Impact of Eleusine Coracana Incorporated with Syzegium Cumini on Lactose Intolerance, Iron Deficiency and Diabetes

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Abstract- **Finger millet halwa is the traditional dish that is familiar in various cuisines but the research on the dish was started to fulfill three demands for three different needs to provide a food that creates satiety and health benefit for the lactose intolerant patient ,, persons who suffer from both type1 and type2 diabetics and the persons who suffer from iron deficiency .finger millet (eleusine coracana)with the protein content of 7.6g,carbohydrate 88g,calcium 370mg, vitamins A : 0.48mg,thiamine(B1): 0.33mg, riboflavin(B2):0.11mg, niacin(B3:1.2mg) and fiber 3g added with lactose free whole milk powder (cow milk).dry powder obtained by evaporating lactose from the whole milk as a part of process. The evaporation temperature of milk to get lactose was depending upon the concentration of lactose they are 70%, 80% and 100% done with the evaporation temperature of 58°C, 60°C and70° C temperatures. And the pine jaggery also helps to cool the body and reduce the iron deficiency is added along with the dried black berry seed powder to control diabetics. All put together serves the purpose for all three and even shelf life was found to be more than 6 month under modified atmospheric packaging by freeze drying the product. The nutritional analysis of the 6 grams of product gave a result of very high amount of dietary fiber (6%), low in saturated fat (1%), zero cholesterol (0%) and no sugar, high in calcium (2%), iron (5%), manganese (2%), zinc (2%), potassium (1%) and vitamin C&A(2%) was present. The total calories found in 6g servings was calculated as 18 calories and would assure to serve the purpose of the deficiencies and control the sugar level to a great extent if consumed at the serving size of 6 grams on the daily basis.**

Index Terms - Eleusine coracana evaporated milk powder, Syzegium cumini seed powder, cardamom powder, pine jaggery, lactose intolerance, diabetes, iron deficiency.

I. INTRODUCTION

The recent technology has emerged in finding the new product development and gives new route lines to make food which has medicinal foods. Among the ethnic foods finger millet plays important role in giving essential nutrients in reducing the malnutrition in many under developed countries. It can be alternate food source to cure the deficiencies like anemia and lactose intolerance. Ragi and pine jaggery added to the product have rich mineral sources of iron supplements which cure the anemia similarly ragi milk extracted by fermentation technique has a good source of calcium and other proteins which can be replaced in the place of milk. Diabetics patients are advised to have ragi in their diets to reduce the sugar level in the blood .in the stressful and depressive environment around us we need stress reducing foods which are to added in the diet, pine jaggery which is sweet and have good flavor components Ragi Halwa or Finger Millet Pudding is a delicious alternative to the Wheat Flour Pudding / Gehun Ka Halwa that we make at home. The distinct taste of the finger millet along with the ghee and almonds make it nutritious and delicious as well. Hence the ragi halwa being appetizing diet serving the needs of three different people with the different health issue. Eating a balanced diet means choosing a wide variety of foods and drinks from all the food groups. It also means eating certain things in moderation, namely saturated fat, Trans fat, cholesterol, refined sugar, salt and alcohol. The goal is to take in nutrients you need for health at the recommended.

A. Finger millet

Scientific name: Eleusine coracana

Eleusine coracana is a annual plant widely grown as a cereal in the arid areas of Asia and Africa. E. coracana is originally native to Ethiopian highlands and was introduced into India approximately 4000 years ago. Finger millet is especially valuable as it contains the amino acid methionine, which is lacking in the diets of hundred million of the poor people who live on starchy staples such as cassava, plantain, polished rice or maize meal. Finger millet is ground and cooked into cakes, puddings or porridge.sripriya et al., in 1997 reported that antinutrients like phytate and tannins in finger millet reduce the bio-availability, which can be improved by suitable processing methods such as germination. The finger millet was germinated for 24 hours. It was observed that the processing decreased the ph from 5.8 to 3.8 and increased the total sugars (2- fold), reducing sugars (13 –fold) and free amino acid (10-fold).the phytate×ca/Zn molar ratio decreased from163 to 66.2, indicative of an increased zn bioavailabililty.thus it was concluded that germination is a potential process for decreasing the antinutrients levels and enhancing digestibility. In 2011 muhimbula et al., also reported that complementary foods prepared using finger millet along with cereals were nutritionally adequate and acceptable in solving some of the nutritional problems facing infants and children in iringa region and other areas of Tanzania. Finger millet is nutritionally a good source of nutrients especially

of calcium, other minerals and fibre.total carbohydrate content of finger millet has been reported to be in the range of 72 to 79.5%, joshi and katoch, 1990;bhatt et al 2003). The growing public awareness of nutrition and health care research substantiates the potential of phytochemical such as polyphenol and dietary fiber on their health beneficial properties. Hence, there is in need to identify newer sources of neutraceuticals and other natural and nutritional materials with the desirable functional characteristics. Finger millet (Eleusine coracana), one of the minor cereals, is known for several health benefits and some of the health benefits are attributed to its polyphenol and dietary fiber contents. It is an important staple food in India for people of low income groups. Nutritionally, its importance is well recognized because of its high content of calcium (0.38%), dietary fiber (18%) and phenolic compounds (0.3–3%). They are also recognized for their health beneficial effects, such as anti-diabetic, anti-tumerogenic, atherosclerogenic effects, and antioxidant and antimicrobial properties. This review deals with the nature of polyphones and dietary fiber of finger millet and their role with respect to the health benefits associated with millet.

B. Jamun fruit

Scientific name-syzygium cumini

Syzygium cumini, jambul, jambolan, jamblang, or Jamun, is an evergreen tropical treeinthe flowering plant family Myrtaceae. Syzygium cumini (S. cumini) (L.) Skeels (jambolan) is one of the widely used medicinal plants in the treatment of various diseases in particular diabetes. The present review has been primed to describe the existing data on the information on botany, phytochemical constituents, traditional uses and pharmacological actions of S. cumini (L.) Skeels (jambolan). Electronic database search was conducted with the search terms of Eugenia jambolana, S. cumini, jambolan, common plum and java plum. The plant has been viewed as an antidiabetic plant since it became commercially available several decades ago. During last four decades, numerous folk medicine and scientific reports on the antidiabetic effects of this plant have been cited in the literature. The plant is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar. The vast number of literatures found in the database revealed that the extracts of different parts of jambolan showed significant pharmacological actions. We suggest that there is a need for further investigation to isolate active principles which confer the pharmacological action. Hence identification of such active compounds is useful for producing safer drugs in the treatment of various ailments including diabetes. All parts of the jambolan can be used medicinally and it has a long tradition in alternative medicine. From all over the world, the fruits have been used for a wide variety of ailments, including cough, diabetes, dysentery, inflammation and ringworm. It is also an ancient medicinal plant with an illustrious medical history and has been the subject of classical reviews for over 100 years. It is widely distributed throughout India and ayurvedic medicine (Indian folk medicine) mentions its use for the treatment of diabetes mellitus. Various traditional practitioners in India use the different parts of the plant in the treatment of diabetes, blisters in mouth, cancer, colic, diarrhea, digestive complaints, dysentery, piles, pimples and stomachache. During last four decades, numerous folk medicinal reports on the antidiabetic effects of this plant have been cited in the literature In Unani medicine various parts of jambolan act as liver tonic, enrich blood, and strengthen teeth and gums and form good lotion for removing ringworm.

