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The Shifts of Acidophilus Milk at the Refrigerator

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ABSTRACT: Acidophilus milk after preparation, with the defined characteristics of pH, titratable acidity and viable counts of *Lb. acidophilus*, was kept at the refrigerator (5 °C) and changes of above mentioned parameters were evaluated during 21 days at 7-day intervals. The obtained results showed that the titratable acidity of product significantly ($p \leq 0.05$) increased over storage period, demonstrating the post acidification ability of *Lb. acidophilus* at low temperatures. The pH values of Acidophilus milk, due to the buffering capacity of milk, didn't show significant ($p \leq 0.05$) decrease after 14 days, which this was contrary to the obtained result at 21 days of storage period. The viability of *Lb. acidophilus* significantly ($p \leq 0.05$) fell down in the first two assessments due to cold shock and increase of acidity and then raised at the last assessment due to the proteolytic activity of *Lb. acidophilus,* causing the liberation of nutritive amino acids.

Keywords: *Acidophilus Milk, Lb. acidophilus, Storage Time.*

Introduction

Fermented milks are salutary products as they represent good sources of vitamins and minerals and generally contain proteins and small amounts of lipids. Fresh fermented dairy products supply a number of lactic acid bacteria (LAB) that might provide additional health benefits. Some of these LAB which are resistant to gastric acidity and bile salts and therefore pass through the gastrointestinal trac, named probiotics (Naidu *et al*., 1999). The action of probiotics on intestinal flora results in vital benefits, including protection against pathogens, development of the immune system (Isolauri *et al*., 2002) and positive effects on colonic health and host nutrition (Falk *et al*., 1998; Uaesaki and Setoyama, 2000). Other important properties that have been attributed to probiotics include prevention and treatment of gastrointestinal disorders (Lewis and Freedman, 1998), reduction of food intolerance (Dunne *et al*., 2001), modulation of the host immune responses (Isolauri *et al*., 2001), prevention of cancer and cardiovascular diseases and reduction of serum cholesterol and lipids (Wallowski *et al*., 1999). The research of novel formulations with newly selected probiotic strains is important to satisfy the increasing request of the market and to obtain functional products in which the probiotic cultures are more active and protected from the gastrointestinal stress. New probiotic strains should be screened by evaluating not only their potential beneficial outcomes, but also for their technological performances, such as growth rate and stability in milk, acidification ability, and favourable organoleptic properties of the final product (Minelli *et al*., 2004). Acidophilus milk is one of well-known dairy probiotic products which is produced in many countries as a functional product. Acidophilus milk contains Lactobacillus acidophilus. The maintenance of the characteristics of this product is very important because, for instant, the count of *Lb. acidophilus* as

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probiotic microorganism shouldn't be less than 10^6-10^7 cfu/ml of product (Gomes and Malcata 1999) besides good maintenance of its chemical characteristics like acidity and pH values that significantly affect its consumption acceptance. Since, mostly, such fermented dairy products are kept at refrigerator till their consumption time, we have investigated some of the chemical and microbial shifts of Acidophilus milk during the 21 day refrigerated storage period.

Materials and Methods

- Probiotic strain

Commercial single strain lyophilized culture of *Lb. acidophilus* known as FD-DVS La-5 was supplied from Chr. Hansen (Horsholm, Denmark).

- Sample preparation

UHT milk of a dairy factory was inoculated, in 37 ˚C, by 0.01% (w/v) of La-5. Inoculated milk aseptically distributed in sterilized 100 ml bottles (one bottle was prepared for each sampling time), then fermentation followed in 5 hours at 37 ˚C. Prepared samples (Acidophilus milk) were kept at 5 °C for 21 days for performance of microbiological and chemical analysis during storage period at 7 day intervals.

