Bifidus milk powder: processing parameter standardization and shelf stability evaluation

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Abstract Spray dried bifidus milk powder was prepared by supplementing cow milk with different level of additives to obtain slurry of desired concentration. The slurry was pasteurized, cooled and inoculated with *Bifidobacterium bifidum*, incubated and dried to produce a bifidus milk powder. Among the various bifidus milk powder prepared, the slurry of mention the level total soluble solids exhibited good organoleptic characteristics and it has been standardized for further analysis. Moisture content, bulk density, insolubility index, hydroxymethyl furfural and thiobarbituric acid value of bifidus milk powder significantly increased, while the reflectance value significantly decreased during storage. The *B. bifidum* count significantly reduced and the bacterium were not detected at the end of the mention storage duration. As such the sentence is not acceptable in the abstract. The reconstituted bifidus milk powder was considered acceptable with an overall acceptability score of 6.97 on a nine-point Hedonic scale and showed a shelf stability of 120 days at ambient temperature condition (27 ± 2 °C).

Keywords *Bifidobacterium bifidum* · Bifidus powder · Hydroxymethyl furfural · Physico-chemical properties · Shelf life · Spray drying

Introduction

Fermented milk products like yogurt, acidophilus milk, kefir, cultured butter milk are being manufactured in various countries and possess significant therapeutic and nutritional characteristics. These products can be prepared by using selected strains of human enteric *Lactobacillus acidophilus* subsp. *Acidophilus* strain (LA), *Lactobacillus bulgaricus* subsp. *Bulgaricus* strain (LB), *Bifidobacterium bifidum* subsp. *Bifidum* strain (BB) and *Lactobacillus casei* subsp. *Casei* strain (LC). BB is gram – positive, non motile bacteria that naturally inhabit the guts of warm – blooded animals and man (Scardovi 1986). These organisms are able to exert beneficial effects including improvement of intestinal microflora by preventing colonization of pathogens, amelioration of diarrhea or constipation, activation of the immune system and increasing protein digestion (Ishibashi and Shimamura 1993). Cow milk and other spray dried cow milk contain less amount of BB strain as compared to human milk. Therefore, it is desirable to prepare a product, which can supply abundant quantity of above probiotic strain.

Spray drying is one of the most effective processes in extending the shelf life of dairy products. This process has the advantages of long-term preservation, convenience in handling, storage, marketing and consumption. Although a number of dried fermented milk products are available as pharmaceutical preparation or as health foods, their viable counts are low. Freeze drying is the most commonly used technique for such products. However, it is a more expensive technique than any other conventional method of drying (Knorr 1998).

Therefore, the present study was undertaken to standardize the processing parameter for the development of bifidus milk powder (BMP) in order to give a product with improved

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nutritional quality and longer shelf-life, as assessed over 120 days storage period by physico-chemical, microbial and sensory analysis.

Materials and methods

Raw materials

Cow milk was collected from Jawaharlal Nehru Krishi Vishwa Vidyalaya Dairy Farm (Jabalpur, India). Skim milk powder was obtained from Jabalpur Dugdha Sangh (Jabalpur, India). Probiotic culture of BB strain No. 255 was purchased from the National Dairy Research Institute (Karnal, India). All other chemical reagents and microbiological media were purchased from Himedia Laboratories Limited (Mumbai, India).

Propagation of starter culture

The freeze-dried culture of BB was propagated in 100 mL sterilized milk by aseptically transferring the contents of ampoules to the flask. The flask was incubated for 24 h at 37 °C, stored at 4 °C and the starter culture was examined for proper coagulation. To attain optimum activity of strain, two to three serial transfers of starter culture was carried out prior to addition in bulk culture. The scale up system of propagation of culture was followed for further day-to-day need.

Development of bifidus milk powder

The BMP was developed according to the procedure as described by Prajapati et al. (1986), as given in the flow chart (Fig. 1). Milk was supplemented with predetermined level of skim milk powder, gelatin, monosodium glutamate and lactose to obtain slurry of desired concentration (Table 1). The different TSS levels of slurry concentration was chosen based on results of preliminary experimental observations by conducting trials. The slurry was stirred well, filtered through sieves, pasteurized and cooled to 37 °C.

