

Preparation and Properties of Probiotic Chocolates Using Yoghurt Powder

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ABSTRACT

Milk chocolates were prepared by replacing skim milk powder in the formulation with yoghurt powder at 50% and 100% levels. The effect of incorporating yoghurt powder on quality of chocolates was studied. No significant changes were observed in fatty acid profile and hardness of the chocolates. Sour taste of chocolate with yoghurt powder due to its acidity was neutralized by adding calculated amount of sodium bicarbonate. Sensory analysis showed that probiotic chocolates were highly acceptable and similar to control chocolate. Microbiological studies of chocolates showed the presence of *Lactobacillus* species to the extent of 3.37 log·cfu/g, which were not present in the control sample prepared only with skim milk powder. Rheological studies showed that milk chocolate prepared using yoghurt powder at 50% showed no significant changes in yield value compared to that of control, but at 100% addition a considerable decrease in yield value was observed. Microstructural properties of chocolate with 50% addition of yoghurt powder showed smaller particles adhering to the cocoa and sugar crystals but at 100% addition of yoghurt powder, the cocoa particles were completely covered by smaller yoghurt powder matrix.

Keywords: Probiotic Chocolates; SEM; Rheology; Yoghurt Powder

1. Introduction

Chocolate is a suspension of fine solid particles of sugar, cocoa and milk powder in a continuous fat phase. Chocolates are solid at ambient (20°C - 25°C) and melt at body temperature (37°C) giving a smooth suspension of particulate solids with a pleasing cooling sensation in the mouth [1]. The continuous phase influences the sensory characteristics such as mouth feel or melt in mouth.

Despite high fat and sugar contents, chocolate consumption makes a positive contribution to human nutrition through provision of antioxidants, principally polyphenols including flavonoids such as epicatechin, catechin and notably, the procyanidins [2]. Chocolates also contain minerals, specifically potassium, magnesium, copper and iron. Due to presence of cocoa, it is rich in natural antioxidants having health benefits. Milk solids added as spray-dried skimmed milk powder or full cream milk powder contributes to flavour, texture and liquid flow properties [3].

Numerous functional foods are consumed as part of a normal diet and they provide consumers with well-documented and physiological benefits such as probiotic bacteria. Probiotics are live microorganisms and proliferate

in the human bowels that confer a health benefit by altering the enteric microflora. The main sources of these organisms are fermented dairy products, for example, yoghurts. However, the functional dairy product must contain a defined number of live probiotic bacteria (usually at least 10⁶ cfu/g). Furthermore, their number at the end of the shelf life is the most important criterion when the health-promoting value of a given foodstuff is evaluated [4-7].

Probiotic bacteria beneficially affect human health by improving the gut micro biota balance and the defenses against pathogens. Additional health benefits attributed to probiotics are the stimulation of the immune system, blood cholesterol reduction, vitamin synthesis, anti-carcinogenesis and anti-bacterial activities. Two other important criteria to determine the efficacy and the success of the product containing probiotics are the acceptance of the product by the consumers and the survival of probiotic microorganisms during its production [8]. *Lactobacilli*, *Bifido* bacteria and several other lactic acid bacteria are regarded as probiotics, as they do not induce mucosal inflammation. The main sources of these organisms are fermented dairy products for example yoghurt (curd). In general, the food industry had applied the recommended level of 10⁶ cfu/gm at the time of consumption of *lacto-*

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bacillus acidophilus, *Bifidobacteria* and other probiotic bacteria [9]. Traditional yoghurt is produced from milk, fermented by strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* [10].

Today, India is the largest producer of milk in the world and the Indian dairy industry is witnessing rapid changes. Yoghurt/curd is the most popular fermented dairy product in India, prepared by the use of mixed mesophilic cultures that ferment lactose to lactic acid. Products like yoghurt are known more for their therapeutic significance than nutritional value [11]. It has a limited keeping quality of 1 - 2 days at ambient temperature and its quality is not retained for more than 1 week under refrigerated conditions [12]. Dried yoghurt powder has enhanced shelf life and it can also be used as a base for formulation of health foods. Nebesny *et al.* [13] have reported that chocolate formulated with isomalt and enriched with viable cells of lactic acid bacteria, introduced in the form of powdered yoghurt, is not only a sucrose-free, low-calorie product but additionally displays nutritional and dietetic attributes, and can be regarded as a functional food additional species in the fingerprints. Possemiers *et al.* [14] have reported bacteria and chocolate to be a successful combination for probiotic delivery. They have showed that coating of the probiotics in chocolate is an excellent solution to protect them from environmental stress conditions and for optimal delivery. The aims of the present study is to incorporate dried probiotic powder for the preparation of probiotic chocolate, and study the effects of replacing skim milk powder with yoghurt powder on the rheological and other quality parameters of milk chocolate.

