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Comparative Studies in the Manufacturing of Acidophilus, Bifidus and Acido-bifidus Milks

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ABSTRACT: The "Encyclopedia of Fermented Fresh Milk" has classified Acidophilus and Bifidus milks as Non-traditional fermented milk products. These products contain *Lactobacillus acidophilus* and *Bifidobacterium* spp., respectively, that are known as probiotic microoganisms. In this study, some aspects of acidity development, pH measurement and bacterial counts were monitored when these micro-organisms were grown in milk. The starter cultures of *L. acidophilus* La-5 and *Bifidobacterium lactis* Bb-12, obtained from Chr. Hansen in Denmark, were each inoculated at the rate of 0.01 g 100 ml^{-1} in UHT milk and incubated at 37ºC for a duration of 5 h (Acidophilus and Bifidus milks). The same procedure was carried out with the same inoculation rates of two mentioned starters as mixed culture (Acidobifidus milk). The titratable acidity measurements and pH values of the single strains fermented milks were similar, but the sour taste of Acido-bifidus milk of the mixed starter culture was more tangible when compared with the two other fermented products. From the limited data available, it was evident from the preliminary studies that the growth of *B. lactis* in mixed cultures was better as compared to the single strain, and it is suggested that a lower inoculum rate of starter cultures or shorter fermentation period might be recommended for the development of Acido-bifidus milk.

Keywords: *Acidophilus Milk, Asido-bifidus Milk, Bifidobacteium lactis, Bifidus Milk, Lactobacillus acidophilus*.

Introduction

Probiotics can be defined as microbial cell preparations or components of microbial cells that have beneficial effects on health and well-being of host (Salminen *et al.,* 1999). Nowadays probiotic foods are firmly established on the market in industrialised countries (Gibson *et al*., 2000; Menrad *et al*., 2000; Sanders & Huis in't Veld, 1999). Here they constitute a substantial part of fermented dairy products, whilst the number of non-dairy products is increasing. According to the claims of the producers, these products are effective in supporting the health of the consumer and are also safe. Considering the challenges and the outlook in research for probotics, our view in respect of food technology, food microbiology and introducing novel foods (Hammes & Hertel, 2002) and constituting some cultured products applied by Lactic acid bacteria (LAB), in particular lactobacilli and bifidobacteria, as probiotic cultures (Richardson, 1999) are momentous affairs. These starter cultures are involved in the production of many fermented milk products that are claimed for probiotic value. The consumption of these products has the potential to aid lactose digestion (Vesa *et al.,* 1996), to prevent traveler's diarrhea (Oksanen *et al.,* 1990), to reduce the duration of rotavirus diarrhea (Guarino *et al.,* 1998), to exert antitumor activity (Kato *et al.,* 1994), to enhance the activity of the immune system (Meydani & Ha., 2000) and to aid in controlling serum cholesterol (Gilliland *et al*., *1985*). Acidophilus and Bifidus milks are the most familier probiotic

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milks among many other probiotic milk products (Table 1).

In this research work some manufacturing aspects of three probiotic milk drinks, by using two of single strains of commercial probiotic starter cultures, has been compared with each other.

Materials and Methods

Two applied probiotic strains, *Lactobacillus acidophilus and* Bifidobacterium lactis with the commercial names of La-5 and Bb-12, respectively, were supplied from Christian Hansen in Denmark and were freeze-dried. These two strains were added to UHT milk in the rate of 0.01% W/V separately and in joint forms for preparing the Acidophilus (A), Bifidus (B) and Acidobifidus (AB) milks respectively. Inoculation has been carried out at 37 ˚C and then prepared inoculated milks were translocated to 37˚C incubator for 5-h incubation period.

- Microbial analysis

Each sample was microbiologically analysed at 0, 2, 4 and 5 h after incubation. One ml of each sample aseptically was diluted in 9 ml of strile ringer solution and after preparation, the proper serial dilutions of each sample, viable numbers of probiotic microorganisms were enumerated by using the pour plate technique.

