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DEVELOPMENT OF OMEGA 3 RICH LINUM USITATISSIMUM (FLAXSEED) YOGHURT BASED MILK CHOCOLATE WITH INCORPORATION OF COCOA & VANILLA FLAVORS

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ABSTRACT

Consumer demand for healthier foods with improved taste and convenience urged to develop of functional foods added with bioactive ingredients that can supplement basic nutrition (food supplement). In this study, a milk chocolate formulation was advanced to serve as a vehicle of Omega-3 (ω3) polyunsaturated fatty acids (PUFAs) and probiotics. In this study, a milk chocolate formulation was developed to serve as a vehicle of Omega-3 (ω3) polyunsaturated fatty acids (PUFAs) and probiotics (L. bulgaricus and S. thermophilus). Flaxseed oil and flaxseed milk & yogurt were incorporated in chocolate as a source of ω3 PUFAs. Probiotics (Prob) and Flaxseeds and their oil were added during preparation, obtaining chocolates with 19 to 21% of ω3 PUFAs, and $>1 \times 10^6$ CFU of Prob per chocolate portion (5 g). Nevertheless, bioactive ingredient addition is a major technological challenge that affects the texture, rheology, and sensory properties of chocolate. The basic aim of the study was "Development of Omega 3 Rich Linum usitatissimum (flaxseed) Yoghurt-Based Probiotic Chocolate with Incorporation of Cocoa & Vanilla Flavors" is prepared as per the standard method using activated culture of Lactobacillus delbrueckii and Streptococcus thermophilus which are added to the Flaxmilk which convert into flax yogurt and also added different levels of cocoa and vanilla flavors i.e., C_1, C_2, V_1 & V_2 while Control sample C_0 was prepared without addition Flavors and Probiotic flax yogurt into the skim milk powder and the product showed adequate overall acceptability. Proximate composition of raw materials (pH, TSS, Carbohydrates, calcium contents, lactose, protein omega 3, and fatty acid profile), Sensory attributes, Physico-chemical constituents, Microbiological analysis, Theoretical Energy Value was analyzed from the prepared product, and data collected from that were tabulated. Incorporating probiotics and omega -3 (ALA) into chocolate could offer a good alternative to common dairy products as it is liked by people of all age groups and is a healthy value-added product. Current thesis reviews recent technologies aimed at incorporating probiotics and omega 3 into chocolate and related ingredients, Flaxseed and flaxseed oil as a functional food, and the ways to enhance or sustain their viability during their processing and storage.

Keywords: Probiotics, Probiotic chocolate, omega 3 (ALA), Flaxseed, Flaxseed oil, skim milk powder, bioactive components, Flavors.

1. INTRODUCTION

Today, the population seeks new intake behaviors and lifestyles to advance their life expectancy. These new consumption habits can help to prevent various diseases such as diabetes, cardiovascular disease, obesity, Alzheimer's, Parkinson's, etc. For this reason, new functional foods are to be developed at the next-generation level that can meet these demands. Next-generation functional foods added with bioactive ingredients have high potential because they can reduce chronic degenerative diseases while providing proper nutrition [1,2]. Similarly, the abundant study focuses on categorizing functional food components as product fortifiers to deliver health benefits beyond the nutritional value. These bioactive complexes are those ingredients that, when supplementary to food, give a health enhancement. The main bioactive ingredients, also known as nutraceuticals, which have been used over time, are prebiotics, probiotics, amino acids, proteins, omega-3s (ω3) polyunsaturated fatty acids (PUFAs), plant extracts, antioxidants, and vitamins, among others. The food industry has attained favorable consequences in the development of new

foodstuffs fortified with Nutrients. Similarly, it is estimated that worldwide the bioactive compounds to be used in the most substantial proportion in the following years are fibers, vitamins, plant extracts, carotenoids, probiotics, and ω3 PUFAs [3].

