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Study of Food Model Enrichment and Sensory Evaluation of Dark Chocolate with Foxnut and Flaxseed

Anushka Goyal and Harish Kumar Dhingra

Dept of Biosciences, SLAS, Mody University of Science & Technology, Lakshmangarh, Rajasthan

Abstract

Formulation of food products which are low on calories and high in nutrients are very much desirable & in demand. Management of weight is the major concern for people in present lifestyle. The enhancement of this food model with highly nutritious components with all major and micronutrients was produced for the individuals who are inadequate in their protein and micronutrient profile. Nowadays every individual is so busy that they do not take important nutrients which are vital for their health and those who are on diet have cravings for sweets and chocolate but cannot eat because of high calories and sugar content. So, this product was developed with incorporation of cocoa powder, cocoa butter, foxnut, flaxseeds and jaggery powder. Flax is also known as Linum usitatissimum is very useful, it comes from family Linacea. Flaxseeds are good source of omega 3 fatty acid and phytoestrogen. They help in treating high blood pressure, diabetes and cholesterol absorption. Jaggery helps in removing toxins out of body, liver detoxification and prevents anemia. Foxnuts are otherwise called Makhana, Euryale ferox, Lotus seed, Gorgon nuts and Phool makhana. Foxnut that is good for heart & overall health since it is low in cholesterol, sodium and saturated fat. Inclusion of foxnut helps in overcoming of major micronutrients deficiency like calcium, phosphate and magnesium and all these are given in the form of chocolate. Carbohydrate, protein, lipid, fiber, ash and moisture were estimated for developed product. Sensory analysis was done to check the overall acceptability and other parameters including taste, after taste, colour, flavour, texture, hardness, aroma, sweetness and mouth-feel of the developed product. Developed product was more accepted than control. Antioxidant activity is more in developed product.

Keywords

Flaxseed, foxnut, jaggery, sensory analysis, antioxidant activity

Introduction

"Every time you eat or drink. You are either feeding disease or fighting it" (Heather Morgan). Each bite is an opportunity to make good choice. Diet or nutritional intake is a vital part in all parts of life. It influences well-being like common cold, risk of diabetes, risk of heart disease, overweight, physical capability. The food straightforwardly impacts energy levels, personality and immune system.

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Chocolate enrichment was prepared by roasted and ground cocoa seeds that are produced in form of paste which is also used for the flavoring ingredients in other foods. The English word “chocolate” originates from the Spanish Nahuati word Xocolati. Theobroma cacao, the cacao tree, produce seed containing flavanols. The seed have been used to make beverages for some 2000 years in Aztec, Olmec and Maya civilization in central and South America. The seeds of the cacao have to be fermented in order to get the flavor because of it's intense bitter taste. To produce cacao nibs the shell is removed, which are grounded then to cacao mass to get rough form of unadulterated chocolate. Cacao mass is then heated and liquefied to get the chocolate liquor. The chocolate liquor is then cooled and processed to two components, i.e., cocoa solids and cocoa butter. There are many different forms of chocolates. Majority of the chocolate used nowadays is the sweet chocolate, which is a combination of cocoa butter, cocoa solids and added vegetables oils and sugars. Milk chocolate is another one which is sweet in taste and that contain milk powder or condensed milk. White chocolate has cocoa butter, sugar and milk however no cocoa solids. Health benefits of chocolate consumption have been mentioned in many researches (Apgar & Tarka, 1999; Ariefdjohan & Savaiano, 2005).

Cocoa powder is used to add flavour to desserts and beverages as an unsweetened chocolate form. Cocoa powder is formed when the fat called cocoa butter is eliminated from the cocoa beans while processing. The additional dried solid gets ground into the item sold as cocoa powder. Cocoa powder contains essentially cocoa solids, with simply around 10-15% cocoa margarine versus the 50% or more in chocolate. Unsweetened cocoa powder has around 12 calories for each tablespoon and not exactly a gram of absolute fat. Cocoa powder has iron, magnesium, zinc and manganese and about 2 g of fiber for each tablespoon. The polyphenolic flavonoids found in cocoa help in lowering blood pressure and improve cholesterol. It additionally contains omega 6 unsaturated fat, which is helpful against heart diseases (Buijsse *et al.*, 2006).

Foxnut are otherwise called Makhana, Euryale ferox, Lotus seed, Gorgon nuts and Phool makhana. This is grown in water and largely found in India. Foxnut are utilized in a couple of Indian dishes like Kheer, Raita, Laddu and also eaten as an evening tea time snack. It is high in nutrition and can be consumed in the fried and roasted form as it helps storage for a longer time. In China it is used as a part of medicine as it has therapeutic properties as well (Ahmed, 2015; Pravin, 2016; Harnoor, 2020). Foxnut is good for the heart health because it is low in cholesterol, sodium and saturated fats. They are also high in magnesium, potassium, manganese, protein and phosphorous. Foxnuts helps in weight loss because it is rich in protein, which keeps one full for longer period of time and it is also low in calories. It also helps in strengthening the bones because it has a good amount of calcium. Fox nuts have good content of sugar, protein, ascorbic acid and phenol which make them as good as dry fruits like almonds, cashew, walnuts etc. Fox nut helps in preventing premature greying of hairs and other signs of ageing as they are high in antioxidants. Being a good source of fiber, foxnuts are found to be helpful in preventing constipation. In men, semen quality can be improved by

foxnuts and in women, fox nuts are helpful in overcoming infertility. Foxnuts are helpful in treating insomnia. It is helpful in the treatment of joint numbness. It helps in maintaining gut health.

