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Chemical analysis of enzymatically modified synbiotic soy milk: pH and acidity determinations

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Abstract

The required acidity of 0.175 ± 0.54 per cent lactic acid and a pH of 5.65 ± 0.15 , suitable for spray drying were reached in three hours by synbiotic milk prepared by substitution of enzymatically modified soymilk to bifidogenic milk at 15 per cent level (SyM₁₅). There was significant difference in acidity between control and synbiotic milk prepared by substitution of enzymatically modified soymilk to bifidogenic milk (SyM) at 10, 15 and 20 per cent level. The pH development at 0h, 1h, 2h, 3h after inoculation of *Bifidobacterium longum* at in modified milk incubated at 37°C anaerobically were estimated and statistical analysis revealed that there exists no significant differences in pH between SyM₁₅, SyM₂₀.

Keywords: Acidity, pH, Synbiotic Milk, *Bifidobacterium longum*

Introduction

A study on the modification of cow milk for the growth of bifidobacteria. The market value and biological potential of both probiotics and prebiotics are enormous. Milk, a mammalian food by itself is a unique diet by its balanced nature. Yet, it needs modification to suit the people of different age groups, health problems acquired or inherited. Combining the probiotics and prebiotics, synbiotic milk, powder from symbiotic milk can be utilized. Modified milk having low fat, 1.5 per cent honey substituted with enzymatically modified soy milk at 10%, 15% and 20% level viz SyM₁₀, SyM₁₅ and SyM₂₀. Four per cent inoculum of *Bifidobacterium longum* was inoculated and incubated up to a maximum of 3 hours. The samples were initially assessed for growth enhancement of *Bifidobacterium longum* by measuring acidity and pH.

Materials and methods**Modification of milk for the growth of Bifidobacteria**

Bifidobacteria are strict anaerobes, fastidious and it grows well in human milk. The casein: whey protein ratio of 80:20, in normally found in bovine milk was modified to 40:60 by addition of whey protein concentrate honey at 1.5% (Suresh, 1994)^[11].

Soy milk was added as the source of prebiotic. Enzymatically modified Soy milk with Neutrased was added in the level of 10 per cent, 15 per cent and 20 per cent (Suresh, 1994)^[11] BIS: 1981. SP 18 (Part XI, 1981)^[2]. Handbook of food analysis Part XI. Dairy Products, New Delhi

Soy milk

Soy flour was procured from market and was used to prepare soy milk. It was treated with proteolytic enzyme for the preparation of EMSM and subsequent addition to SyM at 10, 15, 20 per cent levels.

Honey

Honey (Agmark Grade A) from the finest apiaries of India, marketed by Dabur India Limited, (West Bengal) was used in the study.

Culture used

Bifidobacterium longum– Bb46 DVS culture was obtained from Chr. Hansen laboratory.

Estimation of developed acidity and pH

The acidity was estimated as per BIS:SP:18(PART XI procedure.)^[2]. Digital pH meter (Control dynamics pH meter) was used to assess the pH at different intervals.

Results

The development of acidity at 0,1,2,3h after inoculation of bifidus culture in modified milk incubated at 37°C anaerobically were estimated. The mean \pm S.E. value of six trials for the acidity values as per cent lactic acid developed in EMSM substituted at three different levels (10, 15 and 20 per cent) after 0,1,2,3h interval using 4% *Bifidobacterium longum* are given in the table – 1 and graphically represented in Fig. 1. The acid production in control, SyM₁₀, SyM₁₅, SyM₂₀ in milk at 0 h, 1h,2h,3h were (0.152, 0.154,0.156,0.157), (0.156, 0.159, 0.163, 0.169), (0.156, 0.169, 0.171, 0.175), (0.154, 0.170, 0.172, 0.176) respectively.

Statistical analysis revealed no significant difference between SyM₁₅, SyM₂₀ and is indicated by superscripts based on one way analysis.

