M.Sc. PROJECT AND DISSERTATION

ON

DEVELOPMENT OF VALUE ADDED PRODUCT FROM FINGER MILLET

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

SCHOOL OF AGRICULTURE

LOVELY PROFESSIONAL UNIVERSITY

Phagwara, jalandhar

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CERTIFICATE

This is to certify that **Manu Narayanan**(Registration no. 11706261) has personally completed M.Sc. pre-dissertation entitled "**Development of value added product from Finger Millet"** under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study. No part of the dissertation has ever been submitted for any other purpose at the university.

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

Date: November, 2017

Signature of Supervisor

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DECLARATION

I hereby declare that the work presented in the pre-dissertation report entitled "***Processing of finger millet***" 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 Dr. Navnidhi Panghal, Assistant professor at school of Agriculture, Lovely professional university, Phagwara, Punjab, India for the award of the degree of master of Food technology and science.

Date: April, 2017

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DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

SCHOOL OF AGRICULTURE

LOVELY PROFESSIONAL UNIVERSITY

PROJECT AND DISSERTATION PLAN PROPOSAL

Of The proposed Research Project for the degree of

MASTER'S OF SCIENCE

IN

Food sciences and technology

Name of the Research Scholar:	**Manu Narayanan.B.S**
Registration Number:	**11706261**
Roll no.	**RH1730A09**
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Title of research:	**Processing of Finger millet**

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Approved by coordinator

INTRODUCTION

Dosa is a fermented dish prepared from a thin batter and consumed mainly in south Indian region (Steinkraus, 1996), but due to globalisation, this product has spread throughout world and demand is increasing day by day. It contains milled rice and dehulled black gram as primary ingredients. The leavened and fermented batter is made crispy by baking on a hot pan and can be consumed with chutney and sambar (Purushothaman et al. 1977). The suspended form of batter is prepared by grinding black gram and wet rice separately using water. The prepared suspensions are mixed together and allowed for 8–20 h fermentation. Here the dosa is prepared by pouring the batter on a hot pan, which is applied with some oil. Within few minutes a crispy product resembling pancake will get ready (Battacharya and Bhat, 1997).

Fermentation process in dosa helps to increase the vitamins like vitamin B1, vitamin B2, folic acid, total acids, proteinase, amylase, etc. it also helps to develop antimicrobial and antioxidant components. Bioaccessibility of zinc and iron are increased with the reduction of Antinutrients. Dosa is a fermented product prepared by controlled fermentation to produce acidity and flavour to a desirable level and is suitable for persons with various allergies and gluten intolerance.it helps to fight against postdiabetic and prediabetic conditions of its less glycemic index value. For neural disorders and rheumatism dosa is considers as a remedy (Mousumi Ray, Kuntal Ghosh, 2016).

The dosa is considered as a potent healthy breakfast and snack since they are rich source for proteins and carbohydrates. In China it is believed that rice has the abilities to strengthen spleen, increase appetite, to develop body elements, digestibility, to regulate blood pressure and to prevent skin diseases (Sulochana, 2011). Fermentation also enhances the flavour and aroma of food and enriches food with proteins, essential fatty acids and amino acid and various vitamins and detoxification of few compounds.

Finger millet

Finger millet (Eleusine coracana) is considered as an important minor cereal and they abundant with phytochemicals with neutraceutical potentials. 70% of polyphenols are present in its seed coat. Millets are different and unique because of their health and nutritional specialities (Hahn et al., 1984). They are commonly known as ‘Ragi’, important minor millets of the Indian and some of the African countries. Millets are not only rich in phytochemicals, dietary fiber and minerals especially calcium but also of their anti-cardiovascular,anti-inflammatory,anti-carcinogenic,antimicrobial,antioxidant, anti-diarrheal and anti-ulcer properties (Hadimani and

Malleshi, 1993) (Sripriya et al., 1996). Beside all these functions polyphenols can also manage several physical conditions such as diabetes mellitus, hypercholesterolemia, prevention of oxidation of low-density lipoproteins (LDLs) ,hypertension, and also helps in improving health and condition of gastrointestinal tract, vascular fragility (Scalbert et al., 2005).