Flax is also known as Linum usitatissimum is a Latin word which mean very useful, it comes from family Linacea. Canada is one of the major suppliers of flax seeds. It was initially grown in Egypt. Flax comes from southern Europe and Asia. Flax seeds are mainly reddish brown in color. In terms of flavors, they are mild and nutty. They are slightly gummy in nature. They are good source of phytoestrogens, omega 3-fatty acid and alpha linolenic acid. Flax seeds are used in treating high blood pressure because they can bind with cholesterol and reduce its absorption in body. It is useful in prevention of atherosclerosis, diabetes, improvement of digestion, prevention of cancers fungal infections. They contain insoluble fiber which delays stomach emptying. Flax seed are good source of Vitamin B1 (Thiamine), copper, molybdenum, phosphorous, magnesium etc. They are great source of protein.

Jaggery has been used as a sweetener in Indian Ayurveda for about 3000 years due to its medicinal properties. It is an unrefined natural sugar which is a great source of several vitamins and minerals namely vitamin B complex, folic acid, phosphorous, calcium, copper, zinc, iron, potassium and magnesium. It is superior to sugar as sugar contains simple carbohydrate which gets absorbed in blood instantly and gives the rapid rise in blood glucose level but jaggery is a complex carbohydrate so its absorption in body is gradual and there is no rapid rise in blood glucose level. Jaggery helps in preventing constipation as it contains digestive enzymes, detoxify liver by removing harmful toxins out of body, boost immunity as it contain rich amount of antioxidants which helps in reducing free radicals from body, eases menstrual pain because when eaten before onset of periods, jaggery releases endorphin. It also prevents anemia because it is a good source of folate and iron. It controls blood pressure because it contains balanced amount of sodium and potassium and helps in weight management as well because it improves metabolism of body. Jaggery helps in purifying blood. Pregnant women should consume jaggery during their term. In every 10 grams of jaggery there is 16 mg magnesium which is good for intestinal health.

Cocoa butter is also known as Theobroma oil, slightly yellow in color, comes from the cocoa beans. Cocoa butter has light chocolate aroma and flavor. Cocoa butter is taken out of cocoa beans by fermenting, drying and roasting. Cocoa butter carries a huge proportion of saturated fat as well as monounsaturated oleic acid. The fatty acid structure of cocoa butter is oleic acid, stearic acid, palmitic acid and linoleic acid. It has lower percentage of lauric acid and myristic acid. The predominant triglycerides found in cocoa butter are POS, SOS, POP, where P is palmitic acid, O is oleic acid and S is stearic acid residues. Cocoa butter is a significant ingredient in the chocolate and other confectionery industries. Chocolate has cocoa butter as the only continuous fat stage. Cocoa butter is kept at 25 degree Celsius and below temperature whereas it softens in the hands and melts in mouth when consumed at 34 degree Celsius. Cocoa butter is a decent source of vitamin E, which supports vision, skin, reproduction and health of the

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brain. Cocoa butter manages the cholesterol level and reduces the chance of heart disease. Cocoa butter also helps in liver disease because it contains choline. Cocoa butter helps in build and maintains bones because it contains low amount of vitamin K.

In today's time chocolate is everyone's favourite. But due to the high content of fat and sugars it has many side effects also. Chocolate can be used in many forms, but if nutritional composition is good, then it may become very much in demand. The current investigation represents the utilization of these components in a set of criteria that defines their composition and nutritional benefits. The enhancement of this food model with highly nutritious component with all the major and important micronutrients and adequate calories and low in sugar content was produced for the individuals who are inadequate in their protein and micronutrient profile. Nowadays every individual is so busy that they do not take important nutrient which are important for the health and those who are on diet have cravings for sweets and chocolate but cannot eat because of high calories and sugar content. To defeat these issues, an item that is handful, nutritious and provide all the important nutrients and low in sugar and adequate in calories was developed.

The product comprised of dark compound chocolate made out of cocoa powder and cocoa butter. Omega fatty acids rich cocoa butter was produced using cocoa beans, unsweetened low caloric cocoa powder, decent amount of calcium and other important micronutrient in foxnut, carbohydrate and vitamins and minerals rich jaggery with very essential antioxidants and good source of phytoestrogens, omega 3 fatty acid and alpha linolenic acid rich flax seeds and good saturated fat rich cocoa butter. Developing a food model that is appealable in terms of high nutritional content, good manufacturing practices, taste, texture and in color. Consumer demands are expanding day by day for food item that is manageable with an average cost. The current study was by detailing a chocolate made out of cocoa powder, cocoa butter, fox nut, flaxseed, jaggery and dark compound chocolate. These were mixed in different proportion to achieve the best quality and acceptable product by the consumer. The chocolate was set up at the research facility level with complete optimization. Temperature critical control point was kept into thoughts. A detailed sensory analysis on 9-score hedonic scale was coordinated for 30 panelist that is taste, texture, color and overall acceptability were the significant parameters. Nutritional value was analyzed for the item.

Objectives

- To form and standardize flaxseeds and fox nut dark chocolate
- To examine the antioxidant activity of the chocolate and its ingredients
- To examine the sensorial parameters of the chocolate and its ingredients
- To examine the nutritional composition of the elements of chocolates

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Research Methodology

The current study was done on development of Dark Chocolate with foxnut and flax seeds and influence on sensorial characteristics. The Materials and Methods for the examination have been discussed as follows -

Phase 1: Materials and Chemical Required: The materials required for the formulation of dark chocolate enriched with foxnut and flax seeds were: Unsweetened cocoa powder (Organic purify), Cocoa butter (Minimal Confections), Dark compound Chocolate (Morde), Flax seed (Vedaka), Foxnut (Rajbhog), Jaggery Powder (Dhampur Green), Silicon Chocolate Molds, Refrigerator, Induction, Flat bottom vessel, Sieve, weighing machine, Measuring Cups, Spoons and Aluminum foil.

