Utilization of kodo millet in flatbread formulation

Dissertation-1 Report

Submitted by: Amandeep Kaur

Registration no. 11707233

Program – M.sc (food technology)

Section – H1730

Department of Food Science and Technology

School of Agriculture

Lovely professional university, Phagwara

Under the guidance of

Dr Navnidhi Chhikkara

Assistant professor

Department of food technology

School of Agriculture

Lovely professional university

CERTIFICATE

This is to certify that Amandeep Kaur (registration No.11707233) has personally completed M.Sc, dissertation entitled, "nutritional qualities and utilization of kodo millet" 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 the 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 condition for the evaluation leading to the award of Master of food technology.

Dr. Navnidhi chikkara

Assistance professor

School of agriculture and food technology

Lovely professional university

Declaration

I hereby declare that the framework presented in the Thesis entitled "Nutritional qualities and utilization of kodo millet" is my own and original. The work will be carried out by me at the school of Agriculture, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree of Master of Science in Food Technology.

Amandeep Kaur

Registration No: 11707233

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

Dr. Navnidhi chhikara

Assistant professor (Food Technology)

School of agriculture

Lovely Professional University

Phagwara, Punjab, India

Table of contents

1.Introduction

Millets are the cereals crops which are available in different size and color according to the variety of crop. The millets are divided into two main categories of major and minor millets. Major millets are pearl millet(Pennisetum glancum), foxtail millet(Setaria italica), proso millet(Panicum miliaceum), finger millet(Elusine coracana). Minor millets are kodo millet(Paspalum scrobiculatum), Barnyard millet(Echinochloa esculenta), little millet(Panicum miliare). Kodo millet (paspalum scrobuculatum) has been identified as a minor millet crop. It is commonly called kodomillet, kodo, varagu rice and kodra. It originates in best Africa. It is a hard crop and drought-tolerant (P. Beauv.). it is an annual millet that varies in height from 30-90cm or 4 feet and has a basal tiller. It requires 25-27°C, after 4 months crop will be ready to harvest after harvesting period the grain occurs in the hard husk which makes debraining of grains difficult. The size of seed is small having 2mm in length, 1.5mm width and colour changes from pale brown to dark grey. (Ragee et .al.,(2006). The kodo millet grain is composed of many nutrients like it provides 11% protein, 37-38% dietary fiber, opposite to rice which provides 0.2/100g and 1.2/100g. an adequate amount of -fiber helps to resist the feeling of hunger. The grains accommodate 66.6g of carbohydrates which provide 353kcal per 100g of grain compared to other millet and fat 3.6g/100g. the grains contain high amount minerals like (calcium 15.27mg, Phosphorus 188mg, iron 2.34mg, copper 0.26, magnesium 147mg, sodium 4.6mg, potassium 144mg, zinc0.7mg), vitamins are thiamin 0.299mg, riboflavin0.20mg,niacin 1.49mg.(Saleh et. al., 2013).Chapatti, a flat baked product which is prepared mainly from wheat flour but by applying different processing methods we are going to prepare kodo millet (paspulam scorbiculatum) flour chapati(100%) or with combination of wheat flour in different ratio to make it more nutritious because the wheat flour is deficient in lysine and trytophan(essential amino acids) on another side kodo millet flour is rich in lysine and trythophan hence, the combination of both flour will provide essental nutrients to the human body(panghal and Chhikara et al., 2017). chapatti is a cheap source of proteins, energy/calories and also rich in dietary fibres because kodo millet is a rich source of dietary fibres. In India, kodo millet is consumed in the form of rice, chapatti, cookies, pasta, noodles etc.(Sharma and Gujral, 2014). Chapatti is mainly prepared and consumed fresh having soft texture. The chapatti consists mainly of crust; with a little crumb.

Chapter 2

2. Problem background:

Kodo millet is not highly utilized in industries for product manufacturing because the size of the grain is very small which makes milling difficult or it takes time for the milling of the grain. The bran of the grain contains antinutritional factors that affect the final quality of the product. In recent years, people were not aware of the nutritional component present in kodo millet grain. It contains important nutrients that are essential for human body. In that time this crop was only used as bird feed not for product manufacturing due to lack of processing techniques. Now, studies are going on the millets to improve the nutritional quality, sensory quality, and palatability of millet by using different methods like germination, fermentation, malting, roasting to also reduce antinutritional factors that inhibit the absorption of nutrient in the body.

Chapter 3

3. Proposed objectives:

1. Physio-chemical and phytochemical characteristics.

2. Effect of processing treatment on anti-nutrient factors(germination, roasting).

3. Development of flatbread from kodo millet flour.

4. Quality evaluation of the final product.

4.Botanical description of the plant (Mabberley, 2010)

Table: 1.

Kingdom	Plantae
Subkingdom	Angiosperms
Order	Poales
Family	Poaceae
Subfamily	Panicoideae
Tribe	Paniceae
Genus	Paspalum
Species	p. scrobiculatum

The kodo millet is a cereal crop which mostly grown in Tamilnadu, Gujarat and Madhya Pradesh regions. It is drought tolerant crop. It requires 25-27^{0}C for optimal growth. The seeds contain essential nutrients and dietary fibers in a higher amount which are good for human digestion. The seeds are consumed in the form of kodomillet rice, idli, noodles, cookies, bread, dosa, weaning foods, etc.

Structure

The grain of kodo millet is looked like this as shown in the figure no. 1

Figure:1. a.(kodo millet whole grain)

figure:1. b (grain after milling)

The seeds of kodo millet different in colour as per the type of the grain (Ragee et al., 2006). The size of the kodo millet grain is 1.5mm in width and 2mm length. The aleurone layer maintains proper development of the seed. The grain has main components like germ, bran and endosperm. The outer covering of the seed is known as a bran or husk which forms a massive proportion of the grain about 37%(Malleshi and Hadimani, 1994). The bran contains about 112 µmol ferulic acids (.Deshpande et al.,2015). The germ which is high in oil content is removed during milling to prevent the flour from rancidity, and next is endosperm which is the main part of the grain used during milling or converting the kernel into flour. (Mohapatra et al,.2015). Due to the small size of the grain, the processing of the grain is difficult hence the whole grain is used for the product formation.