MRS-agar with aerobic and anaerobic incubations, at 37 ˚C for 72 hours, were applied respectively for enumeration of *L. acidophilus* content of A and AB samples and *B. lactis* content of B sample. While for

Table 1. Commercial products containing Bifidobacterium spp. and Lactobacillus acidophilus

Product	**Country of origin**	**Microorganisims**
A-38	Denmark	*Lactobacillus acidophilus, Bifidobacterium bifidum, Leuconostoc mesenteroides spp. cremoris, mesophilic lactococci*
Acidophilus buttermilk	USA	*Lactobacillus acidophilus, Leuconostoc mesenteroides spp. cremoris, mesophilic lactococci*
Acidophilus milk	Several countries	*Lactobacillus acidophilus*
Cultura	Denmark	*Bifidobacterium spp., Lactobacillus acidophilus*
Biomild	Several countries	*Bifidobacterium spp., Lactobacillus acidophilus*
Biogarde	Germany	*Lactobacillus acidophilus, Bifidobacterium bifidum, Streptococcus thermophilus*
Bi®dus milk	Several countries	*Bifidobacterium bifidum, B. longum*
Bi®ghurt	Germany	*Bifidobacterium bifidum, Streptococcus thermophilus*
Biogurt	Germany	*Lactobacillus acidophilus, Streptococcus thermophilus*
Biokys	Czech Republic	*Bifidobacterium bifidum, Lactobacillus acidophilus, Pediococcus acidilactici*
Mil-Mil	Japan	*Lactobacillus acidophilus, Bifidobacterium bifidum, B. breve*
Akult	Japan	*Lactobacillus acidophilus, Bifidobacterium bifidum, B. breve, L. casei subsp. casei*

Adapted from Kurmann (1998) and Hoier (1992) enumeration of viable number of *B. lactis* in AB sample, MRS-NNL (neomysin soulfate, nulidixic acid and lithium chloride) media (Laroia & Martin, 1991) was used beside the above mentioned anaerobic incubation conditions. After incubation, bacterial colonies between 30 and 300 were counted and the results expressed as bacterial count per milliliter (cfu/ml) of the samples. The data presented are the means of the results obtained from duplicate plates of the samples analysed in cfu/ml.

- *Chemical analysis*

Beside the microbial assessments, the titratable acidity and the pH values of the samples were measured at regulated time intervals over the fermentation period. The pH values of the samples were measured at $20\text{-}25^{\circ}$ C using an Crison pH meter after calibration with standard buffers and the titratable acidity was determined by titration of 10 ml of samples with 0.1 N NaOH using a 0.5% phenolphthalein as indicator to an end point of faint pink color.

- *Sensory evaluation*

For the evaluation of the acceptability level of these produced milks, such a tastes, according to the one – way graduation test, a questioner was designed and along with the A, B and AB milks and UHT milk (as control) were given to 20 assessors as evaluating group. The extracted results from the questioners were statistically (Kramer, 1966) analyzed.

- *Statistical analysis*

All the experiments and analysis were carried out in triplicate order. All data were analysied using one-way analysis of variance and Tukey's pairwise comparisons procedures of MINITAB.

Results and Discussion

The shifts of viable counts of *L. acidophilus* and development of acidity and pH of sample A has been shown in Table 2.

The titratable acidity, in all assessments, during the fermentation period increased significantly (Fig. 1). Likewise pH values of sample, except in the last hour of incubation, significantly decreased (Fig. 2). The viable counts of *L. acidophilus* was rising up over the 5-h fermentation period but this trend was significant only between the last two assessments in the 4^{th} and 5^{th} hour of incubation. So it can be a symbol of high activity of *L. acidophilus* in this stage (Fig. 3).

The shifts of viable counts of *B. lactis* and development of acidity and pH of sample B has been shown in Table 3.

Table 2. Titratable acidity, pH, and probiotic counts of Acidophilus milk during fermentation period

Sample A (Acidophilus milk)	Incubation Time (h)			
	0	2	4	5
Titratable acidity (Dornic)	13.5	16	19	21.3
pH	6.63	6.36	6.15	6.08
Viable *L. acidophilus* (cfu/ml)	2.50×10^{7}	4.60×10^{7}	2.70×10^{7}	1.35×10^{8}

Table 3. Titratable acidity, pH, and probiotic counts of Bifidus milk during fermentation period.

Sample B (Bifidus milk)	Incubation Time (h)			
	0	2	4	5
Titratable acidity (Dornic	15	17.16	19.83	22
pH	6.61	6.45	6.26	6.10
Viable *B. lactis* (cfu/ml)	1.08×10^{8}	1.18×10^{8}	1.62×10^{8}	2.32×10^{8}

As shown, significant increases of acidity occurred along with the significant decreases of the pH values (Figures 1 and 2). The viable counts of *B. lactis* in sample B only in the 5th hour of incubation showed a significant increase (Fig. 3).