C. Palm jaggery

Palm sugar was originally made from the sap of the Palmyra palm, the date palmor the sugar date palm. Now it is also made from the sap of the Arenga pinnata (sugar palm) and the nipa palm, and may therefore also be sold as "arenga sugar". Palm sugar is often labeled under various other names reflecting the several different species of palm utilized and its wide production area across Africa and Asia. Palm sugar is produced by tapping the sap from the inflorescence of the tree and boiling it down to produce syrup, which is then sold as is, or allowed to crystallize into various shapes and sizes. In some instances the tree itself is tapped rather than the flowering spikes, but this is an isolated production method. Often the distinction is made between coconut sugar and palm sugar, but this only reflects the different species from which the sugar is sourced, i.e. coconut sugar is produced in an identical way. Thailand is one place where the distinction is made and the difference is due to palm sugar being produced there from the tree trunk of the sugar palm, whilst coconut sugar is tapped from the inflorescences of the coconut palm. The differences are semantic, as all the sugars under their various names are still produced from the sucrose rich sap of a palm species' infection of the head.

D. Evaporated milk powder

Evaporated milk is fresh, homogenized milk from which 60 percent of the water has been removed. After the water has been removed, the product is chilled, stabilized, packaged and sterilized. It is commercially sterilized at 240-245 °F (115-118 °C) for 15 minutes. A slightly caramelized flavor results from the high heat process, and it is slightly darker in color than fresh milk. The evaporation process also concentrates the nutrients and the food energy. Thus, for the same weight, undiluted evaporated milk contains more food energy than fresh milk. The milk powder is obtained by spray drying technique.

E. Almonds:

The almond is a nutritionally dense food and is a rich source of vitamin E, containing 26 mg per 100 g. They are also rich

in dietary fiber, B vitamins, essential minerals such as magnesium, copper, manganese, calcium, and potassium as well as monounsaturated fats and polyunsaturated fats (see nutrient table), fats which potentially may lower LDL cholesterol. Typical of nuts and seeds, almonds also contain phytosterols suchasBetasitosterol, stigmasterol, campesterol, sitostanol, and campstool, which have been associated with cholesterol-lowering properties. Potential health benefits, which have not been scientifically validated, include improved complexion and possibly a lower risk of cancer. Preliminary research associates consumption of almonds with elevating blood levels of high density lipoproteins and lowering low density lipoproteins. A preliminary trial showed that using them in the daily diet might lower several factors associated with heart disease, including cholesterol and blood lipids. Almonds contain polyphenol in their skins consisting in a combination of flavonols, flavan-3-ols, hydroxybenzoic acids and flavanones analogous to those of certain fruits and vegetables. The almond contains about 26% carbohydrates (12% dietary fiber, 6.3% sugars, 0.7% starch and the rest miscellaneous carbohydrates), and may therefore be made into flour for cakes and cookies (biscuits) for low-carbohydrate diets. A standard serving of almond flour, 1 cup, contains 20 grams of carbohydrates, of which 10 g is dietary fiber. This makes almond flour very desirable for use in cake and bread recipes by people on carbohydrate-restricted diets. Almonds may cause allergy or intolerance. Cross-reactivity is common with peach allergens (lipid transfer proteins) and tree nut allergens. Symptoms range from local symptoms (e.g., oral allergy syndrome, contact urticaria) to systemic symptoms includinganaphylaxis (e.g., urticaria, angioedema, gastrointestinal and respiratory symptoms).

II. THE MACHINE USED
X- RAY FLOUROSCENCE SPECTROMETRY

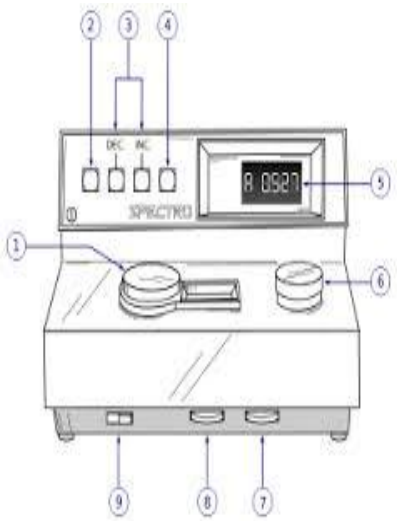
A. Principle of Working

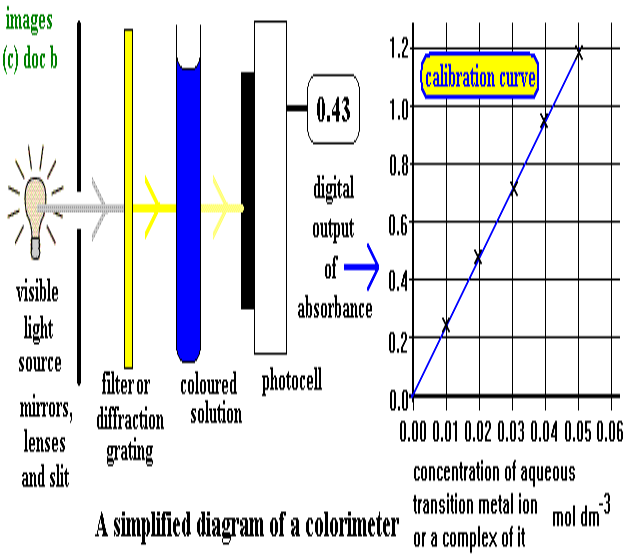
An inner shell electron is excited by an incident photon in the x-ray region during the de-excitation process, an electron is moving from higher energy level to fill the vacancy created by an ejection. The energy difference between the two shells appears as an x-ray emitted by the atom. The x-ray spectrum acquired during the above process reveals a number of characteristics peaks leads to the identification of the elements present in the sample (qualitative analysis).While the peak intensity provides the relevant or absolute elemental concentration (semi-quantitative or quantitative analysis). Use of xrf in milk formula:

Milk and dairy products are balanced complex systems containing essential nutrients for human being such as proteins, lipids, carbohydrates, vitamins and minerals. Milk

Contains 0.7-0.8% inorganic elements, mainly associated with casein micelles (ca, mg, p, Zn), citrate and phosphate complexes (ca, mg, Na, k), chlorides (Na, k), milk fat membrane (fe, cu), enzymes (fe, mn, zn) (gorbatova 2004).broadly used methods of elemental analysis of milk, e.g. flame and graphite furnace atomic absorption spectrometry, inductively coupled plasma optical emission and mass spectrometry, include either preliminary dry or wet ashing(saracoglu et al.,2007).such techniques of sample prepared are time consuming and require large amounts of expensive reagents, which can produce hazardous waste and might contaminate samples with analytes. The x-ray fluorescence analysis (xrf) of milk and dairy product has not yet become widespread in dairy industry, although the method has a great potential s the dried samples can be analyzed directly without any chemical treatment and xrf equipments is rather accessible. Xrf spectrometry is a comparative technique and it requires a set calibration standards in order to perform its quantitative measurements. The calibration samples must be representative of the matrix and elemental concentration ranges of the sample to be analyzed. Taking into account the wide diversity and difference in chemical composition of milk –based products, it is not easy to collect a relevant calibration set. The determination of in-organic elements in biological and food material using x-ray fluorescence spectrometric methods has reported by several workers (jastrzebska et al., 2003; perring and andrey 2003; yellepeddi and Thomas 2006).x-ray fluorescence spectrometry being a very important analytical technique has been extensively used for elemental analysis at trace to percentage concentration level, in many solid materials, alloys, metals, ores, powder and ceramics. The main advantage of the technique is faster analysis and significant reduction in overall analysis cost because of no chemical treatment of the samples.