Materials required for the performance of sensory analysis were: Developed product, survey format, pen, paper napkin, plate.

The Materials required for the proximate and quality analysis of the product were as per the following: Petri plates, Weighing machine, Measuring cylinders, Test tubes, Test tube stand, Glass rod, Beakers, Conical flask, Crucibles, Mortar and Pestle, Funnels, Burette, Pipette, Whatman filter paper, Muslin cloth, Centrifugal tubes, Thermometer, Hot air oven, Spectrophotometer, Water bath, Centrifuge, Magnetic rotor, Shaker, Refrigerator, Water bath shaker, Spectrophotometer, Colorimeter, pH meter.

Chemicals required for the proximate and quality analysis: Acetone, Monosodium Phosphate, Disodium phosphate, Sodium Hydroxide, Sulfuric acid, Sodium carbonate, Copper sulphate, Sodium potassium tartrate, Folin Ciocalteu reagent, Dinitro-salicylic-acid, Proteinase-K, phenolphthalein indicator, Distilled water.

Phase 2: Product Development Using Cocoa Powder, Flax seeds and Foxnut: Dark compound chocolate – 100 grams Flax seeds – 25 grams Foxnut powder – 25 grams Jaggery Powder – 30 grams Cocoa Powder – 30 grams Cocoa butter – 50 grams All the measurements were performed as per standard proportions. For 100 gram of milk compound, the proportion for foxnut powder, flax seeds, cocoa powder, jaggery powder and cocoa butter were appropriately balanced in terms of taste, texture, mouth feel, amount and consistency. Preparation method is as follows -

Foxnut were roasted at a temperature between 150-180 degree Celsius to acquire crunchy texture. Later the foxnut squashed and finely powdered. Flax seed were likewise roasted at a temperature between 70-80 degree Celsius. These seeds squashed and finely powdered. 100 grams of morde dark compound was melted in a pan by double boiler method by continually controlling the temperature. Optimum temperature for the chocolate was 34-37 degree Celsius. 50 grams of cocoa butter were added to the melted chocolate compound. 30 grams of jaggery powder were all the while added to the compound chocolate. After complete melting of cocoa butter and jaggery powder, 30-gram cocoa powder was added to the chocolate mixture. Continuously blending the mixture with the temperature control to prevent the lumps and adhering of the chocolate to the vessel until the dark color was acquired. Foxnut

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and flax seeds powders were added to the mixture. The mixing was done continuously for approx. 10-15 minutes at temperature 32-37 degree Celsius. The chocolate mixture was poured into the silicon molds and kept at temperature between - 4 to - 6 degree Celsius for about 20 minutes and allowed to set.

The summarized flow chart of the product developed is as following -

Flax seed and foxnut roasted And finely powdered

↓

100-gram Dark chocolate melted at 34 degree Celsius

↓

Addition of 50 gram of cocoa butter to the chocolate

↓

Addition of 30 gram of jaggery powder to the chocolate

↓

Addition of 30 gram of cocoa powder to the mixture

↓

Continuous stirring at temperature 34-37 degree Celsius

↓

Addition of foxnut and flax seeds powder to the mixture

↓

Mixture poured into molds

↓

Set at temperature -4 to -6 degree Celsius for 20 minutes

Preparation of the control with Dark Chocolate:

Foxnut were roasted at a temperature between 150-180 degree Celsius to acquire a crunchy texture. Later the foxnut squashed and finely powdered. Flax seed likwise roasted at a temperature between 70-80 degree Celsius. These seeds crushed and finely powdered. 100 gram of Amul Dark Chocolate was melted in a pan by double boiler method. Foxnut and flax seeds powdered were added to the mixture. The

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mixing was done continuously for approx. 10-15 minutes at temperature 32-37 degree Celsius. The chocolate mixture was poured into the silicon moulds and kept at temperature between - 4 to - 6 degree Celsius for about 20 minutes and allowed to set.

Figure1 : Developed Product

Figure 2: control

Figure 3: Raw Materials Used for Chocolate making

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The summarized flow chart of the control is as follows:

Fox nut and flaxseed roasted and finely powdered
↓
100 grams of dark chocolate was melted
↓
Add fox nut and flax seed powder to the melted chocolate
↓
Mixture poured into moulds
↓
Set at temperature -4 to -6 degree Celsius for 20 minutes

Phase 3: Determination of Chemical Properties of Cocoa Powder, Flax seeds, Foxnut and Jaggery:

Phase 3.1: Determination of proximate composition: In this, estimations were done for carbohydrate, protein, fat, fiber, moisture and ash.

