“FORTIFICATION AND NUTRIENT ASSESSMENT OF CHOCOLATE MILKSHAKE WITH PEANUT POMACE PROTEIN”

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By

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BONAFIDE CERTIFICATE

This is to certify that SIVAPRAKASH S(19349006) student of B.Sc., Food Technology, Department of Food Technology, Hindustan Institute of Technology and Science, has done this dissertation work entitled"FORTIFICATION AND NUTRIENT ASSESSMENT OF CHOCOLATE MILKSHAKE WITH PEANUT POMACE PROTEIN" for the partial fulfilment of the degree of B.Sc., in Food Technology, during the academic period 2021-2022 and I certify that the work is original.

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1.

2.

DECLARATION

I hereby declare that the thesis entitled **"FORTIFICATION AND NUTRIENT ASSESSMENT OF CHOCOLATE MILKSHAKE WITH PEANUT POMACE PROTEIN"**is a partial submission to the Hindustan Institute of Technology and Science for the award of degree of Bachelor of Science in Food Technology is my own work carried out under the Guidance and Supervision of MR.GURUPRASATH NARASIMHAN Assistant Professor, Department of Food Technology, Hindustan Institute of Technology and Science, Chennai and that, to the best of my knowledge and belief, it contains no material previously published or written by another person or material which has been accepted for the award of any other Degree or Diploma of the University or other Institute of higher learning or any University.

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SIVAPRAKASH

CHAPTER NO	TITLE	PAGE NO
	Abstract	7
1	**INTRODUCTION**	8
2	**REVIEW OF LITERATURE**	11
2.1	**Nutritional composition of groundnut**	11
2.2	**Peanut as functional food**	14
2.3	**Issues related peanut consumption**	14
2.4	**Role of protein in the body**	15
2.5	**Protein and athletic performance**	16
2.6	**High protein diet**	17
2.7	**Groundnut cake and its products**	17
2.8	**Preparation of groundnut protein isolate using acid precipitation**	18
2.9	**Extraction of protein from various products**	18
3	**AIM AND OBJECTIVES**	23
4	**MATERIALS AND METHODOLOGY**	24
4.1	**PROTEIN EXTRACTION METHOD**	24
4.2	**NITROGEN DETERMINATION BY KJELDAHL**	26
4.3	**DETERMINATION OF MOISTURE**	28
4.4	**DETERMINATION OF CRUDE FIBER-MUSLIN CLOTH METHOD**	29

4.5	**ESTIMATION OF ETHER EXTRACT USING SOXHLET**	32
5	**RESULTS AND DISCUSSION**	34
6	**CONCLUSION**	35
7	**FUTURE THURST**	36
8	**REFERENCES**	37

Abstract

Protein is one of the important macronutrient essential for the body. The protein intake of many people is below recommended dietary allowance. Protein present in groundnut pomace is very high which is used as cattle feed or manure in many countries for centuries. This project is about utilising the protein present in groundnut pomace and fortifying the protein in chocolate milk shake. This project involves Extraction of protein from pomace , Fortification of extracted protein from to chocolate milk shake, Nutrient content assessment of Fortified Chocolate milkshake and comparison of fortified and non-fortified chocolate milk shake. The extraction method involves stirring and centrifugation. The result shows that the percentage of protein present in fortified sample is very high compared to non fortified sample. The protein content of chocolate milk shake is raised by 7% after fortification.

KEYWORDS: Protein, groundnut pomace, fortification, extraction, nuts, Defatted groundnut cake.

CHAPTER 1

INTRODUCTION

Consumption of nuts found to be increasing due to its high nutritional contents. Adding nuts to mediterranean diet is recommended to all the people in the world as nut has very good nutritional value.Almond, cashew nut, hazel nuts, brazil nuts, macadamias, walnuts and pistachios are considered as tree nuts and peanut is considered as legume. However all nuts are considered to be nutritional dense food.(De Souza,.et al, 2017).

Peanut or groundnut are considered as edible legume all over the world and used either raw or processed food such as peanut butter, roasted peanut etc . India produces approximately 7 million metric tonnes of peanut per year and thereby considered as the 2nd largest producer of peanut in the world(Arya .,et al 2015).

In India, the most important growing countries for peanuts are Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, Madhya Pradesh, Uttar Pradesh and Rajasthan. Crops are cultivated in all three seasons, rain, after rain and during the summer. It is mainly cultivated in the rain. Only about 10-15% of the cultivated land is irrigated(Prasad.,et al 2010).

Peanuts are consumed all over the world due to its availability and affordability compared to other nuts.peanuts are very rich in protein, fibers, vitamins and minerals(Bonku and Yu 2019).

Protein content in peanut ranges from 20.7 - 25.3 % , crude fats 31 – 46 % ,Ash 1.2 - 2.3%, crude fibers 1.4 - 3.9% , Carbohydrates 21 – 37% and remaining 4.9 – 6.8 % will be the moisture content(Bonku and Yu 2019).

Soy beans contains high amount of protein compared to peanut on the other hand peanuts contain higher amount of oil than soy bean . protein content in peanut is higher and fat content is slightly higher than cashew and pistachio but significantly lower than other nuts (Purohit and Rajyalakshmi, 2011).

Roasted peanuts , peanut butter, peanut oil, peanut paste, peanut sauce, peanut flour, peanut milk, peanut beverages, peanut snacks(salted and sweet bars) are some of the most popular peanut products (Arya.,et al 2015).

The product obtained after the extraction of oil from peanut is called defatted groundnut cake or groundnut pomace. It contains 45 – 60 % of protein , 22 – 30% carbohydrates , 3.8 – 7.5% crude fiber and 4 – 6 % minerals. The pomace with is very rich in protein is offen used as cattle feed or manure(Purohit and Rajyalakshmi, 2011).

By using peanut flour or defatted peanut meal bakery products can also be prepared such as Bread, biscuits ,crackers, and other products. This can be a great way to increase the use of peanut protein in the diet of malnourished people in developing countries (Nautiyal,2002).

Peanut cakes or flour can be used for human consumption after partial hydrolysis of the constituent proteins by fermentation with certain molds. Such products are racially digestible and nutritious. Spray-dried peanut protein isolates can be used to replace the non-fat milk solids in ice cream. A chocolate-flavored peanut beverage containing 3.5% peanut protein, 3.5% fat, 8% sugar, 0.7% cocoa powder, 0.1% stabilizer, can also be prepared using peanut protein isolates(Nautiyal,2002).