The incorporation of bioactive ingredients such as ω3 fatty acids in children's diet is essential because they play a crucial role in cognitive development [4]. Fatty acids of the n-3 family, such as docosahexaenoic acid (C22:6, DHA) and eicosapentaenoic acid (C20:5, EPA), are fundamental for early-stage development and growth. These fatty acids' deficiencies affect brain performance, inducing behavior and cognitive impairments [5]. Similarly, it has been exposed that there is a connection between the cognition of the brain with marginal functions through the interaction of the central nervous system in the brain and the enteric nervous system in the intestine [4]. Consequently, targeting gut microbiota is vital for the expansion of strategies for health care. In this context, Prob has potential in the treatment of neurological problems [4]. Similarly, it has been initiated that the addition of lactic acid probiotic bacteria in functional products assisted in improving cognitive function in Alzheimer's patients [6]. In addition, the reduction of probiotic strains, such as L. Plantarum has been associated with cognitive impairment [7]. Therefore, the addition of probiotic strains such as L. Plantarum has been found to advance cognitive operational in patients with major depression [8]. In addition, other lactic bacteria such as L. Bulgaricus have shown improvements in children's and adolescents' cognitive function, causing the reduction of behavior and communication disorders such as autism [9]. Consequently, the progress of next-generation functional foods is vital to make accessible to consumers products with advantageous health effects.

The development of new products containing bioactive ingredients can be challenging because various parameters need to be considered, such as the vehicle, the compound’s safety, product stability, and possible interactions between the ingredients [3]. The acceptance of these foods depends mainly on the vehicle type used to carry the main compound [9]. Chocolate, combined with a healthy lifestyle, has been considered a functional food [10]. For this reason, it has been found that milk chocolate can function as a vehicle for the easy and efficient administration of compounds as it has been confirmed to be suitable for carrying both ω3 PUFAs and probiotics.

In the present study, probiotics (Lactobacillus. Bulgaricus, and Streptococcus. thermophilus), were incorporated by adding them into flaxseed milk which convert to flax yogurt, using flaxseed and flaxseed oil to make the omega 3 rich product combined with the skim milk powder, and the study was conducted on the microbial evaluation, physicochemical characteristics and sensory acceptability of milk chocolate were evaluated. Probiotic flax yogurt is added in different ratios i.e., 1:2, and flavors like cocoa and vanilla are added in different concentrations to the milk chocolate to improve the taste since flax oil gives an off-flavor to the product.

Probiotics - Probiotics square measure outlined as live microorganism cells, that promote the health of customers by maintaining or up their gut microorganism balance (Fuller, 1989). Throughout the past 20 years, health-promoting microorganisms are progressively incorporated into business food products in an exceedingly response to the consumers' demand for healthy food choices which will strengthen overall health, viscus performance, and absorption (Menrad, 2002). Consumption of foods supplemented with live cells of Lactic acid bacterium (LAB), especially and their probiotic strains is believed to learn human health because of their well-documented positive impact on the performance of the digestive tube and system, reduction of cholesterin, and considerable anticancer activity (Yoon et al., 2006).

Prebiotics - One product in this category is ‘Prebiotics’ which are non-digestible carbohydrates selectively fermented to stimulate bacteria in the gut. Prebiotics once combined with probiotics type of synbiotics that offer the body with smart bacterium and additionally act as a nutrition supply for them. The synbiotic idea has additionally been used while modeling ice-creams. the thought was to require the advantage of a stable texture in ice-cream (owing to the employment of prebiotic) (El-Nagar et al., 2002) together with augmented survival of probiotics within the intestines whereas the merchandise is digestible within the body.

Flaxseed - Flaxseed is utilized as a versatile ingredient in various types of food products, among the functional foods, flaxseed has emerged as a potential functional food is a good source of alpha-linolenic acid, lignans, high-quality protein, soluble fiber, and phenolic complexes (Oomah 2001). Flaxseed gaining popularity because of its high content of polyunsaturated fatty acids, protein, soluble fiber, and phytochemicals (Kajla et al., 2015). The flaxseed is 35% oil, of which 55% is ALA, a polyunsaturated, n-3 type fatty acid. Also, the

seed is 35% fiber, 28 to 30% protein, and 6% ash, or minerals (J. F. Carter 1993). The presence of ALA provides nevertheless health benefits.