B. Colorimeter: A colorimeter works by measuring the absorbance of particular wavelengths of light by a specific solution. It is used to determine the concentration of colored compounds in solutions, such as constituents in urine lab tests.





A simplified diagram of a colorimeter

Materials required:

Gas stove

Weighing balance

Vessels

Spoon

Ingredients required:

Finger millet

Blackberry seed powder

Olive oil

Evaporated milk powder

Pine jaggery

Cardamom powder

Almonds

C. Method of preparation:

1) Ragi (finger millet)is soaked in the water for more than seven hours and the ragi is allowed to germinate until the antinutrients are eliminated. Ragi is grinded using grinder and the ragi milk is extracted from the mixture.
2) Stove is ignited first and the vessel which is kept to heat for making the halwa
3) The other ingredients such as pine jaggery shavings, cardamom powder and blackberry seed powder are kept ready for the preparation.
4) The virgin olive oil is poured into the kadai, when the kadai is heated.
5) Pour the ragi milk and stir it well along with the evaporated milk powder and stir it well without clumps formation.
6) In the separate vessel heat the jaggery shaving until it melts and after it has melted strain it to remove coarse particles and add it to the hot ragi preparation along with the evaporated milk powder.
7) Stir the mixture well until you get the gelatinous texture transparent appearance achieved in the mixture
8) Finally the cardamom powder is sprinkled to enhance the flavor of the mixture.

Finally the mixture is garnished with almonds

D. Formulation of standard base:

The standard base materials selected for product formation for 200gram net weight of the product.

ingredient	Weight (grams)
Finger millet	65
Pine jaggery	85
Evaporated milk powder	30
Cardamom powder	10

E. Nutritional analysis:

test	Results obtained
moisture	7.67%
Total ash	2.70%
Crude fibre	1.46%
fat	3.95%
protein	10.54%
Carbohydrates(by difference)	73.38%
Energy(food calories per 100g sample)	392.8%

F. Proximate analysis:

A proximate analysis of food is a kind of analysis that seeks to determine all the various components of the food material. The analysis is performed by extracting components such as fiber, minerals, and soluble carbohydrates.

Protein

Proteins are polymers of amino acids. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and physiochemical properties. Proteins are important constituents of foods for a number of different reasons. They are a major source of *energy*, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins are also the major structural components of many natural foods, often determining their overall texture, *e.g.*, tenderness of meat or fish products. Isolated proteins are often used in foods as ingredients because of their unique functional properties, *i.e.*, their ability to provide desirable appearance, texture or stability. Typically, proteins are used as gelling agents, emulsifiers, foaming agents and thickeners. Many food proteins are enzymes which are capable of enhancing the rate of certain biochemical reactions. These reactions can have either a favorable or detrimental effect on the overall properties of foods. Food analysts are interested in knowing the total concentration, type, molecular structure and functional properties of the proteins in foods.

III. DETERMINATION OF OVERALL PROTEIN CONCENTRATION

Kjeldhal method

The Kjeldhal method was developed in 1883 by a brewer called Johann Kjeldhal. A food is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the food. The same basic approach is still used today, although a number of improvements have been made to speed up the process and to obtain more accurate measurements. It is usually considered to be *the* standard method of determining protein concentration. Because the Kjeldhal method does not measure the protein content directly a *conversion factor (F)* is needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications, however, this is only an average value, and each protein has a different conversion factor depending on its amino-acid composition. The Kjeldhal method can conveniently be divided into three steps: digestion, neutralization and titration.

Principles

Digestion

The food sample to be analyzed is weighed into a *digestion flask* and then digested by heating it in the presence of sulfuric acid (an oxidizing agent which digests the food), anhydrous sodium sulfate (to speed up the reaction by raising the boiling point) and a catalyst, such as copper, selenium, titanium, or mercury (to speed up the reaction). Digestion converts any nitrogen in the food (other than that which is in the form of nitrates or nitrites) into ammonia, and other organic matter to $C0_2$ and H_20. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of the ammonium ion (NH_4^+) which binds to the sulfate ion (SO_4^{2-}) and thus remains in solution:

$$N(food) \circledcirc (NH_4)_2SO_4 \quad (1)$$

Neutralization

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

$$(NH_4)_2SO_4 + 2\ NaOH \quad 2NH_3 + 2H_2O + Na_2SO_4 \quad (2)$$

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

$$NH_3 + H_3BO_3 \text{ (boric acid)} \circledcirc NH_4^+ + H_2BO_3^- \text{ (borate ion)} \quad (3)$$

Titration

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

$$H_2BO_3^- + H^+ \circledcirc H_3BO_3 \quad (4)$$

The concentration of hydrogen ions (in moles) required to reach the end-point is equivalent to the concentration of nitrogen that was in the original food (Equation 3). The following equation can be used to determine the nitrogen concentration of a sample that weighs *m* grams using a *x*M HCl acid solution for the titration:

$$\%N = \frac{x \text{ moles}}{1000 \text{ cm}^3} \times \frac{(v_s - v_b) \text{ cm}^3}{m \text{ g}} \times \frac{14 \text{ g}}{\text{moles}} \times 100$$

Where *v*s. and *v*b are the titration volumes of the sample and blank, and 14g is the molecular weight of nitrogen N. A blank sample is usually ran at the same time as the material being analyzed to take into account any residual nitrogen which may be in the reagents used to carry out the analysis. Once the nitrogen content has been determined it is converted to a protein content using the appropriate conversion factor: %Protein = F**?**%N.

IV. DETERMINATION OF TOTAL LIPID CONCENTRATION SOLVENT EXTRACTION

The fact that lipids are soluble in organic solvents, but insoluble in water, provides the food analyst with a convenient method of separating the lipid components in foods from water soluble components, such as proteins, carbohydrates and minerals. In fact, solvent extraction techniques are one of the most commonly used methods of isolating lipids from foods and of determining the total lipid content of foods.

Sample Preparation

The preparation of a sample for solvent extraction usually involves a number of steps:

Drying sample. It is often necessary to dry samples prior to solvent extraction, because many organic solvents cannot easily penetrate into foods containing water, and therefore extraction would be inefficient.

Particle size reduction. Dried samples are usually finely ground prior to solvent extraction to produce a more homogeneous sample and to increase the surface area of lipid exposed to the solvent. Grinding is often carried out at low temperatures to reduce the tendency for lipid oxidation to occur.

Acid hydrolysis. Some foods contain lipids that are complexed with proteins (lipoproteins) or polysaccharides (glycolipids). To determine the concentration of these components it is necessary to break the bonds which hold the lipid and non-lipid components together prior to solvent extraction. Acid hydrolysis is commonly used to release bound lipids into easily extractable forms, *e.g.* a sample is digested by heating it for 1 hour in the presence of 3N HCl acid.