Table 4 shows the viable counts of probiotic bacteria, separately and in joint form, and the changes of titratable acidity and pH values of sample AB.

The titratable acidity of sample AB had significantly increased in all the assessments over the five hours of incubation and its pH values has shown significant decreases in all the assessments except in the 4th hour of incubation (Figures 1 and 2). The development of viable counts of probiotic bacteria (*L. acidophilus* and *B. lactis*) in the sample AB, from the first up to the last enumeration had significant growing trend but the individual enumeration of each bacteria in mixed culture has shown that the significant increase in the number of viable *L. acidophilus* and *B. lactis* has occurred at exactly the 4th hour and around the 4th hour of incubation respectively, (Fig. 3).

Comparison of the growth rates of *B. lactis* in B and AB (Fig. 4) milks illuminated some positive or stimulating effects of *L. acidophilus* on the growth rate of *B. lactis* therefore the growth curve of *B. lactis* in AB milk was significantly different from its growth curve in B. milk, but the growth rate of *L. acidophilus* in A and AB milks were similar, explaining very low, or if any, positive co-existance effects of these two bacteria on the growth rate of *L. acidophilus* (Fig. 5).

The degree of sour taste perception has been evaluated by one-way graduation test.

The extracted results (Table 5) from designed questioners for estimating the degree of sour taste perception in A,B, AB and D milks, indicated that the perception of sour taste in AB milk was significantly higher than other milks (Fig. 6).

Table 4. Titratable acidity, pH, and probiotic counts of Acidobifidus milk during fermentation period

Sample AB (Bifidus milk)	Incubation Time (Hour)			
	0	2	4	5
Titratable acidity (Dornic)	14..3	16	18	21
pH	6.67	6.64	6.32	6.04
Total viable probiotic bacteria (cfu/ml)	6.30×10^7	9.00×10^7	1.41×10^8	2.52×10^8
Viable *L. acidophilus* (cfu/ml)	1.83×10^7	3.57×10^7	4.70×10^7	9.10×10^7
Viable *B. lactis* (cfu/ml)	2.33×10^7	4.03×10^7	6.83×10^7	1.29×10^8