Milk powder - Powdered milk, conjointly known as dried milk, could be a pre-heated foodstuff created by evaporating milk to a concentration of 45-47% TS and contains less than 2% moisture content. Skim milk powder imparts on food products not only a milky flavor but also desirable physical properties, thereby satisfying preference requirements. The nutritional value is also expected to be increased through the addition of skim milk powder because it is rich in nutrients such as lactose, proteins, and calcium. Chemical and physical variations in skim milk powder components are essential during the manufacturing course.

Flavors - A excellent quality cocoa powder must be relatively free-flowing, stable and uniform in color and flavor, of good microbiological quality, and easy to handle by the user (Vu et al, 2003). Artificial vanilla extracts are less complex and typically contain vanillin, ethyl vanillin, and other related complexes that are prepared from inexpensive starting resources. Numerous liquid chromatographic methods have been developed to quantitate coumarin, vanillin, and ethyl vanillin in vanilla essence. Because of advancements in chemistry and pharmacology, most of the earlier uses of vanilla have given way to functional uses of vanillin, vanilla's main constituent.

2. MATERIALS AND METHODS

2.1 Collection of Raw Materials

Freeze-dried probiotic live culture (Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus.), Flaxseeds, Flaxseed oils, Cocoa Powder & Vanilla essence purchased are through online and Milk Powder, Powdered sugar & Roasted Flaxseed & Almond powder are used to make the omega 3 rich flaxseed yogurt-based probiotic chocolate with the incorporation of cocoa and vanilla flavors.

2.2 Extraction of Probiotics from Culturing of Freeze-dried L. bulgaricus and S. thermophilus

To Prepare a Lactobacillus MRS broth (100ml) – take 100ml to distill water in a clean and sterilized 500ml conical flask. Weigh 6.75 gms of MRS Powder and add to the taken distill water in a conical flask. Close the mouth of the conical flask with cotton cork and autoclave for 15 min at 121°c. Select the freeze-dried culture of Lactobacillus delbrueckii and Streptococcus thermophilus and weigh 2gm and inoculate the culture in MRS broth - Add the weighed freeze-dried culture to the sterilized broth and incubate at 37℃ for 68 - 72 hrs. After Obtaining growth in broth centrifugation is done to collect the bacteria pellets at 4500rpm for 30 mins and store the pellets in Eppendorf tubes at ≤ 4℃. Then the addition of bacteria pellets to the pasteurized flaxseed milk to convert into yogurt.

2.3 Preparation of Flaxmilk and Flaxmilk Yogurt

Take 1/4th cup of flaxseeds in a clean container and add 1/2 cup of water to it for soaking (1:2 Ratio) and close the lid partially. Soak it Overnight. The Flaxseeds double their volume and obtain 1/2 cup of flaxseed. Pour the soaked flaxseed into a strainer and wash it through running water until the stickiness is gone. Transfer the flaxseeds into a clean blender and blend it with the addition of water (For 1/2 cup of flaxseed add 2-3 cups of water). Now take an empty clean stainless vessel and keep the strainer on it with muslin cloth on top of it and strain the liquid. Now we get Flaxseed Milk & Flaxseed Meal. Collect the Flaxseed Milk and boil it by continuous stirring till the temperature reaches 70-72℃ (Pasteurization) and mix continuously till it cools. Cool the flax milk (35-45℃). The addition of Probiotics to the flax milk is the key part to making probiotic flax yogurt, add 2gm of extracted culture and mix it thoroughly until it combines well. It contains 2°X brix. Fermentation undergoes at Room temperature, after 6-8hrs we will obtain flax milk yogurt and store it in a refrigerator at a temperature of ≤ 4°c.

