MULTIGRAIN BROCOOLI BREAD

Dissertation Report-1

Submitted By

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CERTIFICATE

This is to certify that **Navreet Kaur (Registration No.11713773)** *has personally completed M.Tech. Dissertation-I entitled,* **"Multigrain Broccoli Bread"** *under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of pre-dissertation has ever been submitted for any other purpose at any University.*

The project report is appropriate for the submission and the partial fulfilment of the conditions for the evaluation leading to the award of Master of Food Technology.

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DECLARATION

I hereby declare that the work presented in the Dissertation- 1 entitled **"Multigrain Broccoli Bread"** is my own and original. The work has been carried out by me at School of Agriculture, Lovely Professional University, Phagwara, Punjab, India under the guidance of **Er. Jasleen Kaur Bhasin**, Assistant Professor (Food Technology) of School of Agriculture, Lovely Professional University, Phagwara, Punjab, India for the award of the degree of Master of Food Technology.

Date: May, 2018 **Navreet Kaur**

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I certified that the above statement made by the student is correct to the best of my knowledge and belief.

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CHAPTER-1 INTRODUCTION

1.1 Bread

Nowadays, the consumption of bread is increasing day by day. It become staple food among all age groups. It contains both macronutrients (Carbohydrates, proteins and fats) and micronutrients (Minerals and vitamins) that fulfil the needs of human health (*S. O. Oluwajoba, 2012*). But in today's urban world, Consumers are more conscious about their health and want to keep themselves away from various diseases (weight gain, diabetes and cardiovascular) to maintain their health. So the various studies are going on, to produce highly nutritious and healthy breads.

Generally bread is formulate by the process of fermentation and is known as confectionery product. It is prepared from dough of different ingredients (wheat flour or multi-grain flour, yeast, salt, sugar and water) as per their requirement of nutritional value and by various processes including mixing, kneading, proofing, shaping and baking etc (*Dewettinck K, 2008*). Different types of breads are prepared all over the world and the most commonly used from them are white bread, brown bread and multigrain bread.

White bread is typically prepared from central core of wheat flour grain. It is the most popular variety of bread. Brown bread is highly nutritious and made of whole wheat flour which contain rich amount of fiber content. Multigrain bread is the healthiest variety because it is prepared from two or more different types of flour which add extra nutrition, protein, fiber, minerals, and vitamins to it. Even though composite breads are made but still it requires at least 70% of wheat flour to give rise to bread (*Satin M, 1988 and Eggleston G, 1992*).

1.2 Grains

1.2.1 Wheat Grain flour

Wheat (*Triticum aestivum*) flour is a powder made from grinding of wheat grains and it is the main ingredient used for bread making. The epidemiological studies concluded that the whole wheat foods could reduce the risk of Cancer (Ferguson LR, 1999 and Slavin JL, 2000), Diabetes (*Hallfrisch J, 2000 and Venn BJ, 2004) and Coronary heart disease (Anderson JW, 2000 and Truswell AS, 2002*). In general three parts of the wheat grain are used in flour –

1. The endosperm (protein / starchy part),

2. The germ (protein / fat / vitamin rich part),

3. The bran (fiber part).

It is estimated that 55% of total processed wheat flour is used in baking and confectionery products, 17% for domestic purpose, 15% for dough making, 11% for cookies or biscuits and rest of 2% is used for drugs, glue and animal feeding (*Abitrigo, 2014*). Changing the wheat flour with other flours has become a major challenge because gluten present in flour is essential structure-building protein, produce elastic and extensible properties to provide good quality bread (*Gallagher, 2003*).

1.2.2 Quinoa Seeds Flour

Quinoa (*Chenopodium quinoa willd*), is a pseudo cereal and cultivated in several countries of south America but nowadays, due to high protein and mineral content it has been attracting toward the technology (*Park, 2005*). The proximate estimation of composition of quinoa varies with cultivar, but protein ranges from 10- 18%, crude fat 9%, carbohydrates 54-64%, fat 2-4%, and rest of 2-5% crude fibre. Quinoa seeds have higher amount of first limiting amino acid, lysine which make its amino acid balance better than that of wheat and corn (*Lorenz, 1991*). By various studies, it is reported that quinoa- wheat composite flour gave better results with less than 10% of substitution (*Chauhan, 1992 and Morita, 2001 and Park, 2005 and Rosell, 2009*).

