# M.Sc. PROJECT AND DISSERTATION ON
# FINGER MILLET INDUSTRIAL UTILIZATION

**DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY**
**SCHOOL OF AGRICULTURE**
**LOVELY PROFESSIONAL UNIVERSITY**
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# CERTIFICATE

This is to certify that **KUMBHAR SHITAL** ( Registration no. 11711921) has personally completed M.Sc. pre dissertation entitled ***"FINGER MILLET INDUSTRIAL UTILIZATION"*** under my guidance and supervision. To the best of my knowledge, the present work is the result of original investigation and study. No part of 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: MAY, 2018

Signature of Supervisor

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# Declaration

I hereby declare that the work presented in the pre- dissertation report entitled "***Fingermillet Industrial Utilisation"*** 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:14 MAY, 2018
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**DEPARTMENT OF FOOR 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: | Kumbhar Shital |
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| Title of research**:** | **Fingermillet** |

**Industrial Utilization**

Signature of the Research Scholar

**Approved by coordinator**

## Introduction

Malt drink is known as non-alcoholic drink obtained from unfermented wort (Obuzor,G.U et al.,2010). It is usually drink in Caribbean Zone and Latin America and its consumed more in the african countries particularly in Nigeria. Historically, malt drink was especially used as baby food and for sick, but now it is accepted by all peoples.Now mostly malt based drinks have good nutritional importance in present conditions , which is mostly good and more beneficial for industry manufacurers to follow this in current strategy of increasing health awareness.

Because of absence of alcohol in malt drink it tends to change in flavor elements and also change in its poor taste and mouth feel. The study is followed by using several proportionality of specialty malts (kilned and roasted) for improving the flavor elements with base malt for developement of its sweetness factor with different proportion of sugar.

P.Pradhanang ., 2013 discovered that malting is process of possessed sprouting which is moved with drying operation and terminating of embryo growth normally , which assist to activate the enzymes of the resting grains result in converting cereal starch into fermentable sugar and other particles, partial hydrolysis of protein and macromolecules into micromolecules (Kent, 1983). It specifically mention to germination proess of barley. Cereals similar to millet, sorghum, wheat, buckwheat, oat, rice, rye, etc are also used for malting purpose.

Malting phenomenon of ragi is suitable for another millet and it comes first in position before to barley malt. Malting of millet helps in evaluating its sensory quality as it releases amylases enzymes which can interrupt starches into many digestible forms including sugars. The end product is to modify, more sweet, and increase in the nutritional amount. Why malting practices are not utilized mostly in period of malnutrition practices is one of the big question for peoples. Possibly the activity is therefore related with barley that the two have become almost similar and because barley will not mature where malnutrition generally occurs, it is not taken in account.

A.Gaddam et al., 2016 discovered that a health food was formulated by combination fingermillet malt with oats flour to provide additive health welfare. Micronutrients are necessary constituents for normal practices of growth and development of kids,which more helpful in correct working of all body parts and are healthful for good health. Ragi is a wealthy origin of iron, dietary fiber and calcium. while oats are massive with , proteins, vitamins, minerals,dietary fiber and phytochemicals.

Ragi malt was combied with oats flour at various proportions with 10%, 20% and 30% respectively. The united products were analysed for sensorial investigation with panel of 30 membersand with use of 5 point Hedonic scale. For control of sample, formula 1 in which 10% oats flour formulation was used for rating . The proper sample was determined and results were matched for malt related foods with FSSAI regulation standards .When packed and stored in metallized polyester the product was shielded by the shelf life of 90 days below dispersive surroundings

A. Gull et al., 2014 explained that historically the millet malt is commonly used for the purpose of new born babies and infants feeding purpose and as well as for the preparation of beverages either with including milk of lukewarm water with adding B.sugar since old times.For malting purpose fingermillet is having good characteristics which is also used to prepare weaning foods. In the areas of Karnataka and some parts of Tamil Nadu malting is more popular. Ragi malting helps in improving its sensory, nutritional quality and digestibility and also has variable effect in decreasing the antinutrients factors.