2.4 Preparation of Omega 3 Rich Flaxseed yogurt-based Probiotic chocolate Incorporated with Cocoa and Vanilla Flavors

Omega 3 rich flavored Probiotic chocolate was prepared with different ratios of 1:2 of probiotic flax yogurt and flavors respectively in 5 different treatments.

C_0: control chocolate sample, doesn't contain any probiotic yogurt, Flaxseed Oil or flavor added.
C_1: Probiotic chocolate sample was added with 5g of probiotic yogurt and 2.5g of cocoa powder.
C_2: Probiotic chocolate sample added with 7.5g of probiotic yogurt and 5g of cocoa powder.
V_1: Probiotic chocolate sample added with 5g of probiotic yogurt and 0.3ml of vanilla essence.
V_2: Probiotic chocolate sample added with 7.5g of probiotic yogurt and 0.6ml of vanilla essence.

Aimed at the preparation of probiotic chocolate, first select the required raw ingredients in required quantities in different bowls as per standardized treatments. Now add all the dry ingredients into the bowl, firstly add Milk Powder into an empty bowl (40g) and addition of powdered sugar (10g) and then the addition of roasted almond 1 tbsp and flax powder ½ tsp. Mix all the dry ingredients well. Now the addition of yogurt in given proportions to make different treatments, to get perfect texture of mix we have to add flax milk and addition of Flaxseed oil and finally add flavors to the mixture in different proportions. While in the control product we only add flax milk instead of yogurt and no flavors are added to it. Mix them well and make a pure control and transfer it to a Molds for giving particular structure Remove chocolate from the mold and place on a plate which is labeled already with a marker for easy identification of the chocolate and cover the plates with aluminum foil and freeze them in the refrigerator for 30 mins to 1 hour at 0 - 4°c. They can also pack in chocolate wrappers/aluminum foil and store it at 4°c.

2.5 Determination of Sensory Analysis

Sensory quality is a combination of different senses of a perception coming into play in choosing and eating a portion of food. This reaction is highly conditioned by a variety of psychological and social factors thus playing a vital role in the acceptance and preference of food. The systematic sensory evaluation was carried out on prepared products having 2 different flavors & probiotic yogurts in two different ratios i.e.,1:2 compared each with the control product. Sensory evaluation of control as well as experimental products was carried out by a 9-point hedonic scale test.

2.6 Determination of Microbial Analysis

2.6.1 Enumeration of total plate count in chocolate (AOAC, 2005)

The second dilutions of probiotic chocolate samples were prepared, have been used for the enumeration of coliforms. The 50 µl from 10^9 dilution were taken in duplicate into Petri plates which containing solidified MRS Agar which was prepared earlier by adding 6.7g in 100ml distilled water and autoclave it for 15 mins at 121° C at 15 lbs pressure and add to Petri plates by pouring method after slightly cooled and then the plates were allowed to solidify. These plates were incubated at 37˚c for 1-2 days.

$$\text{Bacteria per milliliter/gram of sample} = \frac{\text{Number of colonies}}{\text{Amount Placed}} \times 100$$

2.6.2 Enumeration of Yeast and Mold Count (AOAC, 2005)

The dilutions 10^9 of probiotic chocolate samples were taken in duplicate into Petri plates, used for enumeration of yeast and mold forms. The 50 µl from 10^9 dilution were taken in duplicate into Petri plates which containing solidified Agar which was prepared earlier by adding 3.9g in 100ml distilled water and autoclave it for 15 mins at 121° C at 15 lbs pressure and add to Petri plates by pouring method after slightly cooled and then the plates were allowed to solidify. It was then poured plated using yeast PDA (Potatoes dextrose agar) add the diluted sample over the plate and spread and then incubates at room temperature 22-25°C in a dark place for 3-4 days.

