reduced saponin content as compared to the raw seeds. With respect to the germinated seeds, although the reduction was statistically non-significant ($P<0.05$) but germinated seeds were reported to have 50% lower saponin content than industrially processed seeds.

Raw seeds were reported to have 6633±7.5 TIU/100g trypsin inhibitor activity.All the processing methods led to significant decrease ($P<0.05$) in trypsin inhibitor activity. Soaking resulted in 4.7% decrease in trypsin inhibitor activity. Industrially processed quinoa seeds exhibited 20.7% decrease in trypsin inhibitor activity.

iv. *In vitro* analysis

Raw quinoa seeds exhibited 75.3±0.33% protein digestibility. Soaking resulted in 1.5% increase in protein digestibility. Germination resulted in 9.1%, significant increase ($P<0.05$) in protein digestibility. Industrially processed seeds exhibited 1.2% increase in protein digestibility value.

Raw quinoa seeds exhibited 65.7±0.15% starch digestibility. Soaking of quinoa seeds resulted in 1.52%, non-significant increase in starch digestibility while

germination led to 1.80%, significant increase with respect to the raw seeds. Industrially processed seeds exhibited 1.05%, non-significant increase ($P<0.05$), in starch digestibility.

Raw quinoa seeds were reported to have 59.6 mgTE /100g and 37.3 TEAC (Trolox equivalent antioxidant capacity) as calculated by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) method and 84.4 mg TE /100g as calculated by FRAP (Ferric reducing antioxidant activity) method. Soaked quinoa seeds exhibited 7% decrease in antioxidant activity as compared to raw seeds. FRAP values of germinated quinoa seeds increased by 89%. However industrial processing of the seeds lead to decline in antioxidant activity. Processed quinoa seeds showed decline of 14% and 19% antioxidant activity as evaluated by FRAP and DPPH respectively

b) On the basis of proximate, nutritional, and phytochemical composition, industrially processed Indian *Chenopodium quinoa* seeds were compared with industrially processed American *Chenopodium quinoa* seeds.

i. Proximate composition

Moisture, carbohydrate, protein and crude fiber content of Indian and American quinoa seeds were significantly different ($P<0.05$) while ash and crude fat contents were statistically non-significant ($P<0.05$). It was noticeable that the protein content of Indian quinoa (13.11±0.08 g/100g) was 7.02% more than the protein content of American quinoa (12.25±0.92 g/100g).

ii. Nutritional composition

A non-significant difference ($P<0.05$) was observed in dietary fiber content of American and Indian quinoa seeds. Indian quinoa seeds exhibited significantly higher ($P<0.05$) β-Carotene content than American seeds. Vitamin C content of American quinoa was, 14.4%, significantly higher ($P<0.05$) than the vitamin C content of Indian quinoa seeds.

Mineral content of American quinoa was found to be significantly higher ($P<0.05$) than the Indian quinoa. Calcium, iron and zinc contents of American quinoa seeds were 15.6, 43.2 and 26.1% higher than Indian quinoa, respectively. Magnesium content of American quinoa was 6.1% higher than Indian quinoa.

iii. *In vitro* analysis

American quinoa seeds exhibited significantly lower ($P<0.05$) protein digestibility (75.15%) than Indian quinoa seeds. American quinoa seeds exhibited significantly higher ($P<0.05$) starch digestibility (67.14%) than Indian quinoa seeds.

2. The objective two of the study was accomplished by a biological trial using animal model.

a) Male wistar rats (42; divided in 7 groups with 6 rats in each group) for a period of 45 days and testing any cholosterolemic effect of supplementing germinated quinoa (debittered) and raw quinoa (bitter) seeds.

i. The total serum cholesterol level of rats fed with basal diet (Group1) was reported as 160.16±16 mg/dl. The total concentration of plasma cholesterol was observed to reduce significantly ($P<0.05$) upon addition of quinoa to the experimental diet. It was observed that, as compared to the Group 2, the positive control (rats fed on hypercholesterolemic diet), concentration of cholesterol reduced by 8.2% in diet fed with statin, while 15.5 and 24.4% reduction in serum cholesterol was observed in rats fed with raw (Group 4) and germinated quinoa (Group 5) along with hypercholesterolemic diet, respectively. The rats fed with raw (Group 6) and germinated quinoa (Group 7) along with basal diet showed 28.2 and 31.9% reduction in total cholesterol level.

ii. The levels of plasma triglycerides showed a declining trend with supplementation of quinoa to the experimental diet. As compared to the positive control, which showed 96.6±2.9mg/dl triglyceride content, the levels were reported to decrease significantly ($P<0.05$) in rats fed with quinoa diet. However, reduction in triglyceride content by quinoa supplemented along with hypercholestrolemic diet was observed to be lower than the reduction induced by statin administered along with hypercholestrolemic diet. In rats fed with quinoa along with hypercholersterolemic diet, the triglyceride levels reduced by 12.5 and 4.5% in Group 4 and Group 5, respectively. Triglyceride level of rats fed with quinoa along with basal diet i.e. in Group 6 and Group 7, reduced by 10.4 and 14.5%, respectively.

iii. The serum HDL level of rats fed on basal diet were reported as 91.15±4.8 mg/dl while of rats fed on hypocholesterolemic diet was 90.1±2.2 mg/dl . The HDL levels were observed to decrease significantly ($P<0.05$) in rats fed with diets supplemented with raw (5.5%) and germinated (3.3%) quinoa along with hypercholesterolemic diet as compared to serum HDL levels of rats fed with hypercholesterolemic diet (positive control) which reveals negative effect of quinoa supplementation along with hyper cholesterolemic diet on serum HDL levels.

iv. The serum (VLDL+HDL) values of rats fed on basal diet was reported as 68.82±7.2 mg/dl while of rats fed on hypercholesterolemic diet was 128.9±3.5 mg/dl. The results were observed to decrease significantly ($P<0.05$), by 21.3 and 39.4% with supplementation of raw quinoaand germinated quinoa to hypercholesterolemic diet, respectively. The results also infer beneficial effect of germinated quinoa over raw quinoa. Lipid lowering effect of quinoa (raw and germinated) was higher than the beneficial effect of statin, which was reported to cause17.7% decline in (VLDL+HDL) values. The reduction was almost almost twice in germinated quinoa. The results infer lipid lowering beneficial effect of quinoa, mainly germinated quinoa.

v. Atherosclerotic index of rats fed with basal diet was reported as 0.75±0.2 mg/dl while of rats fed on hypercholestrolemic diet was reported as 1.43±0.3 mg/dl. The drug statin administered to rats along with hypercholesterolemic diet, caused 23.7% reduction in atherosclerotic index, while supplementation of raw and germinated quinoa along with hypercholeterolemic diet resulted in 18.4 and 37.2% reduction in atherosclerotic index. The results infer beneficial effect of quinoa, mainly the germinated one, in hypercholesterolemia over the statin drug. The rats fed on raw and germinated quinoa supplemented with basal diet also showed 18.6 and 34.5% reduction in atherosclerotic index.

3. Functional foods, namely, quinoa bar, quinoa cracker and quinoa beverages were prepared from quinoa grains and evaluated for proximate composition, total phenolic content and antioxidant activity. Sensory evaluation and storage studies of functional food products developed in this study was also done. Beverages

prepared from quinoa were additionally analyzed for pH, viscosity, total soluble solids and serum separation.

a) Bars, control bar and quinoa bar, were prepared using chickpea flour and germinated quinoa flour, respectively. Formulated bars were carefully packed in zip lock bags and stored in refrigerator for 30 days. Bars were evaluated for different parameters at an interval of 15 and 30 days.

i. Proximate composition: Moisture content of formulated bars ranged from 15.21±0.18 to 15.19±0.12 g/100gm. Moisture content of control bar (CB) and quinoa bar (QB) were almost similar and statistically non-significant to each other ($P<0.05$). Ash, fat, protein and fiber content of quinoa bar was significantly higher ($P<0.05$) than control bar.

ii. Total phenolic content and antioxidant activity: Total phenolic content of quinoa bar was significantly higher ($P<0.05$) than the control bar. Antioxidant activity of quinoa bars, as evaluated by DPPH and FRAP method, was reported significantly higher ($P<0.05$) than the control bars.

iii. Sensory evaluation: The data analysis of sensory evaluation revealed significant difference ($P<0.05$) in appearance, colour, mouthfeel, flavor and taste of control bars and quinoa bars, while difference in texture was revealed statistically non-significant ($P<0.05$). According to scoring on nine point hedonic scale, overall acceptability of quinoa snack bars indicated "like very much" while control bars which indicated "like slightly." Highest score was scored by taste aspect ($8.4\pm0.09^{)}$ of quinoa bar, which indicated, "like extremely" on a nine point hedonic scale while lowest score was scored by colour aspect of control bar which indicated "like moderaltely" on a nine point hedonic scale.

iv. Effect of storage: A significant increase ($P<0.05$), in moisture content was observed in both control bar and quinoa bar at an interval of 15 and 30 days. However, moisture content at the end of 30 days was highest as compared to the initial moisture content of snack bars. Ash content of snack bars was observed to decrease non-significantly ($P<0.05$) upon storage for 15 days while a significant decrease in ash content was observed after storage period of 30 days. A non significant change

(P<0.05) in fat content was observed after storage period of 15 days while a significant decrease (P<0.05) was observed after storage period of 30 days. A non-significant decrease (P<0.05) was observed in protein content of snack bars after storage period of 15 days while significant decrease (P<0.05) was observed after storage period of 30 days.The carbohydrate content of control snack bars was observed to decrease significantly (P<0.05) after storage period 15 and 30 days as compared to the initial carbohydrate content while quinoa snack bars exhibited non significant decrease (P<0.05) in carbohydrate content after 15 days and a significant decrease after 30 days.

A significant decrease (P<0.05) in total phenolic content and antioxidant activity of snack bars was observed after storage period of 15 and 30days.

A significant decrease (P<0.05) in over acceptability of snack bars was observed after storage period of 15 and 30 days. In general, although the overall acceptability was observed to decrease with storage period in both snack bars but overall acceptability of quinoa bar was more than the control bar after different storage intervals. Bars exhibited least overall acceptability after storage period of 30 days. According to the scoring on nine point hedonic scale, overall acceptability of quinoa bars indicated "like very much" initially and decreased after 15 and 30 days indicating "neither like nor dislike" and "dislike slightly", respectively, while the overall acceptability of control bar decreased from "like slightly" initially to "neither like nor dislike" to "dislike moderately" after 15 and 30 days, respectively.

b) Crackers, control and quinoa cracker, were prepared from wheat flour and quinoa flour, respectively. Crackers made from quinoa flour were formulated in by incorporation of quinoa flour to wheat flour in ratio 80:20 (T_1) and 60:40 (T_2). Formulated crackers were carefully packed in zip lock bags and stored in refrigerator for 15 days. Bars were evaluated for different parameters after 15 days.

i. Proximate composition: Moisture content of crackers ranged from 3.51±0.07 g/100g to 3.55±0.10g/100g. Although the moisture content of

quinoa incorporated crackers was less than the wheat (control, T_0) crackers, but the difference observed was statistically non-significant ($P<0.05$). Ash, fat and protein was observed to increase with increase in the ratio of quinoa incorporation to the crackers. Ash content of wheat cracker was significantly lower ($P<0.05$) than the 20 and 40% quinoa incorporated crackers but was statistically similar in crackers incorporated with 20 and 40% quinoa flour. Protein content of quinoa crackers was higher than that of wheat crackers. Highest carbohydrate content, 80.10±1.03 was reported in wheat crackers. Carbohydrate content was found to decrease with the increase in the ratio of quinoa incorporation to the crackers. Fiber content was lowest, 2.10±0.21g/100g in wheat (control, T_0) cracker and was found to increase with incorporation of quinoa but was statistically similar ($P<0.05$) in all the crackers.

ii. Total phenolic content and antioxidant activity: The total phenolic content of wheat cracker (control, T_0) was observed as 88.16±0.02 mg GAE/ 100g. The TPC was found to increase significantly ($P<0.05$) with incorporation of quinoa flour to the crackers and was directly related to ratio of quinoa flour added. Crackers incorporated with quinoa flour showed 3.5 to 7% higher TPC than the wheat cracker (control). The antioxidant activity of wheat cracker (control, T_0) was observed as 41.68±0.46 % (by DPPH method) and 96.29±0.30 mgTE/100g (by FRAP method). Antioxidant activity was observed to increase significantly ($P<0.05$) with incorporation of quinoa flour. Highest antioxidant activity was observed in crackers incorporated with 40% quinoa flour (50.13±0.90 % by DPPH method and 118.08±0.15 mg TE/ 100g by FRAP method).

iii. Sensory evaluation: The data analysis of sensory evaluation revealed non-significant difference ($P<0.05$) in appearance and colour of all crackers. As compared to the wheat cracker (T_0, control), texture of quinoa incorporated crackers was more acceptable.). Similarly, mouthfeel, flavor, taste of quinoa incorporated crackers (20 and 40%, respectively) was significantly highly ($P<0.05$) acceptable than the wheat crackers. Cracker incorporated with 40% quinoa flour received highest score for texture,

mouthfeel, flavor and taste on nine point hedonic scale which was although non-significant to the scores received by crackers incorporated with 20% quinoa flour. Overall acceptability was lowest for the wheat ($T_{0,}$ control) crackers and highest for crackers incorporated with 40% quinoa flour (T_2).

iv. Effect of storage: A non-significant increase ($P<0.05$), in moisture content was observed in wheat cracker (control, T_0) while the moisture content of quinoa incorporated crackers increased significantly ($P<0.05$) after 15 days. Highest increase in moisture content was observed in T_2 (3.6%) followed by T_1(3.3%) and T_0 (0.6%). Ash content of all the crackers was observed to decrease non-significantly ($P<0.05$) upon storage for 15 days. Highest decrease in ash content was observed in T_1 (1.99%) followed by T_2 (1.24%) and T_0 (1.04%). . Fat content. Fat content of wheat cracker (control, T_0) and cracker incorporated with 20% quinoa (T_1) decreased non-significantly by 0.9 and 0.4%, respectively, while a significant decrease of 2.6% was observed in cracker incorporated with 40% quinoa (T_2). . In general, 1 to 1.5% non-significant decrease ($P<0.05$) was observed in protein content of crackers after storage period of 15 days. Overall, carbohydrate content of crackers was observed to decrease after storage period of 15 days. A non-significant decrease ($P<0.05$), in carbohydrate content was observed in wheat cracker (control, T_0) while the carbohydrate content of quinoa incorporated crackers decreased significantly ($P<0.05$) after 15 days.A significant decrease ($P<0.05$) in total phenolic content of crackers was observed during storage period of 15 days. The highest decrease in total phenolic content was reported in crackers incorporated with 20% quinoa (2.3%) followed by 40% quinoa incorporated cracker (2.1%) and wheat (control, T_0) cracker (1.5%).

A significant decrease ($P<0.05$) was also observed in antioxidant activity of crackers during storage period of 15 days.

Storage period of 15 days had no significant effect ($P<0.05$) on overall acceptability of wheat (control, T_0) cracker while overall acceptability of crackers incorporated with 20 and 40% quinoa decreased significantly ($P<0.05$). In general, highest decrease in overall acceptability, (4.1%) was

observed in overall acceptability of 20% quinoa incorporated cracker followed by 3.9% decrease in 40% quinoa incorporated cracker and 1.5% decrease in wheat (control, T_0).

c) Beverages, namely raw quinoa beverage (RQB), soaked quinoa beverage (SQB) and germinated quinoa beverage (GQB) were prepared from raw, soaked and germinated quinoa seeds, respectively. All quinoa beverages were stored at 4°C for further analysis.

i. Physico-chemical analysis: pH of quinoa beverages ranged from 6.2± 0.01 to 6.5 ± 0.10. Statistically, no significant difference ($P<0.05$) was observed in pH content of all quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Total soluble solid content ranged from 9.08± 0.02 to 9.45 ± 0.01%. No significant difference ($P<0.05$) was observed in total solid of quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Viscosity of quinoa beverages ranged from 15.02 ± 0.01 to 15.31 ± 0.04 cp. No significant difference ($P<0.05$) was observed in viscosity of quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Addition of 0.5% (*w/v*) xanthan gum increased the viscosity and helped in textural stabilization of the beverages by reducing serum separation. Serum separation reduction ranged from 64.7 to 71.6%.

   Moisture content of raw, soaked and germinated quinoa beverage ranged from 84.01± 0.01 to 84.07±0.13 g/100ml. Protein content of quinoa beverages ranged from 0.68 to 1.5 g/100 ml. Significant increase ($P<0.05$) in protein content was reported in GQB as compared to RQB. Carbohydrate content of quinoa beverages ranged from 14.9±0.1 to 16.2±0.02g/ 100 ml with trend in increasing order being GQB<SQB<RQB. Statistically, the difference between carbohydrate content of all quinoa beverages was significant ($P<0.05$). Carbohydrate content of SQB and GQB was observed to be 4 and 8% to be lower with respect to RQB, respectively. Fat content of beverages ranged from 0.23 to 0.93 g/ 100ml. GQB was reported with least fat content. RQB and SQB were reported with significantly higher ($P<0.05$) fat content as compared to GQB. Ash content of quinoa beverages ranged from

0.11±0.01 to 0.28 gm/100ml. A significant increase in ash content ($P<0.05$) was reported in GQB as compared to RQB.

ii. Total phenolic content and Antioxidant activity: Predominantly, all quinoa beverages had total phenolic content well correlated to its anti oxidant activity. Total phenolic content of quinoa beverages ranged from 1.9 to 2.4 mg GAE/ g. Germinated quinoa beverage (GQB) showed highest phenolic content (2.4 ± 0.2 mg GAE/g), followed by SQB (2.1 ± 0.2 mg GAE/g) and RQB (1.0 ± 0.3 mg GAE/ g).

Antioxidant activity of quinoa beverages, as determined by DPPH method ranged from 52 to 92%. Potential to inhibit DPPH free radical i.e. the anti oxidant activity of quinoa beverages followed the same trend as their total phenolic content i.e. GQB>SQB>RQB. Also, the antioxidant activity was found to have good correlation with the total phenolic content ($r = 0.95$, $P<0.05$), which suggests potential contribution of phenolic compounds in quinoa to its antioxidant activity.

iii. Sensory evaluation: Color aspect of all quinoa beverages ranged from 5.1±0.02 to 5.3±0.11 indicating "neither like nor dislike". Flavor of quinoa beverages ranged from 5.3±0.03 to 7.1±0.12. Flavor of RQB was rated as "dislike very much" as compared to SQB ("like slightly") and GQB ("like moderately"). Flavor aspect of GQB was rated similar to the soya beverage used as control. Texture and consistency of all quinoa beverages was rated as "like slightly." Among all the beverages, RQB indicated lowest score on nine point hedonic scale for after taste (1.2±0.1), indicating "dislike extremely" and least good mouth feel indicating "dislike very much" (2.3±0.4). The after taste of raw quinoa beverage, as stated by panelists was "bitter." After taste aspect SQB and GQB was better than RQB and marked as "neither liked nor disliked" and "like slightly," respectively. In general, it was observed that GQB qualified all positive aspects and was rated similarly to the commercial soya milk used as control.

On evaluation of panelists choice of favorite and least favorite beverage, it was observed that among quinoa beverages, RQB was rated as least

favorite beverage by 88% panelists (22 out of 25) and GQB was most favorite beverage of 28% (7 out of 25) panelists. Overall acceptability of quinoa beverages, ranged from dislike very much to like moderately with acceptability trend in increasing order being RQB<SQB<GQB. RQB was disliked because of its bitter after taste and beany flavor while GQB was most liked among all quinoa beverages with overall acceptability score significantly similar ($P<0.05$) to that of commercial soya milk.

BIBLIOGRAPHY

Abugoch James, L. E. (2009). Quinoa (*Chenopodium quinoa* Willd.): composition, chemistry, nutritional, and functional properties. Advances in Food and Nutrition Research (1st ed., Vol. 58). Elsevier Inc.

ACCCI. (2011). Approved Methods of the American Association of Cereal Chemists International, 11th Ed., AACC International, St. Paul, MN. USA.

Adawiyah, D. R., Soekarto, T. S., & Hariyadi, P. (2012). Fat hydrolysis in a food model system: Effect of water activity and glass transition. International Food Research Journal, 19(2): 737–741.

Adekanmi Oladele, A., Osundahunsi, of, & Adebowale, Y. (2009). Effect of processing techniques on the nutrients and antinutrients contents of tigernut (*Cyperus esculentus l.*). Nigerian Food Journal, 27(2): 88–93.

Afiffy, A. E. M. M. R., El-Beltagi, H. S., Abd El-Salam, S. M., & Omran, A. a. (2012). Biochemical changes in phenols, flavonoids, tannins, vitamin E, β - carotene and antioxidant activity during soaking of three white sorghum varieties. Asian Pacific Journal of Tropical Biomedicine, 2(3): 203–209.

Afrose, S., Tsujii, H., Hossain, M. S., Salma, U., & Miah, A. G. (2010). Dietary karaya saponin and rhodobacter capsulatus exert hypocholesterolemic effects by suppression of hepatic cholesterol synthesis and promotion of bile acid synthesis in laying hens. Cholesterol, 8(2010): 1-7.

Agrahar-Murugkar, D., & Jha, K. (2011). Influence of storage and packaging conditions on the quality of soy flour from sprouted soybean. Journal of Food Science and Technology, 48(3), 325–328.

Ahn, J., Kang, E., Kim, E., and Chung, I. (2012). Variation of β -carotene Concentration in Soybean Seed and Sprout. Korean Journal of Plant Science, 57(4), 324–330.

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature Protocols, 2(4): 875–877.

Akin-Idowu, P. E., Asiedu, R., Maziya-Dixon, B., Odunola, A., & Uwaifo, A. (2009). Effects of two processing methods on some nutrients and anti-nutritional factors in yellow yam (*Dioscorea cayenensis*). African Journal of Food Science, 3(1): 022–025.

Alvarez-Jubete, L., Arendt, E. K., & Gallagher, E. (2010). Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. Trends in Food Science and Technology, 21(2): 106–113.

Amir Ahmad Nassiri, Monir Sadat Hakemi (2013). Serum magnesium level and cardiovascular disease in dialysis patients. Iranian Journal of Kidney Diseases, 7(1): 14-22.

Ando, H., Chen, Y.-C., Tang, H., Shimizu, M., Watanabe, K., & Mitsunaga, T. (2002). Food components in fractions of quinoa seed. Food Science and Technology Research, 8(1): 80–84.

Angiosperm Phylogeny Group (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society, 141(4): 399-436.

AOAC, (1995). Official Methods of Analysis, 16th Ed., Association of Official Analytical Chemists, Washington, DC.

AOAC, (2000). Official Methods of Analysis, 16th Ed., Association of Official Analytical Chemists, Washington, DC, USA.

Araujo-Farro, P. C., Podadera, G., Sobral, P. J. a, & Menegalli, F. C. (2010). Development of films based on quinoa (*Chenopodium quinoa*, Willd.) starch. Carbohydrate Polymers, 81(4): 839–848.

Araujo-Farro, P. C., Podadera, G., Sobral, P. J. a, & Menegalli, F. C. (2010). Development of films based on quinoa (*Chenopodium quinoa*, Willd.) starch. Carbohydrate Polymers, 81(4): 839–848.

Arneja, I., Tanwar, B., & Chauhan, A (2015). Nutritional composition and health benefits of golden grain of 21 st century quinoa (*Chenopodium quinoa* Willd.): A Review. Pakistan Journal of Nutrition, 14(12): 1034–1040.

Asao, M., & Watanabe, K. (2010). Functional and bioactive properties of quinoa and amaranth. Food Science and Technology Research, 16(2): 163–168.

Asensi-Fabado, M. A.; Munné-Bosch, S. (2010) Vitamins in plants: Occurrence, biosynthesis and antioxidant function. Trends in Plant Science, 15: 582−592.

Atef, A., & Emam, H. (2014). Use of Quinoa Meal to Produce Bakery Products to Celiac and Autism Stuffs. International Journal of Science and Research, 3(9): 1344–1354.

Azeke, M. A., Egielewa, S. J., Eigbogbo, M. U., & Ihimire, I. G. (2011). Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). Journal of Food Science and Technology, 48(6): 724–729.

Barakat, M.Z., M.F.A. El-Wahab, and M.M. El-Sadr. (1955). Action of N-bromosuccinimide on ascorbic acid. Biochemistry Department, Faculty of Medicine, Abbassia, Cairo, Egypt. Analytical Chemistry, 27: 536-540.

Baruah K, Sahu NP, Pal AK, Jain KK, Debnath D, Yengkokpam S. (2007) Interactions of dietary microbial phytase, citric acid and crude protein level on mineral utilization by Rohu, Labeo rohita (Hamilton), Juveniles. Journal of World Aquaculture Society, 38: 238–249.

Benitez, V., Cantera , S., Aguilera ,Y., Mollá ,E., Esteban ,M.R., Díaz ,F.M., & Martín- Cabrejas,A.M. (2013). Impact of germination on starch, dietary fibre and physicochemical properties in non-conventional legumes. Food Research International, 50: 64 – 69.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as measurement of ‘‘antioxidant power’’: the FRAP assay. Analitical Biochemistry, 239, 70–76.

Berti, C., Riso, P., Monti, L. D., & Porrini, M. (2004). *In vitro* starch digestibility and in vivo glucose response of gluten-free foods and their gluten counterparts. European Journal of Nutrition, 43(4): 198–204.

Bertrandt, J. (2013). Minerals and polyphenols content of quinoa (*Chenopodium quinoa* Willd.) plant, Problem High Epidemiology, 94(2): 300–304.

Bhargava, A., Shukla, S., & Ohri, D. (2006). *Chenopodium quinoa* - An Indian perspective. Industrial Crops and Products, 23(1): 73–87.

Bhargava, A., Shukla, S., Rajan, S., & Ohri, D. (2007). Genetic diversity for morphological and quality traits in quinoa (*Chenopodium quinoa* Willd.) germplasm. Genetic Resources and Crop Evolution, 54(1): 167–173.

Bianchi, F., Rossi, E., Gomes, R., & Sivieri, K. (2015). Potentially synbiotic fermented beverage with aqueous extracts of quinoa (*Chenopodium quinoa* Willd.) and soy. Food Science and Technology International, 21(6): 403–415.

Blessing, I. A., & Gregory, I. O. (2010). Effect of processing on the proximate composition of the dehulled and undehulled mungbean [*Vigna radiata* (L.)] flours. Pakistan Journal of Nutrition, 9(10): 1006–1016.

Borges, R.M., Valença, S.S., Lopes, A.A., Barbi, N.S., Silva, A.J.R. (2013). Saponins from the roots of *Chiococca alba* and their *in vitro* anti-inflammatory activity. Phytochemistry Letters, 6: 96–100.

Borneo, R., & León, A. E. (2012). Whole grain cereals: functional components and health benefits. Food Functions, 3(2): 110–119.

Brend, Y. (2012). Total Phenolic Content and Antioxidant Activity of Red and Yellow Quinoa (*Chenopodium quinoa*; Willd.) Seeds as Affected by Baking and Cooking Conditions. Food and Nutrition Sciences, 03(08): 1150–1155.

Butt MS, Batool R (2010). Nutritional and Functional Properties of Some Promising Legumes Protein Isolates. Pakistan Journal of Nutrition, 9(4): 373-379

Calderelli, V. A. S., de Benassi, M. T., Visentainer, J. V., & Matioli, G. (2010). Quinoa and flaxseed: Potential ingredients in the production of bread with

functional quality. Brazilian Archives of Biology and Technology, 53(4): 981–986.

Capriles, V.D., Areas, J.A.G., (2013). Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance, and glycemic response of gluten-free breads. Food Functions, 4: 104-110.

Caprita Rodica and Adrian, C. (2011). Chemical Methods for the Determination of Soluble and Insoluble Non-Starch Polysaccharides - Review. *Usab*, *44*(2), 73–80.

Carciochi, R. a., Manrique, G. D., & Dimitrov, K. (2014). Changes in phenolic composition and antioxidant activity during germination of quinoa seeds (*Chenopodium quinoa* Willd.). International Food Research Journal, 21(2): 767–773.

Carciochi, R. a., Manrique, G. D., & Dimitrov, K. (2014a). Changes in phenolic composition and antioxidant activity during germination of quinoa seeds (*Chenopodium quinoa* Willd.). International Food Research Journal, 21(2): 767–773.

Carciochi, R. A., Manrique, G. D., & Dimitrov, K. (2014b). Optimization of antioxidant phenolic compounds extraction from quinoa (*Chenopodium quinoa*) seeds. Journal of Food Science and Technology, 52(7): 4396–4404.

Cardador-Martinez, A., Loarca-Pila, G., Oomah, B. D., (2002). Antioxidant activity in common beans (*Phaseolus vulgaris* L.), Journal of Agriculture and Food Chemistry, 50, 6975–6980.

Cevallos-Casals, B. A., & Cisneros-Zevallos, L. (2010). Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Food Chemistry, 119(4): 1485–1490.

Chaieb, I. (2010). Saponins as Insecticides : a Review. Tunisian Journal of Plant Protection, 5(1): 39–50.

Chaparro, D., Pismag, R., & Elizalde, A. (2011). Efecto de la germinación sobre el contenido de hierro y calcio en amaranto, quinua, guandul y soya. Biotecnologia En El Sector Agropecuario Y Agroindustrial, 9(1): 51–59.

Chauhan, G. S., Eskin, N. A. M., & Tkachuk, R. (1992). Nutrients and antinutrients in quinoa seed. Cereal Chemistry, 69(1): 85-88.

Chawla, R., & Patil, G. R. (2010). Soluble dietary fiber. Comprehensive Reviews in Food Science and Food Safety, 9(2): 178–196.

Chikwendu and Justina Ndirika, (2015). Comparative Evaluation of Chemical Composition of Fermented Ground Bean Flour (*Kerstingella geocarpa*), Cowpea Flour (Vigna unguiculata) and Commercial Wheat Flour (*Triticum* spp.). Pakistan Journal of Nutrition, 14: 218-224.

Chillo, S., Laverse, J., Falcone, P. M., & Del Nobile, M. a. (2010). Quality of spaghetti in base amaranthus wholemeal flour added with quinoa, broad bean and chick pea. Journal of Food Engineering, 84(1): 101–107.

Chillo, S., Mastromatteo, M., Iannetti, M., Civica, V., & Del Nobile, M. A. (2010). Formulation optimisation of gluten-free functional spaghetti based on quinoa, maize and soy flours. International Journal of Food Science and Technology, 46(6): 1201–1208.

Chiremba, C., Taylor, J. R. N., & Duodu, K. G. (2009). Phenolic content, antioxidant activity, and consumer acceptability of sorghum cookies. Cereal Chemistry, 86(5): 590–594.

Chirinos, R., Pedreschi, R., Rogez, H., Larondelle, Y., & Campos, D. (2015). Phenolic compound contents and antioxidant activity in plants with nutritional and/or medicinal properties from the Peruvian Andean region. Industrial Crops and Products, 47: 145–152.

Chlopicka, J., Pasko, P., Gorinstein, S., Jedryas, A., & Zagrodzki, P. (2012). Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT - Food Science and Technology, 46(2): 548–555.

Chung, H. J., Cho, D. W., Park, J. D., Kweon, D. K., & Lim, S. T. (2012). *In vitro* starch digestibility and pasting properties of germinated brown rice after hydrothermal treatments. Journal of Cereal Science, 56(2): 451–456.

Chung, H.J., Cho, A., & Lim, S.T. (2014). Utilization of germinated and heat-moisture treated brown rices in sugar-snap cookies. LWT - Food Science and Technology, 57: 260 – 266.

Coda, R., Rizzello, C. and Gobbetti, M. (2010). Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of functional bread enriched of γ- aminobutyric acid (GABA). International Journal of Food Microbiology, 137: 236-245.

Coelho, I. R., Gueifão, S., Nascimento, A. C., Lobo, M., Gimenez, A., Samman, N., … Fibre, D. (2011). Proximate and Mineral profile of Quinoa (*Chenopodium quinoa*), and kiwicha (*Amaranthus caudatus*) consumed in north of Argentina, EuroFIR Nexus consortium, Norwich, 21(2): 2010.

Comai, S., Bertazzo, A., Bailoni, L., Zancato, M., Costa, C. V. L., & Allegri, G. (2007). The content of proteic and nonproteic (free and protein-bound) tryptophan in quinoa and cereal flours. Food Chemistry, 100(4): 1350–1355.

Coulibaly A, Kouakou B, Chen J (2012) Extruded Adult Breakfast Based on Millet and Soybean: Nutritional and Functional Qualities, Source of Low Glycemic Food. Journal of Nutrition and Food Science. 2: 151.

Cowieson, A. J., Acamovic, T., and Gilbert, C. E. (2006). Lupins in poultry nutrition. In E. van Santen,&G. D. Hill (Eds.), Wild and cultivated lupins from the Trop- ics to the Poles. Proceedings of the 10th International Lupin Conference, Laugarvatn, Iceland, 19-24 June 2002 (pp. 314–318). International Lupin Association. Canterbury.

Dasgupta, (2015). The Indian Express. Retrieved from http://indianexpress.com/article/opinion/columns/indias-malnutrition-shame/

Day, R.A. and A.L. Underwood, (1986). Quantitative analysis. 5 th ed. Prentice-Hall publication.

De, C. F. G., S, S. R. De, De, C. A. L., Iannetta, O., & Js, M. (2015). Quinoa or Corn Flakes to Prevent Peripheral Inflammation after Menopause ? Journal of Obesity & Eating Disorders, 1(2): 1–5.

Decker, K.J. (2010). Shape of Things to Come: Innovations in Pasta. Food Products Design. Retrieved from http://www.foodproductdesign.com/articles/2012/01/shape-of-things-to-come-innovations-in-pasta.aspx

Desalegn, B. B. (2016). Effect of Soaking and Germination on Proximate Composition , Mineral Bioavailability and Functional Properties of Chickpea Flour Food and Public Health 2015, 5(4): 108-113.

Desplanques, S., Renou, F., Grisel, M., & Malhiac, C. (2012). Impact of chemical composition of xanthan and acacia gums on the emulsification and stability of oil-in-water emulsions. Food Hydrocolloids, 27(2), 401–410.

Dhingra, D., Michael, M., Rajput, H., & Patil, R. T. (2012). Dietary fibre in foods: A review. Journal of Food Science and Technology, 49(3): 255–266.

Dick Mastebroek, H., Limburg, H., Gilles, T., Marvin, H.J.P., (2000). Occurrence of sapogenins in leaves and seeds of quinoa (*Chenopodium quinoa* Willd). Journal of the Science of Food and Agriculture, 80: 152-156.

Dini, I., Tenore, G. C., & Dini, A. (2005). Nutritional and antinutritional composition of Kancolla seeds: An interesting and underexploited andine food plant. Food Chemistry, 92(1): 125–132.

Dini, I., Tenore, G. C., & Dini, A. (2005). Nutritional and antinutritional composition of Kancolla seeds: An interesting and underexploited andine food plant. Food Chemistry, 92(1): 125–132.

Dini, I., Tenore, G. C., & Dini, A. (2013). Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. LWT - Food Science and Technology, 53(1): 382–385.

Du Thanh Hang, Leo Vanhanen, Geoffrey Savage. (2014). Effect of simple processing methods on oxalate content of taro petioles and leaves grown in central Vietnam. LWT - Food Science and Technology, 50(1): 259-262.

Duenas, M., Hernández, T., Estrella, I., & Fernández, D. (2009). Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (Lupinus angustifolius L.). Food Chemistry, 117(4): 599–607.

Durazzo, A., Gabrielli, P., & Manzi, P. (2015). Qualitative Study of Functional Groups and Antioxidant Properties of Soy-Based Beverages Compared to Cow Milk Antioxidants, 4(3), 523–532.

Echendu, C. A., Obizoba, I. C., & Anyika, J. U. (2009). Effects of germination on chemical composition of groundbean (*Kerstingiella geocarpa*) seeds. Pakistan Journal of Nutrition, 8(12): 1849–1854.

El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Foods for Human Nutrition, 57: 83–97.

El-Hady, E. A. A., & Habiba, R. A. (2003). Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. LWT - Food Science and Technology, 36(3): 285–293.

Elsohaimy, S. A., Refaay, T. M., & Zaytoun, M. A. M. (2015). Physicochemical and functional properties of quinoa protein isolate. Annals of Agricultural Sciences, 60(2): 297–305.

Embaby, H. E. S. (2010). Effect of soaking, dehulling, and cooking methods on certain antinutrients and *in vitro* protein digestibility of bitter and sweet lupin seeds. Food Science and Biotechnology, 19(4): 1055–1062.

Enes, B. N., Galdino Alves, N. E., Valdes, S. T., Martins Silveira, C. M., Brunoro Costa, N. M., Rocha Ribeiro, S. M., Duarte Martino, H. S. (2014). Evaluation of iron bioavailability in a mixture of cereals, seeds, and grains ("Human Ration"). Food Science and Technology, 34(1): 24–31.

Enriquez, N., Peltzer, M., Raimundi, A., Tosi, V., & Pollio, M. L. (2003). Characterization of wheat and quinoa flour blends in relation to their breadmaking quality. Anales Des La Asociacion Quimica Argentina, 91(4-6): 47–54.

Estevez, A. M., Figuerola, F., Hurtado, M. L., Chiffelle, I., & Asenjo, F. (2005). Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chemistry, 91, 395–401.

FAO (2011). Quinoa: An ancient crop to contribute to world food security. In: A. Bojanic ed. Regional Office for Latin America and the Caribbean, pp. 63. Food and Agriculture Organization, Rome, Italy

Farinazzi-Machado, F. M. V., Barbalho, S. M., Oshiiwa, M., Goulart, R., & Pessan Junior, O. (2012). Use of cereal bars with quinoa (*Chenopodium quinoa* W.) to reduce risk factors related to cardiovascular diseases. Ciência E Tecnologia de Alimentos, 32(4): 15-20.

Fernandez-Orozco R, Piskula MK, Zielinski H, Kozowska H, Frias J, Vidal-Valverde C (2006). Germination as a process to improve the antioxidant capacity of Lupinus angustifolius L. var. Zapaton. European Food Research and Technology, 223: 495–502.

Föste, M., Elgeti, D., Brunner, A.-K., Jekle, M., & Becker, T. (2015). Isolation of quinoa protein by milling fractionation and solvent extraction. Food and Bioproducts Processing, 96: 20–26.

Foucault, A.-S., Mathé, V., Lafont, R., Even, P., Dioh, W., Veillet, S., … Quignard-Boulangé, A. (2012). Quinoa Extract Enriched in 20-Hydroxyecdysone Protects Mice From Diet-Induced Obesity and Modulates Adipokines Expression. Obesity, 20(2): 270–277.

Frontela, C., Ros, G., & Martinez, C. (2011). Phytic acid content and "*in vitro*" iron, calcium and zinc bioavailability in bakery products: The effect of processing. Journal of Cereal Science, 54(1): 173–179.

Gawlik-Dziki, U., Świeca, M., Sułkowski, M., Dziki, D., Baraniak, B., & Czyz, J. (2013). Antioxidant and anticancer activities of *Chenopodium quinoa* leaves extracts - *In vitro* study. Food and Chemical Toxicology, 57: 154–160.

Genkawa, T., Uchino, T., Inoue, A., Tanaka, F., & Hamanaka, D. (2008). Development of a low-moisture-content storage system for brown rice: Storability at decreased moisture contents. Biosystems Engineering, 99(4): 515–522.

Gesinski K, Nowak K (2011) Comparative analysis of the biological value of protein of *Chenopodium quinoa* Willd. and Chenopodium album L. Part I Amino

acid composition of the seed protein. Acta Scientiarum Polonorum Agricultura, 10: 47-56.

Ghavidel, R. A., & Prakash, J. (2007). The impact of germination and dehulling on nutrients, antinutrients, *in vitro* iron and calcium bioavailability and *in vitro* starch and protein digestibility of some legume seeds. LWT - Food Science and Technology, 40: 1292–1299.

Global Nutrition Report (2015). International Food Policy Research Institute. Retrieved from www.ifpri.org/publication/global-nutrition-report-2015.

Gomez-Caravaca, A. M., Iafelice, G., Verardo, V., Marconi, E., & Caboni, M. F. (2014). Influence of pearling process on phenolic and saponin content in quinoa (*Chenopodium quinoa* Willd). Food Chemistry, 157: 174–178.

Gomez-Caravaca, A. M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Caboni, M. F. (2011). Simultaneous determination of phenolic compounds and saponins in quinoa (*Chenopodium quinoa* Willd) by a liquid chromatography-diode array detection-electrospray ionization-time-of-flight mass spectrometry methodology. Journal of Agricultural and Food Chemistry, 59(20): 10815–10825.

Gonzalez Martín, M. I., Wells Moncada, G., Fischer, S., & Escuredo, O. (2014). Chemical characteristics and mineral composition of quinoa by near-infrared spectroscopy. Journal of the Science of Food and Agriculture, 94(5): 876–881.

Gordillo-Bastidas E, Díaz-Rizzolo DA, Roura E, Massanés T and Gomis R (2016). Quinoa (*Chenopodium quinoa* Willd), from Nutritional Value to Potential Health Benefits : An Integrative Review, 6(3).

Gorenstein, S.; Caspi, A.; Libman, I.; Katrich, E.; Lerner, H. Z.; Trakhtenberg, S. (2004). Fresh Israeli Sweetie juice consumption improves lipid metabolism and increases antioxidant capacity in hypercholesterolemic patients suffering from coronary artery disease: studies *in vitro* and in human and positive changes in albumin and fibrinogen fractions. Journal of Agriculture and Food Chemistry, 52: 5215-5222.

Graf, B. L., Poulev, A., Kuhn, P., Grace, M. H., Lila, M. A., & Raskin, I. (2014). Quinoa seeds leach phytoecdysteroids and other compounds with anti-diabetic properties. Food Chemistry, 163: 178–185.

Graf, B. L., Poulev, A., Kuhn, P., Grace, M. H., Lila, M. A., & Raskin, I. (2014). Quinoa seeds leach phytoecdysteroids and other compounds with anti-diabetic properties. Food Chemistry, 163: 178–185.

Gunness P, Gidley MJ.(2010). Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. Food Functions, 1(2):149-55.

Hager, A.-S., A. Wolter, F. Jacob, E. Zannini and E.K. Arendt. (2012). Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. Journal of Cereal Science, 56: 239-247.

Hahm, T.-S., Park, S.-J., & Martin Lo, Y. (2009). Effects of germination on chemical composition and functional properties of sesame (Sesamum indicum L.) seeds. Bioresource Technology, *100*(4): 1643–7.

Halvorsen, B.L.; Holte, K.; Myhrstad, M.C.; Barikmo, I.; Hvattum, E.; Remberg, S.F.; Wold, A.B.; Haffner, K.; Baugerød, H.; Andersen, L.F. (2002). A systematic screening of total antioxidants in dietary plants. Journal of Nutrition, 132, 461–471.

Han, H., & Baik, B. K. (2008). Antioxidant activity and phenolic content of lentils (Lens culinaris), chickpeas (Cicer arietinum L.), peas (Pisum sativum L.) and soybeans (Glycine max), and their quantitative changes during processing. International Journal of Food Science and Technology, 43(11): 1971–1978.

Harra NM, Lemm T, Smith C (2011) Quinoa flour is an acceptable replacement for all purpose flour in a peanut butter cookie. Journal of American Dietetic Association, 111:SA45.

Haugh, W.; Lantzsch, H. J. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. Journal of Science, Food, and Agriculture, 34: 1423-1426.

Hejazi Maha A. (2016). Preparation of different formulae from quinoa and different sources dietary fiber to treat obesity in rats. Nature and Science, 14(2): 55–65.

Hemalatha, P., Bomzan, D. P., Sathyendra Rao, B. V., & Sreerama, Y. N. (2016). Distribution of phenolic antioxidants in whole and milled fractions of quinoa and their inhibitory effects on α-amylase and α-glucosidase activities. Food Chemistry, 199: 330–338.

Hemalatha, S., Platel, K., & Srinivasan, K. (2006). Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains. European Journal of Clinical Nutrition, 61(3): 342–348.

Hirose, Y., Fujita, T., Ishii, T., & Ueno, N. (2010). Antioxidative properties and flavonoid composition of *Chenopodium quinoa* seeds cultivated in Japan. *Food Chemistry*, 119(4): 1300–1306.

Holm, J., Björck, I., Asp, N.-G., Sjöberg, L.-B., & Lundquist, I. (1985). Starch availability *in vitro* and in vivo after flaking, steam-cooking and popping of wheat. Journal of Cereal Science, 3(3): 193–206.

Horton, J.D.; Shimomura, I.; Brown, M.S.; Hammer, R.E.; Goldstein, J.L.; Shimano, H. (2006). Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. Journal of Clinical Investigation, 101: 2331–2339.

Hotz, C., & Gibson, R. S. (2007). Traditional Food-Processing and Preparation Practices to Enhance the Bioavailability of Micronutrients in Plant-Based Diets. The Journal of Nutrition , 137 (4): 1097–1100.

Hsu, H., & Vavak, D. (1977). A multienzyme technique for estimating protein digestibility. Journal of Food Science, 45(5): 1269–1273.

Hubner F, Arendt EK (2013) Germination of cereal grains as a way to improve the nutritional value: a review. Critical Review Food Science and Nutrition, 53: 853–861

Ibrahim Abd El-Gawad, I. A., El-Sayed, E. M., Hafez, S. A., El-Zeini, H. M., & Saleh, F. A. (2005). The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. International Dairy Journal, 15(1): 37–44.

Iglesias-Puig, E., Monedero, V., & Haros, M. (2015). Bread with whole quinoa flour and bifidobacterial phytases increases dietary mineral intake and bioavailability. LWT - Food Science and Technology, 60(1): 71–77.

Inglett, G. E., Chen, D., & Liu, S. X. (2015). Antioxidant Activities of Selective Gluten Free Ancient Grains. Food and Nutrition Sciences, 6: 612–621.

Inyang, C.U. and Zakari, U.M. (2008). Effect of germination and fermentation of pearl millet on proximate, chemical and sensory properties of instant "Fura"- a Nigerian cereal food. Pakistan Journal of Nutrition, 7 (1): 9-12.

Isengard, H.-D. (2011). Water content, one of the most important properties of food. Food Control, 12(7): 395–400.

Jackson, C. J. C., Dini, J. P., Lavandier, C., Rupasinghe, H. P. V, Faulkner, H., Poysa, V., DeGrandis, S. (2002). Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. Process Biochemistry, 37(10): 1117–1123.

Jacobsen, S. E. (2011). The Situation for Quinoa and Its Production in Southern Bolivia: From Economic Success to Environmental Disaster. Journal of Agronomy and Crop Science, 197(5): 390–399.

Jancurova, M., Minarovicova, L., & Dandár, A. (2009). Quinoa – a Review. Czech Journal of Food Science, 27(2): 71–79.

Jenkins, D. J. a, Kendall, C. W. C., McKeown-Eyssen, G., Josse, R. G., Silverberg, J., Booth, G. L., … Leiter, L. a. (2008). Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes: a randomized trial. JAMA: The Journal of the American Medical Association, 300(23): 2742–2753.

Kadereit G, Gotzek D, Jacobs S and Freitag H (2005). Origin and age of Australian Chenopodiaceae. Organisms, Diversity and Evolution. International Journal of Plant Science, 164(6): 959–986.

Kajihausa, O. E., Fasasi, R. A., & Atolagbe, Y. M. (2014). Effect of Different Soaking Time and Boiling on the Proximate Composition and Functional Properties of Sprouted Sesame Seed Flour. Nigerian Food Journal, 32(2): 8–15.

Kanensi, O. J., Ochola, S., Gikonyo, N. K., & Makokha, A. (2011). Optimization of the Period of Steeping and Germination for Amaranth Grain, Journal of Agriculture and Food Technology, 1(6): 101–105.

Kang, Y., Khan, S., & Ma, X. (2009). Climate change impacts on crop yield, crop water productivity and food security - A review. Progress in Natural Science, 19(12), 1665–1674.

Kaur, I., & Tanwar, B. (2016). Quinoa Beverages: Formulation, Processing and Potential Health Benefits. Romanian Journal of Diabetes Nutrition and Metabolic Diseases. 23(2): 215–225.

Kaur, I., Tanwar, B., Reddy, M., & Chauhan, A. (2016). Vitamin C , total polyphenols and antioxidant activity in raw , domestically processed and industrially processed Indian *Chenopodium quinoa* seeds, *6*(13): 139–145.

Kavitha, S., & Parimalavalli, R. (2014). Effect of Processing Methods on Proximate Composition of Cereal and Legume flours. Journal of Human Nutrition and Food Science, 2(6): 1051.

Kayembe, N. C., & Jansen van Rensburg, C. (2013). Germination as a processing technique for soybeans in small-scale farming. South African Journal of Animal Sciences, 43(2): 167–173.

Kayembe, N. C., & Jansen van Rensburg, C. (2013). Germination as a processing technique for soybeans in small-scale farming. South African Journal of Animal Sciences, 43(2): 167–173.

Khandelwal, S., Udipi, S. a., & Ghugre, P. (2010). Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. Food Research International, 43(2): 526–530.

Khattab, R. Y., & Arntfield, S. D. (2009). Nutritional quality of legume seeds as affected by some physical treatments. Antinutritional factors. LWT - Food Science and Technology, 42: 1113–1118.

Khattak A.B., A. Zeb, M. Khan, N. Bibi, I. Ihsanullah and M.S. Khattak (2007). Influence of germination techniques on sprout yield, biosynthesis of ascorbic acid and cooking ability, in chickpea (*Cicer arietinum* L.). Food Chemistry, 103, 115-120.

Khattak, A. B., Zeb, A., Bibi, N., Khalil, S. A., & Khattak, M. S. (2007). Influence of germination techniques on phytic acid and polyphenols content of chickpea (Cicer arietinum L.) sprouts. Food Chemistry, 104(3): 1074–1079.

Khursheed P Navder, S. B. (2014). Freeze Dried Blueberry Powder Fortification Improves the Quality of Gluten Free Snacks. Journal of Food Processing & Technology, 5(12): 38-41.

Kim, D. M., Lee, H., & Yoo, S. H. (2012). Compositional changes and physical properties of soymilk prepared with pre-soaked-fermented soybean. Journal of the Korean Society for Applied Biological Chemistry, 55(1): 121–126.

Kokanova-Nedialkova, Z., Nedialkov, P.T., Nikolov, S.D., (2009). The genus chenopodium: phytochemistry, ethnopharmacology and pharmacology. Pharmacognosy Review, 3: 280–306.

Koksoy, A., & Kilic, M. (2004). Use of hydrocolloids in textural stabilization of a yoghurt drink, ayran. Food Hydrocolloids, 18(4): 593–600.

Kong, X., and Bertoft, E. (2010). Fine structure characterization of amylopectins from grain amaranth starch. Carbohydrate Research, 344: 1701–1708.

Konishi, Y., Hirano, S., Tsuboi, H., & Wada, M. (2004). Distribution of minerals in quinoa (*Chenopodium quinoa* Willd.) seeds. Bioscience, Biotechnology, and Biochemistry, 68(1): 231–234.

Konishi, Y., Hirano, S., Tsuboi, H., & Wada, M. (2004). Distribution of minerals in quinoa (*Chenopodium quinoa* Willd.) seeds. Bioscience, Biotechnology, and Biochemistry, 68(1): 231–234.

Konishi, Y., Hirano, S., Tsuboi, H., & Wada, M. (2004). Distribution of minerals in quinoa (*Chenopodium quinoa* Willd.) seeds. Bioscience, Biotechnology, and Biochemistry, 68(1): 231–234.

Kuljanabhagavad, T., & Wink, M. (2009). Biological activities and chemistry of saponins from *Chenopodium quinoa* Willd. Phytochemistry Reviews, 8(2): 473–490.

Kumar Ashok, P., & Upadhyaya, K. (2012). Tannins are Astringent. Journal of Pharmacognosy and Phytochemistry, 1(3): 45–50.

Kumar, A., Goel, B. K., Karthikeyan, S., Asgar, S., Gedda, a K., Choudhary, K. K., & Uprit, S. (2013). Protein and Calcium Rich Malted Health Drink Power. Journal of Food Process Technology, 04(03): 3–7.

Laetitia, B., Qi, P., Wickham, E., & Tomasula, P. (2014). Enrichment and Purification of Casein Glycomacropeptide from Whey Protein Isolate Using Supercritical Carbon Dioxide Processing and Membrane Ultrafiltration. Foods, 3(1): 94–109.

Lamothe, L. M., Srichuwong, S., Reuhs, B. L., & Hamaker, B. R. (2015). Quinoa (*Chenopodium quinoa* W.) and amaranth (Amaranthus caudatus L.) provide dietary fibres high in pectic substances and xyloglucans. Food Chemistry, 167: 490–496.

Laux, M. (2013). Quinoa. USDA Agricultural Marketing Resource Center. Revised by Mykel Taylor, 2012.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. Journal of Food Engineering, 79(3), 1033–1047.

Lee, J., Hwang, Y. S., Lee, J. D., Chang, W. S., & Choung, M. G. (2013). Metabolic alterations of lutein, ??-carotene and chlorophyll a during germination of two soybean sprout varieties. Food Chemistry, 141(3): 3177–3182.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology, 20(3): 207–220.

Lee, S.K., and A.A. Kader. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology 20: 207-22.

Lee, Y. J., Kim, K. J., Park, K. J., Yoon, B. R., Lim, J. H., & Lee, O. H. (2013). Buckwheat (Fagopyrum esculentum M.) sprout treated with methyl jasmonate (MeJA) improved anti-adipogenic activity associated with the oxidative stress system in 3T3-L1 adipocytes. International Journal of Molecular Sciences, 14(1): 1428–1442.

Lestienne, I., Buisson, M., Lullien-Pellerin, V., Picq, C., & Trèche, S. (2007). Losses of nutrients and anti-nutritional factors during abrasive decortication of two pearl millet cultivars (Pennisetum glaucum). Food Chemistry, 100(4): 1316–1323.

Letelier, M. E., Rodríguez-Rojas, C., Sánchez-Jofré, S., & Aracena-Parks, P. (2011). Surfactant and antioxidant properties of an extract from *Chenopodium quinoa* Willd seed coats. Journal of Cereal Science, 53(2): 239–243.

Li, G., and Zhu, F. (2016). Physicochemical properties of quinoa starch. Carbohydrate Polymers, 137: 328–338.

Liang, J., Han, B.-Z., Nout, M. J. R., & Hamer, R. J. (2008). Effects of soaking, germination and fermentation on phytic acid, total and *in vitro* soluble zinc in brown rice. Food Chemistry, 110(4): 821–828.

Lindeboom, N., Chang, P. R., Falk, K. C., & Tyler, R. T. (2005). Characteristics of starch from eight quinoa lines. Cereal Chemistry, 82(2): 216–222.

Lindeboom, N., Chang, P. R., Falk, K. C., & Tyler, R. T. (2005). Characteristics of starch from eight quinoa lines. Cereal Chemistry, 82(2): 216–222.

Lopes, C. O., Dessimoni, G. V., Da Silva, M. C., Vieira, G., and Pinto, N. A. V. D. (2009). Aproveitamento, composição nutricional e antinutricional da farinha de quinoa (Chenoipodium quinoa). Alimentos e Nutrição, 20(4): 669-675.

Lopez-Rubio, A., Flanagan, B. M., Gilbert, E. P., and Gidley, M. J. (2008). A novel approach for calculating starch crystallinity and its correlation with double helix content: A combined XRD and NMR study. Biopolymers 89(9): 761–768.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with folin phenol reagent. Journal of Biochemistry, 193: 265 – 275.