1.2.3 Chia seeds

Chia (*Salvia Hispanica L*), is a pseudo cereal and known as "Super food". It is highly nutritious, contains 15-25% of protein, 30-33% of fats, 26-41% of carbohydrates, 18-30% of dietary fiber, 4-5% of ash and rich amount of vitamins, minerals and antioxidants (*Ali N M, 2012*). Chia seeds have 18 amino acids, including good amount of all nine essential amino acids that could be help the body to make use of the proteins (*Sandoval-Oliveros M R, 2013*) . Chia seeds are an excellent source of omega-3 fatty acids which are essential for body. The three main omega-3 fatty acids ALA (Alpha- linolenic acid), EPA (Eicosapentaenoic acid), and DHA (Docosahexaenoic acid). Our body converts ALA to EPA and DHA which is beneficial to our health and reduces the risk factor of heart disease, high blood pressure (Fish and Omega-3 fatty acids).

1.2.4 Broccoli

Broccoli (*Brassica oleracea var. italic*), is a green leafy vegetable which possesses various medicinal properties. It is a good source of vitamin A, C, Riboflavin, Iron and Calcium. It contains multiple nutrients with potent anti-cancer properties, anti-viral and anti- bacterial activity. It is proximate that a single serving of broccoli provides more than 30mg of vitamin C and a half cup provides 52mg of vitamin C (*Liu, 2009*).

Due to high nutrient density and benefits, broccoli is known as one of the "top powerhouses". The low glycemic content of broccoli helps to maintain normal blood sugar level. The energy content in broccoli is about 34kcl and the other contents estimated as- Carbohydrates 6.64gm, Proteins- 2.82, Fats-0.37gm, Dietary fiber- 2.60gm, Cholesterol content is nil but rich in Vitamin A and C (*Daniells S, 2009*) .

Broccoli grows best in cool weather ranging from 18°C to 23°C, hot summer weather is not suitable for growth of broccoli plant. Broccoli have cluster of flowers appears in centre of head usually green in colour and it should harvested before the flowers turn bright yellow. It is closely appears like cauliflower, 3 varieties of broccoli are grown annually in cool season.

Sprouting broccoli has thin stalks with large number of head and is harvested during winter. Romanesco broccoli has heads of distinctive fractal appearance and is yellow-green in color. Technically, it is a cultivar of botrytis (cauliflower) group. Purple cauliflower (broccoli) is grown in southern Italy, Spain and United Kingdom. The head of purple cauliflower looks like shape of cauliflower but actually it consists of tiny flower buds. Chinese broccoli (Alboglabra Group) is also a cultivar of brassica oleracea group (*Mishra, pk,vommika and mukherjee, 2012*).

Fig1. Calabrese broccoli

Fig2. Sprouting broccoli

Fig4. Romanesco broccoli

Fig4. Purple broccoli

Fig5. Chinese broccoli

1.2.5 Medicinal value of broccoli

Broccoli contains multiple nutrients with powerful anti- cancer properties, such as Di-indolylmethane and small amount of selenium. Broccoli contains 3, 3-di-indolylmethane which is a powerful regulator of immune system with anti-viral, anti-bacterial and anti-cancer activity. It also contains “glucoraphanin” compound which can formed into an anti-cancer compound “sulforaphane”, although boiling of vegetable reduced the benefits of broccoli.

It is a good source of “indole-3-carbinol”, this chemical helps to repair the cells and stop growth of cancer cells. It is found that, prostate cancer has been reduced by high intake of broccoli. Broccoli is also beneficial for prevention of heart disease. The chemical compound “indole-3-carbinol” present in broccoli can oppose breast cancer by converting a cancer promoting estrogen into a protective variety (*Phillip, 2011*). Broccoli sprouts, also contain phytochemical compound sulforaphane, it is a product of glucoraphanin that is believed to helpful in preventing some types of cancer, like colon and rectal cancer (*Benson, 2011*).

Certain enzymes are induced by Sulforaphane compound that can deactivate free- radicals and carcinogens. Other beneficial property of broccoli is, it is considered as low-glycemic food which helps to maintain blood sugar level. It helps to detoxify the body and hence, detoxification helps to weight loss and prevent certain diseases (*Stanley and provost, 2010*). The study shows that, the risk of bladder cancer was reduced (upto 40%) by intake of 3 servings a month of raw broccoli (*Liu, 2009*). The American journal of clinical nutrition reported that, “Broccoli was among the top foods that may prevent colon cancer”. The Harvard scientists (*Kim and Berges, 2009*) reported that “healthy broccoli may help protect against strokes”.

CHAPTER- 2 PROBLEM BACKGROUND

Bread is a staple food among all age groups. It contains both macronutrients (Carbohydrates, proteins and fats) and micronutrients (Minerals and vitamins) but today's consumers are more conscious about their health and want to keep themselves away from various disease. In present scenario most people are suffering from gluten intolerance, which include celiac disease, non-celiac gluten sensitivity, gluten ataxia.