The distinctive qualities of finger millet make it proper matching with cereals and as well as superior preparation of malted eatables and for malting purpose. It is also good for resisting fungal infection and expansion of alpha,beta amylase at the time of germination practices and when preferable odour is formed during practices of roasting/kilning which helps in making it as an ideal food grain for malt based foods. Milk based beverages are also made by use of mixing the weaning food which is mixed in milk powder, whole milk or powdered sugar with different type of flavouring agents. All these preparations are well suited for all peoples as well as it is a great origin of nutrition. Most favorably this preparation of combination is known as‘ragi malt’ and especially it is used as purpose of health drink or energy drink.

## Industrial Utilization

Finger millet is an essential minor cereal in the region of semi-arid of Africa and India and made one of the essential cereals for a wide range of the population (Majumder *et al*., 2006).This is special instead of all minor cereals for its superior and great nutritional properties and it has more and more health importance. Fingermillet layer of seed coat is formed with including about 15% of the kernel,which is a good origin of combination polyphenols,calcium and dietary fibre.

Nutritionally fingermillet is a essential component which include nutrients like fibre, minerals and calcium.72 to 95 % of total dietary fibre content included in the fingermillet.Carbohydrate consisits of starch element which is main constituent contain 59.4 to 70.2 % amount.80 to 85 % of starch is amylopectin and other 15 to 20 % is amylose content (wankhede et al. 1979). Bhatt et al.(2003) told that non starch polysaccharide contains 20 to 30 % of the carbohydrate in ragi.Ramulu and rao (1997) discovered that insoluble dietary fibre,soluble dietary fibre and total dietary fibre include in fingermillet are 12,11 and 2 %. Fingermillet consists of high content of phytates (0.48%),carbohydrates (65-75%), minerals (2.5-3.5), tannins (0.61%),calcium (0.38%), protein (6-13%), dietary fiber (18%), phenolic compounds (0.3-3%) and trypsin inhibitors are identified for good welfare of healthlike anti-diabetic,anti-diarrheal and antioxidant properties.Dinesh Chandra (2016).

Finger millet has great value in industrial utilization of food products for better use and consumption by the human being.Finger millet is essential constituent of calcium and phytochemicals,dietary fibre with nutraceutical potential.There are many industrial products made by finger millet like biscuits, bread, weaning food and other like pasta,vermicelli.etc

There is mostly need of industrial utilization of finger millet in current status for consumption by the peoples.It has great value for the health and development.Consumption of fingermillet malt drinks helps in improving blood sugar level of the human body.Ragi is historically consumed in the form of pudding,preparation of flour, porridge , papad, mudde, chapatti, sweet halwa, kolukattai, ambali and flat breads.Biscuit is one of the product made from fingermillet .

## PROBLEM BACKGROUND

According to risk and utility maximization, problem is totally taken into consideration as now a days there is less utilization of fingermillet all over the country and there is high competition of other millets as compared to fingermillet.Therefore it is ensure that in current agriculture production and consumption fingermillet utilization is highly important for poverty reduction and economic growth.This research states that valuable importance for determining profitability of finger millet production.finger millet remains one of the most known cereal crop wealthy in food nutrients for human body. It also explains about the group action for food security and it is stored for a mortal time to provide food for hungry populations. Its other good benefits it is a great a source of income for social unit and hosuehold peoples. There is some more beneficial action from finger millet is it has a low glycaemic index and it is also bettre for diabeticely affected patients.

## OBJECTIVES -

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

# REVIEW OF LITERATURE

## BAKERY PRODUCTS

There are different type of bakery products like nan khatai, muffins, breads and biscuit are prepared by the utilization of fingermillet flour are made for improving receipe and attribute of product. Bakery products are essential in terms of fiber content, micronutrients as well as importance in the bakery world for no. of value added commodity.

R.Krishnan et al., 2011 reported that fingermillet biscuits are made up with the flour of seed coat matter of finger millet**.** Seed coat matter contains edible source of material, good amount of dietary fiber, minerals and phytochemicals. Chethan & Malleshi, 2007. Layer of millet seed coat is a good origin of polyphenols, dietary fiber and calcium which forms 15% of the kernel . It is the biscuit which is made with combination of wheat flour and fingermillet flour at various compositions. C.Shimray et al., 2011 reported that wheat flour was arranged with native finger millet flour (NFMF) and germinated finger millet flour (GFMF) at various proportion of 30–50% to make soft dough biscuits. Dough rheological properties and baking characteristics of the blends were evaluated.