The 24 h old bulk culture of BB strain was inoculated into slurries at 10 % and incubated at 37 °C for 12 h, immediately cooled to 10 °C, stirred well to get uniform viscous paste and the bifidus milk slurries were dried in SMST lab model spray dryer (SM Sciencetech Private Ltd., Calcutta, India). To achieve best-quality product, the spray dryer was operated at constant spray drying conditions, with various slurry concentration levels i.e. inlet/outlet air temperature of (165/75 °C), air pressure of (2.5 kg/cm²), slurry concentration of (21–30 %) and flow rate of (30 rpm). The BMP was packed in high density polypropylene pouches (HDPP) and stored at ambient temperature and 37 °C.

Fig. 1 Flow diagram for development of spray dried bifidus milk powder

Physico- chemical analysis

The composition of cow milk viz., moisture, fat, protein, lactose, ash and total solids were estimated as per the procedure of BIS (1961). Moisture content of the spray dried BMP was determined as per the method of IDF standards (1991). The total solids and ash content of the BMP was determined by BIS (1961). The fat content of BMP was determined by the Mojonnier method according to MIF (1959) and the protein content was assessed by the semi-microkjeldahl method.

Table 1 Composition of slurries used for spray drying

Component	Slurry concentration TSS (%)			
	B1	B2	B3	B4
Whole milk	13.0	13.0	13.0	13.0
Skim milk powder	4.0	7.0	10.0	13.0
Lactose	3.0	3.0	3.0	3.0
Gelatin	0.7	0.7	0.7	0.7
Monosodium glutamate	0.3	0.3	0.3	0.3
Total	21.0	24.0	27.0	30.0

B1 - bifidus milk powder prepared from cow milk slurry of 21 % TSS; B2 - bifidus milk powder prepared

From cow milk slurry of 24 % TSS; B3- bifidus milk powder prepared from cow milk slurry of 27 % TSS; B4- bifidus milk powder prepared from cow milk slurry of 30 % TSS; TSS - total soluble solids

Table 2 Composition¹ of cow milk

Component	Content
Moisture (%)	86.58 ± 0.06
Fat (%)	4.55 ± 0.36
Protein (%)	3.36 ± 0.62
Lactose (%)	4.83 ± 0.41
Ash (%)	0.68 ± 0.03
Total solids (%)	13.42 ± 0.28

¹ Mean value ± standard deviation (*n* = 3)

according to Maneffe and Overman (1940). Thiobarbituric acid (TBA) content of the BMP was estimated by the method described by Sidwell et al. (1955). Hydroxymethyl furfural (HMF) content of powder was estimated as suggested by Keeney and Bassette (1959). Bulk density of BMP was tested by the method of Sjollem (1963). Insolubility index was determined by the procedure of ADMI standards (1965).

Sensory evaluation

The BMP (100 g) was reconstituted in 660 mL of water with an addition of 100 g sucrose in order to convert it into a fluid form and it was served in glass to a panel of 20 selected judges after labeling them with three-digit random codes. Panelists were provided with distilled water to clean their mouths between samples. All evaluations were conducted at room temperature on the same day in the Department of Food Science and Technology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur.

The reconstituted BMP samples were presented in random order and panelists were asked to rate their assessment of colour, aroma, taste and overall acceptability on a 1–9 point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely) (Amerine et al. 1965). A score of 6 was considered as a limit of acceptability for all sensory attributes tested.