Nutritional composition

Kodo millet (paspalum scrabiculatum) is a minor millet belongs to Poaceae family Kodo millet is mainly divided in damp habitats across the tropics and subtropics of the world. It is widely grown in Uttar Pradesh in the North Kerala, Tamil Nadu in South. This cereal is also known as Varagu, kodo, haraka(Mall and Tripathi, 2016). Kodo millet is a nutritious grain as compare to wheat and rice. The protein, mineral content, and fiber content is higher than rice(Ohariya, 2013).

Kodo millet makes digestion easier because it contains good amount of lecithin and strengthen the nervous system. Kodo millet contains good amount of vitamins especially niacin, folic acid. It also contains minerals such as calcium, iron,magnesium, potassium and zinc, manganese, phosphorus, essential amino acids and vitamin E, makes millets an important nutritional bio- source(Mishra et.al.,2014).

According to Chandel et al.,2014 studies the kodo millet contains 60-70% carbohydrate, 6-19% proteins, 1.5-5% fat content, 10g dietary fibers (37-38%), and 2.6% minerals the iron content in kodo millet ranges from 25.86ppm to 39.60ppm. Among the millets, it has the least amount of phosphorus content. Hedge and Chandra, 2005 reported that the gelatinization temperature of kodo millet flour is 13°C. It has less resistant to gelatinization, and can be used for baking ofcakes and bread, soup, porridge, instant powders, and modified flour and starches for special foods. phytochemicals such as phenolic, lignans, beta-glucan, inulin, resistant starch, phytates, sterol, tocopherol and carotenoids are their in millet. The main polyphenolic components are phenolic acid and tannin while flavonoids are present in small quantities. They act as an antioxidant and prevent from oxidation reactions (Shinoj et al.,2006).

Carbohydrates

In millets 60-70% dietary carbohydrates are present. Sugar and Starches the free sugars found in millets are glucose, fructose, sucrose, raffinose and their content ranges from 1-1.4% with sucrose (0.3-1.2)being the predominant sugar starch is a mainly useful raw materialin foods industries and pharmaceutical, textile, and paper industries(Deshpande, Mohapatra, and Tripathi 2014-2015)**.** The structure, physio-chemical properties of millet starch has been different as compare to other cereal grains(Tsao 2010). It is mainly divided into two main components i.e amylose and amylopectin having ratio of 26:74 %, it plays important role to give structure to the final product. Amylose has linear structure alpha 1-4 linkage while amylopectin is branched having alpha 1-4 linkage and alpha 1-6 linkage. it helps to determine the physical structure, functionality and uses of starch. Acc to Bangoura et.al studies he tells about that the resistance starch content decrease during cooking but amylose content increase which provide gel consistency to the starch. Starch is a major component of all types of cereals including kodo millet, finger millet and little millet, which plays an important role in determining the quality of grain after processing like gel forming properties,digestion and absorption, structure of amylose and amylopectin.

Protein

Kodo millet contains most of the essential amino acids, like arginine, histidine, tryptophan, phenylalanine, isoleucine, methionine, cystine, theronine, leucine, valine, sulphur containing amino acids the ratio of leucine to isoleucine is about 2 (Ravindran, 1992; Antony et al., 1996). According to the different studies of L.Sudharshana and P.V.Monterio, the prolamin fraction of millet grain is 6.5-11.1mg per g of whole grain flour(6.4-10.9% of total proteins). The glutelin ranges between 8.2and 10.3mg/g of whole flour. The true glutelin fraction is largest protein fraction of kodo millet and ranges from 40.7- 54.4 mg/g of whole flour(40.4-52.1% of total protein). The essential amino acids isoleucine, phenylalanine, tryptophan and valine, leucine is present more than 0.33mg. excess leucine impede with the utilization of isoleucine. The leucine: lysine ranges from 5.2-6 in kodo millet.The non-essential amino acids such as aspartic and glutamic acids proline and alanine are present in large amount The high ration of leucine, lysine results in inefficient utilization of lysine nutritionally safe ration of leucine: lysine should be less than 4.6. Kodo millet has the highest free radicals (DPPH) quenching activity followed by great millet.

Table 3: Essential amino acids(mg/100)(Ragee et.a,. 2006)

Parameter	Composition(mg/100g)
Arginine	270
Histidine	120
Lysine	150
Tryptophan	50
Phenylalanine	430
Isoleucine	360
Methionine	180
Cystine	110
Threonine	200
Leucine	650

Fat

The fat content value is 1.4g in kodo millet grain. The fat contains same amount of linoleic and oleic acid which making up 70% of total fatty acids of the major lipid fraction. Due to the presence of less fat content, there are fewer chances of the rancidification after product manufacturing. It contains less fat content as compared to wheat and rice.

Micronutrient

Vitamins and minerals are significantly required by human body. The kodo millet grains contain 2.3mg/100g which is higher as compare to other cereals. Kodo millet is a good source of potasium , phosphorus, copper, magnesium, zinc, iron, manganese. Mature and dried grain does not contain vitamin C but vitamin B is present in sufficient amount in aleurone layer and the germs. Dehulling is used for the removal of hull from the grain that results in reduction of niacin, riboflavin, and thiamine to the limit of 50% in flour.

Dietary fibers

Millets contains both insoluble and soluble dietary fiber and have comparable or even higher total dietary fiber than other cereals. Decorticating significantly decreases millet total dietary fiber. studies on kodo millet have reported the insoluble dietary fiber as 18-30%and soluble dietary fiber as 0.6-2% in the whole form and decortication decreased the amount of insoluble dietary fiber to 1.5-3% and soluble dietary fiber to 0.3-0.9% (Geervani and Eggum 2009). Dietary fiber are polysaccharides which are indigestible and they include hemicellulose, cellulose, oligosaccharide, pectins, gums, and other lignified components. Kodo millet has 37-38% of dietary fiber which is the highest among the cereals and though low in fat.