The pH development at 0,1,2,3h after inoculation of *Bifidobacterium longum* at in modified milk incubated at 37°C anaerobically were estimated. The mean \pm SE value of six trials of pH values in soy milk substituted at three different levels (10,15 and 20 per cent) after 0,1,3h interval using 4% *Bifidobacterium longum* are given in table 2 and Fig.2. The pH values observed in control, SyM₁₀, SyM₁₅, SyM₂₀ at 0h, 1h, 2h, 3h were (6.62, 6.53, 6.39, 6.31), (6.53, 6.18, 5.92, 5.67), (6.57, 5.79, 5.72, 5.65), (6.59, 5.76, 5.70, 5.64).

Statistical analysis revealed that there exists no significant differences between SyM₁₅, SyM₂₀ and are denoted by same superscripts.

Table 1

Influence of EMSM on the activity of *Bifidobacterium longum* in SyM on acidity Development*

Sample	Time in Hours			
	0	1	2	3
Control	0.152 \pm 0.02	0.154 \pm 0.14	0.156 \pm 0.45	0.157 ^a \pm 0.32
SyM ₁₀	0.156 \pm 0.24	0.159 \pm 0.24	0.163 \pm 0.34	0.169 ^b \pm 0.05
SyM ₁₅	0.156 \pm 0.63	0.169 \pm 0.18	0.171 \pm 0.25	0.175 ^c \pm 0.54
SyM ₂₀	0.154 \pm 0.35	0.170 \pm 0.34	0.172 \pm 0.05	0.176 ^c \pm 0.41
F Value	2001.518**			

* Mean \pm Standard error values from six trials. Mean values bearing different superscripts in a column differs

** Highly significant (P \leq 0.01)

EMSM - Enzymatically modified soymilk.

SyM - Synbiotic Milk prepared by substitution of enzymatically modified soymilk to bifidogenic milk.

Table 2: Influence of EMSM on the activity of *Bifidobacterium longum* in SyM on pH *

Sample	Time in Hours			
	0	1	2	3
Control	6.62 \pm 0.10	6.53 \pm 0.23	6.39 \pm 0.24	6.31 ^a \pm 0.25
SyM ₁₀	6.53 \pm 0.06	6.18 \pm 0.51	5.92 \pm 0.36	5.67 ^b \pm 0.36
SyM ₁₅	6.57 \pm 0.21	5.79 \pm 0.00	5.72 \pm 0.12	5.65 ^c \pm 0.15
SyM ₂₀	6.59 \pm 0.10	5.76 \pm 0.33	5.70 \pm 0.28	5.64 ^c \pm 0.05
F Value	2611.932**			

* Mean \pm Standard error values from six trials. Mean values bearing different superscripts in a column differs

** Highly significant (P \leq 0.01)

EMSM - Enzymatically modified soymilk.

SyM - Synbiotic Milk prepared by substitution of enzymatically modified soymilk to bifidogenic milk.

SyM₁₀ - Synbiotic Milk prepared by substitution of enzymatically modified soymilk to bifidogenic milk at 10 per cent level.

SyM₁₅ - Synbiotic Milk prepared by substitution of enzymatically modified soymilk to bifidogenic milk at 15 per cent level.

SyM₂₀ - Synbiotic Milk prepared by substitution of enzymatically modified soymilk to bifidogenic milk at 20 per cent level.

