M.SC PROJECT ON DISSERTATION

ON

MODIFICATION OF STARCH OF ERAGROSTIS TEFF

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

SCHOOL OF AGRICULTURE

LOVELY PROFESSIONAL UNIVERSITY

INDIA

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CERTIFICATE

This is to certify that **Megha Suri**(Registration no. 11708656) has personally completed M.Sc. pre-dissertation entitled "**Modification Of Starch Of Eragrostid tef"** 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**

Dr. Navnidhi Panghal

Signature:-

Dr. Navnidhi Panghal
Assistant Professor
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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.

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:	**Megha Suri**
Registration Number:	**11708656**
Roll no.	**RH1730A18**
Name of the Supervisor	**Dr. Navnidhi Mam**
Title of research**:**	**"Modification of starch in Eragrostis of tef"**

Signature of the Research Scholar

Approved by coordinator

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Introduction

Teff [Eragrostis tef ZUCC.) Trotter] is known to be originated in Ethiopia and is used for human consumption. Teff is believed to be originated in Ethiopia (Mariam Idreis Osman Mohammed (2004) between 1000 BC and 4000 BC (Letizia Saturni 2010**) (**Jay Davison et.al, 2009**)**. Teff is also known as William's lovegrass, annual bunch grass (Mike Hunter et.al, 2007)as it is fine stemmed, tufted, characterized by a large crown, many shoots and a shallow, diverse root system. The word teff have been derived from Amharic word "teffa" which means 'lost', as the size of grain is so small it can be lost easily if drop. There are about 350 species of Eragrostis consisting of annuals and perennials, which are found over a wide geographic range. Teff is also beginning to gain popularity in other countries like Canada, USA, South Africa and Australia. People in India would know a Ragi, a close cousin of teff. Teff has a nutty flavor (Dr. Arnold Dijkstra, 2008) and can be grown in drier zones because it needs minimum level of water to grow. Teff varies from white to dark brown color. The white teff has a chestnut-like flavor and the darker varieties are earthier and taste more like Hazelnuts (Dr. Arnold Dijkstra, 2008). The extremely small grain are 1-1.5 mm long and there are 2500-3000 seeds to the gram. It is known to have an excellent amino acid composition with lysine levels higher than wheat or barley but slightly lesser than rice and contains very little gluten and is being evaluated in gluten-free food systems (Richard J. Roseberg et.al, 2005). It has a high levels of minerals, calcium content, high level of phosphorus (Mariam Idreis Osman Mohammed, 2004) (Railey, 2017) and important source of water soluble vitamins (Piccinin 2002). Teff is also a good source of essential fatty acids and phytochemicals such as polyphenols and phytates. Teff requires large variety of environmental conditions and grows up to 2800m or above the sea level. Teff comes in a variety of colors from white and red to dark brown. White teff is more valued than brown/red teff. The growth period is 90-120 days. Brown/red teff is more reliable for human consumption. Teff is similar to millets as its size is small and cooks faster using less fuel Teff is almost always produced as whole-grain flour, it is difficult to separate the bran and germ because of the relatively small size of the grain. It is estimated that 100grams of teff a day compensates for lack of iron in one's diet. Being a C4 plant(**Don Miller1,2009),** the

crop responds well to warm temperatures and can be grown in areas experiencing moisture stress as well as in waterlogged areas, as it has the ability to withstand anaerobic conditions better than any other cereal crop. Teff grain is mainly use for human food in Ethiopia as a bread (injera) and beer known as 'Tela'and katikala (Bultosa, 2007; Ketema, 1997).

PROBLEMS BACKGROUND

Teff is having very attractive nutritional profile, medical advantages, and restorative advantages. Big scale production of teff is not yet happened. Teff used product development and its business sides as value added products also impressive. To find the unexplored facts based on teff and teff incorporated products efficiently engaged research is required. It is a best home cure which battles with different everyday life medical issues like diabetes, Relieves Menstrual Problems, Boosts Digestive Health Supports, celiac support, thus numerous.

OBJECTIVES

1. Physicochemical and phytochemical analysis of grain
2. Quality evaluation of formulated product
3. Final Product

REVIEW OF LITERATURE

Red Teff Grains

White Teff Grains

[Source: - Google]

Brief description of the crop

It has fibrous root system with erect stems. The sheaths of teff are smooth, open and distinctly shorter than the internodes. Its spikelets have 2-12 florets. Each floret has a lemma, three stamens, an ovary and mostly two, in exceptional cases three, feathery stigmas. Eragrostis species are classified based on characteristics of culms, spike lets, lateral veins, pedicels, panicle, and flowering Scales, and flower scale colors (Dr. Arnold Dijkstra, 2008).