Table 4: Nutritional composition g/100g (Deshpande, Mohapatra, Tripathi 2014-2015)

Composition	**Kodo millet**	**Wheat**	**Rice**
Carbohydrates	65.9g	71.2g	78.2g
Proteins	11g	11.8g	6.8g
Fat	1.4g	1.5g	2.2g
Fiber	10g	1.2g	0.2g
Minerals	2.6mg	1.5mg	0.5mg
Iron	0.5µg	5.3µg	0.7µg
Phosphorus	188µg	306µg	160µg
Calcium	27µg	41µg	45µg
Thiamin	0.33µg	0.41µg	0.41µg
Riboflavin	0.09µg	0.10µg	0.04µg
Niacin	0.2µg	5.1µg	4.3µg

Anti-Nutrients and Phytochemicals:-

Phytochemicals

Phytochemicals are the chemicals which are produced by the plants during metabolic reactions. Phytochemicals are mainly classified into different categories, such as carotenoids and polyphenols, which include phenolic acids, flavonoids, and stilbenes/lignans (Sharma and Riar et.al.,2016). phenolic compounds shows antioxidant activity that are good for human body at certain level. The availibility of good amount of phenolic and flavonoid content in kodo millet grain helps to produce antioxidants that act against diseases such as cardiovascular diseases, diabetes, cancer. The antioxidants, like polyphenols and flavonoids are the most common active ingredients of nutritional and functional foods. It prevents against oxidation and cellular damage by delaying the oxidative process. The consumption of sprouts is assumed very important in reducing human diseases associated with oxidative stress (Silva et.al., 2013). The free radical quenching activity is very high among the millets tested. The antioxidant activity is by the synergistic action of the phytochemicals since breakdown into husk or endosperm caused substantial loss in activity. The extent of antioxidant activity of phenolics depends on the position and extent of hydroxylation of the phenolic rings (Miyake & Shibamoto, 1997). There are many other structural features that play a significant role in evaluating the content of antioxidant activity (Bravo, 1998). Since extracts of natural products have several phenolic compounds, synergistic activity is common. The structure-activity relationships (SARs) seem most important for contributing antioxidative property to any plant product (Bors, Michel, & Stettmaier, 2001; Heim, Tagliaferro, & Bobilya, 2002). Kodo millet had the highest DPPH quenching activity followed by other millets. Methanol extracts of the kodo millet flour are 70% DPPH (1, 1, Diphenyl-2-picrylhydrazyl) quenching in comparison to other millet extracts which showed 15–53%.

Phenolic compounds:

The raw Kodo millet contained the phenolic compounds such as arachidonic amide, N[5hydroxynpentyl] (0.46%), pterin-6-carboxylic acid (0.50%), N-(3,5Dinitropyridin2yl), laspartic acid ester (0.19%), 9,Octadecenoic acid (0.94%), methyl 10-trans, 12,cisoctadecadienoate (0.43%), stigmasterol (0.35%), Ç-Sitosterol (0.25%), pregnenolone (1.02 %), campesterol (0.31%). These compounds were reported to possess carcinogenic, antimicrobial, antiasthma, anti-inflammatory, anticancer, diuretic, aminoglycoside antibiotic, antioxidant, remove toxins, reduce fat, diuretic, anti-coronary, hepatoprotective, antieczemic, nematicide, hypocholesterolemic, antieczemic, nematicide, anti-stiffness, Alzheimer's disease, enhancing memory and trauma factors(Olçera & Mecit, 2012).

Due to the presence of additional bioactive compounds with increased concentration as a result of germination, the Kodo millet can be considered as a useful raw material in various pharmaceutical and industrial applications.

Table 5:- Phenolic compounds and their concentration. (Sharma and Saxena and Riar, 2016)

PHENOLIC COMPOUND	Concentration in raw (in %)
Arachidonic amide, N[5hydroxynpentyl]	0.46
Pterin6carboxylic Acid	0.50
N(3,5-Dinitropyridin,2yl) Laspartic acid ester	0.19
9, Octadecenoic acid	0.94
Methyl 10-trans, 12-cisoctadecadienoate	0.43
Stigmasterol	0.35
Ç-Sitosterol	0.25
Pregnenolone	1.02
Campesterol	0.31

Tannin:

Tannins polyphenolic components naturally present in the plant. Tannins reduce apparent digestibility of protein and energy. Tannins are known to have a direct effect on metabolism. The astringent taste of the grain is due to the presence of tannin in seeds which makes them unpalatable and affect the digestibility of the proteins.(Barry and Blaney, 1987). Tannins react not only with dietary protein but also with enzymes of the gut wall and proteins in the saliva. In vitro protein, digestibility values of low tannin samples were higher than those of the high tannin samples. Dehulling had the effect of removing most of the phenolics from kodo millet grain with a concomitant increase in in-vitro protein digestibility. It has been found that tannins reduce feed

intake, impair nutrient digestibility and nitrogen retention thus causing growth depression of poultry. Elkins et.al., 1978 studies chickens fed high tannins sorghums develop leg abnormalities. (Chang SI, Fuller HL, 1964).

Phytate:

Phytate is a naturally occurring phosphorus compound which significantly influences the functional and nutritional properties of foods. It is the main phosphorus store in mature seeds. Phytate interferes with mineral absorption especially calcium and zinc. The high quantities of this anti-nutrient in the seed reduce their food value hence causing low utilization unless processed prior to us

Fig.3: Phytic acid

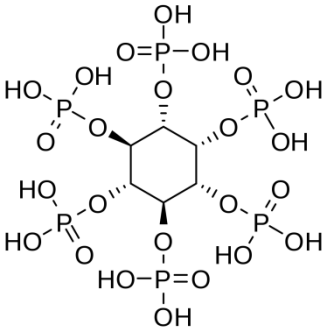

Fig.4: Tannin

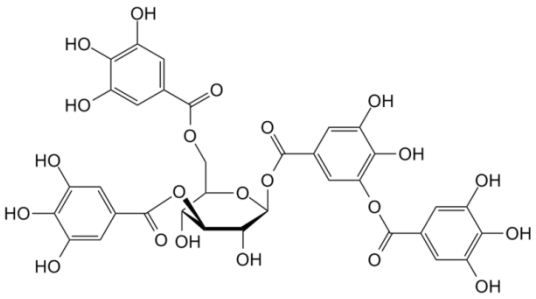

Fig.3: Flavon

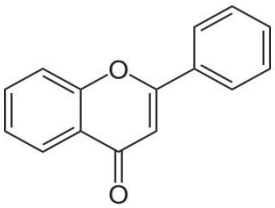

Table 6: Chemical composition, phenolic contents, flavonoid contents, antinutritional factors and mineral contents of Kodo millet flours.