Foods with higher glycemia index such as most white breads, leads to higher risk of type 2 diabetes which raise blood glucose level and release more insulin that become a reason for developing cancer. To minimize the effect of gluten, Quinoa flour and chia flour blends are used in product development that is naturally gluten-free and contains high amount of protein. Broccoli contains multiple nutrients with potent anti-cancer properties, anti-viral and anti- bacterial activity.

So, the present study has been designed to produce highly nutritious and healthy bread with multigrain flour (wheat, quinoa and chia) and broccoli.

CHAPTER-3 OBJECTIVES

1. To standardize the formulation for the preparation of bread by using multigrain flour.

Table.3.1-Basic ingredients required for bread

Ingredients	Control	Formulation(g)
Compressed yeast (g)	3	3
Salt (g)	1.5	2
Sugar(g)	4	5
Milk powder(g)	5	5
Whole wheat flour(g)	100	86
Quinoa flour(g)	-	5
Chia flour(g)	-	5
Broccoli(g)	-	4
Water(ml)	60	60

2. To study the physiochemical, microbiological, sensory and nutritional properties of bread.

3. To study the shelf life of formulated bread.

3.1 Study on breads nutritive, phytochemical and functional properties

Supriya et al., (2015) conducted a study on 4 different compositions of multigrain mix flour (using sorghum, buckwheat, chickpea, sprouted wheat and sprouted barley) and determined their nutritional, phytochemical and functional properties. For further analysis, only 2 of the composite formulations were used. For improving bread’s crumb structure additional flours (Corn and defatted flours) were used. Nutritional analysis gave better results that shows high amount of proteins, low fat in fiber, moderate carbohydrates and rich in mineral contents. Apart from nutritive value, Phytochemical analysis shows that the composite flour and products have good source of phenolics, flavonoids and antioxidant. In further analysis, the effect of high temperature and extraction of final accepted bread product was checked. Phytochemical anaylsis revealed that there was no effect of heat or temperature on all the phenolics and flavonoids. Besides these analyses, chromatographic techniques like HPLC and GCMS was also used on composite flour and on bread extract. HPLC analysis shows that flavonoid compounds (rutin, quercetin, epicatechin and chlorogenic acid) were present. Physiochemical and physical characteristics of bread were also estimated like height of loaf, volume of loaf, weight of loaf and baking loss.

3.2 Anticancer and Antioxidant Activity of Bread

Urszula Gawlik-Dziki et al., (2014) studied the ability of antioxidant and anticancer properties of bread enriched with brocooli sprouts. Usually, addition of bread increased the antioxidant capacity of product; although the increase was not related to the percentage of brocooli sprouts. Upto 2% replacement of broccoli sprouts gives satisfactory results from overall consumer’s acceptability and desirable increase of antioxidant potential. Significantly, the effective phenolics concentration for buffer extract was about 12 μM, for extracts after digestion in vitro was 13 μM and for extracts after absorption in vitro was 7 μM. Therefore, their data confirms that bread enriched with brocooli sprouts have chemopreventive activity and specify that Brocooli sprouts contain valuable food supplement of chemopreventive for stomach cancer.

3.3 A Study of a Multigrain Gluten Free and Protein Rich Flour

The study was conducted by Divinity Malshe et al., (2014) who studied to understand the acceptance of blended healthy gluten free and protein rich flour; with an aim to make it cost effective by standardize an innovative, preservative free, simple, nutritional blend. The product was formulate by multigrain flour, having a unique mixture of chick pea, oats, soya, Indian chia- sabja and fenugreek. This unique blend of multigrain flour makes the product gluten free and protein rich with high fiber content or having good amounts of antioxidants.

The study of acceptance was conducted by sensory evaluation using the scoring method based on five point scales. Tests were performed on sensory attributes like appearance, color, taste, texture, aroma, and overall acceptability of the product. To check the shelf life of product, microbial analysis was carried out. Besides that they focused on packaging, nutritional labelling, budgeting and marketing.