Teff is a tetraploid plant 2n = 40. (Stallknecht, 1997). Teff chromosomes are very small. Fertilization was found to occur in the basal floret of a spikelet. Maturation of flowers is basipetal on the panicle and on each branch, while acropetal on spikelet basis. The flowers of teff are hermaphroditic with both the stamens and pistils being found in the same floret. Teff is a fine stemmed, tufted annual grass characterized by a large crown, many shoots, and a shallow fibrous diverse root system. The plants germinate quickly and are adapted to Environments ranging from drought stress to water logged soil conditions. The inflorescence is an open panicle and produces small seeds (1.000 weigh 0.3 to 0.4 g). The florets consist of a lemma, 3 stamens, two stigma and two lodicules. Floret colors vary from white to dark brown. Plant height

of teff varies from 25–135 cm which dependents on cultivar type and growing environments. Panicle length 11–63 cm, with spike lets numbers per panicle varying from 190–1410. Panicle types vary from loose, lax, compact, multiple branching multi-lateral and unilateral loose to compact forms. Maturity varies from 93–130 days (Stallknect, 1999).

History of teff

Teff is an endemic in Ethiopia. The word tef might have been derived from the Semitic 'Thaf' applied in Yemen to a wild harvested cereal. However, 'Thaf' is a plant growing in Yemen, whose grains resemble those of red mustard which are eaten during famine (Costanza (1974), Haudricourt (1941).

USDA Nutrition Fact for Teff:-

Serving Size	**1 Cup**
Calories	101
Protein	3g
Fat	0g
Total Carbohydrates	19g
Cholesterol	0mg

Classification/Taxonomy

Tef belongs to the Poaceae or Grass family as do all economically important cereals. It is closely related to finger millet as both are in the subfamily Chloridoideae. The genus Eragrostis comprises about 350 species from which only tef is cultivated for human consumption. Teff is a self-pollinating, c4 plant which utilizes the carbon dioxide during photosynthesis. Tef is an

allotetraploid (2n = 4x = 40). Teff grows in wider ecology, lower risk cereal, can tolerate harsh environmental conditions where most others cereals are viable.

Classification of teff is as follows (USDA, Natural resources conservation service)

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super Division	Spermatophyta (seed plants)
Division	Magnoliophyta (flowering plants)
Class	Liliopsida (monocotyledons)
Sub class	Commelinidae
Order43w77	Cyperales
Sub family	Chloridoideae
Genus	Eragrostis von wolf (lovegrass)
Species	E. tef

Synonyms: E. pilosa (L.) P. Beauv. Var. tef (Zucc.); E. pilosa (L.) P. Beauv. Subspecies: - abyssinica (Jacq.); E. abyssinica (Jacq.) Link. Cynodon abyssinicus (Jacq.) Rasp. Poa cerealis Salisb. Poa abyssinica Jacquin; Poa tef Zuccagni (Gilbert F. Stallknecht, Montana State University)

Common Names: Tef, Teff, Lovegrass, Annual Bunch Grass Teff, Annual Bunch Grass (Australia), Warm Season Annual Bunch Grass, Williams Lovegrass, Abyssinian Lovegrass, Teff grass.

Categories of teff:

Teff is categorized into 3 types: - white, red (brown) and mixed (white, red, and brown). White teff is favored and merely grows in specific region of Ethiopia. It requires accurate environmental conditions and also expensive. Red teff is preferred as least type but it contains higher iron (Fe) content. It contains essential amino acid particularly Lysine that is most deficient in grains.

Production Requirement:-

Tef, as an indigenous cereal crop of Ethiopia is having a largest share of area about 22.7 %, 2.4 million hectares under cereal cultivation and third (i.e. after maize and wheat) in terms of grain production 16.3 %, 24.4 million quintals (Geremew Bultosa, 2007).

Teff is daylight sensitive as it flowers best with 12 hours daylight. Teff germinates rapidly when planted at an average depth of 0.3 to 0.6 cm, however the initial growth is slow until a good root system has been established. It is a low input crop and would require as little as 32-46 kg/ha of nitrogen fertilizer to boost production but excessive application would result in lodging of crop.

Due to high yield during a relatively short growing season teff has an excellent forage quality.

In Ethiopia, teff performs well with annual rainfall of 750-850 mm and 450-550 mm during its growing season, but reasonable yield can be obtained with 300 mm during the plant cycle. It grown at altitude of 1000 to 2500 m and a mean temperature range of 10 °C to 27 °C.