Characteristic	**Raw**
Free phenolics	16.46±0.25
Bound phenolics	38.08±0.36
Total phenolics	83.01±0.61
Free Flavonoids	19.25±0.06
Bound flavonoids	33.34±0.09
Total flavonoids	52.59±0.15
Tannins	1.603±0.004
Phytates	1.342±0.003
DPPH radical scavenging activity (%)	67.35±0.10

Processing of kodo millet

The processing of kodo millet grain is complex due to its small grain structure (Prasad, 2013). The main region why industries not use millets in product manufacturing in large amount because the processing of millets are difficult and time-consuming and another region it also contains anti-nutritional factors due to which millets are not brought forward in the food industries but now more studies are going on the millet processing to decrease the antinutritional factors by different processing methods like germination, fermentation, malting, soaking, decortication, dehulling etc. According to the studies of Amadou, Mahamadou and Gounga et.al., 2012 The grain mainly contain bran, germ, and endosperm. The bran part of the grain contain minerals and vitamins, and germ part contains fat that is removed during processing because they interrupt the milling and also responsible for rancidity of flour and decrease the shelf life of flour. The whole grain of the kodo millet contain

phytate and phytin 135mg/100g, dehulling reduce phytate content around 27-53%, and phytin content reduces around 23%. After decortication total protein and lysine reduced by about 9-21% but that also improve the utilization of other proteins(Rathore and Singh et.al., 2016).

Milling:

Milling is done to eliminate the hull. This is usually done by beating tailed by sieving at numerous stages to sift fine particles, coarse particles and bran. The grains are either moistened with water or soaked overnight in preparation for milling (FAO, 1995). When pounding soft grains, the endosperm breaks into small particles and is separated by sieving and screening while in hard grains, the endosperm remains intact and is removed by winnowing. Hand-pounding is labour intensive, inefficient and time-consuming. Traditional methods of grinding make use of two round grinding stones that rotate horizontally against each other or with a mortar and pestle. The recent technology involves the use of abrasive disks in mechanical dehullers (emery boards) or attrition type dehullers. To extend the shelf-life of the flour, the milling process should be able to remove most of the germ. Wet milling process includes soaking overnight followed by grinding into a batter (McDonough et al., 2000; FAO, 1995).

In milling processing coarse fibrous bran or seed coat of the grain is removed, results in significant nutrient losses particularly of B-vitamins and minerals. The extent of the losses depends upon the degree of milling and the distribution pattern of nutrients in the grain. Although the nutrient content of food grains is relatively poor after milling, the bio-availability of certain nutrients appears to improve considerably.

Malting:

Malting is a process in which germination is done in a controlled way which helps to activate the enzymes of the grain which are at rest, as a result it convert starch to simple sugars and also helps in the partial hydrolysis of proteins and other macromolecules (Potter, 1995). Malting is done to obtain soluble starch from insoluble starch, thus it decrease the complex proteins into simpler proteins and acts as food for yeast and helps in the enzyme development (Goldammer, 2008). According to many studies malting has been the active and beneficial way for value addition of cereals (Adeyemo, Olayode and Odutuga, 1992; Akpapunam, Igbedioh and Aremo, 1996). Presently there is a good fortune in developing food products by blending composite malted cereals and legumes as a pathway

towards refining the nutritional quality of the product suitable for improving children's health (Agu and Aluva, 2004).

The main aim of malting is to break down cell walls surrounding starch granules and to produce enzymes. Degradation of the proteinaceous matrix that surrounds the starch granules within the cells of the endosperm and their conversion into soluble peptides and amino acids to provide substrates for the synthesis of proteins in the growing embryo is one of the important physicochemical changes that takesplace during malting (Enejeet al., 2003).

Popping and Puffing:

During popping, expansion process and starch gelatinization occurs simultaneously, where for short time the grains are exposed to high temperatures. In popping process, the vapour super-heated inside the grains produced by heating cooks the grain and expands the endosperm suddenly and breaks the outer skin. Puffing process is similar to popping process. In puffing expansion of kernels are done in a controlled manner, the vapour present inside gets escaped through the grain micropores due to high pressure or thermal gradient. To the snacks, desirable aroma and acceptable taste can be obtained by popping and puffing. Different methods of popping/puffing was used viz., the conventional method of dry heat, popping in hot oil, hot air popping, sand and salt treated, gun puffing and by microwave heating. Even though a most of the cereals and millets such as rice, wheat, sorghum, corn, ragi, millets are used for popping and puffing but only some of them will pop well. This is due to the factors which influence popping qualities of grains, such as varietal difference, season, grain characteristics such as the composition of grain, moisture content, physical characteristics, the method of popping, and also types of endosperm.

Generally, cereal grains are puffed or popped with hot air, hot sand, frying in hot oil, microwave heating and by gun puffing methods. Roasting may lead to burning of grain and producing injuries, whereas the oil from frying can be adsorbed and easily turns rancid. In comparison, high-temperature short time (HTST) fluidized bed air puffing has higher puffing efficiency as the product are uniformly exposed to the medium of heating (Brito-De La Fuente and Tovar, 1995). To avoid the limitations of conventional popping of puffing methods, electromagnetic waves such as microwaves are used nowadays, which provides better energy efficiency in very short time. Microwave energy is worldwide used for producing popcorn.

Superheated vapour formed inside the grains by instant heating, which cooks the grain and expand the endosperm while escaping with great power through the pores of the grain cell wall.

Most of the moisture content in the grain is superheated during puffing and helps for expanding the grain once pericarp ruptures.

Soaking and cooking

Soaking of grains is a popular food preparation technique used for reducing antinutritional compounds such as phytic acid to improve the bioavailability of minerals. The degradation and leaching of phytates, phytase activity, and iron and zinc concentrations have been studied after soaking of whole seeds, dehulled seeds, and flours of millet. The results indicated that dehulling and milling before soaking facilitated the leaching of phytates and phytases in an aqueous medium, and hence, phytate degradation. However, cooking of flours with water used for soaking did not increase phytate degradation (Lestienne, 2007). Soaking of kodo millet grains resulted in a 25% loss of iron, but also leads to the degradation of phytates. particularly when combined with milling and cooking. According to Eyzaguirre, 2006 studies mineral contents, like phosphorus, calcium, and iron, were reduced with an increase in the period of soaking of kodo millet in acid, but HCl-extractability improved to varying extents. The reduction in minerals content of kodo millet may be attributed to leaching out of these minerals into the soaking medium. However, improvement in HCl-extractability, which is an index of the bioavailability of minerals, may be explained by the acid treatment possibly released these minerals from mineral–antinutrient complexes to free form, thereby increasing their HCl-extractability (Arora, 2003). The principal reason for soaking is to gelatinize the starch into the grain. The variation in free sugars and non-starch polysaccharides in finger millet grain was observed upon germination (Nirmala et al., 2000). Water temperature amongst other factors influences the absorption of moisture into the grain during soaking. The changes in carbohydrates, free amino acids, organic acids, phytate and HCL– extractable minerals during germination and fermentation have been reported (Nirmala et al., 2000).