Determination of Carbohydrate content: For the estimation of reducing sugar, 3-5-dinitrosalicylic acid (DNSA) is extensively used method. By this method, presence of carboxyl group of reducing sugar can be determined, where is oxidation of ketone and aldehyde functional group. In this method there is reduction of DNSA into 3-amino 5 nitro salicylic acid that is, ANSA, due to which reddish brown colored complex is formed because of conversion of alkaline condition. Thus formed reddish brown colored complex has maximum absorbance at 540 nm. The procedure is as follows:

- 1 gram of sample was weighed (Developed and Control) and grinded in mortar and pestle
- Further, it was defatted 4-5 times using Acetone
- Added 20 ml distilled water in two beakers, added 0.477M Sodium mono sulphate and 0.523 Di sodium hydrogen ortho phosphate in respective beakers. After this step, pH of solution was checked. It should be 6.8
- Later defatted sample was taken in a clean test tube and heated for 1 minute.
- The sample was then taken in a conical flask. 10 ml of prepared buffer solution was added in this conical flask. Then this conical flask was place on vortex stirrer for 2 hours
- After 2 hours sample was centrifuged at 6000 rpm for 5 minutes
- 0.8-gram sodium hydroxide was mixed in 20 ml distilled water
- In it mixed 0.5 gram 3,5- dinitrosalicyclic acid (DNS). Added 5 ml distilled water in this beaker
- Mixed 15-gram sodium potassium tartarate in 25 ml distilled water in another beaker
- Further, mixed solution prepared of DNS and sodium hydroxide with prepared sodium potassium tartarate solution. Later 8 test tubes were taken (1 for blank).

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- For stock solution, 0.31 mg/ml dextrose was required. To prepare it 1 gram dextrose was added in 100 ml distilled water. For blank, 1 ml DNS was added in 1 ml distilled water
- In taken 7 test tubes added, 1ml DNS solution and 1 ml stock solution
- Extracted 100 micro liter of supernatant formed in centrifugal tube into 3 test tubes. In these, added 900 micro liter distilled water and 1 ml DNS solution
- All the test tubes were heated in water bath at 90 degree Celsius for 10 minutes
- After 10 minutes test tube was taken out of water bath and allow to cool.
- Lastly, absorbance was taken at 540 nm in spectrophotometer. (Warwick L Marsden, 1982)

Determination of protein content: The protein was determined by the Lowry Method. The copper in Lowry Reagent respond with peptide bonds. These bonds become apparent when copper is reduced because this forms a blue coloured solution. The procedure is as follows:

- 1 gram of sample was weighed (Developed and Control) and grinded in mortar and pestle
- Further, it was defatted 4-5 times using Acetone
- Added 20 ml distilled water in two beakers, added 0.477M Sodium mono sulphate and 0.523 Di sodium hydrogen orthophosphate in respective beakers. After this step, pH of solution was checked. It should be 6.8
- Later defatted sample was taken in a clean test tube and heated for 1 minute.
- The sample was then taken in a conical flask. 10 ml of prepared buffer solution was added in this conical flask. Then this conical flask was placed on vortex stirrer for 2 hours
- After 2 hours sample was centrifuge at 6000 rpm for 5 minutes
- Reagent preparation: 2% Na_2CO_3 (2 gm) in 0.1 M NaOH (0.4 gm) in 100 ml distilled water was taken. 1% sodium potassium tartarate (1 gm) was added in 10 ml distilled water. 0.5% copper sulphate (0.05 gm) was added in 10 ml distilled water. Took 48 ml of A+1 ml B + 1 ml C, this was marked as reagent 1.
- 8 test tubes were taken (1 for blank)
- To make stock solution. Added 0.033 mg BSA in 33 ml distilled water
- Extracted 100 micro liter of supernatant formed in centrifugal tube into 3 test tubes. In these, added 900 micro liter distilled water and 1 ml Reagent 1
- All the test tubes were heated in water bath at 90 degree Celsius for 10 minutes
- After 10 minutes test tube was taken out of water bath and allow to cool
- Preparation of Reagent 2: Folin ciocalteau reagent (2N) with equal volume of water
- Added 1 ml of Reagent 2 in each test tube.
- Lastly, absorbance was taken at 660 nm in spectrophotometer (Jakob H Waterborg, 2013)

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Determination of fat content: Fat estimation of the developed product was performed by gravimetric method. The procedure is follows as: One gram sample was taken (Developed and Control) and grinded them separately in mortar and pestle. Two conical flasks were taken and marked them R1 and R2 and added 10 ml acetone in each flask. Two petri plates were taken, marked them A1 and A2 and checked their initial weight. Conical flask was swirled 5 times and emptied in respective petri plates. Third step was repeated 3 times with each sample. Total 30 ml acetone was collected in each petri plates. Petri plates were kept at room temperature till acetone evaporated. Lastly final weight of petri plates was checked (Katherine M Phillips, 1997).

Determination of Fiber content: Fiber analysis of the developed product was done by Acid Base technique. By this technique, indigestible fiber present in the developed product can be estimated. Determination is carried out by sequential digestion of sample with sulphuric acid and sodium hydroxide. In a muslin cloth, the residue is collected, dried, weighed and ashing was done to check for contamination of minerals. By the acid base technique, pectin and hemicellulose cannot be determined because they cannot be collected as they are digested by acid and alkali. The procedure is as follows: Three gram of sample was taken (Developed and Control). Then, 200 ml of 0.313 N sodium hydroxide was added in it. It was boiled for 30 minutes at 37 degree Celsius. Further, it was filtered through muslin cloth for digestion and washed the sample 2-3 times with boiled water. It was repeated thrice with each sample. Washed content was transferred in a fresh beaker. Added 200 ml of sulphuric acid 0.255 N and it was boiled for 30 minutes. Further, it was filtered through muslin cloth for digestion and the sample was washed 2-3 times with boiled water. It was also done thrice with each sample. Two petri plates were taken and marked as R1 and R2. Whatman filter paper was kept in both petri plates and again weight was checked. Sample was placed on petri plate having Whatman filter paper and checked and recorded the initial weight. Petri plates were kept in Hot air oven at 105 degree Celsius for overnight. Weight of petri plates containing fiber was again checked next day. Two empty crucibles were taken and checked their weight. Sample was added in respective crucibles and weighed their initial weight. Further, these were placed in Muffle furnace at 550 degree Celsius for 3 hours. Lastly final weight of crucibles was checked and recorded (Gregory D Miller, 1999).