In the United States, consumers are slowly familiarizing themselves with this unique grain, which is nutritious enough to be sold in health food stores and exotic enough to be marketed in ethnic food stores (Lovis, 2003) (Mariam Idreis Osman Mohammed, 2004).

Application of planting of *teff* is still performed largely by traditional methods in Ethiopia. Farmers normally use hand-broadcasting method for broadcasting *teff*. In this method, *teff* cannot be uniformly distributed in the field. As a result, low efficiency and high cost are being incurred. To obtain satisfactory crop yield one should plant particular amount of *teff* in a given area uniformly. The principal disadvantage of traditional hand-broadcasting method is the non-uniform

distribution of material and thus non-optimal use of resources. Furthermore, it requires experienced manpower to broadcast.

According to Burt-Davy (1913), the chief value of Teff as a hay crop lies in its palatability, high nutritive value, narrow albumin ratio (for a grass hay), high yield, Rapid growth, drought resistance and ability to smother weeds (in Seyfu Ketema Tef Eragrostis tef Zucc.) Trotter 1997). Dr. Don Miller (Teff breeder and Researcher, Since 1995) said that Teff is having a combination of excellent forage quality with high yield during a short growing season. It has Low Input Warm Season Annual grass that requires frost free growing season. It is a drought and heat tolerant crop which is adaptable to different soil types – from dry to wet. It is having a Rapid seed germination and seedling development with very few disease or pest problems. It has very small seed 1.2 million seeds per lbs with Fine stemmed bunch grass which produces a large crown with numerous tillers with Shallow, massive, fibrous root system - can take advantage of light rains

Tolerance to biotic and abiotic stresses

Tef has shown tolerance to a number of biotic and abiotic stresses. The main biotic stresses affecting tef productivity are caused by species of fungi, of which few have had an economic impact, mostly during specific growth and production years or in certain local regions. The most growth and grain limiting abiotic stresses are caused by environmental conditions such as drought, water-logging and increased soil acidity and salinity.

- **Biotic stresses**

In comparison to other cereal crops grown in Ethiopia, tef has shown relative tolerance or resistance to biotic stress conditions caused by the attack of pests, insects and weeds. Of the fungi and pests known to causes disease in the humid areas of Ethiopia and affect tef productivity, leaf rust head smudge and damping are the most important. The use of fungicides have been shown to be effective in limiting fungal diseases under experimental conditions. Although biotic stresses

have been known to cause grain losses and are a growing concern to farmers and breeders alike, the loss of tef grain due to abiotic stress factors.

- **Abiotic stresses**

The major abiotic stress factors affecting tef growth and production include drought, soil salinity and acidity (Tadele et al., 2010). While some research has shown that different varieties of tef exhibit relative tolerance to increased salinity and soil acidity (Abate et al., 2013).

- **Salt stress**

Tef has been subjected to increased salinity in the lowland and Rift Valley areas in Ethiopia, especially the awash valley and lower plains (Asfaw and Dano, 2011). Asfaw and Dano (2011) investigated the effects of increased salinity on tef yields and tef components by screening 15 lowland tef genotypes (10 accessions and 5 varieties) at different salinity levels. They found grain yield per main panicle (GY/MP) to be the most affected by increased salinity and, although there were differences in genetic variation among tef varieties and accessions, salt tolerance was observed in accession. Increased soil salinity conditions affecting grain yield during cultivation are a growing concern in the general areas of Ethiopia, particularly in the awash valley, the authors have encouraged further investigations to help alleviate the problem.

- **Drought stress**

Tolerance to drought stress can be defined as the ability of plants to grow, develop and produce sufficient yields under a limited water supply as a consequence of periodical, environmental or simulated drought conditions. In most parts of Ethiopia, tef is grown under non-irrigated field conditions during the seasons June to September and February to May. As a result, tef crops are regularly subjected to dry-spells where rainfall is limited and yield productivity is affected. Although tef is well suited to growth and development in semi-arid areas often prone to drought conditions. Water-deficit stress or environmental drought is one of the main limiting factors of tef product