3.4 Thermo mechanical and Bread making Properties of Wheat flour

E. Rodriguez-Sandoval et al (2011) studied the thermo mechanical properties of dough and evaluated the physical characteristics of formulated bread from composite flours of Quinoa-Wheat and Potato- Wheat at 10 and 20 % Substitution Level. The functional properties i.e. the water absorption index (WAI), water solubility index (WSI) and swelling power (SP) of flours were measured. By using Mixolab, the thermo mechanical properties of wheat and composite flours were determined and check the baking quality characteristics of breads by weight, height, width and specific volume. The results for potato flour showed the higher values of WAI (4.48), WSI (7.45%), and SP (4.84). The composite flour of quinoa- wheat showed lower problems and cooking stability data, which was a good indicator of shelf life of bread. On the contrary, the composite flour of potato- wheat showed lower stability, minimum torque and peak torque, and higher water absorption. At 10% substitution level the samples of potato- wheat composite flours were similar to weight, height, width, and specific volume of wheat bread.

3.5 Effect of Chia Seeds in the Development of Bread

Shagun Pal et al., (2017) conducted a study to develop suitable bread for all type of persons having celiac disease or such type of diseases. Available data shown that celiac disease was most often in northern india (Punjab, Haryana, Delhi, Rajasthan and Uttar Pradesh) because in these areas wheat is commonly used in the diet. So, the study was carried out to check the effect of supplementation of chia seeds in the deveoplment of gluten free buckwheat bread.

Chia seeds considered a pseudo cereal and belong to the mint family (Labiatae). They are rich in omega-3 (ALA), proteins, fats, and also contains high levels of minerals such as calcium, iron, magnesium and phosphorus. Firstly different proportions of buckwheat was optimized with rice flour (50:50, 60:40, 70:30, 80:20) and conducted their sensory analysis, which shows that overall 50:50 proportion was acceptable as compare to other proportions, Therefore 50:50 proportion was taken as control in which chia seeds were supplemented at three different ratios (5%, 10%, 15%). The investigation of shelf life was conducted at two different temperatures i.e. at room temperature (30-32 °) and refrigeration temperature (7°C) in which total antioxidant activity, moisture content and microbiological quality were also assessed. Overall results showed that proximate composition of bread like protein, ash content, total antioxidant activity and specific volume of bread increases at 10% supplementation of chia seeds. Therefore 10% incorporation of chia seeds was suggested. The shelf life was estimated 6 days at room temperature and 14 days at refrigeration temperature.

Daria Romankiewicz et al., (2017) also carried out a study to analyse the effect of added chia seeds (0, 2, 4, 6 and 8%) on quality and nutritional value of wheat bread. The chemical composition, fatty acid composition, total phenolics content, volume, baking losses, crumb texture, color and various sensory properties of bread was determined. The volume of bread and baking losses was decreased by addition of chia seeds. The crumb color of bread having chia seeds was often darker than control sample. The texture analyser determined that the hardness of crumb was also decreased by addition of chia seeds. Most significant feature of chia seeds was, it increase the nutritional value of the bread. Besides, it has been observed that Bread with chia seeds contained high amount of dietary fiber, mineral components, fatty acids composition and also higher level of phenolic compounds. The study also showed that, the 6% substitution of chia seeds with wheat flour did not affect the quality and acceptance of final product.

3.6 Effects on Chemical, rheological and bread characteristics properties

Marie hruskova et al., (2015), carried out a study to check the influence of different forms of chia seeds on chemical, rheological and bread characteristics of wheat flour. In this study, tests were performed on chia flour obtained white and brown seeds in dry and hydrated form. The ash and protein content of composite flours were raised by both forms of chia seeds (near to 2%). As the Sucrose have solvent retention capacity the non- starch polysaccharides content also increased. Decline in zeleny value measures the protein quality which was affected by alternative plant materials.During amylograph and extensigraph tests, water suspension viscosity and dough elasticity also increased. It was determined that, the composite flours of higher hydrated form chia have higher elasticity and it shows positive results in baking test by raised the bread volume about 20%. Deeper penetration rate shows that the morphology of bread crumb was changed moderately. The crumb of hydrated chia form was firmer than that of dry chia flour because of higher elasticity of hydrated chia form flour which leads to form small pores to larger ones.

Sandor tomaskozi et al., (2011) conducted a study on rheological properties of wheat flour dough and bread using new recording instrumentation, the micro Z-arm mixer(for dough) and SMS-texture analyser(for bread crumb) substitute with amaranth and quinoa flour. The rheological properties did not cause any change when substitution done with 10% amaranth or quinoa flour. However, the significant changes observed in stability, the degree of softening and elasticity with substitution of 20% and 30%. Increase in water absorption capacity of wheat flour substitute with amaranth and quinoa flour was observed. The bread prepared from higher substitution of amaranth and quinoa flour resulted in significant decrease in specific volume but an increase of resistance to firmness of the crumb. The rheological properties of dough and bread crumb did not influence by addition of protein isolates.