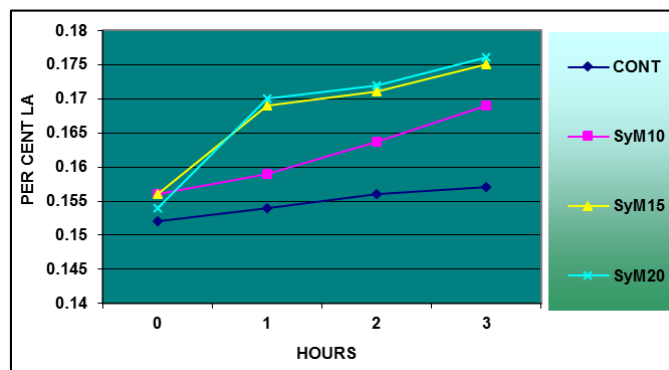


Fig 1: Change in acidity with respect to time

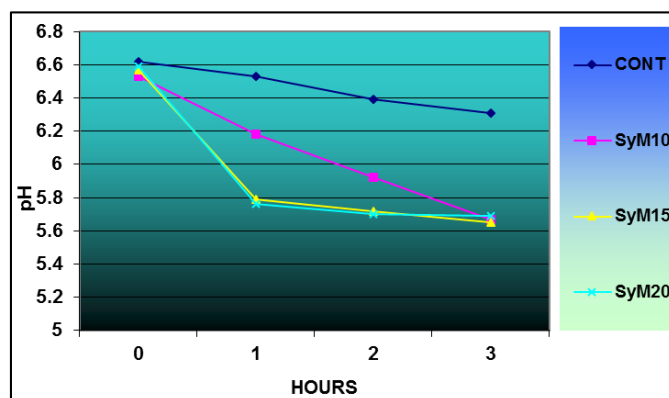


Fig 2: Change in pH with respect to time

Discussion

Influence of EMSM on the activity of *Bifidobacterium longum* in SyM on acidity development

There was significant difference in acidity between control and SyM at 10, 15 and 20 per cent level given in Table 1. Among the treatments the maximum acidity (0.176) was reached when 20 per cent EMSM was substituted and minimum acidity (0.169) was reached when 10 per cent EMSM was added to Bifidogenic milk (Fig.1). These observations were found to be similar to observations of Miloud *et al.*, (2005)^[7]. The maximum acidity of SyM₁₅ in 3 h (0.175) which was not significantly different from SyM₂₀ was due to enhancement of growth of *Bifidobacterium longum* which is a slow acid producer of lactic acid (Hoover)^[6]. Thus addition of 15 per cent EMSM is sufficient. Soy milk has been added as an adequate substrate media for growth and biochemical activities of lactic acid bacteria (Angeles and Marth, 1971^[1]; Wang *et al.*, 1974^[11]; Salem *et al.*, 1994)^[10]. The presence of oligosaccharides such as raffinose, stachyose and aldehydes in soy milk were effectively metabolized by *Bifidobacterium longum* which increases the acidity (Dimitri and Nagendrashah, 2004)^[4].

Influence of EMSM on the activity of *Bifidobacterium longum* in SyM on pH

The minimum pH of 5.64 and maximum pH of 5.67 was reached in 3 h could be due to enhancement of growth of *Bifidobacterium longum* by the probiotics, soy proteins (Dubey and Mistry, 1996)^[5]. As, it is not feasible to ferment milk using only probiotics, due to longer fermentation time required to reduce the pH of milk (Dave and Shah 1997)^[2], Whey proteins and EMSM has been added for providing adequate substrate media for growth and biochemical activities of lactic acid bacteria (Angeles and Marth, 1971)^[1]. The statistical interpretation furnished in table 2 and graphically (Fig 2). These observations were found to be similar to Patricia and Salvador (2006)^[8].

The growth of *Bifidobacterium longum* in SyM due to addition of EMSM to BM, lowered the pH of SyM₁₀, SyM₁₅, SyM₂₀ during 1hr, 2hr, 3hr (6.18, 5.92, 5.67) (5.79, 5.72, 5.65) (5.76, 5.70, 5.65) respectively as compared to the control (6.53, 6.39, 6.31). Whey proteins had stronger influence on bifidobacterial growth (Rajka and Tratnik, 2001)^[9]. The pH values are directly correlated to colony count of *Bifidobacterium longum*.

Thus it was concluded that the required acidity of 0.175±0.54 per cent lactic acid and a pH of 5.65±0.15, suitable for spray drying were reached in three hours by synbiotic milk prepared by substitution of enzymatically modified soymilk to bifidogenic milk at 15 per cent level.

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