Physical properties of tef

Teff is the smallest cereal grain with an average length of 1 mm. The average thousand `kernel weight of 12 teff varieties tested was 0.264 g. The minuteness of teff grains has nutritional and technological implications. For instance, as teff grains are difficult to decorticate, the cereal is consumed as a wholegrain, improving nutrient intake for consumers. The color of teff can vary from white (ivory) to dark brown (black) depending on the variety. In Ethiopia, three major categories can be identified: white, red and mixed. It is also common for wholesalers to further subdivide white teff into very white (magna) and white (nech). However, given that these classifications are imprecise and subjective, what may be referred as magna by some may be considered as nech by others. White teff generally grows only in the Ethiopian highlands and require relatively good growing conditions. This, along with its higher consumer preference, may justify why white teff is the most expensive type of teff. However, in recent years, red teff, which is believed to be more nutritious, is also gaining popularity among health conscious consumers in Ethiopia. Teff is a moisture sensitive cereal. There are 2 mycotoxins occurring in tef grain. The presence of alfatoxin B1 occurred for 22.9% of teff grain. Another important mycotoxin is ochratoxin A, occurring in 27.3% of teff grain. During milling no mycotoxins are destroyed but this prepration step redistributes the mycotoxins in the product. Effects of thermal processing is depending on the heating type and type of mycotoxins. Most mycotoxins are moderate stable in most food processing. Cooking, Roasting, Extrusion cooking at temperatures above 150 degree Celsius lowers the amount of mycotoxins. Teff can be cultivated under harsh environmental conditions where most other cereals cannot be cultivated.

Nutritional composition of teff

Although similar to wheat in food value, teff has a higher vitamin and mineral content. When wheat is processed, the germ (the embryo of the berry, which contains a high concentration of vitamins) is removed from the endosperm. The endosperm is then milled into flour. In contrast,

teff is almost always produced as whole-grain flour. When it is milled, it is difficult to separate the bran and germ because of the relatively small size of the grain. Because there is a greater portion of germ in milled flour, the nutrient content of teff flour is also higher (Piccinin, 2002 and Lovis, 2003).

Macro components

The concentration, relationship and rates between the different macro-components are essential to determine the texture, appearance and physical characteristics of a food. In relation with this composition, Food Engineers we have to design food products, and to select machinery, additives, package materials, etc. The shelf life and then, the storage systems are defined in function of the food macro composition; Protein, fat, ash and carbohydrate content are given as 9.6%, 2, 0%, 2, 9% and 73, 0%, respectively.

- **Carbohydrates**

Carbohydrates are the major source of energy for human nutrition and play an important role in metabolism and homeostasis. Based on the molecular size and degree of polymerization, carbohydrates can be classified into sugars, oligosaccharides, starch (amylose, amylopectin), and non-starch polysaccharides. Complex carbohydrates make up 75 percent of the teff grain. It has a starch content of approximately 73 percent, making teff a starchy cereal. The amylose content of 13 teff varieties tested ranged from 20 to 26 percent, comparable to other grains, such as sorghum.

Starch content in teff

The principal carbohydrates of all cereals is starch, the amount of starch contained in a cereal grain varies but is generally between 60 -75% of weight of the grain. Thus much of the food that humans consume is in the form of starch an excellent source of energy. Cereal starches are similar in

composition, having 74 – 79% amylopectin, 25 – 30% amylose and 1% lipids. 22 – 26% for sorghum and 17% for millet amylose (Whistler and Smart,--).

Protein

The average crude protein content of teff is in the range of 12-14% percent, similar to other more common cereals such as wheat. Teff's fractional protein composition suggests that glutelins (45 percent) and albumins (37 percent), prolamins are a minor constituent (~ 12 percent)---ref.

Teff's amino acid composition is well-balanced. A relatively high concentration of lysine, a major limiting amino acid in cereals, is found in teff. Similarly, compared to other cereals, higher contents of isoleucine, leucine, valine, tyrosine, threonine, methionine, phenylalanine, arginine, alanine, and histidine are found in teff. Another important feature of teff is that it has no gluten. The digests were analyzed for the presence of T-cell–stimulatory epitopes. In contrast to known gluten containing cereals, no T-cell stimulatory epitopes were detected in the protein digests of all the teff varieties, thus confirming the absence of gluten in teff. This makes teff a valuable ingredient for functional foods destined for celiac patients who are gluten intolerant.

- **Fats**

Cereals are not the best source of fat, but as they are often consumed in large quantities, cereals can contribute a significant amount of essential fatty acids to the diet. Fatty acids are potentially beneficial to growth, development and long-term health. The crude fat content of teff is higher than that of wheat and rice, but lower than maize and sorghum. Rice, wheat and maize contain negligible amount of linoleic acid (LA) and only traces of α-linoleic acid (ALA).

- **Minerals**

The difference in mineral content between and within teff varieties is wide ranging. Red teff has a higher iron and calcium content than mixed or white teff. On the other hand, white teff has a higher copper content than red and mixed teff.