Solvent Selection. The *ideal* solvent for lipid extraction would completely extract all the lipid components from a food, while leaving all the other components behind. In practice, the efficiency of solvent extraction depends on the *polarity* of the lipids present compared to the polarity of the solvent. Polar lipids (such asglycolipids or phospholipids) are more soluble in polar solvents (such as alcohols), than in non-polar solvents (such as hexane). On the other hand, non-polar lipids (such as triacylglycerols) are more soluble in non-polar solvents than in polar ones. The fact that different lipids have different polarities means that it is impossible to select a single organic solvent to extract them all. Thus the total lipid content determined by solvent extraction depends on the nature of the organic solvent used to carry out the extraction: the total lipid content determined using one solvent may be different from that determined using another solvent. In addition to the above considerations, a solvent should also be inexpensive, have a relatively low boiling point (so that it can easily be removed by evaporation), be non-toxic and be nonflammable (for safety reasons). It is difficult to find a single solvent which meets all of these requirements. Ethyl ether and petroleum ether are the most commonly used solvents, but pentane and hexane are also used for some foods.

Batch Solvent Extraction

These methods are based on mixing the sample and the solvent in a suitable container, *e.g.*, a separatory funnel. The container is shaken vigorously and the organic solvent and aqueous phase are allowed to separate (either by gravity or centrifugation). The aqueous phase is then decanted off, and the concentration of lipid in the solvent is determined by evaporating the solvent and measuring the mass of lipid remaining: %Lipid = 100 **?**(M_{lipid}/M_{sample}). This procedure may have to be repeated a number of times to improve the efficiency of the extraction process. In this case the aqueous phase would undergo further extractions using fresh solvent, then all the solvent fractions would be collected together and the lipid determined by weighing after evaporation of solvent. The efficiency of the extraction of a particular type of lipid by a particular type of solvent can be quantified by an equilibrium partition coefficient, $K = cs_{solvent}/c_{aqueous}$, where $c_{solvent}$ and $c_{aqueous}$ are the concentration of lipid in the solvent and aqueous phase, respectively. The higher the partition coefficient the more efficient the extraction process.

Semi-Continuous Solvent Extraction

Semi-continuous solvent extraction methods are commonly used to increase the efficiency of lipid extraction from foods. The Soxhlet method is the most commonly used example of a semi-continuous method. In the Soxhlet method a sample is dried, ground into small particles and placed in a porous thimble. The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent and below a condenser. The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. Eventually, the solvent builds up in the extraction chamber and completely surrounds the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. As the solvent passes through the sample it extracts the lipids and carries them into the flask. The lipids then remain in the flask because of their low volatility. At the end of the extraction process, which typically lasts a few hours, the flask containing the solvent and lipid is removed, the solvent is evaporated and the mass of lipid remaining is measured (M_{lipid}). The percentage of lipid in the initial sample (M_{sample}) can then be calculated: %Lipid = 100 $\times$ (M_{lipid}/M_{sample}). A number of instrument manufacturers have designed modified versions of the Soxhlet method that can be used to determine the total lipid content more easily and rapidly (*e.g.* Soxtec).

V. ANALYSIS OF POLYSACCHARIDES AND FIBER

A wide variety of polysaccharides occur in foods. Polysaccharides can be classified according to their molecular characteristics (*e.g.*, type, number, bonding and sequence of monosaccharides), physicochemical characteristics (*e.g.*, water solubility, viscosity, surface activity) and nutritional function (*e.g.*, digestible or non-digestible). Most polysaccharides contain somewhere between 100 and several thousand monosaccharides. Some polysaccharides contain all the same kind of monosaccharide (homopolysaccahride), whereas others contain a mixture of different kinds of monosaccharide (heteropolysaccharides). Some polysaccharides exist as linear chains, whereas others exist as branched chains. Some polysaccharides can be digested by human beings and therefore form an important source of energy (*e.g.*, starch), whereas others are indigestible (*e.g.*, cellulose, hemicelluloses and pectin). These indigestible polysaccharides form part of a group of substances known as *dietary fiber*, which also includes lignin (which is a polymer of aromatic molecules). Consumption of many types of dietary fiber has been shown to have beneficial physiologically functional properties for humans, *e.g.*, prevention of cancer, heart disease and diabetes.

Analysis of Starch

Starch is the most common digestible polysaccharide found in foods, and is therefore a major source of energy in our diets. In its natural form starch exists as water-insoluble granules (3 - 60 □ m), but in many processed foods the starch is no longer in this form because of the processing treatments involved (*e.g.*, heating).It consists of a mixture of two glucose homopolysaccahride: *amylose* (500-2000 glucose units) which is linear, and *amylopectin* (>1,000,000 glucose units) which is extensively branched. These two kinds of starch have different physiochemical properties and so it is often important to determine the concentration of each individual component of the starch, as well as the overall starch concentration.

Sample preparation. The starch content of most foods cannot be determined directly because the starch is contained within a structurally and chemically complex food matrix. In particular, starch is often present in a semi-crystalline form (granular or retrograded starch) that is inaccessible to the chemical reagents used to determine its concentration. It is therefore necessary to isolate starch from the other components present in the food matrix prior to carrying out a starch analysis.In natural foods, such as legumes, cereals or tubers, the starch granules are usually separated from the other major components by drying, grinding, steeping in water, filtration and centrifugation. The starch granules are water-insoluble and have a relatively high density (1500 kg/m^3) so that they will tend to move to the bottom of a container during centrifugation, where they can be separated from the other water-soluble and less dense materials. Processed food samples are normally dried, ground and then dispersed in hot 80% ethanol solutions. The monosaccharides and oligosaccharides are soluble in the ethanol solution, while the starch is insoluble. Hence, the starch can be separated from the sugars by filtering or centrifuging the solution. If any semi-crystalline starch is present, the sample can be dispersed in water and heated to a temperature where the starch gelatinizes (> 65 ^{o}C). Addition of perchloric acid or calcium chloride to the water prior to heating facilitates the solubilization of starches that are difficult to extract.

Analysis methods. Once the starch has been extracted there are a number of ways to determine its concentration:

- Specific enzymes are added to the starch solution to breakdown the starch to glucose. The glucose concentration is then analyzed using methods described previously (*e.g.*, chromatography or enzymatic methods). The starch concentration is calculated from the glucose concentration.
- Iodine can be added to the starch solution to form an insoluble starch-iodine complex that can be determined *gravimetrically* by collecting, drying

and weighing the precipitate formed or *titrimetrically* by determining the amount of iodine required to precipitate the starch.

- If there are no other components present in the solution that would interfere with the analysis, then the starch concentration could be determined using physical methods, *e.g.,* density, refractive index or polarimetry.

The amylose and amylopectin concentrations in a sample can be determined using the same methods as described for starch once the amylose has been separated from the amylopectin. This can be achieved by adding chemicals that form an insoluble complex with one of the components, but not with the other, *e.g.* some alcohols precipitate amylose but not amylopectin. Some of the methods mentioned will not determine the concentration of resistant starch present in the sample. If the concentration of resistant starch is required then an additional step can be added to the procedure where dimethylsulfoxide (DMSO) is added to dissolve the resistant starch prior to carrying out the analysis.

Analysis of Fibers

Over the past twenty years or so nutritionists have become aware of the importance of fiber in the diet. Liberal consumption of fiber helps protect against colon cancer, cardiovascular disease and constipation. Adequate intake of dietary fiber is therefore beneficial to good health. Dietary fiber is defined as plant polysaccharides that are indigestible by humans, plus lignin. The major components of dietary fiber are cellulose, hemicelluloses, pectin, hydrocolloids and lignin. Some types of starch, known as *resistant starch*, are also indigestible by human beings and may be analyzed as dietary fiber. The basis of many fiber analysis techniques is therefore to develop a procedure that mimics the processes that occur in the human digestive system.

