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Preparation of Probiotic Chocolate using Camel Milk powder and Ragi malt

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Abstract

Consumer demand for foodstuffs supplemented with live LAB, preferentially probiotic ones, prompted research into the enrichment of some other foods with these microorganisms. Despite its high fat and sugar content, chocolate consumption benefits human nutrition by providing antioxidants, primarily polyphenols and flavonoids such as epicatechin, catechin, and, most notably, procyanidins. The main objective of this work is to Develop of probiotic chocolate using camel milk powder and ragi malt. Probiotic Bar was developed using raw materials such as buffalo milk, Microbial strain, Ragi malt, Camel milk powder, honey, chocolate . Honey was added as sweetener and stabilizing material (binder). The raw materials were assessed for physicochemical properties such as moisture, Ash, crude protein and fat content and microbial analysis . Four different formulation of chocolate was made. After preparation of chocolate physicochemical properties, microbiological and sensory evaluation was carried out. The chocolate showed 4.0.% moisture content, 1.84 % Ash content 7.81 % protein content, 26.90 %fat content, 59.45 % carbohydrates content . Chocolate is willingly consumed by children and teenagers. The supplementation of this product with encapsulated live probiotic cells can enrich their snacks.

Keywords: Probiotic chocolate, camel milk powder, ragi malt

Introduction

Due to their nutritional and biological value, milk and dairy products are an important part of the human diet. Dairy desserts (cream, puddings, cocktails, whipped cream) are a world-famous group of dairy products, and the most significant element for this group of products is their rheological properties (viscosity and jelly). (Peter and Glyn, 2014).

Creamy milk chocolate dessert, as a popular dairy product, could be an option for incorporating probiotics and prebiotics (Amna et al., 2015; Valencia et al., 2016) After passing through thermal processes such as pasteurisation, pasteurisation with extended shelf life, and sterilisation, airy dessert contains at least 50% fresh

milk or reconstituted milk and food additives (e.g. flavourings, sweeteners, thickeners, and stabilisers) (Ibrahim et al., 2015)

The Arabian dromedary (Camelus drumderius) and the Bactrian camel (Camelus bactrianus), a mountain camel, are the two most common camel species. According to the Food and Agriculture Organization (FAO), more than 5.3 million tonnes of camel milk are produced worldwide. Camel milk contains a significant amount of antioxidants, which help protect cells from damage, which can lead to serious diseases like cancer, diabetes, and heart disease.

It also is full of vitamins and minerals, such as:

Calcium, Vitamin A, Vitamin B, Vitamin C, Vitamin D, Vitamin E

Camel milk has 107 calories per cup.

- Protein content: 5.4 g
- Fat content: 4.6 g
- Saturated fat (three grammes)
- Carbohydrate content: 11 g
- Sugar content: 8 g

Camel milk aids in the reduction of oxidative stress. Oxidative stress occurs when the body's antioxidants and radicals are out of balance. Camel milk also aids in the treatment of autism spectrum disorder and other brain conditions. It assist in proper digestion and reduces acid reflux. This milk is high in nutrients that are beneficial to your gut health. Camel milk is a nutritious alternative to other types of milk. You can actually consume it plain or add it to tea, coffee, baked goods, smoothies, macaroni and cheese, soups, and pancake and waffle batters. Remember that camel milk from different regions may taste differently. American camel milk, for example, is slightly salty, sweet, and creamy.

Milk from camels in the Middle East, on the other hand, has a smokey and nutty flavour. Raw and pasteurised camel milk are both easily digestible. Because it is difficult to process camel milk, butter, yoghurt, soft cheese, and other camel milk products are not widely available. Camel milk is acidic due to the presence of vitamin C. This facilitates nutrient absorption in the body.

Ragi, also known as finger millet (Eleusine coracana L.), is a common millet in many parts of India. It is also identified as Korean in Sri Lanka or by various names throughout Africa, and has historically been an essential millet staple food in parts of central and eastern Africa, as well as India. (FAO, 1995). Ragi/ Finger millet is rich in carbohydrate and low in fat, which can help control obesity. Ragi malt/ Finger millet can be a good substitute for people suffering from milk allergies.