Nutrient	**Per 100 gm. (Teff)**	**Per 100 gm. (Wheat)**
Proximates		
Water	8.82 g	10.94g
Energy	367 Kcal	339 Kcal
Protein	13.3 g	13.68 g
Lipids (Fats)	2.38 g	2.47 g
Carbohydrates	73.13 g	1.78 g
Dietary Fibers	8 g	71.13 g
Total Sugars	1.84 g	-
Starch	36.56 g	-
Ash	2.37 g	-
Minerals		
Calcium	**180 mg**	34 mg
Iron	7.63 mg	3.52 mg
Magnesium	184 mg	144 mg
Phosphorus	429 mg	508 mg
Potassium	427 mg	431 mg
Sodium	12 mg	2 mg
Zinc	3.63 mg	4.16 mg
Copper	0.81 mg	0.553 mg
Manganese	9.24 mg	3.012 mg
Selenium	4.4 µg	89.4 µg
Vitamins		
Thiamin	0.39 mg	0.419 mg
Riboflavin	0.27 mg	0.121 mg
Niacin	3.363 mg	6.738 mg
Pantothenic Acid	0.942 mg	0.935 mg
Vitamin B6	0.482 mg	0.419 mg
Vitamin A,IU	**9 IU**	0 IU

Lipids		
Fatty acids Total saturated	0.449 g	0.454 g
Fatty acids, Total monounsaturated	0.589 g	0.344 g
Fatty acids, Total Polyunsaturated	1.071 g	0.978 g
Amino Acid		
Tryptophan	0.139 g	0.176 g
Threonine	0.51 g	0.366 g
Isoleucine	0.501 g	0.533 g
Leucine	1.068 g	0.934 g
Lysine	0.376 g	0.303 g
Methionine	0.428 g	0.221 g
Phenylalanine	0.698 g	0.681 g
Valine	0.686 g	0.594 g
Histidine	0.301 g	0.322 g

[Source: - USDA]

Amino acid content of tef (g/16 g N) compared with wheat, rice and whole egg

Amino Acid	**Teff**	**Wheat**	**Rice**	**Whole Egg**
Lysine	3.68	2.08	3.79	6.6
isoleucine	4.00	3.68	3.81	7.5
Leucine	8.53	7.04	8.22	9.5
Valine	5.46	4.13	5.50	7.2
Phenylalanine	5.69	4.86	5.15	5.8
Tyrosine	3.84	2.32	3.49	4.4
Tryptophan	1.30	1.07	1.25	1.4
Threonine	4.32	2.69	3.90	4.2
Histidine	3.21	2.08	2.50	2.1
Arginine	5.15	3.54	8.26	6.9
Methionine	4.06	1.46	2.32	3.8
Cystine	2.05	-	-	2.4

Source: - † Jansen et al. (1962).

‡ Amount of amino acid content considered adequate by FAO standards.

§ Alemayehu

Antinutrional Factors

Antinutrional Factors are synthetic or natural compounds that blocks the absorption of the nutrients and act as a toxins, exerting a negative effects on the body. They are found at some level in almost all foods.

Nutritional inhibitor

Certain nutritional inhibitors are associated with teff grains. Anti-nutritional factors classified broadly as those naturally present in the grains. These factors modify the nutritional. Value of the individual grains. For minerals to be used for normal metabolic Functions, they need to be absorbed through the small intestine. The bioavailability of minerals depends on dietary factors. Among dietary factors, phytochemicals, such as polyphenols and phytates, constitute major mineral absorption inhibitors and hence were, for a long time, referred to as anti-nutritional factors. However, in recent years, the recognition of their health promoting effects including anti-diabetic, anti-cancer and antioxidant properties made the term anti-nutritional factor obsolete.

- **Phytates**

Phytates are a common constituent of cereals and legumes. **It is the primary form of phosphorus storage in seeds and accounts for 60-90 percent of the total phosphorus**. It can constitute as much as 1.5 percent of the dry weight of cereals. Teff contains high amounts of phytates with a wide range of variability, probably due to differences in varieties and growing conditions. Phytates Phytic acid is commonly called myo-inositol hexaphosphoric acid or scientifically, 1, 2, 3, 4, 5, 6-hexakiss (dihydrogen phosphate) myo-inositol (IUPAC-IUB, 1968) phytates represents a complex class of naturally occurring phosphorus compounds that can significantly influence the functional and nutritional properties of foods . Phytic acid has strong binding capacity readily forming complexes with mono and multivalent cations (K+, Ca+ and mg+2); it is a form of storage of cations as well as phosphorus in many seeds (Cosgrove, 1966). In cereal grains, it is distributed on both the bran and the germ, expect for corn in the germ. **The amount of Phytic varies from. 5 – 6% in cereal, legumes and oilseeds and accounts for 60 – 90% of their total phosphorus content** Phytic acid have glucose lowering and antioxidant properties.