Major Components of Dietary Fiber

Cell Wall Polysaccharides

Cellulose occurs in all plants as the principal structural component of the cell walls, and is usually associated with various hemicelluloses and lignin. The type and extent of these associations determines the characteristic textural properties of many edible plant materials. Cellulose is a long linear homopolysaccahride of glucose, typically having up to 10,000 glucose subunits. Cellulose molecules aggregate to form micro fibrils that provide strength and rigidity in plant cell walls. Hemicelluloses are a heterogeneous group of branched heteropolysaccharides that contain a number of different sugars in their backbone and side-chains. By definition hemicelluloses are soluble in dilute alkali solutions, but insoluble in water. Pectin are another form of heteropolysaccharides found in cell walls that are rich in uronicacids, soluble in hot water and that are capable of forming gels.

Non Cell Wall Polysaccharides

These groups of substances are also indigestible carbohydrates, but they are not derived from the cell walls of plants. Non-cell wall polysaccharides include hydrocolloids such as guar and locust bean gum, gum Arabic, agar, alginates and caragenans which are commonly used in foods as gelling agents, stabilizers and thickeners.

Lignin

Lignin is a non-carbohydrate polymer that consists of about 40 aromatic subunits which are covalently linked. It is usually associated with cellulose and hemicelluloses in plant cell-walls.

Common Procedures in Sample Preparation and Analysis

There are a number of procedures that are commonly used in many of the methods for dietary fiber analysis:

- *Lipid removal.* The food sample to be analyzed is therefore dried, ground to a fine powder and then the lipids are removed by solvent extraction.
- *Protein removal.* Proteins are usually broken down and solubilized using enzymes, strong acid or strong alkali solutions. The resulting amino acids are then separated from insoluble fiber by filtration or from total fiber by selective precipitation of the fiber with ethanol solutions.
- *Starch removal.* Semi-crystalline starch is gelatinized by heating in the presence of water, and then the starch is broken down and solubilized by specific enzymes, strong acid or strong alkali. The glucose is then separated from insoluble fiber by filtration or separated from total fiber by selective precipitation of the fiber with ethanol solutions.
- *Selective precipitation of fibers.* Dietary fibers can be separated from other components in aqueous solutions by adding different concentrations of ethanol to cause selective precipitation. The solubility of monosaccharides, oligosaccharides and polysaccharides depends on the ethanol concentration. *Water*: monosaccharides, oligosaccharides, some polysaccharides and amino acids are soluble; other polysaccharides and fiber are insoluble. *80% ethanol solutions:* monosaccharides, oligosaccharides and amino acids are soluble; polysaccharides and fibers are insoluble. For this reason, *concentrated ethanol*

solutions are often used to selectively precipitate fibers from other components.

- *Fiber analysis.* The fiber content of a food can be determined either *gravimetrically* by weighing the mass of an insoluble fiber fraction isolated from a sample or *chemically* by breaking down the fiber into its constituent monosaccharides and measuring their concentration using the methods described previously.

Gravimetric Methods

A. Crude Fiber Method

The crude fiber method gives an estimate of indigestible fiber in foods. It is determined by sequential extraction of a defatted sample with 1.25% H_2SO_4 and 1.25% NaOH. The insoluble residue is collected by filtration, dried, weighed and ashed to correct for mineral contamination of the fiber residue. Crude fiber measures cellulose and lignin in the sample, but does not determine hemicelluloses, pectin and hydrocolloids, because they are digested by the alkali and acid and are therefore not collected. For this reason many food scientists believe that its use should be discontinued. Nevertheless, it is a fairly simple method to carry out and is the official AOAC method for a number of different foodstuffs.

B. Total, insoluble and soluble fiber method

The basic principle of this method is to isolate the fraction of interest by selective precipitation and then to determine it's mass by weighing. A gelatinized sample of dry, defatted food is enzymatically digested with □ □ amylase, amyloglucosidase and protease to break down the starch and protein components. The total fiber content of the sample is determined by adding 95% ethanol to the solution to precipitate all the fiber. The solution is then filtered and the fiber is collected, dried and weighed. Alternatively, the water-soluble and water-insoluble fiber components can be determined by filtering the enzymatically digested sample. This leaves the *soluble* fiber in the filtrate solution, and the *insoluble* fiber trapped in the filter. The insoluble component is collected from the filter, dried and weighed. The soluble component is precipitated from solution by adding 95% alcohol to the filtrate, and is then collected by filtration, dried and weighed. The protein and ash content of the various fractions are determined so as to correct for any of these substances which might remain in the fiber: Fiber = residue weight - weight of (protein + ash).

This method has been officially sanctioned by the AOAC and is widely used in the food industry to determine the fiber content of a variety of foods. Its main disadvantage is that it tends to overestimate the fiber content of foods containing high concentrations of simple sugars, *e.g.*, dried fruits, possibly because they get trapped in the precipitates formed when the ethanol is added.

Englyst-Cummings Procedure

A defatted food sample is heated in water to gelatinize the starch. Enzymes are then added to digest the starch and proteins. Pure ethanol is added to the solution to precipitate the fiber, which is separated from the digest by centrifugation, and is then washed and dried. The fiber is then hydrolyzed using a concentrated sulfuric acid solution to break it down into its constituent monosaccharide's, whose concentration is determined using the methods described previously, *e.g.*, calorimetrically or chromatographically. The mass of fiber in the original sample is assumed to be equal to the total mass of monosaccharide's present. The concentration of insoluble and soluble dietary fiber can also be determined by this method, using similar separation steps as for the *total, insoluble and soluble* gravimetric method mentioned above.

This method can be used to determine the total, soluble and insoluble fiber contents of foods, but does not provide information about the *lignin* content. This is because lignin is not a polysaccharide, and so it is not broken down to monosaccharides during the acid digestion. For most foods this is not a problem because they have low lignin concentrations anyway. If a food does contain significant amounts of lignin then another method should be used, *e.g.*, the gravimetric method or more sophisticated chemical methods (*e.g.*, the Theander-Marlett method).

Chemical Methods

In chemical methods, the fiber content is equal to the sum of all nonstarch monosaccharide plus lignin remaining once all the digestible carbohydrates have been removed. Monosaccharides are measured using the various methods described previously.

Moisture:

The moisture content of a food material is defined through the following equation:

$$\%\text{Moisture} = (m_w/m_{sample}) \times 100$$

Where m_w is the mass of the water and ms_{ample} is the mass of the sample. The mass of water is related to the number of water molecules (n_W) by the following expression: $m_w = n_w M_w / N_A$, where M_w is the molecular weight of water (18.0 g per mole) and N_A is Avogadro's number (6.02×10^{23} molecules per mole). In principle, the moisture content of a food can therefore be determined accurately by measuring the number or mass of water molecules present in a known mass of sample. It is not possible to directly measure the number of water molecules