Luthria, A., & Singh, K. (2014). *In vitro* antioxidant activity of black gram , cowpea , desi chickpea and yellow mustard as affected by sprouting. Journal of Global Biosciences, 3(1): 385–389.

Luthria, A., & Singh, K. (2014). *In vitro* antioxidant activity of black gram, cowpea, desi chickpea and yellow mustard as affected by sprouting, Journal of Global Biosciences, 3(1), 385–389.

Lutz, M., Martínez, A., & Martínez, E. a. (2013). Daidzein and Genistein contents in seeds of quinoa (*Chenopodium quinoa* Willd.) from local ecotypes grown in arid Chile. Industrial Crops and Products, 49: 117–121.

Ma, L., Li, B., Han, F., Yan, S., Wang, L., & Sun, J. (2015). Evaluation of the chemical quality traits of soybean seeds, as related to sensory attributes of soymilk. Food Chemistry, 173: 694–701.

Madhu, A. N., N. Amrutha, and S. G. Prapulla. (2012). Characteriza- tion and antioxidant property of probiotic and synbiotic yogurts. Probiotics Antimicrobial Proteins, 4:90–97.

Madhujith T, Shahidi F (2006) Optimization of the extraction of antiox- idative constituents of six barley cultivars and their antioxidant properties. Journal of Agriculture and Food Chemistry, 54:8048–8057.

Maisuthisakul, P., Gordon, M. H., Pongsawatmanit, R., & Suttajit, M. (2007). Enhancing the oxidative stability of rice crackers by addition of the ethanolic extract of phytochemicals from Cratoxylum formosum Dyer. Asia Pacific Journal of Clinical Nutrition, 16(1): 37–42.

Makinde, F. M., & Akinoso, R. (2013). Nutrient composition and effect of processing treatments on anti nutritional factors of Nigerian sesame (Sesamum indicum Linn) cultivars. International Food Research Journal, 20(5): 2293–2300.

Manikandan, R., Anand, A. V., & Muthumani, G. D. (2013). Phytochemical and *in vitro* anti-diabetic activity of methanolic extract of Psidium guajava leaves. International Journal of Current Microbiology Applied Sciences, 2(2): 15–19.

Maradini Filho, A. M., Pirozi, M. R., Da Silva Borges, J. T., Pinheiro Sant'Ana, H. M., Paes Chaves, J. B., & Dos Reis Coimbra, J. S. (2015). Quinoa: Nutritional,

Functional and Antinutritional Aspects. Critical Reviews in Food Science and Nutrition, (ahead of print).

Marmouzi, I., El Madani, N., Charrouf, Z., Cherrah, Y., & El Abbes Faouzi, M. Y. (2015). Proximate analysis, fatty acids and mineral composition of processed Moroccan *Chenopodium quinoa* Willd. and antioxidant properties according to the polarity. Phytothérapie, 13(2): 110–117.

Martin, J., & Diaz, R. (2011). Use of amaranth, quinoa and kañiwa in extruded corn snacks. University of Helsinki, Department of Food and Environmental Sciences EKT Series, 1522: 1–86.

Martin-Cabrejas, M. A., Ariza, N., Esteban, R., Molli, E., Waldron, K., & Lopez-Andreu, F. J. (2003). Effect of germination on the carbohydrate composition of the dietary fiber of peas (Pisum sativum L.). Journal of Agricultural and Food Chemistry, 51(5): 1254–1259.

Martinez, L. C., Vargas, M. A. P., del Angel, A. I. C., Bermudez, F. C., & Avalos, H. A. J. (2013). Total Phenolic Content and Antioxidant Capacity of Germinated, Popped, and Cooked Huauzontle (Chenopodium berlandieri spp. nuttalliae) Seeds. Cereal Chemistry Journal, 90(3): 263–268.

Masood, T., Shah, H. U., & Zeb, A. (2014). Effect of Sprouting Time on Proximate Composition and Ascorbic Acid Level of Mung Bean (Vigna Radiate L .) and Chickpea (Cicer Arietinum L .) Seeds. The Journal of Animal & Plant Sciences, 24(3): 850-859.

Matiacevich, S., Castellión, M., Maldonado, S., Buera, P., (2006). Water-dependent thermal transitions in quinoa embryos. Thermochimica Acta 448, 117–122.

Matos, M., Timgren, A., Sjoo, M., Dejmek, P., & Rayner, M. (2013). Preparation and encapsulation properties of double Pickering emulsions stabilized by quinoa starch granules. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 423: 147–153.

Megat, R. M. R., Azrina, A., & Norhaizan, M. E. (2016). Effect of germination on total dietary fibre and total sugar in selected legumes. International Food Research Journal, 23(1): 257–261.

Meneguetti, Q. A., Brenzan, M. A., Batista, M. R., Bazotte, R. B., Silva, D. R., & Garcia Cortez, D. A. (2011). Biological effects of hydrolyzed quinoa extract from seeds of *Chenopodium quinoa* Willd. Journal of Medicinal Food, 14(6): 653–657.

Milovanovic, M., Demin, M., Vucelic-Radovic, B., Zarkovic, B., & Stikic, R. (2014). Evaluation of the nutritional quality of wheat bread prepared with quinoa, buckwheat and pumpkin seed blends. Journal of Agricultural Sciences, Belgrade, 59(3): 319–328.

Miranda, M., Delatorre-herrera, J., Vega-gálvez, A., Jorquera, E., Quispe-fuentes, I., & Martínez, E. A. (2014). Antimicrobial Potential and Phytochemical Content of Six Diverse Sources of Quinoa Seeds (*Chenopodium quinoa* Willd .), (September), 1015–1024.

Miranda, M., Vega-Gálvez, A., López, J., Parada, G., Sanders, M., Aranda, M., Di Scala, K. (2010). Impact of air-drying temperature on nutritional properties, total phenolic content, and antioxidant capacity of quinoa seeds (*Chenopodium quinoa* Willd.). Industrial Crops and Products, 32(3): 258–263.

Miranda, M., Vega-gálvez, A., Martínez, E. a, López, J., Marín, R., Aranda, M., & Fuentes, F. (2013). Influence Of Contrasting Environments on Seed Composition of Two Quinoa Genotypes : Nutritional And Functional Properties. Chilean Journal of Agriculture Research, 73.

Miranda, M., Vega-gálvez, A., Martínez, E. a, López, J., Marín, R., Aranda, M., & Fuentes, F. (2013). Influence of contrasting environments on seed composition of two quinoa genotypes : nutritional and functional properties. Chilean Journal of Agriculture and Research, 73 (2): 108–116.

Miranda, M., Vega-Gálvez, A., Quispe-Fuentes, I., Rodríguez, M. J., Maureira, H., & Martínez, E. a. (2012). Nutritional Aspects of Six Quinoa (*Chenopodium quinoa* Willd.) Ecotypes from three Geographical Areas of Chile. Chilean Journal of Agricultural Research, 72(2): 175–181.

Miranda, M., Vega-Gálvez, A., Uribe, E., López, J., Martínez, E., Rodríguez, M. J., … Di Scala, K. (2011). Physico-chemical analysis, antioxidant capacity and vitamins of six ecotypes of chilean quinoa (*Chenopodium quinoa* Willd). Procedia Food Science, 1: 1439–1446.

Mittal, R., Nagi, H., Sharma, P., & Sharma, S. (2012). Effect of Processing on Chemical Composition and Antinutritional Factors in Chickpea Flour. Journal of Food Science and Engineering, 2(3): 180–186.

Mittal, R., Nagi, H., Sharma, P., & Sharma, S. (2012). Effect of Processing on Chemical Composition and Antinutritional Factors in Chickpea Flour. Journal of Food Science and Engineering, 2(3): 180–186.

Moongngarm, A., & Saetung, N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. Food Chemistry, 122(3), 782–788.

Mota, C., Nascimento, A. C., Santos, M., Delgado, I., Coelho, I., Matos, A. S., Castanheira, I. (2016). The effect of cooking methods on the mineral content of quinoa (*Chenopodium quinoa*), amaranth (Amaranthus sp.) and buckwheat (Fagopyrum esculentum). Journal of Food Composition and Analysis. (Under publication).

Mubarak, A. E. (2005). Food Chemistry Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus aureus) as affected by some home traditional processes. Food Chemistry, 89: 489–495.

Mujica, A., Jacobsen, S.E., Ezquierdo, J., Marathee, J.P., (2001). Resultados de la Prueba Americana y Europes de la Quinua. FAO, UNA-Puno, CIP, 51.

N.K.Rao, Shahid Mohammed (2012). Quinoa- A Promising Crop For Arabian Peninsula. American-Eurasian Journal of Agriculture and Environmental Science., 12(10): 1350-1355.

Nadarajah, S. Gordon, M. H., Pongsawatmanit, R., & Suttajit, M (2015). Influence of Storage Conditions on the Quality Characteristics of Wheat-Defatted Coconut Flour Biscuits Packed in Metalized Polypropylene. International Journal of Engineering Research and Technology, 4(07): 948–951.

Nascimento, A. C., Mota, C., Coelho, I., Gueifão, S., Santos, M., Matos, A. S., Castanheira, I. (2014). Characterisation of nutrient profile of quinoa (*Chenopodium quinoa*), amaranth (Amaranthus caudatus), and purple corn (Zea mays L.) consumed in the North of Argentina: Proximates, minerals and trace elements. Food Chemistry, 148: 420–426.

Nergiz, C., & Gökgöz, E. (2007). Effects of traditional cooking methods on some antinutrients and *in vitro* protein digestibility of dry bean varieties (Phaseolus vulgaris L.) grown in Turkey. International Journal of Food Science and Technology, 42(7): 868–873.

Ng, T.B., He, J.S., Niu, S.M., Zhao, L., Pi, Z.F., Shao, W., Liu, F. (2007). A gallic acid derivative and polysaccharides with antioxida- tive activity from rose (Rosa rugosa) flowers. Journal of Pharmacy and. Pharmacology; 56 (4): 537–545.

Nidhi Chaudhary and Swati Vyas (2014). Effect of germination on proximate composition and anti nutritional factor of millet (ragi) based premixes. Internatonal Journal of Food and Nutritional Sciences, 3(4): 72-79.

Nonogaki, H., G.W. Bassel and J.W. Bewley (2010). Germination-still a mystery. Plant Science, 179, ISSN 574- 581.

Nowak, V., Du, J., & Charrondière, U. R. (2015). Assessment of the nutritional composition of quinoa (*Chenopodium quinoa* Willd.). Food Chemistry. (Under publication).

Nwosu, J. N. (2010): Effect of soaking, blanching and cooking on the antinutritional properties of asparagus bean (Vigna sesquipedis) flour. Nature Science, 8 (8): 163-167.

Oghbaei, M., Prakash, J., & Yildiz, F. (2016). Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. Cogent Food & Agriculture, 2(1): 1136015.

Oliver, E., McGillicuddy, F. C., Harford, K. A., Reynolds, C. M., Phillips, C. M., Ferguson, J. F., & Roche, H. M. (2012). Docosahexaenoic acid attenuates macrophage-induced inflammation and improves insulin sensitivity in adipocytes-

specific differential effects between LC n-3 PUFA. Journal of Nutritional Biochemistry, 23(9): 1192–1200.

Onwulata, C., Thomas, A., Cooke, P. et al. (2010). Glycemic potential of extruded barley, cassava, corn, and quinoa enriched with whey proteins and cashew pulp. International Journal of Food Properties, 13: 338–359.

Owusu, D., & Oduro, I. (2011). Development of crackers from cassava and sweetpotato flours using Moringa oleifera and Ipomoea batatas leaves as fortificant. American Journal of Food and Nutrition, 1(3): 114–122.

Pagno CH, Costa TMH, de Menezes EW, Benvenutti EV, Hertz PF, Matte CR, Tosati JV, Monteiro AR, Rios AO, Flores SH. (2015). Development of active biofilms of quinoa (*Chenopodium quinoa*W.) starch containing gold nanoparticles and evaluation of antimicrobial activity. Food Chemistry, 173: 755–62.

Pagno CH, Costa TMH, de Menezes EW, Benvenutti EV, Hertz PF, Matte CR, Tosati JV, Monteiro AR, Rios AO, Flores SH. (2015). Development of active biofilms of quinoa (*Chenopodium quinoa* W.) starch containing gold nanoparticles and evaluation of antimicrobial activity. Food Chemistry, 173: 755–62.

Pal, R. S., Bhartiya, A., Arun Kumar, R., Kant, L., Aditya, J. P., & Bisht, J. K. (2016). Impact of dehulling and germination on nutrients, antinutrients, and antioxidant properties in horsegram. Journal of Food Science and Technology, 53(1), 337–347.

Palombini, S. V., Claus, T., Maruyama, S. A., Gohara, A. K., Henrique, A., Souza, P., Matsushita, M. (2013). Evaluation of nutritional compounds in new amaranth and quinoa cultivars. Food Science and Technology, 33(6): 339–344.

Pandey, H., & Awasthi, P. (2013). Effect of processing techniques on nutritional composition and antioxidant activity of fenugreek (Trigonella foenum-graecum) seed flour. Journal of Food Science and Technology, 1–7.

Panovska, Z., Váchová, A., & Pokorný, J. (2012). Effect of thickening agents on perceived viscosity and acidity of model beverages. Czech Journal of Food Sciences, 30(5), 442–445.

Pasko, P., Barton, H., Zagrodzki, P., Izewska, A., Krosniak, M., Gawlik, M., and Gawlik, M., et al., 2010b. Effect of diet supplemented with quinoa seeds on oxidative status in plasma and selected tissues of high fructose-fed rats. Plant Foods for Human Nutrition 65(2): 146-151.

Paśko, P., Sajewicz, M., Gorinstein, S., & Zachwieja, Z. (2008). Analysis of selected phenolic acids and flavonoids in *Amaranthus cruentus* and *Chenopodium quinoa* seeds and sprouts by HPLC. Acta Chromatographica, 20(4), 661–672.

Pasko, P., Zagrodzki, P., Barton, H., Chlopicka, J., and Gorinstein, S., 2010a. Effect of quinoa seeds (*Chenopodium quinoa*) in diet on some biochemical parameters and essential elements in blood of high fructose-fed rats. Plant Foods for Human Nutrition 65(4): 333-338

Pawar, V. D., & Machewad, G. M. (2006). Processing Of Foxtail Millet for Improved Nutrient Availability. Journal of Food Processing and Preservation, 30(3): 269–279.

Perez, G., Docena, G., Curciarello, R., (2009). Productos de panificación dirigidos agrupos especiales de la población. In: Lutz, M., León, A.E. (Eds.), Aspectos nutri-cionales y saludables de los productos de panificación. Universidad de ValparaísoEditorial, Valparaíso, Chile, pp. 174–189.

Pesoti, Aline Regiele, Oliveira, Bruno Menezes de, Oliveira, Augusto Cesar de, Pompeu, Dávia Guimarães, Gonçalves, Daniel Bonoto, Marangoni, Sérgio, Silva, José Antonio da, & Granjeiro, Paulo Afonso. (2015). Extraction, purification and characterization of inhibitor of trypsin from *Chenopodium quinoa* seeds. Food Science and Technology (Campinas), 35(4): 588-597.

Phillippy, B.Q., (2016). Transport of calcium across Caco-2 cells in the presence of inositol hexakisphosphate. Nutrition Research, 26: 146-149.

Pineli, L. de L. de O., Botelho, R. B. A., Zandonadi, R. P., Solorzano, J. L., de Oliveira, G. T., Reis, C. E. G., & Teixeira, D. da S. (2015). Low glycemic index and increased protein content in a novel quinoa milk. LWT - Food Science and Technology, 63(2): 1261–1267.

Piper, C. S., (1996). Soil and Plant Analysis. Han Publications, Bombay.

Population Refernce bureau (2015). World population data sheet. Retrieved from www.prb.org/pdf15/2015-world-population-data-sheet_eng.pdf.

Preet, K., & Punia, D. (2000). Antinutrients and digestibility (*in vitro*) of soaked, dehulled and germinated cowpea. Nutrition and Health, 14(2): 109–117.

Prego, I. (1998). Seed Structure and Localization of Reserves in*Chenopodium quinoa*. Annals of Botany, 82(4): 481–488.

Pushparaj, F. S. (2011). Influence of Processing on Dietary Fiber, Tannin and <i>*in vitro*</i> Protein Digestibility of Pearl Millet. Food and Nutrition Sciences, 02(08): 895–900.

Raamsdonk, L. W. D. Van, Pinckaers, V., Ossenkoppele, J., Houben, R., Lotgering, M., & Groot, M. J. (2010). Quality assessments of untreated and washed Quinoa (*Chenopodium quinoa*) seeds based on histological and foaming capacity investigations. Microscopy: Science, Technology, Applications and Education, 4(2): 1033–1038.

Raboy,V. (2009). Approaches and challenges to engineering seed phytate and total phosphorus. PlantScience, 177: 281–296.

Raghuvanshi, R. S., Singh, S., Bisht, K., & Singh, R. (2011). Processing of mungbean products and its nutritional and organoleptic evaluation. International Journal of Food Science & Technology, 46: 1378–1387.

Ranganna S (1995) Manual analysis of fruits and vegetable products. Tata Mc Graw Hill publishing Co. Ltd, New Delhi.

Ranilla, L. G., Apostolidis, E., Genovese, M. I., Lajolo, F. M., & Shetty, K. (2009). Evaluation of Indigenous Grains from the Peruvian Andean Region for Antidiabetes and Antihypertension Potential Using *In vitro* Methods. Journal of Medicinal Food, 12(4): 704–713.

Rasane, P., Jha, A., Kumar, A., & Sharma, N. (2015). Reduction in phytic acid content and enhancement of antioxidant properties of nutricereals by processing for developing a fermented baby food. Journal of Food Science and Technology, 52: 3219–3234.

Rayner, M., Timgren, A., Sjoo, M., & Dejmek, P. (2012). Quinoa starch granules: A candidate for stabilising food-grade Pickering emulsions. Journal of the Science of Food and Agriculture, 92(9): 1841–1847.

Reichert, R. D., Tatarynovich, J. T., & Tyler, R. T. (1986). Abrasive Dehulling of Quinoa (*Chenopodium quinoa*): Effect on Saponin Content as Determined by an Adapted Hemolytic Assay. Cereal Chemistry, 63: 471-480.

Repo-Carrasco-Valencia, R. a M., Encina, C. R., Binaghi, M. J., Greco, C. B., & de Ferrer, P. a R. (2010). Effects of roasting and boiling of quinoa, kiwicha and kaniwa on composition and availability of minerals *in vitro*. Journal of the Science of Food and Agriculture, 90(12): 2068–2073.

Repo-Carrasco-Valencia, R. A.-M., & Serna, L. A. (2011). Quinoa (*Chenopodium quinoa*, Willd.) as a source of dietary fiber and other functional components. Ciência E Tecnologia de Alimentos, 31(1): 225–230.

Ridout, C., Price, L. R., DuPont, M. S., Parker, M. L. & Fenwick, G. R. (1991). Quinoa saponins--analysis and preliminary investigations into effects of reduction by processing. Journal of Food Science and Agriculture, 54: 165-76.

Rodríguez-Sandoval, E., Lascano, A., Sandoval, G., Rodríguez-Sandoval, E., Lascano, A., & Sandoval, G. (2012). Influence of the partial substitution of wheat flour for quinoa and potato flour on the thermomechanical and breadmaking properties. Brazilian Journal of Chemical Engineering, 15(03): 199–207.

Rosa M, Hilal M, Gonzalez JA, Prado FE (2009). Low-temperature effect on enzyme activities involved in sucrose–starch partitioning in salt-stressed and salt-acclimated cotyledons of quinoa (*Chenopodium quinoa* Willd.) seedlings. Plant Physiology and Biochemistry, 47: 300–307.

Rose, T. J., Liu, L., & Wissuwa, M. (2013). Improving phosphorus efficiency in cereal crops: Is breeding for reduced grain phosphorus concentration part of the solution? Frontiers in Plant Science, 4(9): 444.

Rosero, O., M. Marounek, N. Břeňová and D. Lukešova. (2013). Phytase activity and comparison of chemical composition, phytic acid P content of four varieties of quinoa grain (*Chenopodium quinoa* Willd.). Acta Agronómica, 62: 13-20.

Roy DN & Rao PS (1971). Evidence, isolation, purification and some properties of a trypsin inhibitor in Lathyrus sativus. Journal of Agriculture and Food Chemistry, 19: 257– 259.

Ruales, J., & Nair, B. M. (2002). Nutritional quality of the protein in quinoa (*Chenopodium quinoa*, Willd) seeds. Plant Foods for Human Nutrition, 42(1): 1–11.

Rumiyati, Anthony P. James, Vijay Jayasena (2012). Effect of Germination on the Nutritional and Protein Profile of Australian Sweet Lupin (*Lupinus angustifolius* L.). Food and Nutrition Sciences, 03(05): 621–626.

Rusydi, M. (2012). Effect of germination on total phenolic, tannin and phytic acid contents in soybean and peanut, 19(2): 673–677.

Ryan, E., Galvin, K., O'Connor, T., Maguire, A. & O'Brien, N. (2007). Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains and legumes. Plant Foods for Human Nutrition, 62: 85-91.

Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: A critical review. Journal of Food Science and Technology, 47(6): 587–597.

Sánchez, C. L., Cubero, J., Sánchez, J., Chanclón, B., Rivero, M., Rodríguez, A. B., & Barriga, C. (2009). The possible role of human milk nucleotides as sleep inducers. Nutritional Neuroscience, 12(1): 2–8.

Sangronis, E., & Machado, C. J. (2007). Influence of germination on the nutritional quality of Phaseolus vulgaris and Cajanus cajan. LWT - Food Science and Technology, 40(1): 116–120.

Sanni, L.O., Adebowale, A.A., Filani, T.A., Oyewole, O.B. and Westby, A. (2006). Quality of flash and rotary dryer dried fufu flour. Journal of Food Agriculture and Environment, 4: 74 – 78.

Santos Calderelli, V. A., de Benassi, M. T., Visentainer, J. V., & Matioli, G. (2010). Quinoa and flaxseed: Potential ingredients in the production of bread with functional quality. Brazilian Archives of Biology and Technology, 53(4): 981–986.

Sattar A., Badshah A., Aurangzeb (1995). Biosynthesis of ascorbic acid in germinating rapeseed cultivars. Plant Foods for Human Nutrition, 47(1): 63–70.

Savage, G.P.; Mårtensson, L.; Sedcole, J.R. (2009). Composition of oxalates in baked taro (Colocasia esculenta var. Schott) leaves cooked alone or with additions of cows milk or coconut milk. Journal of Food Composition and Analysis, 22: 83–86.

Savelkoul, F.H., A.F. Vander Poel and S. Tamminga (1992). The presence and inactivation of trypsin inhibitors, tannins, lectins and amylase inhibitors in legume seeds during germination A review. Plant Foods for Human Nutrition, 42: 71-85.

Schoenlechner R, Sarawong C, Sekiguchi K, Berghofer E, Ng PKW. (2010). Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. Food Chemistry, 14333-9.

Segev, A. (2011). Total Phenolic Content and Antioxidant Activity of Chickpea as Affected by Soaking and Cooking Conditions. Food and Nutrition Sciences, 02(07): 724–730.

Sharma, M., & Mridula, D. (2015). Development and Quality Evaluation of Maize-Based Fortified Nutritious Bar. Agricultural Research, 4(1), 93–101.

Shin SS, Jung YS, Yoon KH, Choi S, Hong Y, Park D, Lee H, Seo BI, Lee HY, Yoon M. (2013) The Korean traditional medicine Gyeongshingangjeehwan inhibits adipocyte hypertrophy and visceral adipose tissue accumulation by activating PPARalpha actions in rat white adipose tissues. Journal of Ethnopharmacology, 127(1): 47-54.

Siah, S., Wood, J. A., Agboola, S., Konczak, I., & Blanchard, C. L. (2014). Effects of soaking, boiling and autoclaving on the phenolic contents and antioxidant activities of faba beans (Vicia faba L.) differing in seed coat colours. Food Chemistry, 142: 461–8.

Siener, R.; Hönow, R.; Seidler, A.; Voss, S.; Hesse, A. (2006). Oxalate contents of species of the Polygonaceae, Amaranthaceae and families. Food Chemistry, 98: 220–224

Singh, J., & Basu, P. S. (2012). Non-Nutritive Bioactive Compounds in Pulses and Their Impact on Human Health : An Overview. Food and Nutrition Sciences, 3: 1664–1672.

Singh, U. And Jambunathan, R. (1990). Studies on desi and kabuli chickpea (Cicer arietinum) cultivars. Level of protease inhibitors, levels of polyphenolic compounds and *in vitro* protein digestibility. Journal of Food Science. 46: 1364-1367.

Slavin, J. (2003). Why whole grains are protective, biological mechanisms. The Proceedings of Nutrition Society, 62(1): 129-134.

Slavin, J., & Lloyd, B. (2012). Health Benefits of Fruits and Vegetables. Advances in Nutrition, 3(4): 506–516.

Soetan, K. O., & Oyewole, O. E. (2009). The need for adequate processing to reduce the anti- nutritional factors in plants used as human foods and animal feeds: A review. African Journal of Food Science, 3(9): 223–232.

Soliz-Guerrero, J.B., Jasso de Rodrı´ guez, D., Rodrı´guez-Garcı´ a, R., Angulo-Sanchez, J.L. & Me´ ndez-Padilla, G. (2002). Quinoa saponins: concentration and composition analysis. Book chapter; Conference paper Trends in New Crops and New Uses. Proceedings of the Fifth National Symposium, Atlanta, GA, Pp. 110– 114.

Steffolani, M. E., León, A. E., & Pérez, G. T. (2013). Study of the physicochemical and functional characterization of quinoa and kañiwa starches. Starch–Stärke, 65: 976–983.

Stikic, R., Glamoclija, D., Demin, M., Vucelic-Radovic, B., Jovanovic, Z., Milojkovic-Opsenica, D., Milovanovic, M. (2012). Agronomical and nutritional evaluation of quinoa seeds (*Chenopodium quinoa* Willd.) as an ingredient in bread formulations. Journal of Cereal Science, 55(2): 132–138.

Sun, T., & Ho, C. (2005). Antioxidant activities of buckwheat extracts. Food Chemistry, 90: 743–749.

Sundarrajan, L. (2014). Effect of extrusion cooking on the nutritional properties of amaranth , quinoa , kañiwa and lupine. Department of Food and Environmental Sciences. University Of Helsinki. ISSN: 0355-1180

Sung HG, Shin HT, Ha JK, Lai H-L, Cheng K-J, Lee JH (2005) Effect of germination temperature on characteristics of phytase production from barley. Bioresource Technology, 96: 1297–1303.

Suryanti, C. (2016). Effect of germination on antioxidant activity , total phenolics , β-carotene , ascorbic acid and α-tocopherol contents of lead tree sprouts (Leucaena leucocephala (lmk.). International Food Research Journal, 23(1): 167–172.

Takahashi Yoko, Tseye-Oidov Odbayar, and Takashi Ide (2009). A comparative analysis of Genistein and Diadzein in affecting lipid metabolism in rat. Journal of Clinical Biochemistry and Nutrition, 44: 223–230.

Takeda, E., Yamamoto, H., Yamanaka-Okumura,H., and Taketani,Y. (2012).Dietary phosphorus in bone health and quality of life. Nutrition Reviews, 70: 311–321.

Tang, Y., Li, X., Chen, P. X., Zhang, B., Hernandez, M., Zhang, H., Tsao, R. (2014). Lipids, Tocopherols, and Carotenoids in Leaves of Amaranth and Quinoa Cultivars and a New Approach to Overall Evaluation of Nutritional Quality Traits. Journal of Agriculture and Food Chemistry. 62: 12610−12619.

Tang, Y., Li, X., Chen, P. X., Zhang, B., Hernandez, M., Zhang, H., … Tsao, R. (2014b). Lipids, Tocopherols, and Carotenoids in Leaves of Amaranth and Quinoa Cultivars and a New Approach to Overall Evaluation of Nutritional Quality Traits. Journal of Agriculture and Food Chemistry, 62: 12610−12619.

Tang, Y., Li, X., Zhang, B., Chen, P. X., Liu, R., & Tsao, R. (2015). Characterisation of phenolics, betanins and antioxidant activities in seeds of three *Chenopodium quinoa* Willd. genotypes. Food Chemistry, 166: 380–388.

Tari, T., Annapure, U., Singhal, R., and Kulkarni, P. (2003). Starch-based spherical aggre- gates: Screening of small granule sized starches for entrapment of a model flavouring compound, vanillin. Carbohydrates Polymer, 53: 45–51.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis, 19(6-7), 669–675.

Thapliyal, P., Sehgal, S., & Kawatra, A. (2014). *In-vitro* digestibility and antinutrients as affected by soaking, dehulling and pressure cooking of chickpea (Cicer *arietinum*) varieties. Asian Journal of Dairy and Food Research, 33(2): 131.

Thilakarathna, S. H., & Vasantha Rupasinghe, H. P. (2013). Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients, 5(9): 3367–3387.

Thuresson, C., & Thuresson, C. (2015). Development and studies on a gluten free , liquid suspension based on quinoa (*Chenopodium quinoa*). Swedish University of Agricultural Sciences, Master Thesis, 30 hec, Advanced A2E (427).

Torres, E. R., Santana, E., Felix, R., Santana, D., & Cordeiro, J. (2016). Cereal Bar Development Using Exotic Fruit. Food Process Engineering in a Changing World, 22(1): 41-45.

Tosi, P., Gritsch, C. S., He, J., & Shewry, P. R. (2011). Distribution of gluten proteins in bread wheat (Triticum aestivum) grain. Annals of Botany, 108(1): 23–35.

United States Department of Agriculture (2013). USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory Home.

United States Department of Agriculture. (2015). USDA National Nutrient Database for Standard Reference. Retrieved from http://www.ars.usda.gov/Services/docs.htm?docid=8964 .

Uppal, V., & Bains, K. (2012). Effect of germination periods and hydrothermal treatments on *in vitro* protein and starch digestibility of germinated legumes. Journal of Food Science and Technology, 49(2): 184–191.

Vadivel, V., Stuetz, W., Scherbaum, V., & Biesalski, H. K. (2011). Total free phenolic content and health relevant functionality of Indian wild legume grains:

Effect of indigenous processing methods. Journal of Food Composition and Analysis, 24(7): 935–943.

Valcárcel-yamani, B., & Caetano, S. (2012). Applications of Quinoa (*Chenopodium quinoa* Willd .) and Amaranth (Amaranthus Spp .) and Their Influence in the Nutritional Value of Cereal Based Foods. Food and Public Health, 2(6): 265–275.

Valencia-Chamorro S.A (2003): Quinoa. In: Cabal- lero B.: Encyclopedia of Food Science and Nutrition. 8: 4895–4902.

Valencia-Chamorro, S. A. (2004). Quinoa. In C. W.Wrigley, H.Corke, & C.Walker (Eds.), Encyclopedia of grain science (Vol. 3, pp. 1–8). Oxford, United Kingdom: Academic Press.

Van Soest, P. J., & Wine, R. H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. Journal of the Association of Official Analytical Chemistry, 50: 50–55.

Varli, S. N., & Şanlıer, N. (2016). Nutritional and health benefits of quinoa (*Chenopodium quinoa* Willd.). Journal of Cereal Science. (under publication)

Vasishtha, H., & Srivastava, R. P. (2013). Effect of soaking and cooking on dietary fibre components of different type of chickpea genotypes. Journal of Food Science and Technology, 50(3): 579–584.

Vega-Gálvez, A., Miranda, M., Vergara, J., Uribe, E., Puente, L., & Martínez, E. a. (2010). Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* willd.), an ancient Andean grain: A review. Journal of the Science of Food and Agriculture, 90(15): 2541–2547.

Vega-Gálvez, A., Miranda, M., Vergara, J., Uribe, E., Puente, L., & Martínez, E. a. (2010). Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* willd.), an ancient Andean grain: A review. Journal of the Science of Food and Agriculture, 90(15): 2541–2547.

Verza SG, Silveira F, Cibulski S, Kaiser S, Ferreira F, Gosmann G, Roehe PM, Ortega GG. (2012). Immunoadjuvant activity, toxicity assays, and determination

by UPLC/Q-TOF-MS of triterpenic saponins from *Chenopodium quinoa* seeds. Journal of Agriculture and Food Chemistry, 60: 3113–8.

Vilche, C., Gely, M. & Santalla, E. (2009). Physical properties of quinoa seeds. Biosystems Engineering, 86: 59–65.

Villa, D. Y. G., Russo, L., Kerbab, K., Landi, M., & Rastrelli, L. (2014). Chemical and nutritional characterization of Chenopodium pallidicaule (cañihua) and *Chenopodium quinoa* (quinoa) seeds. Emirates Journal of Food and Agriculture, 26(7): 609–615.

Villacrés, E., Pástor, G., Quelal, M. B., Zambrano, I., & Morales, S. H. (2013). Effect of processing on the content of fatty acids , tocopherols and sterols in the oils of quinoa (*Chenopodium quinoa* Willd), lupine (Lupinus mutabilis Sweet), amaranth (Amaranthus caudatus L .) and sangorache (Amaranthus quitensis L.). Global Advanced Research Journal of Food Science and Technology, 2(4): 44–53.

Wang, N., Hatcher, D. W., Toews, R., & Gawalko, E. J. (2009). Influence of cooking and dehulling on nutritional composition of several varieties of lentils (Lens culinaris). LWT - Food Science and Technology, 42(4): 842–848.

Wang, S., & Zhu, F. (2016). Formulation and Quality Attributes of Quinoa Food Products. Food and Bioprocess Technology, 9(1): 49–68.

Welch,A.A., Fransen,H., Jenab,M., Boutron-Ruault,M.C., Tumino, R., Agnoli,C., (2009). Variation in intakes of calcium, phosphorus, magnesium, iron and potassium in 10 countries in the European prospective Investigation into Cancer and Nutrition study. European Journal of Clinical Nutrition, 63: 101–S121.

Wolter, A., Hager, A. S., Zannini, E., & Arendt, E. K. (2014). *In vitro* starch digestibility and predicted glycaemic indexes of buckwheat, oat, quinoa, sorghum, teff and commercial gluten-free bread. Journal of Cereal Science, 58(3), 431–436.

Wong, J. H., Lau, T., Cai, N., Singh, J., Pedersen, J. F., Vensel, W. H., Buchanan, B. B. (2009). Digestibility of protein and starch from sorghum (Sorghum bicolor) is linked to biochemical and structural features of grain endosperm. Journal of Cereal Science, 49(1): 73–82.

Wu, Z., Song, L., Feng, S., Liu, Y., He, G., Yioe, Y., Huang, D. (2012). Germination dramatically increases isoflavonoid content and diversity in chickpea (Cicer arietinum L.) seeds. Journal of Agricultural and Food Chemistry, 60(35): 8606–8615.

Xu, B., & Chang, S. K. C. (2010). Antioxidant capacity of seed coat, dehulled bean, and whole black soybeans in relation to their distributions of total phenolics, phenolic acids, anthocyanins, and isoflavones. Journal of Agricultural and Food Chemistry, 56(18): 8365–8373.

Xu, M. J., J. D. Dong and M. Y. Zhu (2005). Effects of germination conditions on ascorbic acid level and yield of soybean sprouts. Journal of Science, Food and Agriculture, 85(6): 943-947.

Yao, Y., Yang, X., Shi, Z., & Ren, G. (2014a). Anti-Inflammatory Activity of Saponins from Quinoa (*Chenopodium quinoa* Willd.) Seeds in Lipopolysaccharide-Stimulated RAW 264.7 Macrophages Cells. Journal of Food Science, 79(5).

Yawadio Nsimba, R., Kikuzaki, H., & Konishi, Y. (2008). Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and Amaranthus spp. seeds. Food Chemistry, 106(2): 760–766.

Zamora, R. & Hidalgo, F.J. (2005). Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. Critical Reviews in Food Science and Nutrition, 45: 49–59.

Zevallos, V. F., Ellis, H. J., Herencia, L. I., & Ciclitira, P. J. (2012). Variable activation of immune response by quinoa (*Chenopodium quinoa* Willd.) prolamins in celiac disease. American Journal of Clinical Nutrition, 96: 337–344.

Zevallos, V. F., Herencia, L. I., Chang, F., Donnelly, S., Ellis, H. J., & Ciclitira, P. J. (2014). Gastrointestinal Effects of Eating Quinoa (*Chenopodium quinoa* Willd.) in Celiac Patients. American Journal of Gastroenterology, 109(2): 270–278.

Zhu, N., Kikuzaki, H., Vastano, B. C., Nakatani, N., Karwe, M. V., Rosen, R. T., & Ho, C. T. (2009). Ecdysteroids of quinoa seeds (*Chenopodium quinoa* Willd.). Journal of Agricultural and Food Chemistry, 49(5): 2576–2578.

Zhu, N., Kikuzaki, H., Vastano, B. C., Nakatani, N., Karwe, M. V., Rosen, R. T., & Ho, C. T. (2014). Ecdysteroids of quinoa seeds (*Chenopodium quinoa* Willd.). Journal of Agricultural and Food Chemistry, 49(5): 2576–2578.

Zhu, N., Sheng, S., Li, D., Lavoie, E. J., Karwe, M. V., Rosen, R. T., Ho, C-T. (2002). Antioxidative flavonoid glycosides from quinoa seeds (*Chenopodium quinoa*). Journal of Food Lipids 8(1): 37–44.

DECLARATION

I, Intelli Kaur, student of Ph.D. Nutrition and Dietetics (regular), Lovely Professional University, hereby declare that the thesis entitled "Nutritional and Biological Activity Evaluation of Debittered *Chenopodium quinoa* for Development of Functional Food Products" has been prepared by me under the esteemed guidance of Dr. Beenu Tanwar (guide) and Dr. Manju Reddy (co-guide). No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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CERTIFICATE

We certify that Intelli Kaur has prepared her thesis titled "Nutritional and Biological Activity Evaluation of Debittered *Chenopodium quinoa* Seeds for Development of Functional Food Products" for the award of PhD degree of Lovely Professional University**,** under our guidance. She has carried out the research work at the School of Agriculture, Lovely Professional University.

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ABSTRACT

The present study, was carried to explore Indian *Chenopodium quinoa* seeds, grown under the project "Anantha", in Anantapur district of Andhra Pradesh, India. The study titled "Nutritional and biological activity evaluation of debittered *Chenopodium quinoa* seeds for development of functional food products," was carried at Lovely Professional University. Towards the accomplishment of first objective, the *Chenopodium quinoa* seeds were evaluated for their nutritional quality. The seeds were subjected to proximate, nutritional, phytochemical and *in vitro* analysis. Indian *Chenopodium quinoa* seeds were processed by the domestic processes, namely, soaking and germination and compared with respect to raw and industrially processed seeds on the basis of proximate, nutritional, and phytochemical composition. Soaking resulted in increase in 8.5% of moisture content while germination resulted in decrease in moisture content by 17.5% and industrial processing led to 8.5% decrease in moisture content. Soaking and germination resulted in decrease of carbohydrate content by 1.6% and 2% respectively. The carbohydrate content of industrially processed and domestically processed seeds was significantly ($P<0.05$) different. Soaking and germination of raw seeds caused significant decrease ($P<0.05$) in fat content by 20.5% and 32.5%. Crude protein content of raw, domestically processed and industrially processed quinoa seeds was significantly ($P<0.05$) different. Soaking resulted in 0.6% increase in ash content which was statistically non-significant ($P<0.05$) with respect to ash content of raw quinoa seeds while significant increase ($P<0.05$) in ash content, by 22.5%, was observed after germination. Industrially processed seeds were reported with 4.7% reduced ash content. The fiber content of all quinoa seeds were significantly ($P<0.05$) different. Domestic and industrial processing of seeds led to non-significant change ($P<0.05$) in contents of β- carotene. Soaking and germination led to 0.1% and 0.8% increase in β- carotene. Domestic processing of seeds i.e. soaking and germination, led to 10% and 31% significant increase ($P<0.05$) in TDF (Total Dietary Fiber) content of quinoa seeds. Vitamin C content increased by 15% in soaked quinoa seeds and by 46% in germinated quinoa seeds. A significant difference in Vitamin C content of raw and industrially processed seeds was also observed. Industrial processing decreased the vitamin C

content by 30%. Soaking and germination led to 50% and 66.6% decrease in tannin content. Soaking and germination led to 1% and 15%, significant decrease ($P<0.05$) in alkaloid content of quinoa. Soaking and germination resulted in 30% and 55% decrease in oxalate content. Industrially processed seeds were also reported with 10% reduction in oxalate content. Germination led to significant decrease ($P<0.05$) in phytic acid content by 68%. Industrial processing led to non-significant decrease ($P<0.05$) in phytic acid content by 3.2%. Industrially processed seeds exhibited 4.6% increase in total phosphorous content, which was non-significant ($P<0.05$) as compared to the total phosphorous content of raw seeds. Soaking resulted in significant decrease ($P<0.05$) of 24% in saponin content. Germination led to 98% decrease in saponin content. Industrially processed seeds exhibited 97% reduced saponin content as compared to the raw seeds. Soaking resulted in 4.7% decrease in trypsin inhibitor activity. Industrially processed quinoa seeds exhibited 20.7% decrease in trypsin inhibitor activity. Germination resulted in 9.1%, significant increase ($P<0.05$) in protein digestibility. Soaking of quinoa seeds resulted in 1.52%, non-significant increase in starch digestibility while germination led to 1.80%, significant increase with respect to the raw seeds. Soaked quinoa seeds exhibited 7% decrease in antioxidant activity as compared to raw seeds. FRAP values of germinated quinoa seeds increased by 89%. However industrial processing of the seeds lead to decline in antioxidant activity.

Industrially processed Indian *Chenopodium quinoa* seeds were also compared with industrially processed American *Chenopodium quinoa* seeds. It was noticeable that the protein content of Indian quinoa (13.11±0.08 g/100g) was 7.02% more than the protein content of American quinoa (12.25±0.92 g/100g). Indian quinoa seeds exhibited significantly higher ($P<0.05$) β-Carotene content than American seeds. Vitamin C content of American quinoa was, 14.4%, significantly higher ($P<0.05$) than the vitamin C content of Indian quinoa seeds. Calcium, iron and zinc contents of American quinoa seeds were 15.6, 43.2 and 26.1% higher than Indian quinoa, respectively. American quinoa seeds exhibited significantly lower ($P<0.05$) protein digestibility (75.15%) than Indian quinoa seeds.

The objective two of the study was accomplished by a biological trial using animal model. Male wistar rats (42; divided in 7 groups with 6 rats in each group) for a period of 45 days and testing any cholosterolemic effect of supplementing

germinated quinoa (debittered) and raw quinoa (unwashed) seeds. The rats fed with raw and germinated quinoa along with basal diet showed 28.2 and 31.9% reduction in total cholesterol level. Triglyceride level of rats fed with bitter and debitterd quinoa along with basal diet reduced by 10.4 and 14.5%, respectively. The HDL levels were observed to decrease significantly ($P<0.05$) in rats fed with diets supplemented with raw (5.5%) and germinated (3.3%) quinoa along with hypercholesterolemic diet as compared to serum HDL levels of rats fed with hypercholesterolemic diet (positive control) which reveals negative effect of quinoa supplementation along with hyper cholesterolemic diet on serum HDL levels. Lipid lowering effect of quinoa (raw and germinated) was higher than the beneficial effect of statin, which was reported to cause 17.7% decline in (VLDL+HDL) values. The reduction was almost almost twice in germinated quinoa. The drug statin administered to rats along with hypercholesterolemic diet, caused 23.7% reduction in atherosclerotic index, while supplementation of raw and germinated quinoa along with hypercholeterolemic diet resulted in 18.4 and 37.2% reduction in atherosclerotic index. The results infer beneficial effect of quinoa, mainly the germinated one, in hypercholesterolemia over the statin drug.

Towards the accomplishment of third objective, functional foods, namely, quinoa bar, quinoa cracker and quinoa beverages were prepared from quinoa grains. Bars, control bar and quinoa bar, were prepared using chickpea flour and germinated quinoa flour, respectively. They were evaluated for different parameters at an interval of 15 and 30 days. Ash, fat, protein and fiber content of quinoa bar was significantly higher ($P<0.05$) than control bar. Antioxidant activity of quinoa bars, as evaluated by DPPH and FRAP method, was reported significantly higher ($P<0.05$) than the control bars. A significant decrease ($P<0.05$) in over acceptability of snack bars was observed after storage period of 15 and 30 days. In general, although the overall acceptability was observed to decrease with storage period in both snack bars but overall acceptability of quinoa bar was more than the control bar after different storage intervals. Crackers, control and quinoa cracker, were prepared from wheat flour and quinoa flour, respectively. Ash, fat and protein was observed to increase with increase in the ratio of quinoa incorporation to the crackers. Protein content of quinoa crackers was higher than that of wheat crackers. Antioxidant activity was observed to increase significantly ($P<0.05$) with

incorporation of quinoa flour. Cracker incorporated with 40% quinoa flour received highest score for texture, mouthfeel, flavor, and taste on nine point hedonic scale. Storage period of 15 days had no significant effect ($P<0.05$) on overall acceptability of wheat (control, T_0) cracker while overall acceptability of crackers incorporated with 20 and 40% quinoa decreased significantly ($P<0.05$). Beverages, namely raw quinoa beverage (RQB), soaked quinoa beverage (SQB) and germinated quinoa beverage (GQB) were prepared from raw, soaked and germinated quinoa seeds, respectively. Addition of 0.5% (*w/v*) xanthan gum increased the viscosity and helped in textural stabilization of the beverages by reducing serum separation. All quinoa beverages had total phenolic content well correlated to its anti oxidant activity. Upon sensory evaluation, it was observed that GQB qualified all positive aspects and was rated similarly to the commercial soya milk used as control.

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Dated: **Intelli Kaur**

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CHAPTER 1

INTRODUCTION

Malnutrition, a wide mouthed demon, advancing at a staggering scale, is a global distress affecting one in nine people on earth (FAO, 2011).The Global Nutrition Report (GNR), 2015, a report card on global nutrition scenario, by International Food Policy research Institute (IFPRI) gives a transparent view of triumph of malnutrition, over inefficacious global initiatives to combat this demon. Although malnutrition is affecting worldwide but the scale of occurrence in developing countries is abysmally high. In India, the Report Survey on Children (RSoC) in collaboration with UNICEF (2013-14) reported 29.4% Indian children as underweight, 15% wasted and 38.7% stunted. The figures portrayed malnutrition as a "National Shame to India" (Dasgupta, 2015).

One of the major reasons behind global malnutrition is shrinkage of world food basket. The world food supply depends mainly on a few crops species, termed as 'Major Crops'. Almost 95% of the world food requirement is met by just 30 such crops. Dependence of world population of 7.3 billion people (Population Reference Bureau, 2015) on 30 crops has created great imbalance between global food availability and global food demand, further leading to food crisis and worsened malnutrition scenario worldwide. Hence, there is a great need to broaden the plant genetic diversity in order to avoid dependence merely on the major food crops.

With increasing interest in finding new alternate sources, researchers worldwide emphasize the need to focus and enhance the use of those crops which are abandoned by research, technology and marketing systems. Such crops have been referred as "Neglected and Underutilized Crops" (NUCS) also known as "Orphan Crops".

One such neglected crop is *Chenopodium quinoa*, an underutilized pseudocereal crop belonging to family Chenopodiaceae. Originated and sprung initially in Andean region of South America (Matiacevich et al., 2006) with Peru and Bolivia being the main producers, *Chenopodium quinoa*, is an ancient crop with modern

perspectives. The genus Chenopodium belongs to a family of flowering plants, Amaranthaceae (APG II System, 2003) and is categorized in subfamily Chenopodiaceae (formerly known as goosefoot family) enclosing about 250 species (Kadereit et al., 2005).

Quinoa plant is annual, 1 to 4 m tall, having erect cylindrical stem, green to pale yellow leaves, flowers with no petals and vibrantly coloured (red, purple, yellow, black) seeds (James, 2009). The main astounding features of *Chenopodium quinoa* crop, viz its extraordinary nutritional composition, versatility to adapt extreme environmental conditions and latent innate potential to triumph over global hunger and malnutrition, the thirty-seventh session of the General Conference of FAO adopted a resolution recommending the declaration of year 2013 as an "International Year of Quinoa."

The proximate composition of quinoa as estimated by USDA, 2013 reports, 13.2 per cent moisture content, 14.12 per cent protein, 6 per cent fat, 2.3 per cent ash , 64.8 per cent carbohydrate and 7% fibre content. Talking about the nutritional aspect, the protein quality of quinoa is found comparable to that of milk protein, casein (Gordillo-Bastidas et al., 2016). It contains all essential amino acids with presence of both lysine (5.4%) and methionine (2.1%), which gives it a unique feature and makes it a complete food (USDA, 2013). The ash content of quinoa is higher than that of common cereals like wheat and rice (Miranda et al., 2012) and fibre content more than that present in corn and wheat (Repo-Carrasco-Valencia, 2010). The seeds are also rich in vitamins and minerals (Konishi et al., 2004). In addition quinoa seeds are found rich in bioactive compounds like polyphenols (Hirose et al., 2010), isoflavones (Lutz, 2013), and known to exhibit good anti oxidant properties (Pasko et al. 2010a). Besides, it has been considered as an oil crop with the fatty-acid composition corresponding to soybean oil (Comai et al., 2007). Presence of prolamine in non quantifiable amounts, gives it an additional advantage of being a "gluten-free grain", making it suitable to be consumed by patients with celiac disease (Zevallos et al., 2012).

Despite being nutritious, the only downside of this grain is bitterness and specific astringency in its taste, due to presence of saponins, mainly in the hulls (Gomez-Caravaca et al., 2014). Depending upon the saponin content present in quinoa

seed, the variety can be classified as “sweet” (Saponin<0.1%) or “bitter” (Saponin>0.1%) variety (Mastebroek, 2000). Saponin content can be reduced by domestic or industrial processing (Soetan and Oyewole, 2009) to make the grain more palatable and acceptable.

Presence of nutrients and bioactive compounds also impart nutraceutical characteristics to quinoa. It is known to possess health potential against various disorders like hypertension, obesity, hypercholesterolemia, diabetes, etc. (Jancurova et al. 2009; Arneja et al. 2015). Presence of phytochemicals (Hirose et al., 2010) is responsible for its anti-oxidative, cardiovascular protective, anti-allergic, anti-inflammatory, antiviral, and anti-carcinogenic activities (James, 2009). *Chenopodium quinoa*, is also known to have potential to assist in controlling appetite (Berti et al., 2004).

Talking about the crop’s versatility to adapt extreme environmental conditions, it has tolerance to stressful environment conditions like soil salinity (pH 6.0 to 8.5), drought, frost, adverse temperatures (as low as -1˚C up to 35˚C) etc. and ability to grow at an altitude of 4500m above sea level (Jacobsen, 2011). Successful propagation of quinoa in field trials at world’s hottest and driest place, Arabian Peninsula, also proves its ability to adapt to adversities (Rao and Shahid 2012). Thus, the versatile features of this crop and its impeccable nutritional benefits permit advancement of quinoa cultivation out from the boundaries of its Andean motherland to different parts of world.

Along with exemplary features, the increasing demand of quinoa across the world has also prompted agriculturists and researchers worldwide for quinoa cultivation. After being successfully promoted in England (1970), Denmark, Europe (1993) and Kenya (Jacobsen, 2011), quinoa has found its way to Asia with keen interest for the crop mainly in the Indian subcontinent (Bhargava et al., 2006). Crop is more widespread in Pakistan, Nepal and India. India being a land of diverse climatic regions (tropical wet, tropical dry, subtropical humid and mountains) and quinoa being a crop profoundly known to adapted well in unusual environmental conditions is found apt to grow in Indian boundaries (Bhargava et al., 2006).

Initially, grown in foots of Himlayan hills in India, the crop has been grown successfully for the first time in year 2013 under the project named "Anantha" in plains of drought prone area of Anantapur district in Andhra Pradesh. The project has highly promoted the cultivation of quinoa among various private companies in South India. The craze and demand for quinoa among Indians has grown far more over than their demand for the staple traditional crops and millets like sorghum, pearl millet, finger millet etc.

As, most of the quinoa saponins are present in the outer layers (40-45 %), post harvesting and pior to marketing, grains undergo industrial processing, mainly the process of de-hulling or de-cortication, to remove the outer layers of the grain. Dehulling is known to improve grain quality by lowering the content of anti nutrients (Lestiene et al., 2007) and enhancing the acceptance and palatability of the grain. Besides these benefits, dehulling, has also been reported to cause loss of nutrients. Thus, to minimize the loss and increase the bioavailability of nutrients from grains, the researchers recommend use of common traditional domestic processing methods (Pawar and Machewar, 2006; Hotz and Gibbson, 2007) like soaking and germination for domestic processing of grain (Hemlatha et al. 2006).

Grain variety, climatic conditions of the area of cultivation and processing methods are known to be great influential factors in determination of nutritional and phytochemical composition of a grain. After many studies revealing nutritional and phytochemical composition of American quinoa (Pasko et al.,2010b; Repo Carrasco Valencia et al., 2010; Miranda et al., 2012 and 2013; Carciochi et al., 2014; Gomez-Caravaca et al., 2014 and Tang et al., 2015), recently quinoa grown in Kenya (Mujica et al. 2001), Japan (Hirose et al. 2010) and Morocco (Marmouzi et al., 2015) was studied for its seed quality, yield level, antioxidative properties, proximate, flavonoid, fatty acid and mineral composition. As per literature available, Indian *Chenopodium quinoa*, being recently introduced in India, has not been much explored for its nutritional composition and health benefits. Thus, the aim of our study is to debitter *Chenopodium quinoa* seeds by removing saponins through domestic processing methods and estimate nutritional and biological activity of debittered *Chenopodium quinoa* seeds, grown in Anantapur district of Andhra Pradesh, under the project "Anantha."

In the light of the above discussion, the present study "Nutritional and biological activity evaluation of debittered *Chenopodium quinoa* seeds for development of functional food products," was undertaken with the following objectives:

Objectives of the study:

1) To evaluate the nutritional quality of *Chenopodium quinoa* seeds

2) To assess the cholesterolemic effect of *Chenopodium quinoa* seeds

3) To develop and analyze value added products from *Chenopodium quinoa* seeds

CHAPTER 2

REVIEW OF LITERATURE

Review of literature provides a sound base for any scientific investigation. Any research study, demands an acquaintance with the studies done already on a particular subject, in order to develop a clear picture of the problem in hand and to comprehend the study correctly. It also helps us to elucidate the correct methodology for the study.

The existing literature on C*henopodium quinoa*, has been extensively referred in order to identify the research gap and formalize the objectives and methodologies for this research. In this chapter, the literatures related to this study are presented under the following heads.

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2.5 Health benefits of *Chenopodium quinoa*

2.5.1 Hypocholesterolemic effect of *Chenopodium quinoa*

2.6 Functional foods developed from *Chenopodium quinoa*

2.1 Proximate analysis

2.1.1 Moisture content

Moisture content refers to the water content of any food. Isengard, 2011, stated importance of quantification of moisture content of food for understanding its thermodyanamic properties and shelf life. Miranda et al., 2013 reported 7.7 to 15.1g/100g moisture content in quinoa seeds grown in northern, central, and southern Chile. Lower moisture content is indicator of longer product shelf life (Sanni et al., 2006). Quinoa moisture content is highly influenced by variation in seed variety. Miranda et al., 2013 reported difference (6.5 to 12.5 g/100gm) in 'Regalona Baer' and 'Vicuna' varieties of quinoa grown in Temuco and Villarrica regions in Chile.