2.7 Proximate Composition Analysis of Omega 3 Probiotic Chocolate

2.7.1 Estimation of Ph (AOAC 2000)

The pH content was determined by (M. tronoic digital-255) pH meter

2.7.2 Estimation of TSS

The TSS was determined by using a Hand refractometer (ERB, ERMA - 0-32°X)

2.7.3 Estimation of Moisture (AOAC 930.15)

Accurately weigh about 5 g of sample in a dish previously dried (at 105°c for 20 min) and weigh. Place the dish in a hot air oven, remove the cover of the dish and dry the material for 2 hours at 135° ± 1°C. Cover dish, transfer to a desiccator, and weigh soon after room temperature is attained. Report percent loss in weight as moisture %.

$$\text{Moisture \%} = \frac{W_1 - (W_3 - W_2)}{W_1} \times 100$$

2.7.4 Estimation of ASH (AOAC 942.05)

Place the clean crucibles in a hot air oven to heat at 105° C for 20 minutes. Remove the crucibles, cool in a desiccator for one hour, and weigh the crucible (W). Weigh 2 g of sample in the crucible (W1). Place the crucibles inside the muffle furnace and heat to 700° C for 2 hours. Let the furnace cool and take out crucibles containing ash, clean and white in appearance. Cool the crucible in a desiccator and reweigh (W2) the crucible containing ash.

$$\text{Ash \%} = \frac{(W_3 - W_2) \times 100}{W_1}$$

2.7.5 Estimation of Crude Protein (AOAC 1980)

To find out the amount of crude protein in a given food sample. The Kjeldahl procedure can be divided into three parts: (1) digestion, (2) distillation, (3) titration

Take 2 gm of sample in a Kjeldahl digestion flask. 10 ml of concentrated sulphuric acid and a pinch of digestion mixture were added to it. After that, the sample was digested and made-up vol.100 ml with distilled water in a volumetric flask. 10 ml aliquot of the digested sample was taken in the Kjeldahl flask. 10ml 50% sodium hydroxide (NaOH) and 10ml distilled water were added to the Kjeldahl flask containing the sample and connected with distillation assembly. The sample was boiled for 10-15mintues. Liberated ammonia was collected in a flask containing 10ml of 2% boric acid with 2-3 drops of mixed indicator. This was titrated with 0.02 N sulphuric acid until pink color appears. For blank 5 ml, diluted sulphuric acid was taken and treated as a sample.

$$\%N = \frac{\text{Tire value (sample – Blank)} \times 0.028 \times 100}{\text{Weight of the sample}}$$

$$\text{Protein (gm \%)} = N\% \times 6.25 \text{ (std Value)}$$

2.7.6 Estimation of Total Carbohydrates

Pipette out into a series of test tubes different volumes of glucose solution from the supplied stock solution (200µg /ml) and make up the volume to 1 mL with distilled water. Consider tube 1 as blank and tubes 2 through 9 for construction of a standard curve. Tubes 10-15 are for the unknown samples. To each tube add 5 mL of the anthrone reagent (supplied) and mix well by vortexing. Cool the tubes and cover the tubes with marbles/ Caps on top and incubate at 90o C for 17 minutes or boiling water bath for 10 minutes. Cool to room temperature and measure the optical density at 620 nm against a blank. Prepare a standard curve of absorbance vs. µg glucose. Determine the amount of glucose in the sample by plotting a standard curve on the Y-axis and µg of Glucose on the X-axis.

$$\text{Reducing Sugars \%} = \frac{\text{Dilution x Factor of Fehling (in gm) X100}}{\text{Weight of sample x Titre value}}$$

2.7.7 Estimation of Lactose

Let V1 = ml of diluted sample filtrate required for the titration of 10 ml of Fehling's solution. Let V = ml of the Standard solution of invert sugar (Reagent C) required for the titration of 10 ml of Fehling's solution