Alkali extraction followed by isoelectric precipitation is the method used to produce peanut protein concentrate and peanut protein isolate as they are well known for its functional properties.Its is said that very less or no significant study is conducted on groundnut protein concentrate preparation using membrane technology(Jain A., et al 2015).

Peanut contains all the 20 amino acids and it is the very good source for “arginine” an essential amino acid .According to PDCAAS (protein digestibility corrected amino acid score)Soy bean and peanut is the only vegetable protein that are nutritionally equivalent to meat and egg (Arya.,et al).

Arginine is a semiessential or conditionally essential amino acid in humans, Arginine is one of the most metabolically versatile amino acids and it act as a precursor for the synthesis of poly-amines, glutamate, nitric oxide, creatine ,urea, agmatine and proline(Morris Jr, 2004).

Peanut protein isolates can be an excellent source of protein fortification for a variety of foods for protein-deficient consumers in developing countries, as well as a functional component of the peanut industry. The production of peanut protein isolates can also add value to defatted peanut flour, a low-value by-product of peanut oil production(Shafiqur.,et al 2018)

Compared to famous foods like green tea and red wine, peanuts have higher antioxidant capacity(Arya., et al 2015).

Peanut grains are usually considered an excellent source of antioxidants and phytosterols. Many phenols, such as hydroxybenzoic acid, ferulic acid, coumaric acid, resveratrol, flavonoids, and flavonols, have been identified in peanut grains, along with significant amounts of total tocopherols(Nile and Park,2013).

CHAPTER 2 : REVIEW OF LITERATURE

2.1Groundnuts (*Arachis hypogaea*), All types, Nutritional value per 100 g.ce: USDA National Nutrient data base

principle	Nutrient value	Percentage of RDA
Energy	567 Kcal	29
Carbohydrates	16.13 g	12
protein	25.80 g	46
Total Fat	49.24 g	165
Cholesterol	0 mg	0
Dietary Fiber	8.5 g	22

2.1.1 Proteins in peanut

Peanuts are actually legumes, containing more protein than any other nut, at levels equal to or better than serving beans. After the peanut oil is extracted, the protein content of the cake can reach 50% (Zhao et al, 2011). Peanuts contain all 20 amino acids in different proportions and are the largest source of the protein "arginine" (USDA, 2011). According to the Protein Digestibility Corrected Amino Acid Score (PDCAAS), other legume plant proteins such as peanut protein and soy protein are nutritionally equivalent to meat and eggs in terms of human growth and health (FAO1991). The amino acid profile of peanut meal indicates that it may be a component of protein fortification (Yuetal ,2007). Peanut protein, unlike animal protein, is plant-based and therefore contains additional ingredients that have health benefits, such as dietary fiber and unique bioactive ingredients. It has been found that peanut protein has excellent emulsifying activity, emulsification stability, foaming ability, excellent water retention and high solubility, and can provide new high protein food ingredient product formulations and protein formulations in the food industry (Wu et al. 2009). Based on these observations, peanut protein has recently been added to noodles (Xiaodong and Ying, 2010)

and infant formula (Nimsate et al., 2010). There is a new interest in research related to kernel and shell flavors.

2.1.2 Fiber

Peanuts also are a very good supply of fiber, in line with the Food and Drug Administration. Sucrose and starch represent the essential whilst decreasing sugars shape the minor percentage of the peanut carbohydrates (Tharanathan et al., 1975). This can also additionally make a contribution to the truth that peanut have a low glycemic index (GI) and glycemic load (GL) (Foster-Powell, 2002).On a 100 –factor scale, the GI of peanuts is 14, and the GL of peanuts is one. Additional studies has proven that after peanuts or peanut butter are brought to a excessive glycemic load meal ,along with with a bagel and a pitcher of juice ,they clearly hold the blood sugar stabilized in order that it does now no longer upward thrust too excessive too quickly(Johnston , 2005). Peanuts incorporate carbohydrates, and all ingredients that incorporate carbohydrates raise blood-glucose levels. Some carbohydrates, along with easy sugars, have a swift, dramatic impact in your blood sugar. Carbohydrates that incorporate fiber or starch, those styles of carbohydrates have a slower, much less reported impact on blood sugar. The American Diabetes Association ranks peanuts and different nuts as diabetes superfoods. To make the listing, ingredients ought to deliver crucial vitamins along with fiber, calcium, potassium, magnesium and nutrients A, E and E. Foods at the listing ought to additionally rank low at the glycemic index. Peanuts make the listing due to the fact they incorporate magnesium, fiber and heart-healthful oils and do now no longer overly have an effect on your blood glucose.

2.1.3 Vitamins

Principle	**Nutrient Value(mg)**	**Percentage of RDA**
Folates	240 µg	60
Niacin	12.066	75
Pantothenic acid	1.767	35
pyridoxine	0.348	27
Riboflavin	0.135	10

thiamin	0.640	53
Vitamin A	0 IU	0
Vitamin C	0	0
Vitamin E	8.33	55.5

Table provides the detail regarding the amounts of vitamin present in 100 g of peanuts and their levels as per the RDA.

According to the table , 100 g peanuts consumption is capable of providing up to 75% RDA of Niacin, 60% RDA of folate, 53% RDA of thiamin, 10% RDA of Riboflavin, 35% RDA of pantothenic acid, 27% RDA of pyridoxine, 55.5% RDA of vitamin E.

It has been recognized as a great source of niacin, which is important for functioning of the digestive systems, skin, nerves, helps in conversion of food to energy and supposed to protect against Alzheimer's disease and cognitive decline (Morris, 2004). Peanut is an excellent source of vitamin E is considered a hard-to-get nutrients as it was shown that over 90% of men and women were not meeting the recommendations for intake (Gao, 2006). New research shows that there is more vitamin E in peanuts that was realized (Shin, 2009). And its consumption in low quantities can lead to benefits against coronary heart disease (Bramley et al., 2000). Peanut also contains good amounts of folate which is especially important in infancy and pregnancy, in production and maintenance of cells. Research shows that people who take in higher dietary folate may have an advantage when it comes to prevention of heart disease (Rimm, 1998).