C.Shimray et al., 2011 conducted study on wheat flour of PBW-343 variety and fingermillet flour of brown variety (GPU 28) used for blending of biscuit. Sagar skim milk spray dried powder, sodium steroyl-2-lactylate,sugar powder procured from local market ,sodium chloride, ammonium and sodium bicarbonate and vanilla essence also used. Sodium chloride 3 g, sodium bicarbonate 1.5 g, ammonium bicarbonate 3 g,wheat flour 300 g, sugar powder 90 g, shortening 60 g, skimmed milk powder 6 g and dextrose 6 g. The water added for biscuit preparation depends upon Research Water Absorption Meter consistency as follows: control – 20%, 30% NFMF – 23%, 40% NFMF – 23% and 50% NFMF – 23%;and 30% GFMF – 22%, 40% GFMF – 22% and 50% GFMF – 22%

S.Sonawane et al., 2017 reported that finger millet malt is used for making biscuits. Finger millet malt biscuit has 50 % higher energy value than the commercial available finger millet biscuits. Biscuits prepared from malted finger millet (70%), rava (15%) and maida (15%) were having acceptability for texture, flavour and colour in organoleptic evaluation. According to sensory evaluation white finger

millet malt was used for preparing this biscuits. As related with brown fingermillet and white fingermillet malt variety it has more demand .

S.Banusha et al., 2014 reported that hydrogenated fat and powdered sugar both creamed by using electric beater and after that wheat flour and baking powder were mixed together and sieved twice. Dough was prepared by adding refined flour with baking powder in creamed paste. Dough was rolled out of 0.6 mm in baking tray and it is cut into round shape of 4.5 cm diameter by biscuit cutter. Biscuits were placed in greases paper tray and baked in preheated oven at 150 C for 10 min finally cooled at room temperature and packed in polyethylene bags for further analysis.

S.Giram et al., 2017 discovered that refined wheat flour is replaced with ragi and oats at various levels by mixing the fat and sugar constant with the 40 and 35 g on 100g flour basis. Fat and ground sugar was creamed in a mixer with the flat beater for 2 min at slow speed. Suitable quantity of milk and 1.5 g ammonium bicarbonate added to creamed mixture and mixed for 8 min at low speed in dough mixer to obtain a homogenous mixture. The batter was placed to a thickness of 4.5 mm with the use of rolling pin and an aluminium frame of standard height. The cookies were cut by using cookie die to suitable diameter with 50 mm and transferred to a lightly greased aluminium baking tray. Baking was done at 1800 $^{\circ}$C for 15 min in a baking oven. The baked cookies were initially cooled and afterthat stored in an air tight container for advance analysis.

H.Choudhary et al., 2013 reported that flour of germinated and non-germinated finger millet was used for preparation of white bread. Provided upto 10% of level of germinated and non-germinated fingermillet flour were found more utilisable and was not differentiate from control bread. Millet utilised bread contained importantly high containment of protein and dietary fiber contents.White bread formulated with white flour which is a good source of protein,energy and carbohydrate. Blending of fingermillet flour was followed with proportion of 10,20,30,40 and 50%.Bread is consumed all over the world by all age people prepared with yeast,fat,sugar,salt,water and white flour by series of operation like mixing,kneading,fermentation,proofing and baking.White flour is obtained.

Chhavi A. 2012 told that from genotypes VL-146 and PRM -601 the protein content values of bread included fingermillet flour 8.74% and 9.91% respectively of a fresh weight and 14.26% and 16.08% respectively on a dry weight basis. A fundamental change was determined between the two finger millet

breads. Foxtail millet bread contained 11.2% protein on a fresh weight basis and 17.28 % protein on a dry weight basis.

Kamaraddi et al., 2003 described that generally the breads are selected by using exploratory trial from ragi flour and refined wheat flour blend were selected as product. The proportion of ingredients in product (70% refined wheat flour:30% finger millet flour) was 175g refined wheat flour, 75g finger millet flour, , 8.75g yeast, 3g salt, 4g gluten and 8.75g fat and 8.75g sugar, and for product code D12 (60% refined wheat flour: 40% finger millet flour) it was 150g refined wheat flour, 100g fingermillet flour,8.75g yeast, 3g salt,8g gluten and 8.75g fat, 8.75g sugar.