Now 10 ml of Fehling's solution = V ml of standard reducing sugar solution

= V1 ml of diluted sample filtrate

V1 ml of diluted sample filtrate =V ml of standard reducing sugar solution

= V x M mg of standard reducing sugar or W1 mg of standard reducing sugar

Therefore, 100 ml of sample filtrate contains = $\frac{W_1}{V_1}$ mg of original reducing sugar

Since 100 ml of this sample filtrate was obtained from 50 ml of B1 solution

Therefore, 250 ml of B1 sample filtrate will contain = $W_1 \times \frac{100}{V_1} \times \frac{250}{50}$ mg of original reducing sugar

Since 250 ml of sample filtrate was obtained from 40 g of SCM

Therefore, Lactose (g) present in 100 gm of SCM (say C1) = $\frac{W_1}{V_1} \times \frac{100}{50} \times 250 \times \frac{100}{W_2} \times \frac{1}{1000}$

2.7.8 Estimation of Crude Fiber

Extract 2g of ground material (WS) with ether or petroleum ether to remove fat (Initial boiling temperature 35 -38°C and final temperature 52°C). if the fat content is below 1%, extraction may be omitted. After extraction with ether boil 2g of dried material with 200mL of sulphuric acid for 30min with bumping chips. Filter through muslin and wash with boiling water until washing is no longer acidic. Boil with 200mL of sodium hydroxide solution for 30min. Filter through muslin cloth again and wash with 25mL of boiling 1.25% H2SO4, three 50mL portions of water, and 25mL alcohol. Remove the residue and transfer it to the ashing dish. Dry the residue for 2h at 130 ±2°C. Cool the dish in a desiccator and weigh (W_1). Ignite for 30min at 600 ±15°C. Cool in a desiccator and reweigh (W_2).

$$\% \text{ Crude fiber in ground sample} = \frac{W_1 - W_2 \times 100}{WS}$$

2.7.9 Estimation of Fat

First of all, rinse all the glass apparatus with petroleum ether and dry it in the oven at 102°c and after removing it keep it in the desiccator. Weigh 5 grams of grounded and dried sample and place it in the thimble. Place the thimble in the Soxhlet extractor. Take a 150ml round bottom flask and clean it and fill the flask with 90 ml petroleum ether. Place the whole set on a heating mantle and allow the petroleum ether to boil. Continue the extraction process for several hours, almost 6 hours. Remove the condensing unit from the extraction unit and allow the sample to cool down. Finally, it removes all the lipid. Collect almost all the solvent after distillation. Place the sample in the oven and after removing it place it in the desiccator. Take the weight of the sample. As a result, we get a defat sample.

$$\text{Then crude fat percentage} = (w2\text{-}w1) / Ws \times 100$$

2.7.10 Estimation of omega 3 by GC-MS standard method

One gram sample weighted in the closed tube then diluted in 2 mL hexane, added by 2 mL BF3/Methanol 14 %. The mixture solution was heated in a water bath within a shaker at a temperature of 75°C for 30 minutes, continue by centrifugation until a two-layer solution yielded. The upper layer was taken and ready to be injected into the GCMS instrument. One microliter fatty acid methylene ester that has been prepared is also injected into the GCMS instrument. The operation conditions of the chromatography device were suitable for fatty acid methylene ester. The resulting chromatography peaks were scanned to read the mass spectra that were then interpreted to determine the type of molecule of every peak.

2.8 Evaluation of Shelf Life

The omega 3 rich probiotic chocolate was packed in Aluminum Foil and stored under refrigeration temperature (4˚c). The packed probiotic chocolate samples confirming to the 5 different treatments (C_0, C_1, C_2, V_1, V_2) were studied for storage stability during a storage period of 30 days under refrigeration temperature (4˚c)

2.9 Theoretical Energy Value

The energy value of omega 3 rich probiotic chocolate was computed from the proximate composition of the Pizza base by taking the value of 4, 4, and 9 kcal for carbohydrates, protein, and fat respectively.