2.1.4 Minerals

Minerals	**Nutrient value(mg)**	**Percentage of RDA**
Calcium	92	9
Copper	1.144	127
Iron	4.58	57
Magnesium	168	42

Manganese	1.934	84
Phosphorus	76	54
Selenium	7.2 µg	13
Zinc	3.27	30

Table illustrates that small amounts of peanut consumption can meet the most part of RDA of many minerals which are crucial for health and proper functioning of the body. It is clear from the dates that 100 g of peanut can provide RDA levels of 127% copper, 84% manganese, 57% iron, 54% phosphorus, 42% magnesium intake is associated with reduced inflammation (Song, 2005) and a decreased risk of metabolic syndrome (Song, 2005) and type II diabetes (Larson, 2007).

2.2 Peanut as a functional food

Research has identified numerous compounds in Groundnut and in their skins that may have added health benefits beyond basic nutrition. Groundnut have been touted as a functional food with numerous functional components such as Coenzyme Q10 which protects the heart during the period of lack of oxygen example high altitudes and clogged arteries. peanuts are also a good source of dietary fiber and provide a good range of essential nutrients, including several B group vitamins, Tocopherol , minerals such as zinc, iron, and potassium, selenium, magnesium manganese and copper,plus other antioxidant compounds such as flavonoids and resveratrol (Gulcin, 2010). These bioactive components have been recognized for having ability to prevent disease and some are antioxidants while other is to promote longevity. The antioxidant capacity is due to the total biological matters seed such as tocopherol in oil or caffeic acid, chlorogenic acid, ferulic acid, coumaric acid, stilbene and flavonoidspresent in it (Yu et al, 2005). Fermented peanut powder (Zhang et al. 2011) is used to study free radical scavenging abilities.

2.3 ISSUES RELATED TO PEANUT CONSUMPTION

Peanut Allergies

Peanut proteins are generally classified as albumin (water-soluble) or globulin (salt-soluble). Most storage proteins are globulin, which makes up 87% of total protein (Johns and Jones, 1916). Globulins are composed of two major proteins, arakin and konaratin. Barnettetal. (1975) We tested the allergenicity of various peanut kernels. The cotyledon (kernel) is probably the

main source of allergens for most people, as the skin and heart are often removed during processing. This is because the heart contains saponins that give a bitter taste, and the skin contains catechol tannins and related compounds that give the final product an unwanted color (Woodroof, 1983).

The exact cause of allergies is unknown. Since peanut allergies are associated with the action of immunoglobulin E (IgE) and other anaphylatoxins, they act on the degranulation of histamine and other mediators from mast cells. Histamine, among other effects, induces vasodilation and the accumulation of bronchioles in the lungs. This is also known as bronchospasm. Symptoms include vomiting, diarrhea, urticaria, angioedema (swelling of the lips, face, throat, and skin), exacerbation of atopic eczema, asthma, and anaphylactic shock. (Anderson et al. 1996)

Allergies can last a lifetime, but studies show that 23.3% of children grow from peanut allergies. It is important to note that peanuts belong to the legume family and have nothing to do with nuts. People who are allergic to peanuts may not be allergic to nuts and vice versa. Please note that peanut oil (refined) does not contain allergens.

A nutritious product, peanuts can only be used in everyone's diet if allergies have been treated with some new techniques. Recently, oral desensitization, anti-IgE therapy, use of probiotics, herbal medicine, soybean-based immunotherapy, cell mediator, artificial allergen immunotherapy, plasmid DNA immunotherapy, bacterial adjuvant, immunostimulatory sequence, oligodeoxynucleotide, etc. Many technologies have emerged. Based immunotherapy (NowakWegrzyn et al., 2011). All of these are still in their infancy and there is still a long way to go before they are accepted on a regular basis.

In 2007, North Carolina Agricultural Technology State University, one of its scientists, Dr. Mohamed Ahmedna, developed a process for producing allergen-free peanuts. In the first test, the peanut allergen was 100% inactivated throughout the roasted grains, and human sera from individuals with multiple allergies showed no reaction when exposed to processed peanuts. Food companies are interested in licensing processes that claim to not affect the testing and quality of processed peanuts and provide easier processing for use as a food ingredient.

Develop better techniques to increase the overall efficiency of the extraction of certain functional ingredients for the production of dietary supplements that may be beneficial to

people suffering from metabolic disorders and allergies who cannot take peanuts directly Therefore, a larger focus is needed.

2.4 Role of Protein in the body

Proteins are nitrogen-containing materials which might be shaped through amino acids. They function the fundamental structural aspect of muscle and different tissues withinside the frame. In addition, they're used to supply hormones, enzymes and hemoglobin. Proteins also can be used as electricity; however, they're now no longer the number one preference as an electricity source. For proteins to be utilized by the frame they want to be metabolized into their most effective form, amino acids. There had been 20 amino acids recognized which might be wanted for human boom and metabolism. Twelve of those amino acids (11 in children) are termed nonessential, which means that they may be synthesized through our frame and do now no longer want to be fed on withinside the diet. The ultimate amino acids can't be synthesized withinside the frame and are defined as critical which means that they want to be fed on in our diets. The absence of any of those amino acids will compromise the cappotential of tissue to grow, be repaired or be maintained.

2.5 Protein and Athletic Performance

The main role of dietary protein is to be used in various anabolic processes of the body. As a result, many athletes and trainers believe that high intensity exercise causes higher protein requirements. This comes from the idea of improving protein synthesis if more protein or amino acids are available to the exercising muscle. Studies tend to support this hypothesis. Within 4 weeks of protein supplementation during subject resistance training (3.3 vs. 1.3 g · kg 1 · day 1), a group of subjects with high protein intake observed significant protein synthesis and weight gain (Fern., et al,1991). Similarly, (lemon etc 1992) We also reported greater protein synthesis in beginners of resistance training at a protein intake of 2.62 for 0.99 g · kg 1 · day 1. Studies examining individuals who have undergone strength training have generally shown that high protein intake has a positive effect on muscle protein synthesis and muscle growth (Lemon, 1995). Tarnapolsky and his colleagues (1992) have shown that intensity-trained individuals require a protein intake of 1.8 g · kg 1 · day 1 to maintain a positive nitrogen balance. This is consistent with other studies showing that a protein intake of 1.4-2.4 g·kg1·day1 maintains a positive nitrogen balance in strength athletes (Lemon, 1995). As a result, the recommended protein intake for strength athletes is generally 1.4-1.8g·kg1·day1.