Chocolate is a typically sweet, usually brown, food preparation of The aroma cocoa seeds, roasted and ground, often flavoured, as with vanilla. It is made in the form of a liquid, paste, or in a block, or used as a flavouring ingredient in other foods. Chocolate possesses unique taste, flavour and texture and is also a source of biologically active substances, such as polyphenols, which display significant antioxidant properties and have a beneficial impact on human health, particularly on the cardiovascular system. (Richelle M, 2001).

Since the relationship between microbial community structure and the health of the host has been illuminated, and interest in the manipulation of gut bacterial populations for improved human health has increased. This can be achieved either by introducing the live advantageous bacteria in the gut, which has strengthened the concept of probiotics, or selectively reinforcing the favourable bacteria inhabiting the gut by the means of food components that are referred to as prebiotics. Synbiotics are probiotic and prebiotic products.. (Haenel and Benddig, 1975; Ferreira and Teshima, 2000).

Probiotics are defined as live microbial cells, which promote the health of consumers by maintaining or improving their gut microbial balance proven to have a much higher survival. Health-promoting microorganisms have been increasingly incorporated into commercial food products over the last two decades in response to consumer preference for nutritious foods that can improve overall health, intestinal function, and absorption. (Menrad, 2002). Due to their well-established positive effects on the health of the gastrointestinal tract and immune system, reduction of cholesterol, and notable anticancer activity, consumption of foods supplemented with live cells of lactic acid bacteria (LAB), in particular and their probiotic strains, is thought to be beneficial to human health. The survivability of these microorganisms during the production processes, storage, and through the gastrointestinal tract have been suggested to be vital in exerting the stated advantages. These health effects are also highly strain-specific. Kefir, yoghurt, and cheese are examples of conventionally fermented foods that can contain probiotic bacteria. However, food manufacturers are attempting to develop probiotic products other than dairy products in order to provide consumers with more options. As the market for these products 38 Chocolate as a probiotic carrier food expands, so will research into the development of new food products containing probiotic bacteria, such as probiotic chocolate. (Boylston, et al., 2004).

The addition of probiotics in to the chocolate could be a great option to common today dairy products while also allowing chocolate-based food products to make more health claims. Indeed, recent market research into functional food has shown that, in relation to chocolate, digestive heath was one of the most important drivers of consumer acceptance (Callebaut 2009).

One of the drawbacks of the probiotics incorporation into food products is connected with the very definition of the probiotics: they are live micro-organisms which have to reach the intestine, their site of action, alive, and in sufficient numbers. Their survival is linked to several factors: first the endogenous properties of the chosen bacteria strain, their environment (other ingredients properties, humidity, temperature, pH, oxygen etc.), the digestive process (gastric acidity, bile salts), as well as various mechanical stresses linked to food processing (Callebaut 2009) The development of probiotics containing chocolate necessitates a thorough understanding of the selected probiotic strains, the chocolate manufacturing process, and various process critical points.

Probiotics survival requires the use of specific protective technology. The current article reviews recent advances in probiotics incorporation technologies into chocolate and related products (freeze dried or microencapsulated cell preparations), the impact of various processing parameters on the viability of probiotics survivability in probiotic milk chocolate, as well as their market potential and future prospects.

MATERIALS AND METHODOLOGY

The present study entitled “Development of Probiotic chocolate using ragi malt and and camel milk powder ” was carried out in the Department of Food Technology, Parul Institute of Applied Sciences, Parul University, Vadodara. This section enlists the material used and elaborates the processing techniques, organoleptic evaluation and analytical procedure following during the research

The raw materials such as Ragi malt, camel milk powder, honey, chocolate compound etc. were procured from the local market.