Determination of moisture content: Theory behind this method involves determination of mass of moisture in sample by measuring the weight of sample before and after removal of water content. Removal of moisture content is done by keeping the weighed sample in hot air oven at a specific temperature for certain period of time. The principle behind this technique is that boiling point of water is low than other components in sample e.g. protein, carbohydrate, fat etc. For the determination of moisture content in product, parameter termed as total solid is reported which means the measurement of content remaining after evaporation of moisture content from product. The procedure is as follows: One gram sample (Developed and Control) was taken and ground separately in mortar and pestle. Two petri plates were taken and their weight was recorded. Added sample in petri plates and recorded the initial weight.

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Kept the petri plates in hot air oven for 2 days at 60 degree Celsius. On 3 days recorded the final weight (F. Leslie Hart, 1971).

Determination of Ash content: The theory behind this method is to burn the organic matter and determine the left inorganic content. For ashing, heating is being done in 2 phases: 1. First phase involves removal of water present in the sample. 2. Second phase involves ashing of the samples in muffle furnace at 550 degree Celsius. The procedure is as follows: One gram sample was taken (Developed and control) and ground separately in mortar and pestle. Weighed empty crucibles and recorded it the weight.

Added 1 gram weighed sample in respective crucibles. Placed these crucibles in Muffle Furnace at the interval of 100 degree Celsius for 1 hour, at 300 degree Celsius for next 1 hour and at 560 degree Celsius for 4-5 hours. Lastly, weighed the crucibles and noted down the final weight (A. Sluiter, 2005).

Determination of Antioxidant level in the product (Aurelia, 2011):
1. DPPH Method
2. FRAP Assay Method

Preparation of extract

Methanol extract:

- 2-gram sample was taken (Developed and Control) and grinded them separately in motor and pestle
- 20 ml methanol added in the sample, covered with the aluminum foil and mix.
- Kept it overnight in the incubator shaker at 100 rpm for overnight.

Water extract:

- 2-gram sample was taken (Developed and Control) and grinded them separately in motor and pestle
- 20 ml distilled water added in the sample, covered with the Aluminium foil and mix
- Kept it overnight in the incubator shaker at 100 rpm for overnight

1. DPPH Method: DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay dependent on electron-transfer that delivers a violet solution in ethanol/methanol. This is diminished in the presence of an antioxidant molecule, giving rise to colorless solution. The DPPH assay is used to give a simple and quick way to evaluate antioxidants by spectrophotometry, so it can be helpful to survey different products.

Methanol extract

Took 10 test tube, added methanol extract in it at different concentration, 1000 microliter, 900 microliter, 800 microliter and so on

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- For make up the solution add methanol in each test tube according to concentration
- Prepared 0.004% (4mg of DPPH)
- 0.002-gram DPPH added in 50 ml of methanol
- 1 ml of methanolic DPPH solution added in each test tube and it gives purple colored
- Incubated it at room temperature for 30 minutes
- Lastly, absorbance was taken at 570 nm in spectrophotometer

Water extract

- Took 10 test tube, added water extract in it at different concentration, 1000 microliter, 900 microliter, 800 microliter and so on
- For make up the solution add methanol in each test tube according to concentration
- Prepared 0.004% (4mg of DPPH)
- 0.002 gram DPPH added in 50 ml of methanol
- 1 ml of methanolic DPPH solution added in each test tube and it gives purple colored
- Incubated it at room temperature for 30 minutes
- Lastly, absorbance was taken at 570 nm in spectrophotometer

2. FRAP Assay Method (Ferric Reducing Antioxidant Power):

Ferric reducing ability of plasma (FRAP, additionally Ferric ion reducing antioxidant power) is an antioxidant capacity assay that utilizes Trolox as a norm. This test is frequently used to measure the antioxidant capacity of foods, beverages and nutritional supplements containing polyphenols.

Methanol extract

- Took 10 test tube, added methanol extract in it at different concentration, 1000 microliter, 900 microliter, 800 microliter and so on
- For make up the solution add methanol in each test tube according to concentration
- Added 30 ml distilled water in two beakers, added 0.477M Sodium mono sulphate and 0.523 Di sodium hydrogen ortho phosphate in respective beakers
- After this step, pH of solution was checked. It should be 6.6
- 2.5 ml phosphate buffer solution added in each test tube
- 1% potassium ferrocyanide i.e., (0.5gram in 50 ml distilled water)
- 2.5 ml potassium ferrocyanide added in each test tube, covered it with aluminium foil and mixed it
- Water bath turned and checked the temperature with the help of thermometer. This should be 50 degree Celsius
- Kept the test tube in water bath at 50 degree Celsius for 20 minutes

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- After 20 minutes test tube was taken out of water bath and allow to cool 10% tri chloro acetic acid i.e., (5gm in 50 ml distilled water).
- 2.5 ml TAC was added in each test tube
- Sample was centrifuge at 3000rpm for 5 minutes
- Extracted 2.5 ml of supernatant formed in centrifugal tube in clean test tube
- 2.5 ml of distilled water was added in each test tube
- 0.1% ferric chloride i.e., (0.05 gram in 50 ml distilled water)
- 0.5 ml ferric chloride was added in each test tube. Bluish colored appears
- Lastly, absorbance was taken at 700 nm in spectrophotometer