Effect of processing on moisture content

Moisture diffusivity of quinoa is dependent on moisture content of surrounding media (Vilche et al., 2009). As influenced by processing methods like soaking and germination, Desalegn, 2016 reported increase in moisture content after soaking and decrease in moisture content after germination of chickpea. The increase in moisture content after soaking may be due to uptake of water by dry seed resulting in cell hydration and cell multiplication within the seed (Nonogaki et al., 2010) while decrease in moisture content on germination may be attributed to utilization of water for the synthesis of metabolites (Wang et al., 2009).

2.1.2 Crude Ash

Ash content is the inorganic part of any food sample left as residue after charring. Ashing method used mainly for the estimation of proximate composition is the dry ashing (Azeke et al., 2011). Ash content of a foodstuff is indicator of its mineral content. While dry ashing method measures the total ash content, the wet ashing method is used to measure individual mineral content constituting total ash content (Azeke et al., 2011). Nascimento et al., 2014 reported 2g/100g ash content of quinoa seeds. According to USDA, 2015 (Table 2.1) ash content of quinoa is more than rice, wheat, barley, corn, rye, and sorghum.

Ash content of quinoa varies with variation geographical area of growth. Miranda et al., 2013 reported 3.45g/100g ash content in 'Regalona baer' quinoa variety

grown in Temuco region while 4.2g/100g ash content was exhibited by same quinoa variety grown in Vicuna region in Chile. Ash content also varies with difference in variety of quinoa seed, despite of similar geographical areas of growth. Miranda et al., 2013 reported 4.2g/100g ash content in 'Regalona baer' quinoa variety and 5g/100g ash content in 'Villarrica' quinoa variety grown in same Temuco region in Chile.

Effect of processing on ash content

Various processing methods like soaking, germination, toasting etc have influential effect on ash content of cereals. Inyang and Zakari, 2008 stated activation of phytase on germination, and soaking, resulting in hydrolysis of protein-enzyme bond which leads to release of minerals, due to lixiviation of anti nutrients, as possible cause for increase in ash content on germination. Blessing ang Gregory, 2010 reported increase in ash content of mung bean after toasting. Kavitha and Primalavalli, 2014 reported decrease in ash content of germinated groundnut, which might be due to lixiviation of some minerals in water, while Chikwendu and Ndirika, 2015, reported increase in ash content of germinated ground bean. Afify et al., 2012 also reported decrease in ash content of different varieties of sorghum after soaking and germination.

Table 2.1: Proximate composition of other grains and quinoa

Content /100g	Quinoa	Rice	Barley	Wheat	Corn	Rye	Sorghum
Crude Ash	2.5	0.19	0.62	1.13	0.67	0.98	0.84
Crude protein	14.12	6.81	9.91	13.68	9.42	10.34	10.62
Crude Fat	6.07	0.55	1.3	2.47	4.74	1.63	3.46
Crude Fiber	7	2.8	15.6	10.7	7.3	15.1	6.7
Carbohydrate	64.16	81.68	77.72	71.13	74.26	75.86	72.09

*(USDA, 2015)

2.1.3 Crude protein

According to USDA, 2015, protein content of quinoa is higher than the commonly used grains (Table 2.1). Quinoa's protein content ranges from 11.32 to 16.10 g/100gm (Miranda et al., 2012). Unlike wheat, barley, maize and corn, quinoa

contains albumin (cystine, arginine, histidine) and globulin (chenopodin) as major proteins constituting 35 and 37% of protein content, respectively (James, 2009).

In addition, the presence of non-quantifiable amounts of glutamic acid and prolamine (<7%), an irritant protein for celiac patients, make quinoa fit for consumption by patients suffering from celiac disease (Zevallos et al., 2012). This also makes quinoa a suitable candidate for formulation of gluten free functional food products (Manikandan et al., 2013). Protein content of food is also known to be inversely related to its glycemic index (Shin et al., 2013), Thus, quinoa is also fit for inclusion in list of low glycemic index foods as higher protein content leads to fullness, delayed gastric emptying and decelerated digestion rate (Pineli et al., 2015). The protein quality of quinoa protein has been found to be almost alike the milk protein, casein (Vega-Galvez et al., 2010). The low sulfur amino acid content of globulins is well balanced by the sulfur rich amino acids in albumins (Mardini Filho et al., 2015). Quinoa is known to be an exceptional grain having both methionine (40 to 100mg/100g) and lysine (510 to 640mg/100g), which are limiting amino acids in legumes and cereals, respectively (Gorenstein et al., 2004, Bhargava et al., 2007, Gesinski and Nowak, 2011). Quinoa contains all essential amino acids except tryptophan (Elsohaimy et al., 2016). Hence, the presence of well-balanced amino acid composition renders quinoa protein as a "complete protein" with high nutritional and biological value (Comai et al., 2007).

Besides bestowing unique nutritional properties, protein content of quinoa is also responsible for its diverse functional properties. It imparts properties like foaming, structural, and thermal stabilization to functional food products developed from quinoa. Addition of quinoa protein to film derived from chitin improved the tensile property and thermal stability of quinoa-chitosan film (Araujo-Farro et al., 2010).

Effect of processing on protein content

Domestic processing methods like soaking, germination, cooking, etc are known to greatly influence the protein content of food items. Increase in protein content after soaking of soyabeans was reported by Kayembe and Rensburg, 2013. Nutritive value of cereals is also known to enhance after germination (Hubner and Arendt, 2013). Moongngarm and Sateung, 2010 reported 29% increase in protein content of rice after germination. Inyang and Zakari, 2008 reported increased protein content in germinated peal millet. The increase in protein content may be due to increased activity of protease leading to degradation of peptides to amino acids and further synthesis of new protein (Laetitia et al., 2014). On the contrary, Martinez et al., 2013 reported decreased protein content in germinated legumes, which may be due to excessive hydrolysis of protein on prolonged germination period, leading to its mobilization from the seeds. Industrial processing methods like dehulling and pearling are also known to influence protein content. Ghavidel and Prakash, 2007 reported increase in protein content of dehulled cow pea, green

gram, chick pea and lentils with respect to the raw ones which may be due to the removal of hulls and concentration of protein in embryo while Tosi et al., 2011 reported decrease in protein content of pearled wheat grains due to removal of aleurone layer during the process of pearling.

2.1.4 Crude fat

Quinoa fat content ranges from 4.6 to 5.7 g/100g (Miranda et al., 2013) and is reported to be similar to the Andean grain, kaniwa (Repo-Carrasco-Valencia et al., 2011). The qualitative and quantitative characteristics of quinoa fat make it suitable to be used as an oil crop. The fat content, (Table 2.1) is higher than the common cereals (USDA, 2015), is uniquely rich in essential fatty acids (James, 2009).

In addition it contains less saturated fatty acids and abundant unsaturated fatty comprised of mono and poly unsaturated fatty acid (Marmouzi et al., 2015). According to Marmouzi et al., 2015, quinoa fat is 25 to 29% oleic acid (MUFA), 59% linoleic acid (PUFA) and 12.3% palmitic acid (SFA). The fatty acid profile of quinoa is also reported to be similar to soyabean and maize (Borges et al., 2013). Higher amounts of PUFA have also been known to be beneficial in cardiovascular diseases and insulin sensitivity (Oliver et al., 2012). In addition, the ratio of ω-6/ ω-3 fatty acid in quinoa (10:1/9:1) is higher than western diets (Marmouzi et al., 2015), which also proves quinoa as a beneficial grain for cardio vascular disorders. The fatty acids in quinoa are accompanied by antioxidants, which serve as savior against oxidative rancity (Ng et al., 2007). Among antioxidants, tocopherols have great contribution in imparting antioxidant activity to quinoa (Tang et al., 2014). Presence of γ-tocopherlos (797 ppm) and α-tocopherols (721 pm) braces the use of quinoa in food applications and enhance the shelf life of quinoa oil (Repo-Carrasco-Valencia et al., 2011). The saponification index of quinoa oil (192%) is lower than butter (242%) and coconut oil (250%) but similar to cottonseed (193%) and soyabean (190%) oil (Sundarrajan, 2014). Besides tocopherols, the unsaponifiable oil fraction of quinoa contains squalene and phytosterols. Presence of squalene (54 to 89 mg/100g), an organic compound also present in shark liver oil, is known to impart cardio vascular protective potential to quinoa seeds (Ryan et al., 2007). Graf et al., 2014 concluded phytoecdysteroid content of quinoa to be directly correlated to its oil content. Phytosterols in quinoa have although not been given much importance, but quinoa grains are known to contain about 118mg/100g of phytosterols (Varli et al., 2016). Presence of phytosterols, mainly campesterol (16 mg/100g), stigmasterol (3.4 mg/100g) and β-sitosterol (64 mg/100g) impart anti-inflammatory and anti carcinogenic and hypo cholestrolemic potential to quinoa (Villacres et al., 2013). Phytosterol content of quinoa is higher than corn and millets (Ryan et al., 2007). Phytosterols are also known to have great impact in lowering cholesterol levels by competitive inhibition of cholesterol absorption due to structural similarity with cholesterol (Graf et al., 2015).

Effect of processing on fat content

As influenced by processing methods like soaking and germination, Kayembe et al., 2013 reported increase in fat content after germination of soyabean while Kajihausa et al., 2014 reported decrease in fat content after germination of sesame seeds. The reported decrease may be due to cell growth during the process. Seed growth because of water imbibition by cells on soaking, consumes required energy from fat, a major carbon source in seeds, which may lead to decrease in fat content after soaking (Rumiyati et al., 2012). Germination of seeds leads to metabolite synthesis. This metabolic change requires energy, which is liberated by oxidation of fatty acid resulting in reduced fat content in germinated seeds (Hahm et al., 2009).

2.1.5 Crude fiber

Crude fiber content is measure of mainly the resistant or insoluble fiber (roughage), which is resistant to digestion in human body (Slavin and Lloyd, 2012). Estimation crude fiber content in food does not measure the soluble fibres (pectins, gums, certain poly- and oligo-saccharides) which have been reported to be associated with various health benefits (Chawla and Patil, 2010). Quinoa fiber content of quinoa seeds (Table 2.1) is greater than rice, corn and sorghum (USDA, 2015), which makes it a potential fiber rich food source and can be included in planning dietary strategy to increase fiber intake through diet.

Effect of processing on crude fiber content

Soaking, germination, cooking, toasting, etc are various processing methods employed to enhance nutritional value of foods (Pandey and Awasthi, 2015). Kanensi et al., 2011, reported increase in crude fiber content of Amaranth seeds with increase in steeping period. Increase in fiber content of chickpea, cowpea and mung bean on germination was also reported by Uppal and bains, 2012. Synthesis of insoluble fibers, which are constituents of cell wall, namely cellulose and hemicelluloses may be the cause for increase in fiber content after germination (Pandey and Awasthi, 2015). Kavitha and Parimalavalli, 2014 reported increase in crude fiber content of roasted and germinated wheat flour. Decrease in fiber content after industrial processing has been reported in mung bean by Blessing and Gregory, 2010 which may be due to removal of hulls during industrial processing, which largely account for the fiber content of seed.

2.1.6 Carbohydrates

Starch, the major macro constituent of carbohydrate content in quinoa ranges from approximately 54 to 70% of total carbohydrate content (Steffolani et al., 2013). Quinoa starch granules, as a unit or aggregate, are found mainly in perisperm and are polygonal in shape having 0.6 to 2.3µm diameter (Li et al., 2016). The starch grains are small in size with respect to the size of starch grains of wheat, rice and

maize (Vega-Galvez et al., 2010). Rich content of quinoa starch makes it fit to be used as rice replacement pseudocereal. According to USDA, 2015, (Table 2.1) quinoa contains lower carbohydrate content as compared to some common cereals.

Starch, in general, comprises of 2 types of molecules, namely, amylose and amylopectin. With difference in quinoa varieties, quinoa starch differs with respect to content, structure, granular size and physiochemical characteristics. The amylose content of quinoa starch ranges from 7.7 to 25.7% (Li et al., 2015) and the amylopectin content comprising approximately 78% (Tari et al., 2003) is composed largely of short chains and some long chains which gives it unique structural and functional characteristics (Araujo-Farro et al., 2010). Lindeboom et al., 2005 demonstarted extensive variation in amylase content of quinoa starches, which was directly related to its functional characteristics. The crystalline polymorphic form of quinoa starch shows diffraction pattern of type A, which corresponds to polymorphic form of the cereal starch (Lopez-Rubio et al., 2008). Quinoa starch is known to be the main player behind the quality attributes of resultant food products (Perez et al., 2009). The excellent functional properties of quinoa starch like small granular size, low pasting temperature, high water absoption capacity, swelling power, low gelatinization temperature, freeze-thaw stability and storage stability make it apt to be used as thickener and textural stabilizer for dressings, sauces and other creamy food stuffs (Wu et al., 2012). Rayner et al., 2012 revealed that on basis of weight, ten times less amount of quinoa starch (maximum coverage 1590mg/m^2) is required to stabilize a pickering emulsion, with respect to the barley starch (maximum coverage 16400mg/m^2). The emulsions stabilized using quinoa starch also showed excellent stability for upto 2 years (Rayner et al., 2012). Pagno et al., 2015 revealed antimicrobial characteristics of an edible biofilm prepared from quinoa starch and gold nanoparticles. Hydrophobic alteration in quinoa starch granules make it apt to be used as a stabilization agent in double Pickering where they were efficient in encapsulation hydrophilic samples and showed stability upto 1 month (Matos et al., 2013). Araujo-Farro et al., 2010 prepared a colorless edible film with quinoa starch and glycerol. The homogenous film produced, exhibited best mechanical properties and a smooth surface. Matos et al., 2013 also demonstrated effective use of quinoa starch for encapsulation of cosmetics and pharmaceutical products.

Besides imparting nutritional and functional properties, quinoa carbohydrates have also proven their potential health benefits. Tang et al., 2014 revealed antioxidative and immunolegulatory characterisctic of quinoa polysachharides in an *in vitro* study using RAW 264.7 cells.

Effect of processing on carbohydrate content

Carbohydrate content of food is influenced by various processing methods. Rosa et al 2009 reported decrease in carbohydrate content after soaking and

germination, which may be due to activation of α-amylase in quinoa seeds and breakdown of starch to simple sugars on hydration during both processes. As influenced by industrial processing, Makinde and Akinoso, 2013, reported decrease in carbohydrate content after dehulling of Nigerian sesame. Miranda et al., 2012 and Miranda et al., 2013 also demonstrated different carbohydrate content of quinoa seeds grown in different environmental conditions, revealing influence of varied environmental constraints on nutritional and quality attributes of grain.

2.2 Nutritional analysis of *Chenopodium quinoa*

2.2.1 Dietary fiber

Dietary fiber is the indigestible component of carbohydrate, other than the starch, which is easily digestible. Dietary fiber is composed of non starch polysaccharides and oligosaccharides. Non-starch polysaccharides may be classified as "soluble dietary fiber" or "insoluble dietary fiber" (AACCI, 2011). On the basis of water solubility, dietary fiber can be classified as, Insoluble dietary fiber (cellulose, hemicelluloses and lignin) and soluble dietary fiber (Pectins, gums and mucilages). Among various methods to determine the dietary fiber content of food, detergent methid i.e., ADF (acid detergent fiber) and NDF (neutral detergent fiber) method measure cell components insoluble in acid and neutral detergent, respectively. ADF includes cellulose and lignin while NDF includes hemicelluloses and ADF, which involves measurement of hemicelluloses by difference method (Caprita and Adrian., 2011). Lamothe et al., 2015 reported 10g/100g dietary fiber content in quinoa seeds. Cereals are one of the important sources of dietary fiber (Vega-Galvez et al., 2010) Marmouzi et al., 2015 reported 72.03% NDF (Neutral detergent fiber) and 27.06% ADF (Acid detergent fiber) in Moroccan quinoa seeds. Miranda et al., 2013 reported 11.5 to 15.07 g/100g total dietary fiber (9.9 to 12.1g/100g insoluble dietary fiber and 0.36 to 2.8 g/100g soluble dietary fiber) content in two genotypes of quinoa. Further, the dietary fiber content of food is known to be associated with various physiological benefits like, hypertension, hypercholesterolemia, cardio vascular diseases, diabetes etc (Dhingra et al., 2012). Lamothe et al., 2015 revealed that dietary fiber content of quinoa is similar to amaranth but greater than maize. In addition, dietary fiber is also known to possess antioxidant properties. Zhu et al., 2009 reported 96.3% antioxidant activity of rice bran polysaccharides concluding its effectiveness similar to ascorbic acid.

Effect of processing on dietary fiber

Different processing methods have different effect on dietary fiber content of foods (Dhingra et al., 2012). Cetrain processing methods involving enzymes, chemicals, heat treatments etc., can also cause structural modification of dietary fibres. Increase in dietary fiber contents upon soaking and germination has been previously reported in cereals and legumes (Vasishtha and Srivastava, 2013, and

Megat et al., 2016).The increase content may be due to enlargement of cell body and growth initiation upon water imbibition during soaking and germination (Martin-Cabrejas et al., 2003). Similarly, extrusion cooking has been also reported to increase dietary fiber content due to chemical interactions that take place under heat and pressure. Pushparaj and Urooj, 2011, have reported detrimental effect of *industrial* processing on dietary fiber in pearl millet.

2.2.2 Vitamin C

Vitamin C content in *Chenopodium quinoa*, as reported by Miranda *et al.,* (2010) ranges from 12 to 23 mg/100g. Miranda et al., 2013, reported even higher vitamin C content (22 to 31 mg/100g) in two quinoa genotypes from Temuco and Vacuna localities in Chile. Vitamin C content may be vary according to different environmental and storage conditions, as factors like light intensity, amount of nitrogen fertilizers, frequency of irrigation and temperature of the region strongly affect the vitamin C content in crops (Lee and Kader, 2000).

Effect of processing on Vitamin C content

Ascorbic acid which is practically absent in dry grain legumes (Xu et al., 2005) increased in significant amount after sprouting (Khattak et al. 2007). The metabolic changes during sprouting affect the bioavailability, palatability and digestibility of essential nutrients. However, the effect of sprouting depends on the types of legume and conditions and duration of sprouting process (Savelkoul et al., 1992). Several enzyme systems become active during sprouting that brings about profound changes in the nutritional quality of cereals and pulses. Masood et al., 2014 revealed significant ($p< 0.01$) effect of sprouting time on ascorbic acid level of mung bean and chickpea seeds. Dry seeds had no ascorbic acid but phenomenal linear increase was observed in mung bean and chickpea with the progress in sprouting.

2.2.3 β-carotene

β- Carotene belong to a group of pigments, carotenoids, commonly found in whole grains (Borneo and Leon, 2011). Total carotenoid index (TCI) of quinoa (leaves), as measured by Tang et al., 2014 is 49.6 to 73.8 g/100 and is more than TCI of amaranth leaves. α- carotene, β- Carotene, cryptoxanthin, leutin and zeaxanthin are the commonly found carotenoids in dietary sources. Quinoa leaves contain β- Carotene, as primary carotenoids with concentration higher than β-Carotenecontant of amaranth leaves (Tang et al., 2014). Lutein, second most dominant and zeaxanthin, in traces, were the two other lipophylic carotenoids isolated by Tang et al., 2015 in quinoa.Carotenoids have been known to be the influencial factors that impart antioxidant activity to quinoa (Tang et al., 2015). α-carotene, β- Carotene lead to synthesis of vitamin A and xanthophylls. Commonly found xanthophylls in quinoa, leutin and zeaxanthin act as antioxidants and

protect the cell membranes. One of the most important contribution of carotenoids in quinoa is protection against light-induced damages by acting as photosensitizers (Asensi-Fabado et al., 2010).

Effect of processing on β- Carotene content

Increase in β- Carotene upon soaking and germination has been reported by Luthariya and Singh, 2014 and Suryanti, 2016. Lee et al., 2013 also repored increased β- Carotene contents in soyabean sprouts as compared to the seeds. This may be attributable to the fact that β- Carotene content in cereals and pulses is directly proportional to the growth progression in the seed (Ahn et al., 2012).

2.2.4 Mineral content

Although, the mineral composition of a grain acquires quantitatively insignificant portion, but its function and chemistry plays a quite significant role in nutritive value of a grain (Singh et al., 2012).

Table 2.2: Mineral composition (mg/100g) of quinoa with respect to oat and barley

Minerals	Quinoa seed[a]	Oat [b]	Barley [b]	Wheat[c]
Iron	5.5	5.4	2.5	3.3
Magnesium	206	235	79	96.4
Calcium	32	58	29	34.8
Zinc	1.8	3.11	2.1	1.2

* [a]Konishi et al., 2004; [b]USDA, 2015; [c]Jubete et al., 2010

Inadequate intake and less bioavailability of minerals can trigger various health related complications. Quinoa is considered as a pseudo cereal rich in minerals (Jubete et al., 2010). It's mineral concentration, shown in Table 2.2 is higher than the mineral content in commonly found grains (USDA, 2015). Vega-Galvez et al., 2010 reported higher magnesium content (0.26%) in quinoa grain with respect to wheat (0.16%) and corn (0.14%).

Moreover, the main minerals in quinoa, i.e. iron, calcium, magnesium and zinc, etc. are found in readily bio-available form, which aids in mineral absorption by human body and are almost adequate to meet the requirements of a balanced diet (Vega-Galvez, 2010). Besides, the mineral content in quinoa genotypes is highly dependent on environmental conditions (Miranda et al., 2013). Miranda et al., 2013 and Nascimento et al., 2014 reported 44 to 110 mg/100g calcium content in quinoa seeds. Nascimento et al., 2014 reported 5.4 g/100g iron content in quinoa seeds. Miranda et al., 2013 and Nascimento et al., 2014 reported 2.9 to 9.5 mg/100g zinc content in quinoa seeds. Miranda et al., 2010, and Marmouzi et al., 2015 reported 176 to 192 mg/100g magnesium content in quinoa seeds.

Effect of processing on mineral content

Various processing methods influence the content of mineral retention and mineral loss in grains. Mota et al., 2016 reported up to 20% decrease in mineral content of cereals and pseudo cereals caused by cooking. The study revealed better retention of minerals under steaming as compare to boiling. Domestic processing of seeds i.e. soaking and germination increase calcium content Chaparro et al., 2011. Hahm et al., 2009 also reported 0.7% increase in calcium content of sesame seeds after germination. Chapparo et al., 2011 reported post germination increase of 11.4% in iron content of quinoa seeds. The apparent reason, for increase in calcium and iron content, may be the decrease in phytic acid content post domestic processing. Phytic acid is known to bind with minerals to form insoluble mineral-phytate complexes and thus, making them less bio-available for proper utilization in body (Coulibaly et al., 2012)

Afiffy et al., 2012 reported 14% and 20% reduction in zinc content of sorghum seeds post soaking and germination. Decrease in magnesium content after germination has been reported in black beans by Sangronis and Machado, 2007. The apparent reason behind decrease in zinc and magnesium contents may be lixiviation of minerals into soaking media during domestic processing of seed.

Konishi et al., 2004, in their study related to depiction of mineral distribution in quinoa stated an industrial processing technique, abrasion, as a potent cause of calcium loss from quinoa seeds, as the latter is located in pericarp, which is usually removed during industrial processing. Reduction in zinc and iron contents of industrially processed seeds has been reported by Pal et al., 2016 who reported losses in Zn and Fe content of dehulled horsegram.

2.3 Phytochemical analysis of *Chenopodium quinoa*

2.3.1 Phytic acid

Phytic acid is an anti nutritional component quinoa seeds (Konishi et al., 2003). Quinoa contains 1.18g/100g phytic acid content (Valencia-Chamorro, 2004). Unlike, wheat and rye, which contain phytic acid only in the outer layers, phytic acid in quinoa is present in embryo and seed coat of the seeds (Bastidas et al., 2016) The negatively charged phytic acid molecules bind with the minerals resulting in formation of mineral-phytate chelates and hence interfers with the mineral bioavailability (Frontela and Martinez, 2011). Phillippy et al., 2014 reported low phyate content in foods to be directly related to their potential health benefit in atherosclerosis, dyslipidemia and hypercholestrolemia.

Effect of processing on content of phytic acid

Domestic processing methods like soaking and germination, tend to decrease the phytic acid content and the reason behind the decrease may be leaching of phytic acid in the soaking media (Vadivel et al., 2011). Liang et al., 2008, also reported

reduction in contents of phytic acid in brown rice upon soaking. Ibrahim et al., 2005, reported reduction in phytic acid content of cereals after germination. The decrease may be due to the increased activity of enzyme, phytase, upon germination, which hydrolyzes phytic acid to release phosphorous (Kumar, 2013). Pal et al., 2016 have also reported decrease in phytic acid content of horsegram after germination.

2.3.2 Total phosphorous

Total phosphorous content has recently been much focused on to create sustainable global phosphorous reserves, which are non-renewable in nature (Rose et al., 2013). Dairy based foods and cereal grains, which are rich in protein have also been known to be rich in dietary phosphorous content (Welch et al., 2009). Phytate, inorganic phosphates, DNA, ATP, RNA are various forms in which phosphorous is found in cereal grains (Raboy, 2009). Phosphorous content in dietary sources is of great importance as both excess and deficiency lead to detoriation of bone health (Takeda et al., 2012). Rosero et al., 2013 reported 0.44-0.5g/100g total phosphorous content in four different varieties of quinoa seeds. Ando et al., 2002 and Konishi et al., 2004 reported that about 60% of phytic acidis localized in embryo of quinoa seeds, and is a major indicative of phosphorous.

Effect of processing on total phosphorous

Various domestic processing methods like soaking and germination lead to increase in total phosphorous content. Hydration of seeds leads to activation of enzyme phytase and thus the release of inorganic phosphorous as consequence of phytic acid degradation (Kumar, 2013). Increase in total phosphorous content of cereals upon germination has also been reported by Azeke et al., 2011. As phytic acid disintegrates upon soaking and germination release of in organic phosphorous results in increase of total phosphorous (Baruah et al., 2007). Mota et al., 2016 reported 100% retention of phosphorous in quinoa after steaming.

2.3.3 Oxalates

Quinoa leaves and stems contain high amounts of total oxalate content (875 to 1960 mg/100g) as compared to the seeds (144 to 234 mg/100g) (Jancurova et al., 2009). Oxalate content of food corresponds to soluble oxalate content, that binds sodium, potassium and ammonium ions while soluble oxalte content binds to calcium, magnesium and iron (Savage et al., 2009). Quinoa seeds have been reported to have 1.8g/100g total oxalate content and soluble oxalate content comprises of 71% (131mgmg/100g) of total oxalate content (Siener et al., 2006). Oxalate content in quinoa is less than spinach, beet, rhubarb, etc., which belong to the same family “Chenopodieceae”. Lopes et al 2009, demonstrated 381 mg/100g oxalic acid content in wholemeal from quinoa variety ‘BRS Paibiru”, which was found to be higher than the oxalic acid content of spinach (823 mg/100) and similar to the oxalic acid content of beet (330 mg/100g). Oxalic acid present in

foods is known to form chelates by binding with mineral content of food and making it less bioavailable during digestion (Nile and Park, 2014). Oxalic acid, upon chelation with divalent calcium, forms calcium oxalate, commonly known as root cause of kidney and gall bladder stone. Hyperoxaluria, is caused due to excessive oxalate excretion in urine (Hang et al., 2014). Hence, low oxalate content foods are beneficial for mineral bioavailability from mineral rich foodstuffs.

Effect of processing on oxalate content

Domestic processing methods like soaking and germination tend to decrease the oxalate content of food due to lixiviation of soluble oxalate content into soaking media (Makinde and Akinoso, 2013). Hang et al., 2014 demonstrated detrimental effect of boiling and soaking on oxalate content of taro leaves and petioles by 84.5 and 69.2%, respectively. Boiling causes disruption of cell wall due to excessive heat, and hence the oxalate leaches out in to boiling media (McEwan et al., 2014). Juajun et al., 2012 reported cooking as an effective method to reduce oxalate content of vegetables.

2.3.4 Tannins

Tannins are biomolecules comprising of carboxyl and hydroxyl groups and are polyphenolic in nature. They form complex linkages with carbohydrates and proteins present in plants (Santos, 2006). Because of their interference with absorption of proteins and certain minerals like iron, tannins are considered as antinutrients (Kumar and Udhayaya, 2012). Tannins are also responsible for astringency and nasty color of food product leading to its decreased palatability. In addition they contribute to undesirable biological effect like inhibition of carbohydrate, mineral and vitamin absoption (Santos, 2006). Quinoa seeds contain 0.53g/100g tannin content (Valencia-Chamorro, 2003).

Effect of processing on tannin content

Khandelwal et al., 2010 stated soaking and germination as effective domestic processing methods to reduce tannin contents in legumes. The decrease might be attributed to leaching out of tannins in soaking media during soaking. Megat and Azrina, 2016 also reported decrease in tannin content of peanuts post germination. Germination is known to trigger the disintegration of tannin-protein-enzyme-mineral complex (Echendu et al., 2009) which might cause decrease in tannin content. Akin-Idowu et al., 2009 reported decrease in tannin content of tubers after boiling. Decrease in tannin content has also been known to increase iron bioavailability (Enes et al., 2014).

2.3.5 Alkaloids

Alkaloids are nitrogen-containing compounds, present mainly in plants. First alkaloid discovered was, Morphine (Laux, 2013). Genus *Chenopodium* has been

reported to contain tropane, piperidine and pyridine alkaloids (Kokanova-Nedialkova et al., 2009). Dini et al., 2006 reported presence of five betaines in *Chenopodium quinoa. Chenopodium murale* was reported to contain alkaloid piperine . Chenoalbacine is an alkaloid isolated from roots of *Chenopodium album* (Dini et al., 2005).

Effect of processing on alkaloid content

Soaking and germination lead to decrease in alkaloid content due to solubility of betaine (an alkaloid present in quinoa) in polar solvents such as water (Wang and Zhu, 2012). Sanchez et al., 2009 reported decrease in alkaloid content upon soaking and germination of pigeon pea and lupin seeds, respectively.

2.3.6 Saponins

Quinoa saponins, the natural detergents, are the major negative factor responsible for its bitter taste, lower palatability, and lower consumer acceptability. Majorly, the triterpene saponins derived from oleonic acid, hederagenin and serjanic acid with galactose, arabinos and glucose as sugar moieties have been located in all parts of quinoa plant (Zhu et al., 2002). Kuljanabhagwad and Wink, 2009 have concluded presence of monodesmosidic (with single carbohydrate chain), didesmosidic (with two carbohydrate chains) and tridesmosidic (with three carbohydrate chains) triterpene saponins in quinoa. In quinoa seeds, saponins are located in the outer layer, i.e. the papillose cells of hulls (Raamsdonk et al., 2010). Saponin content in bitter varieties of quinoa is 0.1 to 5 g/100g while sweet varieties are known to contain 0.02 to 0.05 g/100g of saponin content (Valencia-Chamorro, 2003 and Mastebroek et al., 2000). Saponin content in quinoa is less than the saponin content of legumes (James, 2009). In addition to cross hybridization as possible solution to production of sweet quinoa varieties for increasing its consumer acceptability, regulation of soil salinity and irrigation level is also known to greatly influence the saponin level in quinoa seed (Gomez-Caravacca et al., 2014). Soliz-Guerrero et al., 2002 revealed that high soil water deficit may also help in yielding quinoa crops with lower saponin content.

Saponins are known to engage in hemolytic activity of red blood cells and result in toxicity (Araujo-Farro et al., 2010).They also interfere in absorption of minerals by forming tight saponin-mineral linkages (Jancurova et al., 2009). Although saponins adversely affect quinoa acceptability in market but they are to have positive influence on crop protection against pests (Chaeib, 2010). In addition, saponins are reported to have anti microbial and cholesterol lowering potential (Afrose et al., 2010). Yao et al., 2014 also demonstrated anti-inflammatory potential of quinoa saponins. Saponins are also known as “cell permeability modifiers” and used as precursors which intensifies the uptake of certain drugs by small intestine, which represents its pharmacological potential (Vega-Galvez et al., 2010).

Effect of processing on saponin content

Domestic processing methods like soaking and germination are known to be effective in removal of saponins. Raamsdonk et al., 2010 demonstrated destruction of papillose cells, the major location site of saponins in quinoa, during washing process. Nwosu, 2010 reported 25% decrease in saponin content of bean after soaking for 24 hours. Adekanmi et al., 2009 and Mittal et al., 2012 have also reported decrease in saponin content after soaking tiger nut and chickpea, respectively.

2.3.7 Trypsin inhibitor activity

Trypsin Inhibitor is a protease inhibitor, which inhibits the action of pancreatic enzyme, trypsin, and hence interfers with the intestinal digestion of proteins. Pesoti et al., 2015 isolated novel trypsin inhibitor "CqTI" from C*henopodium quinoa* seeds. Quinoa trypsin inhibitor activity is not of much concern as the content of trypsin inhibitors in quinoa (1.38 to 5.1 TIU/mg) is quite less and lower than trypsin inhibitor activity of lentils (18 TIU/mg), beans (20 TIU/mg) and soyabean (40 TIU/mg) (Jancurova et al., 2009). Presence of trypsin inhibitors interferes with the action of trypsin on proteins and may lead to gastric and pancreatic distress (Horton et al., 2006)

Effect of processing on Trypsin inhibitor activity

Mubarak, 2005, reported decrease in trypsin inhibitor activity, by 5.2%, after soaking mung bean seeds. El-Adawy, 2002 reported 33.9% decrease in trypsin inhibitor activity of chickpeas after soaking. McEwan et al., 2014 reported reduction in trypsin inhibitor activity of tubers after cooking due to heat sensitivity of trypsin inhibitors.

2.3.8 Total phenolic content

Quinoa total phenolic content depends on grain variety and environmental conditions (Miranda et al., 2011). Nasimba et al., 2008 reported higher phenolic content of Japanese quinoa (150 mg/g tannic acid equivalent) as compared to the Bolivian quinoa, which exhibited 94mg/g tannic acid equivalent of total phenolic content. Hirose et al., 2010 suggested the presence of quercitin glycosides as major responsible factors for higher antioxidant activity of Japanese quinoa as compared to the South American quinoa. Quinoa seeds and sprouts have been known to be rich in total phenolics and antioxidant activity (Pasko et al., 2010a). The study also revealed higher total phenolic content of quinoa as compared to the amaranth grain and positive correlation between total phenlic content and antioxidant activity, suggesting antioxidative potential of quinoa phenolics.

Effect of processing on total phenolic content

Xu and Chang, 2008 reported 26-56% loss in total phenolic content of black beans (*Phaseolus vulgaris* L.). Lower phenolic content was also reported in soaked faba beans by Siah et al., 2015. Hydrolysis and leaching of some condensed polyphenols during prolonged soaking period into water used for soaking may be the reason for reduction in TPC in soaked seeds (Segev, 2011). Germination leads to increase in phenolic content of seeds (Duenas et al., 2009) as synthesis of phenolic acid is enhanced by seed growth during germination (Cevallos-Casals and Cisneros-Zevallos, 2010). Increase in total phenolic content in germinated quinoa has also been reported by Carciochi et al., 2014a (56 % after 48 hours and 101.2% after 72 hours of germination) and Jubete et al., 2010 (107% after 82 hours of germination). Difference in total phenolic increase can be explained on the basis of varying germination time and techniques (Khattak et al., 2007). Among common processes involved in industrial seed processing is decortication, also known as pearling, which removes its saponins present mainly in outer layer of quinoa (Bastidas et al., 2016). Decrease in phenolic compounds of pearled quinoa (abrasion degree of 30%) was reported by Gomez-Caravaca et al., 2014 with 21.5% and 35.2% decrease in free and bound phenolic compounds, respectively. Decrease in TPC after decortication (industrial abrasive dehulling) has also been reported in soyabean (11%), chickpeas (37%), and yellow peas (34%) by Xu and Chang, 2010. Han, Baik, 2008 (in wheat), Madhujith and Shahidi, 2006 (in barley), and Cardador-Martinez et al., 2002 (in common beans) have also reported decrease in TPC after decortication of seeds.

2.4 *In vitro* analysis of *Chenopodium quinoa*

2.4.1 *In vitro* starch digestibility

Starch, is the major constituent of carbohydrate content in quinoa and corresponds to 54 to 70% of total carbohydrate content (Steffolani et al., 2013). Repo-Carrasco-Valencia and Serna, 2011 reported 65.1 to 68.6% starch digestibility in 4 different varieties of quinoa seeds. High starch digestibility of quinoa can also be attributed to the small size (0.3 to 2µm) of quinoa starch granules (Kong and Bertoft, 2010). Li and Zhu, 2016 reported significant amount of rapidly digestible starch (RDS) content in quinoa, which is readily susceptible to enzyme action.

Effect of processing on in vitro starch digestibility

Soaking and germination are known to increase the in vitro starch digestibility due to better subjection of seed starch matrix to degradation upon hydration and action of α-amylase on soaking and germination (Chung et al., 2012). It also results in starch gelatinization and increased activity of α-amylase, an enzyme responsible for disintegration of starch into sugars, hence, reducing starch into readily digestible form (Preet and Punia, 2000). Kaur et al., 2016 reported increased glycemic index and starch hydrolysis of germinated (glycemic index: 26.8, starch hydrolysis: 54.4) and soaked (glycemic index: 19.1, starch hydrolysis: 50.2%) mungbean as compared to the raw (glycemic index: 17, starch hydrolysis: 49.15)

mugbean . Capriles et al., 2014 also reported 7.02% increase in starch digestibility of industrially processed (extruded) amaranth seeds. Raghuvanshi et al., 2011 and Oghbaei and Prakash, 2016 reported improvement in starch digestibility of dehulled and milled cereals. Thapliyal et al., 2014 also reported 9.8 to 14% increase in starch digestibility of industrially processed (dehulled) chickpeas of different varieties. Removal of bran and outer fibrous tissue of cereals during dehulling and milling leads to concentration of starch rich fraction during separation, hence whole and refined flours have higher *in vitro* starch digestibility (Oghbaei and Prakash, 2016).

2.4.2 *In vitro* protein digestibility

Interactions between proteinic and non proteinic components like starch, polyphenol, lipids, phytates affect the digestibility of dietary protein (Wong et al., 2009). Repo-Carassco-Valencia and Serna, 2011 reported 76.32 to 80.54% protein digestibility in different quinoa varieties. Presence of anti nutrients likes tannin, trypsin inhibitors, etc. impede the digestibility and solubility of protein (Pushparaj, 2011). Phytic acid, anti nutrient present in quinoa seeds is known to interfere by binding with protein and suppressing proteolysis hence, lowering protein digestibility (Cowieson et al., 2006). Protein digestibility of cereals and legumes varies with the variation in cultivar (Torres et al., 2016).

Effect of processing on in vitro protein digestibility

El-Sayed Embaby, 2010, reported 0.9 to 1.4 % increase in protein digestibility of lupin seeds after soaking. Ghavidel and Prakash, 2007 also reported 14 to 15% increase in protein digestibility of various legumes after germination. Increase in protein digestibility after soaking, germination and industrial processing may be due to decrease in anti nutrients. Nergiz and Gokgoz, 2007 reported pressure cooking as an effective method to increase protein digestibility of foods. Decrease in contents of antinutrients like tannins and phytate lead to increase in protein digestibility as otherwise they bind with endogenous proteins and make them unavailable for digestion (Blessing and Gregory, 2010). Madhu et al., 2012reported an increase in *in vitro* protein digestibility of rice to increase with increase in degree of flaking in rice. Khattab et al., 2009 reported increase in in vitro protein digestibility of soaked and fermented legumes. Rasane et al., 2015 reported improvement in protein quality of oats after germination.Raghuvanshi et al., 2011 reported 69 to 75% improved protein digestibility of dehulled and fried mung beans. Ghavidel and Prakash, 2007 reported 2.2 to 13.2% increase in protein digestibility of dehulled cow pea, green gram and chickpea. Removal of hulls and depletion of protein binding anti nutrients during dehulling leads to concentration of proteins into endosperm (Ghavidel and Prakash, 2007).

2.5 Health benefits of *Chenopodium quinoa*

Quinoa, due to its impeccable nutritional characteristics holds a great health potential (Arneja et al., 2015). Presence of high protein content and richness of bioactive compounds make quinoa an interesting dietary source with health benefits (Nowak et al., 2015).

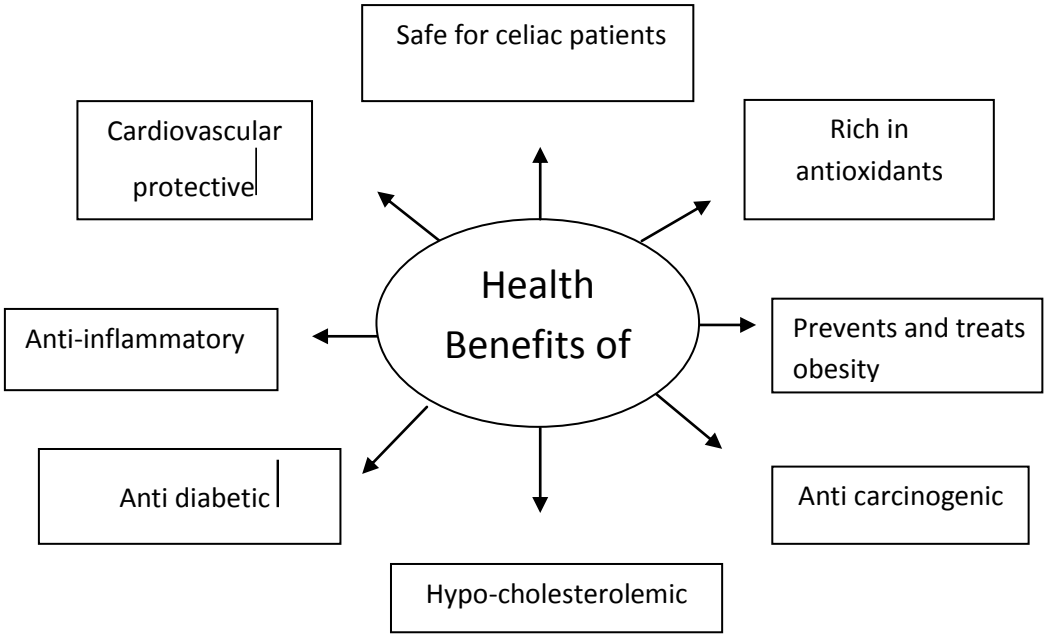

Figure 2.1: Various health benefits of *Chenopodium quinoa*

Non-detectable amount of prolamines in quinoa seed is beneficial for consumption by celiac patients as the grain consumption doesnot cause any intestinal or gastric irritation to individuals allergic to gluten (Zevallos et al., 2014). Querctin, kaempferol and rutin are most abundant phenolic compounds in quinoa (Carciochi et al., 2014b). Being rich in antioxidants quinoa is known to be helpful in various degenerative metabolic disorders like cancer, obesity, diabetes, cardio vascular diseases, etc. (Hejazi, 2016). Researches show both *in vitro* and *in vivo* evidences of health potential of quinoa. Kaur and Tanwar, 2016 revealed anti diabetic and anti hypertensive potential of quinoa beverage prepared from soaked, germinated, and malted quinoa seeds. Some of the work done to explore health benefits of quinoa in *in vitro* and *in vivo* models has been tabulated in Table 2.3 below.

Table 2.3: Health benefits of quinoa in *in vitro* and *in vivo* models.

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
1	***In vitro studies***				
	Quinoa seeds	α-amylase, α-glucosidase inhibition and angiotensin converting enzyme inhibition	Dose dependant	• Antioxidative potential • Treatement of typeII diabetes • Anti hypertensive	Ranilla et al., 2009
	Quinoa milled fractions	α-amylase, α-glucosidase inhibition	Dose dependant	• Dehulled and milled quinoa seed showed increased metal chelating activity • Bran and hull showed anti diabetic potential	Hemalatha et al., 2016
	Soaked and germinated quinoa seed (in form of beverage)	α-amylase, α-glucosidase inhibition and angiotensin converting enzyme inhibition	Dose dependant	• Anti diabetic and antihypertensive potential of malted quinoa beverages.	Kaur and Tanwar, 2016
	Quinoa flour	angiotensin converting enzyme inhibition		• Antihypertensive potential • Antioxidative potential	Asao and Watanabe, 2010

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
	Quinoa saponin fractions	Cell line (RAW 264.7)	50, 100, 200µg/ml of saponins	• Anti inflammatory potential • Inhibition of inflammatory mediators and cytokines	Yao et al., 2014 a
	Quinoa polysachharide	Cell line (RAW 264.7 cells)	10, 50, 150 200µg/ml of polysachharide	• Antioxidant • Immunoregulator	Yao et al., 2014 b
	Quinoa leaf extract	Dunning rat model (Rat prostate cancer cells)	NK	• Chemopreventie • Anticarcinogenic	Gwalik-Dziki et al., 2013
	Quinoa leaf extract	ABTS decolorization And degree of linoleic acid peroxide inhibition	NK	• Antioxidative potential	Gwalik-Dziki et al., 2013
2	***In vivo studies***				
a	Animal model				
	Milled Quinoa flour	Diet induced obese 30 male albino rats	30,40,50,60% quinoa flour incorporated in a formulation	• Increase in hemoglobin and platelets • Decrease in cholesterol, LDL • Decreased liver function parameters in rats fed with 60% quinoa	Hejazi, 2016

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
	Saponin	Mice	300µg	• Safe to consume • Non lethal	Verza et al., 2012
	Quinoa seed extract	Diet induced obese mice	250 and 500 mg/kg quinoa leachate (0.86% 20-hydroxyecdysone, 1% phytoecdysterois, 2.59%flavonoid glycoside, 11.9% oil, 20.4% protein)	• Lower fasting glucose level	Graf et al., 2014
	Quinoa seeds	Fructose fed 24 male wistar rats	310g quinoa seeds/kg fodder for 5 weeks	• Decrease in serum cholesterol • Decrease in LDL • Reduction in blood glucose levels • Inhibition of HDL decrease which was initially induced by fructose	Pasko et al., 2010 a
	Quinoa seeds	Male wistar rats	310g quinoa seeds/kg fodder for 5 weeks	• Decreased MDA (malondialdehyde) in plasma • Reduced lipid peroxidation • Enhanced antioxidant activity of body organs	Pasko et al., 2010b

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
	Quinoa seed extract	Obese male mice	Quinoa seed extract + 20 hydroxyecdysterone (6mg/day/kg body weight) for 3 weeks	• Reduction in adipose tissue development • Inhibition of lipid storage linked gene expression • Reduction in fatty acid uptake	Foucault et al., 2012
	Saponin rich quinoa seed coat	Adult male Sprague-dawley rats	25, 50,75,100µl/10^7RBC	• RBC hemoglobin release at lower concentration • Surfactant at low concentration • Disulfide reducing agent • Antioxidant potential due to presence of thiol compounds	Letelier et al., 2011
	Hydrolyzed quinoa extract	64 adult wistar rats	2000mg/kg body weight for 30 days	• Nill hepatic/renal toxicity • Decreased body weigh • Low fat accumulation and triglygeride level	Meneguetti et al., 2011
b	**Clinical trial**				
	Quinoa seeds	19 celiac patients	50g quinoa/day for 6 weeks	• Well tolerated by celiac patients • Better histology and serology relate parameters • Hypocholestrolemic	Zevallos et al., 2014

S. No.	Quinoa component	Model	Dosage	Health benefit	Reference
	Quinoa flakes	35 overweight women	25g quinoa flakes for 4 weeks	• Decrease in triglycerides and LDL • Higher level of glutathione (antioxidant)	De Carvalho et al., 2013
	Quinoa cereal bar	9 males;13 females	19.5g quinoa/day for 30 days	• Lower cholesterol, triglyceride and LDL-c • Normal urea, aspartate transaminase (AST) and alanine transaminase (ALT) levels	Farinazzi-Machado et al., 2012
	Quinoa seeds (in milk form)	12 volunteers	312.5ml	• Low glycemic index • Beneficial for diabetics	Pineli et al., 2015
	Quinoa seeds	Boys (50-65 months)	100g quinoa + baby food, twice a day, for 15 days	• Increase in plasma inulin growth factor • Potential to fight malnutrition	Ruales and Nair, 2002
	Low glycemic index diet including quinoa	Diabetic patients	210 diabetic participants	• Decreased HbA1c parameters • Increased HDL	Jenkins et al., 2008

*NK: not known

2.5.1 Hypocholesterolemic effect of *Chenopodium quinoa*

Cholesterol level in human body is mainly dependant on *de novo* synthesis (by liver), and diet intake. Cholesterol elimination process includes conversion of cholesterol to bile salts, steroids, excretion through stools etc. Liver is mainly associated with cholesterol homeostasis in body. Disturbance or interference in cholesterol homeostasis may lead to increased serum cholesterol levels, which have been known to be directly linked to cardio vascular diseases (Gunness and Gidley, 2010). Researches so far have shown positive effect of presence of dietary fibres and bioactive compounds on cholesterol (Gunness and Gidley, 2010; Thilakarathna and Rupasinghe, 2013). Being rich in dietary fiber, magnesium content, phenols, flavanoids and antioxidants quinoa is known to exhibit cholesterol lowering health potential (Pasko et al., 2010; Hejazi, 2016). Dietary fibres, mainly indigestible soluble dietary fibres, are fermented by the colon and bacteria, which results in production of short chain fatty acids (acetate, butyrate and propionate). These short chain fatty acids, mainly propionate, interefere with cholesterol metabolism in liver and result in depletion of cholesterol levels (Gunness and Gidley, 2010). In addition, high magnesium content of quinoa (250mg/100g) as compared to wheat (170mg/100g) and barley (75mg/100g) impart hypocholestrolemic effect to quinoa (Jubete et al., 2010 and Valencia-Chammaro, 2003). Magnesium influences cholesterol level by dilating the blood vessels and leading to lowering of serum cholesterol level (Nasseri and Hakemi, 2013). Quinoa has also been reported to contain low phytate (1 to 1.8g/ 100 g) content (Valencia-Chamarro, 2003). Phillippy et al., 2014 reported low phytate content of foods to be directly related to their potential health benefit in hypercholesterolemia. Flavanol glycosides, main polyphenols in quinoa have been reported to possess hypeocholestrolemic health potential (Gomez-Caravaca et al., 2011). Diadzein and genistein, the bioactive compounds in quinoa, isolated by Lutz et al., 2013, have been reported to greatly influence the lipid metabolism and have cholesterol lowering properties (Takahashi et al., 2009). Diadzein and genistin enhance translocation of fatty acid and deecreases the synthesis of Low density lipoprotein (LDL) in body (Takahashi et al., 2009). Pasko et al. 2009 reported presence of anthocyanins in coloured quinoa seeds. Anthocyanin supplementation have been reported to be beneficial in increasing serum HDL level and help in proper regulation of cholesterol homeostasis (Zhu et al., 2014).

Recently, Hejazi, 2016 reported beneficial effect of quinoa incorporation at 60% level in diet fed to 30 obese male albino rats. The study concluded hypocholestrolemic effect of quinoa incorporation. Konishi et al. 2004 reported that quinoa pericarp incorporation in diet at 3% level led to significant decrease in serum cholesterol level in mice. Takao et al., 2005 reported hypocholesterolemic effect of incorporation of quinoa protein (at 0, 2.5 and 5% level) to mice fed with 0.5 % cholesterol diet 4 weeks. Pasko et al., 2010a reported 26% lower serum cholesterol, 57% lower LDL, 11% lower triglycerides, 10% lower glucose level in high fructose fed male wistar rats after incorporation of 310g/kg quinoa seeds in rat fodder for 5 weeks. De Carvalho, 2013 reported decrease in cholesterol (191 ± 35 to 181 ± 28 mg/dl) and LDL (129 ±

35 to 121 ± 26 mg/dl) level of 35 obese women after consumption of 25g quinoa flakes in 4 week clinical trial on 35 women with weight excess who consumed 25 grams of quinoa flakes (QF) or corn flakes (CF) daily during a period of four consecutive weeks. Jenkins et al., 2008 reported increased HDL level of diabetic patients who were fed with quinoa incorporated low glycemic index diet. Farinazzi-Machado et al., 2012 reported lower cholesterol, triglyceride and LDL levels of subjects who consumed 19.5g quinoa per day in form of a quinoa cereal bar for 30 days.

2.6 Functional foods developed from *Chenopodium quinoa*

Owing to varied functional properties and an additional advantage of being a gluten free pseudocereal, quinoa has been extensively used in formulation of functional food products. Breads (fermented/steamed), biscuits, snacks, pasta, edible films, beverages, etc., are some of the recently developed food products using quinoa as an ingredient (Wang and Zhu, 2016). Quinoa being rich in dietary fiber and a good source of anti oxidants, holds a great potential in today's food industry (James, 2009). It is also an ideal candidate for inclusion in "composite flour technology" which is based on incorporation of cereal/legume/millet flour to wheat flour for extended bioavailability of nutrients (Valcarel-Yamani and Caetano, 2012). In corporation of quinoa has known to affect both, functional and nutritional properties of the resultant food product (Wang and Zhu, 2016).

Jubete et al., 2010, incorporated 50% quinoa flour (QF) in wheat flour bread formulation and revealed better nutritional and textural properties of the resultant bread. Rodriguez-Sandoval et al., 2012 demonstrated decreased volume and bulkiness of bread incorporated with 10 and 20% QF. The quality characteristics of quinoa food products largely depend on the physiochemical attributes of its protein and starch components (Kong and Bertoft, 2010). Addition of quinoa protein to film derived from chitin improved the tensile property and thermal stability of quinoa-chitosan film (Araujo-Farro et al., 2010). Modification in quinoa starch granules make it apt to be used as a stabilization agent in Pickering emulsions as well as production of edible films (Matos et al., 2013). Incorporation of quinoa to the wheat dough can modify its thermo mechanical properties (Hager et al., 2012). This so because addition of quinoa flour to gluten containing flour causes weakening of cohesive bonds in gluten matrix leading to lower springiness and cooking stability of dough (Rodriguez-Sandoval et al., 2012).

Quinoa incorporation also extends the longevity and reduces microbial spoilage of resultant food product. Hager et al., 2012 and Wolter et al., 2014 demonstrated 95 and 400% reduced staleness in quinoa flour bread as compared to the wheat flour bread, respectively. Pronged longevity of quinoa food products is due to lower degradation rate of its starch molecule (Lindeboom et al., 2005) while presence of phytochemicals like phenols inhibit mould growth in quinoa based food products (Wang et al., 2015).

Quinoa is known to add on to nutritional aspects of food products due to its markable nutritional profile (Arneja et al., 2015). Stikic et al., 2012 revealed 16% improvement in protein quality of bread incorporated with 20% quinoa seeds as compared to wheat flour bread. Chlopika et al., 2012 found increased antioxidant activity, phenolic and flavonoid content by 11, 11 and 36%, respectively upon addition of 15% quinoa flour to wheat bread.