Table 3 Mean sensory scores¹, 2 for different spray dried reconstituted bifidus milk powder

Type of reconstituted bifidus milk powder				
Organoleptic characteristics	B1	B2	B3	B4
Colour	6.23 ± 1.30 ^{ba}	8.09 ± 1.21 ^a	7.04 ± 0.82 ^a	6.13 ± 1.26 ^{ba}
Aroma	6.30 ± 1.31 ^{ba}	8.11 ± 0.70 ^a	7.12 ± 0.93 ^a	6.61 ± 0.83 ^{ba}
Taste	6.59 ± 1.02 ^b	8.10 ± 0.30 ^a	6.60 ± 0.81 ^b	7.09 ± 1.07 ^a
Overall acceptability	6.69 ± 0.53 ^b	8.02 ± 0.30 ^a	7.01 ± 0.85 ^b	6.94 ± 1.01 ^b

^a mean value ± standard deviation (*n* = 20)^b Means in a row bearing the same superscript letters do not differ significantly (*P* > 0.05)

B1 - bifidus milk powder prepared from cow milk slurry of 21 % TSS; B2 - bifidus milk powder prepared from cow milk slurry of 24 % TSS; B3- bifidus milk powder prepared from cow milk slurry of 27 % TSS; B4- bifidus milk powder prepared from cow milk slurry of 30 % TSS

Microbial analysis

The total BB count in the BMP was analyzed initially and once in 20 days, upto a period of 120 days. The total BB count was enumerated by serial dilution as per the method described by Prajapati et al. (1987).

Statistical analysis

The results were subjected to ANOVA using the Microsoft Office Excel 2000 (Microsoft Corporation, Washington, USA) and the paired comparisons of means using Duncan's test (Steel and Torrie 1980).

Results and discussion

Composition of cow milk

The composition of cow milk used in the present investigation was given in Table 2. The milk was found to contain more amount of moisture, total solids and organoleptic taste of milk samples was also acceptable, which shows that the fresh cow milk was utilized for development of spray dried BMP during the entire experimental process.

Sensory evaluation

Mean scores for the organoleptic attributes (Table 3), showed that the spray dried BMP made from slurry containing 24 % total soluble solids (TSS) i.e. B2 had the highest organoleptic scores because of using optimum slurry concentration. In contrast, the BMP made from slurry of 21 % TSS (B1) recorded the lowest values except colour. No significant difference in organoleptic scores were observed (*P* > 0.05) in case of spray dried BMP made from slurry of TSS 21 % (B1) and 30 % (B4) except taste.

Physico-chemical properties of BMP prepared from cow milk slurry of 24 % TSS

The organoleptically rated best BMP i.e. which was prepared from cow milk slurry of 24 % TSS was chosen for further analysis. The BMP had moisture level within an acceptable limit. It also found to contain more amounts of nutrients viz., fat and protein (Table 4). The powder also exhibited good insolubility index level with low HMF content (503 μ mol/kg) and showed negligible browning during spray drying. TBA represents the rate of auto-oxidative deterioration, which was low (0.07 o.d.) in BMP. The high reflectance value (86.0 %) signified that there had been a low rate of browning during the spray drying of the bifidus milk.

Storage of spray dried BMP made from cow milk slurry of 24 % TSS

Moisture

The initial moisture content of the BMP (4.82 %) significantly increased ($P < 0.05$) to 6.23 and 5.62 %, after 120 days of storage at ambient temperature (27 ± 2 °C) and 37 °C, respectively (Fig. 2). Sudhir et al. (2003) also reported an increase in moisture content of spray dried mushroom whey soup powder during storage period at above temperature conditions. The powder began to cake after 120 days of storage at elevated temperature. This might be due to exposure of α -lactose monohydrate to a higher temperature and also as a result of occurrence of crystallization which finally leads the powder to a hardened form by forming cakes at above condition (Harper 1992).

Bulk density

Bulk density controls the volume of the storage container and affects the packaging costs of the dried products. The bulk density of the BMP slightly increased from 0.27 to 0.34 and

Table 4 Physico-chemical properties¹ of bifidus milk powder prepared from milk slurry of 24 % TSS

Component	Content
Moisture (%)	4.82 $\pm$ 0.03
Protein (%)	23.20 $\pm$ 0.28
Ash (%)	48.17 $\pm$ 0.35
Bulk density (g/mL)	5.40 $\pm$ 0.15
Insolubility index (mL)	0.27 $\pm$ 0.12
Hydroxymethyl furfural (μ mol/kg)	3.80 $\pm$ 0.41
Thiobarbituric value (o.d.)	503.00 $\pm$ 0.07
Reflectance value (%)	86.00 $\pm$ 0.04