Ingredients

Urad dal (split black lentils)

Fenugreek (methi)

Raw rice (chawal)

Parboiled rice (urkda chawal)

Oil for cooking

OBJECTIVES

1. Physicochemical and phytochemical analysis of grain
2. Optimisation of ingredients and process parameters
3. Quality evaluation of formulated product

PROBLEM BACKGROUND

Millets can be grown in even harsh climates because of its capacity to tackle adverse weather conditions like limited nutrition and rainfall. Also, they are a good source of protein for lots of people in dry regions with many nutrition and medical functions. It is an important cereal because they are superior to other principle crops like rice, wheat, barley etc. with their good source of micronutrients like iron, phosphorous, Calcium, potassium and Zinc. The micro nutrients are less bio-accessible due to the presence of anti-nutrients such as tannins and phytates. However, by different processing techniques like germination, fermentation, dehulling, popping these anti-nutrients can be removed etc. Malting of the grain can significantly reduce the phytin phosphorus in finger millet and reduction was accompanied by significant increase in ionisable iron and soluble zinc, indicating the improved availability of these two elements. The higher amount of micronutrients will help to compensate or nullify the lower bioavailability. The health benefits of dietary fibers are reduction in sugar and blood glucose level (less glycemic index), good bowel movement. Besides fiber, millets are also packed with Phytochemical like lignans, Phyto-oestrogens, phytosterols, phycocyanin, and polyphenols.

REVIEW OF LITERATURE

Finger millet is cultivated the largest among the other small millets. Finger millet stands unique and superior among the cereals (principle crops) such as rice, wheat barley, rye, and oats with higher nutritional contents and has outstanding properties like drought resistant, can tolerate lack of nutrients etc. finger millet is potent in calcium (0.34%), phenolics (0.3-3%), minerals (2.5-305%) , phytates (0.48%), dietary fiber (18%) and proteins (6-18%). They are also rich source of leucine thiamine, methionine, riboflavin, and other essential amino acids. The plenty of these phytochemicals enhances the nutraceutical potential of finger millet and in the world, finger millet ranks 4th among other millets after, pearl millet, foxtail and millet sorghum in importance.

MILLING

The most common primary processing method is milling where grains are convert flour by grinding or pulverising. Many modern machines and conventional methods are there to covert grains to flour. As soft grains are subjected to milling the endosperm breaks into small particles and then separated by sieving or screening while in hard grains the endosperm stay intact and will be removed by winnowing. Hand-pounding can be time-consuming,inefficien and labour intensive. Grains are pulverized or grinded in an iron disc, stone mill or emery coated disc mills. Dehusking in finger millet is done by pearling or decortication. (Amir Gull, Gulzar Ahmad N, 2016).

While doing milling processing seed coat of the grain is removed resulting in significant loss of nutrients like B-vitamins and other minerals. Degree of milling and their nutrition distribution in the grain influences the loss. Even though the nutrient content of food grains will get relatively poor after milling, the bio-availability of the nutrients improves considerably. Complex polysaccharides, polyphenolic compounds such as phytates and tannins will limit the availability of iron and minerals. So by milling these complex compounds can be removed and increase iron availability eventually. Finger millets are rich in polyphenols compared rice, barley, wheat and maize, barley because of its brown seed coat. These polyphenols are mainly concentrated in outer layers such as pericarp, testa, aleurone layer which are the main components of the bran fraction. (S. Patel & Veenu Verma, 2015)

Milling of finger millet increases its value in terms of its marketability, acceptability, and profitability. However hydrothermal processing is better to decorticate finger millet which involves h steaming, hydration and drying (Shankar M and Chowde Gowda, 2013).

Tannins and flavonoids in millet seed coat are multifunctional and they act as metal chelators, free radical terminators, and singlet oxygen quenchers. Higher than that of wheat, rice, and other millets finger millet show high radical-scavenging activity because of its anti-oxidant content. Finger millet seed coat involves high polyphenolic content with high anti-bacterial and antifungal as compared to its whole flour. (Chandrasekara &. Shahidi2010). The reduction was 74.7% and 39.8% in polyphenols and phytate phosphorus contents on decortications of millet which increased the bio-availability of minerals and protein. (S. Patel & Veenu Verma, 2015).

MALTING

Malting helps in converting complex proteins to simpler, insoluble starch to soluble starch, development of enzymes and generating nutrients for yeast development (Goldammer, 2008). Developing food products by blending combined malted legumes and cereals increases the nutritional quality of the product which are good in improving children's health (Agu and Aluva, 2004).One of the main purpose of malting is to release enzymes and break down cell walls of starch granules and degradation of the proteinaceous matrix that surrounds the starch granules (Enejeet al., 2003).