Fermentation:-

Fermentation is the most effective and traditionally used methods of preserving and producing foods by reducing the antinutritional components in millets. According to studies it is reported that natural fermentation of kodomillet decreased the polyphenols and phytic acid but there was no any effect on tannin content. It also helps to increase the vitamins contents like niacin, riboflavin and thiamine.

Food applications

It has a wider food application in food industries consumed in the form of biscuits, cookies, bread, pasta, idli and noodles etc. The food products are made with the combination of wheat flour and other millets flour with kodo millet flour to enhance the sensory profile.

1. Bread: According to Malathi and Sindhumathi, 2012 they made the bread with the incorporation of kodo millet flour and foxtail millet flour at 10%, 20%, 30%, 40%, 50%,60%, and 70% level. The bread developed was evaluated for the sensory attribute, At 20% level, it was highly accepted of small millet incorporation. The developed products were analyzed for the physio-chemical properties. The millet flour incorporation had increased the bread characteristics such as bulk density, height, weight, water absorption, specific volume, and decrease the dough extensibility. As the substitution level increased it enhanced the colour and for the developed millet bread yellow colour index had decreased. The staleness of breadcrumb was enhanced on storage. Texture profile like cohesiveness, resilience and springiness were decreased. The fiber content was 1.31g, 1.46g, and 1.53g for kodo millet and foxtail millet bread. Under the ambient conditions, shelf life of the bread was 7 days in various packaging materials and during the storage periods, microbial population was kept within the safe limit.

2. Noodles: According to Himabindu and Devanna, 2015 studies they develop noodles in the Combinations of wheat and malted kodo millet flour in different ratio of (90:10, 80:20, 70:30, 60:40 and 50:50) and other ingredient like spinach paste (for green color), eggs, salt and water are kept constant for all formulations to get good results after the formulation of noodles and in the sensory evalutaion the more score was given to the 70:30 ratio combination of wheat flour and kodo millet flour. Thus, the nutrient-rich noodles will be a good source of instant food for children, teenagers, sports person, pregnant and lactating women.

3. Pasta: Pasta is a staple food of traditional Italian cuisine. Several studies have been reported in the value addition for different millets (Begum et al.2003). The dehusked kodo millet rice grain and wheat flour were used as a raw material for the development of pasta. (Palanimuthu and Kumar, 2014)**.** A domestic grain pulverizer was used to mill kodo millet rice grains into desired particle size flour suitable for developing cold extruded pasta product (Veena et al. 2004). According to some studies they observe that maximum 70% of millet flour could be used to produce commercial quality pasta product if the millet flour proportion exceeds 70 percent than the remarkable loss in texture and structure of the end pasta product. The suitable composition of kodo millet flour and wheat flour is 70:30 On this ratio the product was liked by most of the people.

4. Cookies: Kumar et al., made an attempt to prepare cookies by using soy flour, kodo millet flour and whole wheat flour. Soy flour is used to increase the protein content of the biscuits. The ratio 20:80, 30:70, and 40:60 of the mixture was used for both sponge cake and butter biscuits, during sensory evaluation 30:70 was most accepted by the people, and there was no such effect on the properties of biscuits.

Material and methods Chapter 5

1. Physical analysis of grains:

Principle: To assess the physical properties of kodo millet grains (1000 grains weight, porosity, bulk density, true density, length, size), Physical analysis indicate about the grains health (sound, plumpy, free from damage, healthy, bulk density, true density, and thousand kernel weight (if more) indicates that the grain is healthy. physical properties of grains such as size, shape, 1000 kernel weight, bulk density, true density, and porosity are useful for their processing and storage. They are important parameters for grading and pricing of the product.

Equipment required: measuring cylinder, weighing balance, beaker, vernier calliper.

Procedure: 1000 kernel weight: 100 grains of kodo millet are collected manually or grains are spread on counting plate with 100 dents equal to the size of the grain. Grains are carefully spread over the mounting plate so that all the dents are filled. Extra grains are removed from the plate the weight of these grains is noted by weighing on an analytical balance. Repeat the experiment at least ten times and then report the average value.

Bulk density: Bulk density determination take a measuring cylinder of 1000ml capacity and fill it with grains for which density is to be measured. The measuring cylinder should be filled to its highest mark. Adjust the level of grains by repeated tapping takes the weight of these grains in a digital/analytical balance. Repeat the reading five times.

True density: For true density determination, take 10 grains randomly from the lot. The exact volume of these grains is found by liquid displacement method. For this take a 100 ml capacity measuring cylinder and fill it with toluene to a predetermined level. Drop randomly selected 10 grams in the cylinder and note the change in volume accurately. This gives the volume of 10 grains. Now weigh these 10 grains in analytical balance/digital balance.

General calculation:

$$\text{Thousand kernel weight} = \text{weight of 1000 grains}$$

$$Bulk\ density\ g/ml = \frac{weight\ of\ 10\ grains\ in\ gram}{volume\ of\ grains(ml)}$$

$$True\ density\ g/ml = \frac{\text{weight of 10 grains (g)}}{\text{Volume of 10 grains (ml)}}$$

$$Porosity = \frac{\text{True density} - \text{Bulk density}}{\text{True density}}$$

$$\text{Porosity\%} = \frac{1 - \text{Bulk density}}{\text{True density}} \times 100$$

2. Chemical analysis

2.1 <u>Estimation of moisture content</u>

Equipment and Apparatus required:

Moisture content: hot air oven (thermostatically controlled), weighing balance, desiccator(with active desiccant), weighing pots.

Theory: Moisture content of flour is an important parameter. It gives an idea about shelf life and milling conditions. The water is present in two forms free form and bound form. The free form of water is water which is freely available for the microbial growth and leads to spoilage of product and decreases the shelf life of the product. In bound form, water is not available for microbial growth.