Similarly, endurance athletes appear to require higher protein consumption to prevent significant loss of adipose tissue (Lemon, 1995). Although the goal of endurance athletes is not necessarily to maximize muscle size and strength, loss of lean tissue can have a significant negative impact on endurance performance. Therefore, these athletes need to maintain muscle mass to ensure proper performance. Some studies have shown that the protein intake of endurance athletes must be 1.2-1.4 g · kg 1 · day 1 to ensure a positive nitrogen balance (Lemon, 1998). It is clear that athletes will benefit from increased protein intake. Next, the focus will be on what type of protein you consume.

2.6 High Protein Diets

Increased protein intake and supplements are generally focused on the athletic population. However, in recent years, high-protein diets have become the method used by the general public to promote weight loss. The low-carbohydrate, high-protein, high-fat diet promoted by Atkins is probably the most popular diet used for weight loss in the United States today (Johnston et al., 2004). The basis of this diet is that protein is associated with a feeling of fullness and a spontaneous decrease in caloric expenditure (Araya et al., 2000). Recent studies have shown that, based on US dietary guidelines, the Atkins diet can result in significantly more weight loss than a low-fat, high-carbohydrate diet at 3 and 6 months (Foster et al., 2003). However, there are potential health concerns regarding the safety of high-protein foods. In 2001, the American Heart Association issued a statement on diet protein and weight loss, and those on such a diet are at potential risk of metabolic, heart, kidney, bone, and liver disease. It suggests a possibility (St. Jeor et al., 2001).

2.7.1 Groundnut cake (groundnut pomace)

Peanut cake is a by-product obtained after extracting the oil. The cake contains 45-60% protein, 22-30% carbohydrates, 3.8-7.5% crude fiber and 4-6% minerals (Desai et al ,1999). India's peanut production was 9.4 tonnes and Andhra Pradesh's peanut production was 0.74 tonnes (Anon2009). Andhra Pradesh is one of the largest producers of peanuts, accounting for about 15% of total production, with more than 80% of production used for oil extraction. Protein-rich cakes are used locally as feed and fertilizer for cattle.

2.7.2 Products with DGC(defatted groundnut cake)

Derived from the Experimental Oil Expeller (LOE), DGC was selected as an added value for traditional and convenient foods such as laddu, chutney powder, fryums, biscuits, noodles and

extruded snacks at various stages of incorporation. For ease of incorporation, it was further ground in a laboratory blender and grinder (Sumeet, India) to give defatted peanut cake flour (DGCF).

2.8 Preparation of groundnut protein isolate using acid precipitation

Solvent degreased peanut flour was mixed with water at a ratio of 1:10 (w / v) and the pH of the solution was adjusted to 8.5 with 1N NaOH. The mixture was stirred at room temperature for 2 hours and then centrifuged at 6000 rpm for 30 minutes. The supernatant was collected and adjusted to pH 4.5 with 1N HCl. The suspension was centrifuged at 6000 rpm for 20 minutes to collect the protein precipitate. The protein precipitate was collected and lyophilized. The dried protein separation powder was stored in the refrigerator until used in the experiment.

2.9 EXTRACTION OF PROTEIN FROM VARIOUS PRODUCTS

2.9.1 Cashew protein

Alkaline extraction-isoelectric precipitation method (IP)

Wagner et al.'S method. It was used for alkaline extraction, isoelectric point precipitation, and protein production. Solvent degreased cashew nut powder was extracted at room temperature (about 200 ° C) using two different cashew nut powder and water ratios of 1: 5 and 1:10. The distilled water used was also Wagner et al. Adjusted to two different pH values, 7.0 and 9.0, with 0.1N NaOH. [13] For the production of soy protein fractions. The suspension was stirred using a magnetic stirrer for 1 hour, then 1 he was cold-centrifuged at 1000 x G, 40 ° C for 30 minutes using SR, Heraeus Multifuge, Germany. The insoluble cashew protein cake was reslurried with distilled water whose pH was adjusted as described above, and cold-centrifuged again. Mix the supernatant together, divide into two parts, adjust to two different pH values of 3.5 and 4.5 by adding 0.1N HCl, leave at 40 ° C for 2 hours, then leave again at 40 ° for 30 minutes. C and 1000 × G. The supernatant was decanted to give the cashew protein slurry at the bottom of the flask. The slurry was resuspended in water at pH 3.5 and 4.5 and the extraction was repeated as described above to obtain a cashew slurry. The cashew protein slurry was lyophilized for 12 hours on the CHRIST lyophilization system (Alpha 24, Martins Christ, Germany). The cashew protein was then ground in an experimental grinder and passed through a standard # 103 mesh screen for packaging.

The proteins were stored at –100C. All reported values are the average of triplicate Experiments.(Henshaw , 2010)

2.9.2Soy proteins:

The majority of proteins are organised in protein bodies of the cotyledon cells. According to Preece et al. (2017), the protein our bodies withinside the cotyledon cells have been observed to be withinside the length variety 2.4 to 13.5 μm while tested the usage of SEM with out pattern hydration. These values fell at the low facet while in comparison to values recorded the usage of transmission electron microscopy (TEM) of two to twenty μm on hydrated soybeans (Rosenthal et al., 1998). It has been pronounced previously (White, Welsby, & Kolar, 2013) that protein our bodies swell upon hydration with water at impartial pH to double their authentic length,confirming those findings.

There are principal garage proteins that account for usually 60–80% of the entire soybean protein: the globulins glycinin (11S) and β-conglycinin (7S) (Murphy, 2008). At impartial pH and ambient temperature, glycinin (11S) is a hexameric complicated produced from acidic and primary polypeptides connected through disulphide bridges to offer a molecular weight withinside the variety 320–375 kDa (Lakemond., et al ,2000).

Soy Protein Extraction

Mix soybean meal and alkaline solution at a ratio of 1:20 (w / v) and mix the suspension continuously at various temperatures and times based on SSI2 incubators (Sheldon Manufacturing, Cornelius, OR, USA) (150). rpm) About the experimental plan. First, the mixture was centrifuged in a 5804 R centrifuge (Eppendorf, Hamburg, HH, Germany) at 15 ° C for 15 minutes at 12,964 xg (10,000 rpm). The pellet was then discarded and the supernatant was adjusted to pH 4.5 using a solution of 7.5% HCl and centrifuged as described above. The pellet was then dried at 60 ° C. for 24 hours, weighed and the protein content was determined using the Micro Kjeldahl method. Finally, to optimize the extraction, the mixture was centrifuged in a SL 40R centrifuge (ThermoFisher Scientific, Langenselbold, HE, Germany) at 15 ° C for 25 minutes at 7827 × g (4700 rpm) for protein. Was frozen and dried for 48 minutes. H for further analysis.