Soaking of grains helps to gelatinize the starch and the distinction in free sugars and non-starch polysaccharides in finger millet grain was witnessed upon germination (Nirmala et al., 2000). Temperature of water among with other factors influences absorption of moisture in grains. The changes in free amino acids, organic acids, carbohydrates, phytate and minerals during germination and fermentation have been reported (Nirmala et al., 2000).

Finger millet is important millet and its malting has been practised both at household and industrial level in India and some of the African countries. Generally, the finger millet seeds are cleaned and steeped for 24 h, then germinated under the controlled condition on moist cloth at room temperature up to 24 h. Germinated seeds are taken out every 24 h and dried at 50^0c in an air oven for 12 h and vegetative growth portion to be removed by gentle brushing (manually) and to be ground for malting. Malted ragi flour, or extract derived from it, is extensively used in the preparation of weaning and infant foods, beverages or other pharmaceutical preparations (Nirmala et al., 2000).

The malt flour is a good source of amylases and is hence can be called as “Amylase-rich food.” Malt flour is a substitute for maltodextrin and can be blended with milk and spray dried to prepare infant food (Malleshi, 2007). While germination the amylases partially hydrolyze the starch to lower molecular weight carbohydrates. Due to this, the refined finger millet malt flour has scope for utilization in infant foods (Malleshi & Gokavi, 1999). The millet malt flour has also been utilized in milk-based beverages, confectionary, and cakes (Desai, Kulkarni, Sahoo, Ranveer, & Dangde, 2010).

Whole raw finger millet flour (WRFMF): Finger millet seeds were thoroughly cleaned, remove foreign material and dirt. Thereafter, they were sundried and ground into fine flour or powder in a mixer and stored. Germinated finger millet flour (GFMF) One portion of finger seeds were soaked overnight. Next day, water was drained and wrapped of seeds in a muslin cloth and hung in a humid atmosphere for germination. After 48 hours of germination, seeds were sundried to make moisture free. Germinated seeds were ground in a mixer and stored in a container for analysis.

Millets contain water-soluble gum and β-glucan that is useful in improving glucose metabolism. Ragi can be used in preparing amylase-rich premixes by germinating technique at household level which can then be administered to children. During germination enzymatic activity gets enhanced due to which hydrolysis of complex molecules like starch, proteins increases which results in the production of their simplified form like dextrin, peptones and peptides (Reddy et.al, 2003)

Nutritional Estimations were carried out with the basic aim to evaluate nutrient content of the prepared premixes by using standard biochemical techniques. All the techniques which were used for estimations were first standardized. Moisture estimation was done by using oven drying method; Ash was determined by muffle furnace, protein by the micro khejaldal method and crude Fiber by acid alkali (NIN, 2010).

The germinated samples showed a significant reduction in ash content. As the duration of germination increased reduction in ash content also increased and it also reported that as the soaking time increases there is a loss of minerals as the seed utilizes then for the emergence of rootlet and hence the ash content in reduced (Nidhi Chaudhary and Swati Vyas, 2014).

total non-protein nitrogen , total nitrogen; protein nitrogen, true protein nitrogen also increased with germination (Nazni 2014, Khatoon and Prakash 2006, et.al.)

The study done by Shah et al (2011) says germination significantly increased the crude fiber content. But with the present investigation, the crude fat percentage decreased with germination time.

Mineral contents

In the present study, the calcium amounts in the standard premixes were estimated to be 189.36 mg/100g and 206.3 mg/100g in 24 h germinated premixes. In premix prepared by using 36 hours germinated grains, the value showed a higher shoot up of 214.30 mg/100g and221.26mg/100g in 48 hours germinated sample.

According to the research done by Mamiro et.al. (2001) in vitro extractability of calcium and other minerals in finger millets and kidney beans increased significantly after germination in comparison to other processing techniques like soaking, autoclaving and fermentation. D'souza (2013) highlighted in his research on field bean that germination or malting enhanced availability iron, calcium & other minerals. Soaking prior to cooking or germination is a simple and more effective method that can be used both in the home and in industries that produce food products.

Anti-nutritional factors (Nidhi Chaudhary and Swati Vyas, 2014)

Under the experimental conduction control premixes had approximately 102.33 mg per 100 g phytic acid, however as duration of germination was increased the phytic acid content reduced to 82.83 mg per 100 g (Sample A); 49.89 mg per 100 g(Sample B); 38.65mg per 100g (Sample C) these findings are similar to results reported by Tizazuet.al., (2011).