Water extract

- Took 10 test tube, added water extract in it at different concentration, 1000 microliter, 900 microliter, 800 microliter and so on
- For make up the solution add methanol in each test tube according to concentration
- Added 30 ml distilled water in two beakers, added 0.477M Sodium mono sulphate and 0.523 Di sodium hydrogen ortho phosphate in respective beakers
- After this step, pH of solution was checked. It should be 6.6
- 2.5 ml phosphate buffer solution added in each test tube
- 1% potassium ferrocyanide i.e., (0.5gram in 50 ml distilled water)
- 2.5 ml potassium ferrocyanide added in each test tube, covered it with aluminium foil and mixed it
- Water bath turned and checked the temperature with the help of thermometer. This should be 50 degree Celsius
- Kept the test tube in water bath at 50 degree Celsius for 20 minutes
- After 20 minutes test tube was taken out of water bath and allow to cool
- 10% tri chloro acetic acid i.e., (5gm in 50 ml distilled water)
- 2.5 ml TAC was added in each test tube
- Sample was centrifuge at 3000 rpm for 5 minutes
- Extracted 2.5 ml of supernatant formed in centrifugal tube in clean test tube
- 2.5 ml of distilled water was added in each test tube
- 0.1% ferric chloride i.e., (0.05 gram in 50 ml distilled water)
- 0.5 ml ferric chloride was added in each test tube. Bluish colored appears
- Lastly, absorbance was taken at 700 nm in spectrophotometer

Phase 4. Sensory Analysis of the Developed Product

Sensory analysis of the product was done by hedonic method. This method includes 9 scale balanced parameter for sensory analysis of the product with 4 positive and 4 negative categories on each side and one neutral option. Product achieving score 7 or more than it is considered to be high acceptability (Lawless and Heymann, 2010)

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For the performance of sensory analysis of developed product, 30 panelists were selected between the 18-50 years of age group. They were asked to taste the product and give score. This method was helpful in finding the overall acceptability of the products. The 9 scale include following parameters: Extreme Likely-9; Very much likely-8; Moderate-7; Slightly likely-6; Neither likely nor dislikely-5; Slight Dislikely-4; Moderate Dislikly-3; Very much dislikely-2; Extreme dislikely-1.

Results and Discussion

Results for Sensory Analysis: Sensory Evaluation of the Developed chocolate and Control is shown in table 1-

S. No.	Characteristics	Average Score for Developed Chocolate	Average Score for Control
1	Taste	9±0.20	8±0.18
2	After Taste	8±0.61	7±0.12
3	Color	8±0.32	7±0.21
4	Aroma	8±0.12	8±0.19
5	Sweetness	8±0.15	7±0.27
6	Flavor	9±0.23	8±0.25
7	Texture	9±0.19	7±0.18
8	Hardness	7±0.22	6±0.11
9	Mouthfeel	8±0.10	7±0.26
10	Overall Acceptability	9±0.17	8±0.14
	Percentage of Overall Acceptability	90.15%	85.65%

Table 1: Sensory Evaluation of Developed Chocolate and Control

As demonstrated by Table 1, the ordinary score for taste, after taste, color, aroma, sweetness, flavor, texture, hardness and mouthfeel had the in general tactile rating of 9. On the parameter of taste, texture and flavor, developed product was like extremely as the sensory evaluation score for this characteristic was 9. In terms of after taste, color, aroma, sweetness and mouthfeel, developed product score 8 which made it like very much product.

The result for control, the normal score for taste, after taste, color, aroma, sweetness, flavor, texture, hardness, mouthfeel had the in general tactile rating of 8. On the parameter of taste, aroma and flavor, control was like very much as the sensory evaluation score for this characteristic was 8. In terms of after taste, color, sweetness, texture, hardness and mouth feel, control score 7 which made it like moderately.

Flavor of the developed product was bitter which was dissatisfactory for some people. Bitter flavor was due to addition of cocoa powder. Aroma was not liked very much because there was tang of cocoa powder. Highest score was 9 indicating liked extremely. It was given for taste, flavor 42 and texture and lowest was 7 indicating like moderately which was given for hardness. For control highest score was 8 indicating

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like very much that was given for taste, aroma and flavor and lowest was 6 indicating like slightly which was given for hardness. Hardness was missing because of unseemly temperature control.

The overall acceptability of the developed product was 9 so the product was like extremely. Developed product was more liked than the control one because developed product was made up of cocoa powder, cocoa butter and dark compound chocolate. Addition of these ingredients was found to be more appealing to sensory evaluation subject. Similar study on sensory evaluation of chocolate was also conducted by Ines, 2008; Meriel and Harwood (2017).

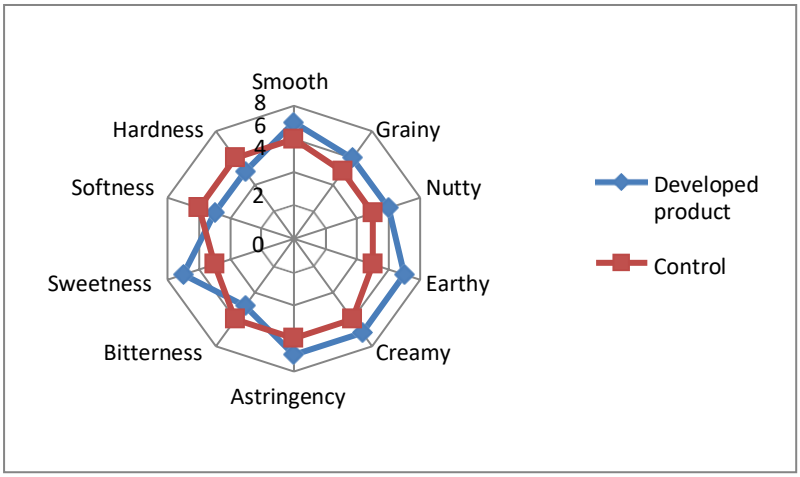

Figure 4: Radial Representation of the taste parameters for developed Chocolate and Control