3.7 Solid waste characterization and application in the bread food matrix

Rafael audino zambelli et al., (2017) conducted a study on possibility of reuse of agroindustrial solid waste that have nutritional benefits. This study evaluates the possibility of reuse of constituents present in beet, broccoli and carrot in bread products. The waste residues of agro waste was dried by vacuum oven and crushed to form powder. The fatty acid and vitamin C content was determined by chromatographic analysis and chemical analysis was done for ash content, fat content, ph and acidity of residues. The residue used to prepare bread in combination with carrot powder was applied to evaluate physiochemical properties of product, by Central Rotational Compound Design (DCCR) for the formulations. This study evaluates the specific volume, density, expansion index, acidity, ph and volume produced and the variance or tukey’s test was analyse statistically. The amount of vitamin C determined in analysed residue is 918.57mg/100g. The result shows the amount fatty acid in sample is

2.04%, ash content is 4.84%, lipid content is 2.13%, ph of residue is 4.7 and total acidity is about 10.63%. Addition of residues results in improvement of parameters of specific volume and decrease on density of product. Therefore, it was concluded that residues from local juice industry (beet, broccoli and carrots) can be used in food industry as a source of nutrients

3.8 Nutritional and Sensory Quality of Bread

Haimanot H. Ayelel et al., (2017) studied the nutritional and sensory quality of wheat bread supplemented with cassava and soybean flours. To analyse nutritional and sensory quality of bread they made sixteen different composite flours of D- optimal constrained mixture design. The range of flours taken in formulation- wheat 40-80%, cassava 10-30% and soybean 10-30%. Using straight dough method (American Association of Cereal Chemists, 2000), bread was prepared from composite flour (cassava flour, soybean flour and wheat flour) and wheat flour for control. Similar results were observed in previous studies (*Mashayekh et al., 2008; Sanful & Darko, 2010*). High mineral content The bread prepared from composite flour of 65% wheat, 25% cassava and 10% soybean flour had least quantity of protein content. The bread formulated from composite flour of 65% wheat, 25% cassava and 10% soybean had minimum (4.96%) fat content however, bread prepared from 60% wheat, 10% cassava and 30% soybean was reported with maximum (11.25%) fat content. The study concluded that, the increase in fat content of bread was due to increase in amount of soybean flour (*Dhingra & jood, 2004; Garg et al., 2016; Noorfarahzilah et al., 2014*). The optimum results of proximate analysis were observed in composite bread of 71% wheat, 10% cassava and 19% soybean flour with fiber, protein, fat, ash, carbohydrate and energy of 3.64%, 17.54%, 7.96%, 4.03%, 60.96% and 400.23kcal/100g, respectively. At the end this research concluded that, the optimum ratio accepted for both nutritional and sensory quality parameters of formulated flour wheat, cassava and soybean flour was in the range of 49.0-71.0%, 10.6-29.0%, and 18.2-22.0% respectively. The quality attributes of bread loaf prepared from less than 70% wheat flour was considered as inferior in this research.

CHAPTER-5 MATERIAL AND METHODS

5.1 Proposed Research Methodology:

The present formulation is planned to prepare “Multigrain Broccoli bread” by combination of Whole wheat flour, Quinoa flour, Chia flour and broccoli.

5.1.1 Procurement of Raw material:

Quinoa seeds and Chia seeds are purchased from Amazon retailers. Wheat flour and Broccoli are purchased from local market of Phagwara, Punjab, India.

5.1.2 Preparation of Multigrain flour:

The whole grains (Quinoa seeds and Chia seeds) were cleaned from dirt by sorting out stones and unwanted material, then washed and dried in oven. After drying, both grains are milled and sieved properly. Different formulations of multigrain flour are made along with other ingredients such as salt, sugar, yeast and milk powder.

5.1.3 Preparation of Bread by straight dough method:

In straight dough method, all the ingredients are mixed at the same time. For activation of yeast, Luke warm water, dry yeast and sugar are mixed properly in a separate bowl and left undisturb. After 10-15 minutes it forms slump and bubbles which indicates that yeast is activated (Bhatt, SM, Gupta & R.K,2015).

Table-5.1 Mixture contents for formulated bread

Ingredients	**Control**	**Formulation(g)**
Compressed yeast (g)	3	3
Salt (g)	1.5	2
Sugar(g)	4	5
Milk powder(g)	5	5
Whole wheat flour(g)	100	86
Quinoa flour(g)	-	5
Chia flour(g)	-	5
Broccoli(g)	-	4
Water(ml)	60	60

5.2.1 Flow Chart for bread preparation

Take weight of all the dry ingredients (whole wheat flour, Quinoa flour, Chia flour, salt and milk powder)

↓

Mix all the weighed ingredient in a bowl

↓

Add water (60% w/w) along with sugar-yeast solution (previously activated yeast) in a bowl to form dough.