POPPING

Popping is a starch gelatinization and expansion process where grains are exposed to high temperatures for short time period. During this process, the heated water vapour produced inside tries to come out through the small pores experiencing a change in temperature and pressure grandniece, breaking out the outer skin and popping out. Puffing is almost same to popping but in puffing a controlled temperature and other conditions are followed. Popping and puffing give satisfactory taste and desirable aroma. The different type of popping are of dry heat, salt treated, sand, hot air popping, popping in hot oil, gun puffing and by microwave heating. Even though a variety of cereals and millets such as rice, corn ,wheat, corn, sorghum, ragi, foxtail millet are used for popping/puffing;

only some of them pop well due to different parameters such as moisture content, the composition of grain, type of endosperm physical characteristics, and also the method of popping.

In comparison, high-temperature short time (HTST) fluidized bed air puffing has better efficiency in puffing as the product uniformly exposed to the heating medium (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. Popping also improves the digestibility of starch as it involves gelatinization of starch and degradation of dietary fibers (Holm et al., 1985; Nyman et al., 1987).

The present review concludes that there is a need to optimize processing methods and factors which govern the popping characteristics cereal grains to get better and good popping yield, less un-popped kernels and higher expansion volume. Popping is a simple and less expensive processing method which improves textural and sensory qualities of cereals and also there are minimal changes with respect to the nutrient composition in the processed product. Traditionally, popped products are prepared only during few specific occasions. This type of home processed ready-to-eat snacks has a great market potential for value-added health products, convenient food, as consumer needs are changing towards more convenient foods as well as less refined or polished grains.

Popped and puffed cereals are Ready-To-Eat whole cereal food. Hence, further needs to be assessed for micronutrients availability, dietary fiber content and in vitro protein and carbohydrate digestibility, to develop value-added healthy foods to meet the community nutritional problems. There is also need for technology development for popping and puffing of different cereals and puff-able non-grains to accomplish the target of achieving consumer satisfaction, such as microwave popping, fluidized bed puffing etc.

FERMENTATION

Fermentation is one of the effective, oldest and most effective methods of preserving and producing foods and lowering the antinutrient in millets. Reported that fermentation of Finger Millet decreased the phytic acid and polyphenols and no change has been reported in tannin contents. Also reported that rabadi prepared from the fermentation of finger millet have low phytic ac polyphenol content. Certain B vitamins like thiamine, riboflavin and niacin got improved by fermentation.

Fermentation is a metabolic process in which carbohydrates and related compounds are oxidised with the release of energy in the absence of any external electron acceptors (Jay, 1978).

Micro-organisms convert sugars to pyruvic acid and NADH is formed and NADH must pass its acquired electrons on to some acceptor if the organism is to continue to metabolise.

Fermentation is not only the oldest method or effective method but it also helps to reduce anti-nutrients, to improve the contents of certain B vitamins like thiamine, riboflavin and niacin.

THE EFFECT OF FERMENTATION ON ANTI-NUTRIENTS

Phenols

Brown varieties of finger millet contains more tannin than white varieties (Ramachandra, Virupaksha & Shadaksharaswamy, 1977). Tannins in cereals have the ability to bind with dietary proteins and forming indigestible protein-tannin complexes (Reddy & Pierson, 1994). Proteins network with tannins by means of hydrophobic interaction, hydrogen bonding, covalent bonding and electrostatic attraction (Butler, Riedl, Lebryk and Blytt, 1984). In addition, tannin is inhibiting digestive enzymes also. Fermentation alone does not decrease the number of tannins but by combination of sprouting, soaking, and fermentation reduces the level of assayable tannins. (Dhankher & Chauhan, 1987a; Khetarpaul & Chauhan, 1990a).

Fermentation has failed or proved to be ineffective in reducing trypsin inhibitor activity by its own even though soaking and boiling are effective (Reddy & Pierson, 1994).

Phytates

Phytic acid is present as a salt of monovalent and divalent cations in certain parts of cereal grains, legumes, some tubers and roots (Ologhobo & Fetuga, 1984; Reddy & Pierson, 1994). The presence of high concentrations of phytic acid in cereals and legumes is of nutritional concern because of its ability to reduce the bioavailability of minerals, particularly divalent cations including zinc, calcium, iron and magnesium (McFeeters, 1988). Phytates also interact with enzymes such as pepsin, ,trypsin, a-amylase and galactosidase resulting in a decrease of their activity. Finger millet fermentation has shown reduction in phytic acid concentrations (Dhanker & Chauhan, 1987a).