present in a sample because of the huge number of molecules involved. A number of analytical techniques commonly used to determine the moisture content of foods are based on determinations of the mass of water present in a known mass of sample. Nevertheless, as we will see later, there are a number of practical problems associated with these techniques that make highly accurate determinations of moisture content difficult or that limit their use for certain applications. For these reasons, a number of other analytical methods have been developed to measure the moisture content of foods that do not rely on direct measurement of the mass of water in a food. Instead, these techniques are based on the fact that the water in a food can be distinguished from the other components in some measurable way.An appreciation of the principles, advantages and limitations of the various analytical techniques developed to determine the moisture content of foods depends on an understanding of the molecular characteristics of water. A water molecule consists of an oxygen atom covalently bound to two hydrogen atoms (H_2O). Each of the hydrogen atoms has a small positive charge (□ +), while the oxygen atom has two lone pairs of electrons that each has a small negative charge (□-). Consequently, water molecules are capable of forming relatively strong hydrogen bonds (O-H□ + □-O) with four neighboring water molecules. The strength and directionality of these hydrogen bonds are the origin of many of the unique physicochemical properties of water. The development of analytical techniques to determine the moisture content of foods depends on being able to distinguish water (the "analytes") from the other components in the food (the "matrix"). The characteristics of water that are most commonly used to achieve this are: its relatively low boiling point; its high polarity; its ability to undergo unique chemical reactions with certain reagents; its unique electromagnetic absorption spectra; and, its characteristic physical properties (density, compressibility, electrical conductivity and refractive index).Despite having the same chemical formula (H_2O) the water molecules in a food may be present in a variety of different molecular environments depending on their interaction with the surrounding molecules. The water molecules in these different environments normally have different physiochemical properties:

- *Bulk water.* Bulk water is free from any other constituents, so that each water molecule is surrounded only by other water molecules. It therefore has physicochemical properties that are the same as those of pure water, *e.g.*, melting point, boiling point, density, compressibility, heat of vaporization, electromagnetic absorption spectra.
- *Capillary or trapped water*. Capillary water is held in narrow channels between certain food components because of capillary forces. Trapped water is held within spaces within a food that are surrounded by a physical barrier that prevents the water molecules from easily escaping, *e.g.*, an emulsion droplet or a biological cell. The majority of this type of water is involved in normal water-water bonding and so it has physicochemical properties similar to that of bulk water.
- *Physically bound water*. A significant fraction of the water molecules in many foods are not completely surrounded by other water molecules, but are in molecular contact with other food constituents, *e.g.* proteins, carbohydrates or minerals. The bonds between water molecules and these constituents are often significantly different from normal water-water bonds and so this type of water has different physicochemical properties than bulk water *e.g.*, melting point, boiling point, density, compressibility, heat of vaporization, electromagnetic absorption spectra.
- *Chemically bound water***.** Some of the water molecules present in a food may be chemically bonded to other molecules as water of crystallization or as hydrates, *e.g.* $NaSO_4.10H_20$. These bonds are much stronger than the normal water-water bond and therefore chemically bound water has very different physicochemical properties to bulk water, *e.g.*, lower melting point, higher boiling point, higher density, lower compressibility, higher heat of vaporization, different electromagnetic absorption spectra.Foods are heterogeneous materials that contain different proportions of chemically bound, physically bound, capillary, trapped or bulk water. In addition, foods may contain water that is present in different physical states: gas, liquid or solid. The fact that water molecules can exist in a number of different molecular environments, with different physicochemical properties, can be problematic for the food analyst trying to accurately determine the moisture content of foods. Many analytical procedures developed to measure moisture content are more sensitive to water in certain types of molecular environment than to water in other types of molecular environment. This means that the measured value of the moisture content of a particular food may depend on the experimental technique used to carry out the measurement. Sometimes food analysts are interested in determining the amounts of water in specific molecular environments (*e.g.*, physically bound water), rather than the total water content. For example, the rate of microbial growth in a food depends on the amount of bulk water present in a food, and not necessarily on the total amount of water present. There are analytical techniques available that can provide some information about the relative fractions of water in different molecular environments (*e.g.*, DSC, NMR, vapor pressure).

Sample preparation

Selection of a representative sample, and prevention of changes in the properties of the sample prior to analysis, is two major potential sources of error in any food analysis procedure. When determining the moisture content of a food it is important to prevent any loss or gain of water. For this reason, exposure of a sample to the atmosphere, and excessive temperature fluctuations, should be minimized. When samples are stored in containers it is common practice to fill the container to the top to prevent a large headspace, because this reduces changes in the sample due to equilibration with its environment. The most important techniques developed to measure the moisture content of foods are discussed below.

Evaporation methods

Principles

These methods rely on measuring the mass of water in a known mass of sample. The moisture content is determined by measuring the mass of a food before and after the water is removed by evaporation:

Here, M_{INITIAL} and M_{DRIED} are the mass of the sample before and after drying, respectively. The basic principle of this technique is that water has a lower boiling point than the other major components within foods, *e.g.*, lipids, proteins, carbohydrates and minerals. Sometimes a related parameter, known as the *total solids*, is reported as a measure of the moisture content. The total solids content is a measure of the amount of material remaining after all the water has been evaporated:

$$\%\text{Total Solids} = \frac{M_{\text{DRIED}}}{M_{\text{INITIAL}}} \times 100$$

Thus, %Total solids = (100 - %Moisture). To obtain an accurate measurement of the moisture content or total solids of a food using evaporation methods it is necessary to remove all of the water molecules that were originally present in the food, without changing the mass of the food matrix. This is often extremely difficult to achieve in practice because the high temperatures or long times required to remove all of the water molecules would lead to changes in the mass of the food matrix, *e.g.*, due to volatilization or chemical changes of some components. For this reason, the drying conditions used in evaporation methods are usually standardized in terms of temperature and time so as to obtain results that are as accurate and reproducible as possible given the practical constraints. Using a standard method of sample preparation and analysis helps to minimize sample-to-sample variations within and between laboratories.

Evaporation Devices

The thermal energy used to evaporate the water from a food sample can be provided directly (*e.g.*, transfer of heat from an oven to a food) or indirectly (*e.g.*, conversion of electromagnetic radiation incident upon a food into heat due to absorption of energy by the water molecules).

Convection and forced draft ovens. Weighed samples are placed in an oven for a specified time and temperature (*e.g.* 3 hours at 100 ^{o}C) and their dried mass is determined, or they are dried until they reach constant mass. The thermal energy used to evaporate the water is applied directly to the sample *via* the shelf and air that surround it. There are often considerable temperature variations within convection ovens, and so precise measurements are carried out using *forced draft ovens* that circulate the air so as to achieve a more uniform temperature distribution within the oven. Samples that contain significant quantities of carbohydrates that might undergo chemical changes or volatile materials other than water should not be dried in convection or forced draft oven. Many official methods of analysis are based on forced draft ovens.

Vacuum oven. Weighed samples are placed under reduced pressure (typically 25-100 mm Hg) in a vacuum oven for a specified time and temperature and their dried mass is determined. The thermal energy used to evaporate the water is applied directly to the sample *via* the metallic shelf that it sits upon. There is an air inlet and outlet to carry the moisture lost from the sample out of the vacuum oven, which prevents the accumulation of moisture within the oven. The boiling point of water is reduced when it is placed under vacuum. Drying foods in a vacuum oven therefore has a number of advantages over conventional oven drying techniques. If the sample is heated at the same temperature, drying can be carried out much quicker. Alternatively, lower temperatures can be used to remove the moisture (*e.g.*70^{o}C instead of 100 ^{o}C), and so problems associated with degradation of heat labile substances can be reduced. A number of vacuum oven methods are officially recognized.

Microwave oven. Weighed samples are placed in a microwave oven for a specified time and power-level and their dried mass is weighed. Alternatively, weighed samples may be dried until they reach constant final mass - analytical microwave ovens containing balances to continuously monitor the weight of a food during drying are commercially available. The water molecules in the food evaporate because they absorb microwave energy, which causes them to become thermally excited. The major advantage of microwave methods over other drying methods is that they are simple to use and rapid to carry out.