- **Polyphenols**

Polyphenols constitute one of the most common and widespread groups of substances in plant. Polyphenols in plants are not directly involved in any metabolic process and are therefore considered secondary metabolites. Some polyphenolic compounds have a role as defense chemicals, protecting the plant from predatory attacks of herbivores, pathogenic fungi and parasitic weeds. Polyphenols in the grains also prevent grain losses from premature germination and damage due to mould and protect seedling from insect attack (Bennick, 2002). Polyphenols traditionally have been considered anti-nutrient by nutritionists, because of the adverse effect of tannins, one type of polyphenols, on protein digestibility. However, recent interest in food phenolic has increased greatly, owing to their antioxidant capacity (free radical scavenging and metal chelating activities) and their possible beneficial implications in human health (Bravo, 1998).

- **Tannins**

Tannins constitute a complex group of naturally occurring polymers, and a rigorous chemical definition is difficult (Bennick, 2002). The term was originally used to describe vegetable components that are responsible for converting animal hides into leather in the process of tanning by forming stable complexes with skin collagen .Thus, tannins are considered to be polyphenolic metaboliters of plant with a molecular weight larger than 500 and with the ability to precipitate gelatin and other proteins from solution (Mehansho et.al. 1987a).

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 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 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

AIM: - To estimate the amount of protein in the given sample by Lowry's Method.

Principle: - concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] Ions under alkaline conditions and the subsequent reduction of the Folin Ciocalteau phosphomolybdic phosphotungstic acid to heteropolymolybdenum Blue by the copper-catalyzed oxidation of aromatic acids. The Lowry method is sensitive to pH changes and therefore the pH of assay solution should be maintained at 10 - 10.5.

The Lowry method is sensitive to low concentrations of protein.

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 H2O

C. 0.5% CuSO4.5 H2O in H2O

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 serve 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.

Principle

Carbohydrate is first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydro methyl furfural. This compound forms with anthrone a green colored product with absorption maximum at 630 nm.

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:-

AIM: - Estimation of Amino Acids. (Ninhydrin method)

Principle: - Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha-amino acids and yields an intensely colored bluish purple product which is calorimetrically measured at 570 nm.

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 chloride in 500 ml of 0.2 M citrate buffer (pH 5.0). Add this solution to 20g of Ninhydrin in 500ml of methylcellosolve (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:-

AIM: - To estimate the fat content by soxhlet method.

Principle :- Lipid in food present in various forms like monoglycerides, diglycerides, triglycerides and sterol and free fatty acid and phospholipid and carotenoids and fat-soluble vitamins. Lipid is soluble in organic solvent and insoluble in water, because of this, organic solvents like hexane, petroleum ether have the ability to solubilize fat and fat is extracted from food in combination with the solvent. Later the fat is collected by evaporating the solvent. Almost all the solvent is distilled off and can be reused.

***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.

AIM: - To Determine the Amylose content in Grain.

Apparatus: -

Dish

Weighing balance

Measuring Cylinder (100 ml)

Spectrophotometer

Reagents: -

Distilled ethanol

1N NaOH

0.1% phenolphthalein

Iodine reagent (dissolve 10g KI + 1gm iodine in water & make the vol. upto 500ml)

Standard Amylose (dissolved 0.1g amylose in 10ml 1N NAOH) (Volume make upto 100ml with demineralized water)

Procedure:-

1. 0.1g sample in powdered form was weighed. To this 1ml of distilled ethanol was added.
2. Add 10ml NAOH and leave it overnight.
3. On the following day, volume was made upto 100ml with distilled water, 2.5ml with distilled water was added, followed by 3 drops of phenolphthalein.
4. To this 0.1N HCl drop was added until pink color just disappeared.
5. 1ml iodine reagent was added, volume was made upto 50ml and color read at 590nm.
6. 0.2, 0.4, 0.6, 0.8 and 1ml of the standard amylose was taken and color was developed as in the case of sample for preparing standard curve.
7. 1ml of iodine reagent was diluted with distilled water for a blank. Record the wavelength at 590nm on spectrophotometer.

Calculations:-

Absorbance corresponds to

2.5ml of test solution = X gm. amylose.

100ml contains = (X/25) * 100gm amylose

= _____ % amylose.

AIM: - 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

AIM: - To Estimate the Flavonoids.