3. RESULT AND DISCUSSION

Here are the results for sensory evaluation, Microbial analysis, Physio-chemical analysis, Theoretical energy value estimation, and the techno-Economical feasibility of the omega 3 rich probiotic chocolate.

3.1 Results obtained for sensory evaluation

Table No: 4.1 Organoleptic Evaluation of Omega Probiotic Chocolate Incorporated with Cocoa & Vanilla Flavors

S. No	PRODUCT CODE	COLOR	FLAVOR	AROMA	TASTE	APPEARANCE	OVERALL ACCEPTABILITY
1.	C_0	7.5	7	7	8.2	8	6
2.	C_1	8	8.5	7.4	8.6	8.5	8.2
3.	C_2	8.5	9	8	9	8.5	8.5
4.	V_1	8	7.9	7.6	8	7	7.4
5.	V_2	8.5	8	7.9	8.5	8	8

3.2 Results obtained for microbial analysis

Table no 4.2 Microbial Analysis of omega 3 probiotic chocolate with cocoa & vanilla Flavors

Storage period	Total Plate Count (CFU/50µl)			Yeast and Mould count (CFU/50µl)		
	C_0	V_1	C_2	C_0	V_1	C_2
1st Day	ND	ND	1×10^2	ND	ND	ND
2nd Day	ND	0.3×10^2	4×10^2	ND	ND	ND
3rd Day	ND	1.9×10^2	7.5×10^2	ND	0.1×10^2	0.35×10^2
4th Day	ND	2×10^2	1.1×10^3	ND	1.7×10^2	1.22×10^2

3.3 Results obtained for Physio-chemical analysis

3.3.1 pH

Table. No.4.3.1 Value of the pH in omega 3 probiotic chocolate prepared with different treatments

Sample Code	pH
C_0	6.61
C_1	6.66
C_2	6.71
V_1	6.69
V_2	6.90

3.3.2 TSS

Table. No.4.3.2 Value of the TSS in omega 3 probiotic chocolate prepared with different treatments

Sample	TSS
C_0	8
C_1	7.5
C_2	9
V_1	8.5
V_2	7.5

3.3.3 Moisture

Table. No.4.3.4 Value of the Moisture% in omega 3 probiotic chocolate prepared with different treatments

Sample Code	Moisture%
C_0	15.1
C_1	13.6
C_2	17.3
V_1	11.8
V_2	15.05

3.3.4 Ash

Table. No.4.3.5 Value of the Ash% in omega 3 probiotic chocolate prepared with different treatments

Sample Code	Ash %
C_0	2.24%
C_1	5%
C_2	4.8%
V_1	5.3%
V_2	2.1%

3.3.5 Protein

Table. No.4.3.6 Value of the protein% in omega 3 probiotic chocolate prepared with different treatments

Treatments	Protein %
C_0	12.58
C_1	12.76
C_2	13.46
V_1	12.9
V_2	13.22

3.3.6 Carbohydrates

Table. No.4.3.7 Value of the CHO in omega 3 probiotic chocolate prepared with different treatments

Treatments	CHO in g
C_0	2.11
C_1	2.24
C_2	2.33
V_1	2.15
V_2	2.19

3.3.7 Lactose

Table. No.4.3.9 Value of the lactose in omega 3 probiotic chocolate prepared with different treatments

Treatments	Lactose In mg
C_0	1.08
C_1	0.98
C_2	1.05
V_1	1.08
V_2	1

3.3.8 Crude fiber

Table. No.4.3.10 Value of the crude fiber% in omega 3 probiotic chocolate prepared with different treatments

Treatments	Crude Fiber %
C_0	8.7
C_1	9.2
C_2	8.5
V_1	9
V_2	9.8

3.3.9 Fat

Table. No.4.3.11 Value of the Fat% in omega 3 probiotic chocolate prepared with different treatments