In vitro studies on the carbohydrate digestibility of finger millet

Study cases on the type of starch, its digestibility, and crystallinity demonstrated that the degree of crystallinity of finger millet and the amount of heat flow required to gelatinize starch was much higher as compared to rice. The molecular weight of the human salivary amylase digests of the finger millet starch was higher than that of rice starch digests (Mohan, Anitha, Malleshi, & Tharanathan, 2005)

Further studies indicate that finger millet starch is the most difficult to be hydrolysed in vitro by fungal a-amylase (Singh & Ali, 2006). These results indicate a higher degree of crystallinity and slightly lower digestibility of finger millet starch by the digestive enzymes in - vitro.

The effect of processing on the starch parts in finger millet is reported in puffing. There will be a increase in digestible starch and decrease in slowly digestible starch parts compared to local finger millet flour. But the studies have also said that resistant starch will be decreased in the process of puffing. The study also indicated that the pressure cooking and roasting processes increased the RS fraction in finger millet compared to other processes. The finger millet product prepared by roasting contained the highest amount of RS compared to other products (S. Shobana, K et al., 2013).

Several in vitro and in vivo cases on the carbohydrate digestibility and glycemic properties of finger millet foods indicated that the rate of starch hydrolysis and glucose release (digestibility index, DI) are affected by the degree of gelatinization (DG), added ingredient components and accompaniments (Urooj, & Puttaraj, 2001). Finger millet puttu (steamed product made out of finger millet flour and consumed with grated coconut and sugar) registered a lower DG as compared to rice puttu and other finger millet and rice preparations (roti, dosa, and dumpling). It is to be noted that wheat chapatis have better DI as compared to finger millet roti (S. Shobana, K. Krishnaswamy, 2013).

Table.1 Anti-nutrients composition of unprocessed and processed finger millet (Rotimi Toyin folasade, 2011)

Parameters (mg/100g)	Unprocessed Seed	Processed finger millet seed			
		Boiled	Soaked	Roasted	Fermented
Saponins	5.40	4.70	4.90	4.00	3.50
Cyanide	5.55	4.25	5.15	4.75	2.75
Tannins	1.60	0.10	0.90	0.20	0.80

Phytates	3.10	2.10	2.20	2.60	2.20
Oxalates	0.68	0.15	0.18	0.07	0.09

Table.2 Nutrient composition of finger millet (Rotimi Toyin folasade, 2011)

Nutrients (100g)		Native finger millet	Malted	Milled	popped	Flaked	Roasted	Fermented
Moisture		9.8	8.2	6.5	3.4	2.9	8.20	14.04
Protein		8.7	4.5	0.8	6.4	5.8	7.50	11.42
Fat		1.5	0.6	81.3	0.9	0.6	6.10	8.90
Starch		72.0	77.9	10.3	69.1	78.9	70.72	58.57
	Soluble	3.5	-	2.8	-	-		
	Insoluble	16.1	-	7.5	-	-		
Ash		2.2	0.9	1.1	2.0	1.5	4.11	3.70
Calcium (mg)		321	350	18.5	250	270	-	-
Phosphorous (mg)		201	190	111	103	146	-	-

Table.3 Ragi Nutrition Chart (Amino Acids)

Amino Acids Content	Amount (mg/g of protein)
Leucine	594
Valine	413
Phenylalanine	325
Isoleucine	275
Threonine	263
Methionine	194
Tryptophan	191
Lysine	181
Cystine	163

eTable.4 PROCESSING EFFECTS

Processing methods	Conditions	Effect	Reference
Germination	6 hours at ambient temperature (30°C) and germinated for a different time of 12, 24 and 36 hours.	• Significant improvement in nutrient density • Increase in Bioavailability of iron (5.60mg/100g to 7.0 mg/100g) • The larger increase in haemoglobin concentration. • decrease in phytate concentration(1.88 mg/100 to 0.33mg/100g) • enzymatic activity gets enhanced due to which hydrolysis of complex molecules like starch, proteins increases which results in the production of their simplified forms like dextrin, peptones and peptides)	(S. Banusha and S. Vasantharuba, 2013) S. Shobana, K. Krishnaswamy, et, al.) (Reddy et.al, 2003)
Popping or puffing	The optimized conditions for popping little millet were obtained at 16% grain moisture and particulate medium temperature of 260°C	• Increased solubility • Since it is thermally processed food also have better organoleptic properties • Lowers viscosity • Higher susceptibility to enzymatic digestibility • Popping and puffing imparts	(Priyanka Kapoor,2013)