Strains of Micro organism: Three strains were used

- *Streptococcus thermophilus*
- *Lactobacillus bulgari*
- *Lactobacillus delbrueckii*

Methods

1. **Preparation Of Probiotic Chakka**
   Buffalo Milk and Camel milk were taken in 90:10 proportion and heated at 100℃ for 20-30 mins and then cooled at 40-45℃ for 20-30 minutes. Temperatures were recorded using thermometer. After cooling microbial strains (*Streptococcus thermophilus, Lactobacillus bulgari, Lactobacillus delbrueckii)* were added and incubated for 80-10 hrs. After incubation the whey was drained using muslin cloth and was hanged for 6 hours. The product obtained was chakka.
2. **Mixing of Ingredients**
   Chakka was used as a base and other ingredients and different ingredients were mixed such as sugar , camel milk powder, ragi malt in different proportion. (Given in table).
3. **Preparation of chocolate**
   Chocolate was melted and added to mould, after it was set the ingredients that were mixed were added and another layer of chocolate was added.

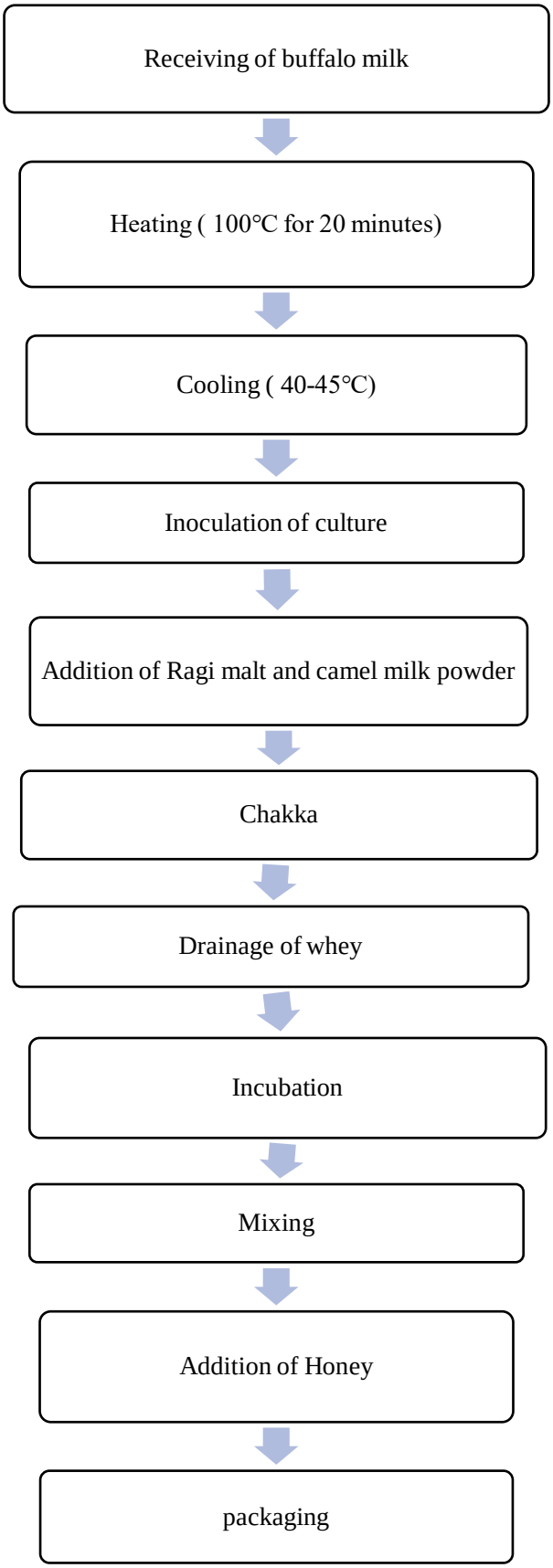

Fig 1: Flowchart on Preparation of Probiotic Chocolate using Camel milk powder and Ragi mal

Table 1: Formulations of probiotic Chocolate

Sr No	Ingredients	T0	T1	T2	T3
1	Chakka	-	10	10	10
2	Milk powder	20	20	30	40
3	Ragi malt	80	70	60	50
4	Honey	1 tbsp	1 tbsp	1 tbsp	1 tbsp
Total		100	100	1001	100