↓

After manual kneading for 10-12 mins, the dough is left for 15-20 mins

for first fermentation (1st proofing time)

↓

During Proofing time, the dough is knocked back to expel the excess gas

↓

Now oil the mold with brush to avoid stickiness of dough to mold

↓

Transfer the dough into mold and placed it in proofer at 35-36 °C temperature for 50-55 min with 85 % relative humidity (2nd proofing time)

↓

After proofing, the dough is placed in preheated oven (200-210°C temperature) for baking for 45-50 min

5.2 Physico- Chemical Analysis

5.2.1 Moisture Content

Weigh about 3 g of the prepared sample in the moisture dish, previously air dried in the oven and weighed. Placed the dish in the oven maintained at the 105±5°C for 4 hours. Cooled in the desiccators and weighed. Repeated the process of drying, cooling and weighing at 30 minute intervals until the difference between the two consecutive weighing is less than 1 mg. record the lowest weight.

$$M.C. = \frac{(\text{Wt. of the sample at desired time}) - (\text{Wt. of bone dry material})}{(\text{Wt. of sample at any time})} * 100$$

It will be analyzed in Hot air oven at 103°C and will be calculated by formula.

5.2.2 Determination of Ash Content by Muffle Furnace (AOAC, 1986)

The ash of the food stuff is the organic residue remaining after the organic matter has been burnt away. When a high ash figure found it suggests the presence of an adulterant, it is often advisable to determine the acid insoluble ash also.

3 g of the sample was weighed. It was tare, cleaned, dried and pre-weighed porcelain dish or silica dish or platinum dish was taken. The sample was ignited in the dish with the flame of a suitable burner (oxidizing agent) for about one minute. Ignition was completed by keeping it in a muffle furnace at 550-6000°C until the grey ash results. It was cool in a desiccator and weigh. The process was repeated till constant weight was obtained. The final weight was noted.

$$\text{Ash \%} = \frac{W2 - W1}{W} * 100$$

Where,

W2 = Final weight of dish + Ash

W1 = Weight of dish

W = Weight of sample

5.2.3 Determination of fat content by Soxhlet method (AOAC, 1986)

Firstly weight of empty round flask. Weight 05 g of pineapple pomace, wheat bran biscuits in a dry cellulose thimble and covered with cotton and kept into the Soxhlet assembly. The fat extraction was carried out with petroleum ether (60°C-80°C) for four hours. After evaporating the solvent, flask were kept into the hot air oven for one hour and cool into desiccators and weight again.

$$\% \text{ Fat Content} = \frac{W1 - W2}{W} * 100$$

Where,

W_1 = Initial weight of round flask

W_2 = Final weight of flask + fat

W = Weight of sample

5.2.4 Determination of Protein Content By Micro-Kjeldhal Method (AOAC, 1986)

Kjeldahl method for determining total nitrogen involves first heating with concentrated sulphuric acid. The reaction rate was accelerated by adding Sodium Sulphate to raise the boiling point. The catalyst used was copper sulphate. The oxidation causes the nitrogen to be converted to ammonium sulphate. After making alkaline with concentrated NaOH, the liberated ammonia was distilled into HCl. The protein content was obtained by multiplying total nitrogen by an empirical factor.

About 2g of sample were weighted accurately and transferred to a kjeldahl flask. Then 4 g of $CuSO_4$ and 10 g of Na_2SO_4 were added to the flask. 25 ml of concentrated H_2SO_4 were added. The flask was heated gently in an inclined position till the light blue color solution was obtained. Then the flask was heated on a high flame for three hours. Then the digestion mixture was cooled at the room temperature. The digest were wash into the distillation flask with distilled water.

The distillation assembly was arranged to the receiving flask and 50 ml of 0.01N HCl with 2-4 drops of methyl red indicator were added. The distillation apparatus were connected with a delivery tube dipping in an HCl solution. Zn metal pieces were added to the distillation flask which was carefully added to the digestion mixture. It was rinse with water. Around 50-60 ml of 50% NaOH was added to it. Sufficient water was added to the flask. The H_2O were started through the condenser. The solution was heated and it liberates NH_3. The liberated NH_3 were distilled into HCl solution. The heating was continuing thrice, the initial volume of HCl in the receiving flask. The tap was open and washes down the condenser and the delivery tube into the receiver. The burner was put off. The distillate with 0.1N NaOH were titrated and slight pink colour were obtained. Conduct with blank determination.