2. Materials and Methods

2.1. Composition of Yoghurt

Yoghurt was procured locally in bulk and used for the studies. The yoghurt had protein content was 3.3%, fat 3.0%, minerals 0.7%, calcium 120 mg, vitamin A 35 ug and carbohydrates (as Lactose) 3.9%.

2.2. Preparation of Yoghurt Powder

The yoghurt was dried in a freeze drier (Lyophilisation Inc. USA, Model LT 5S): Freezing cycle : -26°C for 2 h, Drying cycle: -25°C to $+25^{\circ}\text{C}$ for 18 h and vacuum from 100,000 m-torr to 250 m-torr were used for a total period of 20 h. The powder obtained was packed in polypro-

pylene covers and stored in refrigerator.

2.3. Composition of Yoghurt Powder

The prepared yoghurt powder had of: 3.0% - 3.5% moisture, 35% protein, 1.5% - 2.0% fat, and total acidity of 5.8% as lactic acid.

2.4. Preparation of Chocolate

Milk chocolate was prepared using the formulation given in **Table 1**. Skim milk powder was replaced at 50% and 100% level by yoghurt powder in milk chocolate formulation and compared with control chocolate prepared with skim milk alone. All the ingredients were mixed and passed through a triple-roll chocolate refiner (Pascal, England), three times, keeping the distance between the rollers and number of passes constant for all the batches. The mass was then conched by adding the remaining cocoa butter and lecithin for 3 h at 50°C - 55°C . The mass after conching was taken for viscosity measurement. The mass was then tempered and moulded. The samples were kept at refrigerated condition for further analyses.

2.5. Texture of Chocolate

The hardness of the chocolate was measured using a Lloyd's texture measuring system (model LR 5K, UK). The samples of uniform dimension ($4 \times 8 \times 1$ cm-lbh), were conditioned by keeping at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for about 3 h before measuring. Penetration was measured at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ using a probe of 3 mm dia at a speed of 50 mm/min and 2 mm deflection using a 50 N load cell; the force required to penetrate was measured as hardness (N) and an average of 12 measurements was reported.

2.6. Fatty Acid Analysis

The fat from chocolate samples was extracted with chloroform and converted to fatty acid methyl esters (FAME) using KOH/Methanol (AOCS, 1993). FAME were analysed by GC (Varian GC 450, Herculesweg, The Netherlands) with FID and using Supelco, SP-2340 (0.25 mm \times 30 m) capillary column, programmed from 50°C to 200°C at $5^{\circ}\text{C}/\text{min}$ and maintaining at 200°C for 10 min; injection temperature 230°C , split ratio 1:20; detector temperature 240°C and nitrogen flow, 0.9 mL/min. The fatty acids were identified by using authentic standards and reported as relative percentage.

Table1. Viscosity of chocolates prepared with yoghurt powder.

Chocolate	Casson Yield Value (Pa) τ_0	Casson Plastic Viscosity (Pa S) η_p	R ²
Control	40.23 ^b	1.87 ^a	0.99
50% Yoghurt Powder	41.02 ^b	2.11 ^a	0.99
100% Yoghurt Powder	28.65 ^a	3.76 ^b	0.99

^{a,b}Mean values (n = 6) bearing different superscripts in a column are significantly different ($p \leq 0.05$).

2.7. Viscosity Measurement

The rheological behaviour of chocolate was measured at 40°C using HAAKE Viscotester, VT550 (Haake, Karlsruhe, Germany), using coaxial cylinder SV, from 0 to 100 shear rate in 3 min, and the curves of shear rate versus shear stress and viscosity were recorded. The shear rate and shear stress curves were subjected to various rheological models to see the best fit and determine viscosity, using Rheo Win (Haake, Karlsruhe, Germany) software. All the measurements were made in triplicate and the average was reported. The basic Casson equation is used to describe the flow behavior of chocolate. Casson yield value and plastic viscosity were used to describe the flow behavior of chocolate.