¹ Mean value $\pm$ standard deviation ($n = 3$)

Fig. 2 Changes in moisture content of spray dried bifidus milk powder during storage

0.37 g/mL after 120 days of storage at ambient temperature (27 ± 2 °C) and 37 °C, respectively (Fig. 3a). The increase in bulk density was found to be non significant ($P > 0.05$) during entire period of storage at above conditions. Sudhir et al. (2003) also reported a similar result during the storage of mushroom whey soup powder at above conditions.

Fig. 3 Changes in bulk density **a** and insolubility index **b** of spray dried bifidus milk Powder during storage

Fig. 4 Changes in hydroxymethyl furfural **a**, thiobarbituric acid value **b** and reflectance. Value **c** of spray dried bifidusmilk powder during storage

Insolubility index

The insolubility index is a measure of insoluble material, which determines how easy it is to reconstitute the dried powder. It is an indication of the degree of denaturation of protein and also indicates how much heat-induced damage to other ingredients mainly carbohydrates has occurred. It varies with the severity of heat treatment during processing and the temperature of storage. The initial insolubility index value for the BMP sample was 3.80 mL, which significantly increased ($P < 0.05$) to 5.67 and 8.74 mL during the entire period of storage at ambient temperature (27 ± 2 °C) and 37 °C. However, the rate of increase in the insolubility index was

most rapid for the samples stored at 37 °C (Fig. 3b). This may be due to degradation of protein and carbohydrate at accelerated temperature condition might have increased the insolubility index level to a greater extent. Similar results were reported in spray dried mushroom whey soup powder samples during storage period at above temperature conditions (Sudhir et al. 2003).

Hydroxy methyl furfural (HMF)

The extent of browning in BMP during storage was measured in terms of the HMF content, the compound formed during the early stages of the Maillard reaction. Production of HMF is

Table 5 Mean sensory scores¹ of reconstituted spray dried bifidus milk powder during storage

Storage period (days)	Colour		Aroma		Taste		Overall acceptability	
	AT	37 °C	AT	37 °C	AT	37 °C	AT	37 °C
0	8.09 ± 1.21	8.09 ± 1.21	8.11 ± 0.70	8.11 ± 0.70	8.10 ± 0.30	8.10 ± 0.30	8.02 ± 0.30	8.10 ± 0.30
20	7.90 ± 0.69	6.10 ± 1.16	7.81 ± 0.65	5.90 ± 0.94	7.97 ± 0.62	5.79 ± 1.21	7.81 ± 0.65	5.82 ± 0.83
40	7.81 ± 1.11	5.34 ± 1.20	7.70 ± 0.65	5.11 ± 1.10	7.79 ± 0.65	4.71 ± 0.90	7.79 ± 0.85	4.91 ± 0.39
60	7.52 ± 0.81	4.79 ± 0.93	7.48 ± 0.56	4.40 ± 0.56	7.41 ± 0.69	4.31 ± 0.62	7.46 ± 0.66	4.49 ± 0.56
80	7.21 ± 0.62	4.28 ± 0.68	7.23 ± 0.81	4.11 ± 0.39	7.29 ± 0.73	4.17 ± 0.30	7.29 ± 0.88	4.13 ± 0.42
100	7.17 ± 0.73	3.90 ± 0.30	7.10 ± 0.72	3.73 ± 0.34	7.19 ± 0.68	3.94 ± 0.30	7.26 ± 0.85	3.75 ± 0.41
120	6.90 ± 1.04	3.43 ± 0.39	6.81 ± 1.10	3.37 ± 0.40	6.92 ± 1.02	3.48 ± 0.51	6.97 ± 1.01	3.37 ± 0.50

¹ Mean value ± standard deviation ($n = 20$)

AT- ambient temperature (27 ± 2 °C)

related to the amount of lactose present in the powder (Mistry and Pulgar 1996). The HMF content significantly increased ($P < 0.05$) from 503 to 563 and 658 $\mu\text{mol/kg}$, after 120 days of storage at ambient temperature ($27 \pm 2^\circ\text{C}$) and 37°C , respectively (Fig. 4a). Kumar and Mishra (2004) also reported an increasing trend of HMF in mango soy fortified yogurt powder during storage at 38°C .