Nevertheless, care must be taken to standardize the drying procedure and ensure that the microwave energy is applied evenly across the sample. A number of microwave oven drying methods are officially recognized.

Infrared lamp drying The sample to be analyzed is placed under an infrared lamp and its mass is recorded as a function of time. The water molecules in the food evaporate because they absorb infrared energy, which causes them to become thermally excited. One of the major advantages of infrared drying methods is that moisture contents can be determined rapidly using inexpensive equipment, *e.g.*, 10-25 minutes. This is because the IR energy penetrates into the sample, rather than having to be conducted and convected inwards from the surface of the sample. To obtain reproducible measurements it is important to control the distance between the sample and the IR lamp and the dimensions of the sample. IR drying methods are not officially recognized for moisture content determinations because it is difficult to standardize the procedure. Even so, it is widely used in industry because of its speed and ease of use.

1. *Sample dimensions.* The rate and extent of moisture removal depends on the size and shape of the sample, and how finely it is ground. The greater the surface area of material exposed to the environment, the faster the rate of moisture removal.
2. *Clumping and surface crust formation*. Some samples tend to clump together or form a semi-permeable surface crust during the drying procedure. This can lead to erroneous and irreproducible results because the loss of moisture is restricted by the clumps or crust. For this reason samples are often mixed with dried sand to prevent clumping and surface crust formation.
3. *Elevation of boiling point.* Under normal laboratory conditions pure water boils at 100 ^{o}C. Nevertheless, if solutes are present in a sample the boiling point of water is elevated. This is because the partial vapor pressure of water is decreased and therefore a higher temperature has to be reached before the vapor pressure of the system equals the atmospheric pressure. Consequently, the rate of moisture loss from the sample is slower than expected. The boiling point of water containing solutes (T_b) is given by the expression, $T_b = T_0 + 0.51m$, where T_0 is the boiling point of pure water and m is the molality of solute in solution (mol/kg of solvent).
4. *Water type.* The ease at which water is removed from a food by evaporation depends on its interaction with the other components present. Free water is most easily removed from foods by evaporation, whereas more severe conditions are needed to remove chemically or physically bound water. Nevertheless, these more extreme conditions can cause problems due to degradation of other ingredients which interfere with the analysis (see below).
5. *Decomposition of other food components.* If the temperature of drying is too high, or the drying is carried out for too long, there may be decomposition of some of the heat-sensitive components in the food. This will cause a change in the mass of the food matrix and lead to errors in the moisture content determination. It is therefore normally necessary to use a compromise time and temperature, which are sufficient to remove most of the moisture, but not too long to cause significant thermal decomposition of the food matrix. One example of decomposition that interferes with moisture content determinations is that of carbohydrates.

$$C_6H_{12}O_6 \xrightarrow{\text{heat}} 6C + 6\,H_2O$$

The water that is released by this reaction is not the water we are trying to measure and would lead to an overestimation of the true moisture content. On the other hand, a number of chemical reactions that occur at elevated temperatures lead to water absorption, *e.g.*, sucrose hydrolysis

$$(\text{sucrose} + H_2O \xrightarrow{\text{heat}} \text{fructose} + \text{glucose}),$$

and therefore lead to an underestimation of the true moisture content. Foods that are particularly susceptible to thermal decomposition should be analyzed using alternative methods, *e.g.* chemical or physical.

6. *Volatilization of other food components.* It is often assumed that the weight loss of a food upon heating is entirely due to evaporation of the water. In practice, foods often contain other volatile constituents that can also be lost during heating, *e.g.*, flavors or odors. For most foods, these volatiles only make up a very small proportion and can therefore be ignored. For foods that do contain significant amounts of volatile components (*e.g.* spices and herbs) it is necessary to use alternative methods to determine their moisture content, *e.g.*, distillation, chemical or physical methods.
7. *High moisture samples.* Food samples that have high moisture contents are usually dried in two stages to prevent "spattering" of the sample, and accumulation of moisture in the oven. Spattering is the process whereby some of the water jumps out of the food sample during drying, carrying other food constituents with it. For example, most of the

moisture in milk is removed by heating on a steam bath prior to completing the drying in an oven.

8. *Temperature and power level variations.* Most evaporation methods stipulate a definite temperature or power level to dry the sample so as to standardize the procedure and obtain reproducible results. In practice, there are often significant variations in temperatures or power levels within an evaporation instrument, and so the efficiency of the drying procedure depends on the precise location of the sample within the instrument. It is therefore important to carefully design and operate analytical instruments so as to minimize these temperature or power level variations.
9. *Sample pans.* It is important to use appropriate pans to contain samples, and to handle them correctly, when carrying out a moisture content analysis. Typically aluminum pans are used because they are relatively cheap and have a high thermal conductivity. These pans usually have lids to prevent spattering of the sample, which would lead to weight loss and therefore erroneous results. Pans should be handled with tongs because fingerprints can contribute to the mass of a sample. Pans should be dried in an oven and stored in a desiccators prior to use to ensure that no residual moisture is attached to them.

Ash

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals (the analytes) can be distinguished from all the other components (the matrix) within a food in some measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components. The three main types of analytical procedure used to determine the ash content of foods are based on this principle: *dry* ashing, *wet* ashing and *low temperature plasma dry* ashing. The method chosen for a particular analysis depends on the reason for carrying out the analysis, the type of food analyzed and the equipment available. Ashing may also be used as the first step in preparing samples for analysis of specific minerals, by atomic spectroscopy or the various traditional methods described below. Ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%, *e.g.*, dried beef.

Sample Preparation

As with all food analysis procedures it is crucial to carefully select a sample whose composition represents that of the food being analyzed and to ensure that its composition does not change significantly prior to analysis. Typically, samples of 1-10g are used in the analysis of ash content. Solid foods are finely ground and then carefully mixed to facilitate the choice of a representative sample. Before carrying out an ash analysis, samples that are high in moisture are often dried to prevent spattering during ashing. High fat samples are usually defatted by solvent extraction, as this facilitates the release of the moisture and prevents spattering. Other possible problems include contamination of samples by minerals in grinders, glassware or crucibles which come into contact with the sample during the analysis. For the same reason, it is recommended to use deionized water when preparing samples.

Dry Ashing

Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600 ^{o}C. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost, *e.g.*, iron, lead and mercury. If an analysis is being carried out to determine the concentration of one of these substances then it is advisable to use an alternative ashing method that uses lower temperatures.

The food sample is weighed before and after ashing to determine the concentration of ash present. The ash content can be expressed on either a *dry* or *wet* basis:

$$\%\ \text{Ash (dry basis)} = \frac{M_{ASH}}{M_{DRY}} \times 100$$

Carbohydrate

A large number of analytical techniques have been developed to measure the total concentration and type of carbohydrates present in foods (see *Food Analysis* byNielssen or *Food Analysis* by Pomeranz and Meloan for more details). The carbohydrate content of a food can be determined by calculating the percent remaining after all the other components have been measured: %carbohydrates = 100 - %moisture - %protein - %lipid - %mineral. Nevertheless, this method can lead to erroneous results due to experimental errors in any of the other methods, and so it is usually better to directly measure the carbohydrate content for accurate measurements.

Minerals:

The amount of minerals i.e. calcium, zinc and iron were also assessed by AOAC methods.