Some quinoa functional food products have also been known to hold therapeutic potential. Pineli et al., 2015 developed protein rich quinoa milk from quinoa seeds with low glycemic index. Kaur and Tanwar, 2016 dveloped quinoa beverages from soaked, germinated, and malted quinoa seeds, which exhibited anti diabetic and anti hypertensive potential. Farinazzi-Machado et al., 2012 developed a quinoa cereal bar which exhibited hypocholesterolemic potential. De Carvalho et al., 2015 demonstrated antioxidative and cardiovascular protective potential of quinoa flakes. Some of the recently developed functional food products from quinoa and their quality characteristics have been summarized below in Table 2.4

Table 2.4: Functional foods developed from *Chenopodium quinoa*

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
1	**BREADS**				
	Non-sourdough breads				
	Wheat and quinoa bread	Wheat flour + 20% and 50% quinoa flour	100% wheat flour bread	• Decrease in loaf volume • Increase in nutritional value • Acceptable by consumers	Iglesias-Puig et al., 2014
	Wheat and quinoa bread	Wheat flour + 10 and 20% quinoa incorporation	100% wheat flour bread	• Decreased water absorption of dough • increased stability of dough • Lower weight of bread • Increased fluffiness of bread	Rodriguez-Sandoval et al., 2012
	Wheat and quinoa bread	Wheat flour + 15 and 30% quinoa incorporation	100% wheat flour bread	• Inrease in total phenolic content, total favonoid content and antioxidant activity • Better sensory characteristics	Chlopika et al., 2012
	Wheat and quinoa bread	100% quinoa flour	100% wheat flour bread	• Decrease in dough development height • Decrease in springiness • Increase in chewiness • Decrease in slice area	Hager et al.,2012
	Wheat and quinoa bread	80, 85, 90, 100% Wheat flour + 10,15 and 20% quinoa flour	100% wheat flour bread	• Increase in proximate and mineral content • Better sensory attributes (sensory score: 5 to 4.98 on nine point hedonic scale)	Stikic et al., 2012
	Wheat and quinoa bread	Wheat flour + 50 and 100% quinoa seeds	100% wheat flour bread	• Increase in antioxidant activity	Jubete et al., 2010

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
	Wheat and quinoa bread	Wheat flour + 6% quinoa seed	Wheat flour+6% flaxseed	• Reduction in levels of saturated fatty acids • Reduction in levels of trans fatty acids.	Calderilli et al., 2010
	Quinoa malt incorporated bread	1,2.5,5% quinoa malt + 50% rice flour + 50 % potato flour	Oat malt bread	• No significant effect on baking properties • No effect on bread density	Manikandan et al., 2013
	Quinoa and wheat bread	40% wheat flour + 15 % quinoa + 15% buckwheat + 5% pumpkin seed kernel	Wheat bread	• Increased protein, fat and fiber content • Higher energy value • Better sensory value	Milovanivic et al., 2014
	Sourdough breads				
	Wheat and quinoa bread	50% quinoa flour + 50% wheat flour	Wheat flour bread	• Increased elasticity • Decreased bake loss • Increase moisture content • Decreased slice area	Wolter et al., 2014
	Quinoa	quinoa flour + buckwheat + amaranth + chickpea flour	Wheat flour bread	• Decrease in specific volume • Increased hardness • Decreased springiness	Coda et al., 2010
2	**Snacks**				
	Quinoa and corn snack	20% quinoa flour+80% corn flour	100% corn flour snack	• More dietary fiber • Increased hardness • Lower moisture content	Martin and Diaz , 2013
	Quinoa and cashew snack	100% quinoa flour+ 12.5% whey protein concentrate +12.5% cashew pulp	Quinoa fried snack	• decreased water absorption index on adding whey protein concentrate • decreased moisture content increased volume expansion	Onwulata et al., 2010

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
	Quinoa cookie	100% quinoa flour	100% Wheat flour cookies	• increased chewiness • increased overall acceptability	Harra et al., 2011
	Quinoa cake	25, 50, 75, 100% quinoa flor	100% wheat cake	• increased water absorption and dough weakening • decreased stability • good quality cake with equal substitution of wheat and quinoa flour	Atef et al., 2012
3	**Pasta/Spaghetti**				
	Quinoa flour Pasta (taglaitell)	69.7,69.8, 69.9% quinoa flour + 5,10, 15% quinoa starch)	semolina	• no difference in rheological properties of quinoa dough	Chillo et al., 2009
	Pre-gelatinized quinoa flour spaghetti	53.3% quinoa flour and 20% quinoa starck	100% quinoa flour spaghetti	• increased dough gelatinization • improved colour • no difference in sensory attributes	Chillo et al., 2010
	Quinoa pasta	20% amaranth flour + 20% quinoa flour + 60% buckwheat flour	20% amaranth flour + 20% quinoa flour + 60% buckwheat flour + egg white	• better agglutination • increased cooking stability • decreased cooking loss	Schoelencher et al., 2010

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
4	**Breakfast cereal**				
	Quinoa flakes	Wheat flour + rice flour + quinoa flour	Wheat flour + rice flour flakes	• No significant difference in gamma-amino butyric acid level • Increased glutamic acid level after roating	De Calvalho et al., 2015
5	**Quinoa edible films**				
	Quinoa starch film	100% Quinoa starch + glycerol	-	• Peelable • Colourless • Transparent and Smooth surface • Best mechanical properties • Homogenous	Araujo-Farro et al., 2010
	Quinoa protein film	Quinoa protein + chitosan	Chitosan film	• Increased thichkness • Good tensile strength • Thermal stability	James, 2009
	Antimicrobial biofilm	Quinoa starch + gold nanoparticles		• Perfect for food packaging • Protective potential against *E.coli* and *S. aureus*	Pagno et al., 2015

S No.	Product	Quinoa incorporated product formulation	Control product	Quality attributes of quinoa incorporated product	Reference
6.	**Emulsion stabilizer**				
	Stabilizer in Pickering emulsion	88.2% octenyl succinic anhydride + 1.8% Quinoa starch	-	• Efficient for encapsulation • Potential to be used used in formulation of food products with reduced fat content	Matos et al., 2013
7	**Beverages**				
	Quinoa milk	White quinoa:water (1:7)	Rice milk	• Increased protein content • Low glycemic index • Sensory acceptance at par with rice milk	Pineli et al., 2015
	Quinoa liquid suspension	White/ red quinoa: water (1:10)	Oat milk	• White quinoa was best variety for quinoa beverage preparation • Smooth beverage • Good in taste • Perfectly gelatinized	Thuresson, 2015
	Fermented quinoa beverage	100,70,50,30 % quinoa extract	Soy extract	• 70% soy and 30% quinoa extract was most acceptable. • Quinoa fermented beverages with >30% quinoa extract were less preferred as compared to the soy extracts	Bianchi et al., 2015

CHAPTER 3

RESEARCH METHODOLOGY

This chapter refers to the research design of this study. It explains in detail the methods used to accomplish the specified research objectives.

3.1 MATERIALS

3.1.1 Sample collection

To conduct this study Indian white C*henopodium quinoa* grains "Royal White", grown under project "Anantha," were procured from TSIPARD (Telangana State Institute of Panchayat Raj and Rural Development), Hyderabad, Telangana, India. The American white *Chenopodium quinoa* grains "Royal White", imported from Bolivia, South America, were procured from Devshree grains and pulses, New Delhi, India.

3.1.2 Sample processing

To debitter the raw white Indian *Chenopodium quinoa*, the grains were subjected to domestic processing methods viz. soaking and germination to obtain debittered *Chenopodium quinoa* seeds. The industrially processed grains refer to the product as available in the market for consumer consumption after undergoing industrial processes for debittering of quinoa.

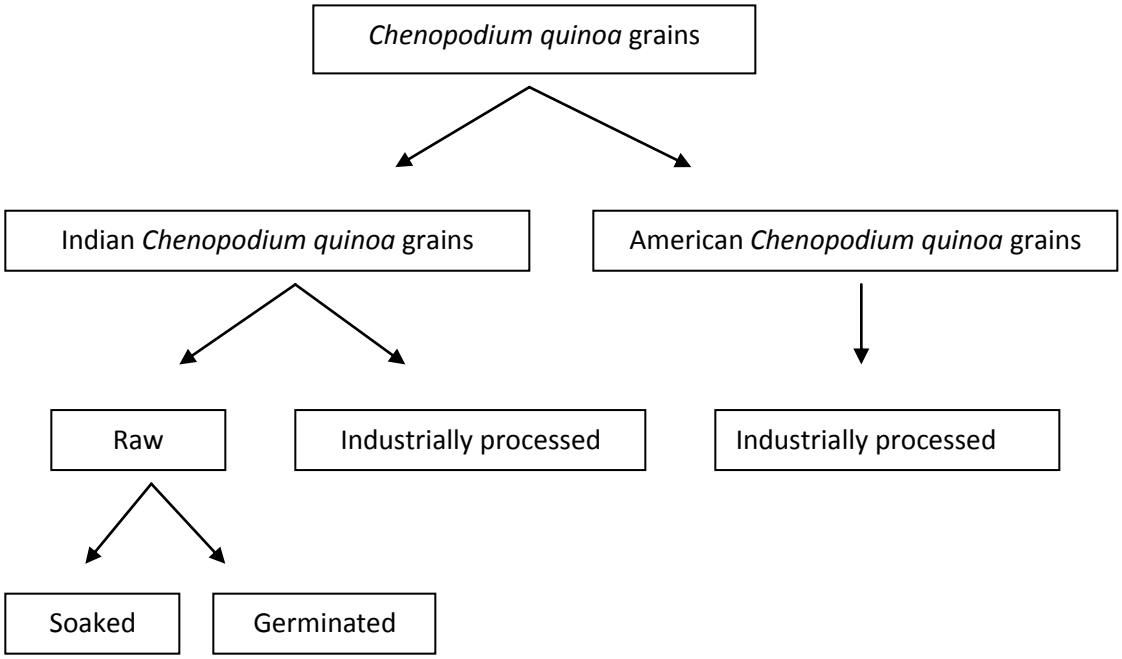

Figure 3.1: Processing of *Chenopodium quinoa* grains

a) Soaking

Raw Indian *Chenopodium quinoa* seeds were soaked for 24 hours in deionized water (1:5 *w/v*) obtained through Millipore (Merck-Milli-Q® Direct 8 Water Purification System, USA). Water used for soaking was changed thrice at regular interval of 8 hours. After 24 hours, the water was discarded and seeds were washed once again with the de ionized water. Soaked seeds were further dried in vacuum drying oven at 40±5°C. Dried seeds were ground to finely powdered flour with a laboratory grinder (Philips HL1606/03 500 W Mixer Grinder). Soaked quinoa seed flour was stored at 4°C for further analysis.

b) Germination

Raw Indian *Chenopdium quinoa* seeds were thoroughly washed with and soaked in de ionized water (1:5 *w/v*) obtained through Millipore (Merck-Milli-Q® Direct 8 Water Purification System, USA) for 24 hours. Seeds were then spread on to petri dishes covered with autoclaved filter paper and incubated at 20°C in an incubator (Biotechnics, India) for 72 hours (Carciochi et al., 2014a) for development of sprouts. Water was changed every 8 hours. Germinated seeds were then dried in vacuum drying oven at 40±5°C. Dried germinated seeds were ground to flour with a laboratory grinder (Philips HL1606/03 500 W Mixer Grinder). Finely powdered germinated quinoa seed flour was stored at 4 °C for further analysis.

3.2 METHODS

Objective 1: To evaluate the nutritional quality of *Chenopodium quinoa.*

Under this objective, nutritional quality of Indian and American *Chenopodium quinoa* was assessed and compared. The sub parts of this objective were:

A) Nutritional evaluation of bittered and debitterd (domestically and industrially processed) Indian *Chenopodium quinoa* seeds

B) Comparison of nutritional quality of industrially processed Indian and American *Chenopodium quinoa* seeds

To accomplish this objective of the study, the samples were analyzed for their proximate, nutritional, and phytochemical composition. Also the *invitro* analysis was carried out to determine antioxidant activity, starch and protein digestibility of samples.

3.2.1 Proximate analysis of *Chenopodium quinoa*

Moisture, crude protein, crude fat, crude fiber and crude ash content were determined according to standard methods given by Association of Official Analytical Chemists AOAC, 2000. The samples were analyzed in triplicates.

3.2.1.1 Moisture content

Moisture content of samples was determined by drying the sample at 105°C until a constant weight was observed. 2g of sample was taken in a previously weighed crucible (W_c). The crucible containing the sample (W_{cs}) was then subjected to drying in oven at 105°C. At an interval of every 4 hours, the sample was taken out of the oven with the help of a pair of tongs. It was then immediately put in a dessicator to avoid moisture (present in air) absorption by the sample and was allowed to cool. The crucible containing the sample after oven drying was reweighed (W_{csd}). The readings were noted and the process was repeated until a constant weight of crucible containing the sample was obtained. Moisture content in 100g of sample was calculated using following formula

$$\text{Moisture content} = \frac{(W_{cs} - W_c) - (W_{csd} - W_c)}{(W_{cs} - W_c)} \times 100$$

Where:

W_c : Weight of emplty crucible

W_{cs} : Weight of crucible + sample prior to drying

W_{csd} : Weight of crucible + sample after drying

3.2.1.2 Crude ash

Crude ash content of samples was determined by placing 2 g of sample in muffle furnace at 550°C for 6 hours. Ash content in 100 g of sample was calculated using following formula

$$\text{Crude ash content} = \frac{W_{ca} - W_c}{W_{cs} - W_c} \times 100$$

Where

W_{ca} : Weight of crucible with ash

W_c : weight of empty crucible

W_{cs} : weight of crucible with sample

3.2.1.3 Crude protein

Kjeldahl method was employed to determine the crude protein content of samples. The analysis involves 3 processes viz. digestion, distillation, and titration. 5g of sample was digested in 10 ml sulphuric acid for about 3 hours to obtain a clear solution. Kjeldahl distillation unit was set up and clear liquid obtained was distilled by adding 40% concentrated sodium hydroxide solution in 50 ml water. The process results in liberation of ammonia which was then collected over 25 ml boric acid solution containing indicator (bromo cresol green and methyl red). It was then titrated against 0.05N sodium hydroxide solution. Blank was prepared similarly without using the sample. Quantification of crude protein content was done by converting nitrogen to protein using conversion factor of 6.25. Crude protein content in 100g of sample was calculated using following formula

$$\text{Crude protein content} = \frac{V_s - V_b}{W_s} \times 14 \times 6.25 \times 100$$

Where,

V_s: Titration volume of sample

$V_{b:}$ Titration volume of blank

14: Molecular weight of nitrogen

6.25: Nitrogen to protein conversion factor

3.2.1.4 Crude fat

Soxhlet method was employed for determination of crude fat content of samples. About 2g of moisture free sample put in a thimble. Petroleum ether (50ml), used as fat extraction solvent was taken in round bottom flask. Soxhlet apparatus was set up. Crude fat content in 100g of sample was calculated using following formula

$$\text{Crude fat content} = \frac{W_{fr} - W_{ir}}{W_{ts} - W_t} \times 100$$

Where

Wt = weight of empty thimble

Wts = weight of thimble + sample

Wir = initial weight of round bottom flask

Wfr = final weight of round bottom flask

3.2.1.5 Crude fiber

Acid base digestion method was employed for determination of cruder fiber content of samples. A 2g of sample was boiled in 0.1M HCl and then treated with 0.3M sodium hydroxide. The samples were then put in muffle furnace at 550°C for 5 hours. Crude fiber content in 100g sample was calculated using following formula

$$\text{Crude fiber content} = \frac{W_{cr} - W_{cs}}{W_s} \times 100$$

Where

W_s = weight of sample

W_{cs} = weight of crucible with ash

W_{cr} = weight of crucible with residue after acid base digestion

3.2.1.6 Carbohydrate

Carbohydrate content in samples was measured using anthrone method. A 100 mg of sample was hydrolyzed with 5ml 0g 2.5N HCl and placed in a boiling water bath for 2 hours. The solution was then neutralized with sodium carbonated till the effervescence ceased. Further, the volume make was done upto 100 ml using distilled water. The resultant solution was centrifuged and standards were prepared using 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard, where 0 served as blank. The volume was further made upto 1 ml in all tubes and 4 ml of ice cold anthrone reagent was added. The solution was further heated for eight minutes in a boiling water bath, cooled rapidly and green colored solution was read at 630 nm. Glucose solution was used as a standard.

3.2.2 Nutritional analysis of *Chenopodium quinoa*

3.2.2.1 Dietary Fiber

Dietary fiber constituents include Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Lignins, Cellulose and Hemicellulose.

a) Neutral detergent Fiber (NDF)

Neutral Detergent Fibre in samples was estimated by the method as suggested by Van Soest and Wine (1967)

Reagents

Neutral Detergent Solution

Sodium borate decahydrate	: 6.18 g
Sodium Lauryl Sulphate	: 30.0 g
2- ethoxy ethanol	: 10.0 ml
Disodium ethylene diamino tetra acetate (EDTA)	: 18.16 g
Water	: 1.0 litre
Disodium Hydrogen Phosphate	: 5.0 g

EDTA and sodium borate decahydrate was weighed together in a large beaker and water was added. The solution was heated till the contents dissolved. Then sodium lauryl sulphate was added to this solution. In a separate beaker disodium hydrogen phosphate was weighed and dissolved in remaining water by heating. The solution was then added to the beaker containing other ingredients and volume was made to 1ltr in a volumetric flask.

Procedure

Air-dried sample (in triplicate) was weighed 500 mg and transferred in to beakers of the refluxing apparatus. To this 100 ml neutral detergent solution was added and heated to boiling. As it will start boiling, the heat was reduced to avoid foaming and allowed to reflux for 60 minutes. The solution was filtered through a weighed gooch crucible with minimum of hot water. Then it was washed with acetone in the same manner. The crucible was dried in hot air oven at 100^{o} C for 8 hours and weighed after cooling.

Calculation

$$\text{NDF (\%)} = \frac{(\text{Wt. of crucible + fiber content}) - \text{Wt. of crucible}}{\text{Weight of sample (g)}} \times 100$$

b) Acid Detergent Fiber (ADF)

Acid Detergent Fibre in samples was estimated on the basis of the method as suggested by Van Soest and Wine (1967).

Reagents

Acid Detergent Solution

Cetyl trimethyl ammonium bromide (CTAB) : 20.0 g 1 N H_2SO_4

Add 20.0 g of CTAB to 1N H_2SO_4 to make total volume of one liter and stir.

Procedure

Air-dried sample was weighed 500mg and transfered in to a beaker of the refluxing apparatus and 100 ml of acid detergent solution was added to it. Mixture was heated to boiling ,refluxed for 60 minutes and was filtered through weighed Gooch crucibles. The samples was rinsed in to crucibles with minimum of hot water (90 to 100^o C). Liquid was filtered and washed repeated. Finally the residue was washed twice with acetone in the same manner. All the lumps was broken, so that the solvent can come in to contact with all particles of the fiber. Acid detergent fiber was dried at 100^o C for 8 hours in hot air oven and weighed.

Calculation

$$\text{ADF (\%)} = \frac{(\text{Wt. of crucible + Fiber content}) - \text{Wt. of crucible}}{\text{Weight of sample (g)}} \times 100$$

Hemicellulose

Hemicellulose was calculated using following formula

Hemicellulose = NDF- ADF

3.2.2.2 Vitamin C

N- bromosuccinimide (NBS) method for determination of vitamin C as given by Barakat et al.1955 and Miranda et al. (2010) was used for determination of vitamin C in quinoa samples. Slight modifications were made in analysis accordingly. The method includes preparation of standard ascorbic acid solution, standardization of NBS with ascorbic acid and estimation of ascorbic acid in sample extract. a) Preparation of standard ascorbic acid: Standard ascorbic acid of concentration0.4mg ml^{-1} was prepared by dissolving 200mg ascorbic acid in 500 ml distilled water. b) Standardization of NBS Solution: Standard ascorbic acid solution (20ml) was added to a flask containing 4 ml of 4% potassium iodide solution (KI), 1.6 ml of 10% acetic acid (CH_3COONa), 4 drops of 1% starch (used as an indicator) and 25 ml distilled water. It was then titrated with NBS (0.2mg ml^{-1}). Appearance of permanent blue colour was considered as end point of titration. c) Estimation of ascorbic acid in sample: Quinoa extracts, acidified with 0.4 g oxalic acid was added to a flask containing 10 ml of 4% potassium iodide solution (KI), 4 ml of 10% acetic acid (CH_3COONa), 4 drops of 1% starch (used as an indicator) and 40 ml distilled water. Final vitamin C content was expressed as mg 100^{-1} using following equation:

$$\text{Vit C content} = \frac{\text{concentration of standard ascorbic acid solution} \times \text{volume of NBS corresponding to quinoa extract (ml)}}{\text{volume of NBS corresponding to std ascorbic acid solution(ml)} \times \text{Sample mass (g)}}$$

3.2.2.3 β-carotene

β carotene in the samples was estimated by the method of Ranganna, (1995).

Reagents

Petroleum ether B.P. (60-80^{o}C)

Acetone

Procedure

One gram sample was extracted with petroleum ether (60-80^{o}C) and acetone (3:2) by grinding with sand in 50 ml silica dish with a glass mortar. Extract was decanted in to a 50 ml volumetric flask and extraction was continued 4-5 times till all fat-soluble pigments was completely dissolved. Volume was adjusted to 50 ml and absorbance was read at 450 nm against a suitable blank. The results was expressed in terms of beta- carotene.

Calculation

$$\beta \text{ Carotene } (\mu g/ 100g) = \frac{\text{O.D.} \times 13.9 \times 10^4 \times 100}{\text{Wt of sample} \times 560}$$

3. 2.2.4 Mineral content

Mineral content of samples was estimated by digestion in Diacid (Piper, 1966)

Reagents

Diacid mixture (25ml) of nitric acid and perchloric acid in the ratio of 3:1 was used for digesting the samples, taking care to prepare the reagent fresh before use.

Procedure

1 g of sample was digested with 25 ml of diacid mixture in a conical flask (100-250 ml). The contents were kept overnight for cold digestion and then heated at low temperature on a hot plate till about clear, colourless liquid with precipitate was left. The contents were allowed to cool and then transferred with deionized water into a 50

ml volumetric flask after repeated washing and then volume was made up to the mark. The digested samples were filtered through Whatman paper No.42 filter paper and stored in the decontaminated dried labeled air tight plastic bottles for the mineral determination. For blank, 25 ml of diacid mixture was digested as in case of sample and volume was made to 50 ml with deionized water.

The digested samples were analysed for iron by using atomic absorption spectrophotometer while calcium, magnesium and zinc were analyzed by using flame photometer

3.2.3 Phytochemical analysis of *Chenopodium quinoa*

3.2.3.1 Phytic Acid

Phytic acid was estimated in the samples by the method of Haugh and Lantzch 1983.

Reagents

Phytate reference solution: Sodium phytate (30.54 mg) was dissolved in 100 ml of 0.2 N HCl, which will give a solution containing 200 ml phytic acid per ml.

Ferric ammonium sulphate solution: Ferric ammonium sulphate (0.2 g) was dissolved in 100 ml 2N HCl and made to 1000 ml with water.

Bipyridine solution: 2', 2' bi-pyridine (10 g) was and 10 ml thioglycollic acid was dissolved in water and made to 1 litre. The solutions are stable for several months at room temperature.

Extraction

Finely ground sample (1g) was extracted with 25 ml of 0.2 N HCl for three hours with continuous shaking in a shaker. After proper shaking, it was filtered through Whatman No.1 filter paper and volume was made 25 ml with 0.2 N HCl.

Estimation

Above mentioned extract (0.5ml) was pipetted into a test tube fitted with a glass stopper and 1 ml of ferric ammonium sulphate solution was added. Tube was heated and centrifuged for 30 minutes at 3,000 rpm. One ml supernatant was transferred to another test tube and 1.5 ml bipyridine solution was added. The absorbance was read at 519 nm against distilled water. For plotting a standard curve different concentration i.e. 0.2 to 1.0 ml of standard sodium phytate solution containing 40-200 µg phytic acid was taken and made to 1.4 ml with water. 0.5 OD will correspond to 120 µg phytic acid.

Calculation

$$\text{Phytic acid (mg/100g)} = \frac{\text{Reading of Graph X} \quad \text{ml of volume made}}{\text{Weight of sample taken X} \quad \text{ml of aliquot taken}} \text{ X } 100$$

3.2.3.2 Total phosphorous

Total phosphorus concentration was determined spectrophotometrically by the molybdo-vanadate reagent after ashing of the sample with HCL according to AOAC, 1995.

Phytate phosphorus was derived by using the following formula:

Phytate phosphorus, mg = A X 28.18/ 100

Where A = the phytate content (mg)

Non-phytate Phosphorus

Non-phytate Phosphorus was calculated as the difference between Total Phosphorus – Phytate Phosphorus.

3.2.3.3 Oxalates

Oxalate was estimated by the method of Day and Underwood 1986.

To 1 g of the ground powder, 75 ml of 15 N H_2SO_4 was added. The solution was carefully stirred intermittently with a magnetic stirrer for 1 hr and filter using Whatman No. 1 filter paper. 25 ml of the filtrate was then collected and titrated against 0.1 N $KMnO_4$ solution, till a faint pink color appears that persists for 30 seconds.

3.2.3.4. Tannins

Sample (100 mg) was mixed with 40 ml distilled water. The suspension was then boiled for 30 min cooled and subsequently centrifuged at 2000 rpm for 10 min and used as a source for tannin estimation. Tannins were estimated using Folin-Denis reagent. After extraction, 1 ml of the clear supernatant was used as a source of tannins and to this 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solution were added followed by dilution to 100 ml with water. The tubes were incubated at room temperature for 30 min and the color thus developed was read at 700nm using a spectrophotometer.

3.2.3.5 Alkaloids

Five grams of the dried powder of each sample was weighed into a 250 ml beaker and 200 ml of 10 per cent acetic acid in ethanol was added. The mixture is covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath until it reaches to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated.

3.2.3.6 Saponins

Twenty grams of sample was placed into a conical flask and 100 ml of 20 per cent aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml 20 per cent ethanol. The combined extracts was reduced to 40 ml over water bath at 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts was washed twice with 10 ml of 5 per cent aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples was dried in the oven to a constant weight and saponin content was calculated as percentage.

3.2.3.7 Trypsin Inhibitor Activity

Trypsin inhibitor activity in the samples was determined by the method given by Roy and Rao 1971.

Reagents

0.1 M phosphate buffer (pH 7.6): Sodium dihydrogen phosphate (16 ml) (0.2 M) and 84 ml Disodium hydrogen phosphate (0.2 M) was diluted to 200 ml with distilled water and pH was adjusted to 7.6.

0.05 M phosphate buffer (pH 7.0): 50 ml of 0.1 M phosphate buffer was diluted to 100 ml with distilled water and the pH adjusted to 7.0.

2 % casein: A suspension of 2 g casein was prepared with phosphate buffer (0.1 M, pH 7.6) and dissolved by warming on steam bath for 10 minutes. The cooled solution was made to 100 ml with phosphate buffer and stored in the refrigerator.

Trypsin solution (5 mg/ml): 125 mg Trypsin (20,000 F gross units/g) was dissolved in 20 ml phosphate buffer (0.1M, pH 7.6)

Trichloroacetic acid solution (5 %): 5 g TCA was dissolved in water to make 100 ml.

Procedure

Extraction

To 5 g sample taken in conical flask add 25 ml of 0.05 M phosphate buffer (pH 7.0). The contents was shaken for 3 hrs and centrifuged for 10,000 rpm for 20 minutes. The following sets of incubation mixture was prepared.

Phosphate	Test	Control	Blank
Buffer (0.1M pH 7.6)	0.1 ml	1.1 ml	1.0 ml
Trypsin (5 mg/ml)	0.5 ml	0.5 ml	0.5 ml
HCl (0.001N)	0.4 ml	0.4 ml	0.4 ml
TCA (5 per cent)	-	-	6.0 ml
Casein (2 per cent)	2.0 ml	2.0 ml	2.0 ml
Extract incubated at 37 ^{o}C for 20 minutes	1.0 ml	-	0.1 ml
TCA (5 per cent)	6.0 ml	6.0 ml	-

Protein Determination

After incubation the contents was centrifuged at 10,000 rpm for 10 minutes. The TCA soluble proteins was determined by the method of Lowry et al. (1951).

Reagents

A: 2 % Na_2CO_3 in 0.1 N NaOH

B: 0.5 % copper sulphate in 1% sodium citrate solution

Alkaline copper sulphate solution: 50 parts of solution A and 1 part of solution B was mixed just before use.

Folin phenol reagents: as indicator

Determination: To 0.5 ml of supernatant 5 ml alkaline copper sulphate solution was added, mixed thoroughly and allowed to stand for 10 minutes at room temperature. After this, 0.5 ml Folin phenol reagent (double diluted the original reagent) was added

and immediately mixed. Water blank was also run side by side. The color intensity was read after 30 minutes at 520 nm.

For preparing standard curve 0.1 ml of 0.5 ml casein solution (400 µg/ml) was taken.

Trypsin inhibitor units: One unit of trypsin was defined as amount of enzyme which converted 1mg casein to TCA soluble component at 37^0 C for 20 minutes at 7.6 pH. One unit of inhibitory activity is that which reduces the activity of trypsin by one unit under assay condition.

3.2.3.8 Total phenolic content

Total phenolic content (TPC) was assessed according to method described by Ainsworth and Gillespie, (2007) using Folin-ciocalteau reagent. Sample extract (0.5ml) was diluted and volume was made upto 1ml. After 2 minutes 2ml 0f 10% folin-ciocalteau reagent and vortex thoroughly. At sixth minute, add 8 ml of 700mM Na_2CO_3 and incubate the mixture for 2 hours. Transfer 2 ml of mixture to quartz cuvette and read the absorbance at 765nm using spectrophotometer (Shimadzu, Japan).The readings were compared to gallic acid standard curve (linearity range 50-250 mg/ml and R^2=0.991). Final total phenolic content expressed as mg of gallic acid equivalent (GAE) per 100 g i.e. mg GAE/100g

3.2.3.9 Total flavonoid

Total flavonoid was determined according to procedure followed by Carciochi et al., 2014a. Quinoa extract (0.5ml) was taken in a test tube. To the test tube 4 ml of distilled water and 0.5 ml of 20% NaNO2 (Sodium nitrite) was added. Mixture was allowed to stand for about 5 minutes and then 0.3ml of 10% $AlCl_3$ (Aluminium Chloride) was added. After 1 minute 0.5 ml 0f 2M NaOH (Sodium hydroxide) was added to the reaction mixture. Absorbance was read at 510nm using spectrophotometer (Shimadzu, Japan). Quercitin was used as standard. Results were expressed as mg of quercitin equivalent/100g i.e. mg QE/100g.

3.2.4 *In vitro* tests

3.2.4.1 Protein *in vitro* digestibility

Digestibility of proteins was determined by an *in vitro* method according to Hsu et al., 1977. The multi-enzymatic method is based on the decrease of pH during 10 minutes. The percentage of digestibility was calculated using the Equation:

$$Y = 210.464 - 18.103\,X \quad (3)$$

Where: X = pH of the protein suspension after 10 minutes of digestion and Y = percentage of protein hydrolysis.

3.2.4.2 *Starch in vitro digestibility*

The digestibility of starch was determined by an *in vitro* method according to Holm et al. (1985). Starch (500 mg) was mixed with phosphate buffer (pH 6.9) and incubated with α-amylase at 37 °C for 1 hour. The sugars released was determined by spectrophotometry.

3.2.4.3 *In vitro* antioxidant activity

In vitro antioxidant activity of samples was determined by following 2 methods:

a) 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) assay

DPPH i.e. 2,2,-diphenyl-2-picryl-hydrazyl assay was proceeded according to method followed by Jubete et al. (2010b) and Sun and Ho, (2005) with some modification. Aliquots of quinoa extract, in increasing trend, (i.e. 100, 200, 400, 800, 1000 µl) were taken for serial dilution. Diluted quinoa extracts (1 ml) from each serial dilution was added to cuvettes respectively. DPPH solution of concentration 200µM (absorbance 1.4) was freshly prepared and 1 ml of this solution was added to each cuvette containing quinoa extract. The mixture was vortexed and incubated in dark for 30 minutes. Absorbance was measured at 517 nm using spectrophotometer (Shimadzu, Japan). DPPH was expressed as mgTE/100g. Trolox (0.02M) was used as standard.

b) Ferric reducing ability of plasma (FRAP) assay

FRAP i.e. Ferric reducing ability of plasma assay was proceeded according to procedure followed by Jubete et al., 2010b and Benzie and Strain, 1996 with some modification. FRAP reagent was prepared by mixing 2.5 ml of 0.01M TPTZ in 0.04M hydrochloric acid, 2.5ml of 0.02M ferric chloride and 25 ml of 0.3M sodium acetate buffer (pH 3.6). Quinoa extracts (100µl-300µl) and 2ml of FRAP reagent was taken in a 5ml volumetric flask. Distilled water was used to make up the volume. Solutions were kept in dark at 37°C for 60 minutes. Absorbance was read at 595nm using spectrophotometer (Shimadzu, Japan). Trolox stock solution of 0.02M was used as standard for the assay FRAP reagent (2ml), made up to 5 ml in a volumetric flask was used as blank.

3.2.4.4 Statistical analysis

The experiments were performed in triplicates and the data was expressed as mean±standard deviation. The data was analyzed on Microsoft office excel, 2007 and Graphpad prism 5 software (La jolla, CA, USA). Means were compared using one way analysis of variance (ANOVA) followed by Tukeys multiple comparison test for comparison between means. The values were considered significant at $p \leq 0.05$.

Objective 2: To assess cholesterolemic effect of *Chenopodium quinoa*

To accomplish objective two of the study was accomplished by a biological trial using animal model

3.2.5 Biological trial

Forty two (42) adult male albino rats, wistar strains, weighing 252± 5 g, aged 28 days, were housed individually in stainless steel mesh cages. They were fed on standard diet, AIN 93G for 10 days before experiments begun (Adaptation period). The Institutional Animal Ethics Committee at Lovely Professional University approved the protocols for animal experiments.

The diets were prepared every week in the laboratory. The water and diets was given *ad libitum*. Induction of hypercholesterolemia was carried out on 4 groups (G2-5; 24). The animals (42) were divided into 7 groups (6 rat/ group) as follows:

Table 3.1: Experimental grouping for biological trial

Group 1	Rats fed on basal diets (Control group, negative control)
Group 2	Hypercholesterolemic rats (positive control)
Group 3	Hypercholesterolemic rats fed on basal diet supplemented with hypocholesterolemic drugs (Statin)
Group 4	Hypercholesterolemic rats fed on basal diet supplemented with Chenopodium (Unwashed)10%
Group 5	Hypercholesterolemic rats fed on basal diet supplemented with Chenopodium (debittered) 10 %
Group 6	Normal rats fed on basal diet supplemented with Chenopodium (Unwashed) 10 %
Group 7	Normal rats fed on basal diet supplemented with Chenopodium (debittered) 10 %

The standard atrovastatin tablets were procured from Lupin Pharmaceuticals Limited, Tarapur, India. At the end of the experimental period (6 weeks after treatment), rats were fasted over night before the blood was collected. The blood was collected in

tubes coated with EDTA through retro orbital puncture. It was then centrifuged; serum was separated and stored at -80°C until analysis.

3.2.5.1 Blood Lipid Profile

The serum total cholesterol (TC), serum high density lipoprotein cholesterol (HDL-C) level, and serum triacylglycerol (TG) level were determined using colorimetric enzymatic kits.

The VLDL+LDL were calculated by an equation reported previously by Ibrahim et al., 2005:

VLDL+LDL cholesterol = total cholesterol-HDL cholesterol.

The atherosclerotic index was calculated by an equation reported previously by Ibrahim et al., 2005:

Atherosclerotic index = (VLDL+LDL-cholesterol)/ (HDL-cholesterol)

3.2.5.2 Statistical analysis

The experiments were performed in triplicates and the data was expressed as mean±standard deviation. The data was analyzed on Microsoft office excel, 2007 and Graphpad prism 5 software (La jolla, CA, USA). Means were compared using one way analysis of variance (ANOVA) followed by Tukeys multiple comparison test for comparison between means. The values were considered significant at $p \leq 0.05$.

Table 3.2 Composition of Experimental Diets (g/100 g)

Ingredients	Control diet	Control diet with cholesterol	Control diet with cholesterol and statin	Control diet with cholesterol and raw *Chenopodium quinoa*	Control diet with cholesterol and debittered *Chenopodium quinoa*	Control diet with raw *Chenopodium quinoa*	Control diet with debittered *Chenopodium quinoa*
Casein	10	10	10	9.38	9.28	9.38	9.28
Ground nut oil	10	10	10	9.78	9.82	9.78	9.82
Sucrose	10	10	10	10	10	10	10
Cellulose	5	5	5	4.64	4.68	4.64	4.68
Mineral mixture	4	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1	1
Cholesterol	0	1	1	1	1	0	0
Starch	60	59	59	55.2	55.2	56.2	56.2
Quinoa	0	0	0	5	5	5	5

* All diets contained 10% protein including the crude protein from C*henopodium quinoa*
** All diets contained 10% fat including the crude fat from C*henopodium quinoa*
*** All diets contained 5% fibre including the crude fibre from *Chenopodium quinoa*

Table 3.3 Composition of Mineral Mixture (AIN 93G)*

Ingredients	g/kg mineral mixture
Calcium Carbonate	357
Sodium chloride	74.00
Potassium citrate monohydrate	70.78
Potassium sulphate	46.6
Magnesium oxide	24.00
Ferric citrate	6.06
Magnesium carbonate	0.63
Zinc carbonate	1.65
Cupric carbonate	0.30
Potassium iodate	0.01
Sodium selenite pentahydrate	1.45
Chromium Potassium sulphate dodecahydrate	0.275
Sucrose	221.02
Boric acid	0.08
Sodium fluoride	0.06
Nickel carbonate	0.03
Ammonium vandate	0.006

*Based on the National Academy of Science recommended levels for rats (BARR committee on Animal Nutrition, 1972)

Table 3.4 Composition of Vitamin Mixture (AIN 93G)*

Ingredients	g/kg vitamin mixture
Thiamine hydrochloride	0.6
Riboflavin	0.6
Pyridoxine hydrochloride	0.7
Nicotinic acid	3.0
Calcium pantothenate	1.6
Folic acid	0.2
Biotin	2
Retinol acetate	0.8
Cholecalciferol	1
D-alpha tocopherol	To provide 5000 IU of Vitamin E activity
Cyanocobalamine	2.5
Sucrose	To make 1000 g

*Based on the National Academy of Science recommended levels for rats (BARR committee on Animal Nutrition, 1972)

Objective 3: To develop and analyze value added gluten free products from *Chenopodium quinoa*

To accomplish objective three functional foods, namely, quinoa bar, quinoa cracker and quinoa beverages were prepared from quinoa grains and evaluated for proximate composition, total phenolic content and antioxidant activity. Sensory evaluation and storage studies of functional food products developed in this study was also done. Beverages prepared from quinoa were analyzed, additionally, for pH, viscosity, total soluble solids, and serum separation.

3.2.6 Development of functional food products

3.2.6.1 Development of snack Bar

Quinoa snack bar was prepared according to method followed by Sharma and Mridula, 2015, with slight modifications.

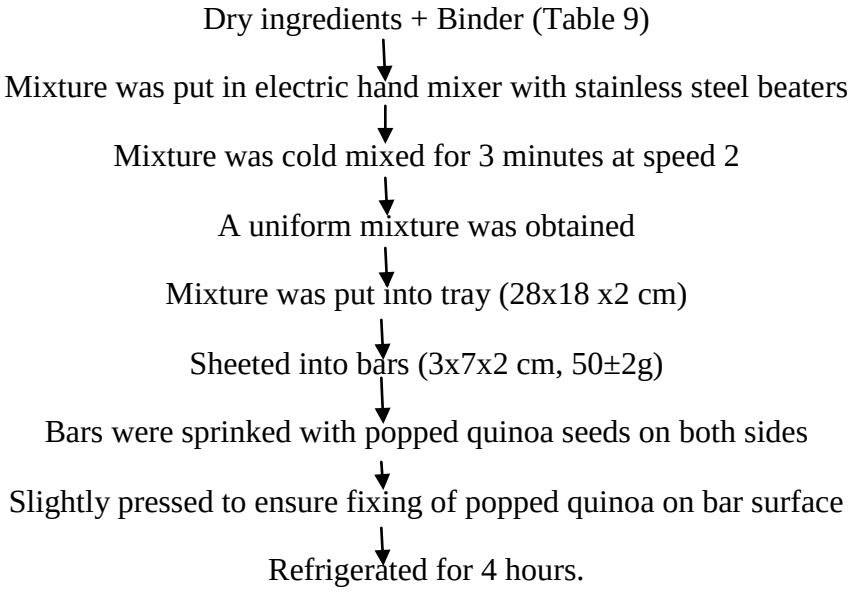

Figure 3.2: Flowchart for method of preparation of quinoa cereal bar

The prepared snack bars were analysed for

a) **Proximate compostion** as described in 3.2.1

b) **Total phenolic content** as described in 3.2.3.8

c) **Antioxidant activity** as described in 3.2.4.3

d) **Sensory evaluation**:

Sensory evaluation was carried out by serving bars to 20 untrained panel of judges for evaluating different sensory attributes like appearance, color, texture, flavor, mouth feel, taste and overall acceptability on a 9-point Hedonic scale grading 9 for extremely like and 1 for extremely disliked samples.

e) Storage study: Formulated snack bars were carefully packed in zip lock bags and stored in refrigerator for 30 days. Bars were evaluated for different parameters (a to e) at an interval of 15 and 30 days

Table 3.5: Proportion of dry ingredients and the binders used for preparation of snack bars

Ingredients	Quantity (%)	
	Control Bar	Quinoa Bar (QB)
Dry Ingredients		
Oat flakes	15	15
Roasted chickpea flour	50	25
Germinated quinoa flour	-	20
Coco powder	5	5
Sugar	5	5
Popped quinoa seeds	-	5
Binders		
Honey	10	10
Vegetable oil	10	10
Water	5	5

3.2.6.2 Development of crackers

The crackers were prepared according to the method described in table below

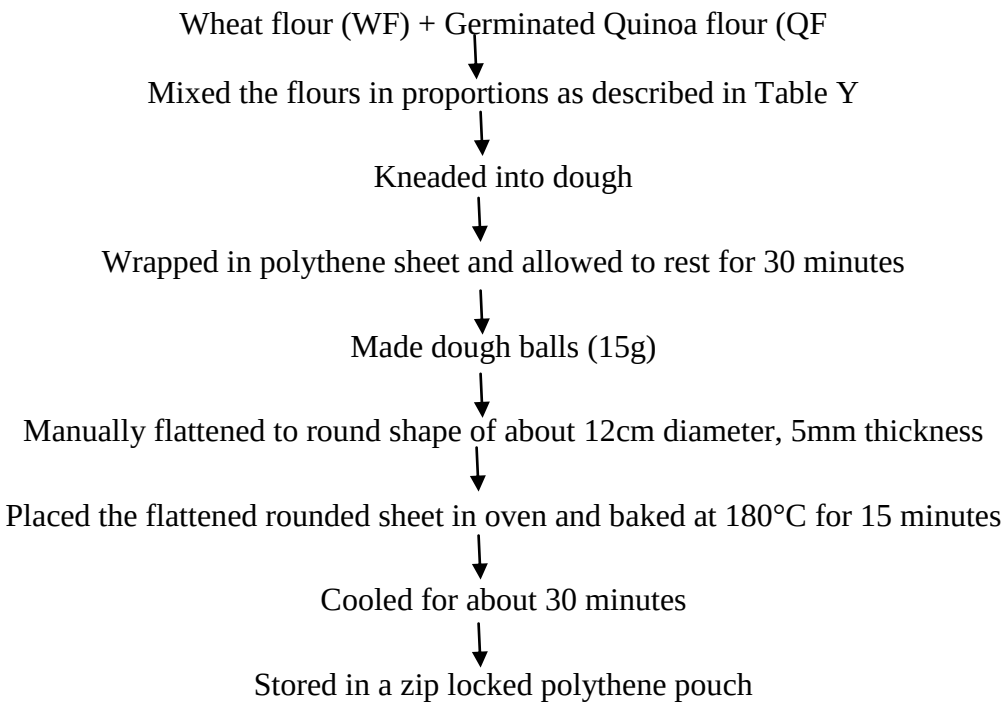

Figure 3.3: Flowchart for method of preparation of cracker (*Khakhra*) incorporated with 20 and 40% quinoa flour

Table 3.6: Basic formulation of quinoa cracker using all ingredients

Ingredients (g)	T_0 (Control)	T_1 (80:20)	T_2 (60:40)
WF	150	120	90
QF	0	30	60
Cumin seeds	5	5	5
Salt	5	5	5
Oil	3	3	3
Turmeric Powder	1.5	1.5	1.5
Baking Powder	2	2	2
Water (ml)	40	40	40

Table 3.7: Substitution ratio of wheat flour and quinoa flour for preparation of quinoa cracker (*Khakhra*)

Sample	Wheat flour (WF) (%)	Quinoa flour (QF) (%)
Control (T_0)	100	0
Quinoa cracker (T_1)	80	20
Quinoa cracker (T_2)	60	40

The prepared crackers were analysed for

a) **Proximate compostion** as described in in 3.2.1

b) **Total phenolic content** as described in 3.2.3.8

c) **Antioxidant activity** as described in 3.2.4.3

d) **Sensory evaluation**:

Sensory evaluation was carried out by serving crackers to 20 untrained panel of judges for evaluating different sensory attributes like appearance, color, texture, flavor,

mouth feel, taste and overall acceptability on a 9-point Hedonic scale grading 9 for like extremely and 1 for disliked extremely samples.

e) Storage study:

Formulated snack bars were carefully packed in zip lock bags and stored in refrigerator for 30 days. Bars were evaluated for different parameters (a to e) at an interval of 15 and 30 days

3.2.6.3 Development of Beverages

Basic grain treatment prior to beverage preparation

Clean *Chenopodium quinoa* seeds were soaked for 10 minutes in 10% sodium hypochlorite solution (NaOCl) for surface sterilization and the washed with distilled water obtained from milli pore (Merck-Milli-Q® Direct 8 Water Purification System). The seeds were then steeped in 0.03 mol/L sodium chloride and maintaining pH 5.0 for better protein yield as described by Pineli et al., 2015. Further raw were used as such, soaked, and germinated for production of their respective quinoa beverage.

Soaked quinoa seeds: Quinoa seeds were soaked in milli Q water for 24 hours at room temperature. Water was changed every 8 hours. Soaked seeds were further processed on the same day for beverage preparation.

Germinated quinoa seeds: Quinoa seeds soaked in milli Q water for 24 hours, were spread on to petri dishes layered with filter paper dipped in 3ml distilled water and incubated at 20°C in an incubator (Biotechnics, India) for 72 hours (Carciochi, 2014b). Water was changed and checked for dryness every 6 hours. Germinated seeds were further processed on the same day for beverage preparation.

Preparation method for raw, soaked and germinated quinoa beverages

Basic process for preparation of raw, soaked, and germinated beverages was followed according to Ma et al., 2015 with some modifications. Flowchart representation of the preparation process has been described in Figure 1. All quinoa beverages were stored at 4°C for further analysis.

The prepared beverages were analysed for

a) **Physical analysis**

pH was measured using a digital pH meter at 20°C.Total soluble solids were measured according to method followed by Kim et al., 2012. Viscosity was measure using a rotational viscometer (Cole-Parmer Basic Viscometer, Cole-Parmer India Pvt. Ltd,

India). To determine the effectiveness of xanthum gum, serum separation was assessed using a graduated cylinder (of volume 50 ml) according to method used by Koksoy and Kilic, (2004).

b) **Proximate compostion** as described in in 3.2.1

c) **Total phenolic content** as described in 3.2.3.8

d) **Antioxidant activity** as described in 3.2.4.3

e) **Sensory evaluation**

Beverages were assessed for organoleptic acceptance using a nine point hedonic scale, from extremely dislike to extremely like. The samples were randomly marked and served at room temperature in white paper cups to a semi-trained panel of 20 members. Commonly available commercial soya milk (Sofit natural unflavored soya milk, Hershey India Pvt. Ltd.) was used as a reference beverage to valuate acceptance. Panellists were asked about their favourite and least favourite beverage and also about positive and negative sensory aspects of each beverage.

CHAPTER 4

RESULT AND DISCUSSION

The results of the data collected for the study on nutritional evaluation of quinoa, its cholesterolemic effect in wistar rats and development of functional food products, were statistically analyzed, critically discussed, and presented in this chapter.

Objective 1: To evaluate the nutritional quality of *Chenopodium quinoa*

A) Nutritional evaluation of bittered and debittered (domestically and industrially processed) Indian *Chenopodium quinoa* seeds

4.1 Proximate Analysis of Indian *Chenopodium quinoa*

4.1.1 Moisture content

The moisture content of raw and processed quinoa seeds is shown in Table 4.1. The raw seeds were reported to have 11.30±0.08 g/100g of moisture content. The result was in agreement with the values reported by Nascimento et al., 2014. A significant ($P<0.05$) difference in moisture content of raw, domestically processed and industrially processed seeds was observed. Soaking resulted in increase in 8.5% of moisture content while germination resulted in decrease in moisture content by 17.5%. Similar trend of variation in moisture content after soaking and germination of chickpea has been reported by Desalegn, 2016. The increase in moisture content after soaking may be due to uptake of water by dry seed resulting in cell hydration and cell multiplication within the seed (Nonogaki et al., 2010) while decrease in moisture content on germination may be attributed to utilization of water in synthesis of metabolites (Chung et al., 2014). Industrial processing of quinoa led to 8.5% decrease in moisture content. This may be due to removal of hulls, during the process. Chauhan, 1992 reported 11.3% moisture content in quinoa hulls which account for about 8% of total seed weight. Lower moisture content is indicator of longer product shelf life (Sanni et al., 2006). Thus, the results suggest better shelf life of germinated quinoa seeds.

Table 4.1: Proximate composition of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		**Moisture (g/100g)**	**Carbohydrate (g/100g)**	**Crude fat (g/100g)**	**Crude protein (g/100g)**	**Ash (g/100g)**	**Crude fibre (g/100g)**
Raw		11.30±0.08[a]	65.11±0.12[a]	5.17±0.18[a]	12.54±0.03[a]	3.19±0.03[a]	2.62±0.01[a]
Domestically Processed	Soaked	12.26±0.34[b]	64.01±0.36[b,d]	4.29±0.28[b]	13.18±0.03[b, e]	3.21±0.16[a, c]	2.9±0.05[b]
	Germinated	9.29±0.15[c]	63.78±0.32[c, d]	3.9±0.04[c,b]	14.96±0.04[c]	3.92±0.05[b, d]	3.3±0.03[c]
Industrially processed		10.35±0.20[d]	64.90±0.09[a]	5.11±0.05[a]	13.11±0.08[d,e]	3.04±0.03[a]	2.49±0.04[d]

4.1.2 Crude Ash

The ash content of raw and processed quinoa seeds is shown in Table 4.1. The raw seeds were reported to have ash content of 3.19±0.03 g/100g which is similar to the ash content reported by Miranda et al., 2012 in different varieties of *Chenopodium quinoa* seeds. Soaking resulted in 0.6% increase in ash content which was statistically non significant ($P<0.05$) with respect to ash content of raw quinoa seeds while significant ($P<0.05$) increase in ash content, by 22.5%, was observed after germination. This may be due to decrease in carbohydrate (0.3 to 2%) and crude fat (15.7 to 32.5%) content of processed seeds which can be accounted for increase in ash content (Chaudhary and Vyas, 2014). Inayang and Zakari, 2008 stated activation of fitase on germination, resulting in hydrolysis of protein-enzyme bond and hence release of minerals as possible cause for increase in ash content on germination. Industrial processed seeds were reported to have 4.7% reduced ash content. The apparent reason behind decreased ash content can be removal of hulls which accounts for 8.5% of total ash content in quinoa seeds (Chauhan et al., 1992).

4.1.3 Crude protein

Crude Crude protein content of quinoa seeds is shown in Table 4.1. Raw quinoa seed was reported to have 12.54±0.03g/100g crude protein content. The result is similar to the protein content of unprocessed quinoa (12.06 g/100g) reported by Coehlo et al., 2007 and protein content of Moroccon quinoa (12.5g/100 gm) reported by Marmouzi et al., 2015. Crude protein content of raw, domestically processed and industrially processed quinoa seeds was significantly ($P<0.05$) different. Soaking and germination led to increase in protein content by 4.8% and 19.2%. Increase in protein content after soaking was also reported in soyabeans by Kayembe and Rensburg, 2013. Nutritive value of cereals is known to enhance after germination (Hubner and Arendt, 2013). Moongngarm and Sateung, 2010 reported 29% increase in protein content of germinated rice. The result is also supported by findings of Inyang and Zakari (2008) in germinated peal millet. This increase in protein content may be due to increased activity of protease leading to degradation of peptides to amino acids and further synthesis of new protein (Laetitia et al., 2014). Increase in protein content of industrially processed quinoa seeds was similar to the increase reported after soaking. As most protein content

of quinoa is located in embryo (Prego et al., 1998), increased protein content of industrially processed seeds as compared to raw seeds may be due to removal of hulls and concentration of protein in embryo (Ghavidel and Prakash, 2007).

4.1.4 Crude fat

Crude fat content of Indian quinoa is shown in Table 4.1. Raw Indian quinoa had 5.17±0.18 g/100g crude fat content which falls within the range of fat content for various quinoa varieties reported by Miranda et al., 2013. Soaking and germination of raw seeds caused significant ($P<0.05$) decrease in fat content by 20.5% and 32.5%. Similar decrease in fat content has been reported in germinated sesame seeds by Kajihausa et al., 2014. Seed growth, because of water imbibition by cells on soaking, consumes required energy from fat, a major carbon source in seeds, which may lead to decrease in fat content after soaking (Rumiyati et al., 2012). Germination of seeds leads to metabolite synthesis. This metabolic change requires energy which is liberated by oxidation of fatty acid resulting in reduced fat content in germinated seeds (Hahm et al., 2009). Industrially processed seeds had 1.6% reduced fat content as compared to the raw seeds. This may be due to removal of hulls which contain 5.7% of crude fat. No significant ($P<0.05$) difference was observed in change in fat content of soaked, germinated and industrially processed quinoa seeds. The results reveal chances of good oil yield from raw quinoa seeds as compared to soaked, germinated and industrially processed seeds.

4.1.5 Crude fiber

The crude fiber content of raw and processed quinoa seeds is shown in Table 4.1. Raw quinoa seeds had crude fiber content of 2.22±0.01 g/100g. The fiber content of all quinoa seeds were significantly ($P<0.05$) different. Soaking led to 31.8% increase in fiber content and germination caused further increase by 13.7%. The findings are supported by increase in fiber content on germination of chickpea, cowpea and mungbean as reported by Uppal and bains, 2012. Synthesis of insoluble fibers, which are constituents of cell wall, namely cellulose and hemicelluloses may be the cause for increase in fiber content after germination (Pandey and Awasthi, 2013). Industrial processing led to 7.6% decrease in fiber content. Chauhan et al., 1992 reported hulls to account for 5.6% fiber content in

quinoa seeds. Decrease in ash content may be attributed to the fact that industrial processing leads to removal of hulls, resulting in decreased fiber content of seeds. Decrease in fiber content after germination has also been reported by Blessing and Gregory, 2010.