Procedure:

Weigh accurately 5 g of the material in a dish previously dried and weighed. Place the dish along with lid in an electric air oven maintained at 105°C. Cool the dish to room temperature in a

desiccator and weigh with the lid on. Repeat the process until three consecutive readings are same. Note down the weight.

General calculation:

$$\text{Percentage moisture in material} = \frac{\text{Quantity of moisture in the material}}{\text{Weight of the material}} \times 100$$

2.2 <u>Estimation of ash content:</u>

Equipment and Apparatus required:

Ash content: crucibles, burner, muffle furnace, tongs, and desiccator, Silica dish, Chemical balance, Hotplate or burner, muffle furnace.

Ash: To determine the inorganic residues present in gain samples. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food.

Procedure

Ash Content: Ignite the dried material in dish left after the determination of moisture with the flame of a burner till charred. Transfer to a muffle furnace maintained at 550-560°C and continue ignition till grey ash is obtained. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing at half hour interval till the difference in weigh in two consecutive weighing in less than 1 mg. Note the lowest weight.

General calculation:

$$\text{Percent of ash in material} = \frac{W2 - W1}{W1 - W} X100$$

2.3 <u>Estimation of proteins</u>:

Material required: kjeldhal flask**,** conc. Sulphuric acid, CuSO4.5H2O, K2SO4(2:1), 40%NAOH, 2%boric acid (10g boric in 470ml hot distilled water, cool and add 2ml of 0.1% alcoholic solution of bromo cresol green and 4 ml of 0.1% methyl red solution, make the volume 500ml), 0.01N HCl.

Principle: Digestion: The food sample to be analyzed is weighted into a digestion flask and then digested by heating in the presence of sulfuric acid (an oxidizing agent which digest the food), anhydrous sodium sulfate (to speed up the reaction by raising the boiling point) and a catalyst, such as copper, selenium, or mercury. Digestion converts any nitrogen in the food (other than that which is in the form of nitrates and nitrites) into ammonia, and other organic matter to CO2, and H2O. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of ammonium ion, which binds to the sulfate ion and thus remains in the solution.

Neutralization: after the digestion has been completed the digestion flask has been connected to a receiving flask by a tube. The solution is in the digestion flask is then made alkaline by addition of sodium hydroxide which converts the ammonium sulfate into ammonia gas:

$$NH_4 + 2SO_4 + 2NAOH \rightarrow 2NH_3 + 2H_2O + Na_2SO_4 (2)$$

The ammonia gas that is formed is liberated from the solution and moves out of the digestion flask and into receiving flask- which contains an excess of boric acid. The low pH of the solution in the solution in the receiving flask converts the ammonia gas into ammonium ion and simultaneously converts the boric acid into borate ion:

$$NH_3 + H_3BO_3(boricacid) \rightarrow NH_4^+ + H_2BO_3(borateions) (3)$$

Titration: The nitrogen content is then estimated by titration of the ammonium borate formed with standard sulfuric and hydrochloric acid, using a suitable indicator to determine the end point of the reaction.

$$H_2BO_3 + H^+ \rightarrow H_3BO_3 (4)$$

Procedure: Weigh 1-2g sample into the kjeldhal flask, add 10ml conc. H2SO4 and 200mg of catalyst mixture and digest the sample until the solution becomes clear. Cool it and transfer into the 50ml volumetric flask. Rinse the digestion flask several times with the small amount of water and pour washing in a volumetric flask. Make the volume 50 ml with distilled water. Take 10ml boric

acid in a 100ml conical flask, place this receiving flask in such a way that outlet of condenser dips into boric acid solution.

Transfer 5ml of the acid-digested sample to the steam chamber. Add 5-8ml of 40% NaOH to the aliquot. Immediately close the stopcock and pass the steam to distil ammonia till about 30-40ml of distillate is collected. Titrate the content against 0.01N HCl till the bluish colour changes to pink. Run a blank.

2.4 <u>Estimation of fat content</u>:

Equipment required: Burette, soxhlet apparatus, extraction filter paper thimble, analytical balance, sample grinder, food sample, organic solvent(hexane, isopropanol, diethyl ether), acetone.

Principle: Fat and oils are soluble in organic solvents like hexane, isopropanol but other constitutes are not. Hence, the fat present in a food sample is dissolved into the solvent and afterwards, the solvent is removed by evaporation and distillation (boiling point of the solvent is much less than that of oils/ fats).

Procedure: Thoroughly wash the boiling flasks and rinse with commercial grade acetone to remove any residual oil/ fat. Dry the flask by placing in a hot air oven for 3-4 hours. Weigh the flask and label them. Weigh the extraction filter paper thimbles (in duplicate) and label them. Transfer 2-5g sample in pre-weighed thimbles and determine their accurate weight. Plugged this thimble with non-adsorbent cotton and place them straight in the Soxhlet extraction tube. Fill the extraction tubes with sufficient amount of solvent so the syphon system starts working. Now fix the soxhlet assembly properly and switch on the heaters. As soon as the initiation of the boiling indicated stars the water connected to condensers and allow the extraction for 8 hours. After 8 hours switch off the heaters and allow cooling.

The solvent is evaporated using vacuum ovens at 50C or a water bath and than flasks with oils are weighed.

General calculation:

The fat/ oil is calculated by the formula

$$\% \text{ of fat content} = \frac{W4 - W1}{W3 - W2} \text{X}100$$

2.5 Estimation of Dietary fibers.

Aim: To analyze dietary fibers by enzymatic methods.

Material required: Protease, Amyloglucosidase, ethanol, acetone, phosphate buffer, filtration assembly, driers.

Theory: An enzymatic-gravimetric method was developed in which the sum of the soluble and insoluble polysaccharides and lignin are measured as a unit and considered to be total dietary fibers.

Procedure: 1g of the defatted sample was taken, and phosphate buffer(50ml) PH-6 and add 0.2 ml alpha-amylase and keep the beaker on boiling water bath for 30 min, shake the beaker. Cool the solution to room temperature and adjust the pH to 7.5 with NaOH (0.2M) and add protease 5mg incubate the content for 30 min. at 60°C cool at room temperature. Add 10ml phosphoric acid (0.2M) and adjust the pH of the solution at approx. 4.5 use NaOH (0.2m) to set the pH if necessary. Then add Amyloglucosidase (0.3ml) and incubation was given at 60°C for 30 min, cool the content. Then wash the precipitate with 4 volume of ethanol. Filtration of the sample.