2.8. Sensory Evaluation

A trained panel was employed for carrying out sensory evaluation of chocolate by following the method of Quantitative Descriptive Analysis (QDA). The definitions of the attributes were discussed and descriptors were developed by asking the panelists to describe the product with the suitable descriptive terms for development of a score card, which consisted of each attribute on a 15-cm line scale. Quantitative descriptive analysis methods were adopted and the panelists were asked to mark the intensity of each attribute [15]. The main desirable sensory attributes of chocolate, such as gloss, snap, melt-in-mouth, sweetness, chocolate flavour and overall quality were assessed by a panel of 15 trained judges. Calculated amount of sodium bicarbonate based on their neutralization equivalent of the total acid present in yoghurt powder was added to the chocolates to avoid the acidic taste in chocolates. The judges were asked to mark by drawing a vertical line on the scale for all quality attributes of the coded samples.

2.9. Scanning Electron Microscope (SEM)

The microstructure of chocolate samples was studied by SEM using LEO Scanning electron model 435 VP (Leo electronics system, Cambridge, UK). The chocolate was defatted with hexane and placed on the sample holder with the help of a double scotch tape and sputter coated with gold (2 min, 2 m Bar) where it was observed at 15 kV.

2.10. Statistical Analysis

Data obtained was statistically analysed using Duncan's Multiple Range Test (DMRT) at significant level of $P \leq 0.05$ [16]. All the analyses were performed in triplicate except for texture ($n = 12$). The mean values with standard deviations (SD) are reported.

2.11. Microbiological Analysis

Number of *Lactobacilli* was counted by pour plate method using 1 ml of appropriate dilution of each sample in De Man Rogosa-Sharpe agar (MRS agar, Hi media, Mumbai). After 2 days of incubation at 37°C the colonies were counted and the results expressed in logarithm of colonies forming units per gram of product ($\log\text{-cfu/g}$). The representative colonies were tested for absence of catalase to confirm the presence of LAB

3. Results and Discussion

3.1. Viscosity of Chocolates

The viscosity of chocolate plays an important role during manufacture of chocolate. The solid ingredients, processing parameters and amount of fat influence viscosity of chocolate mass. The rheological behavior of chocolate followed non-Newtonian flow, as reported in literature, due to presence of solids in molten fat and followed the Casson model, which is the best fit as shown by the correlation coefficient (**Table 1**). Casson model is recommended by IOCCC [17] and has been used as an internationally accepted standard model for determination of viscosity of chocolates. It is now accepted and applied as an appropriate mathematical model for predicting flow behavior and rheological analysis of different kinds of chocolates [18,19].

Milk chocolate prepared using yoghurt powder at 50% by weight of milk powder showed no significant changes in yield value compared to that of control chocolate prepared with milk powder. However, chocolate with 100% addition of yoghurt powder showed considerable decrease in yield value. Plastic viscosity of chocolate increased with increase in addition of yoghurt powder from 50% to 100% (**Table 1**), which may be due to finer particles of yoghurt powder compared to milk solids.

3.2. Texture of Chocolate

Only marginal differences in hardness were observed among the chocolates, *i.e.*, control and with added yoghurt powder, ranging from 34 to 36 N.

3.3. Fatty Acid Composition

No significant differences in fatty acid composition were observed among the chocolates prepared with yoghurt powder as all samples contain cocoa butter and milk fat as fat sources (**Table 2**). Short chain fatty acids are from milk fat from milk powder or yoghurt powder. The product contains about 30% of monounsaturated (oleic) fatty acids and equal quantity of stearic acid, which is neutral with respect to affecting serum cholesterol [20].

Table 2. Fatty acid composition (%) of fat in chocolates prepared with yoghurt powder.

Fatty Acid	Chocolate with		
	Control	50% Yoghurt Powder	100% Yoghurt Powder
C4:0	2.50	2.45	2.50
C10:0	1.50	-	-
C14:0	0.42	0.71	1.32
C16:0	24.63	25.18	25.87
C18:0	34.61	35.07	34.56
C18:1	32.2	32.32	32.82
C18:2	4.00	3.00	2.96

Mean values (n = 3).