4.1.6 Carbohydrate

The carbohydrate content of quinoa seeds is shown in Table 4.1. Carbohydrate content of raw quinoa seeds was 65.11±0.12 g/100g, which is in accordance with the value reported reported by Marmouzi et al., 2015. The results were significantly ($P<0.05$) different between raw and domestically processed seeds. However no significant difference ($P<0.05$) in carbohydrate content of seeds subjected to soaking and germination was observed. Soaking and germination resulted in decrease of carbohydrate content by 1.6% and 2% respectively. This decrease may be due to activation of α-amylase in quinoa seeds and breakdown of starch to simple sugars on hydration during soaking and germination (Rosa et al., 2009). Although non significant ($P<0.05$) but 0.3% decrease in carbohydrate content of industrialy processed seeds was observed. Also, the carbohydrate content of industrially processed and domestically processed seeds was significantly ($P<0.05$) different. Industrial processing of grains leads to removal of hulls (Slavin, 2003). Chauhan, 1992 reported that quinoa hulls account for 55% carbohydrate content. Hence lower carbohydrate content of industrially processed seeds may be because of removal of hulls. Decrease in carbohydrate content after dehulling was also reported by Makinde and Akinoso, 2013.

4.2 Nutritional analysis of Indian *Chenopodium quinoa*

4.2.1 Dietary fiber

Total dietary fiber content of quinoa seeds is shown in Table 4.2 . Raw quinoa seeds were reported with 10.26±0.17 g/100g of dietary fiber content. The results are similar to the dietary fiber content in quinoa seeds as reported by Lamothe et al., 2015. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) constituted about 71.9 to 73.3% and 26.6 to 28% of total dietary fiber, respectively. The results correspond well to the findings of Marmouzi et al., 2015 who reported 72.03% NDF and 27.06% ADF in Moroccan quinoa seeds. Different processing methods have different effect on dietary fiber content of foods

(Dhingra et al., 2012). Domestic processing of seeds i.e. soaking and germination, led to 10% and 31% significant increase ($P<0.05$) in TDF content of quinoa seeds, respectively. Both the constituents of dietary fiber i.e. NDF (Lignin, cellulose and hemicellulose) and ADF increased upon soaking and germination. Increase in dietary fiber contents upon soaking and germination has also been previously reported in cereals and legumes (Vasishtha and Srivastava, 2013, Benitez et al., 2013 and Megat et al., 2016). The increased content may be due to enlargement of cell body and growth initiation upon water imbibition during soaking and germination (Martin-Cabrejas et al., 2003). Industrially processed seeds exhibited 9.9%, significant decrease ($P<0.05$) in TDF content. Pushparaj and Urooj, 2011, have also reported detrimental effect of industrial processing on dietary fiber in pearl millet.

4.2.2 Vitamin C

The vitamin C content of Indian quinoa seeds is shown in Table 4.2. Vitamin C content of raw quinoa seeds was found to be 13mg/100g, which is within the range of vitamin c content as reported by Miranda et al., 2010 (12-23mg/100g) and is close to the vitamin C content of Cahuil variety (13.8 mg/100g) among six chilean quinoa ecotypes studied in his study. The value reported in this study is greater than the values reported by Koziol, 1992 (4mg/100g) and less than as reported by Ruales and Nair, 2002 (16.4 mg/100g), Miranda et al., 2013 (22-31 mg/100g) in two quinoa genotypes from Temuco and Vacuna localities in Chile. This difference may be due to different environmental and storage conditions, as factors like light intensity, amount of nitrogen fertilizers, frequency of irrigation and temperature of the region strongly affect the vitamin C content in crops (Lee and Kader, 2000).

Also there is significant difference in vitamin C content of raw and domestically processed Indian quinoa seeds ($P<0.05$). As depicted in this study, vitamin C content increased by 15% in soaked quinoa seeds and by 46% in germinated quinoa seeds. Higher increase in germinated seeds observed might be due to synthesis of vitamin C during the process of germination (Sattar et al., 1995 and Fernandez-Orozco et al., 2006). Tang et al., 2015 reported vitamin C content in sprouted mungbean where as nil vitamin C content was reported in raw seeds, which confirms vitamin C synthesis during germination process.

Table 4.2 Nutritional composition of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		**Dietary fiber (g/100g)**						**β- Carotene (µg/100g)**	**Vitamin C (mg /100gm)**
		NDF	**ADF**	**Lignin**	**Cellulose**	**Hemi-cellulose**	**Total dietary fibre**		
Raw		7.38±0.07	2.88±0.02	2.4±0.04	3.6±0.03	1.38±0.01	10.26±0.17[a]	535.9±3.6[a]	13.43±0.4[a]
Domestically Processed	**Soaked**	8.24±0.11	3.05±0.02	2.6±0.04	3.9±0.03	1.74±0.03	11.29±0.23[b]	536.4±2.07[a]	15.09±0.17[b]
	Germinated	9.87±0.06	3.59±0.03	2.8±0.02	4.2±0.06	2.87±0.02	13.46±0.19[c]	540.6±1.5[a]	19.38±0.28[c]
Industrially processed		6.69±0.03	2.50±0.07	2.2±0.02	3.3±0.04	1.19±0.05	9.24±0.21[d]	535.6±2.8[a]	9.45±0.35[d]

NDF: Neutral Dtergent Fiber; ADF: Acid Detergent Fiber

Increased vitamin C content in germinated Indian quinoa seeds is also supported by the findings of Khattak et al., 2007 where linear relationship was observed between germination time and content of vitamin C in chickpea seeds. In addition, a significant difference in Vitamin C content of raw and industrially processed seeds was also observed. Industrial processing decreased the vitamin C content by 30%. De hulling, pearling, shelling etc are post harvest industrial treatments applied to cereal grains, which lead to loss of their nutritional content (Singh and Jambunathan, 1990).

4.2.3 β- Carotene

The β- Carotene content of quinoa seeds is shown in Table 4.2. Raw quinoa seeds were reported to have 535.9±3.6 µg/100g of β- Carotene content. The results are in accordance with the β- Carotene content in *Chenopodium quinoa* as reported by Sharma et al., 2012. Domestic and industrial processing of seeds led to non-significant change ($P<0.05$) in contents of β- Carotene. Soaking and germination led to 0.1% and 0.8% increase in β- Carotene, respectively. Increase in β- Carotene upon soaking and germination has also been reported by Luthriya and Singh, 2014 and Suryanti, 2016. Lee et al., 2013 also repored increased β- Carotene contents in soyabean sprouts as compared to the seeds. This may be attributable to the fact that β- Carotene content in cereals and pulses is directly proportional to the growth progression in the seed (Ahn et al., 2012). Industrially processed seeds exhibited almost similar content of β- Carotene as compared to the raw seeds.

4.2.4 Mineral Content

Mineral content of Indian quinoa seeds is reported in Table 4.3 . Minerals like calcium, iron, zinc and magnesium were assessed in raw, domestically processed and industrially processed Indian quinoa seeds. Raw quinoa seeds were reported with 85.3±0.25 mg/100g calcium content. The results are in accordance with the values of calcium content (44 to 110 mg/100g) in quinoa seeds reported by Miranda et al., 2013 and Nascimento et al., 2014. Domestic processing of seeds i.e. soaking and germination led to 0.59% and 0.94%, non-significant increase ($P<0.05$) in calcium content, respectively, which is supported by findings of Chaparro et al., 2011. Hahm et al., 2009 also reported 0.7% increase in calcium

content of sesame seeds after germination. The iron content in raw Indian quinoa seeds was 5.2±0.01 mg/ 100g. The results are in agreement with the findings of Nascimento et al., 2014 who reported 5.4 g/100g iron content in quinoa seeds. A significant increase (P<0.05) of 1.9% and 13.4% was observed in quinoa seeds subjected to domestic processing methods i.e. soaking and germination, respectively. Chaparro et al., 2011 also reported a similar post germination increase of 11.4% in iron content of quinoa seeds. The apparent reason, for increase in calcium and iron content, may be the decrease in phytic acid content post domestic processing as reported in this study. Phytic acid is known to bind with minerals to form insoluble mineral-phytate complexes and thus, making them less bio-available for proper utilization in body (Coulibaly et al., 2010).

Table 4.3: Mineral content of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		**Calcium (mg/100g)**	**Iron (mg/100g)**	**Zinc (mg/100g)**	**Magnesium (mg/100g)**
Raw		85.3±0.25 a	5.2±0.01 a	6.6±0.04 a	182.4±0.11 a
Domestically Processed	**Soaked**	85.8±0.70 a	5.3±0.02 b	5.9±0.12 b	180.2±0.14 b
	Germinated	86.1±0.05 a, b	5.9±0.01 c	5.7±0.06 c	179±0.16 c
Industrially processed		85.06±0.15 a, c	5.1±0.02 d	6.5±0.13 a	182.2±0.15 a

The zinc content of raw quinoa seeds was 6.6±0.04 mg/100g. The results lie within the range of zinc content in quinoa seeds (2.9 to 9.5 mg/100g) reported by Miranda et al., 2013 and Nascimento et al., 2014. Zinc content was significantly reduced (P<0.05) by 10.6% and 13.5% after soaking and germination, respectively. Afiffy et al., 2012 also reported 14% and 20% reduction in zinc content of sorghum seeds post soaking and germination. Raw quinoa seeds were reported to have magnesium content of 182.4±0.11 mg/100g. The values are consistent with the magnesium content in quinoa seeds (176 to 192 mg/100g) reported by Miranda et al., 2010, Gonzales Martin et al., 2014 and Marmouzi et al., 2015. Domestic processing methods like soaking and germination led to 1.21% and 1.8% significant decrease (P<0.05) in magnesium content,

respectively. Decrease in magnesium content after germination has also been reported in black beans by Sangronis and Machado, 2007. The apparent reason behind decrease in zinc and magnesium contents may be lixiviation of minerals into soaking media during domestic processing of seed.

Overall, industrially processed seeds exhibited lower mineral content as compared to the raw seeds. A non-significant reduction ($P<0.05$) was observed in calcium (0.3%), zinc (1.5%) and magnesium (0.1%) content of industrially processed seeds while a significant depreciation ($P<0.05$) was observed in iron (2%) content. Konishi et al., 2004, in their study related to depiction of mineral distribution in quinoa stated an industrial processing technique, abrasion, as a potent cause of calcium loss from quinoa seeds, as the latter is located in pericarp, which is usually removed during the process. The study also supports non-significant reduction in magnesium content as the latter is located in the embryo of the seeds and hence, least affected by the industrial processing methods. The observed reduction in zinc and iron contents of industrially processed seeds are supported by the findings of Pal et al., 2016 who reported losses in Zn and Fe content of dehulled horsegram.

4.3 Phytochemical analysis of Indian *Chenopodium quinoa*

4.3.1 Phytic acid

The phytic acid content of raw and processed quinoa seeds is shown in Table 4.4. Raw quinoa seeds were reported to have 1.25±0.22 g/100g phytic acid. The results were similar to the phytic acid content reported by Ruales and Nair, 2002 (1g/100g) and Valencia-Chamorro, 2003 (1.18 g/100g). Soaking resulted in reduction of phytic acid content by 2.5%, which was statistically non-significant ($P<0.05$) as compared to raw seeds. In quinoa seeds, phytic acid is present in seed coat as well as the embryo (Konishi et al., 2004). The reason behind the decrease in phytic acid content after soaking may be leaching of the same in the soaking media (Vadivel et al., 2011). Liang et al., 2008, also reported reduction in contents of phytic acid in brown rice upon soaking. Germination led to significant decrease ($P<0.05$) in phytic acid content by 68%. The present results are supported by findings of Ibrahim et al., 2002, who reported reduction in phytic acid content of cereals after germination. The decrease may be due to increased activity of enzyme, phytase, upon germination, which hydrolyzes phytic acid to release

phosphorous (Kumar et al., 2013). Pal et al., 2016 have also reported decrease in phytic acid content of horsegram after germination. Industrial processing led to non- significant decrease ($P<0.05$) in phytic acid content by 3.2% which might be due to removal of seed coat during the process. Decrease in phytic acid content after industrial processing has also been reported by Ghavidel and Prakash, 2007.

4.3.2 Total phosphorous

Total phosphorous content of quinoa seeds is indicated in Table 4.4. Total phosphorous content of raw Indian quinoa seeds was 0.43±0.22 g/100g. The result is in accordance with Rosero et al., 2013 who reported 0.44-0.5g/100g total phosphorous content in four different varieties of quinoa seeds. Soaking resulted in a non-significant increase ($P<0.05$) in total phosphorous content by 7%. The result coincides with the non-significant reduction ($P<0.05$), in phytic acid content of quinoa seeds as reported earlier. Hydration of seeds leads to activation of enzyme phytase and thus the release of inorganic phosphorous as consequence of phytic acid degradation (Kumar et al., 2013). The results are in accordance with the findings of El-Hady and Habiba, 2003. Germination of quinoa seeds was observed to cause a significant increase ($P<0.05$) in total phosphorous content. The results correlate well with the significant decrease ($P<0.05$) in phytic acid content as reported earlier, based on the fact that increased activity of phytase during germination, breaks down phytic acid to release phosphorous (Sung et al., 2005). Increase in total phosphorous content of cereals upon germination has also been reported by Azeke et al., 2011. Konishi et al., 2004 reported that about 60% of phytic acid is localized in embryo of quinoa seeds, and is a major indicative of phosphorous. As phytic acid disintegrates upon soaking and germination release of in organic phosphorous results in increase of total phosphorous. Industrially processed seeds exhibited 4.6% increase in total phosphorous content, which was non-significant ($P<0.05$) as compared to the total phosphorous content of raw seeds. This may be due to decrease in phytic acid content as reported earlier. The results infer better phosphorous bioavailability of germinated quinoa seeds because of increased phytase activity and phytic acid breakdown (Baruah et al., 2007).

Table 4.4: Phytochemical composition of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		Total phosphorous (g/100 g)	Phytic acid/ Phytate (g/100g)	Phytate phosph-orous	Non phytate phosphorous	Saponin (g/100g)	Trypsin Inhibitor Activity TIU/100g	Oxalates (g/100g)	Alkaloids (g/100gm)	Tannins (g/100g)
Raw		0.43±0.22[a]	1.25±0.22[a]	0.35	0.8	2.01±0.15[a]	6633±7.5[a]	0.2±0.02 [a]	2.11±0.01[a]	0.6±0.08[a]
Domestically Processed	**Soaked**	0.46±0.06[a]	1.22±0.14[a]	0.34	1.94	1.52±0.19[b]	6321±10[b]	0.14±0.03[b]	2.09±0.04 [a,c]	0.33±0.03[b]
	Germinated	0.68±0.07[b]	0.40±1.21[b]	0.11	3.41	0.03±0.01[c]	5116.3±5.6[c]	0.09±0.02 [c]	1.78±0.00 [b,c]	0.23±0.03[b]
Industrially processed		0.45±0.01[a]	1.21±0.31[a]	0.26	1.62	0.06±0.02[d, c]	5254.6±11.5[d]	0.18±0.04 [d]	1.9±0.02 [b,c]	0.35±0.0[b]

significant decrease ($P<0.05$) in phytic acid content by 3.2% which might be due to removal of seed coat during the process. Decrease in phytic acid content after industrial processing has also been reported by Ghavidel and Prakash, 2007.

4.3.3 Oxalates

Total oxalate content of quinoa seeds is shown in Table 4.4. Total oxalate content of raw Indian quinoa seeds was 2.11±0.01 g/100g. Results are in agreement with Siener et al., 2006, who reported 1.8g/100g oxalate content in quinoa seeds. All the processing methods led to reduction in oxalate content. Soaking and germination resulted in 30% and 55% decrease in oxalate content. Savage and Dubois, 2006 also reported 25% decrease in oxalate content of Taro leaves after soaking. This may be due to presence of 71% (131mg/100g) of soluble oxalates quinoa with respect to total oxalate content (182 mg/100g) in quinoa seeds (Siener et al., 2006), which may leach out upon soaking and germination. Industrially processed seeds were also reported with 10% reduction in oxalate content. The results are in agreement with findings of Makinde and Akinoso, 2013 who reported decrease in oxalate content of dehulled sesame seeds.

4.3.4 Tannin

Tannin content of quinoa seeds is depicted in Table 4.4. Raw quinoa seeds were reported to have 0.6±0.08g/100g of tannin content. The findings are close to the results reported by Valencia-Chamorro, 2003 (0.53g/100g). Soaking and germination led to 50% and 66.6% decrease in tannin content. Khandelwal et al., 2010 stated soaking and germination as effective domestic processing methods to reduce tannin contents in legumes. The decrease might be attributed to leaching out of tannins in soaking media during soaking. Megat and Azrina 2012 also reported 58% decrease in tannin content of germinated peanuts. Germination triggers disintegration of tannin-protein-enzyme-mineral complex (Echendu et al., 2009) which might cause decrease in tannin content. Industrially processed seeds were reported to have reduction in tannin content similar to the reduction observed after soaking. This might be due to removal of hulls during industrial processing which also account for tannin content in quinoa seeds (Valencia-Chammaro, 2003).

4.3.5 Alkaloids

Total alkaloid content of quinoa seeds is shown in Table 4.4. Total alkaloid content of raw Indian quinoa seeds was 2.11±0.01 g/100g. Sanchez et al., 2004, have also reported similar results for total alkaloid content in lupin seeds. Genus *Chenopodium* has been reported to contain tropane, piperidine and pyridine alkaloids (Kokanova-Nedialkova et al., 2009). Dini et al., 2005 reported presence of five betaines in *Chenopodium quinoa.* Soaking and germination led to 1% and 15%, significant decrease ($P<0.05$) in alkaloid content of quinoa. This might be due to solubility of betaine (an alkaloid present in quinoa) in polar solvents such as water (Wang et al., 2012). The result is in agreement with reported decrease in alkaloid content upon soaking and germination in pigeon pea and lupin seeds, respectively (Sanchez et al., 2002). Industrialy processed seeds exhibited 10% decrease in alkaloid content.

4.3.6 Saponins

Saponin content of quinoa seeds is shown in Table 4.4. Raw quinoa seeds were reported to contain 2.01±0.15 g/100g saponin content. The results are supported by findings of Valencia-Chamorro, 2003 who reported 0.1 to 5 g/100g saponin content in bitter varieties of quinoa. Soaking resulted in significant decrease ($P<0.05$) of 24% in saponin content. The present result is supported by the findings of Nwosu, 2010 who reported 25% decrease in saponin content of bean after soaking for 24 hours. Adekanmi et al., 2009 and Mittal et al., 2012 have also reported decrease in saponin content after soaking in tigernut and chickpea, respectively. As saponin is located in outer covering of quinoa seeds (Chauhan et al., 1999), the decrease may be due to leaching of saponin in water during soaking period. Germination led to 98% decrease in saponin content. The saponin content reported after germination (0.03±0.01 g/100g) was within the range of saponin content of sweet quinoa varieties (0.02 to 0.05 g/100g) as reported by Mastebroek et al., 2000. Thus, germination can be a preferred method to debitter raw quinoa seeds. Lorenz and Nayanzi, 1989 also stated saponin lowering effect of wet processing methods in quinoa seeds. Industrially processed seeds exhibited 97% reduced saponin content as compared to the raw seeds. Riechert et al., 1986 also reported decrease in saponin content after industrial processing of quinoa. Ridout et al., 1991 also reported similar effect of wet processing and industrial processing

on saponin content in quinoa seeds. The reason behind reduction in saponin content after industrial processing may be removal of outer coat, containing 34% saponins (Chauhan et al., 1992), during the process. With respect to the germinated seeds, although the reduction was statistically non-significant ($P<0.05$) but germinated seeds were reported to have 50% lower saponin content than industrially processed seeds. Thus, the results demonstrate the efficacy of domestic processing, mainly germination, over industrial processing in removal, saponin, the major anti nutrient present in quinoa seeds.

4.3.7 Trypsin Inhibitor Activity

Trypsin Inhibitor activity of quinoa seeds is shown in Table 4.4. Raw seeds were reported to have 6633±7.5 TIU/100g trypsin inhibitor activity. The results are almost similar to and supported by values of trypsin inhibitor activity (6890 TIU/ 100g) reported by Ando et al., 2002 in quinoa seeds. All the processing methods led to significant decrease ($P<0.05$) in trypsin inhibitor activity. Soaking resulted in 4.7% decrease in trypsin inhibitor activity, which may be due to leaching of trypsin inhibitors from seed coat to soaking media (Sharma and Sehgal, 1992). Mubarak, 2005, reported almost similar decrease in trypsin inhibitor activity, by 5.2%, after soaking mung bean seeds. Decrease in trypsin inhibitor activity, by 22.8%, was also observed in germinated seeds. The result is in agreement with findings of El-Adawy, 2002 who reported 33.9% decrease in trypsin inhibitor activity of chickpeas after soaking. Industrially processed quinoa seeds exhibited 20.7% decrease in trypsin inhibitor activity. This may be due to removal of hulls from seeds during industrial processing, which accounts for 10 to 12% of whole seed weight (Hemlatha et al., 2016) and have major participation (16%) in trypsin inhibition (Chauhan et al., 1992) as compared to the whole seed. Highest decrease in trypsin inhibitor activity in germinated seeds also infers better protein digestibility of the same as compared to raw, soaked, and industrially processed seeds.

4.3.8 Total phenolic content

Total phenolic content (TPC) of Indian *Chenopodium quinoa* is shown in Table 4.5. TPC of raw Indian quinoa seeds, was reported to be 43.2±0.28 mgGAE/ 100g which corresponds well to TPC content reported by Repo-Carrasco-Valencia et

al., 2010 (42 mg GAE 100^{-1}) and Vollamannova et al., 2013 (45mgGAE /100g) in Carmen variety of quinoa.

Also the value of TPC content found in this study lies close to reported the values of TPC in raw quinoa seeds by Carciochi et al., 2014 (39 mgGAE /100g), Pasko et al., 2009 (38mgGAE /100g), higher than as reported by Miranda et al., 2010 (28mg GAE/100g). Higher reported values in this study can be explained as raw seeds (direct from the field) used were with the seed coat while the quinoa seeds procured by these authors were as available in the local market, which might be industrially processed for removal of seed coat which leads to decrease in phenolic content . Significant difference in phenolic contents reported by various other authors may be due to different environment conditions for growth, extraction solvents (Marmouzi et al., 2015), quinoa varieties with coloured testa (Tang et al., 2015). Total phenolic content reported in soaked quinoa seeds was significantly less (28%, $P \leq 0.05$) as compared to raw seeds. The result corresponds well to 26-56% loss in total phenolic content of soaked black beans (*Phaseolus vulgaris* L.) reported by Xu and Chang 2008. Germinated seeds were found to exhibit 134% increase in total phenolic content as compared to the raw quinoa seeds. This is because germination leads to increase in phenolic content of seeds as synthesis of phenolic acid is enhanced by seed growth during germination (Cevallos-Casals and Cisneros-Zevallos, 2010). Increase in total phenolic content in germinated quinoa has also been reported by Carciochi et al., 2014 (56 % after 48 hours and 101.2% after 72 hours of germination) and Jubete et al., 2010 (107% after 82 hours of germination). Industrial processed quinoa seeds exhibited 20% decrease in total phenolic content (34 mgGAE /100g). Similar decrease in phenolic compounds of pearled quinoa (abrasion degree of 30%) was reported by Gomez-Caravaca et al., 2014 with 21.5% and 35.2% decrease in free and bound phenolic compounds respectively.

4.3.9 Total flavonoids

Total flavonoid content (TFC) of quinoa seeds is shown in Table 4.5. Total flavonoid content of raw Indian quinoa was reported as 11.40 mg QE /100g. Results agree with the findings of Carciochi et al., 2014 (11.06 mg QE /100g) and Chirinos et al., 2013 (11 mg QE /100g). The total flavonoid content reported in our study is significantly different to the values reported by Marmouzi et al.,

2015; Carciochi et al., 2014 and Chlopika et al., 2012. This might be due to different solvents used for extraction, difference in temperature during extraction process and different methods of flavonoid analysis used (HPLC or spectrophotometry). Total flavonoid content of soaked quinoa seeds decreased by 36% (7.2 mg QE /100g). Similar decrease in flavonoid content after soaking has been found in white sorgum (Afiffy et al., 2012). Germination of quinoa seeds lead to significant increase (56%) in flavoniod content (18 mg QE /100g). Similar increase in flavonoid content (59%) has been reported by Carciochi et al., 2014 in germinated quinoa seeds. The increase in flavonoid content on germination of seeds is due to synthesis of metabolites like flavonoids by phenylproponoid pathway, common to all plants, during process of seed germination (Wu et al. 2011). Industrial processing of quinoa seeds led to reduction in flavonoid content by 47%. The findings may be attributed to the fact that most of the flavonoids are contained in the seed coat and industrial processing involves removal of outer layer of seed thus causing decrease in the flavonoid content (Xu and Chang, 2008).

Table 4.5: Total phenolic content and total flavonoid content (TFC) of Raw, domestically and industrially processed Indian *Chenopodium quinoa* seeds

Indian *Chenopodium quinoa*		**Total Phenolic Content (mg GAE/100g)**	**Total Flavonoids (mg QE/100g)**
Raw		43.2±0.28[a]	11.4±0.08[a]
Domestically Processed	**Soaked**	7.2±0.08[a]	31.1±0.35[b]
	Germinated	18.02±0.2[a]	101.2±0.29[c]
Industrially processed		34.6±0.33[a]	5.8±0.10[a]

4.4 *In-Vitro* analysis

3.4.1 In vitro starch digestibility

The starch digestibility of quinoa seeds is shown in Table 4.6. Raw quinoa seeds exhibited 65.7±0.15% starch digestibility. The results are in agreement with the findings of Repo-Carassco-Valencia, 2011 who reported 65.1 to 68.6% starch

digestibility in 4 different varieties of quinoa seeds. Soaking of quinoa seeds resulted in 1.52%, non-significant increase in starch digestibility while germination led to 1.80%, significant increase with respect to the raw seeds. This can attributed to the fact that soaking and germination result in starch gelatinization and increased activity of α-amylase, an enzyme responsible for disintegration of starch into sugars, hence, reducing starch into readily digestible form (Preet and Punia, 2000). High starch digestibility can also be attributed to small size (0.3 to 2µm) of quinoa starch granules (Kong and Bertoft, 2010). Li and Zhu 2016 also reported significant amount of rapidly digestible starch (RDS) content in quinoa, which is readily susceptible to enzyme action. Industrialy processed seeds exhibited 1.05%, non-significant increase ($P<0.05$), in starch digestibility. Capriles et al., 2014 also reported 7.02% increase in starch digestibility of industrially processed (extruded) amaranth seeds. Thapliyal et al., 2014 also reported 9.8 to 14% increase in starch digestibility of industrially processed (dehulled) chickpeas of different varieties. Starch digestibility was higher after soaking and germination as compared to industrial processing, this may be due to better subjection of seed starch matrix to degradation upon hydration and action of α-amylase on soaking and germination (Chung et al., 2012).

4.4.2 *In vitro* Protein digestibility

The protein digestibility of raw and processed Indian quinoa seeds is shown in Table 4.6. Raw quinoa seeds exhibited 75.3±0.33% protein digestibility. Repo-Carassco-Valencia and Serna, 2011 also reported 76.32 to 80.54% protein digestibility in different quinoa varieties. Among all quinoa seeds (raw and processed), raw quinoa seeds had lower protein digestibility value. This may be attributed to presence of anti nutrients like tannin, trypsin inhibitors, etc. which impede the digestibility and solubility of protein (Pushparaj and Urooj, 2011). Soaking resulted in 1.5% increase in protein digestibility. The results are supported by findings of El-Sayed Embaby, 2010, who reported 0.9 to 1.4 % increase in protein digestibility of lupin seeds after soaking. Germination resulted in 9.1%, significant increase ($P<0.05$) in protein digestibility. Ghavidel and Prakash, 2007 also reported 14 to 15% increase in protein digestibility of various legumes after germination. Industrially processed seeds exhibited 1.2% increase in

protein digestibility value. Increase in protein digestibility after soaking, germination and industrial processing may be due to decrease in anti nutrients. Phytic acid, anti nutrient present in quinoa seeds is known to interfere by binding with protein and suppressing proteolysis hence, lowering protein digestibility (Cowieson et al., 2006).

Table 4.5: In vitro analysis of Indian *Chenopodium quinoa*

Indian *Chenopodium quinoa*		**Invitro protein digestibility (%)**	**In vitro Starch Digestibility (%)**	**Antioxidant activity**	
				FRAP mg TE/ 100g	**DPPH mg TE/ 100g**
Raw		75.3±0.33[a]	65.7±0.15[a]	84.46±5.9[a]	59.61±0.39[a]
Domestically Processed	**Soaked**	76.5±0.32[b]	66.7±0.05[a]	96.46±1.5[a]	53.51±0.56[b]
	Germinated	82.2±0.36[c]	66.9±0.2[a,b]	159.23±0[.b]	61.41±1.89[c]
Industrially processed		76.2±0.1[d, b]	66.4±0.6[b]	72.35±1.82[a]	49.69±1.5[a]

4.4.3 *In vitro* antioxidant activity

Antioxidant activity of Indian *Chenopodium quinoa* seeds is shown in Table 4.6. Raw quinoa seeds were reported to have 59.6 mgTE /100g as calculated by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) method and 84.4 mg TE /100g as calculated by FRAP (Ferric reducing antioxidant activity) method. The results were close to antioxidant activity according to Jubete et al., 2010 (57.7 mg TE/100g and 34.8 TEAC by DPPH method and 84.1 mg TE /100g by FRAP method). Antioxidant activity of quinoa reported by Ranilla et al., 2009 (by DPPH) was much higher (86 mg TE /100g) than as reported in this study (59.6 mgTE /100g). This is because red quinoa were used by Ranilla et al., 2009 and difference in color of seeds strongly effects antioxidant activity with dark colored seed coats exhibiting highest antioxidants activities (Tang et al., 2015). Quinoa seeds exhibit higher antioxidant activity (evaluated by FRAP and DPPH) as compared to grain Amaranth (Nsimba et al., 2008 and Pasko et al., 2009, Vollmannova et al., 2013)

and some Peruvian Andean fruit like Tuna and grain Kwicha (Chirinos et al. 2015). It is found to exhibit lower antioxidant activity as compared to buckwheat (Jubete et al., 2010 and Vollmannova et al., 2013), oat and rice (Halvorsen et al., 2002), higher than amaranth (Jubete et al., 2010) and almost similar to wheat (Jubete et al., 2010).

Antioxidant activity of quinoa also depends on intensity of colour of seed coat (Tang et al. 2015). Soaked quinoa seeds exhibited 7% decrease in antioxidant activity as compared to raw seeds. The results is supported by findings of Afiffy et al., 2012, who reported decrease in antioxidant activity in soaked white sorghum. Xu and Chang, 2008 also reported decrease in antioxidant activity of soaked green pea (9%), yellow pea (8%), and lentil (7%). As phenols and flavonoids contribute significantly to antioxidant activity (Thaipong, 2006), the decrease may be due to leaching of phenols and flavonoids in water used for soaking the seeds (Afiffy et al., 2012). Quinoa seeds germinated in day light for 7 days exhibit significantly high antioxidant activity than raw seeds (Pasko et al., 2010a). In our study antioxidant activity of germinated seeds (after 4 days or 48 hours) was found to increase by 90% (calculated by DPPH method). The result is supported by findings of Carciochi et al., 2014a which showed 100% increase in antioxidant activity of germinated quinoa seeds as evaluated by DPPH method. Similarly, increase in antioxidant activity of germinated quinoa seeds has also been reported by Pasko et al., 2008. FRAP values of germinated quinoa seeds increased by 89%. The result is supported by increase in FRAP values of quinoa sprouts (79%) as reported by Jubete et al., 2010b. Quinoa (*Chenopodium quinoa*) sprouts have lower antioxidant activity (evaluated by FRAP) as compared to Amaranth (*Chenopodium album*) sprouts (Pasko et al., 2009). However industrial processing of the seeds lead to decline in antioxidant activity. Processed quinoa seeds showed decline of 14% and 19% antioxidant activity as evaluated by FRAP and DPPH, respectively. The result is supported by decrease in antioxidant activity of wheat (Hung et al., 2009), after undergoing industrial processing like decortications and pearling. Decline in antioxidant activity after processing can be due to removal of hulls, which are majorly responsible for antioxidant activity (Cardador-Martinez et al., 2002).

B) Comparison of nutritional quality of industrially processed Indian and American *Chenopodium quinoa* seeds

4.5 Proximate Composition of industrially processed Indian and American *Chenopodium quinoa* seeds

Proximate composition of quinoa seeds grown in India and South America is shown in Table 4.7. Moisture, carbohydrate, protein and crude fiber content of quinoa seeds grown at two different countries were significantly different ($P<0.05$) while ash and crude fat contents were statistically non-significant ($P<0.05$). The results of proximate composition of American quinoa reported in this study are in agreement with proximate composition of Peruvian quinoa (grown in Peru, South America) and Argentinean quinoa (grown in Argentina, South America) reported by Nascimento et al., 2013 and Villa et al., 2014, respectively. Significant difference ($P<0.05$), observed in moisture contents of grains might be due to varying climatic conditions and soil water holding capacities, which highly affect constitutional aspects of the crops (Kang et al., 2009).

Table 4.7: Proximate Composition of industrially processed Indian and American

Chenopodium quinoa	Moisture (g/100g)	Carbohydrate (g/100g)	Crude fat (g/100g)	Crude protein (g/100g)	Ash (g/100g)	Crude fibre (g/100g)
Indian	10.35±0.20 [a]	64.90±0.09 [a]	5.11±0.05 [a]	13.11±0.08 [a]	3.04±0.03 [a]	2.49±0.04 [a]
American	11.23±0.32 [b]	67.93±0.84 [b]	4.63±0.15 [a]	12.25±0.92 [b]	3.18±0.08 [a]	2.14±0.31 [b]

It was noticeable that the protein content of Indian quinoa (13.11±0.08 g/100g) was 7.02% more than the protein content of American quinoa (12.25±0.92 g/100g). Foste et al., 2015 reported similar protein content of Bolivian quinoa (grown in Bolivia, South America). Gonzalez et al., 2014 also reported different protein content of quinoa seeds grown at different sites. Although, protein content of grain varies with the variation in genotype but environmental elements are also

known to exert a pronounced effect on the protein content (Kumar et al., 2006). Marmouzi et al., 2015 reported lower protein content of Moroccan quinoa as compared to American quinoa, which shows effect of environmental conditions and their interactions on protein content of grain.

A significant difference ($P<0.05$) observed in crude fiber contents of grain might be attributed to the different processing methods and the extent of processing, applied to grains for saponin removal, which involves removal of outer fibrous covering of seeds (Ghavidel and Prakash , 2007).

4.6 Nutritional Composition of industrially processed Indian and American *Chenopodium quinoa* seeds

Dietary fiber and β-Carotene contents of American and Indian quinoa seeds is given in Table 4.8. Dietary fiber content of American and Indian quinoa seeds is given in Table . The results are in accordance with the values reported by Marmouzi et al., 2015. A non-significant difference ($P<0.05$) was observed in dietary fiber content of American and Indian quinoa seeds. Miranda et al., 2013 also reported non-significant difference ($P<0.05$) in quinoa seeds grown at different places.

Indian quinoa seeds exhibited significantly higher ($P<0.05$) β-Carotene content than American seeds. The results are in accordance with the β-Carotene content of American chenopodium species reported by Sharma et al., 2012. According to Gastol et al., 2012, plant macro and micronutrients are highly dependent on soil composition, method of farming and management practices. Hence, the difference in β-Carotene contents of quinoa seeds observed in this study might be due to variation in these factors.

Vitamin C content of Indian and American quinoa seeds is given in Table 4.8. Vitamin c content of American quinoa was, 14.4%, significantly higher ($P<0.05$) than the vitamin C content of Indian quinoa seeds. The results are in agreement with the vitamin c content of South American (Chilean) quinoa varieties reported by Miranda et al., 2010. The difference might be due to certain influential factors like fertilizer quality, regional temperature, soil water management etc., which highly affect the vitamin c content of crop (Lee and Kader, 2000).

Table 4.8: Nutritional Composition of industrially processed Indian and American

Indian *Chenopodium quinoa*	Vitamin C (mg/100g)	β-Carotene (µg/100g)	Dietary fiber (g/100g)					
			NDF	ADF	Lignin	Cellulose	Hemi-cellulose	Total dietary fibre
Indian	13.43±0.4 [a]	535.6±2.8 [a]	6.69±0.03	2.50±0.07	2.2±0.02	3.3±0.04	1.19±0.05	9.24±0.21 [a]
American	15.38±0.16 [b]	165.24±1.2 [b]	6.93±0.31	2.45±0.46	2.13±0.18	3.52±0.10	1.22±0.01	9.41±0.73 [a]

Mineral composition of Indian and American quinoa is shown in Table 4.9. Mineral content of American quinoa was found to be significantly higher ($P<0.05$) than the Indian quinoa. Calcium, iron and zinc contents of American quinoa seeds were 15.6, 43.2 and 26.1% higher than Indian quinoa, respectively. The results are in agreement with the findings of Miranda et al, 2013 who reported similar calcium, iron and zinc content in South American quinoa grown in Argentina. Magnesium content of American quinoa was 6.1% higher than Indian quinoa. The resultsa re in agreement with the findings of Coelho et al., 2011 who reported similar results for magnesium content in Argentinean quinoa. Miranda et al., 2013 also demonstrated different soil characteristics of different regions where quinoa was cultivated. The difference in mineral content of quinoa grains grown at two different place may be due to difference in soil composition, as soil type is known to have great influence on crop characteristics (Baratasevec et al., 2013).

4.7 Phytochemical analysis of industrially processed Indian and American *Chenopodium quinoa* seeds

The content of phytochemical in Indian and American quinoa grain is shown in Table 4.10. A significant difference ($P<0.05$) was observed in antinutritional content of both grains grown at different places. The difference observed in phytic acid

Table 4.9: Mineral content of industrially processed Indian and American C*henopodium quinoa* seeds

Indian *Chenopodium quinoa*	Calcium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Magnesium (mg/100g)
Indian Quinoa	85.06±0.15 [a]	5.1±0.02 [a]	6.5±0.13 [a]	182.2±0.15 [a]
American quinoa	98.38±0.22 [b]	7.32±0.15 [b]	8.24±0.76 [b]	193.34±0.65 [b]

Table 4.10: P**hytochemical Composition of industrially processed Indian and American *Chenopodium quinoa* seeds**

Indian *Chenopodium quinoa*	Total phosphorous (g/100 g)	Phytic acid/ Phytate (g/100g)	Phytate phosphorous	Non phytate phosphorous	Saponin (g/100g)	Trypsin Inhibitor Activity TIU/100g	Oxalates (g/100g)	Alkaloids (g/100gm)	Tannins (g/100g)
Indian	0.45±0.01 [a]	1.21±0.31[a]	0.26	1.62	0.06±0.02 [a]	5254.6±11.5 [a]	0.18±0.04 [a]	1.9±0.02 [a]	0.35±0.0 [a]
American	0.62±0.16 [a]	1.29±0.15[b]	0.33	0.96	0.02±0.15 [b]	5249.3±9.32 [b]	0.24±0.16 [b]	1.2±0.41 [b]	0.41±0.18 [b]

contents may be due to environmental conditions and difference in crop variety that are known to have influential effect on crop's phytic acid content (Mahmood et al., 2010). In addition, the crop harvesting time is also known to influence phytic acid content, which further influences its content of total phosphorous (Kim et al., 2002). Difference in saponin, trypsin inhibitor activity, oxalate, alkaloid and tannin contents may be due to difference in extent and type of processing technique applied to the grain for increasing its palatability and acceptability by consumers (Preedy, 2014). The antinutrients are largely located and congregated in outer layers of grains, which get removed during various industrial processes applied to them post harvesting (Mao et al., 2011), so extent of processing applied largely affects the quantity of anti nutrient removed and retained.

Total phenolic content and total flavonoid content of Indian and American quinoa (Table 4.11) was significantly ($P<0.05$) different. Significant difference in phenolic contents due to different environment conditions for growth has also been rep orted by Miranda et al., 2013.

4.11 Total phenolic content and total flavonoid content of industrially processed Indian and American *Chenopodium quinoa* seeds

Indian *Chenopodium quinoa*	Total phenolic content	Total flavonoid content
Indian quinoa	34.6 ± 0.33^{a}	5.8 ± 0.10^{a}
American quinoa	30.16 ± 0.49^{b}	4.6 ± 0.18^{b}

4.8 *In vitro* analysis of industrially processed Indian and American *Chenopodium quinoa* seeds

***3.8.1 In vitro* starch digestibility**

Invitro starch digestibility of quinoa seeds is shown in Table 4.12. American quinoa seeds exhibited significantly higher ($P<0.05$) starch digestibility (67.14%) than Indian quinoa seeds. The results lie within the range of values of in vitro starch digestibility of four different South American quinoa varieties reported by

Repo Carassco-Valencia and Serna, 2011. The difference in *invitro* protein and starch digestibility of Indian and American quinoa seeds may be due to varietal difference of the grain because of different environmental conditions of cultivation and also different processing methods used for saponin removal from raw seed. Shimelis and Rakshit, 2007 demonstrated effect of processing on *invitro* protein digestibility of different varities of kidney beans. Souilah et al., 2015 reported different *invitro* starch digestibility in different varieties of sorghum.

4.8.2 *In vitro* protein digestibility

Invitro protein digestibility of quinoa seeds is shown in Table 4.12. American quinoa seeds exhibited significantly lower ($P<0.05$) protein digestibility (75.15%) than Indian quinoa seeds. The results are in agreement with findings of Rehman and Shah, 2001 and Repo Carassco-Valencia and Serna, 2011 who reported 75.3 to 80.4% protein digestibility of different variety of quinoa seeds procured from Peru, South America.

Table 4.12: *In vitro* analysis of industrially processed Indian and American *Chenopodium quinoa* seeds

Indian *Chenopodium quinoa*	Invitro protein digestibility (%)	In vitro Starch Digestibility (%)
Indian quinoa	76.22±0.13 [a]	66.48±0.62 [a]
American quinoa	75.15±0.35 [b]	67.14±0.38 [b]

4.8.3 *In vitro* antioxidant activity

The total antioxidant activity of Indian *Chenopodium quinoa* (Table: 4.13) was 72.35±1.82 mgTE/100g (by FRAP) and 49.69±1.50 (by DPPH) while American seeds were reported with antioxidant activity of 70.53±0.15 (by FRAP) and 46.38±0.41 (by DPPH). Miranda et al., 2013 also reported 41 to 73% difference in antioxidant activities of quinoa seeds grown in contrasting environmental regions. Nsimba et al., 2008, revealed antioxidant activity of two quinoa genotypes with

mean values of 72.1% of radical scavenging activities for Bolivian genotype and 59.2% for Japan sea-level type. The results suggest in that case that non phenolic compounds might also play an important role in the free radicals scavenging activity.

Table 4.13: Antioxidant activity of industrially processed Indian and American *Chenopodium quinoa* seeds

Indian *Chenopodium quinoa*	***In vitro* antioxidant activity**	
	FRAP mgTE/100g	**DPPH mgTE/100g**
Indian quinoa	72.35±1.82[a]	49.69±1.5[a]
American quinoa	70.53±0.15[b]	46.38±0.41[b]

Objective 2: To assess cholesterolemic effect of *Chenopodium quinoa* seeds.

Plasma lipid levels of rats fed with experimental diet for 45 days is shown in Table 4.14.

4.9.1 Effect on serum chlosterol levels

The total serum cholesterol level of rats fed with basal diet (Group1) was reported as 160.16±16 mg/dl. The total concentration of plasma cholesterol was observed to reduce significantly ($P<0.05$) upon addition of quinoa to the experimental diet. It was observed that, as compared to the Group 2, the positive control (rats fed on hypercholesterolemic diet), concentration of cholesterol reduced by 8.2% in diet fed with statin, while 15.5 and 24.4% reduction in serum cholesterol was observed in rats fed with raw (Group 4) and germinated quinoa (Group 5) along with hypercholesterolemic diet, respectively. The rats fed with raw (Group 6) and germinated quinoa (Group 7) along with basal diet showed 28.2 and 31.9% reduction in total cholesterol level. The results are in agreement with the findings

of Foucault et al., 2012 who reported reduction in serum cholesterol levels of mice fed with quinoa extract for 3 weeks. The results reveal higher hypercholesterolemic effect of germinated quinoa seeds as compared to the raw quinoa seeds

4.9.2 Effect on plasma triglycerides level

The levels of plasma triglycerides showed a declining trend with supplementation of quinoa to the experimental diet. As compared to the positive control, which showed 96.6±2.9mg/dl triglyceride content, the levels were reported to decrease significantly ($P<0.05$) in rats fed with quinoa diet. However, reduction in triglyceride content by quinoa supplemented along with hypercholestrolemic diet was observed to be lower than the reduction induced by statin administered along with hypercholestrolemic diet. El-gawad et al., 2005, have also reported similar trend. In rats fed with quinoa along with hypercholersterolemic diet, the triglyceride levels reduced by 12.5 and 4.5% in Group 4 and Group 5, respectively. Triglyceride level of rats fed with quinoa along with basal diet i.e. in Group 6 and Group 7, reduced by 10.4 and 14.5%, respectively. Takao et al., 2005 also reported similar reduction in triglyceride level of rats fed with quinoa 2.5 and 5% quinoa protein. Mithila et al., 2015 also reported reduction of triglycerides in rats fed with quinoa supplemented diet. This could be due to the wide variety of phytoconstituents present in quinoa which offer a synergistic effect in exerting hypolipidimic effects (González and Rodriguez 2011).

4.9.3 Effect on HDL level

The serum HDL level of rats fed on basal diet were reported as 91.15±4.8 mg/dl while of rats fed on hypocholesterolemic diet was 90.1±2.2 mg/dl . The HDL levels were observed to decrease significantly ($P<0.05$) in rats fed with diets supplemented with raw (5.5%) and germinated (3.3%) quinoa along with hypercholesterolemic diet as compared to serum HDL levels of rats fed with hypercholesterolemic diet (positive control) which reveals negative effect of quinoa supplementation along with hyper cholesterolemic diet on serum HDL levels. Mithila and Khanum, 2015, have reported similar results. Supplementation of raw and quinoa seeds with basal diet showed an increase in HDL level by 6.5 and 9.8%, respectively effect as compared to the supplementation of quinoa with basal diet.

The results are in accordance with the results reported by Pasko et al., 2010 and Hejazi, 2016.

4.9.4 Effect on serum (VLDL+HDL) level

The serum (VLDL+HDL) values of rats fed on basal diet were reported as 68.82±7.2 mg/dl while of rats fed on hypercholesterolemic diet was 128.9±3.5 mg/dl. The results were observed to decrease significantly ($P<0.05$), by 21.3 and 39.4% with supplementation of raw quinoa and germinated quinoa to

Table 4.14: Effect of quinoa supplementation (raw and germinated) on blood lipid profile

	Cholesterol (mg/dl)	**Triglyceride (mg/dl)**	**HDL (mg/dl)**	**(LDL+VLDL) (mg/dl)**	**Atherosc-lerotic Index (mg/dl)**
Group 1	160.16±4.8 [a]	86.3±1.6 [a]	91.15±4.8 [a]	68.82±7.2 [a]	0.75±0.2 [a]
Group 2	219.10±11.3 [b]	96.6±2.9 [b]	90.1±2.2 [b]	128.9±3.5 [b]	1.43±0.3 [b]
Group 3	201.13±2.9 [c]	84±2.5 [c]	96±1.7 [c]	105.13±5.4 [c]	1.09±0.4 [c]
Group 4	185.5±2.8 [d]	92.3±1.6 [d]	85.1±2.2 [d]	100.4±1.9 [d]	1.17±0.16 [d]
Group 5	165.18±2.7 [e]	88±3.5 [e]	87.02±1.4 [e]	78.16±1.3 [e]	0.89±0.1 [e]
Group 6	157.14±3.1 [f]	86.6±2.8 [f]	97.33±1.6 [f]	59.81±1.1 [f]	0.61±0.1 [f]
Group 7	149.5±1.0 [g]	82.16±1.4 [g]	100±1.5 [g]	49.5±1.2 [g]	0.49±0.1 [g]

VLDL: Very low-density lipoprotein-cholesterol; LDL: low-density lipoprotein-cholesterol; VLDL+LDL-cholesterol=Total cholesterol- HDL.
Atherosclerotic index= (VLDL+LDL-cholesterol)/(HDL-cholesterol)

hypercholesterolemic diet, respectively. The results also infer beneficial effect of germinated quinoa over raw quinoa. This may be due to higher phenolic content of germinated quinoa which might be responsible for lipid lowering beneficial effects (Carciochi et al., 2013). Also, it is noteworthy that lipid lowering effect of quinoa (raw and germinated) was higher than the beneficial effect of statin, which was reported to cause17.7% decline in (VLDL+HDL) values. The reduction was almost almost twice in germinated quinoa. The rats fed on diet containing raw and germinated quinoa supplemented with basal diet also reported 13.2 and 27.3% reduction in (VLDL+HDL) values. The results infer lipid lowering beneficial effect of quinoa, mainly germinated quinoa.

4.9.5 Effect on Atherosclerotic index

Atherosclerotic index of rats fed with basal diet was reported as 0.75±0.2 mg/dl while of rats fed on hypercholestrolemic diet was reported as 1.43±0.3 mg/dl. The drug statin administered to rats along with hypercholesterolemic diet, caused 23.7% reduction in atherosclerotic index, while supplementation of raw and germinated quinoa along with hypercholeterolemic diet resulted in 18.4 and 37.2% reduction in atherosclerotic index. The results infer beneficial effect of quinoa, mainly the germinated one, in hypercholesterolemia over the statin drug. The rats fed on raw and germinated quinoa supplemented with basal diet also showed 18.6 and 34.5% reduction in atherosclerotic index. This may be due high dietary fiber and phenolic content of germinated quinoa which may be responsible for lipid lowering effect (Carciochi et al., 2013 and Marmouzi et al., 2016). The results also are in line with the lipid lowering and beneficial effects of quinoa supplementation, mainly the germinated quinoa, reported in this study. To our knowledge, no study till now has reported effect of quinoa supplementation on atherosclerotic index.

Objective 3: To develop and analyze value added products from *Chenopodium quinoa*

4.10 Development of Snack bar

4.10.1 Proximate composition of snack bars

Proximate composition of snack bars is shown in Table 4.15. Moisture content of formulated bars ranged from 15.21±0.18 to 15.19±0.12 g/100gm. The results are in

agreement with the moisture content of snack bars (15.56 to 18.52 g/100gm) formulated by Nadeem et al., 2012. Moisture content of control bar (CB) and quinoa bar (QB) were almost similar and statistically non-significant to each other ($P<0.05$). Moisture content of snack bar formulated in this study was found to be lower than flaxseed incorporated cereal bars prepared by Khouryeih and Aramouni, 2013 which also indicates better shelf life of CB and QB. Ash, fat, protein and fiber content of quinoa bar was significantly higher ($P<0.05$) than control bar. The results are supported by findings of Slinkard, 2014 who reported better nutritional value of pasta formulated using combination of quinoa and chickpea flour with respect to the pasta formulated with quinoa flour alone.

Table 4.15: Proximate composition of snack bars

Proximate composition	**Quantity (g/100g)**	
	Control Bar (CB)	**Quinoa Bar (QB)**
Moisture	15.21±0.18 [a]	15.19±0.12 [a]
Ash	2.03±0.25[a]	3.16±0.92[b]
Fat	6.19±0.57[a]	7.31±0.13[b]
Protein	8.14±0.43[a]	13.41±0.53 [b]
Carbohydrate	67.14±0.13 [a]	60.87±0.73 [b]
Fiber	2.16±0.93 [a]	3.03±0.16 [b]

4.10.2 Total phenolic content and antioxidant activity

The total phenolic content and antioxidant activity of quinoa bar is shown in Table 4.16. Total phenolic content of quinoa bar was significantly higher ($P<0.05$) than the control bar. The reported results reveal higher total phenolic content of quinoa bar in comparison to the total phenolic content (30.7mgGAE/ 100g) of quinoa breads as reported by Jubete et al., 2010. Carciochi et ., 2014 reported rich polyphenolic content of quinoa. Total phenolic content of quinoa bars reported in this study is also higher than the total phenolic content of crackers (84 to 148mg GAE/100g) reported by Sedej et al., 2011.

Antioxidant activity of quinoa bars, as evaluated by DPPH and FRAP method, was reported significantly higher ($P<0.05$) than the control bars. The results are supported by the findings of Bhaduri and Navder, 2014 who reported 137% in antioxidant activity of muffins incorporated with quinoa. Carciochi et al., 2016 reported significantly higher antioxidant activity of germinated quinoa seeds, which might be the probable reason behind higher antioxidant activity of quinoa bars because of incorporation of germinated quinoa flour in formulated bar. Antioxidant activity of quinoa snack bars formulated in this study is higher than the snack bars formulated by Bailek et al., 2016 using popped amaranth seeds and oat flakes.

Table 4.16: Total phenolic content and antioxidant activity

Bar	**Antioxidant Activity**		**Total phenolic Content (mg GAE/100g)**
	DPPH (%)	**FRAP (mg Te/100g)**	
CB	59.34±0.31 [a]	101.22±0.66 [a]	89.43±0.34 [a]
QB	62.12±0.12 [b]	103.05±0.21 [b]	96.59±0.65 [b]

4.10.3 Sensory evaluation of bars

The data analysis of sensory evaluation (Table 4.17), revealed significant

Table 4.17: Sensory evaluation of snack bars

Bars	**Appearance**	**Colour**	**Texture**	**Mouth feel**	**Flavour**	**Taste**	**Overall Acceptability**
CB	7.1±0.70 [a]	6.2±0.70 [a]	6.7±0.18 [a]	6.9±0.14 [a]	6.5±0.2 [a]	7.7±0.13 [a]	6.4±0.03 [a]
QB	7.9±0.17 [b]	6.9±0.14 [b]	6.9±0.98 [a]	7.2±0.21 [b]	7.1±0.6 [b]	8.4±0.09 [b]	7.5±0.13 [b]

difference (P<0.05) in appearance, colour, mouthfeel, flavor and taste of control bars and quinoa bars, while difference in texture was revealed statistically non-significant (P<0.05).

Similarly in texture of both snack bars may be due to similar processing methods used for formulation of bars. The results infer better consumer acceptability of quinoa bars rather than control bars formulated with chickpea four and are parallel to the findings of Slinkard, 2014 who reported better consumer acceptability of quinoa-chickpea composite pasta. Revelations by Bahaduri and Navder, 2014 who demonstrated positive effect of incorporation of quinoa flour to food products for better sensory characteristics and higher consumer acceptability, also support the results in this study. According to scoring on nine point hedonic scale, overall acceptability of quinoa snack bars indicated "like very much" while control bars which indicated "like slightly". Highest score was scored by taste aspect (8.4 ± 0.09^{j}) of quinoa bar, which indicated "like extremely" on a nine point hedonic scale while lowest score was scored by colour aspect of control bar which indicated "like moderaltely" on a nine point hedonic scale.

4.10.4 Storage study

4.10.4.1 Effect on moisture content

Figure 4.1 represents moisture content of snack bars as affected during storage period of 30 days. The figure depicts increase in moisture content of snack bars upon storage. A significant increase (P<0.05), in moisture content was observed in both control bar and quinoa bar at an interval of 15 and 30 days. However, moisture content at the end of 30 days was highest as compared to the initial moisture content of snack bars. Increased moisture content of quinoa bars over the period may be due to the changes in water holding capacity of quinoa during the storage as already reported by Abugoch et al., 2009. Inglett et al., 2015 also reported increased water holding capacity of quinoa-oat composites. Increase in moisture content might also be due to water vapour transmission through the polythene packaging material used to store the bars (Bertrand et al., 2013). As, the moisture content of food is inversely related to its shelf life (Genkawa et al., 2008), the results depict degradation in shelf life of formulated bars with time.