2.9.3 Sesame protein

Isolation of protein sesame seeds meal:

The protein is from sesame seed meal, using different solvents (distilled water, NaOH 0.5N, NaOH 0.1N, NaCl 1%, NaCl 0.5%) at different pH values (2-13), sample to solvent ratio. Was prepared by changing. (1:10, 1:20, 1:30, and 1:40) Different temperatures (25, 30, 35, 40, 45, and 50 ° C) and different periods (30, 45, 60, 75,) And 90) minutes). protein

The grade recovered from each step was determined using the A.O.A.C (2000) method.

Optimal conditions have been applied for maximum production. The recovered protein was centrifuged based on its isoelectric point and dried at low temperature.

(40 ° C) Then grind and pass through a 100 mesh sieve. The powdered sample was stored in a polyethylene bag at room temperature until use.

2.9.4 Milk protein extraction methods

Figure Figure11 outlines the experimental design. Three extraction methods were tested on skim milk samples in triplicates (coded e1 to e3), thus yielding 18 protein extracts

Method A (urea)

Skim milk samples were divided into 3x0.5 mL aliquots in 2.0 mL tubes. Add an equal volume (0.5 mL) of solubilization buffer [SB: 6 M urea, 10 mM DTT, 10 mM TrisHCl pH 8.0, 75 mM NaCl, 0.05% SDS (w: w: v: w: w) in H2O]. bottom. The mixture was vortexed for 1 minute. The tubes were incubated at 30 ° C for 60 minutes. A 1 M iodoacetamide (IAA) solution was added to a final concentration of 20 mM and the tubes were incubated in the dark at room temperature for 60 minutes. The tube was centrifuged at 13,000 rpm for 5 minutes at room temperature. The protein extract (hereinafter referred to as A) was stored at -80 ° C until use.

Method B (TCA/acetone)

Skim milk samples were divided into 3x0.5 mL aliquots in 2.0 mL tubes. 1.5 mL of 10% TCA, 10 mM DTT in ice-cold acetone (w: w: v) was added to produce a precipitate. Then vortex the tube for 1 minute and

Incubated overnight at -20 ° C for precipitation. The tube was centrifuged at 13,000 rpm at -6 ° C for 10 minutes. The supernatant was discarded. 1.5 mL of 10 mM DTT in ice-cold acetone (w: v) was added. The pellets were first ground using a spatula and then further ground by vortexing the tube for 1 minute. The tubes were incubated at -20 ° C for 60 minutes and then centrifuged at 13,000 rpm at -6 ° C for 10 minutes. The supernatant was discarded. Washing the pellets was repeated once more. The pelleted protein was vacuum dried in a Speedvac concentrator (SPD2010 model, Savant) for 60 minutes without heating and

completely resuspended in 0.5 ml SB by vortexing. 1 M IAA solution was added to a final concentration of 20 mM and the tubes were incubated at room temperature.

60 minutes in the dark. The protein extract (hereinafter referred to as B) was stored at -80 ° C until use.

Method C (methanol/chloroform)

Skim milk samples were divided into 3x0.5 mL aliquots in 50 mL tubes. We carried out a phase separation extraction method applying Taylor and Savage (2006). Briefly, chloroform (1: 2) (v: v) in 7.5 mL methanol was added to the aliquot of skim milk and the mixture was vortexed for 1 minute. Chloroform (5.0 mL) was added and the mixture was vortexed for 1 minute. NaCl solution [2.0 mL, (1:10) (w: v)] was added and the mixture was vortexed for 1 minute. This resulted in a three-phase solution containing the interprotein phase. To maximize phase separation, the tube was centrifuged at 5100 rpm for 30 minutes at room temperature using a swing bucket rotor. Both the upper and lower phases were carefully discarded and the remaining moist intermediate phase was transferred to a new 1.5 ml tube. The mesophase was dried under vacuum for 60 minutes using a SpeedVac concentrator. During the dry interphase Add 0.5 ml of SB and resuspend and slowly reabsorb interphase SB while incubating overnight at 4 ° C. Resuspension of the intermediate phase was completed by vortexing at full speed for 30 minutes at room temperature using a multi-tube vortex mixer (model MTV1, Ratek). A 1 M IAA solution was added to a final concentration of 20 mM and the tube was incubated in the dark at room temperature for 60 minutes. The protein extract (hereinafter referred to as C) was stored at -80 ° C until use. (Dolphin Vincent, 2016).

2.9.5 Walnut protein

Walnuts (Juglans regia) are considered high quality foods that are beneficial to human health because they are rich in proteins, unsaturated fatty acids, phospholipids, vitamins, minerals, essential fatty acids and other nutrients. Walnuts have important medicinal and health functions in the prevention and alleviation of cardiovascular disease, diabetes and obesity. Common walnuts (Juglans regia L.) are economically important species grown worldwide in both high quality wood and nuts. Walnut cultivation has a long history and is widely cultivated in most parts of the Eurasian continent. However, it is difficult to determine the original geographic extent due to the relatively small amount of large-scale molecular phylogenetic geography research on walnuts. China is an important center of walnut genetic diversity and serves as a source of genetic resources for walnut breeding efforts. Beginning in the Western Han dynasty (206 BC-9 AD), walnuts were cultivated by selecting seedlings from

geographically diverse natural populations and spreading them through trade and military conquest. Many classic studies on the origins of cultivated plants in the world have investigated the original cultivated plants of China and suggested that China is one of the largest original centers of agriculture and cultivated plants. In the genus Walnut (Juglans), three species are of Chinese origin (Geng., et al 2021). Walnut Juglansregia L. was born outside of China. Walnut resources are widely distributed in China, Yunnan, Hebei, Shandong, Shandong, Liaoning, Sichuan, Shaanxi and Tibet. Many studies have reported significant differences in the appearance, value, and nutritional composition of walnuts in different growing regions. These differences can be due to changes in the growing environment or genetic changes. To address the root causes of these differences, a better understanding of the origin, genetic diversity, and structure of the wild walnut population in China is needed.