Thiobarbituric acid value (TBA)

The thiobarbituric acid value (TBA) is a measure of the formation of secondary lipid oxidation products (Truyen 1975). The TBA values of BMP significantly increased ($P < 0.05$) from 0.07 to 0.150 o.d. at ambient temperature ($27 \pm 2^\circ\text{C}$) and 0.26 o.d. at 37°C after 120 days of storage in HDPP pouches (Fig. 4b). Kumar and Mishra (2004) also observed an increase in TBA value during storage of mango soy fortified yogurt powder samples at above temperature conditions.

Reflectance value (RV)

The reflectance value (RV) indicated the extent of browning in the BMP. These values significantly decreased ($P < 0.05$) from 86 to 78 % after 120 days of storage at ambient temperature ($27 \pm 2^\circ\text{C}$). However, the decrease was at a faster rate from (86 to 62 %) in the BMP samples after 120 days of storage at 37°C (Fig. 4c). This indicates that the rate of browning was higher in BMP samples stored at 37°C when compared to ambient temperature ($27 \pm 2^\circ\text{C}$) stored samples.

Sensory evaluation of stored spray dried BMP

The sensory scores of spray dried BMP samples stored at ambient temperature ($27 \pm 2^\circ\text{C}$) and 37°C in HDPP pouches

are presented in Table 5. Initially the spray dried BMP had an overall acceptability score of 8.02, on a nine-point Hedonic scale and a score of 6.97, liked slightly was taken as the limit of shelf life in the storage studies. Spray dried BMP samples remained acceptable to a selected panel of judges for up to 120 days of storage at ambient temperature ($27 \pm 2^\circ\text{C}$). However colour, aroma, taste and overall acceptability were found to be reduced drastically at the end of 40 days of storage period at 37°C . This might be due to chemical reactions which leads to the formation of brown pigments which might have decreased the colour score, loss of volatile aromatic substances during storage period at above condition might have decreased the aroma score. The decrease in overall acceptability score might be due to the certain induced biochemical changes in the product by elevated temperature storage. Changes in the spray dried BMP had a significant effect ($P < 0.05$) on colour, aroma, taste and overall acceptability during storage period for 120 days at above temperature conditions.

BB count

The BB count of spray dried BMP samples during storage is provided in Fig. 5. The freshly prepared BMP contains 225.0×10^6 cfu/g of BB count which decreased to 51.46×10^6 cfu/g after 120 days of storage at ambient temperature ($27 \pm 2^\circ\text{C}$), while it was found to be not detectable at the end of 60 days storage period at 37°C . Significant decrease ($P < 0.05$) in BB count of the BMP samples stored at above temperature conditions were observed. Cultured product which claims health benefits should contain 106 cfu/g or mL and in this case, the product stored at elevated temperature has showed <106 BB count after 40 days of storage. Failure of BB to survive for a longer period in spray dried BMP may be because of achievement of higher water activity during the storage period at above condition. Kumar and Mishra (2004) also reported a decline in probiotic bacterial count during storage of mango soy fortified yogurt powder at accelerated storage conditions.

Conclusion

BMP can be developed by utilizing cow milk as a sole source and it can be stored in a stable form for a longer periods. Spray drying techniques can be employed as an effective processing tool to produce such products in large quantities with relatively low expense. The acceptable quality of BMP can be standardized by preparing it from cow milk with a TSS of mention concentration. The predicted shelf life of BMP was found to be 120 days at ambient temperature ($27 \pm 2^\circ\text{C}$) in HDPP pouches. Therefore, this bifidus milk powder can be used as a baby formula for infants.

Fig. 5 Changes in *B. bifidum* count of spray dried bifidus milk powder during storage

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