4.10.4.2 Effect on crude ash

Figure 4.2 represents ash content of formulated snack bars as affected during storage period of 30 days. Ash content of snack bars was observed to decrease non-significantly ($P<0.05$) upon storage for 15 days while a significant decrease in ash content was observed after storage period of 30 days. Decrease in ash content during storage period has also been reported by Nadarajah et al., 2015 in coconut cookies. Decrease in ash content may also be due to increase in moisture content, which favours microbiological growth. During their growth period, the microbes utilize minerals and other nutrients, which result in decrease in ash content (Adams and Moss, 2005).

4.10.4.3 Effect on crude fat

Figure 4.3 represents changes in fat content of snack bars during the storage period of 30 days. A non significant change ($P<0.05$) in fat content was observed after storage period of 15 days while a significant decrease ($P<0.05$) was observed after storage period of 30 days. Decrease in fat content of cereals over a period during storage has also been reported by Sharma et al., 2014. This might be attributed to the lipid oxidation due to their larger surface area of snack bar which is in contact with air and moisture (Maisuthisakul et al., 2007). Decrease in fat content can also be attributed to increased activity of lipase which is highly influenced by moisture content of food (Agrahar-Murgkar and Jha, 2011). Lipase is responsible for oxidative rancidity leading to hydrolysis of fat present in food matrix and formation of free fatty acids which also imparts off flavor to the food product (Adawiyah et al., 2012).

4.10.4.4 Effect on crude protein

Figure 4.4 represents effect of storage period on protein content of snack bars. A non-significant decrease ($P<0.05$) was observed in protein content of snack bars after storage period of 15 days while significant decrease ($P<0.05$) was observed after storage period of 30 days.. Decrease in protein content may be attributed to increase in proteolyic activity due to increase in moisture content (Butt et al., 2010).

4.10.4.5 Effect on carbohydrate content

Figure 4.5 represents effect of storage period on carbohydrate content of snack bars. The carbohydrate content of control snack bars was observed to decrease significantly ($P<0.05$) after storage period 15 and 30 days as compared to the initial carbohydrate content while quinoa snack bars exhibited non significant

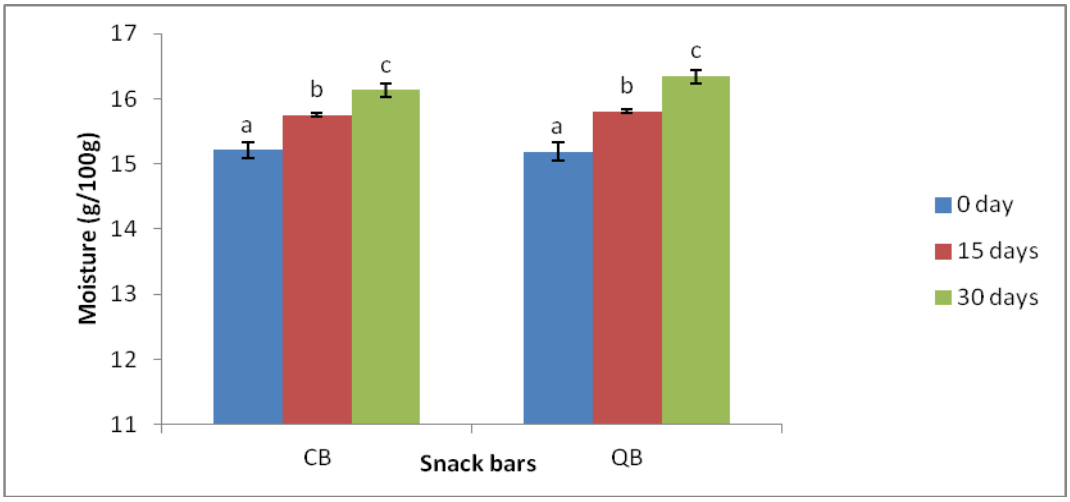

Figure 4.1: Effect of storage period on moisture content of snack bars.

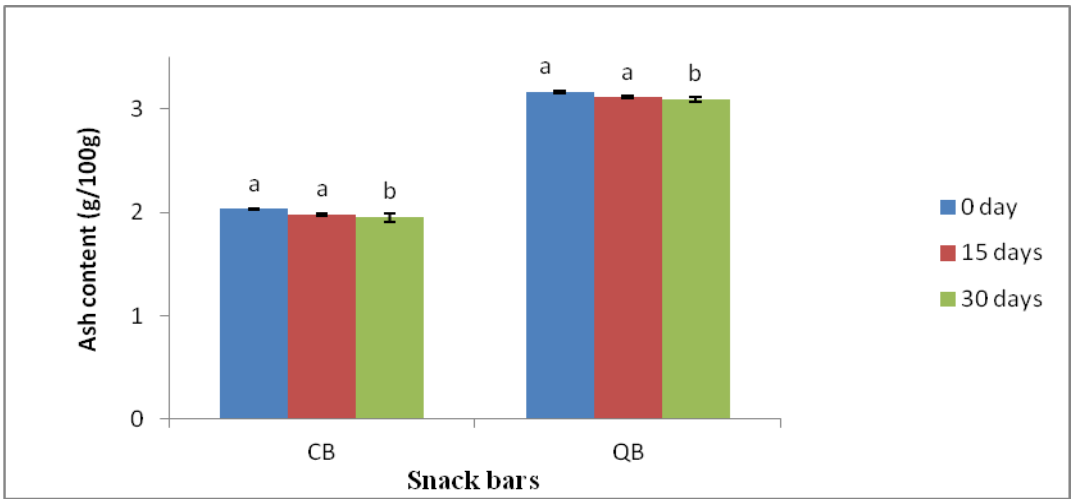

Figure 4.2: Effect of storage period on ash content of snack bars.

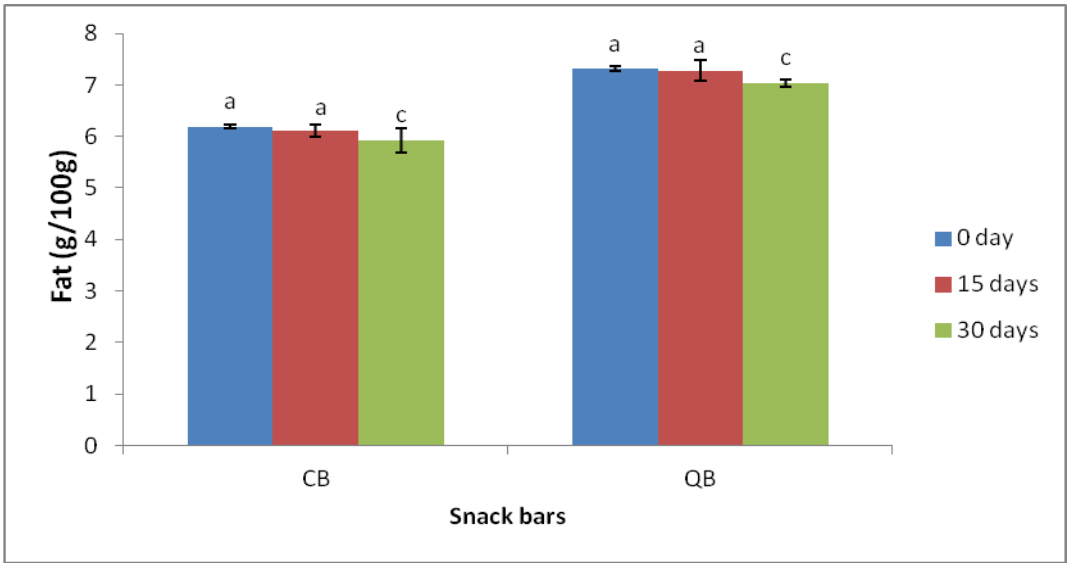

Figure 4.3: Effect of storage period on fat content of snack bars.

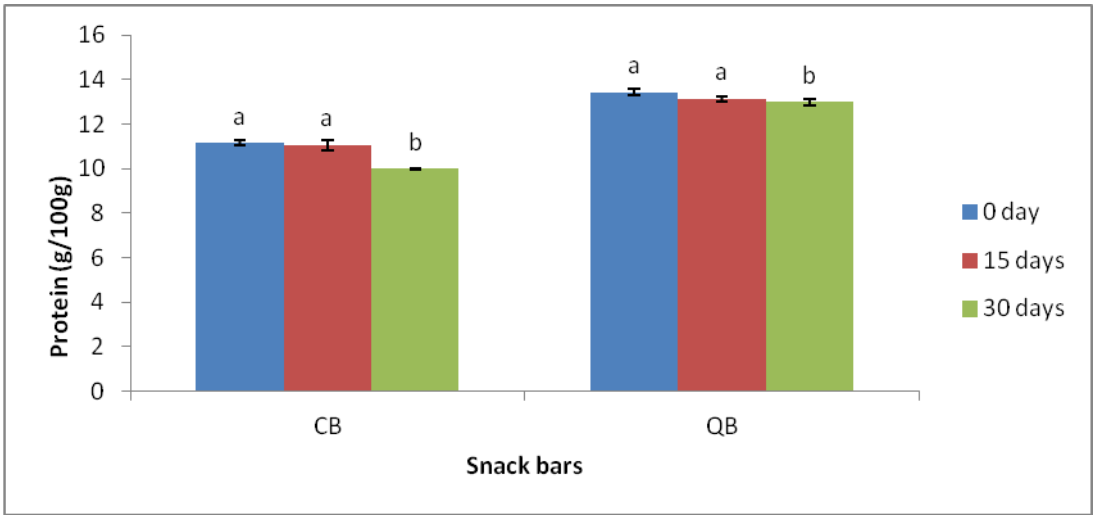

Figure 4.4: Effect of storage period on protein content of snack bars.

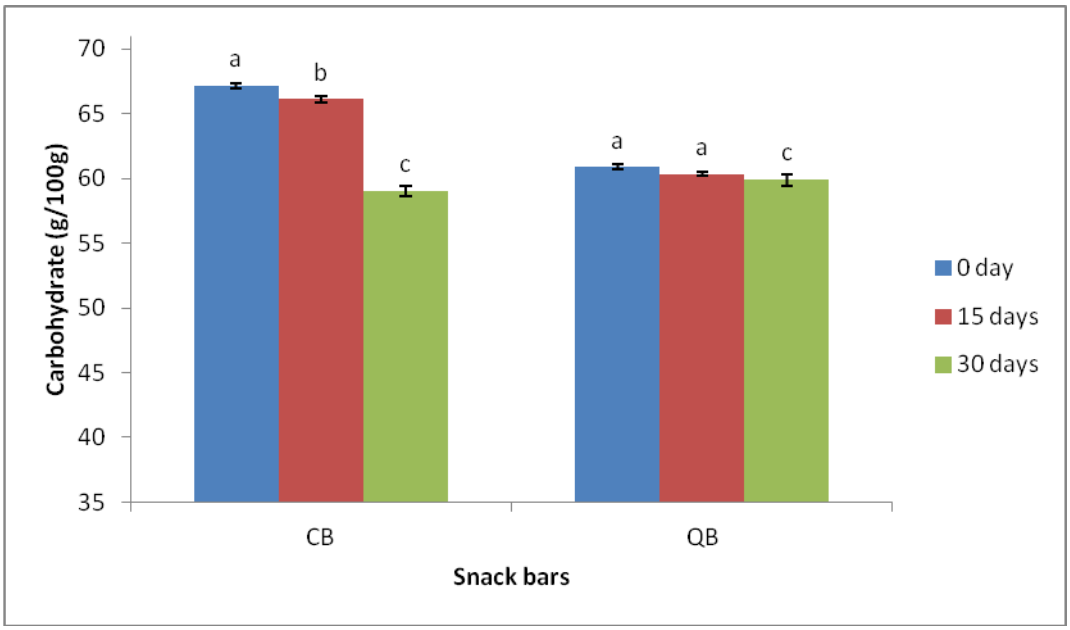

Figure 4.5: Effect of storage period on carbohydrate content of snack bars.

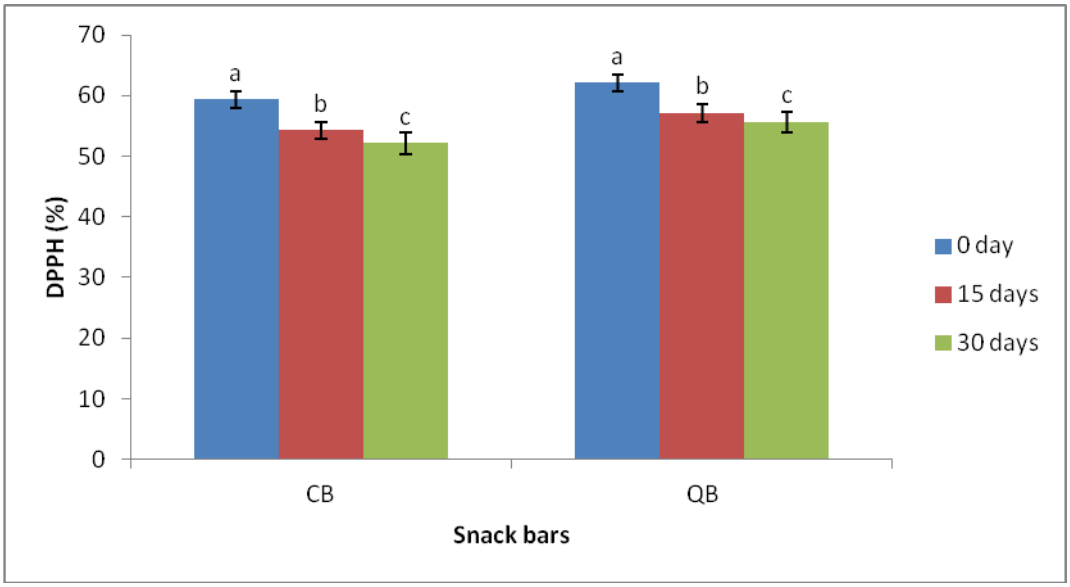

Figure 4.6: Effect of storage period on antioxidant activity (DPPH) of snack bars.

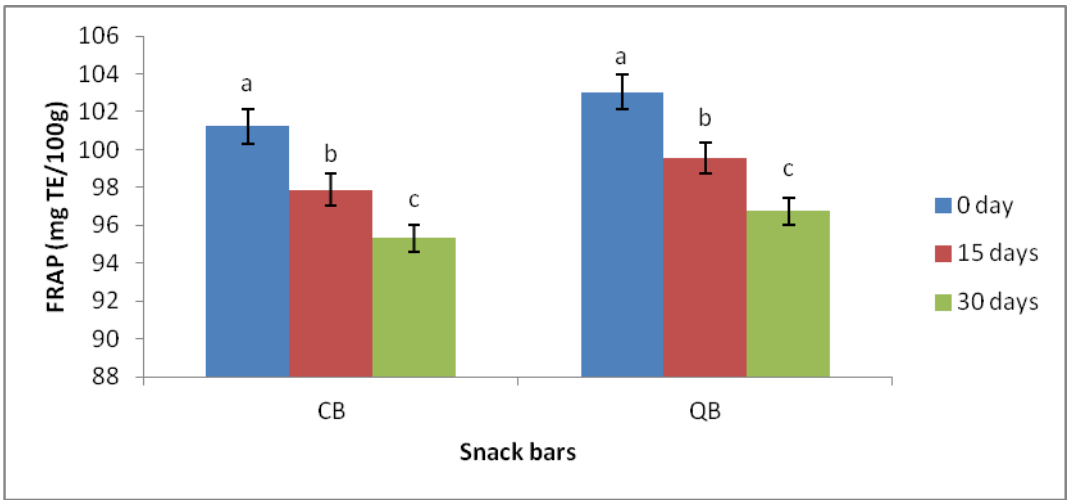

Figure 4.7: Effect of storage period on antioxidant activity (FRAP) of snack bars.

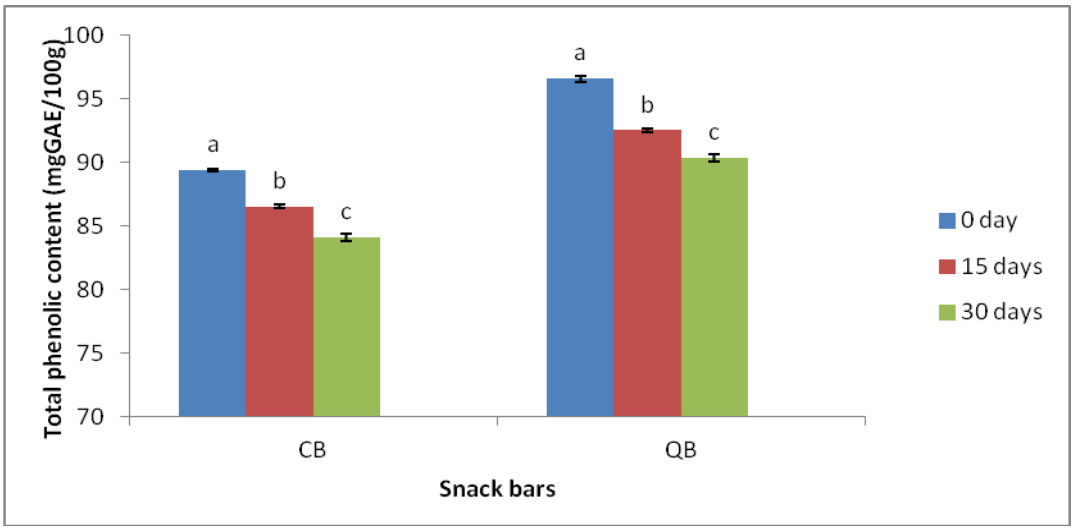

Figure 4.8: Effect of storage period on total phenolic content of snack bars.

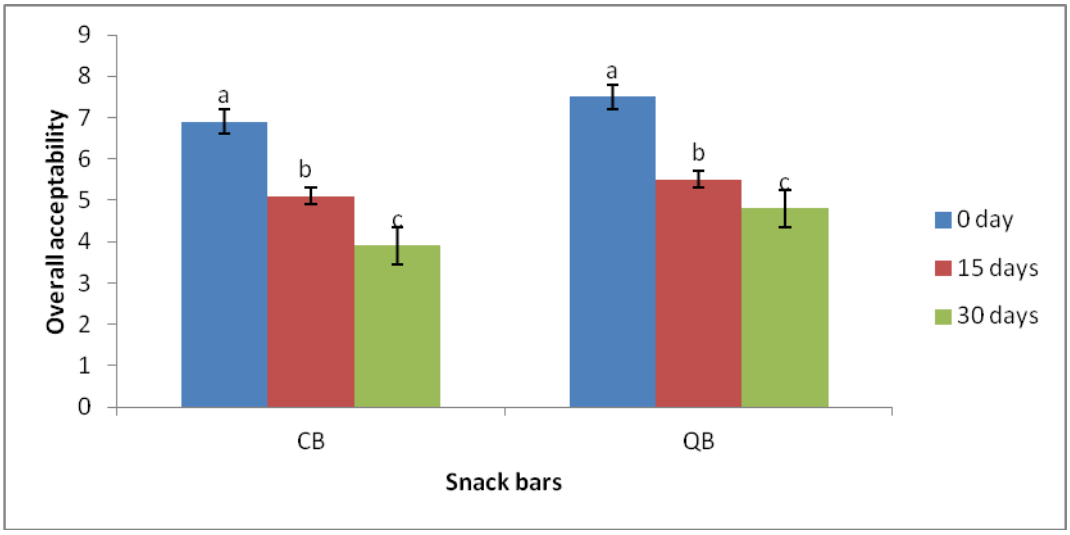

Figure 4.9: Effect of storage period on overall acceptability of snack bars.

decrease (P<0.05) in carbohydrate content after 15 days and a significant decrease after 30 days. This may be due to activation of enzyme, α-amylase, upon increase in moisture content, which results in degradation of starch (Rosa et al., 2004).

4.10.4.6 Effect on antioxidant activity

Figure 4.5 and Figure 4.6 represent effect of storage on antioxidant activity measured by DPPH and FRAP method, respectively. A significant decrease (P<0.05) in antioxidant activity of snack bars was observed after storage period of 15 and 30days. Antioxidant activity of a product depends on storage temperature, moisture content and surface area of food product in contact with air (Sharma et al., 2015). The observed decrease in antioxidant activity may be due to oxidation of lipids due to large surface area of snack bars (Maisuthisakul et al., 2007). The results correspond well with the decrease in fat content of snack bars due to increased lipid oxidation, which is mainly due to retarded antioxidant activity.

4.10.4.7 Effect on total phenolic content

Figure 4.7 depicts effect of storage period on total phenolic content of snack bars. A significant decrease (P<0.05) in total phenolic content of snack bars was observed after storage period of 15 and 30days. The decrease in total phenolic content upon storage may be due to hydrolysis of phenolic acids present in snack bars (Wong et al., 2009).

4.10.4.8 Effect on overall acceptability

Figure 4.8 represents overall acceptability of snack bars. A significant decrease ($P<0.05$) in over acceptability of snack bars was observed after storage period of 15 and 30 days. The apparent reason behind decrease in overall acceptability is unpleasant mouthfeel caused by oxidative rancidity of the food product. The free radicals released after fat oxidation also result in colour detoriation of the product (Zamora and Hidalgo, 2005). Another reason behind decreased overall acceptability might be cross linkaging of oxidized lipids with protein present in food resulting in modification of product texture (Estevez et al., 2005). In general, although the overall acceptability was observed to decrease with storage period in both snack bars but overall acceptability of quinoa bar was more than the control bar after different storage intervals. Bars exhibited least overall acceptability after storage period of 30 days. According to the scoring on nine point hedonic scale, overall acceptability of quinoa bars indicated "like very much" initially and decreased after 15 and 30 days indicating "neither like nor dislike" and "dislike slightly", respectively, while the overall acceptability of control bar decreased from "like slightly" initially to "neither like nor dislike" to "dislike moderately" after 15 and 30 days, respectively.

4.11 Development of crackers

4.11.1 Proximate composition of crackers

Proximate composition of wheat and quinoa incorporated crackers are shown in Table 4.18. Moisture content of crackers ranged from 3.51±0.07 g/100g to 3.55±0.10g/100g. The results are in agreement with the moisture content of various types of crackers reported by Owusu et al., 2011. Although the moisture content of quinoa incorporated crackers was less than the wheat (control, T_0) crackers, but the difference observed was statistically non-significant ($P<0.05$). This may be due to similar cooking and temperature conditions while preparation of all the crackers. Ash, fat and protein was observed to increase with increase in the ratio of quinoa incorporation to the crackers. Ash content of wheat cracker was significantly lower ($P<0.05$) than the 20 and 40% quinoa incorporated crackers but was statistically similar in crackers incorporated with 20 and 40% quinoa flour. Increase in ash content of wheat-quinoa blend with increase in quantity of quinoa incorporation has also been reported by Enriquez et al., 2003.

Protein content of quinoa crackers was higher than that of wheat crackers. The results are supported by the reported higher protein content in quinoa seeds as compared to the other common cereals like wheat (Arneja et al., 2015). Enriquez et al., 2003 also reported better protein quality of food products after incorporation of quinoa to wheat flour.

Table 4.18: Proximate composition of crackers.

Crackers	Moisture (g/100g)	Ash (g/100g)	Fat (g/100g)	Protein (g/100g)	Carbohydrate (g/100g)	Fiber (g/100g)
T_0	3.55±0.10 [a]	1.93±0.31 [a]	4.28±0.03 [a]	10.6±0.08 [a]	80.10±1.03 [a]	2.10±0.21 [a]
T_1 (80:20)	3.51±0.07 [a]	3.01±0.08 [b]	4.31±0.10 [a]	11.9±0.05 [b]	77.13±0.01 [b]	2.17±0.26 [a]
T_2(60:40)	3.53±0.01 [a]	3.22±0.08 [c,b]	4.46±0.35 [a]	12.21±0.08 [c]	77.5±0.35 [c,b]	2.29±0.04 [a]

Highest carbohydrate content, 80.10±1.03 was reported in wheat crackers. Carbohydrate content was found to decrease with the increase in the ratio of quinoa incorporation to the crackers. This may be due to observed increase in fat and protein content. The result correspond well to the findings of Ibrahium, 2015 who reported higher carbohydrate content in wheat biscuits as compared to biscuits supplemented with 20 and 40% quinoa flour.

Fiber content was lowest, 2.10±0.21g/100g in wheat (control, T_0) cracker and was found to increase with incorporation of quinoa but was statistically similar ($P<0.05$) in all the crackers. The results are in line with the findings of Jancurova et al., 2009 who reported higher crude fiber content in quinoa as compared to the wheat.

4.11.2 Total phenolic content (TPC) and antioxidant activity

The total phenolic content (TPC) and antioxidant activity of crackers is shown in Table 4.19. The total phenolic content of wheat cracker (control, T_0) was observed

as 88.16±0.02 mg GAE/ 100g. The results are similar to the findings of Sedej, 2011 who reported 84 to 148 mg GAE/ 100gm total phenolic content in wheat crackers used as experimental control in their study. The TPC was found to increase significantly ($P<0.05$) with incorporation of quinoa flour to the crackers and was directly related to ratio of quinoa flour added. Crackers incorporated with quinoa flour showed 3.5 to 7% higher TPC than the wheat cracker (control).The results correspond well to the findings of Jubete et al., 2010 who reported higher total phenolic content of quinoa as compared to the wheat. Brend et al., 2012 also reported increase in total phenolic content of quinoa as influenced by baking. Highest TPC (94.25±0.14 mg GAE/100g) was observed in cracker incorporated with 40% quinoa flour (T_2).

Table 4.19: Total phenolic content (TPC) and antioxidant activity of crackers

Cracker	**Antioxidant Activity**		**Total phenolic Content (mg GAE/100g)**
	DPPH (%)	**FRAP (mg Te/100g)**	
T_0	41.68±0.46 [a]	96.29±0.30 [a]	88.16±0.02 [a]
T_1	45.93±0.14 [b]	109.12±0.04 [b]	91.32±0.51 [b]
T_2	50.13±0.90 [c]	118.08±0.15 [c]	94.25±0.14 [c]

The antioxidant activity of wheat cracker (control, T_0) was observed as 41.68±0.46 % (by DPPH method) and 96.29±0.30 mgTE/100g (by FRAP method). Antioxidant activity was observed to increase significantly ($P<0.05$) with incorporation of quinoa flour. The results are supported by the findings of Sedej et al., 2011 who reported increased antioxidant activity of buckwheat crackers as compared to the wheat crackers. Highest antioxidant activity was observed in crackers incorporated with 40% quinoa flour (50.13±0.90 % by DPPH method and 118.08±0.15 mg TE/ 100g by FRAP method). Increase in antioxidant activity upon incorporation of cereal and pseudocereal flours like sorghum and buckwheat has also been reported earlier by Chiremba et al., 2009 and Sedej et al., 2011.

4.11.3 Sensory evaluation

The data analysis of sensory evaluation, Table 4.20, revealed non-significant difference ($P<0.05$) in appearance and colour of all crackers. As compared to the wheat cracker ($T_{0,}$ control), texture of quinoa incorporated crackers was more acceptable. This may be due to better water holding capacity of quinoa as compared to wheat, which enhances evenness in consistency and hence imparts better texture to resultant product (Inglett et al., 2015).

Table 4.20: Sensory evaluation of crackers

	Appearance	**Colour**	**Texture**	**Mouthfeel**	**Flavour**	**Taste**	**Overall Acceptability**
T_0	7.3±0.70 ^{a}	6.5±0.8 ^{a}	6.9±0.5 ^{a}	7.1±0.78 ^{a}	6.2±0.9 ^{a}	6.1±1.05 ^{a}	6.5±0.94 ^{a}
T_1	7.7±0.40 ^{a}	7.2±0.6 ^{a}	7.2±0.4 ^{b}	7.5±0.5 ^{b}	7.7±0.4 ^{b}	8.0±0.5 ^{b}	7.6±0.58 ^{b}
T_2	7.6±0.50 ^{a}	7.3±0.7 ^{a}	7.3±0.52 ^{c,b}	7.4±0.72 ^{c,b}	8.2±0.6 ^{c,b}	8.44±0.52 ^{c,b}	7.94±0.71^{c,b}

Similarly, mouthfeel, flavor, taste of quinoa incorporated crackers (20 and 40%, respectively) was significantly highly ($P<0.05$) acceptable than the wheat crackers. The results are supported by findings of Elsohiamy et al., 2015 who reported water and oil absorption characteristics of quinoa better than the wheat and similar to soy, which is responsible for better mouthfeel and taste of the product. Cracker incorporated with 40% quinoa flour received highest score for texture, mouthfeel, flavor and taste on nine point hedonic scale which was although non-significant to the scores received by crackers incorporated with 20% quinoa flour. The results also indicated that the increase in ratio of quinoa incorporation resulted in increase in panelist's rating for texture, mouthfeel, flavor and taste of the resultant quinoa cracker. Overall acceptability was lowest for the wheat ($T_{0,}$ control) crackers and highest for crackers incorporated with 40% quinoa flour (T_2). The findings are in

line with the results reported by Bhathal et al., 2015 who revealed higher overall acceptability of food products prepared with quinoa than the control products. Statistically, the results indicated significantly lower ($P<0.05$) overall acceptability of wheat cracker ($T_{0,}$ control) as compared to quinoa incorporated crackers (20 and 40%), while overall acceptability of cracker incorporated with 40% quinoa flour was although higher but statistically non-significant to the cracker incorporated with 20% quinoa flour. The results are supported by Bahaduri and Navder, 2014 who demonstrated positive effect of use of quinoa flour in baked food products for better sensory characteristics and higher consumer acceptability.

4.11.4 Storage study

4.11.4.1 Effect proximate composition of crackers

Figure 4.10 represents moisture content of crackers as affected during storage period of 15 days. The figure depicts increase in moisture content of all the crackers upon storage. A non-significant increase ($P<0.05$), in moisture content was observed in wheat cracker (control, T_0) while the moisture content of quinoa incorporated crackers increased significantly ($P<0.05$) after 15 days. Highest increase in moisture content was observed in T_2 (3.6%) followed by T_1(3.3%) and T_0 (0.6%). Increased moisture content of quinoa crackers over the period may be due to the changes in water holding capacity of quinoa during the storage as already reported by James et al., 2009. Increase in moisture content might also be due to water vapour transmission through the polythene packaging material used to store the crackers (Bertrand et al., 2013). As, the moisture content of food is inversely related to its shelf life (Genkawa et al., 2008), the results depict lower shelf life of formulated wheat and quinoa crackers.

4.4.11.2 Effect on crude ash

Figure 4.11 represents ash content of crackers as affected during storage period of 15 days. Ash content of all the crackers was observed to decrease non-significantly ($P<0.05$) upon storage for 15 days. Decrease in ash content during storage period has also been rported by Nadarajah et al., 2015 in coconut cookies. Highest decrease in ash content was observed in T_1 (1.99%) followed by T_2 (1.24%) and T_0 (1.04%). This decrease in ash content may be due to mineral binding properties of by products formed during millard reaction (Nadarajah et al., 2015). Decrease in

ash content may also be due to increase in moisture content, which favours microbiological growth. During their growth period, the microbes utilize minerals and other nutrients, which result in decrease in ash content (Adams and Moss, 2005).

4.11.3 Effect on crude fat

Figure 4.12 represents changes in fat content of crackers during the storage period of 15 days. Fat content of wheat cracker (control, T_0) and cracker incorporated with 20% quinoa (T_1) decreased non-significantly by 0.9 and 0.4%, respectively, while a significant decrease of 2.6% was observed in cracker incorporated with 40% quinoa (T_2). Decrease in fat content of cereals over a period of time during storage has also been reported by Sharma et al., 2015. Fat in presence of moisture causes tenderness in baked food products. Crackers are highly susceptible to lipid oxidation due to their larger surface area which is in contact with air and moisture (Maisuthisakul et al., 2007). Decrease in fat content can be attributed to increased activity of lipase which is highly influenced by moisture content of food (Agrahar-Murgkar and Jha, 2011). Lipase is responsible for oxidative rancidity leading to hydrolysis of fat present in food matrix and formation of free fatty acids which also imparts off flavor to the food product (Adawiyah et al., 2012).

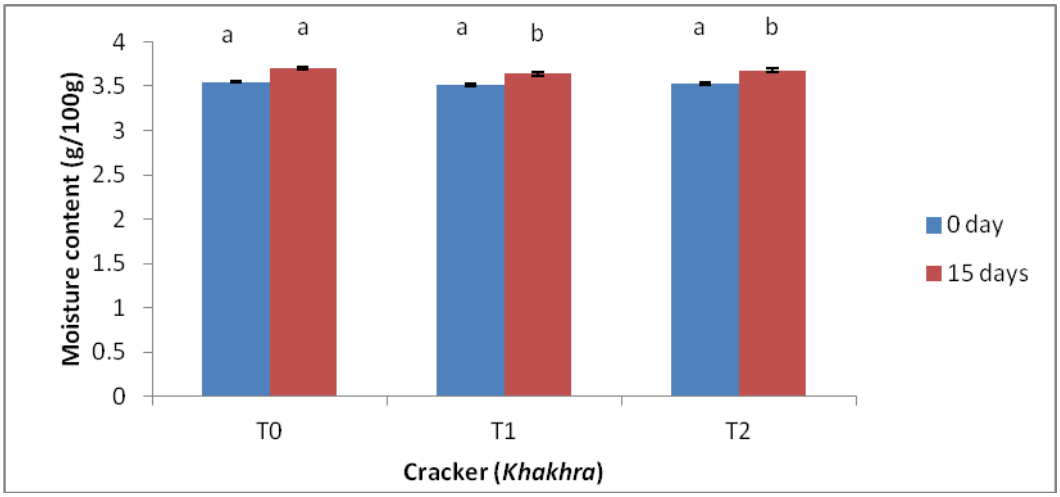

Figure 4.10: Effect of storage period on moisture content of crackers

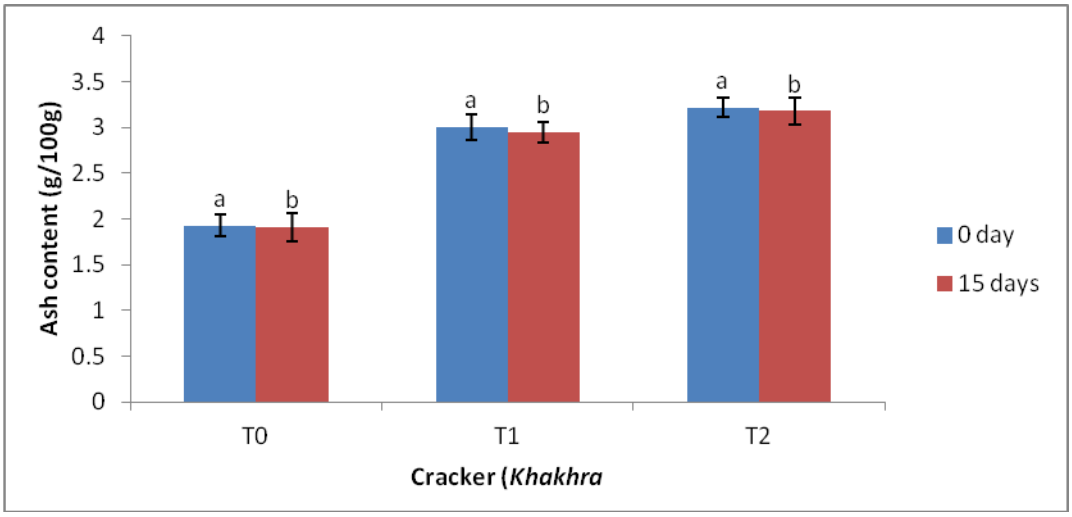

Figure 4.11: Effect of storage period on ash content of crackers.

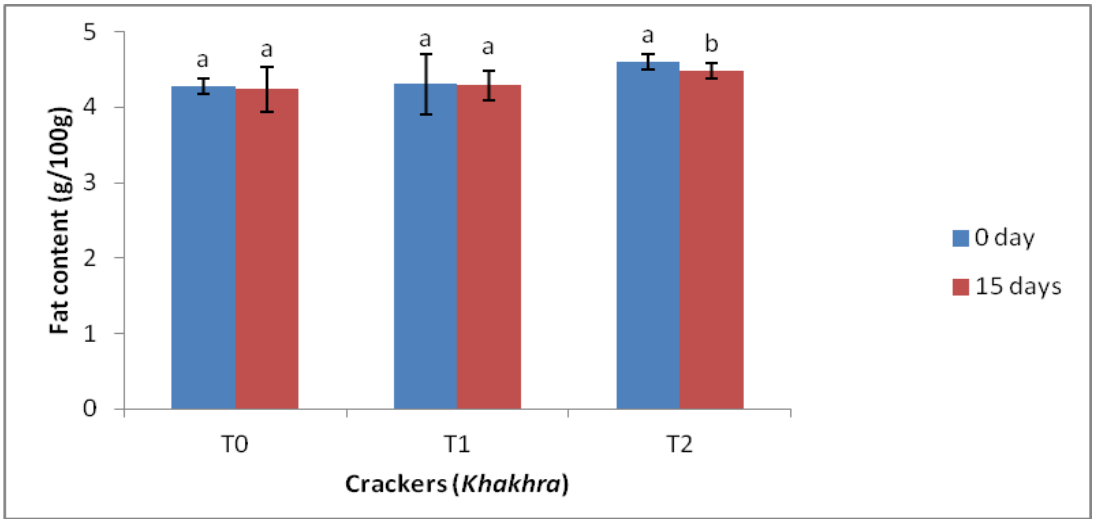

Figure 4.12: Effect of storage period on fat content of crackers.

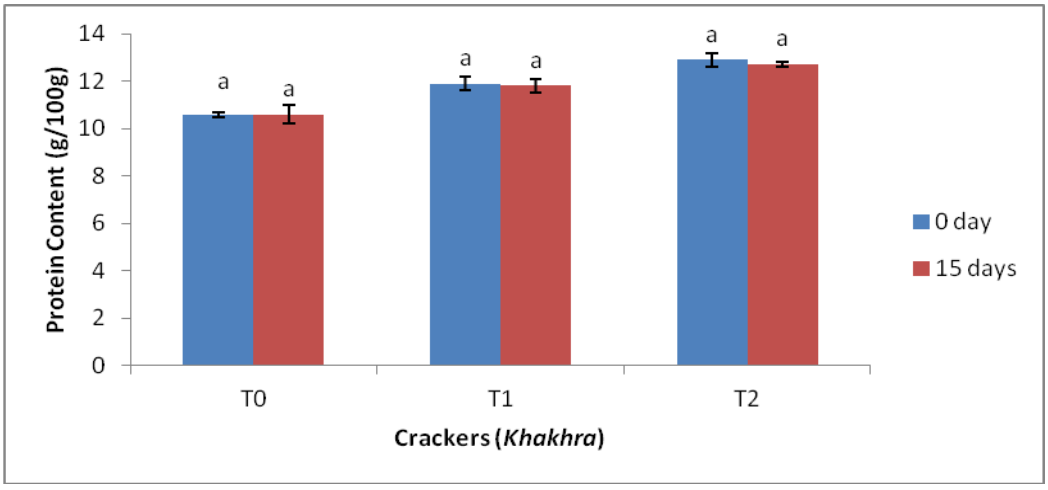

Figure 4.13: Effect of storage period on protein content of crackers.

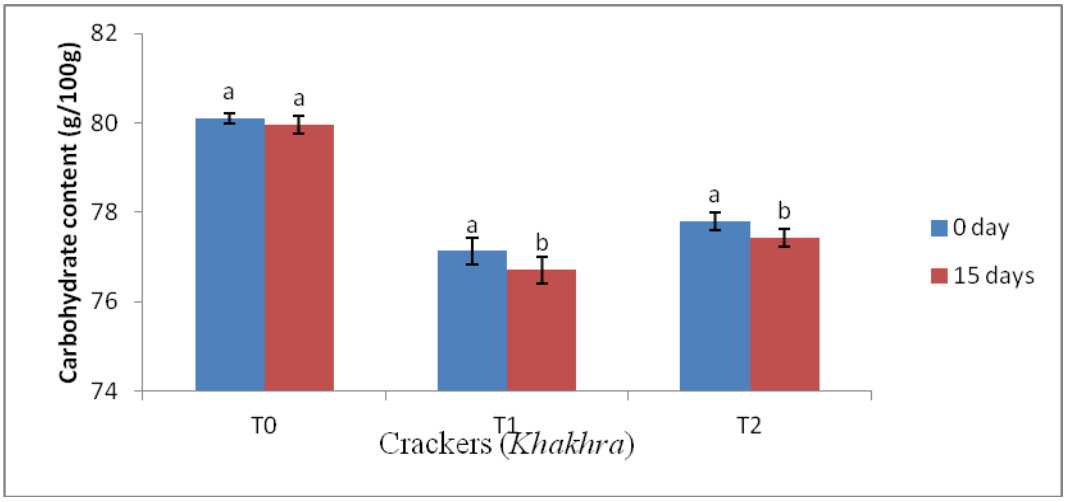

Figure 4.14: Effect of storage period on carbohydrate content of crackers

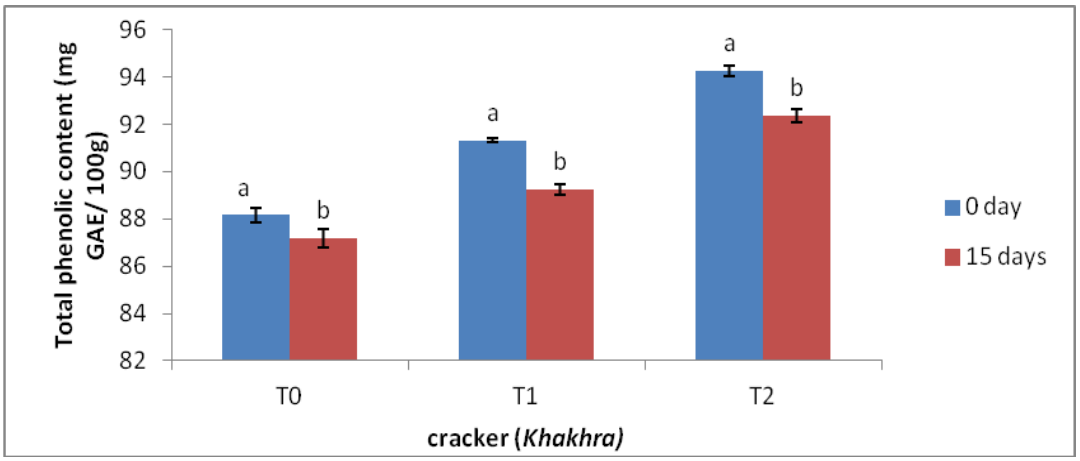

Figure 4.15: Effect of storage period on total phenolic content of crackers.

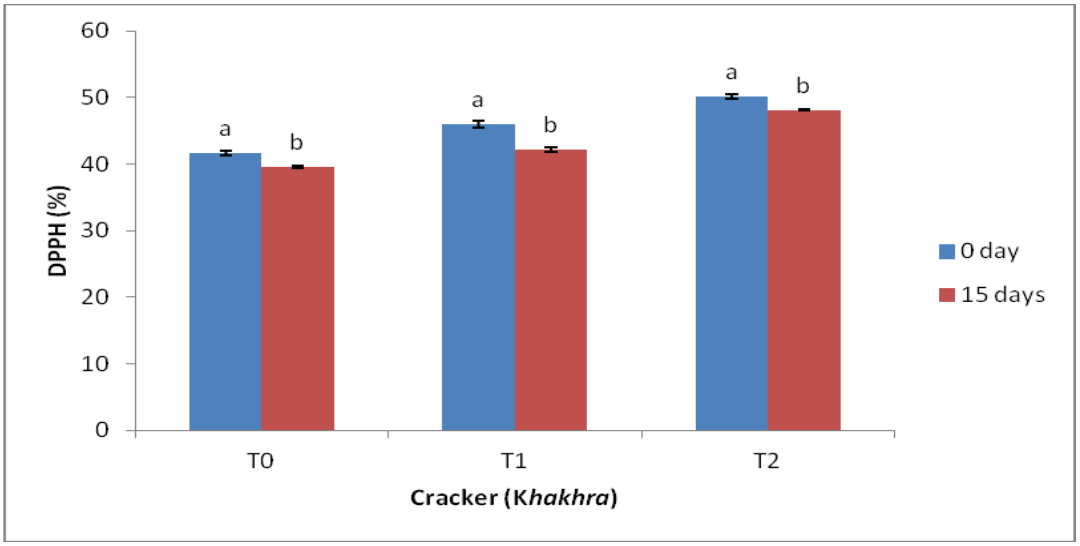

Figure 4.16: Effect of storage period on antioxidant activity (DPPH) of crackers.

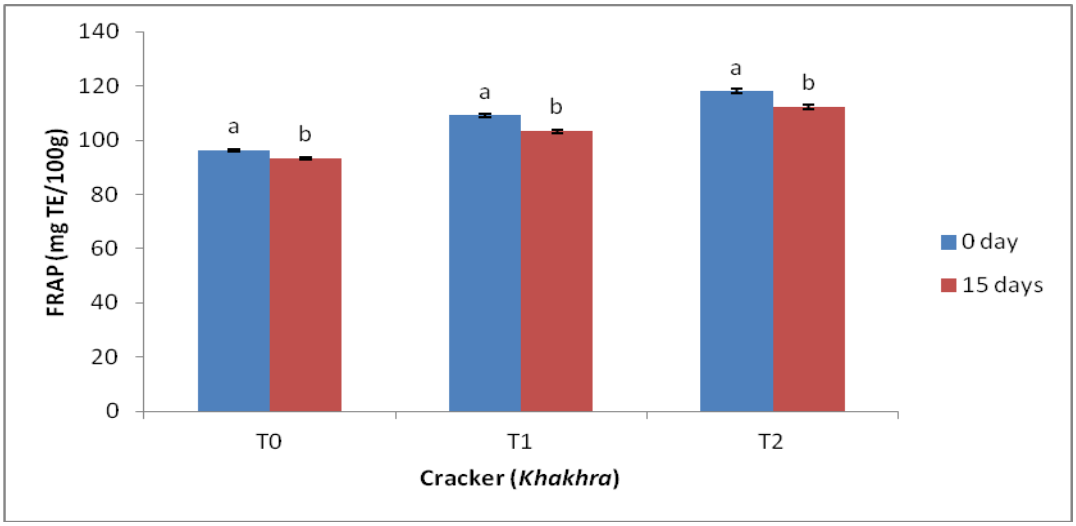

Figure 4.17: Effect of storage period on antioxidant activity (FRAP) of crackers.

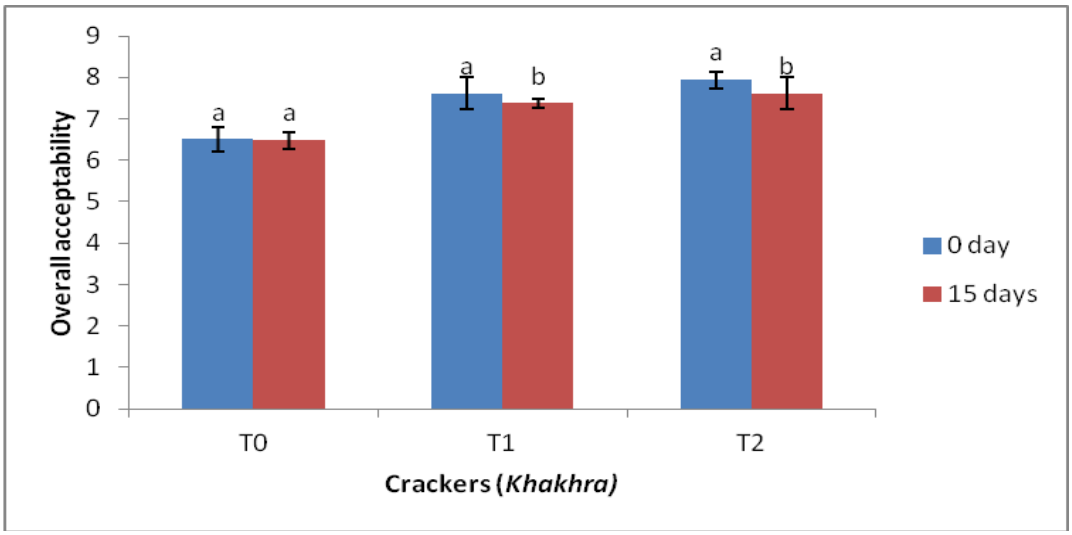

Figure 4.18: Effect of storage period on overall acceptability of crackers.

4.11.4 Effect on protein

Figure 4.13 represents effect of storage period on protein content of crackers. In general, 1 to 1.5% non-significant decrease ($P<0.05$) was observed in protein content of crackers after storage period of 15 days. Decrease in protein content during storage of pumpkin incorporated cake has also been reported by Bhat and Bhat, 2013. Decrease in protein content may be attributed to increase in proteolyic activity due to increase in moisture content (Butt et al., 2004). Another cause of decrease in protein content can also be ascribed to susceptibility of baked food products to millard reaction, which results in protein detoriation (Nadarajah et al., 2015).

4.11.5 Effect on carbohydrate

Figure 4.14 represents effect of storage period on carbohydrate content of crackers. Overall, carbohydrate content of crackers was observed to decrease after storage period of 15 days. A non-significant decrease ($P<0.05$), in carbohydrate content was observed in wheat cracker (control, T_0) while the carbohydrate content of quinoa incorporated crackers decreased significantly ($P<0.05$) after 15 days. This may be due to activation of enzyme, α-amylase, upon increase in moisture content, which results in degradation of starch (Rosa et al., 2004). Furthermore, decrease in carbohydrate of crackers was observed to follow statistically similar trend to increase in moisture content after storage period of 15 days.

4.11.6 Effect of total phenolic content (TPC)

Figure 4.15 depicts effect of storage period on total phenolic content of crackers. A significant decrease ($P<0.05$) in total phenolic content of crackers was observed during storage period of 15 days. The highest decrease in total phenolic content was reported in crackers incorporated with 20% quinoa (2.3%) followed by 40% quinoa incorporated cracker (2.1) and wheat (control, T_0) cracker (1.5%). The decrease in total phenolic content upon storage may be due to hydrolysis of phenolic acids present in cracker (Wong et al., 2006).

4.11.7 Effect on antioxidant activity

Figure 4.16 and Figure 4.17 represent effect of storage on antioxidant activity measured by DPPH and FRAP method, respectively. A significant decrease ($P<0.05$) in antioxidant activity of crackers was observed during storage period of 15 days. Antioxidant activity of a product depends on storage temperature, moisture content and surface area of food product in contact with air (Decker et al., 2010 and Sharma et al., 2015). The observed decrease in antioxidant activity may be due to oxidation of lipids due to large surface area of cracker (Maisuthisakul et al., 2007). The results correspond well with the decrease in fat content of cracker, reported in this study, which may be probably due to increased lipid oxidation, generally caused by decreased antioxidant activity. Thus, reduction in total phenolic content might be the apparent reason behind decrease in antioxidant activity of crackers during storage.

4.11.8 Effect on overall acceptability

Figure 4.18 represents overall acceptability of crackers. Overall acceptability of wheat (control, T_0) had no significant effect ($P<0.05$) after storage period period of 15 days while overall acceptability of crackers incorporated with 20 and 40% quinoa decreased significantly ($P<0.05$). The apparent reason behind decrease in overall acceptability unpleasant mouthfeel caused by oxidative rancidity of the food produt. The free radicals released after fat oxidation also result in colour detoriation of the product (Zamora and Hidalgo, 2005). Another reason behind decreased overall acceptability might be cross linkaging of oxidized lipids with protein present in food resulting in modification of product texture (Estevez et al., 2005). In general, highest decrease in overall acceptability, 4.1% was observed in overall acceptability of 20% quinoa incorporated cracker followed by 3.9% decrease in 40% quinoa incorporated cracker and 1.5% decrease in wheat (control, T_0).

4.12 Beverages

4.12.1 Physical analysis

Physical analysis Parameters are tabulated in Table 4.21. pH ranged from 6.2± 0.01 to 6.5 ± 0.10. Statistically, no significant difference ($P<0.05$) was observed in pH content of all quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Thuresson, 2015 also reported 5.4 to 6.4 pH value of quinoa beverage formulated from white *Chenopodium quinoa* seeds.

Total soluble solid content ranged from 9.08± 0.02 to 9.45 ± 0.01%. No significant difference ($P<0.05$) was observed in total solid of quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Results reported in our study are quite similar to total solid content (%) in soymilk as reported by Kim et al., 2012.

Viscosity of quinoa beverages ranged from 15.02 ± 0.01 to 15.31 ± 0.04 cp. No significant difference ($P<0.05$) was observed in viscosity of quinoa beverages prepared from raw, soaked and germinated quinoa seeds. The results are similar to the viscosity (13 cp) of quinoa beverage reported by Thuresson, 2015. The slight difference noted, on higher side, in viscocity of quinoa beverage prepared in this

study may be due to addition of hydrocolloid, xanthan gum, which tends to thicken and increase the viscosity of the resultant product (Saha and Bhattacharya 2010).

4.12.2 Proximate analysis of beverages

Moisture content of raw, soaked and germinated quinoa beverage (Table 4.21) ranged from 84.01± 0.01 to 84.07±0.13 g/100ml. The results are similar to the moisture content of fermented quinoa bevereges reportd by Bianchi et al., 2014. The results also correspond well to the 88.9 g/100ml moisture content of soy beverage reported by Jackson et al., 2002. Moisture content of all the formulated quinoa beverages was found to be statistically similar ($P<0.05$). Moisture content of quinoa beverage reported in this study is less than the moisture content of quinoa beverage reported by Thuresson, 2015, which may be due addition of hydrocolloid, xanthan gum, which serves as a thickening agent (Panovska et al., 2012).

Protein content of quinoa beverages ranged from 0.68 to 1.5 g/100 ml. The values of protein content in quinoa beverages reported in this study are similar to the protein content in quinoa milk (0.49- 1.72 g/100g, *wb*) reported by Pineli et al., 2015. The results are also supported by findings of Thuresson, 2015 who reported 1.43g/100ml protein content in quinoa beverage. Significant increase ($P<0.05$) in protein content was reported in GQB as compared to RQB. This may be due to increased activity of enzyme protease, during germination of seed, leading to degradation of peptides to amino acids and further synthesis of new protein (Laetitia et al., 2005). Protein content of quinoa beverage was observed to be less than the protein content of soy beverages (1.68 to 2.36 g/100ml) as reported by Terhaag et al., 2013. This may be due to higher protein content of soyabean as compared to quinoa (Jancurova et al., 2009).

Carbohydrate content of quinoa beverages ranged from 14.9±0.1 to 16.2±0.02g/ 100 ml with trend in increasing order being GQB<SQB<RQB. Statistically, the difference between carbohydrate content of all quinoa beverages was signinificant ($P<0.05$). The results were close to the the carbohydrate content in quinoa milk (14.7 g/100g) reported by Pineli et al., 2015. Carbohydrate content of SQB and GQB was observed to be 4 and 8% to be lower with respect to RQB, respectively. Similar decrease in carbohydrate content upon soaking and germination has been

reported by Uppal and Bains, 2012. This decrease may be accounted to hydrolysis of starch during process of soaking and germination (Tang et al., 2015).

Fat content of beverages ranged from 0.23 to 0.93 g/ 100ml. GQB was reported with least fat content which may be due to use of fat as energy during grain sprouting (Kayembe and Rensburg, 2013). RQB and SQB were reported with significantly higher ($P<0.05$) fat content as compared to GQB. The results were similar to the lipid content in quinoa milk reported by Pineli et al., 2015.