Treatments	Fat %
C_0	28.89
C_1	29.22
C_2	29.57
V_1	28.96
V_2	30.02

3.3.10 Omega 3

Table. No.4.3.12 Value of the omega 3% in omega 3 probiotic chocolate prepared with different treatments

Treatments	Omega 3 %
C_1	19
C_2	21
V_1	19.5
V_2	20.5

3.4 Results obtained for the theoretical energy value

Table no.4.13 Measurement of the theoretical energy value of omega 3 probiotic chocolate with cocoa & vanilla Flavors

Sample	Nutrients per 5g			Energy Value of product K. Cal/5g
	CHO/g	Protein/g	Fat/g	
C_0	2.11	0.31	0.72	16.16
C_1	2.24	0.32	0.73	16.81
C_2	2.33	0.34	0.74	17.34
V_1	2.15	0.32	0.72	16.36
V_2	2.19	0.33	0.75	16.83

According to the sensory analysis, all different treatments were accepted but mostly liked one is C_2 which is cocoa-flavored of omega 3 enriched Probiotic chocolate. The results obtained after conducting physio-chemical analysis were satisfactory and the omega 3 enrich probiotic chocolate showed good quality characteristics on pH, Acidity, Moisture, Total solid, Ash, Fat, Protein, Lactose, omega 3, calcium. The overall acceptability was found with C_2 (cocoa-flavored omega 3 enriched probiotic chocolate). A high pH value from treatment V_2 (6.90), an increase in the percent of moisture which was high in V_2 (17.3%), level of total solid content was higher in C_2 (9). Ash content was higher with treatment V_1 (5.31%), higher fat content was observed in V_2 (30.02%), higher content of protein in C_2 (13.46%), maximum lactose content was found in treatments V_1 and control sample is 1.08 g and other samples shows nearly similar content of lactose, The crude fiber estimates high in the sample V_2 (9.8%), the estimation of total carbohydrate content shown the highest value in the C_2 probiotic chocolate with high cocoa content of 2.33g and the highest percent of omega 3 content present in omega 3 enrich probiotic chocolate is C_2 of 21%. Efforts have been made to study the shelf life of the omega 3 rich-flavored probiotic chocolate in refrigeration conditions that can last for 30 days. The data about the theoretical energy value of the omega 3 enrich flavored probiotic chocolate was found to be nearly 17k.cal per 5 grams of chocolate (1 small chocolate bar).

4. CONCLUSION

Reviewed data revealed that value-added omega 3 enrich flavored probiotic chocolate with its good quality and more acceptability can be prepared by the addition of Cocoa powder. All sample of omega 3 enrich flavored probiotic chocolate was found most acceptable in the terms of sensory as well as physiochemical scores whereas treatment C_2 obtained more satisfactory results as they were within the acceptable limit. omega 3 enrich flavored probiotic chocolate can be stored for 30 days due to limited growth of coliforms and yeast and mold was also lower, and it can be recommended as healthy confectionary and milk-based food for everyone due to its high amount of omega 3 fatty acids and being a probiotic will be

beneficial to health-conscious people and also good for the skin. The results obtained after conducting physio-chemical analysis were satisfactory and the omega 3 enrich probiotic chocolate showed good quality characteristics on pH, Acidity, Moisture, Total solid, Ash, Fat, Protein, Lactose, omega 3, carbohydrates & crude fiber. The overall acceptability was found with C_2 (cocoa-flavored omega 3 enriched probiotic chocolate).

Hence, from the research work, it was concluded that value-added omega 3 enrich probiotic chocolate which is incorporated with cocoa and vanilla flavors which are high in omega 3 fatty acids, protein and even contains a small amount of dietary fiber as compared to the normal probiotic chocolates. The study revealed that probiotic chocolate fortified omega 3 rich flaxseed oil was found to have high overall acceptability with good nutritional value. Consumption of foods formulated with omega 3 and probiotics would be an important step towards relieving problems related to lack of omega 3 fatty acids and also improve the gut health and good absorption of the nutrition into blood and body that which makes health.

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