Reagents: - Reagents

1. Vanillin reagent -1% vanillin in 70% conc.H2SO4

2. Catechin standard 110 µg/ml

Procedure

An aliquot of the extract was pipette into a test tube and evaporated to dryness. Then added 4 ml of vanillin reagent and heated for 15 min in a boiling waterbath. A standard was also treated in the same manner. Then the optical density was read at 340 or 360 nm

Phytochemical Analysis of Grain

AIM: Estimation of Tannins by Folin- Denis method.

Principle

Tannins like compounds reduce phosphotungstic molybdic acid in alkaline solution to produce a blue color complex and the color intensity is proportional to the concentration of Tannin and measured at 700nm.

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 litre 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)

Procedur

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:-

AIM: - Estimation of total antioxidant Activity.

Principle

The total antioxidant activity was determined by phosphomolybdenum method, it is based on the reduction of MO (VI) to MO (V) by the sample and subsequence formation of a green Phosphate/ MO (V) complex at acidic ph. The absorbance is measured at 695nm using an UV/Vis spectrophotometrically. The antioxidant capacity was expressed as Ascorbic acid equivalent (AAE) by using the standard Ascorbic acid.

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:-

AIM: - To estimate the Phytic acid content in grain.

Materials: - 1. Trichloroacetic acid (TCA), 3%. Weigh 3 g TCA and dissolve in 100 mL distilled water.

2. Sodium sulfate (3%) in 3% TCA. Weigh 3 g sodium sulfate and dissolve in 100 mL of 3% TCA.

3. NaOH (1.5M). Weigh 6 g sodium hydroxide and dissolve in 100 mL distilled water.

4. HNO_3 (3.2N). Take 20.5 mL nitric acid and make the volume up to 100 mL with distilled water.

5. $FeCl_3$ solution. Dissolve 583 mg $FeCl_3$ in 100 mL of 3% TCA.

6. Potassium thiocyanate (KSCN), 1.5M. Dissolve 29.15 g of potassium thiocyanate in 200 mL distilled water.

7. Stock standard $Fe(NO_3)_3$ solution. Weigh 433 mg $Fe(NO_3)_3$ and dissolve in 100 mL of distilled water in a volumetric flask.

Procedure:-

1. Weigh a finely ground (40 mesh, ground preferably using a ball mill) sample estimated to contain 5 to 30 mg phytates-P into a 125-mL Erlenmeyer flask. Generally, the amount weighed for cereals and legumes is 500 to 700 mg.

2. Extract phytates in 50 mL of 3% TCA by shaking on a magnetic stirrer for 30 min or with occasional swirling by hand for 45 min.

3. Centrifuge the suspension (3000 g, 10 min) and transfer a 10-mL aliquot of the supernatant to a 40-mL conical centrifuge tube.

4. Add rapidly 4 mL of FeCl3 solution to the aliquot in the centrifuge tubes. Heat the contents in a boiling water bath for 45 min. If the supernatant is not clear after 30 min, add one or two drops of 3% sodium sulfate in 3% TCA and continue heating.

5. Centrifuge (3000 g, 10–15 min) and carefully decant the clear supernatant. Wash the precipitate twice by dispersing it well in 20 to 25 mL 3% TCA. Heat it in boiling water for 5 to 10 min and then centrifuge (3000 g, 10 min). Repeat the washing of the precipitate with distilled water.

6. Disperse the precipitate in a few milliliters of water and add 3 mL of 1.5N NaOH with mixing. Bring volume to approximately 30 mL with distilled water and heat in boiling water for 30 min.

7. Filter hot (quantitatively) through a moderately retentive paper (Whatsman No. 2). Wash the precipitate with 60 to 70 mL of hot distilled water and discard the filtrate.

8. Transfer and dissolve the precipitate that is on the filter paper into the 100 mL volumetric fl ask containing 40 mL of hot 3.2N HNO3. Wash paper with several portions of distilled water and collect the washings in the same flask.

9. Cool flask and contents to room temperature and bring the volume to 100 mL with distilled water.

10. Transfer a 5-mL aliquot to another 100-mL volumetric flask and dilute to approximately 70 mL with distilled water.

11. Add 20 mL of 1.5M KSCN and bring the volume to 100 mL with distilled water, and read the color immediately (within 1 min) at 480 nm using a spectrophotometer.

12. Run a reagent blank with each set of samples.

Preparation of Fe (NO3)3 Calibration Curve

Take 2.5 mL of the stock Fe (NO3)3 solution and make the volume up to 250 mL in a volumetric flask. Pipette 2.5-, 5-, 10-, 15- and 20-mL aliquots of this working standard into a series of 100-mL volumetric flasks and dilute them to approximately 70 mL with distilled water. Then proceed from step 11 from the above procedure.