		acceptable taste and desirable aroma to the snacks	
Fermentation	A moderate temperature of 25°C to 50°C. Air must be excluded(absence of oxygen)	• Lowering the antinutrient in millets • Decreases the polyphenol, phytic acid by 20 %, tannin by 52% and trypsin inhibitor activity by 32% at the end of 24 h • Total sugar concentration remained static. • The observed significant higher concentration of riboflavin (0.62 mg/100g), pantothenic acid (1.6 mg/100g), and niacin (4.2 mg/100g) in the fermented finger millet	(Geetha Thirumangaimannan and Kalaichelavan Gurumurthy, 2013) (Mbithi et al., 2000)
Milling	grains are either moistened with water or soaked overnight in preparation for milling Three plate clearances (0.3, 0.5 &0.7 mm), three plate speeds (450, 600 &700 rpm) and three feed rates (90, 100 & 115 kg\hr)	• Helps in removing bran and germ layers • Increases the functionality(inhibitory properties, antioxidant activity, antifungal activity, glycation property) • For developing value-added products. • Polyphenols and phytate phosphorus contents after decortications of millet were 74.7% and 39.8%.	(Shankar M, Chowde Gowda M, 2013) (Issoufou Amadou,2012) (S. Patel & Veenu Verma, 2015)

Finger millet has got good malting properties and hence is being used in the preparation of weaning foods. Ragi malt and millet-based beverages are very popular in South India. Many

traditional foods are made from finger millet. It is one of most nutritious millet and it is easy to digest as well. Since it does not contain gluten, it is a wonderful grain alternative for people who are gluten-sensitive. It is low-fat cereal and most of the fats are in the unsaturated form.

DOSA

Ingredients

Finger millet - 780g

Black gram dhal - 200g

Fenugreek - 20g

Salt - as required

Method

• Soak finger millet rice and black gram dhal separately for 4 hours and grind into a fine batter

• Add salt, mix it evenly and allow it to ferment overnight.

• Apply oil on the hot plate and pour batter and cook till crisp dosa is obtained

• Serve hot with chutney.

Table.5 Nutritional Composition of Finger millet Dosa (Dr D. Malathi et al., 2012)

Nutrient content per 100g	
Carbohydrate	68.96 g
Protein	11.02 g
Fat	1.4 g
Fiber	3.12 g
Iron	3.93 mg
Calcium	302.32 mg
Phosphorus	305.14 mg
Niacin	1.27 mg
Folic acid	42.35 µg

MATERIALS AND METHODS

Physical analysis of grain

Bulk Density:

The bulk density of a powder is the ratio of the mass of an entrapped powder sample and its volume including the contribution of the interparticulate void volume. Hence the bulk density depends on both the density of powder particles and spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per millilitre (g/ml) although the international unit is kilogram per cubic meter (1 g/ml = 1000 kg/m^3) because the measurements are made using cylinders.

It's may also have expressed in grams per cubic centimetre.

True density:

Determination takes 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 grains in the cylinder and note the change in volume accurately. This gives the volume of 10 grains. Now weigh these 10 grains in an analytical balance (or) digital balance.

1000 kernel weight:

One hundred grains of any cereal/pulse is collected manually or grains are spread on a counting plate with 100 dents equal to the size of the grains. Grains are carefully spread over the counting plate so that all the dents are filled. Extra grains are removed from the plate; grains are collected by turning plate upside down. The weight of these grains is noted by weighing on an analytical balance or digital balance. Repeat the experiment at least ten times and report the average value.

Chemical Analysis of grain

To estimate the protein content in the given simple:

Equipment

- Test tubes
- Graduated cylinder
- Weight Balance
- UV spectrophotometer

Reagents:-

- 2% Na2CO3 in 0.1 N NaOH
- 1% NaK Tartrate in H2O
- 0.5% CuSO4.5 H2O in H2O
- Reagent I: 48 ml of A, 1 ml of B, 1 ml C
- Reagent II- 1 part Folin-Phenol [2 N]: 1 part water
- BSA Standard - 1 mg/ ml

Procedure: -

- 0.2 ml of BSA working standard in 5 test tubes and makeup to 1ml using distilled water.
- The test tube with 1 ml distilled water serves as blank.
- Add 4.5 ml of Reagent I and incubate for 10 minutes.
- After incubation add 0.5 ml of reagent II and incubate for 30 minutes
- Measure the absorbance at 660 nm and plot the standard graph.
- Estimate the amount of protein present in the given sample from the Standard graph.