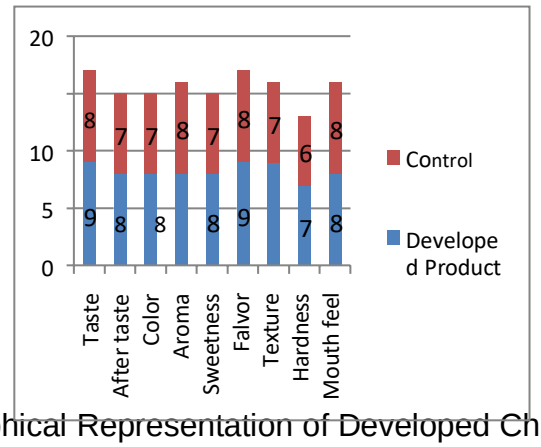

Figure 5: Graphical Representation of Developed Chocolate and Control

Results for Proximate Analysis

4.2. Results for Proximate Analysis

Proximate composition of the developed chocolate and control -

Nutrients	Developed chocolate	Control
Carbohydrate	31.45% ± 0.06	29.5%±0.09
Protein	28.32% ±0.02	26.34±0.07

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Fat	63.78% ±0.47	66.34%±0.032
Moisture	4.85% ± 0.41	5.15%±0.04
Fiber	1.05%± 0.022	1.10%±0.028
Ash	1.15%± 0.02	1.25%± 0.07

Table 2: Proximate composition of Developed chocolate and Control

Carbohydrate content is average in developed product because cocoa powder has higher carbohydrate composition as compared to control. Control was noted to have 29.15%±0.09 carbohydrate and developed product was known to have 31.45%±0.06 carbohydrate. Developed products are average in fat and higher in control. Developed product was noted to have 63.78%±0.47 fat and control was known to have 66.34%±0.032 fat. Protein content is high in developed product as compare to control. Developed product was noted to have 28.32%±0.02 protein and control was known to have 26.34%±0.07 protein. Fiber percentage for developed product 1.05%±0.022 and control 1.10%±0.028 and for the Ash is 1.15%±0.02 for developed product and 1.25%±0.07 for control. It was made known in a study that nutrient composition chiefly protein and carbohydrate and protein content varies when product is stored in low temperature. Carbohydrate reduces due to hydroxylation and protein produces due to neutralization. Moisture percentage was high in control compared to the developed product. Control was noted to have 5.15%±0.04moisture and developed product was known to have a 4.85% ± 0.41 moisture.

4.3. Results for Antioxidant activity

Antioxidant capacity of the Developed product and Control.

For Methanolic Extract	**DPPH**	**FRAP**
Developed Product	45.5±0.11	46.3±0.44
Control	35.5±0.23	33.2±0.32
For Water Extract		
Developed Product	38.5±0.12	42.6±0.44
Control	31.3±0.21	31.2±0.28

Table 3: Antioxidant activity of Developed product and Control.

The main characteristic of an antioxidant is its capacity to wipe up free radicals available in biological system. These free radicals may oxidize nucleic acid, proteins, lipids or DNA and can possibly start degenerative diseases. Table 3 show that developed product have high antioxidant activity because of cocoa powder and cocoa butter in it and it is 45.5±0.11 and Control has average antioxidant activity 35.5±0.23 by DPPH. By FRAP in developed product it is 46.3±0.44 and in control has 33.2±0.32 for methanolic extract. For water extract developed product show antioxidant activity i.e., 38.5±0.12 and for control 31.3±0.21 by DPPH. By FRAP developed product have antioxidant activity is 42.6±0.44 and control is 31.2±0.28. The product has cocoa butter, cocoa powder, flaxseed, foxnut and jaggery which also has decent amounts of antioxidants in it. Kondrashov & Sevcik, 2015 also depicted antioxidant properties in chocolate and cocoa products. Allgrove *et al*., 2011 presented in their study the

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reduction in oxidative stress after consumption of dark chocolate. Antioxidant properties of foxnut was depicted by Lee *et al*. (2002)

Conclusion

The enhancement of food item is a significant plan to manage weight or prevent specific nutritional deficiency. Keeping a healthy weight is significant for well-being. As well as well as bringing down the risk of heart disease, stroke, diabetes, and hypertension, it can likewise lower the risk of many different cancers. The product was made using cocoa powder, cocoa butter, jaggery, flaxseed and fox nut. The item was developed by optimizing the substance of these ingredients in order to acquire an appealing taste. Cocoa and chocolate have been acclaimed for a quite long years for their conceivable health benefits. Fox nut are also known as Makhana, Euryale Ferox, lotus seed, is utilized as a part of medicine as it has a medicinal properties as well. Foxnut is good for heart health also as it is low in cholesterol, sodium and saturated fat. It is rich in magnesium, potassium, magnesium and phosphorus. Flaxseed also known as Linum Usitatissimum, comes from family Linacea. Flaxseed is a good source of phytoestrogen, omega-3 fatty acid and alpha linolenic acid. Flaxseed is utilized in treating hypertension, atherosclerosis, diabetes, found to be beneficial in lowering the risk of several cancer. Jaggery is unrefined natural sugar which is great source of a few vitamins and minerals to be specific vitamin B complex, folic acid, phosphorus etc. It helps in detoxifying liver, blood pressure control and improvement of metabolism also. Cocoa butter is also known is Theobroma oil. It has beneficial properties in heart disease and bone strength.