2.9.6 Hazelnuts protein

After winter weather, nuts spread through small mammals, birds, and human translocations. There are two types of nuts grown around the world: nuts and peanuts. Of course, the nuts are single-seeded dried fruits such as almonds, hazelnuts and walnuts. Almonds, hazelnuts, walnuts, Brazilian nuts, pine nuts and pistachios are common and necessary edible nuts. Hazelnuts are a rich source of proteins, carbohydrates, unsaturated fatty acids, vitamins and essential minerals. They have been useful as human food since ancient times and have been cultivated since at least Roman times. Peanuts are an example of peanuts and have a nutritional structure similar to nuts (Dobhal ., et al). The source of hazelnuts, the genus Corylus, contains a variety of deciduous shrubs and tree species that are important components of many temperate forests in the Northern Hemisphere. It is widespread in Europe and Asia(Dobhal.,et al).

Extraction of Protein hazelnuts

Protein is lost in alkaline extraction and acid precipitation processes Hazelnuts, the effect of concentration and Determines the temperature of the functional properties of a protein and its isoelectric point. The optimum manufacturing conditions for proteins are as follows: Solid-liquid ratio 1: 6.40 ° C, 0.5 hours alkaline extraction at pH 8.0, and acid rain at pH 4.5. Its effervescent properties are highest at 4% concentration and highest at 2% concentration for foam stability and emulsification. The stability of the emulsion is largely unaffected by the standing temperature. The oil adsorption capacity is 3.72 ml / g. The protein dispersibility index (PDI) is highest at 50 ° C, the PDI value decreases above 50 ° C and remains stable above 70 ° C. Water retention is best at 1% concentration, 60 ° C and 1.5 hours. Its isoelectric point is measured as 4.52.

CHAPTER 3

AIM

To fortify and assess the nutrient content of chocolate milkshake with the protein obtained from pomace

OBJECTIVES

1.To extract protein from groundnut pomace .

2.To fortify the extracted pomace protein with chocolate milkshake.

3. To assess nutrient quality attributes of the fortified sample.

4. To analysis the sensory attributes of fortified chocolate milk shake.

CHAPTER 4 :MATERIALS AND METHODOLOGY

4.1 PROTEIN EXTRACTION METHOD

4.1.1 MATERIALS:

Groundnut pomace: 1kg ground nut pomace is bought from local commercial oil mill located in Murukkeri ,Villupuram district .

4.1.2 EQUIPMENTS REQUIRED

Centrifuge

pH meter

Mechanical stirrer

4.1.3 EXTRACTION OF PROTEIN FROM POMACE

Defatted groundnut flour+ water (ratio 1:10)

pH adjustment

NaOH (1N) is added until the ph reaches 8.5

The mixture is stirred for 30 minutes in mechanical stirrer

Centrifuged at 6000rpm for 30min

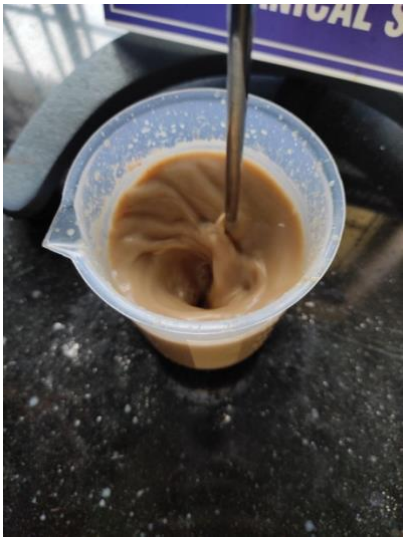
The obtained protein is stored in refrigerator until use

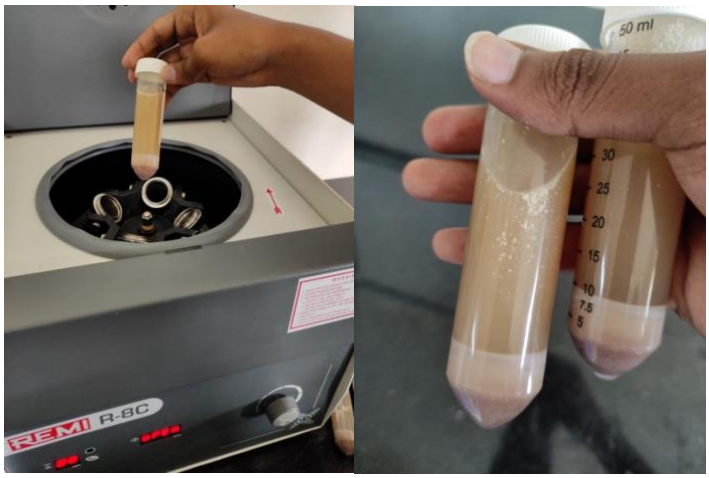
4.1.4 PROCEDURE

1. 20g of pomace(defatted groundnut flour) was taken in a beaker.
2. 180ml of water was added to the pomace.

3. 1N NaOH was added until the pH become 8.5 to all the four tubes.
4. The solution was stirred in mechanical stirrer for 30mins.
5. Then the solution was transferred to 4 separate centrifuge tubes.
6. Then centrifuged at 4500 rpm for 30mins.
7. The supernatant was discarded.
8. The protein precipitate was collected and stored in refrigerator
9. The obtained protein was added to chocolate milk shake at the ratio of 1:10
10. Then stirred using mechanical for 5 minutes.

DEFATTED GROUNDNUT CAKE


STIRRING



CENTRIFUGED

OBTAINED PROTEIN ON WET BASIS

4.2 NITROGEN DETERMINATION BY KJELDAHL

4.2.1 Basic Principle

The Kjeldahl method is the standard method of nitrogen determination dating back to its development in the late 1800's. The method consists of three basic steps:

1) Digestion of the sample in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia;

2) Distillation of the ammonia into a trapping solution; and

3) Quantification of the ammonia by titration with a standard solution.

The sample was digested by sulphuric acid in the presence of a catalyst. The acid solution was made alkaline with sodium hydroxide solution. The ammonia was distilled and collected an excess of boric acid solution, followed by titration with standard sulphuric acid solution.

4.2.2 Equipment

Kjeldahl flasks, 500 to 800 mL

Kjeldahl digestion unit with fume removal manifold

Kjeldahl distillation apparatus - Kjeldahl flask connected to distillation trap by rubber stopper. Distillation trap is connected to condenser with low-sulfur tubing. Erlenmeyer flask, 250 ml

Analytical balance, sensitive to 0.5 mg

4.2.3 Reagents

Sulfuric acid, concentrated, 95-98%, reagent grade Sodium hydroxide

Potassium sulfate (K_2SO_4), anhydrous

Copper sulfate ($CuSO_4$), anhydrous

boric acid, 2% (w/v)
standardized HCl (C= 0.1000 mol/l)

4.2.4 Safety Precautions

Handle acid safely: use acid resistant fumehood. Always add acid to water unless otherwise directed in method. Wear face shield and heavy gloves to protect against splashes. If acids are spilled on skin, immediately wash with large amounts of water. Sulfuric acid and sodium hydroxide can burn skin, eyes and respiratory tract severely.