AIM: - To Estimate the number of carbohydrates in the grain sample.

Reagents:-

1. Glucose stock standard: 100 mg of glucose was dissolved in 100 ml of water in a standard flask.

2. Working standard: 10 ml of the stock was diluted to 100 ml. 1.0 ml of this solution contains 100µg of glucose.

3. Anthrone reagent: 0.2% anthrone was dissolved in ice-cold concentrated sulphuric acid. Prepared fresh before use

4. 2.5 N HCl.

Procedure:-

1. Weigh 100mg of the sample into a boiling tube, hydrolyze by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cool to room temperature.
2. Neutralize it with solid sodium carbonate until the effervescence ceases. make up the volume to 100 ml and centrifuge.
3. Collect the supernatant and take 0.2 to 1.0 ml for analysis.
4. Prepare the standards by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank make up the volume to 1.0 ml in all the tubes with distilled water, then add 4.0 ml of anthrone reagent, heated for eight minutes in a boiling water bath.
5. Cool rapidly and read the green to dark green colour at 630 nm.

Calculations:-

Estimation of Amino Acids (Ninhydrin method)

Reagents:-

i. Dissolve 50mg leucine in 50ml of water in a volumetric flask. Take 10ml of this stock standard and dilute to 100ml in another volumetric flask for working standard solution. A series of volume from 0.1-1 ml of this standard solution gives a concentration range 10 μg-100μg. Proceed as that of the sample and read the colour.

ii. Ninhydrin: Dissolve 0.8 stannous chlorides in 500 ml of 0.2 M citrate buffer (pH 5.0). Add this solution to 20g of Ninhydrin in 500ml of methyl cellosolve (2 methoxyethanol)

Iii. 0.2M Citrate buffer pH 0.5

iv. Diluent solvent: Mix equal volumes of water and n-propanol and use.

Procedure

1. To 0.1 ml of extract, add 1ml of Ninhydrin solution
2. Make up the volume to 2ml with distilled water
3. Heat the tube in a boiling water bath for 20min.
4. Add 5ml of the diluents and mix the contents.
5. After 15min read the intensity of the purple colour against a reagent blank in a colourimeter at 570 nm. The colour is stable for 1h.
6. Prepare the reagent blank as above by taking 0.1ml of 80% ethanol instead of the extract.

Calculations: -

To estimate the fat content by Soxhlet method.

Requirements:

- Weighing balance
- Soxhlet apparatus
- Drying Oven
- Thimble
- Heating mantle
- Glass rod
- Desiccator with silica gel
- Petroleum ether (Boiling temperature 60°-80°c)
- Cotton plugs

Procedure:

1. First of all, rinse all the glass apparatus by petroleum ether and dry it in the oven at 102°c and `after removing it keep in the desiccator.
2. Weigh 5 gram of grounded and dried sample and place it in the thimble.
3. Place the thimble in the Soxhlet extractor.
4. Take a 150ml round bottom flask and clean it and fill the flask with 90 ml petroleum ether.
5. Place the whole settings on a heating mantle and allow the petroleum ether to boil.
6. Continue the extraction process for several hours, almost 6 hours.
7. Remove the condensing unit from extraction unit and allow the sample to cool down. Finally, it removes all the lipid.
8. Collect almost all the solvent after distillation.
9. Place the sample in the oven and after removing its place in the desiccator.
10. Take the weight of the sample.
11. As a result, we get a defat sample.

To determine moisture content in grain sample

Requirements: -

Hot air oven (thermo statistically controlled)

Weighing balance

Desiccator (with active desiccant)

Weighing pots

Procedure:-

1. Weigh accurately 5g of material in a dish previously dried and weighed.
2. Place the dish along with lid in an electric oven maintained at 105˚C.
3. Cool the dish to room temperature in a desiccator and weigh with the lid on.
4. Repeat the process until three consecutive readings are same.
5. Note down the weight.

Calculations:-

The weight of the weighing dish with lid = W1 = …….g.

The weight of the dish with lid and material = W2 = …….g.

The weight of the dish with a lid and dried material = W3 = …….g.

The weight of the material = (weight of the sample – the weight of the dish) = (W2-W1) =…….g.

Quantity of the moisture in the material = (weight of the material before drying – the weight of the material after drying) = (W2-W3) =……g.