In present study, dark chocolate was made utilizing cocoa butter, cocoa powder, jaggery, flaxseeds and fox nut. The item was for overall acceptability through sensory evaluation. The assessment was performed by 30 specialists in 18 to 50 years of age group. The score was given for taste, aftertaste, flavor, texture, mouthful, sweetness, hardness, aroma, overall acceptability on a 9-point hedonic scale. Hedonic scale for the developed chocolate was high for the most of the characteristics. The maximum average score was 9 and was given for taste, texture, and flavor. The overall acceptability was 90.15%. On the other hand, the overall acceptability for control group was 85.65%. The composition of nutrients for proximate estimation had 29.15%±0.09 carbohydrate, 63.78%±0.47 fat, 28.32%±0.02 protein, 1.05%±0.022 fiber, 1.15%±0.02 ash and 4.85%±0.41 moisture in developed product. For the control the result was follows as: 31.45%±0.06 carbohydrate, 66.34%±0.032 fat, 26.34%±0.07 protein, 1.10%±0.028 fiber, 1.25%±0.07 ash and 5.15%±0.04 moisture content. Antioxidant level of the product was detected by DPPH and FRAP. The reading recorded for developed chocolate is 45.5±0.11 and Control has average antioxidant activity 35.5±0.23 by DPPH. By FRAP in developed product it is 46.3±0.44 and in control has 33.2±0.32 for methanolic extract. For water extract developed product show antioxidant activity i.e., 38.5±0.12 and for control 31.3±0.21 by DPPH. By FRAP developed product have antioxidant activity is 42.6±0.44 and control is 31.2±0.28. Thus the developed product was overall nutritiously good and found fairly acceptable also.

References

1. Kondrashov, A. & Sevcik R. (2015). The major determinants of Antioxidant Capacities in chocolate and cocoa products. *Vitamins, Antioxidants and Minerals,* **34(1)**, S123.

2. Sluiter, A. (2005). Determination of Ash in biomass. *Bettelle*: National Renewable Energy Laboratory.

3. Ahmed, D. (2015). Antidiabetic, antioxidant, antihyperlipidemic effect of extract of Euryle ferox Salisb. with enhanced histopathology of pancreas, liver and kidney in streptozotocin induced diabetic rats. *Springerplus*, **3(4)**, 315. doi: 10.1186/s40064-015-1059-7.

4. Allgrove, J., Farrell, E., Glesson, M., Williamson, G. & Cooper, K. (2011). Regular Dark Chocolate Consumption's Reduction of Oxidative Stress and Increase of Free-Fatty-Acid Mobilization in Response to Prolonged Cycling. *Int. J. Sport Nutr. Exerc. Metab*., **21(2)**, 113 – 23.

5. Apgar, J. L. & Tarka, S. M. (1999). Methylxanthines. In: Chocolate & Cocoa: Health and Nutrition. Knight, I., Ed., Blackwell Science Ltd, Oxford.

6. Ariefdjohan, M. W. & Savaiano, D. A. (2005). Chocolate and cardiovascular health: is it too good to be true? Nutrition Reviews.

7. Aurelia Magdalena Pisoschi, G. P. (2011). Methods for Total Antioxidant Activity Determination: A Review. Biochemistry and Analytical Biochemistry.

8. Buijsse, B., Feskens, E. J. & Kok, F. J. (2006). Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study.

9. F. Leslie Hart, H.J. (1971). Introduction- General methods for Proximate and Minerals Analysis. Springer.

10. Gregory D Miller, J. K. (1999). Handbook of Dairy Food and Nutrition. CRC Press.

11. Harnoor Channi-Tiwary (2020). Health Benefits of Makhana: The Desi Snack That's Making a Comeback.

12. Ines Thamke, K.D. (2008). Sensory description of dark chocolates by consumers. Elsevier Ltd.

13. Jakob, H. & Waterborg, H. R. (2013). The Lowry Method for Protein Quantitation. In H. H. Carmen Buchrieser, Methods in Molecular Biology. Hertfordshire: Springer.com.

14. Katherine, M. & Phillips, M.T. (1997). Simplified Gravimetric determination of Total Fat in food composites after Chloroform-methanol Extraction. *Journal American Oil Chemists Society*, **74(2)**, 137 – 142.

15. Lawless, H. T. & Heymann, H. (2010). Sensory evaluation of food: principles and practices: Springer Science & Business Media.

16. Lee, S.E., Ju, E.M. & Kim, J.H. (2002). Antioxidant activity of extracts from Euryale ferox seed. Exp. Mol. Med., **34(2)**, 100 – 6.

17. Meriel, L. & Harwood, J. E. (2017). Sensory evaluation of chocolate and cocoa products. In M. S. Stephen T. Beckett, Beckett's Industrial Chocolate Manufacture and Use.

ISSN: 2583-3499 (online)
Volume 1, Issue 3, Dec 2021

18. Pravin, M. (2016). Makhana (Euryle ferox Salisb.) A Review. *International Journal of Ayurveda and Pharmaceutical Chemistry*, **4(2)**, 69 – 76.

19. Warwik, L. Marsden (1982). Evaluation of the DNS Method for Analyzing Lignocellulosic Hydrolysates. *Journal of Chemical Technology and Biotechnology*. **32 (7-12)**, 1016 – 22.