Thuresson, 2015 also reported 1.43g/100ml of fat content in quinoa beverage. The difference may be due to varietal difference in *Chenopodium quinoa* seeds used for the formulation of beverage.

Ash content of quinoa beverages ranged from 0.11±0.01 to 0.28 gm/100ml. The results are similar to the ash content of beverages prepared with 100% quinoa extracts, reported by Bianchi et al., 2015. A significant increase in ash content ($P<0.05$) was reported in GQB as compared to RQB. This may be due to increase in ash content upon germination as reported by Echendu et al., 2009.

4.12.3 Total phenolic content and Antioxidant activity

Predominantly, all quinoa beverages, as indicated in Figure 4.19, had total phenolic content well correlated to its anti oxidant activity. Total phenolic content of quinoa beverages ranged from 1.9 to 2.4 mg GAE/ g. The results are close to total phenolic content in quinoa beverage (1.52 mg GAE/ g) reported by Thuresson, 2015. Germinated quinoa beverage (GQB) showed highest phenolic content (2.4 ± 0.2 mg GAE/g), followed by SQB (2.1 ± 0.2 mg GAE/g) and RQB (1.0 ± 0.3 mg GAE/ g). Megat et al., 2016, also reported an increment in total phenolic content upon germination. Higher phenolic content in germinated quinoa beverage might be attributed to better liberation of bound phenolic contents from the cereal matrix during the process of germination (Sharma et al., 2015).

Antioxidant activity of quinoa beverages (Figure 4.19), as determined by DPPH method ranged from 52 to 92%. Potential to inhibit DPPH free radical i.e. the anti oxidant activity of quinoa beverages followed the same trend as their total phenolic content i.e. GQB>SQB>RQB. Also, the antioxidant activity was found to have good correlation with the total phenolic content ($r = 0.95$, $P<0.05$), which suggests

potential contribution of phenolic compounds in quinoa to its antioxidant activity. Good pearson coefficient correlation between antioxidant activity and total phenolic content has also been reported in soy beverages by Durazzo et al., 2015. The findings are also supported by linear correlation between antioxidant activity and phenolic compounds in germinated quinoa reported by Carciochi et al., 2014b.

Table 4.21: Physical and proximate analysis of beverages

Quinoa bevera-ges	pH	Total soluble Solid (% Brix)	Viscocity (cp)	Serum Separation		Moisture (g/100ml)	Protein (g/100ml)	Carbohydrate (g/100ml)	Fat (g/100ml)	Ash (g/100 ml)
				Water phasewithout xanthan Gum (cm)	Water phase with Xanthan gum					
RQB	6.2± 0.01[a]	9.08± 0.02 [a]	15.31 ± 0.04[a]	15.3±0.02	5.4±0.04	84.01±0.02 [a]	0.68± 0.01[a]	16.2 ± 0.02[a]	0.93 ± 0.02[a]	0.13± 0.04[a]
SQB	6.5 ± 0.10[b]	9.45 ± 0.01[b]	15.12± 0.01[b]	14.8±0.13	4.2±0.11	84.05±0.11 [a]	1.2 ± 0.10[b]	15.5± 0.01[b]	0.81 ± 0.01[b]	0.11± 0.01[b]
GQB	6.3 ± 0.03[a,c]	9.39 ± 0.04[c]	15.02 ± 0.01[c]	14.5±0.11	4.3±0.16	84.07±0.13 [a]	1.5 ± 0.01[c]	14.90± 0.1[c]	0.23 ± 0.04[c]	0.28 ± 0.01[c]

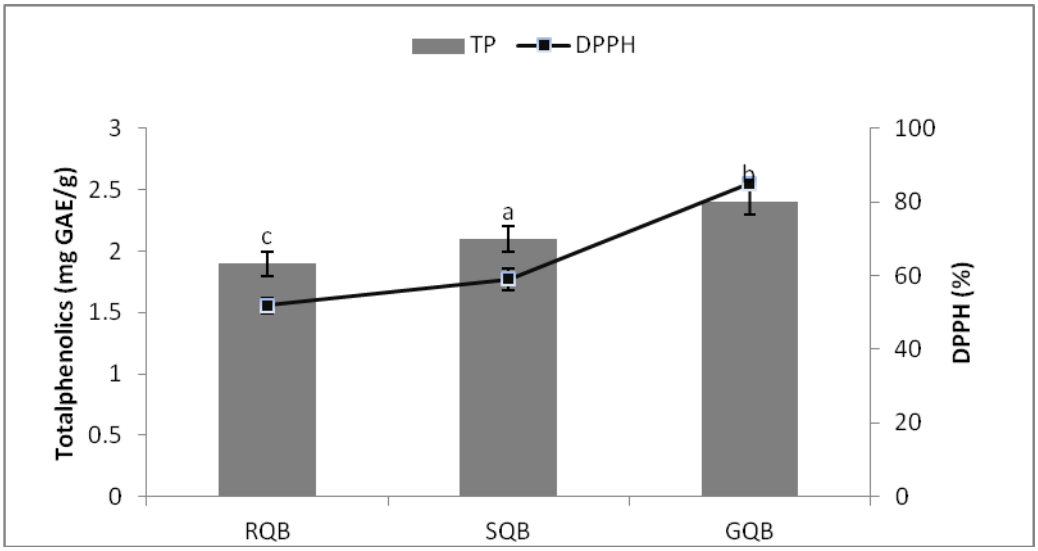

Figure 4.19: DPPH and total phenolic content of quinoa beverages.

4.12.4 Effect of addition of xanthan gum in quinoa beverages:

Xanthan gum has been used extensively in gluten free formulations (Lazaridou et al., 2007). Addition of 0.5% (*w/v*) xanthan gum increased the viscosity and helped in textural stabilization of the beverages by reducing serum separation. Serum separation reduction ranged from 64.7 to 71.6%. The results are supported by findings of Panovska et al., 2010 who reported reduced rate of phase separation on addition of 0.1-0.8% (*w/w*) xanthan gum in beverage. Xanthum gum has been reported as successful hydrocolloid for textural stabilization of beverages due to its peculiar structural characteristics (Desplanques et al., 2012).

4.12.5 Sensory Evaluation

Panelists opinion on positive (Color, flavor, texture, mouthfeel, taste, consistency) and negative aspect (after taste), of formulated quinoa beverages is indicated in spider diagram in Figure 4.20. Color aspect of all quinoa beverages ranged from 5.1±0.02 to 5.3±0.11 indicating "neither like nor dislike". Flavor of quinoa beverages ranged from 5.3±0.03 to 7.1±0.12. Flavor of RQB was rated as "dislike very much" as compared to SQB ("like slightly") and GQB ("like moderately"). Low consumer acceptance of quinoa beverage prepared from raw quinoa seeds due to the presence of strong beany flavor of quinoa has also been reported by Thuresson, 2015. Flavor aspect of GQB was rated similar to the soya beverage used as control. Texture and consistency of all quinoa beverages was rated as "like slightly." This may be due to addition of xanthan gum which is known to

Table 4.22: Sensory evaluation of quinoa beverages

Quinoa Beverage	Favourite (%)	Least favourite (%)	Overall acceptability
RQB	0	88	2.2 ± 0.1^{a}
SQB	24	4	3.9 ± 1.4^{b}
GQB	28	4	6.8 ± 2.5^{c}
Commercial Soya milk	32	4	6.9 ± 1.4^{c}

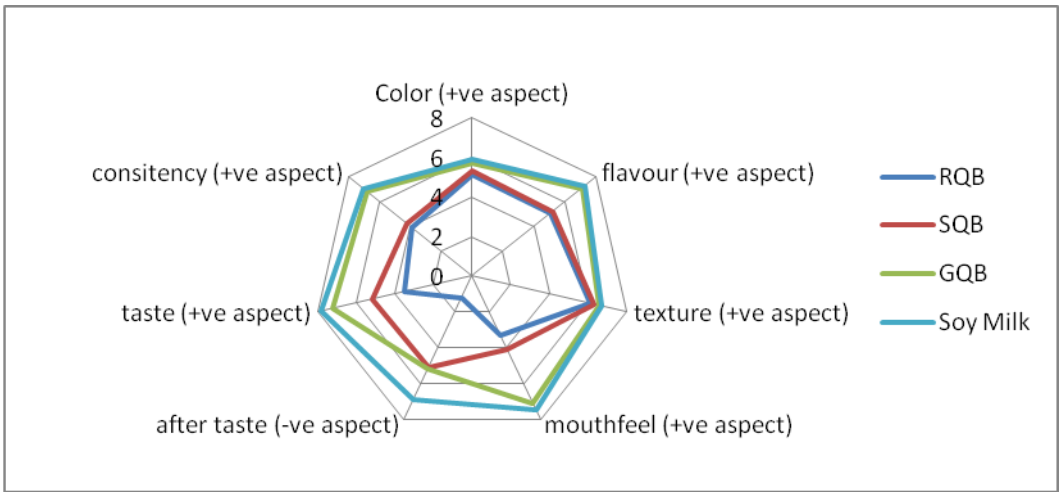

Figure 4.20: Panelists opinion on positive and negative aspects of quinoa beverages with respect to commercial soy milk.

improve texture and stabilize consistency of food products (Desplanques et al., 2012). Among all the beverages, RQB indicated lowest score on nine point hedonic scale for after taste (1.2±0.1), indicating "dislike extremely" and least good mouth feel indicating "dislike very much" (2.3±0.4). The after taste of raw quinoa beverage, as stated by panelists was "bitter". This may be due to presence of saponins in raw quinoa, which imparts bitter taste to quinoa (Miranda et al., 2014) and their lixiviation into water used for formulation of beverage during preparation. Similar negative comments about quinoa beverages from raw quinoa seeds have also been stated by Thuresson, 2015. After taste aspect SQB and GQB was better than RQB and marked as "neither liked nor disliked" and "like slightly," respectively. This is due to reduction in content of bitter taste imparting

antinutrients like saponins post processing methods like soaking and germination (Kayembe and Rensburg, 2011). In general, it was observed that GQB qualified all positive aspects and was rated similarly to the commercial soya milk used as control.

On evaluation of panelists choice of favorite and least favorite beverage (Table 4.22), it was observed that among quinoa beverages, RQB was rated as least favorite beverage by 88% panelists (22 out of 25) and GQB was most favorite beverage of 28% (7 out of 25) panelists. Overall acceptability of quinoa beverages, as indicated in Table 2, ranged from dislike very much to like moderately with acceptability trend in increasing order being RQB<SQB<GQB. RQB was disliked because of its bitter after taste and beany flavor while GQB was most liked among all quinoa beverages with overall acceptability score significantly similar ($P<0.05$) to that of commercial soya milk.

CHAPTER 5

SUMMARY AND CONCLUSION

1. Towards the accomplishment of objective one, the *Chenopodium quinoa* seeds were evaluated for their nutritional quality. The seeds were subjected to proximate, nutritional, phytochemical and *in vitro* analysis.

a) Indian *Chenopodium quinoa* seeds were processed by the domestic processes, namely, soaking and germination and compared with respect to raw and industrially processed seeds and evaluated for proximate, nutritional, and phytochemical composition.

i. **Proximate composition**:

The raw seeds were reported to have 11.03±0.08 g/100g of moisture content. A significant ($P<0.05$) difference in moisture content of raw, domestically processed and industrially processed seeds was observed. Soaking resulted in increase in 8.5% of moisture content while germination resulted in decrease in moisture content by 17.5%. Industrial processing of quinoa led to 8.5% decrease in moisture content.

The Carbohydrate content of raw quinoa seeds was 65.11±0.12 g/100g. The results were significantly ($P<0.05$) different between raw and domestically processed seeds. However no significant difference ($P<0.05$) in carbohydrate content of seeds subjected to soaking and germination was observed. Soaking and germination resulted in decrease of carbohydrate content by 1.6% and 2% respectively. The carbohydrate content of industrially processed and domestically processed seeds was significantly ($P<0.05$) different.

The raw Indian quinoa had 5.17±0.18 g/100g crude fat content. Soaking and germination of raw seeds caused significant decrease ($P<0.05$) in fat content by 20.5% and 32.5%. Industrially processed seeds had 1.6% reduced fat content as compared to the raw seeds. No significant ($P<0.05$) difference was observed in change in fat content of soaked, germinated and industrially processed quinoa seeds.

The raw quinoa seed was reported to have 12.54±0.03g/100g crude protein content. Crude protein content of raw, domestically processed and industrially processed quinoa seeds was significantly ($P<0.05$) different. Soaking and germination led to increase in protein content by 4.8% and 19.2%. Increase in protein content of industrially processed quinoa seeds was similar to the increase reported after soaking.

The raw seeds were reported to have ash content of 3.19±0.03 g/100g. Soaking resulted in 0.6% increase in ash content which was statistically non-significant ($P<0.05$) with respect to ash content of raw quinoa seeds while significant increase ($P<0.05$) in ash content, by 22.5%, was observed after germination. Industrially processed seeds were reported with 4.7% reduced ash content.

The raw quinoa seeds had crude fiber content of 2.22±0.01 g/100g. The fiber content of all quinoa seeds were significantly ($P<0.05$) different. Soaking led to 31.8% increase in fiber content and germination caused increase by 4%. Industrial processing led to 7.6% decrease in fiber content.

ii. Nutritional composition:

Raw quinoa seeds were reported to have 535.9±3.6 μg/100g of β- Carotene content. Domestic and industrial processing of seeds led to non-significant change ($P<0.05$) in contents of β- carotene. Soaking and germination led to 0.1% and 0.8% increase in β- carotene.

Raw quinoa seeds were reported with 10.26±0.17 g/100g of dietary fiber content. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) constituted about 71.9 to 73.3% and 26.6 to 28% of total dietary fiber, respectively. Domestic processing of seeds i.e. soaking and germination, led to 10% and 31% significant increase ($P<0.05$) in TDF content of quinoa seeds. Both the constituents of dietary fiber i.e. NDF (Lignin, cellulose, and hemicellulose) and ADF increased upon soaking and germination. Industrially processed seeds exhibited 9.9%, significant decrease ($P<0.05$) in TDF content.

Vitamin C content of raw quinoa seeds was found to be 13mg/100g. There was significant difference in vitamin C content of raw and domestically processed Indian quinoa seeds ($P<0.05$). Vitamin C content increased by 15% in soaked

quinoa seeds and by 46% in germinated quinoa seeds. A significant difference in Vitamin C content of raw and industrially processed seeds was also observed. Industrial processing decreased the vitamin C content by 30%.

Raw quinoa seeds were reported with 85.3±0.25 mg/100g calcium content. Domestic processing of seeds i.e. soaking and germination led to 0.59% and 0.94%, non-significant increase ($P<0.05$) in calcium content. The iron content in raw Indian quinoa seeds was 5.2±0.01 mg/ 100g. A significant increase ($P<0.05$) of 1.9% and 13.4% was observed in quinoa seeds subjected to domestic processing methods i.e. soaking and germination, respectively. The zinc content of raw quinoa seeds was 6.6±0.04 mg/100g. Zinc content was significantly reduced ($P<0.05$) by 10.6% and 13.5% after soaking and germination, respectively. Raw quinoa seeds were reported to have magnesium content of 182.4±0.11 mg/100g. Domestic processing methods like soaking and germination led to 1.21% and 1.8% significant decrease ($P<0.05$) in magnesium content, respectively. Overall, industrially processed seeds exhibited lower mineral content as compared to the raw seeds. A non-significant reduction ($P<0.05$) was observed in calcium (0.3%), zinc (1.5%) and magnesium (0.1%) content of industrially processed seeds while a significant depreciation ($P<0.05$) was observed in iron (2%) content.

iii. Phytochemical composition

Raw quinoa seeds were reported to have 0.6±0.08g/100g of tannin content. Soaking and germination led to 50% and 66.6% decrease in tannin content. Industrially processed seeds were reported to have reduction in tannin content similar to the reduction observed after soaking.

Total alkaloid content of raw Indian quinoa seeds was 2.11±0.01 g/100g. Soaking and germination led to 1% and 15%, significant decrease ($P<0.05$) in alkaloid content of quinoa. Industrialy processed seeds exhibited 10% decrease in alkaloid content

Total oxalate content of raw Indian quinoa seeds was 2.11±0.01 g/100g. All the processing methods led to reduction in oxalate content. Soaking and germination resulted in 30% and 55% decrease in oxalate content. Industrially processed seeds were also reported with 10% reduction in oxalate content.

Raw quinoa seeds were reported to have 1.25±0.22 g/100g phytic acid. Soaking resulted in reduction of phytic acid content by 2.5%, which was statistically non-significant ($P<0.05$) as compared to raw seeds. Germination led to significant decrease ($P<0.05$) in phytic acid content by 68%. Industrial processing led to non-significant decrease ($P<0.05$) in phytic acid content by 3.2%

Total phosphorous content of raw Indian quinoa seeds was 0.43±0.22 g/100g. Soaking resulted in a non-significant increase ($P<0.05$) in total phosphorous content by 7%. Germination of quinoa seeds was observed to cause a significant increase ($P<0.05$) in total phosphorous content. Industrially processed seeds exhibited 4.6% increase in total phosphorous content, which was non-significant ($P<0.05$) as compared to the total phosphorous content of raw seeds.

Raw quinoa seeds were reported to contain 2.01±0.15 g/100g saponin content. Soaking resulted in significant decrease ($P<0.05$) of 24% in saponin content. Germination led to 98% decrease in saponin content. Industrially processed seeds exhibited 97% reduced saponin content as compared to the raw seeds. With respect to the germinated seeds, although the reduction was statistically non-significant ($P<0.05$) but germinated seeds were reported to have 50% lower saponin content than industrially processed seeds.

Raw seeds were reported to have 6633±7.5 TIU/100g trypsin inhibitor activity.All the processing methods led to significant decrease ($P<0.05$) in trypsin inhibitor activity. Soaking resulted in 4.7% decrease in trypsin inhibitor activity. Industrially processed quinoa seeds exhibited 20.7% decrease in trypsin inhibitor activity.

iv. *In vitro* analysis

Raw quinoa seeds exhibited 75.3±0.33% protein digestibility. Soaking resulted in 1.5% increase in protein digestibility. Germination resulted in 9.1%, significant increase ($P<0.05$) in protein digestibility. Industrially processed seeds exhibited 1.2% increase in protein digestibility value.

Raw quinoa seeds exhibited 65.7±0.15% starch digestibility. Soaking of quinoa seeds resulted in 1.52%, non-significant increase in starch digestibility while

germination led to 1.80%, significant increase with respect to the raw seeds. Industrially processed seeds exhibited 1.05%, non-significant increase ($P<0.05$), in starch digestibility.

Raw quinoa seeds were reported to have 59.6 mgTE /100g and 37.3 TEAC (Trolox equivalent antioxidant capacity) as calculated by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) method and 84.4 mg TE /100g as calculated by FRAP (Ferric reducing antioxidant activity) method. Soaked quinoa seeds exhibited 7% decrease in antioxidant activity as compared to raw seeds. FRAP values of germinated quinoa seeds increased by 89%. However industrial processing of the seeds lead to decline in antioxidant activity. Processed quinoa seeds showed decline of 14% and 19% antioxidant activity as evaluated by FRAP and DPPH respectively

b) On the basis of proximate, nutritional, and phytochemical composition, industrially processed Indian *Chenopodium quinoa* seeds were compared with industrially processed American *Chenopodium quinoa* seeds.

i. Proximate composition

Moisture, carbohydrate, protein and crude fiber content of Indian and American quinoa seeds were significantly different ($P<0.05$) while ash and crude fat contents were statistically non-significant ($P<0.05$). It was noticeable that the protein content of Indian quinoa (13.11±0.08 g/100g) was 7.02% more than the protein content of American quinoa (12.25±0.92 g/100g).

ii. Nutritional composition

A non-significant difference ($P<0.05$) was observed in dietary fiber content of American and Indian quinoa seeds. Indian quinoa seeds exhibited significantly higher ($P<0.05$) β-Carotene content than American seeds. Vitamin C content of American quinoa was, 14.4%, significantly higher ($P<0.05$) than the vitamin C content of Indian quinoa seeds.

Mineral content of American quinoa was found to be significantly higher ($P<0.05$) than the Indian quinoa. Calcium, iron and zinc contents of American quinoa seeds were 15.6, 43.2 and 26.1% higher than Indian quinoa, respectively. Magnesium content of American quinoa was 6.1% higher than Indian quinoa.

iii. *In vitro* analysis

American quinoa seeds exhibited significantly lower ($P<0.05$) protein digestibility (75.15%) than Indian quinoa seeds. American quinoa seeds exhibited significantly higher ($P<0.05$) starch digestibility (67.14%) than Indian quinoa seeds.

2. The objective two of the study was accomplished by a biological trial using animal model.

a) Male wistar rats (42; divided in 7 groups with 6 rats in each group) for a period of 45 days and testing any cholosterolemic effect of supplementing germinated quinoa (debittered) and raw quinoa (bitter) seeds.

i. The total serum cholesterol level of rats fed with basal diet (Group1) was reported as 160.16±16 mg/dl. The total concentration of plasma cholesterol was observed to reduce significantly ($P<0.05$) upon addition of quinoa to the experimental diet. It was observed that, as compared to the Group 2, the positive control (rats fed on hypercholesterolemic diet), concentration of cholesterol reduced by 8.2% in diet fed with statin, while 15.5 and 24.4% reduction in serum cholesterol was observed in rats fed with raw (Group 4) and germinated quinoa (Group 5) along with hypercholesterolemic diet, respectively. The rats fed with raw (Group 6) and germinated quinoa (Group 7) along with basal diet showed 28.2 and 31.9% reduction in total cholesterol level.

ii. The levels of plasma triglycerides showed a declining trend with supplementation of quinoa to the experimental diet. As compared to the positive control, which showed 96.6±2.9mg/dl triglyceride content, the levels were reported to decrease significantly ($P<0.05$) in rats fed with quinoa diet. However, reduction in triglyceride content by quinoa supplemented along with hypercholestrolemic diet was observed to be lower than the reduction induced by statin administered along with hypercholestrolemic diet. In rats fed with quinoa along with hypercholersterolemic diet, the triglyceride levels reduced by 12.5 and 4.5% in Group 4 and Group 5, respectively. Triglyceride level of rats fed with quinoa along with basal diet i.e. in Group 6 and Group 7, reduced by 10.4 and 14.5%, respectively.

iii. The serum HDL level of rats fed on basal diet were reported as 91.15±4.8 mg/dl while of rats fed on hypocholesterolemic diet was 90.1±2.2 mg/dl . The HDL levels were observed to decrease significantly ($P<0.05$) in rats fed with diets supplemented with raw (5.5%) and germinated (3.3%) quinoa along with hypercholesterolemic diet as compared to serum HDL levels of rats fed with hypercholesterolemic diet (positive control) which reveals negative effect of quinoa supplementation along with hyper cholesterolemic diet on serum HDL levels.

iv. The serum (VLDL+HDL) values of rats fed on basal diet was reported as 68.82±7.2 mg/dl while of rats fed on hypercholesterolemic diet was 128.9±3.5 mg/dl. The results were observed to decrease significantly ($P<0.05$), by 21.3 and 39.4% with supplementation of raw quinoaand germinated quinoa to hypercholesterolemic diet, respectively. The results also infer beneficial effect of germinated quinoa over raw quinoa. Lipid lowering effect of quinoa (raw and germinated) was higher than the beneficial effect of statin, which was reported to cause17.7% decline in (VLDL+HDL) values. The reduction was almost almost twice in germinated quinoa. The results infer lipid lowering beneficial effect of quinoa, mainly germinated quinoa.

v. Atherosclerotic index of rats fed with basal diet was reported as 0.75±0.2 mg/dl while of rats fed on hypercholestrolemic diet was reported as 1.43±0.3 mg/dl. The drug statin administered to rats along with hypercholesterolemic diet, caused 23.7% reduction in atherosclerotic index, while supplementation of raw and germinated quinoa along with hypercholeterolemic diet resulted in 18.4 and 37.2% reduction in atherosclerotic index. The results infer beneficial effect of quinoa, mainly the germinated one, in hypercholesterolemia over the statin drug. The rats fed on raw and germinated quinoa supplemented with basal diet also showed 18.6 and 34.5% reduction in atherosclerotic index.

3. Functional foods, namely, quinoa bar, quinoa cracker and quinoa beverages were prepared from quinoa grains and evaluated for proximate composition, total phenolic content and antioxidant activity. Sensory evaluation and storage studies of functional food products developed in this study was also done. Beverages

prepared from quinoa were additionally analyzed for pH, viscosity, total soluble solids and serum separation.

a) Bars, control bar and quinoa bar, were prepared using chickpea flour and germinated quinoa flour, respectively. Formulated bars were carefully packed in zip lock bags and stored in refrigerator for 30 days. Bars were evaluated for different parameters at an interval of 15 and 30 days.

i. Proximate composition: Moisture content of formulated bars ranged from 15.21±0.18 to 15.19±0.12 g/100gm. Moisture content of control bar (CB) and quinoa bar (QB) were almost similar and statistically non-significant to each other ($P<0.05$). Ash, fat, protein and fiber content of quinoa bar was significantly higher ($P<0.05$) than control bar.

ii. Total phenolic content and antioxidant activity: Total phenolic content of quinoa bar was significantly higher ($P<0.05$) than the control bar. Antioxidant activity of quinoa bars, as evaluated by DPPH and FRAP method, was reported significantly higher ($P<0.05$) than the control bars.

iii. Sensory evaluation: The data analysis of sensory evaluation revealed significant difference ($P<0.05$) in appearance, colour, mouthfeel, flavor and taste of control bars and quinoa bars, while difference in texture was revealed statistically non-significant ($P<0.05$). According to scoring on nine point hedonic scale, overall acceptability of quinoa snack bars indicated "like very much" while control bars which indicated "like slightly." Highest score was scored by taste aspect ($8.4\pm0.09^{)}$ of quinoa bar, which indicated, "like extremely" on a nine point hedonic scale while lowest score was scored by colour aspect of control bar which indicated "like moderaltely" on a nine point hedonic scale.

iv. Effect of storage: A significant increase ($P<0.05$), in moisture content was observed in both control bar and quinoa bar at an interval of 15 and 30 days. However, moisture content at the end of 30 days was highest as compared to the initial moisture content of snack bars. Ash content of snack bars was observed to decrease non-significantly ($P<0.05$) upon storage for 15 days while a significant decrease in ash content was observed after storage period of 30 days. A non significant change

(P<0.05) in fat content was observed after storage period of 15 days while a significant decrease (P<0.05) was observed after storage period of 30 days. A non-significant decrease (P<0.05) was observed in protein content of snack bars after storage period of 15 days while significant decrease (P<0.05) was observed after storage period of 30 days.The carbohydrate content of control snack bars was observed to decrease significantly (P<0.05) after storage period 15 and 30 days as compared to the initial carbohydrate content while quinoa snack bars exhibited non significant decrease (P<0.05) in carbohydrate content after 15 days and a significant decrease after 30 days.

A significant decrease (P<0.05) in total phenolic content and antioxidant activity of snack bars was observed after storage period of 15 and 30days.

A significant decrease (P<0.05) in over acceptability of snack bars was observed after storage period of 15 and 30 days. In general, although the overall acceptability was observed to decrease with storage period in both snack bars but overall acceptability of quinoa bar was more than the control bar after different storage intervals. Bars exhibited least overall acceptability after storage period of 30 days. According to the scoring on nine point hedonic scale, overall acceptability of quinoa bars indicated "like very much" initially and decreased after 15 and 30 days indicating "neither like nor dislike" and "dislike slightly", respectively, while the overall acceptability of control bar decreased from "like slightly" initially to "neither like nor dislike" to "dislike moderately" after 15 and 30 days, respectively.

b) Crackers, control and quinoa cracker, were prepared from wheat flour and quinoa flour, respectively. Crackers made from quinoa flour were formulated in by incorporation of quinoa flour to wheat flour in ratio 80:20 (T_1) and 60:40 (T_2). Formulated crackers were carefully packed in zip lock bags and stored in refrigerator for 15 days. Bars were evaluated for different parameters after 15 days.

i. Proximate composition: Moisture content of crackers ranged from 3.51±0.07 g/100g to 3.55±0.10g/100g. Although the moisture content of

quinoa incorporated crackers was less than the wheat (control, T_0) crackers, but the difference observed was statistically non-significant ($P<0.05$). Ash, fat and protein was observed to increase with increase in the ratio of quinoa incorporation to the crackers. Ash content of wheat cracker was significantly lower ($P<0.05$) than the 20 and 40% quinoa incorporated crackers but was statistically similar in crackers incorporated with 20 and 40% quinoa flour. Protein content of quinoa crackers was higher than that of wheat crackers. Highest carbohydrate content, 80.10±1.03 was reported in wheat crackers. Carbohydrate content was found to decrease with the increase in the ratio of quinoa incorporation to the crackers. Fiber content was lowest, 2.10±0.21g/100g in wheat (control, T_0) cracker and was found to increase with incorporation of quinoa but was statistically similar ($P<0.05$) in all the crackers.

ii. Total phenolic content and antioxidant activity: The total phenolic content of wheat cracker (control, T_0) was observed as 88.16±0.02 mg GAE/ 100g. The TPC was found to increase significantly ($P<0.05$) with incorporation of quinoa flour to the crackers and was directly related to ratio of quinoa flour added. Crackers incorporated with quinoa flour showed 3.5 to 7% higher TPC than the wheat cracker (control). The antioxidant activity of wheat cracker (control, T_0) was observed as 41.68±0.46 % (by DPPH method) and 96.29±0.30 mgTE/100g (by FRAP method). Antioxidant activity was observed to increase significantly ($P<0.05$) with incorporation of quinoa flour. Highest antioxidant activity was observed in crackers incorporated with 40% quinoa flour (50.13±0.90 % by DPPH method and 118.08±0.15 mg TE/ 100g by FRAP method).

iii. Sensory evaluation: The data analysis of sensory evaluation revealed non-significant difference ($P<0.05$) in appearance and colour of all crackers. As compared to the wheat cracker (T_0, control), texture of quinoa incorporated crackers was more acceptable.). Similarly, mouthfeel, flavor, taste of quinoa incorporated crackers (20 and 40%, respectively) was significantly highly ($P<0.05$) acceptable than the wheat crackers. Cracker incorporated with 40% quinoa flour received highest score for texture,

mouthfeel, flavor and taste on nine point hedonic scale which was although non-significant to the scores received by crackers incorporated with 20% quinoa flour. Overall acceptability was lowest for the wheat ($T_{0,}$ control) crackers and highest for crackers incorporated with 40% quinoa flour (T_2).

iv. Effect of storage: A non-significant increase ($P<0.05$), in moisture content was observed in wheat cracker (control, T_0) while the moisture content of quinoa incorporated crackers increased significantly ($P<0.05$) after 15 days. Highest increase in moisture content was observed in T_2 (3.6%) followed by T_1(3.3%) and T_0 (0.6%). Ash content of all the crackers was observed to decrease non-significantly ($P<0.05$) upon storage for 15 days. Highest decrease in ash content was observed in T_1 (1.99%) followed by T_2 (1.24%) and T_0 (1.04%). . Fat content. Fat content of wheat cracker (control, T_0) and cracker incorporated with 20% quinoa (T_1) decreased non-significantly by 0.9 and 0.4%, respectively, while a significant decrease of 2.6% was observed in cracker incorporated with 40% quinoa (T_2). . In general, 1 to 1.5% non-significant decrease ($P<0.05$) was observed in protein content of crackers after storage period of 15 days. Overall, carbohydrate content of crackers was observed to decrease after storage period of 15 days. A non-significant decrease ($P<0.05$), in carbohydrate content was observed in wheat cracker (control, T_0) while the carbohydrate content of quinoa incorporated crackers decreased significantly ($P<0.05$) after 15 days.A significant decrease ($P<0.05$) in total phenolic content of crackers was observed during storage period of 15 days. The highest decrease in total phenolic content was reported in crackers incorporated with 20% quinoa (2.3%) followed by 40% quinoa incorporated cracker (2.1%) and wheat (control, T_0) cracker (1.5%).

A significant decrease ($P<0.05$) was also observed in antioxidant activity of crackers during storage period of 15 days.

Storage period of 15 days had no significant effect ($P<0.05$) on overall acceptability of wheat (control, T_0) cracker while overall acceptability of crackers incorporated with 20 and 40% quinoa decreased significantly ($P<0.05$). In general, highest decrease in overall acceptability, (4.1%) was

observed in overall acceptability of 20% quinoa incorporated cracker followed by 3.9% decrease in 40% quinoa incorporated cracker and 1.5% decrease in wheat (control, T_0).

c) Beverages, namely raw quinoa beverage (RQB), soaked quinoa beverage (SQB) and germinated quinoa beverage (GQB) were prepared from raw, soaked and germinated quinoa seeds, respectively. All quinoa beverages were stored at 4°C for further analysis.

i. Physico-chemical analysis: pH of quinoa beverages ranged from 6.2± 0.01 to 6.5 ± 0.10. Statistically, no significant difference ($P<0.05$) was observed in pH content of all quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Total soluble solid content ranged from 9.08± 0.02 to 9.45 ± 0.01%. No significant difference ($P<0.05$) was observed in total solid of quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Viscosity of quinoa beverages ranged from 15.02 ± 0.01 to 15.31 ± 0.04 cp. No significant difference ($P<0.05$) was observed in viscosity of quinoa beverages prepared from raw, soaked and germinated quinoa seeds. Addition of 0.5% (*w/v*) xanthan gum increased the viscosity and helped in textural stabilization of the beverages by reducing serum separation. Serum separation reduction ranged from 64.7 to 71.6%.

   Moisture content of raw, soaked and germinated quinoa beverage ranged from 84.01± 0.01 to 84.07±0.13 g/100ml. Protein content of quinoa beverages ranged from 0.68 to 1.5 g/100 ml. Significant increase ($P<0.05$) in protein content was reported in GQB as compared to RQB. Carbohydrate content of quinoa beverages ranged from 14.9±0.1 to 16.2±0.02g/ 100 ml with trend in increasing order being GQB<SQB<RQB. Statistically, the difference between carbohydrate content of all quinoa beverages was significant ($P<0.05$). Carbohydrate content of SQB and GQB was observed to be 4 and 8% to be lower with respect to RQB, respectively. Fat content of beverages ranged from 0.23 to 0.93 g/ 100ml. GQB was reported with least fat content. RQB and SQB were reported with significantly higher ($P<0.05$) fat content as compared to GQB. Ash content of quinoa beverages ranged from

0.11±0.01 to 0.28 gm/100ml. A significant increase in ash content ($P<0.05$) was reported in GQB as compared to RQB.

ii. Total phenolic content and Antioxidant activity: Predominantly, all quinoa beverages had total phenolic content well correlated to its anti oxidant activity. Total phenolic content of quinoa beverages ranged from 1.9 to 2.4 mg GAE/ g. Germinated quinoa beverage (GQB) showed highest phenolic content (2.4 ± 0.2 mg GAE/g), followed by SQB (2.1 ± 0.2 mg GAE/g) and RQB (1.0 ± 0.3 mg GAE/ g).

Antioxidant activity of quinoa beverages, as determined by DPPH method ranged from 52 to 92%. Potential to inhibit DPPH free radical i.e. the anti oxidant activity of quinoa beverages followed the same trend as their total phenolic content i.e. GQB>SQB>RQB. Also, the antioxidant activity was found to have good correlation with the total phenolic content ($r = 0.95$, $P<0.05$), which suggests potential contribution of phenolic compounds in quinoa to its antioxidant activity.

iii. Sensory evaluation: Color aspect of all quinoa beverages ranged from 5.1±0.02 to 5.3±0.11 indicating "neither like nor dislike". Flavor of quinoa beverages ranged from 5.3±0.03 to 7.1±0.12. Flavor of RQB was rated as "dislike very much" as compared to SQB ("like slightly") and GQB ("like moderately"). Flavor aspect of GQB was rated similar to the soya beverage used as control. Texture and consistency of all quinoa beverages was rated as "like slightly." Among all the beverages, RQB indicated lowest score on nine point hedonic scale for after taste (1.2±0.1), indicating "dislike extremely" and least good mouth feel indicating "dislike very much" (2.3±0.4). The after taste of raw quinoa beverage, as stated by panelists was "bitter." After taste aspect SQB and GQB was better than RQB and marked as "neither liked nor disliked" and "like slightly," respectively. In general, it was observed that GQB qualified all positive aspects and was rated similarly to the commercial soya milk used as control.

On evaluation of panelists choice of favorite and least favorite beverage, it was observed that among quinoa beverages, RQB was rated as least

favorite beverage by 88% panelists (22 out of 25) and GQB was most favorite beverage of 28% (7 out of 25) panelists. Overall acceptability of quinoa beverages, ranged from dislike very much to like moderately with acceptability trend in increasing order being RQB<SQB<GQB. RQB was disliked because of its bitter after taste and beany flavor while GQB was most liked among all quinoa beverages with overall acceptability score significantly similar ($P<0.05$) to that of commercial soya milk.

BIBLIOGRAPHY

Abugoch James, L. E. (2009). Quinoa (*Chenopodium quinoa* Willd.): composition, chemistry, nutritional, and functional properties. Advances in Food and Nutrition Research (1st ed., Vol. 58). Elsevier Inc.

ACCCI. (2011). Approved Methods of the American Association of Cereal Chemists International, 11th Ed., AACC International, St. Paul, MN. USA.

Adawiyah, D. R., Soekarto, T. S., & Hariyadi, P. (2012). Fat hydrolysis in a food model system: Effect of water activity and glass transition. International Food Research Journal, 19(2): 737–741.

Adekanmi Oladele, A., Osundahunsi, of, & Adebowale, Y. (2009). Effect of processing techniques on the nutrients and antinutrients contents of tigernut (*Cyperus esculentus l.*). Nigerian Food Journal, 27(2): 88–93.

Afiffy, A. E. M. M. R., El-Beltagi, H. S., Abd El-Salam, S. M., & Omran, A. a. (2012). Biochemical changes in phenols, flavonoids, tannins, vitamin E, β - carotene and antioxidant activity during soaking of three white sorghum varieties. Asian Pacific Journal of Tropical Biomedicine, 2(3): 203–209.

Afrose, S., Tsujii, H., Hossain, M. S., Salma, U., & Miah, A. G. (2010). Dietary karaya saponin and rhodobacter capsulatus exert hypocholesterolemic effects by suppression of hepatic cholesterol synthesis and promotion of bile acid synthesis in laying hens. Cholesterol, 8(2010): 1-7.

Agrahar-Murugkar, D., & Jha, K. (2011). Influence of storage and packaging conditions on the quality of soy flour from sprouted soybean. Journal of Food Science and Technology, 48(3), 325–328.

Ahn, J., Kang, E., Kim, E., and Chung, I. (2012). Variation of β -carotene Concentration in Soybean Seed and Sprout. Korean Journal of Plant Science, 57(4), 324–330.

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature Protocols, 2(4): 875–877.

Akin-Idowu, P. E., Asiedu, R., Maziya-Dixon, B., Odunola, A., & Uwaifo, A. (2009). Effects of two processing methods on some nutrients and anti-nutritional factors in yellow yam (*Dioscorea cayenensis*). African Journal of Food Science, 3(1): 022–025.

Alvarez-Jubete, L., Arendt, E. K., & Gallagher, E. (2010). Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. Trends in Food Science and Technology, 21(2): 106–113.

Amir Ahmad Nassiri, Monir Sadat Hakemi (2013). Serum magnesium level and cardiovascular disease in dialysis patients. Iranian Journal of Kidney Diseases, 7(1): 14-22.

Ando, H., Chen, Y.-C., Tang, H., Shimizu, M., Watanabe, K., & Mitsunaga, T. (2002). Food components in fractions of quinoa seed. Food Science and Technology Research, 8(1): 80–84.

Angiosperm Phylogeny Group (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society, 141(4): 399-436.

AOAC, (1995). Official Methods of Analysis, 16th Ed., Association of Official Analytical Chemists, Washington, DC.

AOAC, (2000). Official Methods of Analysis, 16th Ed., Association of Official Analytical Chemists, Washington, DC, USA.

Araujo-Farro, P. C., Podadera, G., Sobral, P. J. a, & Menegalli, F. C. (2010). Development of films based on quinoa (*Chenopodium quinoa*, Willd.) starch. Carbohydrate Polymers, 81(4): 839–848.

Araujo-Farro, P. C., Podadera, G., Sobral, P. J. a, & Menegalli, F. C. (2010). Development of films based on quinoa (*Chenopodium quinoa*, Willd.) starch. Carbohydrate Polymers, 81(4): 839–848.

Arneja, I., Tanwar, B., & Chauhan, A (2015). Nutritional composition and health benefits of golden grain of 21 st century quinoa (*Chenopodium quinoa* Willd.): A Review. Pakistan Journal of Nutrition, 14(12): 1034–1040.

Asao, M., & Watanabe, K. (2010). Functional and bioactive properties of quinoa and amaranth. Food Science and Technology Research, 16(2): 163–168.

Asensi-Fabado, M. A.; Munné-Bosch, S. (2010) Vitamins in plants: Occurrence, biosynthesis and antioxidant function. Trends in Plant Science, 15: 582−592.

Atef, A., & Emam, H. (2014). Use of Quinoa Meal to Produce Bakery Products to Celiac and Autism Stuffs. International Journal of Science and Research, 3(9): 1344–1354.

Azeke, M. A., Egielewa, S. J., Eigbogbo, M. U., & Ihimire, I. G. (2011). Effect of germination on the phytase activity, phytate and total phosphorus contents of rice (*Oryza sativa*), maize (*Zea mays*), millet (*Panicum miliaceum*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*). Journal of Food Science and Technology, 48(6): 724–729.

Barakat, M.Z., M.F.A. El-Wahab, and M.M. El-Sadr. (1955). Action of N-bromosuccinimide on ascorbic acid. Biochemistry Department, Faculty of Medicine, Abbassia, Cairo, Egypt. Analytical Chemistry, 27: 536-540.

Baruah K, Sahu NP, Pal AK, Jain KK, Debnath D, Yengkokpam S. (2007) Interactions of dietary microbial phytase, citric acid and crude protein level on mineral utilization by Rohu, Labeo rohita (Hamilton), Juveniles. Journal of World Aquaculture Society, 38: 238–249.

Benitez, V., Cantera , S., Aguilera ,Y., Mollá ,E., Esteban ,M.R., Díaz ,F.M., & Martín- Cabrejas,A.M. (2013). Impact of germination on starch, dietary fibre and physicochemical properties in non-conventional legumes. Food Research International, 50: 64 – 69.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as measurement of ‘‘antioxidant power’’: the FRAP assay. Analitical Biochemistry, 239, 70–76.

Berti, C., Riso, P., Monti, L. D., & Porrini, M. (2004). *In vitro* starch digestibility and in vivo glucose response of gluten-free foods and their gluten counterparts. European Journal of Nutrition, 43(4): 198–204.

Bertrandt, J. (2013). Minerals and polyphenols content of quinoa (*Chenopodium quinoa* Willd.) plant, Problem High Epidemiology, 94(2): 300–304.

Bhargava, A., Shukla, S., & Ohri, D. (2006). *Chenopodium quinoa* - An Indian perspective. Industrial Crops and Products, 23(1): 73–87.

Bhargava, A., Shukla, S., Rajan, S., & Ohri, D. (2007). Genetic diversity for morphological and quality traits in quinoa (*Chenopodium quinoa* Willd.) germplasm. Genetic Resources and Crop Evolution, 54(1): 167–173.

Bianchi, F., Rossi, E., Gomes, R., & Sivieri, K. (2015). Potentially synbiotic fermented beverage with aqueous extracts of quinoa (*Chenopodium quinoa* Willd.) and soy. Food Science and Technology International, 21(6): 403–415.

Blessing, I. A., & Gregory, I. O. (2010). Effect of processing on the proximate composition of the dehulled and undehulled mungbean [*Vigna radiata* (L.)] flours. Pakistan Journal of Nutrition, 9(10): 1006–1016.

Borges, R.M., Valença, S.S., Lopes, A.A., Barbi, N.S., Silva, A.J.R. (2013). Saponins from the roots of *Chiococca alba* and their *in vitro* anti-inflammatory activity. Phytochemistry Letters, 6: 96–100.

Borneo, R., & León, A. E. (2012). Whole grain cereals: functional components and health benefits. Food Functions, 3(2): 110–119.

Brend, Y. (2012). Total Phenolic Content and Antioxidant Activity of Red and Yellow Quinoa (*Chenopodium quinoa*; Willd.) Seeds as Affected by Baking and Cooking Conditions. Food and Nutrition Sciences, 03(08): 1150–1155.

Butt MS, Batool R (2010). Nutritional and Functional Properties of Some Promising Legumes Protein Isolates. Pakistan Journal of Nutrition, 9(4): 373-379

Calderelli, V. A. S., de Benassi, M. T., Visentainer, J. V., & Matioli, G. (2010). Quinoa and flaxseed: Potential ingredients in the production of bread with

functional quality. Brazilian Archives of Biology and Technology, 53(4): 981–986.

Capriles, V.D., Areas, J.A.G., (2013). Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance, and glycemic response of gluten-free breads. Food Functions, 4: 104-110.

Caprita Rodica and Adrian, C. (2011). Chemical Methods for the Determination of Soluble and Insoluble Non-Starch Polysaccharides - Review. *Usab*, *44*(2), 73–80.

Carciochi, R. a., Manrique, G. D., & Dimitrov, K. (2014). Changes in phenolic composition and antioxidant activity during germination of quinoa seeds (*Chenopodium quinoa* Willd.). International Food Research Journal, 21(2): 767–773.

Carciochi, R. a., Manrique, G. D., & Dimitrov, K. (2014a). Changes in phenolic composition and antioxidant activity during germination of quinoa seeds (*Chenopodium quinoa* Willd.). International Food Research Journal, 21(2): 767–773.

Carciochi, R. A., Manrique, G. D., & Dimitrov, K. (2014b). Optimization of antioxidant phenolic compounds extraction from quinoa (*Chenopodium quinoa*) seeds. Journal of Food Science and Technology, 52(7): 4396–4404.

Cardador-Martinez, A., Loarca-Pila, G., Oomah, B. D., (2002). Antioxidant activity in common beans (*Phaseolus vulgaris* L.), Journal of Agriculture and Food Chemistry, 50, 6975–6980.

Cevallos-Casals, B. A., & Cisneros-Zevallos, L. (2010). Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Food Chemistry, 119(4): 1485–1490.

Chaieb, I. (2010). Saponins as Insecticides : a Review. Tunisian Journal of Plant Protection, 5(1): 39–50.

Chaparro, D., Pismag, R., & Elizalde, A. (2011). Efecto de la germinación sobre el contenido de hierro y calcio en amaranto, quinua, guandul y soya. Biotecnologia En El Sector Agropecuario Y Agroindustrial, 9(1): 51–59.

Chauhan, G. S., Eskin, N. A. M., & Tkachuk, R. (1992). Nutrients and antinutrients in quinoa seed. Cereal Chemistry, 69(1): 85-88.

Chawla, R., & Patil, G. R. (2010). Soluble dietary fiber. Comprehensive Reviews in Food Science and Food Safety, 9(2): 178–196.

Chikwendu and Justina Ndirika, (2015). Comparative Evaluation of Chemical Composition of Fermented Ground Bean Flour (*Kerstingella geocarpa*), Cowpea Flour (Vigna unguiculata) and Commercial Wheat Flour (*Triticum* spp.). Pakistan Journal of Nutrition, 14: 218-224.

Chillo, S., Laverse, J., Falcone, P. M., & Del Nobile, M. a. (2010). Quality of spaghetti in base amaranthus wholemeal flour added with quinoa, broad bean and chick pea. Journal of Food Engineering, 84(1): 101–107.

Chillo, S., Mastromatteo, M., Iannetti, M., Civica, V., & Del Nobile, M. A. (2010). Formulation optimisation of gluten-free functional spaghetti based on quinoa, maize and soy flours. International Journal of Food Science and Technology, 46(6): 1201–1208.

Chiremba, C., Taylor, J. R. N., & Duodu, K. G. (2009). Phenolic content, antioxidant activity, and consumer acceptability of sorghum cookies. Cereal Chemistry, 86(5): 590–594.

Chirinos, R., Pedreschi, R., Rogez, H., Larondelle, Y., & Campos, D. (2015). Phenolic compound contents and antioxidant activity in plants with nutritional and/or medicinal properties from the Peruvian Andean region. Industrial Crops and Products, 47: 145–152.

Chlopicka, J., Pasko, P., Gorinstein, S., Jedryas, A., & Zagrodzki, P. (2012). Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT - Food Science and Technology, 46(2): 548–555.

Chung, H. J., Cho, D. W., Park, J. D., Kweon, D. K., & Lim, S. T. (2012). *In vitro* starch digestibility and pasting properties of germinated brown rice after hydrothermal treatments. Journal of Cereal Science, 56(2): 451–456.

Chung, H.J., Cho, A., & Lim, S.T. (2014). Utilization of germinated and heat-moisture treated brown rices in sugar-snap cookies. LWT - Food Science and Technology, 57: 260 – 266.

Coda, R., Rizzello, C. and Gobbetti, M. (2010). Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of functional bread enriched of γ- aminobutyric acid (GABA). International Journal of Food Microbiology, 137: 236-245.

Coelho, I. R., Gueifão, S., Nascimento, A. C., Lobo, M., Gimenez, A., Samman, N., … Fibre, D. (2011). Proximate and Mineral profile of Quinoa (*Chenopodium quinoa*), and kiwicha (*Amaranthus caudatus*) consumed in north of Argentina, EuroFIR Nexus consortium, Norwich, 21(2): 2010.

Comai, S., Bertazzo, A., Bailoni, L., Zancato, M., Costa, C. V. L., & Allegri, G. (2007). The content of proteic and nonproteic (free and protein-bound) tryptophan in quinoa and cereal flours. Food Chemistry, 100(4): 1350–1355.

Coulibaly A, Kouakou B, Chen J (2012) Extruded Adult Breakfast Based on Millet and Soybean: Nutritional and Functional Qualities, Source of Low Glycemic Food. Journal of Nutrition and Food Science. 2: 151.

Cowieson, A. J., Acamovic, T., and Gilbert, C. E. (2006). Lupins in poultry nutrition. In E. van Santen,&G. D. Hill (Eds.), Wild and cultivated lupins from the Trop- ics to the Poles. Proceedings of the 10th International Lupin Conference, Laugarvatn, Iceland, 19-24 June 2002 (pp. 314–318). International Lupin Association. Canterbury.

Dasgupta, (2015). The Indian Express. Retrieved from http://indianexpress.com/article/opinion/columns/indias-malnutrition-shame/

Day, R.A. and A.L. Underwood, (1986). Quantitative analysis. 5 th ed. Prentice-Hall publication.

De, C. F. G., S, S. R. De, De, C. A. L., Iannetta, O., & Js, M. (2015). Quinoa or Corn Flakes to Prevent Peripheral Inflammation after Menopause ? Journal of Obesity & Eating Disorders, 1(2): 1–5.

Decker, K.J. (2010). Shape of Things to Come: Innovations in Pasta. Food Products Design. Retrieved from http://www.foodproductdesign.com/articles/2012/01/shape-of-things-to-come-innovations-in-pasta.aspx

Desalegn, B. B. (2016). Effect of Soaking and Germination on Proximate Composition , Mineral Bioavailability and Functional Properties of Chickpea Flour Food and Public Health 2015, 5(4): 108-113.

Desplanques, S., Renou, F., Grisel, M., & Malhiac, C. (2012). Impact of chemical composition of xanthan and acacia gums on the emulsification and stability of oil-in-water emulsions. Food Hydrocolloids, 27(2), 401–410.

Dhingra, D., Michael, M., Rajput, H., & Patil, R. T. (2012). Dietary fibre in foods: A review. Journal of Food Science and Technology, 49(3): 255–266.

Dick Mastebroek, H., Limburg, H., Gilles, T., Marvin, H.J.P., (2000). Occurrence of sapogenins in leaves and seeds of quinoa (*Chenopodium quinoa* Willd). Journal of the Science of Food and Agriculture, 80: 152-156.

Dini, I., Tenore, G. C., & Dini, A. (2005). Nutritional and antinutritional composition of Kancolla seeds: An interesting and underexploited andine food plant. Food Chemistry, 92(1): 125–132.

Dini, I., Tenore, G. C., & Dini, A. (2005). Nutritional and antinutritional composition of Kancolla seeds: An interesting and underexploited andine food plant. Food Chemistry, 92(1): 125–132.

Dini, I., Tenore, G. C., & Dini, A. (2013). Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. LWT - Food Science and Technology, 53(1): 382–385.

Du Thanh Hang, Leo Vanhanen, Geoffrey Savage. (2014). Effect of simple processing methods on oxalate content of taro petioles and leaves grown in central Vietnam. LWT - Food Science and Technology, 50(1): 259-262.

Duenas, M., Hernández, T., Estrella, I., & Fernández, D. (2009). Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (Lupinus angustifolius L.). Food Chemistry, 117(4): 599–607.

Durazzo, A., Gabrielli, P., & Manzi, P. (2015). Qualitative Study of Functional Groups and Antioxidant Properties of Soy-Based Beverages Compared to Cow Milk Antioxidants, 4(3), 523–532.

Echendu, C. A., Obizoba, I. C., & Anyika, J. U. (2009). Effects of germination on chemical composition of groundbean (*Kerstingiella geocarpa*) seeds. Pakistan Journal of Nutrition, 8(12): 1849–1854.

El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Foods for Human Nutrition, 57: 83–97.

El-Hady, E. A. A., & Habiba, R. A. (2003). Effect of soaking and extrusion conditions on antinutrients and protein digestibility of legume seeds. LWT - Food Science and Technology, 36(3): 285–293.

Elsohaimy, S. A., Refaay, T. M., & Zaytoun, M. A. M. (2015). Physicochemical and functional properties of quinoa protein isolate. Annals of Agricultural Sciences, 60(2): 297–305.

Embaby, H. E. S. (2010). Effect of soaking, dehulling, and cooking methods on certain antinutrients and *in vitro* protein digestibility of bitter and sweet lupin seeds. Food Science and Biotechnology, 19(4): 1055–1062.

Enes, B. N., Galdino Alves, N. E., Valdes, S. T., Martins Silveira, C. M., Brunoro Costa, N. M., Rocha Ribeiro, S. M., Duarte Martino, H. S. (2014). Evaluation of iron bioavailability in a mixture of cereals, seeds, and grains ("Human Ration"). Food Science and Technology, 34(1): 24–31.

Enriquez, N., Peltzer, M., Raimundi, A., Tosi, V., & Pollio, M. L. (2003). Characterization of wheat and quinoa flour blends in relation to their breadmaking quality. Anales Des La Asociacion Quimica Argentina, 91(4-6): 47–54.

Estevez, A. M., Figuerola, F., Hurtado, M. L., Chiffelle, I., & Asenjo, F. (2005). Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chemistry, 91, 395–401.

FAO (2011). Quinoa: An ancient crop to contribute to world food security. In: A. Bojanic ed. Regional Office for Latin America and the Caribbean, pp. 63. Food and Agriculture Organization, Rome, Italy

Farinazzi-Machado, F. M. V., Barbalho, S. M., Oshiiwa, M., Goulart, R., & Pessan Junior, O. (2012). Use of cereal bars with quinoa (*Chenopodium quinoa* W.) to reduce risk factors related to cardiovascular diseases. Ciência E Tecnologia de Alimentos, 32(4): 15-20.