Percentage moisture in the material

$$= \frac{Quantity\ of\ the\ moisture\ in\ the\ material}{The\ weight\ of\ the\ material} \times 100$$

Determination of Ash Content:

Method:

Take a clean crucible which was dried in the oven, then cool it and weigh it (W_1). then take 5g of the sample in the crucible and weigh it again (W2). the sample will be churned and placed into muffle furnace for proper ashing at 550°C and left for 5-6 hours. The crucible containing the remaining ash is removed and weighed (W2).

Calculations:

$$\% \, Ash = \frac{W3 - W1}{W2 - W1} \times 100$$

Materials:

Crucible, muffle furnace

Estimation of Tannins by Folin- Denis method

Reagents

1. Folin-Denis reagent: Dissolve 100g of sodium tungstate and 20 g phosphomolybdic acid in 750ml distilled water in a suitable flask and add 50ml phosphoric acid. Reflux with the mixture for 2 hours and makeup to one litre of distilled water, protect the reagent from exposure to light.

2. Sodium carbonate solution: Dissolve 350g sodium carbonate in one litre of water at 70ºC-80ºC. Filter through glass wool after allowing it to stand overnight.

3. Tannic acid solution:

Stock standard: Dissolve 100mg tannic acid in 100ml of distilled water.

Working standard: Dissolve 5ml of stock solution in 100ml with distilled water (concentration 50µg/ml)

Procedure:

1. Extraction of Tannin: Weigh 0.5g of the powdered sample and transfer to the 250ml conical flask. Add 75ml of water. Heat the flask gently and boil for 30mins. Centrifuge at 2000rpm for 20mins and collect the supernatant in a 100ml volumetric flask and make up the volume.

2. Transfer 1ml of the sample extract to 100ml volumetric flask containing 75ml water.

3. Add 5ml of Folin-Denis reagent, 10ml of sodium carbonate solution and dilute to 100ml with water.

4. Shake well. Read the absorbance at 700nm after 30mins.

5. Prepare a standard graph using 0-100µg tannic acid.

Calculations:-

Estimation of total antioxidant Activity

Reagents required

1. Standard solution:-

50mg of Ascorbic acid is dissolved in the 50ml standard flask using distilled water. (conc., 1mg/ml)

2. Extract solution:-

50mg of methanol dried extract is dissolved in the 50ml standard flask using distilled water. (conc., 1mg/ml).

3. Phosphomolybdenum Reagent:-

0.6M H2S04.

28mM sodium phosphate.

4mM ammonium molybdate.

Procedure

1. Prepare (50-250µg) concentration of standard & extract solution, from that, take 0.3ml of each sample respectively.

2. To all the tubes add 3.0ml of phosphomolybdenum reagent.

3. 0.3ml of water and 3.0 ml of reagent alone serves as blank.

4. All the tubes incubate at 97oC for 90minutes.

5. Cooled and the absorbance was measured at 695nm using a UV/Vis spectrophotometrically against the blank. The antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard Ascorbic acid.

Calculations:-

Determination of polyphenols:

Method:

To an aliquot of the extract, 15ml of 20% sodium carbonate was added, mixed well, and after 15min 5ml of Folin-ciocalteus reagent was added and the reaction mixture incubated for 30min at room temperature. The contents were diluted to 100 ml distilled water and the absorbance was measured at 760 nm using UV Spectrophotometer.

Determination of phytate:

Method:

We have to take a particular weight of the sample and ground and it has to be soaked into 100ml of 2% HCl for 5hours and filter. Take 25ml of the filtrated sample into a conical flask, add 50ml of 0.3% potassium thiocyanate solution. The mixture was titrated with a standard solution of $FeCl_3$ until a brown-yellow colour persisted for 5 minutes. The concentration of the $FeCl_3$ was 1.04% w/v calculations; mole ratio of Fe to phytate = 1:1.

Determination of total phenols:

Method:

Take 2gm finely grounded sample was extracted with 5-10ml of 80% alcohol in a pestle mortar and the homogenate was boiled in water bath for 5-10 minutes, centrifuged and supernatant was collected and volume made up to 200ml in the same flask (T), then 1.0 ml Folin-ciocalteu's reagent and 0.8ml sodium carbonate (7.5%) were added into 'T' test tube. The absorbance of the sample was measured at 760nm after incubating at 30°C for 1.5hr.

Calculation:

Results were expressed as milligram of gallic acid equivalent (GAE) per gram of fresh weight. A standard curve was drawn by plotting the absorbance against concentration of gallic acid.

PROPOSED WORK WITH PLAN TIMELINE

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											√

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