Proximate analysis

All samples were analyzed for moisture, crude protein, crude fat, total ash and total carbohydrate contents according to their respective standard methods as described in (A.O.A.C., 2000)

Moisture content:

Moisture content was estimated adopting AOAC (1990) method. The following equation was used to measure moisture content.

$$\text{Moisture content (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

Were, W1=Weight of the container with lid, g
W2=Weight of the sample before drying +weight of the container with lid, g
W3=Weight of the sample after drying+ weight of the container with lid, g

Ash Content

AOAC (1990) method using muffle furnace was used to determined ash content of the samples. The per cent ash was calculated using following formula

$$\text{Ash content (\%)} = \frac{W3-W2}{W1} \times 100$$

Where, W1= Weight of the sample, g
W2= Weight of the crucible before combustion, g
W3=Weight of the crucible after combustion, g

Protein content:

Determination of Protein content: Protein content was determined by Micro-Kjeldhal method.

- Digestion: 200mg of defatted ground sample was accurately weighed and a pinch of catalyst mixture K2So4:CuSo4:HgO red (91:8.2:0.8g) was added and then it was transferred to the digestion flask, digestion was carried out with 5ml of concentrated H2So4 for 2-3hrs at 450 C till the content becomes colorless.

- Neutralization and Distillation: Digested sample was diluted to the 50ml in volumetric flask and made final volume to 50ml with distilled water. Then the 5ml of aliquot was neutralized with 30% HCL and 40% of NaOH containing 5g of sodium thiosulphate. Distillation was carried and liberated ammonia was absorbed in 2% boric acid solution containing methyl red as indicator.

- Titration: The collected ammonia was titrated against 0.01N H2SO4. Titer reading was noted, Nitrogen was calculated by using following formula and % protein was calculated by multiplying 6.25. Simultaneously

a blank sample was also run. Crude Protein % = (Sample titre – Blank titre) × 0.0014 × 6.25 / Sample weight × 100

Fat content

AOAC (1990) method using Soxhlet apparatus was used to determined crude fat content of the samples. The percent of crude fat was expressed as follows

$$\text{Fat content (\%)} = \frac{\text{Final Weight of flask}}{\text{Intital weight of flask}} \text{x} 100$$

Total Carbohydrate

The total carbohydrate content of the samples was calculated by subtracting the protein, fat, ash, and moisture from 100 (Pearson, 1976)

% Carbohydrate =100- (% Moisture +% Ash +%Fat + % Protein)

Microbial Analysis

In food products quality analysis, microbial examination is the perfect quality assessment protocol performed. The microbial quality of prepared probiotic chocolate was determined. In the present study different microbial parameters such as Total Plate Count was examined also the samples were examined during the storage at ambient temperature. Microbial examinations were carried out as per the methods given by APHA, (1992).

Determination of total plate count

• Preparation of nutrient agar medium: 28g of nutrient agar was added in 1000ml of distilled water and it was heated till it dissolved properly. Its mouth was plugged with cotton and it was sterilized in an autoclave for 20min at 120℃ and 15lbs pressure.

• Preparation of sample solution (serial dilution): Nine sterilized test tubes were taken and numbered. In each tube 9ml of distilled water was poured. The test tubes were plugged with cotton plugs and were sterilized in an autoclave at 121℃ for 15min with 15lbs pressure. 1ml of sample was added inn 9ml distilled water of sterile test tube serially.

• Preparation of plates: Petri plates and pipettes were sterilized by hot air oven (dry heat treatment) or by autoclave (moist heat treatment). Sterilized petri dishes were taken to the laminar airflow cabinet and ultraviolet light was switched on for 30min. After 30min UV light was switched off and then blower was switched on, and the working surface was cleaned by 70% alcohol. Plates were properly marked then 1ml of samples were poured into the plates. 15-20ml of molten media was poured into each plate. This was done near a flame to prevent contamination of the plate by microbes. The plates were firmly swirled and kept for solidification. The plates were then placed into the incubator for 48hrs at 37℃ and then observed for the colonies on the plates.