2.6 Estimation of Antioxidant activity (DPPH assay):

Aim: To estimate antioxidant activity using DPPH assay**.**

Materials Required: Ethanol, DPPH(1, 1, Diphenyl-2-picryl hydrazyl), methanol**.**

Theory: Kodo millet had the highest DPPH quenching activity followed by great millet and finger millet. Methanol extracts of the kodo millet flour are 70% DPPH (1, 1, Diphenyl-2-picrylhydrazyl) quenching in comparison to other millet extracts which showed 15–53%.

$$\text{Antioxidant activity}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Procedure: Take 0.1g of the sample. Add 5ml of ethanol. Leave for 3 min. Take 0.1ml of it in another test tube. Add 3.9ml of DPPH. Keep in dark for 30min. Check O.D at 517nm. DPPH-0.0236g in 1000ml methanol (23.6mg in 1litre).

2.7 <u>Determination of Vitamin C</u>:

Aim: To estimate the Vitamin C present in the sample.

Materials Required: 3% Metaphosphoric acid, Dye: 0.05g dye (2,6-dichloroindophenol) + (150ml of hot distilled water + 0.042g of Sodium bicarbonate). Cool and dilute to 200ml. Std. ascorbic acid.

Procedure:

For Dye factor: Take 5ml of HPO_3. Add 5ml of std. Ascorbic acid. Titrate against dye.

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}}$$

For sample: Take a sample. Extract of with 100ml of 3% HPO_3. Filter it. Take 5ml extract (filtered) and 10ml of 3%HPO_3 and titrate it against the dye.

2.8 <u>Estimation of Tannin</u>:

Aim: To determine the total tannin present in the sample.

Materials Required: Folins Denis reagent: 100g of Sodium tungstate + 20g of phosphomolybdic acid in 750ml of distilled water + 50ml of phosphoric acid. Reflex the mixture for 2hr and make up the vol. to 1ltr with water (Protect from light). Sodium carbonate solution: 350g Na_2Ca_3 in 1ltr of water at 70^0c. Leave for overnight standing.

Standard: 0.1g tannic acid in 100ml of distilled water (dilute 5ml of stock solution to 100ml with distilled water. 1ml contains 50µg tannic acid).

Theory: Tannins can be determined by the Vanillin-HCL modified method of Chang et al, 1994. The vanillin-HCl method is widely used for the determination of condensed tannins. The chemistry of the reaction involved, its specificity, and various assay procedures have been reviewed by Deshpande et al. (1986). Its unit is mg/100g.

$$\text{Tannin} = \frac{C \times 10 \times X}{200}$$

Where,

C is Concentration corresponding to the optical density,

10 is the volume of extract (ml)

X is the sample weight (mg)

Procedure: Take 0.1g of the powdered sample in the 250ml conical flask. Add 75ml of water. Heat & boil for 30min and filter it. Collect the supernatant and make volume to 100ml in a volumetric flask. Transfer 1ml of this extract to 100ml volumetric flask having 75ml of water. Add 5ml of Folin Denis reagent + 10ml of Sodium carbonate solution and dilute to 100ml with distilled water. Shake well and read absorbance at 700nm after 30min.

2.9 <u>Estimation of Phytates</u>:

Aim: To estimate the total phenolic content.

Materials Required: Folin reagent, 20% Na_2CO_2,

Theory: Phytates can be determined by the method described by Wheeler & Ferrel (1971).

$$\text{Phytate content}(^{\text{mol}}/_{\text{kg}}) = \frac{\text{T}\times 564.11}{\text{M}}$$

Where,

T = Titre value,

M = Molar mass of phytates

Procedure: Take 0.2 - 2ml of the sample in a test tube. Makeup to 3ml with distilled water. Add 0.5ml of Folin reagent. After 3min, add 2ml of 20% Na_2CO_2 solution to each tube. Mix thoroughly. Place the tubet in boiling water for 1min and cool. Measure the absorbance at 650nm against a reagent blank. Prepare a standard curve at different concentration.

2.10 <u>Determination of Flavonoid</u>

Materials Required: Ethanol, 5% Sodium nitrate, 10% $AlCl_3$, 1N NaOH,

Theory: Flavonoid content is determined using spectrophotometric methods.

Procedure: Weigh 0.1g of the sample. Macerate with 5ml of 80% ethanol. Centrifuge the extract at 6000rpm for 30 min. Take 0.25ml of extract in a test tube. Add 0.75ml of 5% Sodium nitrate. Allow standing for 5min. Add 0.15ml of 10% $AlCl_3$. Let it stand for 6min. Add 0.5ml of 1N NaOH. Add 0.275ml of distilled water. O.D is taken at 510nm.

2.11 Reducing sugars:

Materials Required: DNS reagent: 2gm DNS + 400mg crystalline phenol + 100mg Sodium sulphite + 200ml 1% NaOH and store 4^0c.

Rochelle salt solution: 40% Sodium potassium tartarate.

Procedure: A known weight of sample macerated with 80% ethanol. Collect supernatant & evaporate it completely. Add 10ml distilled water and dissolve the residue. Pipette out 1ml of extract and make up the volume to 3ml with distilled water. Add 3ml of DNS reagent. Heat the content in boiling water bath for 5min. Take out samples and add 1ml of 40% Rochelle salt. Allow to cool and take O.D at 510nm.

Health benefits of kodo millet(Chunk KT.et.al 2009)

Kodo millet is a nutritious grain having a high content of proteins, dietary fibers, and minerals, vitamins, phytochemicals(tannin, antioxidants, phytic acid), as compared to other crops like wheat, rice.it is a good substitute to wheat and rice. Kodo millet is good for the diabetic patient because it is rich in dietary fibers and it does not cause drastic spikes in blood sugar levels, hence it acts as a good food for a diabetic patient. Kodo millet is gluten-free it is good for the people who are gluten intolerant or suffering from celiac disease.it is also rich in phenolics and antioxidants, flavonoid content in which the bioactive components are present and protect the body from cancer, promote ageing and oxidative stress. Regular consumption of kodo millet is very beneficial for postmenopausal women suffering from symptoms of the cardiovascular disease like high blood pressure and high cholesterol levels.