Fernandez-Orozco R, Piskula MK, Zielinski H, Kozowska H, Frias J, Vidal-Valverde C (2006). Germination as a process to improve the antioxidant capacity of Lupinus angustifolius L. var. Zapaton. European Food Research and Technology, 223: 495–502.

Föste, M., Elgeti, D., Brunner, A.-K., Jekle, M., & Becker, T. (2015). Isolation of quinoa protein by milling fractionation and solvent extraction. Food and Bioproducts Processing, 96: 20–26.

Foucault, A.-S., Mathé, V., Lafont, R., Even, P., Dioh, W., Veillet, S., … Quignard-Boulangé, A. (2012). Quinoa Extract Enriched in 20-Hydroxyecdysone Protects Mice From Diet-Induced Obesity and Modulates Adipokines Expression. Obesity, 20(2): 270–277.

Frontela, C., Ros, G., & Martinez, C. (2011). Phytic acid content and "*in vitro*" iron, calcium and zinc bioavailability in bakery products: The effect of processing. Journal of Cereal Science, 54(1): 173–179.

Gawlik-Dziki, U., Świeca, M., Sułkowski, M., Dziki, D., Baraniak, B., & Czyz, J. (2013). Antioxidant and anticancer activities of *Chenopodium quinoa* leaves extracts - *In vitro* study. Food and Chemical Toxicology, 57: 154–160.

Genkawa, T., Uchino, T., Inoue, A., Tanaka, F., & Hamanaka, D. (2008). Development of a low-moisture-content storage system for brown rice: Storability at decreased moisture contents. Biosystems Engineering, 99(4): 515–522.

Gesinski K, Nowak K (2011) Comparative analysis of the biological value of protein of *Chenopodium quinoa* Willd. and Chenopodium album L. Part I Amino

acid composition of the seed protein. Acta Scientiarum Polonorum Agricultura, 10: 47-56.

Ghavidel, R. A., & Prakash, J. (2007). The impact of germination and dehulling on nutrients, antinutrients, *in vitro* iron and calcium bioavailability and *in vitro* starch and protein digestibility of some legume seeds. LWT - Food Science and Technology, 40: 1292–1299.

Global Nutrition Report (2015). International Food Policy Research Institute. Retrieved from www.ifpri.org/publication/global-nutrition-report-2015.

Gomez-Caravaca, A. M., Iafelice, G., Verardo, V., Marconi, E., & Caboni, M. F. (2014). Influence of pearling process on phenolic and saponin content in quinoa (*Chenopodium quinoa* Willd). Food Chemistry, 157: 174–178.

Gomez-Caravaca, A. M., Segura-Carretero, A., Fernández-Gutiérrez, A., & Caboni, M. F. (2011). Simultaneous determination of phenolic compounds and saponins in quinoa (*Chenopodium quinoa* Willd) by a liquid chromatography-diode array detection-electrospray ionization-time-of-flight mass spectrometry methodology. Journal of Agricultural and Food Chemistry, 59(20): 10815–10825.

Gonzalez Martín, M. I., Wells Moncada, G., Fischer, S., & Escuredo, O. (2014). Chemical characteristics and mineral composition of quinoa by near-infrared spectroscopy. Journal of the Science of Food and Agriculture, 94(5): 876–881.

Gordillo-Bastidas E, Díaz-Rizzolo DA, Roura E, Massanés T and Gomis R (2016). Quinoa (*Chenopodium quinoa* Willd), from Nutritional Value to Potential Health Benefits : An Integrative Review, 6(3).

Gorenstein, S.; Caspi, A.; Libman, I.; Katrich, E.; Lerner, H. Z.; Trakhtenberg, S. (2004). Fresh Israeli Sweetie juice consumption improves lipid metabolism and increases antioxidant capacity in hypercholesterolemic patients suffering from coronary artery disease: studies *in vitro* and in human and positive changes in albumin and fibrinogen fractions. Journal of Agriculture and Food Chemistry, 52: 5215-5222.

Graf, B. L., Poulev, A., Kuhn, P., Grace, M. H., Lila, M. A., & Raskin, I. (2014). Quinoa seeds leach phytoecdysteroids and other compounds with anti-diabetic properties. Food Chemistry, 163: 178–185.

Graf, B. L., Poulev, A., Kuhn, P., Grace, M. H., Lila, M. A., & Raskin, I. (2014). Quinoa seeds leach phytoecdysteroids and other compounds with anti-diabetic properties. Food Chemistry, 163: 178–185.

Gunness P, Gidley MJ.(2010). Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. Food Functions, 1(2):149-55.

Hager, A.-S., A. Wolter, F. Jacob, E. Zannini and E.K. Arendt. (2012). Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. Journal of Cereal Science, 56: 239-247.

Hahm, T.-S., Park, S.-J., & Martin Lo, Y. (2009). Effects of germination on chemical composition and functional properties of sesame (Sesamum indicum L.) seeds. Bioresource Technology, *100*(4): 1643–7.

Halvorsen, B.L.; Holte, K.; Myhrstad, M.C.; Barikmo, I.; Hvattum, E.; Remberg, S.F.; Wold, A.B.; Haffner, K.; Baugerød, H.; Andersen, L.F. (2002). A systematic screening of total antioxidants in dietary plants. Journal of Nutrition, 132, 461–471.

Han, H., & Baik, B. K. (2008). Antioxidant activity and phenolic content of lentils (Lens culinaris), chickpeas (Cicer arietinum L.), peas (Pisum sativum L.) and soybeans (Glycine max), and their quantitative changes during processing. International Journal of Food Science and Technology, 43(11): 1971–1978.

Harra NM, Lemm T, Smith C (2011) Quinoa flour is an acceptable replacement for all purpose flour in a peanut butter cookie. Journal of American Dietetic Association, 111:SA45.

Haugh, W.; Lantzsch, H. J. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. Journal of Science, Food, and Agriculture, 34: 1423-1426.

Hejazi Maha A. (2016). Preparation of different formulae from quinoa and different sources dietary fiber to treat obesity in rats. Nature and Science, 14(2): 55–65.

Hemalatha, P., Bomzan, D. P., Sathyendra Rao, B. V., & Sreerama, Y. N. (2016). Distribution of phenolic antioxidants in whole and milled fractions of quinoa and their inhibitory effects on α-amylase and α-glucosidase activities. Food Chemistry, 199: 330–338.

Hemalatha, S., Platel, K., & Srinivasan, K. (2006). Influence of germination and fermentation on bioaccessibility of zinc and iron from food grains. European Journal of Clinical Nutrition, 61(3): 342–348.

Hirose, Y., Fujita, T., Ishii, T., & Ueno, N. (2010). Antioxidative properties and flavonoid composition of *Chenopodium quinoa* seeds cultivated in Japan. *Food Chemistry*, 119(4): 1300–1306.

Holm, J., Björck, I., Asp, N.-G., Sjöberg, L.-B., & Lundquist, I. (1985). Starch availability *in vitro* and in vivo after flaking, steam-cooking and popping of wheat. Journal of Cereal Science, 3(3): 193–206.

Horton, J.D.; Shimomura, I.; Brown, M.S.; Hammer, R.E.; Goldstein, J.L.; Shimano, H. (2006). Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. Journal of Clinical Investigation, 101: 2331–2339.

Hotz, C., & Gibson, R. S. (2007). Traditional Food-Processing and Preparation Practices to Enhance the Bioavailability of Micronutrients in Plant-Based Diets. The Journal of Nutrition , 137 (4): 1097–1100.

Hsu, H., & Vavak, D. (1977). A multienzyme technique for estimating protein digestibility. Journal of Food Science, 45(5): 1269–1273.

Hubner F, Arendt EK (2013) Germination of cereal grains as a way to improve the nutritional value: a review. Critical Review Food Science and Nutrition, 53: 853–861

Ibrahim Abd El-Gawad, I. A., El-Sayed, E. M., Hafez, S. A., El-Zeini, H. M., & Saleh, F. A. (2005). The hypocholesterolaemic effect of milk yoghurt and soy-yoghurt containing bifidobacteria in rats fed on a cholesterol-enriched diet. International Dairy Journal, 15(1): 37–44.

Iglesias-Puig, E., Monedero, V., & Haros, M. (2015). Bread with whole quinoa flour and bifidobacterial phytases increases dietary mineral intake and bioavailability. LWT - Food Science and Technology, 60(1): 71–77.

Inglett, G. E., Chen, D., & Liu, S. X. (2015). Antioxidant Activities of Selective Gluten Free Ancient Grains. Food and Nutrition Sciences, 6: 612–621.

Inyang, C.U. and Zakari, U.M. (2008). Effect of germination and fermentation of pearl millet on proximate, chemical and sensory properties of instant "Fura"- a Nigerian cereal food. Pakistan Journal of Nutrition, 7 (1): 9-12.

Isengard, H.-D. (2011). Water content, one of the most important properties of food. Food Control, 12(7): 395–400.

Jackson, C. J. C., Dini, J. P., Lavandier, C., Rupasinghe, H. P. V, Faulkner, H., Poysa, V., DeGrandis, S. (2002). Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. Process Biochemistry, 37(10): 1117–1123.

Jacobsen, S. E. (2011). The Situation for Quinoa and Its Production in Southern Bolivia: From Economic Success to Environmental Disaster. Journal of Agronomy and Crop Science, 197(5): 390–399.

Jancurova, M., Minarovicova, L., & Dandár, A. (2009). Quinoa – a Review. Czech Journal of Food Science, 27(2): 71–79.

Jenkins, D. J. a, Kendall, C. W. C., McKeown-Eyssen, G., Josse, R. G., Silverberg, J., Booth, G. L., … Leiter, L. a. (2008). Effect of a low-glycemic index or a high-cereal fiber diet on type 2 diabetes: a randomized trial. JAMA: The Journal of the American Medical Association, 300(23): 2742–2753.

Kadereit G, Gotzek D, Jacobs S and Freitag H (2005). Origin and age of Australian Chenopodiaceae. Organisms, Diversity and Evolution. International Journal of Plant Science, 164(6): 959–986.

Kajihausa, O. E., Fasasi, R. A., & Atolagbe, Y. M. (2014). Effect of Different Soaking Time and Boiling on the Proximate Composition and Functional Properties of Sprouted Sesame Seed Flour. Nigerian Food Journal, 32(2): 8–15.

Kanensi, O. J., Ochola, S., Gikonyo, N. K., & Makokha, A. (2011). Optimization of the Period of Steeping and Germination for Amaranth Grain, Journal of Agriculture and Food Technology, 1(6): 101–105.

Kang, Y., Khan, S., & Ma, X. (2009). Climate change impacts on crop yield, crop water productivity and food security - A review. Progress in Natural Science, 19(12), 1665–1674.

Kaur, I., & Tanwar, B. (2016). Quinoa Beverages: Formulation, Processing and Potential Health Benefits. Romanian Journal of Diabetes Nutrition and Metabolic Diseases. 23(2): 215–225.

Kaur, I., Tanwar, B., Reddy, M., & Chauhan, A. (2016). Vitamin C , total polyphenols and antioxidant activity in raw , domestically processed and industrially processed Indian *Chenopodium quinoa* seeds, *6*(13): 139–145.

Kavitha, S., & Parimalavalli, R. (2014). Effect of Processing Methods on Proximate Composition of Cereal and Legume flours. Journal of Human Nutrition and Food Science, 2(6): 1051.

Kayembe, N. C., & Jansen van Rensburg, C. (2013). Germination as a processing technique for soybeans in small-scale farming. South African Journal of Animal Sciences, 43(2): 167–173.

Kayembe, N. C., & Jansen van Rensburg, C. (2013). Germination as a processing technique for soybeans in small-scale farming. South African Journal of Animal Sciences, 43(2): 167–173.

Khandelwal, S., Udipi, S. a., & Ghugre, P. (2010). Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. Food Research International, 43(2): 526–530.

Khattab, R. Y., & Arntfield, S. D. (2009). Nutritional quality of legume seeds as affected by some physical treatments. Antinutritional factors. LWT - Food Science and Technology, 42: 1113–1118.

Khattak A.B., A. Zeb, M. Khan, N. Bibi, I. Ihsanullah and M.S. Khattak (2007). Influence of germination techniques on sprout yield, biosynthesis of ascorbic acid and cooking ability, in chickpea (*Cicer arietinum* L.). Food Chemistry, 103, 115-120.

Khattak, A. B., Zeb, A., Bibi, N., Khalil, S. A., & Khattak, M. S. (2007). Influence of germination techniques on phytic acid and polyphenols content of chickpea (Cicer arietinum L.) sprouts. Food Chemistry, 104(3): 1074–1079.

Khursheed P Navder, S. B. (2014). Freeze Dried Blueberry Powder Fortification Improves the Quality of Gluten Free Snacks. Journal of Food Processing & Technology, 5(12): 38-41.

Kim, D. M., Lee, H., & Yoo, S. H. (2012). Compositional changes and physical properties of soymilk prepared with pre-soaked-fermented soybean. Journal of the Korean Society for Applied Biological Chemistry, 55(1): 121–126.

Kokanova-Nedialkova, Z., Nedialkov, P.T., Nikolov, S.D., (2009). The genus chenopodium: phytochemistry, ethnopharmacology and pharmacology. Pharmacognosy Review, 3: 280–306.

Koksoy, A., & Kilic, M. (2004). Use of hydrocolloids in textural stabilization of a yoghurt drink, ayran. Food Hydrocolloids, 18(4): 593–600.

Kong, X., and Bertoft, E. (2010). Fine structure characterization of amylopectins from grain amaranth starch. Carbohydrate Research, 344: 1701–1708.

Konishi, Y., Hirano, S., Tsuboi, H., & Wada, M. (2004). Distribution of minerals in quinoa (*Chenopodium quinoa* Willd.) seeds. Bioscience, Biotechnology, and Biochemistry, 68(1): 231–234.

Konishi, Y., Hirano, S., Tsuboi, H., & Wada, M. (2004). Distribution of minerals in quinoa (*Chenopodium quinoa* Willd.) seeds. Bioscience, Biotechnology, and Biochemistry, 68(1): 231–234.

Konishi, Y., Hirano, S., Tsuboi, H., & Wada, M. (2004). Distribution of minerals in quinoa (*Chenopodium quinoa* Willd.) seeds. Bioscience, Biotechnology, and Biochemistry, 68(1): 231–234.

Kuljanabhagavad, T., & Wink, M. (2009). Biological activities and chemistry of saponins from *Chenopodium quinoa* Willd. Phytochemistry Reviews, 8(2): 473–490.

Kumar Ashok, P., & Upadhyaya, K. (2012). Tannins are Astringent. Journal of Pharmacognosy and Phytochemistry, 1(3): 45–50.

Kumar, A., Goel, B. K., Karthikeyan, S., Asgar, S., Gedda, a K., Choudhary, K. K., & Uprit, S. (2013). Protein and Calcium Rich Malted Health Drink Power. Journal of Food Process Technology, 04(03): 3–7.

Laetitia, B., Qi, P., Wickham, E., & Tomasula, P. (2014). Enrichment and Purification of Casein Glycomacropeptide from Whey Protein Isolate Using Supercritical Carbon Dioxide Processing and Membrane Ultrafiltration. Foods, 3(1): 94–109.

Lamothe, L. M., Srichuwong, S., Reuhs, B. L., & Hamaker, B. R. (2015). Quinoa (*Chenopodium quinoa* W.) and amaranth (Amaranthus caudatus L.) provide dietary fibres high in pectic substances and xyloglucans. Food Chemistry, 167: 490–496.

Laux, M. (2013). Quinoa. USDA Agricultural Marketing Resource Center. Revised by Mykel Taylor, 2012.

Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations. Journal of Food Engineering, 79(3), 1033–1047.

Lee, J., Hwang, Y. S., Lee, J. D., Chang, W. S., & Choung, M. G. (2013). Metabolic alterations of lutein, ??-carotene and chlorophyll a during germination of two soybean sprout varieties. Food Chemistry, 141(3): 3177–3182.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology, 20(3): 207–220.

Lee, S.K., and A.A. Kader. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology 20: 207-22.

Lee, Y. J., Kim, K. J., Park, K. J., Yoon, B. R., Lim, J. H., & Lee, O. H. (2013). Buckwheat (Fagopyrum esculentum M.) sprout treated with methyl jasmonate (MeJA) improved anti-adipogenic activity associated with the oxidative stress system in 3T3-L1 adipocytes. International Journal of Molecular Sciences, 14(1): 1428–1442.

Lestienne, I., Buisson, M., Lullien-Pellerin, V., Picq, C., & Trèche, S. (2007). Losses of nutrients and anti-nutritional factors during abrasive decortication of two pearl millet cultivars (Pennisetum glaucum). Food Chemistry, 100(4): 1316–1323.

Letelier, M. E., Rodríguez-Rojas, C., Sánchez-Jofré, S., & Aracena-Parks, P. (2011). Surfactant and antioxidant properties of an extract from *Chenopodium quinoa* Willd seed coats. Journal of Cereal Science, 53(2): 239–243.

Li, G., and Zhu, F. (2016). Physicochemical properties of quinoa starch. Carbohydrate Polymers, 137: 328–338.

Liang, J., Han, B.-Z., Nout, M. J. R., & Hamer, R. J. (2008). Effects of soaking, germination and fermentation on phytic acid, total and *in vitro* soluble zinc in brown rice. Food Chemistry, 110(4): 821–828.

Lindeboom, N., Chang, P. R., Falk, K. C., & Tyler, R. T. (2005). Characteristics of starch from eight quinoa lines. Cereal Chemistry, 82(2): 216–222.

Lindeboom, N., Chang, P. R., Falk, K. C., & Tyler, R. T. (2005). Characteristics of starch from eight quinoa lines. Cereal Chemistry, 82(2): 216–222.

Lopes, C. O., Dessimoni, G. V., Da Silva, M. C., Vieira, G., and Pinto, N. A. V. D. (2009). Aproveitamento, composição nutricional e antinutricional da farinha de quinoa (Chenoipodium quinoa). Alimentos e Nutrição, 20(4): 669-675.

Lopez-Rubio, A., Flanagan, B. M., Gilbert, E. P., and Gidley, M. J. (2008). A novel approach for calculating starch crystallinity and its correlation with double helix content: A combined XRD and NMR study. Biopolymers 89(9): 761–768.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Protein measurement with folin phenol reagent. Journal of Biochemistry, 193: 265 – 275.

Luthria, A., & Singh, K. (2014). *In vitro* antioxidant activity of black gram , cowpea , desi chickpea and yellow mustard as affected by sprouting. Journal of Global Biosciences, 3(1): 385–389.

Luthria, A., & Singh, K. (2014). *In vitro* antioxidant activity of black gram, cowpea, desi chickpea and yellow mustard as affected by sprouting, Journal of Global Biosciences, 3(1), 385–389.

Lutz, M., Martínez, A., & Martínez, E. a. (2013). Daidzein and Genistein contents in seeds of quinoa (*Chenopodium quinoa* Willd.) from local ecotypes grown in arid Chile. Industrial Crops and Products, 49: 117–121.

Ma, L., Li, B., Han, F., Yan, S., Wang, L., & Sun, J. (2015). Evaluation of the chemical quality traits of soybean seeds, as related to sensory attributes of soymilk. Food Chemistry, 173: 694–701.

Madhu, A. N., N. Amrutha, and S. G. Prapulla. (2012). Characteriza- tion and antioxidant property of probiotic and synbiotic yogurts. Probiotics Antimicrobial Proteins, 4:90–97.

Madhujith T, Shahidi F (2006) Optimization of the extraction of antiox- idative constituents of six barley cultivars and their antioxidant properties. Journal of Agriculture and Food Chemistry, 54:8048–8057.

Maisuthisakul, P., Gordon, M. H., Pongsawatmanit, R., & Suttajit, M. (2007). Enhancing the oxidative stability of rice crackers by addition of the ethanolic extract of phytochemicals from Cratoxylum formosum Dyer. Asia Pacific Journal of Clinical Nutrition, 16(1): 37–42.

Makinde, F. M., & Akinoso, R. (2013). Nutrient composition and effect of processing treatments on anti nutritional factors of Nigerian sesame (Sesamum indicum Linn) cultivars. International Food Research Journal, 20(5): 2293–2300.

Manikandan, R., Anand, A. V., & Muthumani, G. D. (2013). Phytochemical and *in vitro* anti-diabetic activity of methanolic extract of Psidium guajava leaves. International Journal of Current Microbiology Applied Sciences, 2(2): 15–19.

Maradini Filho, A. M., Pirozi, M. R., Da Silva Borges, J. T., Pinheiro Sant'Ana, H. M., Paes Chaves, J. B., & Dos Reis Coimbra, J. S. (2015). Quinoa: Nutritional,

Functional and Antinutritional Aspects. Critical Reviews in Food Science and Nutrition, (ahead of print).

Marmouzi, I., El Madani, N., Charrouf, Z., Cherrah, Y., & El Abbes Faouzi, M. Y. (2015). Proximate analysis, fatty acids and mineral composition of processed Moroccan *Chenopodium quinoa* Willd. and antioxidant properties according to the polarity. Phytothérapie, 13(2): 110–117.

Martin, J., & Diaz, R. (2011). Use of amaranth, quinoa and kañiwa in extruded corn snacks. University of Helsinki, Department of Food and Environmental Sciences EKT Series, 1522: 1–86.

Martin-Cabrejas, M. A., Ariza, N., Esteban, R., Molli, E., Waldron, K., & Lopez-Andreu, F. J. (2003). Effect of germination on the carbohydrate composition of the dietary fiber of peas (Pisum sativum L.). Journal of Agricultural and Food Chemistry, 51(5): 1254–1259.

Martinez, L. C., Vargas, M. A. P., del Angel, A. I. C., Bermudez, F. C., & Avalos, H. A. J. (2013). Total Phenolic Content and Antioxidant Capacity of Germinated, Popped, and Cooked Huauzontle (Chenopodium berlandieri spp. nuttalliae) Seeds. Cereal Chemistry Journal, 90(3): 263–268.

Masood, T., Shah, H. U., & Zeb, A. (2014). Effect of Sprouting Time on Proximate Composition and Ascorbic Acid Level of Mung Bean (Vigna Radiate L .) and Chickpea (Cicer Arietinum L .) Seeds. The Journal of Animal & Plant Sciences, 24(3): 850-859.

Matiacevich, S., Castellión, M., Maldonado, S., Buera, P., (2006). Water-dependent thermal transitions in quinoa embryos. Thermochimica Acta 448, 117–122.

Matos, M., Timgren, A., Sjoo, M., Dejmek, P., & Rayner, M. (2013). Preparation and encapsulation properties of double Pickering emulsions stabilized by quinoa starch granules. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 423: 147–153.

Megat, R. M. R., Azrina, A., & Norhaizan, M. E. (2016). Effect of germination on total dietary fibre and total sugar in selected legumes. International Food Research Journal, 23(1): 257–261.

Meneguetti, Q. A., Brenzan, M. A., Batista, M. R., Bazotte, R. B., Silva, D. R., & Garcia Cortez, D. A. (2011). Biological effects of hydrolyzed quinoa extract from seeds of *Chenopodium quinoa* Willd. Journal of Medicinal Food, 14(6): 653–657.

Milovanovic, M., Demin, M., Vucelic-Radovic, B., Zarkovic, B., & Stikic, R. (2014). Evaluation of the nutritional quality of wheat bread prepared with quinoa, buckwheat and pumpkin seed blends. Journal of Agricultural Sciences, Belgrade, 59(3): 319–328.

Miranda, M., Delatorre-herrera, J., Vega-gálvez, A., Jorquera, E., Quispe-fuentes, I., & Martínez, E. A. (2014). Antimicrobial Potential and Phytochemical Content of Six Diverse Sources of Quinoa Seeds (*Chenopodium quinoa* Willd .), (September), 1015–1024.

Miranda, M., Vega-Gálvez, A., López, J., Parada, G., Sanders, M., Aranda, M., Di Scala, K. (2010). Impact of air-drying temperature on nutritional properties, total phenolic content, and antioxidant capacity of quinoa seeds (*Chenopodium quinoa* Willd.). Industrial Crops and Products, 32(3): 258–263.

Miranda, M., Vega-gálvez, A., Martínez, E. a, López, J., Marín, R., Aranda, M., & Fuentes, F. (2013). Influence Of Contrasting Environments on Seed Composition of Two Quinoa Genotypes : Nutritional And Functional Properties. Chilean Journal of Agriculture Research, 73.

Miranda, M., Vega-gálvez, A., Martínez, E. a, López, J., Marín, R., Aranda, M., & Fuentes, F. (2013). Influence of contrasting environments on seed composition of two quinoa genotypes : nutritional and functional properties. Chilean Journal of Agriculture and Research, 73 (2): 108–116.

Miranda, M., Vega-Gálvez, A., Quispe-Fuentes, I., Rodríguez, M. J., Maureira, H., & Martínez, E. a. (2012). Nutritional Aspects of Six Quinoa (*Chenopodium quinoa* Willd.) Ecotypes from three Geographical Areas of Chile. Chilean Journal of Agricultural Research, 72(2): 175–181.

Miranda, M., Vega-Gálvez, A., Uribe, E., López, J., Martínez, E., Rodríguez, M. J., … Di Scala, K. (2011). Physico-chemical analysis, antioxidant capacity and vitamins of six ecotypes of chilean quinoa (*Chenopodium quinoa* Willd). Procedia Food Science, 1: 1439–1446.

Mittal, R., Nagi, H., Sharma, P., & Sharma, S. (2012). Effect of Processing on Chemical Composition and Antinutritional Factors in Chickpea Flour. Journal of Food Science and Engineering, 2(3): 180–186.

Mittal, R., Nagi, H., Sharma, P., & Sharma, S. (2012). Effect of Processing on Chemical Composition and Antinutritional Factors in Chickpea Flour. Journal of Food Science and Engineering, 2(3): 180–186.

Moongngarm, A., & Saetung, N. (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. Food Chemistry, 122(3), 782–788.

Mota, C., Nascimento, A. C., Santos, M., Delgado, I., Coelho, I., Matos, A. S., Castanheira, I. (2016). The effect of cooking methods on the mineral content of quinoa (*Chenopodium quinoa*), amaranth (Amaranthus sp.) and buckwheat (Fagopyrum esculentum). Journal of Food Composition and Analysis. (Under publication).

Mubarak, A. E. (2005). Food Chemistry Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus aureus) as affected by some home traditional processes. Food Chemistry, 89: 489–495.

Mujica, A., Jacobsen, S.E., Ezquierdo, J., Marathee, J.P., (2001). Resultados de la Prueba Americana y Europes de la Quinua. FAO, UNA-Puno, CIP, 51.

N.K.Rao, Shahid Mohammed (2012). Quinoa- A Promising Crop For Arabian Peninsula. American-Eurasian Journal of Agriculture and Environmental Science., 12(10): 1350-1355.

Nadarajah, S. Gordon, M. H., Pongsawatmanit, R., & Suttajit, M (2015). Influence of Storage Conditions on the Quality Characteristics of Wheat-Defatted Coconut Flour Biscuits Packed in Metalized Polypropylene. International Journal of Engineering Research and Technology, 4(07): 948–951.

Nascimento, A. C., Mota, C., Coelho, I., Gueifão, S., Santos, M., Matos, A. S., Castanheira, I. (2014). Characterisation of nutrient profile of quinoa (*Chenopodium quinoa*), amaranth (Amaranthus caudatus), and purple corn (Zea mays L.) consumed in the North of Argentina: Proximates, minerals and trace elements. Food Chemistry, 148: 420–426.

Nergiz, C., & Gökgöz, E. (2007). Effects of traditional cooking methods on some antinutrients and *in vitro* protein digestibility of dry bean varieties (Phaseolus vulgaris L.) grown in Turkey. International Journal of Food Science and Technology, 42(7): 868–873.

Ng, T.B., He, J.S., Niu, S.M., Zhao, L., Pi, Z.F., Shao, W., Liu, F. (2007). A gallic acid derivative and polysaccharides with antioxida- tive activity from rose (Rosa rugosa) flowers. Journal of Pharmacy and. Pharmacology; 56 (4): 537–545.

Nidhi Chaudhary and Swati Vyas (2014). Effect of germination on proximate composition and anti nutritional factor of millet (ragi) based premixes. Internatonal Journal of Food and Nutritional Sciences, 3(4): 72-79.

Nonogaki, H., G.W. Bassel and J.W. Bewley (2010). Germination-still a mystery. Plant Science, 179, ISSN 574- 581.

Nowak, V., Du, J., & Charrondière, U. R. (2015). Assessment of the nutritional composition of quinoa (*Chenopodium quinoa* Willd.). Food Chemistry. (Under publication).

Nwosu, J. N. (2010): Effect of soaking, blanching and cooking on the antinutritional properties of asparagus bean (Vigna sesquipedis) flour. Nature Science, 8 (8): 163-167.

Oghbaei, M., Prakash, J., & Yildiz, F. (2016). Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. Cogent Food & Agriculture, 2(1): 1136015.

Oliver, E., McGillicuddy, F. C., Harford, K. A., Reynolds, C. M., Phillips, C. M., Ferguson, J. F., & Roche, H. M. (2012). Docosahexaenoic acid attenuates macrophage-induced inflammation and improves insulin sensitivity in adipocytes-

specific differential effects between LC n-3 PUFA. Journal of Nutritional Biochemistry, 23(9): 1192–1200.

Onwulata, C., Thomas, A., Cooke, P. et al. (2010). Glycemic potential of extruded barley, cassava, corn, and quinoa enriched with whey proteins and cashew pulp. International Journal of Food Properties, 13: 338–359.

Owusu, D., & Oduro, I. (2011). Development of crackers from cassava and sweetpotato flours using Moringa oleifera and Ipomoea batatas leaves as fortificant. American Journal of Food and Nutrition, 1(3): 114–122.

Pagno CH, Costa TMH, de Menezes EW, Benvenutti EV, Hertz PF, Matte CR, Tosati JV, Monteiro AR, Rios AO, Flores SH. (2015). Development of active biofilms of quinoa (*Chenopodium quinoa*W.) starch containing gold nanoparticles and evaluation of antimicrobial activity. Food Chemistry, 173: 755–62.

Pagno CH, Costa TMH, de Menezes EW, Benvenutti EV, Hertz PF, Matte CR, Tosati JV, Monteiro AR, Rios AO, Flores SH. (2015). Development of active biofilms of quinoa (*Chenopodium quinoa* W.) starch containing gold nanoparticles and evaluation of antimicrobial activity. Food Chemistry, 173: 755–62.

Pal, R. S., Bhartiya, A., Arun Kumar, R., Kant, L., Aditya, J. P., & Bisht, J. K. (2016). Impact of dehulling and germination on nutrients, antinutrients, and antioxidant properties in horsegram. Journal of Food Science and Technology, 53(1), 337–347.

Palombini, S. V., Claus, T., Maruyama, S. A., Gohara, A. K., Henrique, A., Souza, P., Matsushita, M. (2013). Evaluation of nutritional compounds in new amaranth and quinoa cultivars. Food Science and Technology, 33(6): 339–344.

Pandey, H., & Awasthi, P. (2013). Effect of processing techniques on nutritional composition and antioxidant activity of fenugreek (Trigonella foenum-graecum) seed flour. Journal of Food Science and Technology, 1–7.

Panovska, Z., Váchová, A., & Pokorný, J. (2012). Effect of thickening agents on perceived viscosity and acidity of model beverages. Czech Journal of Food Sciences, 30(5), 442–445.

Pasko, P., Barton, H., Zagrodzki, P., Izewska, A., Krosniak, M., Gawlik, M., and Gawlik, M., et al., 2010b. Effect of diet supplemented with quinoa seeds on oxidative status in plasma and selected tissues of high fructose-fed rats. Plant Foods for Human Nutrition 65(2): 146-151.

Paśko, P., Sajewicz, M., Gorinstein, S., & Zachwieja, Z. (2008). Analysis of selected phenolic acids and flavonoids in *Amaranthus cruentus* and *Chenopodium quinoa* seeds and sprouts by HPLC. Acta Chromatographica, 20(4), 661–672.

Pasko, P., Zagrodzki, P., Barton, H., Chlopicka, J., and Gorinstein, S., 2010a. Effect of quinoa seeds (*Chenopodium quinoa*) in diet on some biochemical parameters and essential elements in blood of high fructose-fed rats. Plant Foods for Human Nutrition 65(4): 333-338

Pawar, V. D., & Machewad, G. M. (2006). Processing Of Foxtail Millet for Improved Nutrient Availability. Journal of Food Processing and Preservation, 30(3): 269–279.

Perez, G., Docena, G., Curciarello, R., (2009). Productos de panificación dirigidos agrupos especiales de la población. In: Lutz, M., León, A.E. (Eds.), Aspectos nutri-cionales y saludables de los productos de panificación. Universidad de ValparaísoEditorial, Valparaíso, Chile, pp. 174–189.

Pesoti, Aline Regiele, Oliveira, Bruno Menezes de, Oliveira, Augusto Cesar de, Pompeu, Dávia Guimarães, Gonçalves, Daniel Bonoto, Marangoni, Sérgio, Silva, José Antonio da, & Granjeiro, Paulo Afonso. (2015). Extraction, purification and characterization of inhibitor of trypsin from *Chenopodium quinoa* seeds. Food Science and Technology (Campinas), 35(4): 588-597.

Phillippy, B.Q., (2016). Transport of calcium across Caco-2 cells in the presence of inositol hexakisphosphate. Nutrition Research, 26: 146-149.

Pineli, L. de L. de O., Botelho, R. B. A., Zandonadi, R. P., Solorzano, J. L., de Oliveira, G. T., Reis, C. E. G., & Teixeira, D. da S. (2015). Low glycemic index and increased protein content in a novel quinoa milk. LWT - Food Science and Technology, 63(2): 1261–1267.

Piper, C. S., (1996). Soil and Plant Analysis. Han Publications, Bombay.

Population Refernce bureau (2015). World population data sheet. Retrieved from www.prb.org/pdf15/2015-world-population-data-sheet_eng.pdf.

Preet, K., & Punia, D. (2000). Antinutrients and digestibility (*in vitro*) of soaked, dehulled and germinated cowpea. Nutrition and Health, 14(2): 109–117.

Prego, I. (1998). Seed Structure and Localization of Reserves in*Chenopodium quinoa*. Annals of Botany, 82(4): 481–488.

Pushparaj, F. S. (2011). Influence of Processing on Dietary Fiber, Tannin and <i>*in vitro*</i> Protein Digestibility of Pearl Millet. Food and Nutrition Sciences, 02(08): 895–900.

Raamsdonk, L. W. D. Van, Pinckaers, V., Ossenkoppele, J., Houben, R., Lotgering, M., & Groot, M. J. (2010). Quality assessments of untreated and washed Quinoa (*Chenopodium quinoa*) seeds based on histological and foaming capacity investigations. Microscopy: Science, Technology, Applications and Education, 4(2): 1033–1038.

Raboy,V. (2009). Approaches and challenges to engineering seed phytate and total phosphorus. PlantScience, 177: 281–296.

Raghuvanshi, R. S., Singh, S., Bisht, K., & Singh, R. (2011). Processing of mungbean products and its nutritional and organoleptic evaluation. International Journal of Food Science & Technology, 46: 1378–1387.

Ranganna S (1995) Manual analysis of fruits and vegetable products. Tata Mc Graw Hill publishing Co. Ltd, New Delhi.

Ranilla, L. G., Apostolidis, E., Genovese, M. I., Lajolo, F. M., & Shetty, K. (2009). Evaluation of Indigenous Grains from the Peruvian Andean Region for Antidiabetes and Antihypertension Potential Using *In vitro* Methods. Journal of Medicinal Food, 12(4): 704–713.

Rasane, P., Jha, A., Kumar, A., & Sharma, N. (2015). Reduction in phytic acid content and enhancement of antioxidant properties of nutricereals by processing for developing a fermented baby food. Journal of Food Science and Technology, 52: 3219–3234.

Rayner, M., Timgren, A., Sjoo, M., & Dejmek, P. (2012). Quinoa starch granules: A candidate for stabilising food-grade Pickering emulsions. Journal of the Science of Food and Agriculture, 92(9): 1841–1847.

Reichert, R. D., Tatarynovich, J. T., & Tyler, R. T. (1986). Abrasive Dehulling of Quinoa (*Chenopodium quinoa*): Effect on Saponin Content as Determined by an Adapted Hemolytic Assay. Cereal Chemistry, 63: 471-480.

Repo-Carrasco-Valencia, R. a M., Encina, C. R., Binaghi, M. J., Greco, C. B., & de Ferrer, P. a R. (2010). Effects of roasting and boiling of quinoa, kiwicha and kaniwa on composition and availability of minerals *in vitro*. Journal of the Science of Food and Agriculture, 90(12): 2068–2073.

Repo-Carrasco-Valencia, R. A.-M., & Serna, L. A. (2011). Quinoa (*Chenopodium quinoa*, Willd.) as a source of dietary fiber and other functional components. Ciência E Tecnologia de Alimentos, 31(1): 225–230.

Ridout, C., Price, L. R., DuPont, M. S., Parker, M. L. & Fenwick, G. R. (1991). Quinoa saponins--analysis and preliminary investigations into effects of reduction by processing. Journal of Food Science and Agriculture, 54: 165-76.

Rodríguez-Sandoval, E., Lascano, A., Sandoval, G., Rodríguez-Sandoval, E., Lascano, A., & Sandoval, G. (2012). Influence of the partial substitution of wheat flour for quinoa and potato flour on the thermomechanical and breadmaking properties. Brazilian Journal of Chemical Engineering, 15(03): 199–207.

Rosa M, Hilal M, Gonzalez JA, Prado FE (2009). Low-temperature effect on enzyme activities involved in sucrose–starch partitioning in salt-stressed and salt-acclimated cotyledons of quinoa (*Chenopodium quinoa* Willd.) seedlings. Plant Physiology and Biochemistry, 47: 300–307.

Rose, T. J., Liu, L., & Wissuwa, M. (2013). Improving phosphorus efficiency in cereal crops: Is breeding for reduced grain phosphorus concentration part of the solution? Frontiers in Plant Science, 4(9): 444.

Rosero, O., M. Marounek, N. Břeňová and D. Lukešova. (2013). Phytase activity and comparison of chemical composition, phytic acid P content of four varieties of quinoa grain (*Chenopodium quinoa* Willd.). Acta Agronómica, 62: 13-20.

Roy DN & Rao PS (1971). Evidence, isolation, purification and some properties of a trypsin inhibitor in Lathyrus sativus. Journal of Agriculture and Food Chemistry, 19: 257– 259.

Ruales, J., & Nair, B. M. (2002). Nutritional quality of the protein in quinoa (*Chenopodium quinoa*, Willd) seeds. Plant Foods for Human Nutrition, 42(1): 1–11.

Rumiyati, Anthony P. James, Vijay Jayasena (2012). Effect of Germination on the Nutritional and Protein Profile of Australian Sweet Lupin (*Lupinus angustifolius* L.). Food and Nutrition Sciences, 03(05): 621–626.

Rusydi, M. (2012). Effect of germination on total phenolic, tannin and phytic acid contents in soybean and peanut, 19(2): 673–677.

Ryan, E., Galvin, K., O'Connor, T., Maguire, A. & O'Brien, N. (2007). Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains and legumes. Plant Foods for Human Nutrition, 62: 85-91.

Saha, D., & Bhattacharya, S. (2010). Hydrocolloids as thickening and gelling agents in food: A critical review. Journal of Food Science and Technology, 47(6): 587–597.

Sánchez, C. L., Cubero, J., Sánchez, J., Chanclón, B., Rivero, M., Rodríguez, A. B., & Barriga, C. (2009). The possible role of human milk nucleotides as sleep inducers. Nutritional Neuroscience, 12(1): 2–8.

Sangronis, E., & Machado, C. J. (2007). Influence of germination on the nutritional quality of Phaseolus vulgaris and Cajanus cajan. LWT - Food Science and Technology, 40(1): 116–120.

Sanni, L.O., Adebowale, A.A., Filani, T.A., Oyewole, O.B. and Westby, A. (2006). Quality of flash and rotary dryer dried fufu flour. Journal of Food Agriculture and Environment, 4: 74 – 78.

Santos Calderelli, V. A., de Benassi, M. T., Visentainer, J. V., & Matioli, G. (2010). Quinoa and flaxseed: Potential ingredients in the production of bread with functional quality. Brazilian Archives of Biology and Technology, 53(4): 981–986.

Sattar A., Badshah A., Aurangzeb (1995). Biosynthesis of ascorbic acid in germinating rapeseed cultivars. Plant Foods for Human Nutrition, 47(1): 63–70.

Savage, G.P.; Mårtensson, L.; Sedcole, J.R. (2009). Composition of oxalates in baked taro (Colocasia esculenta var. Schott) leaves cooked alone or with additions of cows milk or coconut milk. Journal of Food Composition and Analysis, 22: 83–86.

Savelkoul, F.H., A.F. Vander Poel and S. Tamminga (1992). The presence and inactivation of trypsin inhibitors, tannins, lectins and amylase inhibitors in legume seeds during germination A review. Plant Foods for Human Nutrition, 42: 71-85.

Schoenlechner R, Sarawong C, Sekiguchi K, Berghofer E, Ng PKW. (2010). Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. Food Chemistry, 14333-9.

Segev, A. (2011). Total Phenolic Content and Antioxidant Activity of Chickpea as Affected by Soaking and Cooking Conditions. Food and Nutrition Sciences, 02(07): 724–730.

Sharma, M., & Mridula, D. (2015). Development and Quality Evaluation of Maize-Based Fortified Nutritious Bar. Agricultural Research, 4(1), 93–101.

Shin SS, Jung YS, Yoon KH, Choi S, Hong Y, Park D, Lee H, Seo BI, Lee HY, Yoon M. (2013) The Korean traditional medicine Gyeongshingangjeehwan inhibits adipocyte hypertrophy and visceral adipose tissue accumulation by activating PPARalpha actions in rat white adipose tissues. Journal of Ethnopharmacology, 127(1): 47-54.

Siah, S., Wood, J. A., Agboola, S., Konczak, I., & Blanchard, C. L. (2014). Effects of soaking, boiling and autoclaving on the phenolic contents and antioxidant activities of faba beans (Vicia faba L.) differing in seed coat colours. Food Chemistry, 142: 461–8.

Siener, R.; Hönow, R.; Seidler, A.; Voss, S.; Hesse, A. (2006). Oxalate contents of species of the Polygonaceae, Amaranthaceae and families. Food Chemistry, 98: 220–224

Singh, J., & Basu, P. S. (2012). Non-Nutritive Bioactive Compounds in Pulses and Their Impact on Human Health : An Overview. Food and Nutrition Sciences, 3: 1664–1672.

Singh, U. And Jambunathan, R. (1990). Studies on desi and kabuli chickpea (Cicer arietinum) cultivars. Level of protease inhibitors, levels of polyphenolic compounds and *in vitro* protein digestibility. Journal of Food Science. 46: 1364-1367.

Slavin, J. (2003). Why whole grains are protective, biological mechanisms. The Proceedings of Nutrition Society, 62(1): 129-134.

Slavin, J., & Lloyd, B. (2012). Health Benefits of Fruits and Vegetables. Advances in Nutrition, 3(4): 506–516.

Soetan, K. O., & Oyewole, O. E. (2009). The need for adequate processing to reduce the anti- nutritional factors in plants used as human foods and animal feeds: A review. African Journal of Food Science, 3(9): 223–232.

Soliz-Guerrero, J.B., Jasso de Rodrı´ guez, D., Rodrı´guez-Garcı´ a, R., Angulo-Sanchez, J.L. & Me´ ndez-Padilla, G. (2002). Quinoa saponins: concentration and composition analysis. Book chapter; Conference paper Trends in New Crops and New Uses. Proceedings of the Fifth National Symposium, Atlanta, GA, Pp. 110– 114.

Steffolani, M. E., León, A. E., & Pérez, G. T. (2013). Study of the physicochemical and functional characterization of quinoa and kañiwa starches. Starch–Stärke, 65: 976–983.

Stikic, R., Glamoclija, D., Demin, M., Vucelic-Radovic, B., Jovanovic, Z., Milojkovic-Opsenica, D., Milovanovic, M. (2012). Agronomical and nutritional evaluation of quinoa seeds (*Chenopodium quinoa* Willd.) as an ingredient in bread formulations. Journal of Cereal Science, 55(2): 132–138.

Sun, T., & Ho, C. (2005). Antioxidant activities of buckwheat extracts. Food Chemistry, 90: 743–749.

Sundarrajan, L. (2014). Effect of extrusion cooking on the nutritional properties of amaranth , quinoa , kañiwa and lupine. Department of Food and Environmental Sciences. University Of Helsinki. ISSN: 0355-1180

Sung HG, Shin HT, Ha JK, Lai H-L, Cheng K-J, Lee JH (2005) Effect of germination temperature on characteristics of phytase production from barley. Bioresource Technology, 96: 1297–1303.

Suryanti, C. (2016). Effect of germination on antioxidant activity , total phenolics , β-carotene , ascorbic acid and α-tocopherol contents of lead tree sprouts (Leucaena leucocephala (lmk.). International Food Research Journal, 23(1): 167–172.

Takahashi Yoko, Tseye-Oidov Odbayar, and Takashi Ide (2009). A comparative analysis of Genistein and Diadzein in affecting lipid metabolism in rat. Journal of Clinical Biochemistry and Nutrition, 44: 223–230.

Takeda, E., Yamamoto, H., Yamanaka-Okumura,H., and Taketani,Y. (2012).Dietary phosphorus in bone health and quality of life. Nutrition Reviews, 70: 311–321.

Tang, Y., Li, X., Chen, P. X., Zhang, B., Hernandez, M., Zhang, H., Tsao, R. (2014). Lipids, Tocopherols, and Carotenoids in Leaves of Amaranth and Quinoa Cultivars and a New Approach to Overall Evaluation of Nutritional Quality Traits. Journal of Agriculture and Food Chemistry. 62: 12610−12619.

Tang, Y., Li, X., Chen, P. X., Zhang, B., Hernandez, M., Zhang, H., … Tsao, R. (2014b). Lipids, Tocopherols, and Carotenoids in Leaves of Amaranth and Quinoa Cultivars and a New Approach to Overall Evaluation of Nutritional Quality Traits. Journal of Agriculture and Food Chemistry, 62: 12610−12619.

Tang, Y., Li, X., Zhang, B., Chen, P. X., Liu, R., & Tsao, R. (2015). Characterisation of phenolics, betanins and antioxidant activities in seeds of three *Chenopodium quinoa* Willd. genotypes. Food Chemistry, 166: 380–388.

Tari, T., Annapure, U., Singhal, R., and Kulkarni, P. (2003). Starch-based spherical aggre- gates: Screening of small granule sized starches for entrapment of a model flavouring compound, vanillin. Carbohydrates Polymer, 53: 45–51.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis, 19(6-7), 669–675.

Thapliyal, P., Sehgal, S., & Kawatra, A. (2014). *In-vitro* digestibility and antinutrients as affected by soaking, dehulling and pressure cooking of chickpea (Cicer *arietinum*) varieties. Asian Journal of Dairy and Food Research, 33(2): 131.

Thilakarathna, S. H., & Vasantha Rupasinghe, H. P. (2013). Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients, 5(9): 3367–3387.

Thuresson, C., & Thuresson, C. (2015). Development and studies on a gluten free , liquid suspension based on quinoa (*Chenopodium quinoa*). Swedish University of Agricultural Sciences, Master Thesis, 30 hec, Advanced A2E (427).

Torres, E. R., Santana, E., Felix, R., Santana, D., & Cordeiro, J. (2016). Cereal Bar Development Using Exotic Fruit. Food Process Engineering in a Changing World, 22(1): 41-45.

Tosi, P., Gritsch, C. S., He, J., & Shewry, P. R. (2011). Distribution of gluten proteins in bread wheat (Triticum aestivum) grain. Annals of Botany, 108(1): 23–35.

United States Department of Agriculture (2013). USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory Home.

United States Department of Agriculture. (2015). USDA National Nutrient Database for Standard Reference. Retrieved from http://www.ars.usda.gov/Services/docs.htm?docid=8964 .

Uppal, V., & Bains, K. (2012). Effect of germination periods and hydrothermal treatments on *in vitro* protein and starch digestibility of germinated legumes. Journal of Food Science and Technology, 49(2): 184–191.

Vadivel, V., Stuetz, W., Scherbaum, V., & Biesalski, H. K. (2011). Total free phenolic content and health relevant functionality of Indian wild legume grains:

Effect of indigenous processing methods. Journal of Food Composition and Analysis, 24(7): 935–943.

Valcárcel-yamani, B., & Caetano, S. (2012). Applications of Quinoa (*Chenopodium quinoa* Willd .) and Amaranth (Amaranthus Spp .) and Their Influence in the Nutritional Value of Cereal Based Foods. Food and Public Health, 2(6): 265–275.

Valencia-Chamorro S.A (2003): Quinoa. In: Cabal- lero B.: Encyclopedia of Food Science and Nutrition. 8: 4895–4902.

Valencia-Chamorro, S. A. (2004). Quinoa. In C. W.Wrigley, H.Corke, & C.Walker (Eds.), Encyclopedia of grain science (Vol. 3, pp. 1–8). Oxford, United Kingdom: Academic Press.

Van Soest, P. J., & Wine, R. H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. Journal of the Association of Official Analytical Chemistry, 50: 50–55.

Varli, S. N., & Şanlıer, N. (2016). Nutritional and health benefits of quinoa (*Chenopodium quinoa* Willd.). Journal of Cereal Science. (under publication)

Vasishtha, H., & Srivastava, R. P. (2013). Effect of soaking and cooking on dietary fibre components of different type of chickpea genotypes. Journal of Food Science and Technology, 50(3): 579–584.

Vega-Gálvez, A., Miranda, M., Vergara, J., Uribe, E., Puente, L., & Martínez, E. a. (2010). Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* willd.), an ancient Andean grain: A review. Journal of the Science of Food and Agriculture, 90(15): 2541–2547.

Vega-Gálvez, A., Miranda, M., Vergara, J., Uribe, E., Puente, L., & Martínez, E. a. (2010). Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* willd.), an ancient Andean grain: A review. Journal of the Science of Food and Agriculture, 90(15): 2541–2547.

Verza SG, Silveira F, Cibulski S, Kaiser S, Ferreira F, Gosmann G, Roehe PM, Ortega GG. (2012). Immunoadjuvant activity, toxicity assays, and determination

by UPLC/Q-TOF-MS of triterpenic saponins from *Chenopodium quinoa* seeds. Journal of Agriculture and Food Chemistry, 60: 3113–8.

Vilche, C., Gely, M. & Santalla, E. (2009). Physical properties of quinoa seeds. Biosystems Engineering, 86: 59–65.

Villa, D. Y. G., Russo, L., Kerbab, K., Landi, M., & Rastrelli, L. (2014). Chemical and nutritional characterization of Chenopodium pallidicaule (cañihua) and *Chenopodium quinoa* (quinoa) seeds. Emirates Journal of Food and Agriculture, 26(7): 609–615.

Villacrés, E., Pástor, G., Quelal, M. B., Zambrano, I., & Morales, S. H. (2013). Effect of processing on the content of fatty acids , tocopherols and sterols in the oils of quinoa (*Chenopodium quinoa* Willd), lupine (Lupinus mutabilis Sweet), amaranth (Amaranthus caudatus L .) and sangorache (Amaranthus quitensis L.). Global Advanced Research Journal of Food Science and Technology, 2(4): 44–53.

Wang, N., Hatcher, D. W., Toews, R., & Gawalko, E. J. (2009). Influence of cooking and dehulling on nutritional composition of several varieties of lentils (Lens culinaris). LWT - Food Science and Technology, 42(4): 842–848.

Wang, S., & Zhu, F. (2016). Formulation and Quality Attributes of Quinoa Food Products. Food and Bioprocess Technology, 9(1): 49–68.

Welch,A.A., Fransen,H., Jenab,M., Boutron-Ruault,M.C., Tumino, R., Agnoli,C., (2009). Variation in intakes of calcium, phosphorus, magnesium, iron and potassium in 10 countries in the European prospective Investigation into Cancer and Nutrition study. European Journal of Clinical Nutrition, 63: 101–S121.

Wolter, A., Hager, A. S., Zannini, E., & Arendt, E. K. (2014). *In vitro* starch digestibility and predicted glycaemic indexes of buckwheat, oat, quinoa, sorghum, teff and commercial gluten-free bread. Journal of Cereal Science, 58(3), 431–436.

Wong, J. H., Lau, T., Cai, N., Singh, J., Pedersen, J. F., Vensel, W. H., Buchanan, B. B. (2009). Digestibility of protein and starch from sorghum (Sorghum bicolor) is linked to biochemical and structural features of grain endosperm. Journal of Cereal Science, 49(1): 73–82.

Wu, Z., Song, L., Feng, S., Liu, Y., He, G., Yioe, Y., Huang, D. (2012). Germination dramatically increases isoflavonoid content and diversity in chickpea (Cicer arietinum L.) seeds. Journal of Agricultural and Food Chemistry, 60(35): 8606–8615.

Xu, B., & Chang, S. K. C. (2010). Antioxidant capacity of seed coat, dehulled bean, and whole black soybeans in relation to their distributions of total phenolics, phenolic acids, anthocyanins, and isoflavones. Journal of Agricultural and Food Chemistry, 56(18): 8365–8373.

Xu, M. J., J. D. Dong and M. Y. Zhu (2005). Effects of germination conditions on ascorbic acid level and yield of soybean sprouts. Journal of Science, Food and Agriculture, 85(6): 943-947.

Yao, Y., Yang, X., Shi, Z., & Ren, G. (2014a). Anti-Inflammatory Activity of Saponins from Quinoa (*Chenopodium quinoa* Willd.) Seeds in Lipopolysaccharide-Stimulated RAW 264.7 Macrophages Cells. Journal of Food Science, 79(5).

Yawadio Nsimba, R., Kikuzaki, H., & Konishi, Y. (2008). Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and Amaranthus spp. seeds. Food Chemistry, 106(2): 760–766.

Zamora, R. & Hidalgo, F.J. (2005). Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. Critical Reviews in Food Science and Nutrition, 45: 49–59.

Zevallos, V. F., Ellis, H. J., Herencia, L. I., & Ciclitira, P. J. (2012). Variable activation of immune response by quinoa (*Chenopodium quinoa* Willd.) prolamins in celiac disease. American Journal of Clinical Nutrition, 96: 337–344.

Zevallos, V. F., Herencia, L. I., Chang, F., Donnelly, S., Ellis, H. J., & Ciclitira, P. J. (2014). Gastrointestinal Effects of Eating Quinoa (*Chenopodium quinoa* Willd.) in Celiac Patients. American Journal of Gastroenterology, 109(2): 270–278.

Zhu, N., Kikuzaki, H., Vastano, B. C., Nakatani, N., Karwe, M. V., Rosen, R. T., & Ho, C. T. (2009). Ecdysteroids of quinoa seeds (*Chenopodium quinoa* Willd.). Journal of Agricultural and Food Chemistry, 49(5): 2576–2578.

Zhu, N., Kikuzaki, H., Vastano, B. C., Nakatani, N., Karwe, M. V., Rosen, R. T., & Ho, C. T. (2014). Ecdysteroids of quinoa seeds (*Chenopodium quinoa* Willd.). Journal of Agricultural and Food Chemistry, 49(5): 2576–2578.

Zhu, N., Sheng, S., Li, D., Lavoie, E. J., Karwe, M. V., Rosen, R. T., Ho, C-T. (2002). Antioxidative flavonoid glycosides from quinoa seeds (*Chenopodium quinoa*). Journal of Food Lipids 8(1): 37–44.