Calculation

Determine the micrograms of iron present in the test from the calibration curve, and calculate the phytates P as per the following equation:

Phytates P mg/100 g sample = [Fe (μg) × 15]/Weight of sample in gm.

Medical/Health Aspects of teff

Teff grain is a very small grain. This makes teff flour high in nutrient value, because the bran and germ are the most nutritious of any grain. Teff has a very high calcium content, and contains high levels of phosphorous, iron, copper, aluminium, barium, and thiamin. It is considered to have an excellent amino acid composition, with lysine levels higher than wheat or barley and slightly less than rice or oats (Stallknecht, 1997). Teff is high in protein, carbohydrates, and fibre. The protein composition offers an excellent balance among the essential amino acids (Yu, 2006). It contains no gluten so it is appropriate for people with gluten intolerance (Stallknecht, 1999). While the reported high iron content of teff seed has been refuted, the lack of anemia in Ethiopia is considered to be due to the available iron from enjera (Mamo and Parsons 1987). Teff is the main staple in the northern, western and central parts of the country (Umeta 2007). Some scientists think that the high results about teff's iron content are due to ferruginous soil ground into the outside surface of the grains. That's why Sukian and Pittwell, 1968, they decided to check the iron content of teff once more. It is concluded from these results that the true iron content of the actual dirt-free teff grain is about 0.0033%. However Melaks has obtained higher values than Almgdrd using teff fresh

from the plant, threshed in the laboratory (Sukian and Pitwell, 1968). iron actually embedded in the grain walls must be considered to be a source of iron along with the actual true iron content of the grain itself. Zinc and iron are two of the micronutrients that are most often deficient in developing countries. Iron deficiency is the most important cause of nutritional anemia. This arises from the low bioavailability of non-haem iron (Hallberg and Hulthén, 2000) caused not only by phytate but also tannins in the diet. Phytic acid, which is present in significant amounts in the seed coat of cereals and legumes (Umeta *et al*, 2007) exerts its inhibitory effect on the absorption of zinc and iron by forming insoluble complexes in the gut under physiological condition (Wise, 1995). The formation of such chelates depends on the ratio of the content of zinc, iron or calcium relative to that of phytates in the food. Other minerals of nutritional importance that are chelated by phytates are copper and manganese (Wise, 1983; Hallberg *et al*, 1987). Following are some health Benefits :-

PROMOTES GROWTH

As there are eight different amino acids, including a slightly rare one called lysine, teff can deliver substantial support for growth and development. Our bodies need protein to create new cells, repair old ones, and general development throughout our lives. While animal proteins can be helpful, our bodies can more easily break down vegetable proteins into their constituent amino acids, which is why having something like teff in your diet is important for everyone, not just vegetarians and health food fans.

Healthy Heart

The low sodium content ensures that the body doesn't clog up the arteries too much, and the grain has been shown to effectively reduce blood pressure in research studies. This can reduce your chances of suffering from heart attacks or strokes, and reduce the overall strain on your cardiovascular system.

Supports Energy Production

Another mineral in high concentrations in teff grain is copper which plays important roles throughout our body, including in energy production, growth and repair, enzymatic reactions,

nervous system function, and red blood cell creation. Without proper copper levels, many of our body's systems will begin to fail, so adding a copper boost with teff is never a bad idea.

Diabetes Control

Teff can keep slow the release of insulin into the bloodstream, teff can help diabetics prevent the dangerous spikes and plunges that can occur if you aren't careful. This is partially due to the fiber content of teff grain, but there are more complex chemical pathways also at work in this regard, although research is still in the relatively early stages.

Relieves Menstrual Problems

Teff grain has been known as an anti-inflammatory and menstrual soothing agent for generations, so if you tend to suffer from heavy menstrual flow, severe cramping, or other physical manifestations of the monthly event, adding teff to your diet could help reduce the severity and live in comfort.

Boosts Digestive Health

Teff grain is a means to speed up the digestive process. This is likely due to the high content of dietary fiber in the grain, which can bulk up stool, stimulate peristaltic motion, and increase the regularity and quality of your bowel movements. Dietary fiber is also able to balance cholesterol levels in the body by eliminating excess omega-6 fatty acids, in addition to relieving constipation, bloating, cramping, and more serious gastrointestinal issues.

Gluten-Free

Being a gluten-free grain, it can be a great alternative for those living with celiac disease, having gluten intolerance.

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