$$\% \text{ crude protein} = \frac{(\text{titre of sample- blank}) * 0.01 * 14.007 * 6.25}{10 * \text{weight of sample}} * 100$$

S = Sample Titrate Reading

B = Blank Titrate Reading

5.2.5 Determination of Fiber (AOAC, 1986)

When a fat free sample of weighed 2g taken in the triplicate and digest with 200 ml of 1.25% sulphuric acid by gentle boiling for half an hour. Now the contents of the sample filtered and policed by a muslin cloth under suction. Then it washed the residue free of acid using hot distilled sodium hydroxide. Digest the content again for half an hour, filtered and wash free of alkali using hot distilled water. Dry the residue in an oven overnight at 105ºC. Now weigh it and place in the muffle furnace at 600ºC for four hrs. The loss in weight after ignition represents the crude fiber in the sample.

$$\% \text{ of Fiber} = \frac{(W2 - W1) - (W3 - W1)}{W}$$

Where,

W =Weight of sample

W1 = Weight of crucible

W2 = Weight of empty crucible + sample before ignition

W3 = Weight of empty crucible + sample after ignition

5.2.6 Swelling capacity (SWC):

SWS was measured using the bed volume technique described by Kuniak and Marchessault (1972) . Approximately 0.2 g of the sample material was weighed into a 50 mL graduated glass cylinder. After making up the volume to 50 mL with de-ionized water and the mixtures were then vigorously stirred, the material was left overnight at room temperature for equilibration. The volume of the swollen sample was noted. Results of SWC were expressed as the ratio of volume (mL) of swollen sample to the weight (g) of dry initial sample. Triplicate measurements were taken for all WHC,OHC and SWC.

5.2.7 Physical characteristics of bread:

The weight (g) for bread was determined individually within one hour after baking the average was recorded. The volume (cm3) of different types of produced bread was determined by rape seeds displacement method according to (AACC, 2000). Specific volume was calculated according to the method of (AACC, 2000) using the following equation

Specific volume = Volume (cm3)/Weight (g).

5.2.8 Texture characteristics:

The texture of the bread was measured objectively using food texturometer (TAHDi, Stable Micro System, UK) as per the standard AACC methods (2000). A test speed of 2.0 mm s-1 was used. A 35 mm diameter cylinder aluminum probe (P-35), was used to measure the required compression force. Force required to compress 25% of the cake slice (2.54 cm) was recorded. All measurements were performed at ambient temperature 25±2°C according to (*Gomez et al., 2007*).

5.2.9 Mechanical properties measurements:

All mechanical properties were made using the Instron Universal Testing Machine (Model 4401) equipped with: 5-mm diameter tip probe for penetration test (with 100, 500, 1000 and 5000 N load cell). All testing was performed at room temperature (25±2°C).

5.2.10 Penetration test (PT):

Each piece of tested bread was placed in a hole of the beveled ring. The pin penetrated with a constant speed 10 mm. min-1 into each piece of bread tested. Three penetration points at different parts for each piece of bread and 5 pieces for each sample were taken for each test condition, recording the force profile at 1 cm of probe penetration. Force – deformation curves were recorded and hardness was derived as indicators of textural properties. Each sample was used for only one measurement (*Femenia et a;., 1997*).

5.3 Mechanical measurements analysis:

1- The force corresponding to the maximum puncture is defined as the maximum force (Fmax). The maximum puncture force (Fmax) was measured in Newton's (N), as mentioned by Sharoba et al. (2012) and Saleh et al. (2012).

2- Maximum deformation: the distance from beginning to distance at maximum force.
3- Hardness = Maximum force (N) / Maximum deformation (mm), as mentioned by Sharoba et al. (2012) and Saleh et al. (2012).

5.4 Sensory analysis:

Sensory evaluation of bread was carried out in a standardized test room in morning sessions (11:00-13:00 h) by a 12 trained sensory panel, and were carried out by a properly well trained panel of 12 panelists. They were selected if their individual scores in 10. Different tests showed a reproducibility of 90%.

The 12 member internal panel evaluated on a five point hedonic scale, scoring was based on a 100 point scale (10-100) where (90-100) = excellent,(70-80) = very good, (50-60) = good, (30-40) =fair and (10-20) = poor. Mineral water was used by the panelists to rinse the mouth between samples, according to the method described by (AACC, 2000); bread samples were left to cool at room temperature for 1 hr. after baking. Then bread was cut with a sharp knife and subjected to panel test. Cells 30 (uniformity 10, size of cells 10, and thickness of walls 10), grain 20, texture 30 (moistness 10, tenderness 10, and softness 10), crumb color 10, flavor 10 and overall acceptability 100 degrees.