Sensory Evaluation

Probiotic chocolate was evaluated by 3 panellists .

The samples were evaluated for appearance, colour, melt in mouth, texture, flavour, taste and overall acceptability, using 9-hedonic scale test as described by Larmond (1991)

Scores to Be Given As Follows

1. Liked extremely – 9

2. Liked very much – 8

3. Liked moderately – 7

4. Liked slightly – 6

5. Neither liked nor disliked – 5

6. Disliked slightly – 4

7. Disliked moderately – 3

8. Disliked very much – 2

9. Disliked extremely – 1

Results and Discussion

The parameters like moisture content, protein content, ash content, fat content, carbohydrates were evaluated for probiotic chocolate and presented in the table 2.

Table 2: Proximate composition of probiotic chocolate

Sr No	Parameter	Per 100 g
1	Moisture	4.0 %
2	Ash	1.84%
3	Protein	7.81g
4	Fat	26.90 g
5	Carbohydrate	59.45 g

Organoleptic evaluation of probiotic chocolates

Sensory evaluation acceptance tests was performed for probiotic chocolates which was formulated by addition of Probiotic Chakka, Camel milk powder, Ragi malt and honey in different proportions to know the acceptability of products prepared. The acceptance scores were assigning for vari

es sensory parameter like colour, flavour, taste, texture, ap

pearance and overall acceptability.

Table 3: Organoleptic evaluation of probiotic chocolate chocolate

Sr No	Parameter	T0	T1	T2	T3
1	Colour	7	6	7	8
2	Flavour	7	7	6	8
3	Texture	7	6	7	8
4	Appearance	7	7	7	8
5	Overall acceptability	7	7	7	8

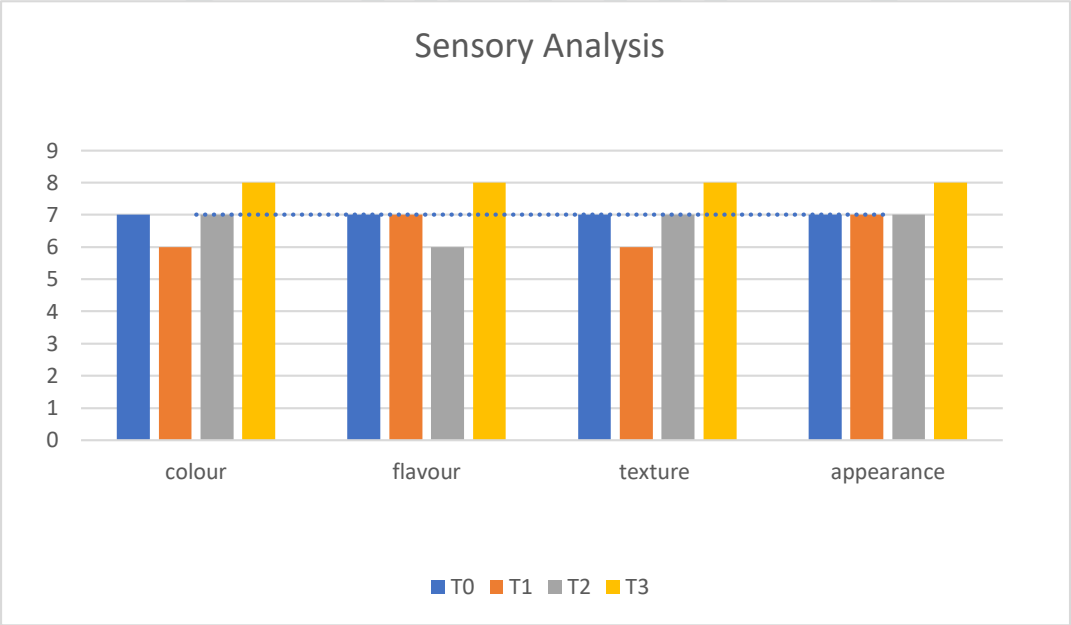

Fig 2: Sensory analysis of prepared probiotic chocolate

It was observed that highest overall acceptability score was awarded for Sample T3 8 because it got acceptable result in colour, flavour, texture, appearance and overall acceptability. So that based on sensory data T3 sample was finalized for the further analysis.