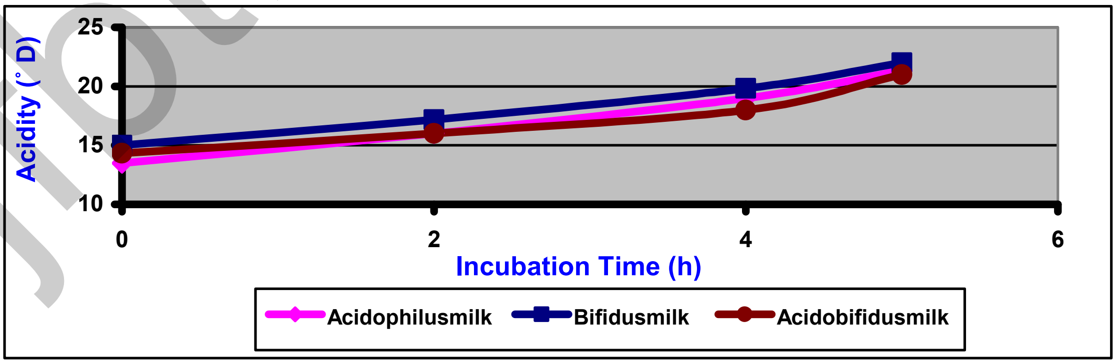

Fig. 1. Evaluation of acidity in A, B and AB milk during fermentation period

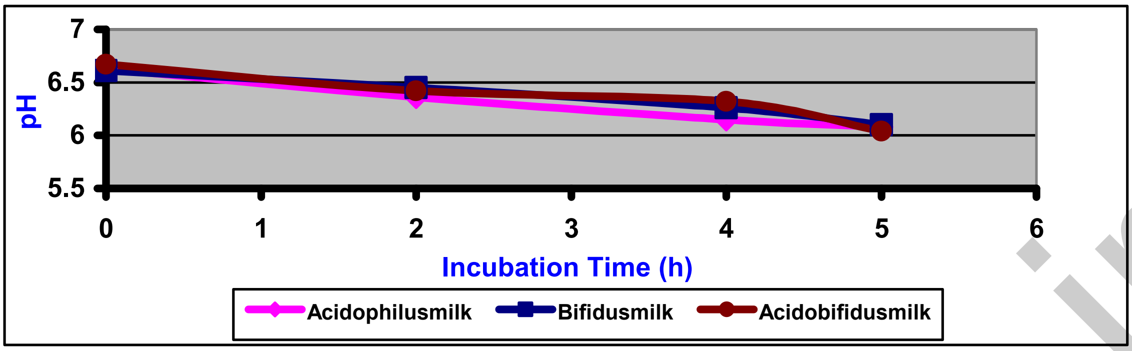

Fig. 2. Evaluation of pH in A, B and AB milk during fermentation period

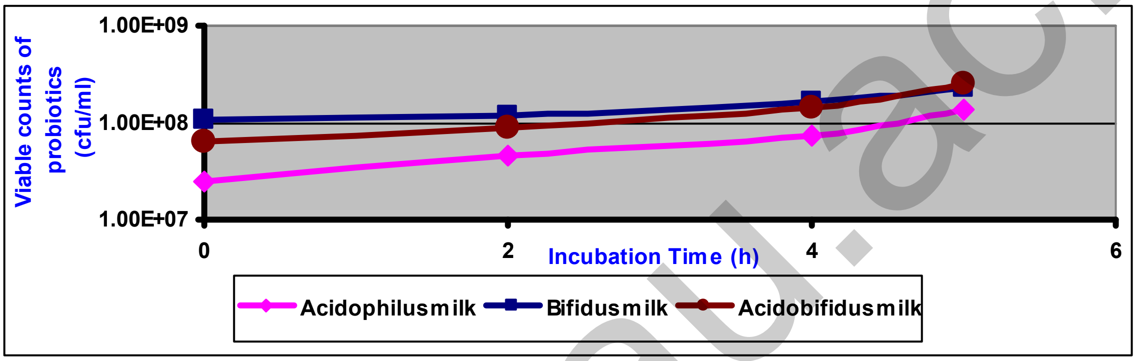

Fig. 3. Viable counts of probiotic bacteria in A, B and AB milks during fermentation period

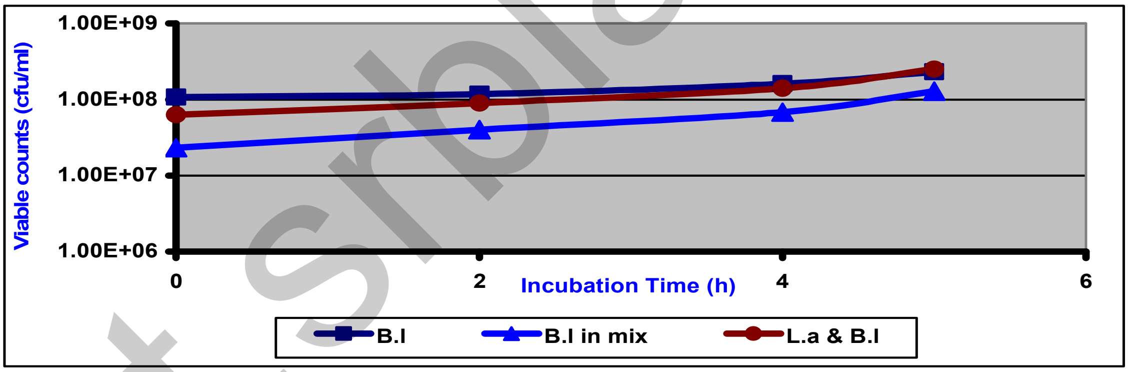

Fig. 4. Comparison of viable counts of B. lactis in B and AB milk during fermentation period