Chhavi A. 2012 prepared breads by incorporation of suitable ingredients like Bran of wheat, ginger,finger millet flour and garlic at various amount of 10, 20, and 30 per cent levels. Breads are formulated with 30 % wheat bran, 20% finger millet flour, 10% ginger and 10% garlic were well accepted.

Devani et al., 2016 described that normally straight dough method was used for the preparation of the bread. Blending of white flour was done with fingermillet flour at various amounts of 10,20,30,40 and 50 percent especially for development of fortified white bread. Different contents like sugar 45gm, yeast 8gm,fat 18gm, salt 5gm, HPMC 3.75gm and water 140-150 ml were added to 250 gm of white or fingermillet flour. After sometime yeast was good rised in the luke warm water for 5-10 minutes for mixing of flour and then flour was gently mixed with ingredients required and slowly kneaded and fermented for one hour. Finally dough was kept in pan of greased surface and put into baking oven at 200c for 15-20 min. Finally cool it and sliced the loaf and packed it and used for analysis.

# Extruded products

Extrusion is the process of moistened starchy or conspicuous food material with combined heat, pressure and mechanical shear in which no. of operations are included like hydration, grinding, kneading, shearing, mixing, aglomeration, compression and forming. Rosson and Miller (1973) reported that ingredients were allowed to flow by force with mixing, heating and shear from a die that forms the ingredients.Extrusion is the medium of producing different type of value added products like pasta, vermicelli, noodles, porridge ,kurkure as well as snacks.

Onyango et al., 2004 explained that uji is a thin lactic porridge prepared by the combination of ragi,sorghum,maize and cassava flour which is drinking as good quality drink in the region of eastern africa.A blend of maize and fingermillet is specially used only for the good quality chocolate brown color of the end product.The blend is diluted with water to give 30-40g/100ml slurry which is fermented in backslop manner for 24h at 25-35 1C.Slurry is again diluted to 10g/100ml,cooked for 30 min,sweetened with sugar and serve normally. Preparation of fermented was followed by addition of 60 gm flour into 100 ml of distilled water.Inoculation of slurry was done with 10 ml/100 ml of previous fermented slurry and incubated with memmert cabinet at 301 C for 24 h.

R.Jaybhaye et al., 2012 conducted study on extruded snacks from millets are made by use of twin screw extruder by using blends of fingermillet, pearlmillet, kodomillet, chickpea and soybean flour blend or ragi, sorghum, rice and soy. Balsubramanian et.al 2012 or ragi, sorghum, soy and rice (42.03,14.95,12.97 and 30%) flour blend (Seth and Rajamanickam, 2012) with desired quality. Expansion index (2.31) and sectional expansion index (5.39) was found to be maximum for feed rate and screw speed combination of 9.5 kg/h and 250 rpm for pearl millet (81.68%), finger millet (7.02%) and decorticated soy bean (11.29%) composite flour.

## PASTA -

Pasta is one of the most popular product next to bread. It contains high amount of starch but low in minerals, vitamins, phenolic compounds and dietary fiber. It is one of the healthier option which is increasing overall the world because of its adaptability,quality and convenience. Pasta is usually prepared by using durum wheat as the main ingredient. Hard and soft wheat flour were also be used with addition of various protein sources.

Caperuto et al., 2001 described that pasta was a stable food product is made mainly with mixing durum semolina and water. Also more commonly another grains have been used for normally replacing of durum semolina (Chillo et al., 2008). Especially, pseudo cereals have also been utilised for preparation of gluten free (GF) or low glycemic index pastas for special nutrition.Pasta is made by using different type of flour (CF) of carrot pomace (4%) and durum wheat semolina (96%) supplemented with pearl millet flour (PMF, 0-30g), finger millet flour (FMF, 0-20g), and carboxy methyl cellulose (CMC, 2-4g). Millets are highly nutritious, wealthy in health supporting phytochemicals and dietary fiber. The main work of it was to modify the proportion of millet flours and carrot pomace for evaluation and development of functional pasta.