1. Moisture Content

The moisture content of chocolate was evaluated using a 5gm capacity infrared moisture meter. The sample was placed on a plate and attached to the meter. After inserting the sample into the meter, the needle was reset to zero because moisture will cause the needle's reading to alter.

2. Ash Content

The ash content of meals shows the total mineral content. The ash content of chocolate was determined using a muffle furnace, in which a 10 g sample was taken and weighed, and then a dried crucible was weighed. It

then burns away the chemical compound in air at temperatures above 500 degrees Celsius for 8 to 10 hours, and then it is cooled in a desiccator and the burning sample is weighed again.

3. Fat Content

The fat content of this product is affected by the addition of chocolate. Chocolate contains a high fat compared to other constituent ingredients. Fat in the body acts as an energy source, especially in sports with moderate intensity in a long time, for example, endurance (Rismayanthi, 2015). It was observed that the fat content of chocolate was higher (26.90 %). The significant increase in the fat content of chocolate was as a result of the contribution of ingredients added in the production of chocolate such as milk powde.rFats, particularly unsaturated fats, are easily oxidized and reduce the shelf-life of food products. (Borchers et al., 2000; Afoakwa et al., 2007) The fat analysis of chocolate was done using Soxhlet. 5g of sample was weighed and took in thimble. The extraction cups were dried in oven at 1300 C for 15 min and took the weight of empty cups. The extraction cups were cooled and 70ml of petroleum ether was added. The instrument was pre-heated and when the temperature was attained, the extraction cups were attached to the instrument and left for boiling for 30min, followed by rising for 20min and last of all recovery of solvent was done for 10 min. The recovered ether was collected and fat contained in extraction cups were estimated.

$$\text{Fat} = (W2 - W1) / W \times 100$$

4. **Protein content**:
   Protein was determined using the AOAC (1995) Kjedahl technique, which consists of three steps: digestion, distillation, and titration.
5. **Carbohydrate:**

Based on the analysis of nutrient found the highest carbohydrate content is 59.45 g. because of the concentration of sugar more than other ingredients

6. Microbial analysis of Probiotic Chocolate

Total plate count (TPC) of Chocolate: The TPC value in T3 was observed as (1.05 Log10 cfu/g). With different treatment there is a significant difference in TPC.

Conclusion:

The trend to enrich new foodstuffs with live microbial cells is a novel and promising approach to the application in the food production. The supplementation of chocolate with encapsulated live cells is one of these new applications (VT Kharat and HW Deshpande, 2017). . It is very difficult to select a probiotic strain having all required properties by applying classical probabilistic approach. The stress adaptation method is another approach; however, stress responses are strain-specific. A number of techniques have been developed to protect the probiotics from environmental stresses in food matrices, processing, and storage, and GI tract passage. Among these, microencapsulation has been found to be, the most suitable and accessible technology to protect the tiny living organisms.(Gadhiya et. al, 2015), Thus in the light of the scientific data of the present investigation, it can be concluded that milk chocolate was a good carrier for *Streptococcus thermophilus, Lactobacillus bulgaris, Lactobacillus delbrueckii*. It can be concluded from above results that from all the formulations containing different concentrations of chakka, camel milk powder, ragi malt and honey T3 was found to be the best among all the different treatments. Probiotic chocolate with T3 formulation contain 4.0% moisture, 1.84 % ash, 7.81 % protein, 26.90 % fats, 59.94 carbohydrates. It contains camel milk powder which can be used as a substitute to mothers milk.Chocolate is willingly consumed by children and teenagers. The supplementation of this product with encapsulated live probiotic cells can enrich their snacks.

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