Table 7: Health benefits

COMPOUND	HEALTH BENEFITS	MECHANISM	REFERENCE
Tannin(water soluble)	It shows health benefits if consumed in	It binds with iron and inhibit the absorption of iron in	(Chung KT et.al 2009).

	required limits otherwise in large amount it acts as harmful to the body, due to antinutritional activity.	the body and leads to iron deficiency.	
Dietary fibers	It lowers the risk of coronary heart disease, obesity, hypertension, diabetes,	It helps to improve the digestion in the human body or faster the metabolic reactions.	Fereidoon Shahidi, Anoma Chandrasekara et.,al. 2013.
Antioxidants	It keeps heart healthy and reduces the risk of infection and some forms of cancer like colon cancer.	Antioxidants bind the free radical formation and inhibit the oxidation reactions.	Dr Edwar et., al. 2015.
Polyphenols	Reduce chances of cardiovascular disease, oxidative stress		Sandhya Khurana, Matthew Piche et.al.,2014.

Expected outcome Chapter 6

According to all the studies, millets were not highly utilized in food industries as a major food source as wheat, rice or other cereals. Now more studies are going on the millet grains to bring forward in the food industries because millet grains are a cheaper source of nutritional composition, it provides all the essential nutrients which are required by the human body. The main reason why millets are not brought forward in the food industries because it contains antinutritional factors and due to its small size, the milling of grain is difficult and time-consuming. Research is going on, in which by

using different methods of processing like germination, fermentation, etc to reduce the anti-nutritional factors.

PROPOSED WORK WITH PLAN TIMELINE Chapter 7

work plan	Jan	Feb	March	April	May	June	July	Aug	Sep	Oct	Nov
Review of literature	√	√	√								
Report submission				√							
Product standardization									√	√	
Product development									√	√	
Product analysis										√	√
Result compilation											√

1. Amadou, I., Gounga, M. E., & Le, G. W. (2013). Millets: Nutritional composition, some health benefits and processing-A review. *Emirates Journal of Food and Agriculture*, *25*(7), 501.
2. Balasubramanian, S., V Vishwanathan, R. Influence of moisture content on physical properties of minor millet. J. Food Science. Technology. 2010, 47, 279- 284.
3. Bora, P. (2014). Nutritional Properties of Different Millet Types and their Selected Products (Doctoral dissertation).
4. Bors, Michel, & Stettmaier, (2001). Structure-activity relationships governing antioxidant capacities of plant polyphenols.
5. Bouis, H. E., & Welch, R. M. (2010). Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science*, *50*(Supplement_1), S-20.
6. Chavan, J. K., Kadam, S. S., & Beuchat, L. R. (1989). Nutritional improvement of cereals by fermentation. *Critical Reviews in Food Science & Nutrition*, *28*(5), 349-400.
7. De Wet, J. M. J., Brink, D. E., Rao, K. P., & Mengesha, M. H. (1983). Diversity in kodo millet, Paspalum scrobiculatum. *Economic Botany*, *37*(2), 159-163.
8. Deshpande, S. S., Mohapatra, D., Tripathi, M. K., & Sadvatha, R. H. (2015). Kodo Millet-Nutritional Value and Utilization in Indian Foods. Journal of Grain Processing and Storage| July-December, 2(2), 16-23.
9. Dr S. Shobana, (2009). Nutritional composition and health benefits of millets;
10. G.Sudha Devi, V.Palanimuthu and P.Arun Kumar, (2014).studies on development and storage of kodomillet based pasta; a journal of processing and post-harvesting technology, 5: 33-40.
11. Heim, Tagliaferro, & Bobilya, (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships.
12. HIMABINDU, P., & DEVANNA, N. (2015). DEVELOPMENT OF NUTRIENT RICH NOODLES BY SUPPLEMENTATION WITH MALTED KODO MILLET FLOUR.
13. Jaybhaye, R. V., Pardeshi, I. L., Vengaiah, P. C., & Srivastav, P. P. (2014). Processing and technology for millet-based food products: a review. *Journal of Ready to Eat Food*, *1*(2), 32-48.
14. Laniel, M., Emond, J.P., & Altunbas, A.E. journal of food measurement and characterization.

15. Mehta, P. S., Negi, K. S., & Ojha, S. N. (2010). Native plant genetic resources and traditional foods of Uttarakhand Himalaya for sustainable food security and livelihood.
16. Mousumi Ray, Kuntal Ghosh, Somnath Singh and Keshab Chandra Mondal,(2016). Folk to functional: An explorative overview of rice-based fermented foods and beverages in India.
17. P. Himabindu, N.Devanna, (2015). Development of nutrient-rich noodles by supplementation with malted kodo millet flour; a journal of advancement in research and technology, volume 4.
18. R.V. Jaybhaye, I. L. Pardeshi, P. C. Vengaiah, P. P. Srivastav, (2014). Processing and technology for millet-based food products; Journal of ready to eat food, 11: 32-48.
19. Rathore, S., Singh, K., & Kumar, V. (2016). Millet Grain Processing, Utilization and Its Role in Health Promotion: A Review. *International Journal of Nutrition and Food Sciences*, *5*(5), 318-329.
20. S.S. Deshpande, D. Mohapatra, M. K. Tripathi, R. H. Sadvatha, (2015). Kodo Millet-nutritional value and utilization in Indian foods; Journal of grain processing and storage, 2: 16-23.
21. Saleh, A.S.M.; Q.; Chen, J, Shen, Q. Millet Grains: Nutritional Quality, processing, and potential Health Benefits. Rev. Food Science. Food Safty. 2013. /3, 281-295.
22. Sarita, S. E. (2016). Potential of millets: nutrients composition and health benefits. *J. Sci. Innov. Res*, *5*, 46-50.
23. Seema Sharma, Dharmesh C Saxena and Charanjit S Riar (2016). Using combined optimization, GC-MS and analytical technique to analyze the germination effect on phenolics, dietary fibers, minerals and GABA contents of Kodo millet.
24. Sudharshana, L., Monteiro, P. V., & Ramachandra, G. (1988). Studies on the proteins of kodo millet (Paspalum scrobiculatum). *Journal of the Science of Food and Agriculture*, *42*(4), 315-323.