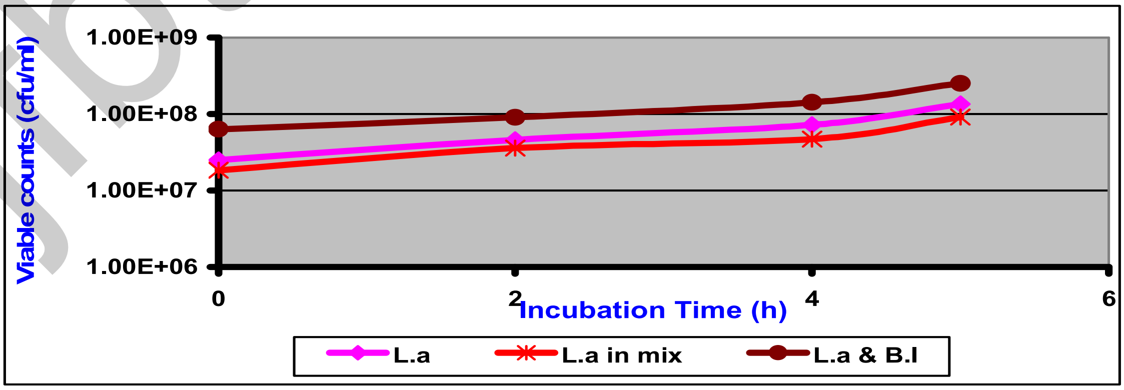

Fig. 5. Comparison of viable counts of L. acidophilus in A and AB milk during fermentation period

Table 5. Extracted scores from the designed questioners of 20-person assessor group for sensory evaluation of A, B and AB milks

Scores allocated from no to very high perception of sour taste	A-milk	B- milk	AB-milk	D-milk
1	12	11	4	13
2	4	7	8	4
3	3	1	1	2
4	1	1	4	1
5	-	-	3	-
6	-	-	-	-
Sums of scores	33	32	54	31
Average of scores	1.65	1.60	2.70	1.55

D: UHT milk as control
* Statistically shown significant differences ($p \leq 0.05$)

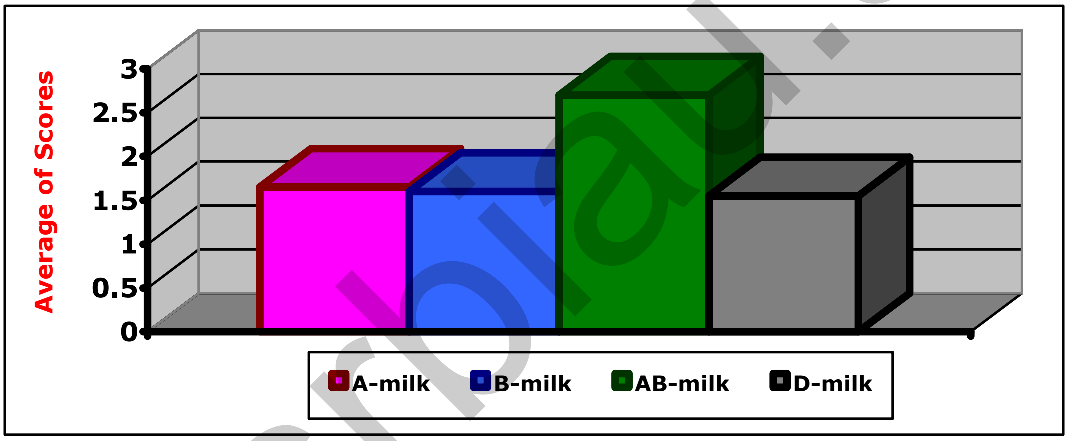

Fig. 6. Perception of sour taste in A, B and AB milks compare with D milk (UHT) as control

Conclusion

Table 6 shows the final characteristic of 3 produced milks.

Considering the same inoculation rate and incubation conditions for the three products, there were not any significant differences between the final chemical characteristics and their final contents of viable probiotic bacteria and the properties were in the range allocated to probiotic products (10^6-10^7 cfu/ml), but the perception of sour taste in AB milk, as explained above, was significantly higher than other milks investigated. Regarding the growth rates of single strains in pure cultures (A and B milks) and mixed culture (AB milk), some changes were observed in the growth rate of *B.lactis* in the presence of *L. acidophilus*. In other words the co-existance of these two strains probably causes the production of some special metabolites that affect the sensory evaluation, therefore insipid of the same acidity and pH, the perception of sour taste in AB milk were considerably more than A and B milks. Thus on the base of these found data the lower inoculum rate of starter cultures and even the shorter incubation time might be recommended for production of AB milk.

Table 6. Final microbial and chemical characteristics of A, B and AB milks

Name of product	Acidity (D)	pH	Viable counts of probiotic bacteria (cfu/ml)	Average of scores in perception of sour taste
A-milk	21.3	6.08	1.35×10^8	1.55
B-milk	22	6.10	3.20×10^8	1.6
AB-milk	21	6.04	2.52×10^8	2.7

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