B.Devaraju et al., 2008 stated that fingermillet pasta was extruded by use of 40% of refined wheat flour, 50% of fingermillet flour and 10% of defatted soy flour in one set.In another one 10% of WPC was used compared to deffated soy flour.Refined fingermillet flour was used for formulation.The composite flour was extruded in shell shape die and dried in hot air drier at 60° C for 3 hours and the final moisture content was 7 percent.

NOODLES -

Noodles are defined as the pasta products and also named as convenience food produced by the cold extrusion method in which noodles are become hard and brittle . VL-149 variety was taken for the preparation of pasta.It is staple food in many cultures.Made with unleavened dough which is extruded,stretched or rolled flat and cut into number of shapes.Also long, thin strips may be most common generally cooked in the boiling water and sometimes in cooking oil and salt.It is deep fried and pan fried and served in accompanying sauce or soup.

Evaluation of refined wheat flour (RWF) and ragi flour were used for nutrient composition. 30 to 50% proportion of blends of fingermillet flour with refined wheat flour were used for the preparation of noodles. Constitution of nutrients for noodles demonstrated that 50% finger millet formulated noodles contained highest amount of ), crude fiber (1.28%),crude fat (1.15% carbohydrate (78.54%), physiological energy (351.36 kcal),soluble dietary fiber (3.71%), total ash (1.40%), insoluble dietary fiber (5.45%), iron (5.58%) and calcium (88.39%), respectively. Sarita Shrivastava ., 2011

K.Shukla. 2011 reported a protein content of 7.1 for finger millet. Refined flour of wheat had 8.45% protein. . Important difference was found in the protein content of FMF and RWF. The received containt of crude protein in ragi should in the range 4.88–15.58% in 16 varieties of finger millet as reported by Singh., 2006. Dietary fiber acts a multifarious role in decreasing a no. of health disorders through their influence in the gastrointestinal tract. Gopalan et al., 2002 reported protein content of 11% in refined wheat flour.

Hadimani. 1993 reported insoluble dietary fiber content of milled fingermillet flour was 5.3% while reasonably higher (6.5%) soluble dietary fibre. Refined wheat flour contained 3.87 and 1.88% insoluble and soluble dietary fiber, respectively which were found importantly lower than respective insoluble dietary fiber of finger millet flour. The total dietary fiber (TDF) content of finger millet flour was 12.27%.

VERMICELLI -

Vermicelli is one of the traditional type of pasta similar as spaghetti and round in section. It has used to describe different type of noodles in asia. It has diameter less than 0.06 inches.Vermicellis are mostly eaten in two varieties mostly rice vermicelli and other is chinese vermicelli.Rice vermicelli are a part of several asian cuisines where it is also eaten as soup dish,salad or in stir fry form.

H.Mamtha et al., 2013 explained that generally fingermillet vermicelli with defatted soy flour was prepared using 45% of wheat flour and 10% of defatted soy flour that were premixed for 2 minutes in a laboratory model of dolly pasta machine known as cold extruder. Mixing till 9-10 min. At 75c with hot water.36% level water was used for dough. Dough was extruded by use of brass die with 1mm holes. Cutter of 3cm long cutting was attached.

M.Begum et al., 2017 discovered combination of different compositions as compared to other compositions.In it generally 50% of fingermillet flour, 40% of whole wheat flour and 10% of defatted soya flour were used for extraction of vermicelli. Initially these three components are premixed in pasta extruder for 2 minutes. Then 360 ml of water was added and remixed for 2 minutes. After that extrusion was done and cutted with attached cutter and oven dry at 60c and finally fingermillet vermicelli was made.

# WEANING FOOD

Weaning is the process of presenting an infant food for the infants.Traditionally millets are malted for feeding of infants. Fingermillet has better malting quality and characteristics. Malting is important for human nutrition, fibre, fat, vitamin B, C, minerals and nutrients.It is totally essential for the normal growth of infants body and overall health.Now a days it is a common need for infants.

Asma et al., 2006 discovered weaning food composed of 20% of legumes,28% of additives,42% sorghum and 10% oil seeds. These are found in good protein of 16.6% to 19.3% having high energy level 405.8 to 413.2 kcal /100g and lysine content.