Wear heavy rubber gloves and face shield to protect against concentrated acid or alkali.

Use effective fume removal device to protect against acid fumes or alkali dusts or vapors. Always add concentrated sulfuric acid or sodium hydroxide pellets to water, not vice versa. Concentrated sodium hydroxide can quickly and easily cause blindness. If splashed on skin or in eyes, flush with copious amounts of water and seek medical attention.

Digests must be cool before dilution water is added to avoid a violent reaction during which the acid can shoot out of the flask. Likewise, the diluted digest must be cool before sodium hydroxide is added to avoid a similarly violent reaction.

4.2.5 Digestion

0.5 g of the sample was weighed to the nearest 0.001 g and transfered to the Kjeldahl flask of the digestion apparatus. 15g of mix of potassium sulphate is added and catalyst (copper (II) sulphate pentahydrate), 12 ml of sulphuric acid and mix.Basic Animal Nutrition, Proximate analysis, BF ZOO, Chair for Nutrition, 2018 . the Kjeldahl flask was heated moderately at first, swirling from time to time if necessary until the mass has carbonized and the foam has disappeared; then heated more intensively until the liquid is boiling steadily. Heating was adequate if the boiling acid condenses on the wall of the flask. The solution was continued boiling for 2 hours even after become green in colour, then cooled.

4.2.6 Distillation into boric acid

the distillation unit was fully automated to include titration of the ammonia content of the distillate, follow the manufacturer's instructions for operation of the distillation unit. In a semi-automatic distillation unit, the addition of excess sodium hydroxide and the steam distillation are performed automatically.

collecting flask is placed containing 60 ml of the boric acid solution under the outlet of the condenser in such a way that the delivery tube is below the surface of the excess boric acid

solution. Operate the distillation unit in accordance with the manufacturer's instructions and distil off the ammonia liberated by the addition of the sodium hydroxide solution. Collect distillate in the boric acid receiving solution.

4.2.7 Titration

Titrate the contents of the collecting flask with the sulphuric acid standard volumetric solution using a burette and read the amount of titrant used.

When colorimetric end-point detection was applied, the end-point was reached when color of the solution changes from green to red. Estimate the burette reading to the nearest 0, 01 ml.

Blank test

To confirm that the reagents were free from nitrogen, carry out a blank test (digestion, distillation and titration) using only reagents (no sample is added).

4.2.8Calculation of results

Nitrogen content in the sample

$$\boldsymbol{m_N\left(\frac{g}{kg}\right) = (Va - Vb).cHCl.\frac{MN}{mvz}.1000}$$

Va = volume of standard HCl solution when titrating sample (l)

Vb = volume of standard HCl solution when titrating blank (l)

cHCl= concentration of HCl (mol/l)

MN = nitrogen molar mass (g/mol)

mvz = weight of sample (g)

CP(g/kg)=mN (g/kg).F

mN = nitrogen content in sample (g/kg)

F = factor (6.25 for feed samples)

4.3 DETERMINATION OF MOISTURE

4.3.1Equipment

Aluminium moisture cup with lid, hot air oven, metal tongs, desiccator, analytical balance.

4.3.2 Procedure

• Dry aluminium cup was kept in oven at 100oC for 15 to 30 minutes.

• Cooled in a dessicator, weighed and recorded

• 5 g of previously ground samples weighed accurately in an aluminium cup and weight was recorded.

• The cup was placed in hot are oven at 100 degree celcius

• Cooled in a desiccator and weighed.

• The process was repeated for average value.

4.3.3 Calculation

Moisture content = M1 -M2

M1=weight of sample before drying

M2=weight of sample after drying

4.4 DETERMINATION OF CRUDE FIBRE – MUSLIN CLOTH METHOD

4.4.1 Principle

The feed sample is subjected to acid digestion followed by alkali digestion and the remaining residue is weighed and ashed. The loss of weight after ashing is the crude fibre content of the feed.

4.4.2 Apparatus

• Lipless beaker 600 ml capacity

• Condensing flask

• Electric heater/hot plate

• Crucible

• Muslin cloth

• Spatula

• Porcelain tile

4.4.3 Reagents

• 0.255 N H SO : 7 ml of conc. H SO is dissolved in distilled water to get one litre of solution. 2 4 2 4

• 0.313 N NaOH : 12.78 g of sodium hydroxide is dissolved in distilled water to get one litre of solution.

4.4.4 Procedure

In a 600 ml lipless beaker. Place it on an electric heater and keep a suitable condensing flask (round bottom) over the beaker. See that the condensing flask fixes well over the beaker leaving no space. The condensing flask is filled with cold water. Now switch on the heater. The purpose of keeping a condensing flask filled with cold water is for condensing back the evaporating acid to the beaker. This maintains the volume of the acid without any reduction.

4.4.5 Digestion in acid

Two gram of feed was accurately weighed. Accurately measure 200 ml of 0.255 N H SO 24 The beaker was heated to bring the acid (0.255 N H SO) to boiling stage. Then 2 g of substance was transferred to the boiling acid. The acid boils and the feed is digested in acid. This boiling and digestion was continued for 30 minutes. After the end of 30 minutes the boiling was stoped and the condenser was removed.

4.4.6 Filtration

A funnel was settled up in a large conical flask. a linen cloth was fixed over the funnel. the contents was transfered from the beaker to the filtering funnel. After all the acid and acid digested residues were transferred to the linen cloth, the beaker was washed with distilled water and the contents were transferred to the filtering funnel. The process is continued until sample become acid free.

4.4.7 Test

This was tested simply by catching one or two drops of filtrate over blue litmus. If the blue litmus remains blue, that means the residue was washed free of acid. After complete washing take the filter cloth along with the residue, squeeze well to remove the water from the residue. Place the cloth over porcelain slab. Scrap gently the adhering residue from the filter cloth and keep the residue in the centre of the filter cloth.