5.5 Microbiological Analysis

5.5.1 Standard Plate Count (SPC) (Govt. Of India, 2012)

The following media and reagents (1-4) are commercially available and are to be prepared and sterilized according to the manufacturer's instructions.

- Plate count agar (PC)/Nutrient Agar (NA)
- Peptone water diluent (0.1%)(PW)
- Sodium 2, 3, 5 triphenyltetrazolium chloride, TTC (0.1%) (optional)
- 1N HCl and 1N NaOH
- pH meter or paper capable of distinguishing to 0.3 to 0.5 pH units within a range of 5.0 to 8.0 Stomacher, blender or equivalent for sample preparation/homogenization.
- Incubator capable of maintaining the growth temperature required for the specific type of aerobic bacteria being enumerated (i.e. for psychrophilic bacteria: 15 – 20°C, for mesophilic bacteria: 30 – 35°C, and for thermophilic bacteria: 55°C) and 45°C waterbath

- Colony counting device (optional)

5.5.2 Coliform Count Analysis (Govt. Of India, 2012)

The following media and reagents are commercially available and are to be prepared and sterilized according to the manufacturer's instructions.

- Violet Red Bile Agar
- Peptone water diluent (0.1%)(PW)/ N-Saline
- pH meter or paper capable of distinguishing to 0.3 to 0.5 pH units within a range of 5.0 to 8.0 Stomacher, blender or equivalent for sample preparation/homogenization.
- Incubator capable of maintaining the growth temperature required for the specific type of aerobic bacteria being enumerated i.e. at 35°C.
- Colony counting device (optional)

5.5.3 Yeast and Mould Count (Govt. Of India, 2012)

The following media and reagents are commercially available and are to be prepared and sterilized according to the manufacturer's instructions.

These agars are suitable for yeast and mould count in food products:

- Chloramphenicol Yeast extract Glucose Agar (CYGA)
- Potato dextrose agar with chloramphenicol (PDA-C)
- 20% sucrose (diluent additive for osmophiles)
- Malt extract agar containing 50% (w/w) sucrose

Other materials:

- Peptone water (0.1%) (PW)
- 1N HCl and 1N NaOH
- Gram stain solutions
- Stomacher, blender or equivalent
- pH meter or paper capable of distinguishing to 0.3 to 0.5 pH units within a range of 5.0 to 8.0
- Light microscope
- Colony counting device (optional)
- Incubator (darkened) capable of maintaining 22 to 25°C

CHAPTER-6 EXPECTED OUTCOME

1. Optimized the formulation for the preparation of multigrain bread.

2. The obtained multigrain bread will have good sensory characteristics.

3. The bread prepared from multigrain flour will serve as good source of nutrients.

CHAPTER-7 RESULT AND DISCUSSION

The present study on 'Development of Multigrain Broccoli Bread' is carried out in the Department of Food Science and Technology, Lovely Professional University, Punjab, India. To evaluate nutritional content of different flours, proximate analysis was done. The results obtained during proximate analysis of different flours (whole wheat, Quinoa and Chia flour) are represented in the table.

Table-2 Nutritional Content of different flour used for preparation of bread

Sample	Moisture %	Ash %	Fibre %	Fat %	Protein%
Whole wheat flour	12.7%±0.05	1.3%±0.03	4.5%±0.10	7.5%±0.11	13.1%±0.30
Quinoa flour	12.9%±0.01	5.6%±0.07	11%±0.01	9.5%±0.02	8.7%±0.03
Chia flour	10.8%±0.04	4.6%±0.08	19%±0.10	31.5%±0.11	21.8%±0.30

The experimental result showed that, the protein content of chia flour i.e 21.8% was significantly higher than whole wheat flour and quinoa flour (13.1% and 8.7%). Protein provides essential nutrients in bread as well as it is responsible for texture and appearance of final product. The fat content of whole wheat flour and quinoa flour was 7.5% and 9.5% respectively which was was less than that of Chia flour i.e 31.5%. This significant increase in fat content of chia flour shouldability to make bread with less or without use of shortening agent. Similarly, Chia flour has high fiber content of 19%. Although fiber is beneficial for digestive system, diabetes (*Dewettinck K, 2008*), blood cholesterol level (*Okarter N, 2010*), reduces constipation, coronary heart disease (*Ferguson LR, 1999*), and obesity (*Slavin JL,2010*) but it decreases dough rheology like texture, volume and dough mixing properties. Moisture content depends on grain quality. Although Quinoa flour reported higher moisture content 12.9% + ash content 5.6% respectively.

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