3.4. Microstructural Properties of Probiotic Chocolates

The outer topography of probiotic milk chocolates were assessed by SEM (**Figures 1(a)-(c)**), in order to study the effect of addition of yoghurt powder into chocolates. **Figure 1(a)** shows coarse particles of cocoa mass sheared during refining and conching process in the manufacture of chocolate. A few sugar particles and skim milk powder can also be seen along with the cocoa mass. Skim milk powder particles are smaller than cocoa and sugar particles and adhere to the larger and coarser cocoa particles.

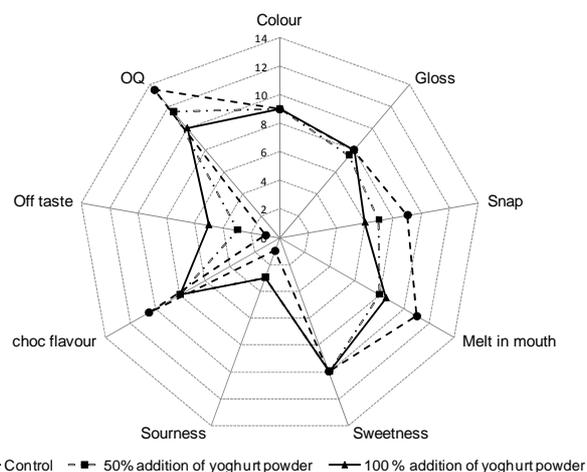
In chocolates with 50% addition of yoghurt powder, the smaller particles adhering to the cocoa and sugar crystals are more in number (**Figure 1(b)**). In chocolate with 100% addition of yoghurt powder, the cocoa particles are completely covered by smaller yoghurt powder matrix (**Figure 1(c)**). Skim milk powder shows surface dents, which can be attributed to the atomization conditions [21] but yoghurt powder which is freeze dried has smaller particle size compared to skim milk powder which forms a cluster around the larger cocoa and sugar particles without dents.

3.5. Microbiological Studies

Microbiological studies revealed that the chocolates prepared with yoghurt powder showed the presence of lactic acid bacteria to the extent of $3.37 \log\text{-cfu/g}$, which were not present in the control sample prepared only with skim milk powder (**Table 3**). There was no significant increase in the viable LAB count in 100% substitution over 50% as the cells were exposed (as seen in SEM) and probably they were more susceptible to processing treatments. It is well established that foods containing viable lactic acid bacteria as probiotics, improve gut health.

3.6. Sensory Analysis

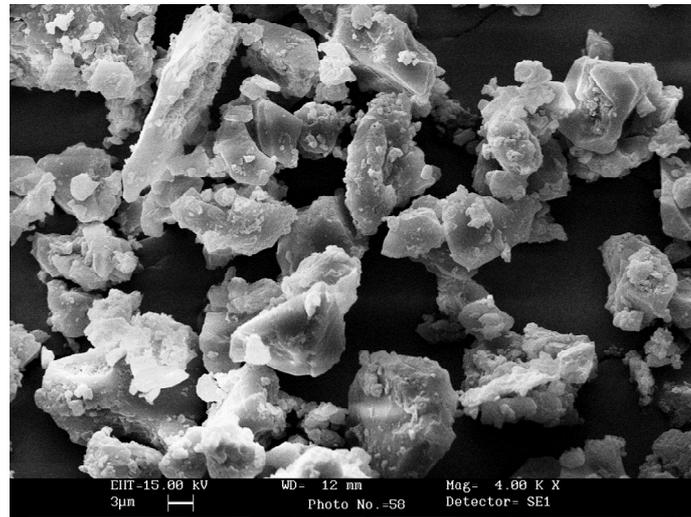
Sensory evaluation revealed that chocolate with 50% addition of yoghurt powder was more acceptable than

**Figure 1. Sensory evaluation of chocolates prepared with yoghurt powder.****Table 3. Microbiological studies of chocolates prepared with yoghurt powder.**