Klopfenstein et al., 1998 conducted study on germinted seeds for improvement of nutrient potential of millets. Flour of malt prepared by using germinated, dried and milled fractions of sorghum, pearlmillet and fingermillet because of moisture malt is conditioning and low fibre malt flour is prepared. Amino acid profile of low fibre malt is better than sorghum and millet was compatible with barley wheat flour.

**Health Benefits of Ragi Malt (Advantages)**

Ragi malt provide plenty of calcium instead of all plant foods. Calcium and Vitamin D improve bones stronger. Good source of calcium for kids as well as for aging people. This also helps in bone development for growing infant kids and in upholding the bone health of seniors. Therefore ragi can be good for solving bone issues at bay and can change the possibility of fractures as well.

**Natural Relaxant:** Ragi contains great source of antioxidants and amino acids that support huamn body to relax natural.Abnormal diseases like insomnia, anxiety, and headaches as well as depression can be addressed by this cereal. Ragi is source of Tryptophan, a form of amino acid and a main provider of relaxing effects of this cereal.

**Reduce the Possibility of Stroke:**At the advance stage of development, while it is still green, this plant can result in avoid high blood pressure. The level of cholesterol present in the blood can be synchronized, which lead to less formation of patch and hindering of blood vessel. Thus, the possibility of stroke and hypertension decreased significantly.

**Super Baby Food:** Ragi is mostly consumed in the southern part of India, and 28 days old babies are fed by this cereal at their christening. Indians believed that it is best for the digestive process. Because it contains iron and calcium, it supports the development of bone in the kids.

**Gluten Free:** Consumption of ragi is good as it is gluten free diet and people who suffer from celiac disease get good benefit from ragi .

**Boosts Lactation:** It is suggested to lactating mother to consider ragi in their daily diet most particularly if it still young. It is considered that it can help to improve milk handling as well assimilate the milk with amino acids, calcium and iron that are needed by babies and mothers for good nutrition. Milk production is affected positively as well. This cereal is highly recommended to mothers who want to improve the amount of milk which they are produced.There are lots of recipies that will support you make the most of the advantages of Ragi such as Nachani pancakes and Jowar and Raji Kanji. On the other side, high intake of this cereal must be avoided due to the fact that it can increase the amount of oxalic acid in your system. So, it is not suggested to those suffering kidney stones as well as urinary calculi.

**Physical analysis of grain**

**Bulk Density:**

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 milliliter (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 centimeter.

**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 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 are 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:**

**A. Equipment**

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

**B. Reagents:-**

**A**. 2% $Na_2CO_3$ in 0.1 N NaOH

**B**. 1% NaK Tartrate in $H_2O$

**C**. 0.5% $CuSO_4.5 H_2O$ in $H_2O$

**D**. **Reagent I:** 48 ml of A, 1 ml of B, 1 ml C

**E**. **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 make up 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.

**Calculations:-**

**2. AIM: -** To Estimate the amount 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 cease. 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 color 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 color.

**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 color against a reagent blank in a colorimeter at 570 nm. The color 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 setting 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 it 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 lid on.
4. Repeat the process until three consecutive readings are same.
5. Note down the weight.

**Calculations:-**

Weight of the weighing dish with lid = W1 = …….g.
Weight of the dish with lid and material = W2 = …….g.
Weight of the dish with lid and dried material = W3 = …….g.
Weight of the material = (weight of the sample – weight of the dish) = (W2-W1) =…….g.

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

**Percent moisture in the material** = quantity of the moisture in the material * 100

Weight of the material

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 sample in the crucible and weigh it again ($W_2$). 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 ($W_3$).

Caluclations:

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

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 suitable flask and add 50ml phosphoric acid. Reflux with mixture for 2 hours and make up to one liter with 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 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 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 50ml standard flask using distilled water. (conc., 1mg/ml)
**2. Extract solution:-**
50mg of methanolic dried extract is dissolved in 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 an 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 have take a particular weight of the sample and groundand it has to be soaked in to 100ml of 2% HCl for 5hours and filter. Take 25ml of filtrated sample in to 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 mintues. 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 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. Standard curve was drawn by plotting the absorbance against concentration of gallic acid.

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