4.4.8 Digestion in alkali

The acid digested residue was then subjected to alkali digestion. For this 0.313 N sodium hydroxide solution was used. As in the acid digestion, 200 ml of sodium hydroxide (0.313 N)was poured into a lipless beaker (600 ml capacity). The placed over the heater and a condensing flask was fixed over that. the alkali solution was brought to boiling stage by

heating. When it starts boiling, the condensing flask was removed and the acid digested residue was transferred to the boiling alkali.

the condensing flask was replaced and the heating was continued . The residue was digested in the boiling NaOH for a period of 30 minutes. After 30 minutes, the condenser was removed and the contents were transferred of the beaker to a filtering funnel. The residue was washed repeatedly with distilled water till it became alkali free.

4.4.9 Test

This was tested by catching one or two drops of the filtrate over red litmus. If it remains red it indicates that the residue is free from alkali. When the residue was free from alkali squeeze the cloth well to dry the residue. Transfer the residue, without any loss, to a clean silica crucible.
Note: The cold water in the condensing flask should never be hot at any time. If the water is hot replace it with cold water. Before removing the condensing flask care should be taken to avoid the loss of any residue sticking to the bottom of the flask.

4.4.10 Drying and washing

The crucible was placed in preheated hot air oven (110oC) over night. This is to drive off the moisture completely. After complete drying, the crucible was cooled in desiccator. It was weighed along with the residue. Heat the crucible with electrical bunsen in order to ash the residue. heating was continued till you obtain a whitish ash. crucible was cooled to room temperature and weighed .

4.4.11 Calculation

Weight of sample = c
Weight of crucible with dry residue = a
Weight of crucible with ash = b

Percentage of crude fibre = $\frac{a-b}{c}100$

4.5 ESTIMATION OF ETHER EXTRACT USING SOXHLET APPARATUS

Principle

The crude fat present in the feed is extracted by petroleum ether using the soxhlet apparatus.

Apparatus

• Spatula

• Weighing balance

• Thimble

• Heating mantle/hot plate.

• Soxhlet apparatus

Reagent

• Petroleum ether

Procedure

The soxhlet apparatus was set in position.Thimble was taken and weighed. about 5 g of sample was tranfered into the thimble and weighed accurately by weighing the thimble with the sample. the mouth of the thimble was plugged with cotton wool, to avoid the escape of material from the thimble during extraction. The thimble was slided with the contents into the soxhlet extractor. the lower end of the soxhlet extractor was fixed to the flask underneath. Then the condenser was fixed above the soxhlet extractor. the water circulation was adjusted for efficient and uniform cooling of the condensing unit.The soxhlet apparatus was placed over an electrical heater/ hot plate . From the top end of the apparatus pour about 100 ml of petroleum ether and plug the mouth with cotton. Run the extraction for 6 hours till the collecting ether in the extractor is clean.

the apparatus was dismantled on the completion of extraction. Remove the extractor with the flask from the condenser. the thimble was removed with its contents. It was placed in the oven for drying. When dried find out the weight of the thimble with the extracted residue.

The soxhlet flask was removed with the extract. It was transferred to a hot air oven (80oC) for evaporating the petroleum ether. The sample was weighed with flask with the dried residue.

Calculation

You can find out the weight of ether extract either directly from weighing the flask with and without ether extract or indirectly by weighing the thimble with the substance before and after extraction. The loss of weight in this case will give the value for ether extract.

Direct

Weight of flask (empty)

Weight of flask + ether extract

Weight of ether extract

g

Percentage of ether extract

100 (W is the weight of sample taken)

= X g = Y g = Y - X

= [(Y-X) /W] x

Indirect

Weight of thimble + feed sample before extraction =X

g Weight of thimble after extraction. =Y

g Weight of the ether extract. =X-

Y g (Loss of weight represents the ether extract)

Percentage of ether extract. $=(X-Y)/W.100$

100 (W is the weight of sample taken).

Precaution: While placing the thimble-containing sample in the soxhlet flask, make sure that the top of the thimble is above the siphon tube. Put a cotton swab on the mouth of the condenser to avoid the loss of ether vapours.

Reference: AOAC official method 920.39, 16th Edition

CHAPTER 5

RESULTS AND DISCUSSION

The nutritional composition of chocolate milk shake

s.no	PARAMETERS	VALUE (%)
1	Carbohydrates	14
2	Fat	3.3
3	Protein	3.4
4	Fibre	0.9
5	Moisture	78.8

The nutrition composition of Chocolate milk shake fortified with groundnut protein

s.no	PARAMETERS	METHODS	RESULTS (%)
1	Carbohydrates	Anthrone method	15.5
2	Fat	Soxhlet method	3.9
3	Protein	Kjeldahl method	10.4
4	Fibre	Muslin cloth method	1.5
5	Moisture	Microwave method	68.7

SENSORY EVALUATION

SENSORY ATTRIBUTES	CONTROL	PROTEIN MILK SHAKE
TEXTURE	8.4	8.6
TASTE	8.4	8.2
FLAVOUR	7.6	8.4
OVERALL ACCEPTANCE	8	8.6

DISCUSSION

The chocolate milk shake itself contains 3.4% of protein with it.After fortification, the protein content content of the milkshake raised to 10.4% .The fat content of the fortfied milkshake increased 0.6 %.The protein content of the protein fortified milk shake increased by 7%.

The fiber content increased by 0.6%. The protein intake per day should be 0.8g per Kg of body weight(Wu,2016). For example, the protein intake of 50 kg man should be 40g per day

CHAPTER 6

CONCLUSION

Children need more protein per pound of body weight than adults because they are growing and building new protein tissue. Pregnant and nursing women need more protein for growth of the baby and to produce milk (Hermann,2019). Protein catalyse reactions in our bodies, transport molecules such as oxygen, keep us healthy as part of the immune system and transmit messages from cell to cell(Raheem,2019). Most of the people in India are not eating enough of protein every day. This milk shake fortified with groundnut protein will partially fulfil the protein need of many people.

CHAPTER 7

FUTURE THURST

Chocolate milk shake is favourite for every Children, when it is fortified with protein, it will be very good for children. Protein is also very important for muscle growth and maintenance. This milk shake can also be consumed by sportsmen, as it improves the performance and muscle growth. Elderly people absorb less protein when compared to young people, they should consume more protein than young people. So, this protein milk shake will also fulfil the protein need of elderly people .This protein obtained from peanut Pomace can also be used to fortify other food products to improve its nutritional value.

CHAPTER 8

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