Functional properties:

The following functional properties were studied for all the prepared samples**.**

Ph

The ph was measured by making a 10 %(w/v) flour suspension of each sample in distilled water. Each sample was then mixed thoroughly in a beaker, and the ph was recorded with an electronic ph meter.

Water and oil absorption capacities

The water and oil absorption were determined by the method of sosulki.2g weaning food sample was mixed with 20 ml distilled water or refined soybean oil and was allowed to stand at ambient temperature(32degree Celsius)for 30 min,then centrifuged (hettich universal II GERMANY)FOR30 MIN AT 2000Xg . water and oil absorption capacity was expressed as percent water or oil bound per gram flour.

Water or oil capacity of the flour=w1/w2

W1=weight of water or oil absorbed

W2= weight of sample

Bulk density

Bulk density is a physical property of a granular solid, such as soil, sand, or powder. It is calculated by dividing the weight of a given volume of the material by the volume it occupies. This includes not only the volume of the particles themselves, but also the volume of space and weight of any material between the particles.

Water solubility index (WSI)

WSI was measured according to the method of Anderson et al.,(1996),2.5g of halwa mix sample was dispersed in 25 ml of distilled water taking are to break up any lumps using a glass rod. After 30 min of stirring ,the dispersion was rinsed into tarred centrifuge tube made up to 32.5 ml and then centrifuged at 3000x g for 10 min.the supernatant was then decanted and the weight of its solid content determined after it had been evaporated to a constant weight. The wsi was then calculated as per the following equation

WSI=wt. of dissolved solids in supernatant/wt of dry flour sample

Sensory analysis

Sensory evaluation consists of using a combination of different senses of perception in choosing and eating a food**.**

VI. SENSORY ANALYSIS FOR OPTIMIZATION OF SWEETENER

The optimization of the sweetener was conducted on 9-point on hedonic scale relating to pleasurable or un-pleasurable experiences.

Two samples are served to the panelist at one session. They were asked to rate the acceptability of the product on a scale ranging

From “like extremely” to “dislike extremely”.

Calculation of yield

The yield of the formulated product was calculated as per

Yield=wt of the powdered sample/wt of feed given

Cost estimation

Cost estimation includes the techniques and processes of ascertaining costs. Costing basically consists of manufacturing the product , cost of providing electricity, labor charges, overheads etc.

VII. RESULTS AND DISCUSSION

Preparation of the trial feed

The trial feed was prepared by seivingthe floor and adding the pine jaggery of 1:3 ratio for giving good taste and other ingredients such as cardamom and almonds . and the dry sample is homogenized in the homogenizer to make it as a fine powder. shows the Proximate Composition of Raw Millet Flour (RWMF), Germinated Millet Flour (GMF), Roasted Millet Flour (RMF) and Fermented Millet Flour (FMF) on dry weight basis. The protein content ranged from 14.0-18.7%. Significant increase ($p<0.05$) was observed in the crude protein content of the processed flours compared with the control. The highest increase was observed in the germinated millet flour (18.7%). The crude protein of the fermented millet flour (17.5%) is similar to value reported for fermentedTreculia africana seed flour (Fasasi et al., 2004). The increase in protein of the fermented and germinated flour may be due to protein synthesis. Fermentation and germination may be a desirable processing technique to increase the protein content of millet seed. The lowest protein content (15.7%) observed in roasted millet may be attributed to the destruction of amino acids as a result of heat (Mauron, 1982).The carbohydrate content of control millet was 76.3%, which is greater than carbohydrate content of germinated millet flour (71.1%) and roasted millet flour (72.6%). Decrease in carbohydrate content of germinated seed flour may be due to the utilization of some of the sugars during the growth metabolic activity. Fermented Millet Flour (FMF) has the

highest carbohydrate content of 76.5%.The fat content of processed millet flour ranged from 2.4-7.2%. The fat content obtained for Germinated Millet Flour (GMF) is 5.6%, which is less than the value reported for African breadfruit seed (11.39%) according to Fasasi et al. (2004). Significant increase ($p<0.05$) was observed in the fat content of Fermented and Germinated Millet Flour (FMF and GMF) respectively. The low fat content recorded in fermented sample will help in increasing the shelf life of the samples by decreasing the chances of rancidity and will also contribute to the low energy value of the sample. Roasted Millet Flour (RMF) with a fat content of 7.2% will get rancid quickly, but will have a high energy value, since heat processing promotes lipid oxidation. The ash content of processed millet flours ranged from 1.9% in FMF to 2.7% in RMF. The ash content obtained for roasted millet flour (RMF) is 2.7% which is higher than that of Germinated Millet Flour (GMF) which is 2.1%. Fermentation was observed to reduce the total ash; this reduction may be due to the leaching of the soluble inorganic salts during fermentation. The ash content indicates a rough estimation of the mineral content of the product. The crude fiber content of the samples ranged from 1.8-2.0%. The Raw Millet Flour (RWMF) has the highest fiber content while germinated and roasted millet flour has fiber content of 1.8%, respectively. The high crude fiber content in Germinated Millet Flour (GMF) and the reduction in the crude fiber content of Fermented Flour (FMF) may be due to sugar utilization in the seed for metabolic sprouting activity leaving fibrous seeds and enzymatic degradation of the fiber during fermentation (Ikenebomah et al., 1986). Similar observations have been reported for fluted pumpkin (Giami and Bekebain, 1992) and cowpea (Padmashree et al., 1987).Energy values obtained for the millet flour samples ranged from 397-418 kcal. Fermented Millet Flour (FMF) has the lowest energy value 397 kcal which can be attributed to the low fat content. Roasted Millet Flour (RMF) gave the highest energy value of 418 kcal when compared to the Raw Millet Flour (RWMF) and other processed millet flour. This indicates that roasting is suitable in increasing the energy value of food to meet the energy requirement by man. Reduction in the energy value of Germinated Millet Flour (GMF) may be due to the decrease in fat and carbohydrate value.

Optimization of sweetener

The feed was prepared using 20g of cane jaggery and 30 g of pinejaggery respectively sensory analysis was conducted to find about the acceptable amount of sweetener in the samples. The mean scores given by 10 untrained panelists to the sample prepared using different concentration of sugar and jaggery . the result from the sensory analysis 30g of pine jaggery was found to be more preferable among the other.

Proximate analysis:

The proximate analysis was carried out for 200g of sample which contain ragi flour or ragi milk powder , pine jaggery , cardamom and almonds were added to it.

Nutritional analysis:

test	Results obtained
moisture	7.67%
Total ash	2.70%
Crude fibre	1.46%
fat	3.95%
protein	10.54%
Carbohydrates(by difference)	73.38%
Energy(food calories per 100g sample)	392.8%

Functional properties:

samples	ph	Water absorption capacity	Oil absorption capacity(mg/l)	Bulk density(g/cm3)	Water solubility index
Mixed ingredients	6.02±.015	1.06±.057	1.64±.005	0.308±0.0	0.284±0.001

VIII. SUMMARY AND CONCLUSION

The study revealed that the pudding prepared from the various suggested ingredients were potentially having many nutrients to give health benefits to our body.when it comes to the question whether this formulation can be substitute for the other pudding which is made traditionally.it can be rehabilitation diet for malnourished children that be more cost effective. The further studies would be undertaken to analyze the antinutritional factors, as well as the bioavailability of micronutrients in an effort to enhance this potential food.

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