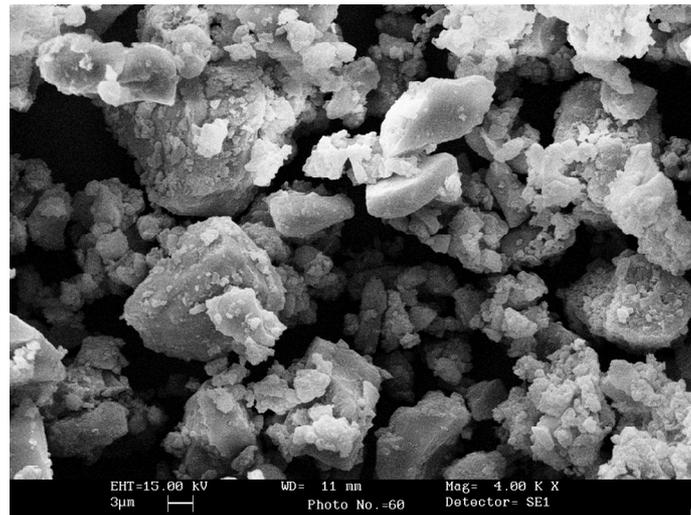
Samples	<i>Lactobacillus sp.</i> (log-cfu/g)
Yoghurt Powder	4.758 ± 0.11^b
50% Yoghurt Powder	3.581 ± 0.23^a
100% Yoghurt Powder	3.586 ± 0.24^a
Control	-Nil-

Mean values (n = 6) bearing different superscripts in a column are significantly different ($p \leq 0.05$).

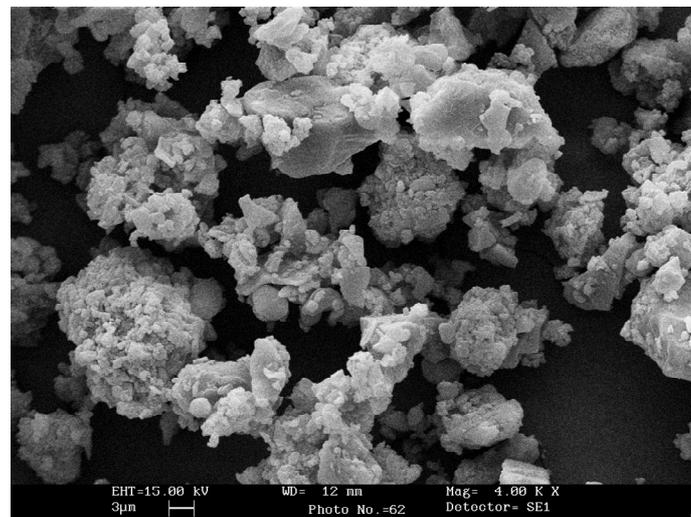
that with 100% addition and both had slightly lower overall quality when compared to control prepared only with skim milk powder (**Figure 2**). No significant differences were observed in attributes such as colour, gloss and sweetness among all the three samples. Snap, melt in mouth and chocolate flavour attributes decreased with addition of yoghurt powder. Chocolates with added yoghurt powder are sour in taste due to acid present in yoghurt powder. However, chocolates with addition of required amount of sodium bicarbonate to neutralize the acid did not show any sour taste. Similar results have been reported by Nebesny *et al.* [13], where sensory



(a) Control chocolate



(b) Chocolate with 50% addition of yoghurt powder



(c) Chocolate with 100% addition of yoghurt powder

Figure 2. Scanning electron micrographs (SEM) of chocolates prepared with yoghurt powder (Mag. 4000×).

attributes of sucrose-free yoghurt-containing dark and milk chocolates and their yoghurt-free counterparts revealed excellent quality. The sensory attributes of yoghurt and isomalt containing dark chocolates received a high average score which was only slightly lower than that of analogous milk chocolates.

4. Conclusion

Milk chocolate with yoghurt powder was prepared replacing milk powder to make it probiotic. Marginal differences in viscosity, texture and sensory evaluation of chocolate with yoghurt were observed in comparison with those of control milk chocolate. Chocolate with yoghurt powder contain probiotic lactobacilli species and thus making it probiotic. Chocolate is reported to contain natural antioxidants and the nutritional quality of this was further enhanced by making it probiotic. Thus, milk chocolate with probiotic yoghurt powder was prepared without affecting the desirable quality of conventional chocolate.

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