-Microbiological analysis

At each sampling interval, one bottle was aseptically withdrawn and after vigorous shaking, 1ml of its content dispensed into 9 milliliter of quarter strength Ringer's solution (Merck, Germany). Following this way appropriate dilutions were made and subsequently pour-plated in duplicate order was performed onto a selective media. *Lb. acidophilus* was counted in MRS (De Man, Rogosa and Sharpe) agar incubated aerobically at 37°C for 3 days. After incubation, bacterial colonies between 30 and 300 were counted and the results expressed as colony forming unit per milliliter (cfu/ml) of the sample. The data presented are the means of results obtained from duplicate plates of the samples analysed in cfu/ml.

-Chemical analysis

Titratable acidity of samples (°D: degree of dornic) was measured by titrating of 10 ml of sample with 0.1 N NaOH using phenol phetalein as indicator (Akin *et al*., 2007). All pH measurements were made using a digital pH meter with combined glass electrode and temperature probe. The pH-meter was calibrated using standard buffer solutions at pH 4.0 and 7.0 (Ostil *et al*., 2005).

- Statistical analysis

All the experiments replicated three times and then statistically analysed using one way analysis of variance with the circle assurance of 95 %, ($p \leq 0.05$), by MINITAB procedures.

Results and Discussion

Table 1 shows the changes of viable counts of *Lb. acidophilus*, titratable acidity and pH values of Acidophilus milk, from the preparation time of samples up to the end of 21 days of refrigerated storage.

Table 1. The shifts of Acidophilus milk from the point of microbiological and chemical views during storage at refrigerator over 21 days

Acidophilus milk	Storage time (Day)			
	0	7	14	21
Viable cell count (cfu/ml)	$1.35\,8 \times 10^{8*}$	$2.90 \times 10^{7*}$	$9.30 \times 10^{6*}$	$7.93 \times 10^{7*}$
Titratable acidity (°D)	21.33*	23.83*	26.50*	28.66*
pH value	6.08	6.05	5.95	5.76*

* This point had statistically significant change ($p \leq 0.05$) rather than its previous point.

Titratiable acidity: The acidity of Acidophilus milk increased significantly in all assessments during the storage period (Fig. 1). As previously reported (Martinez-Villaluenge *et al.*, 2006), the increase in acidity is a common characteristic of fermented milks after their refrigerated storage. The retention of β-galactosidase activity by non viable cells and enzyme stability upon refrigerated storage might explain the post acidification in fermented milks during their refrigerated storage (Hughes and Hoover, 1995).

pH: The pH value of Acidophilus milk after 14 days of storage was almost constant. This observation, considering the increase of the acidity in these intervals, might explain the buffering capacity of milk proteins repressing the falling of pH value. Finally, with the increase of the acidity during the last 7 days of the storage period, the pH value of Acidophilus milk fell down significantly in the final assessment (Fig. 2).

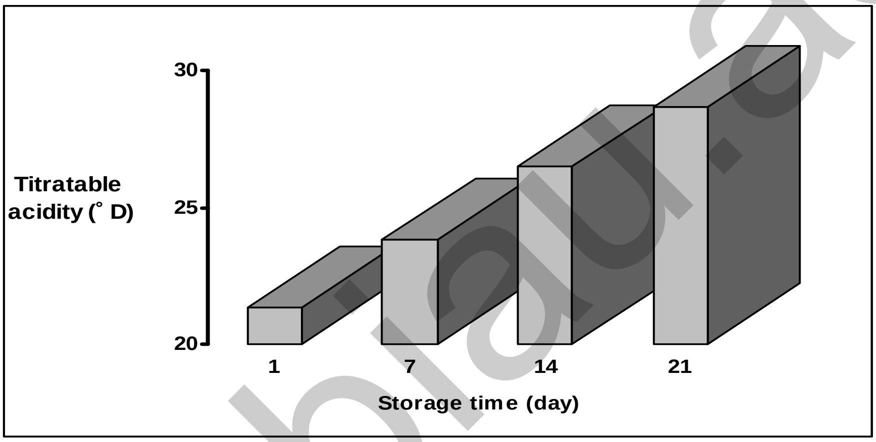

Fig. 1. Variation of titratable acidity in Acidophilus milk during 21days at refrigerator

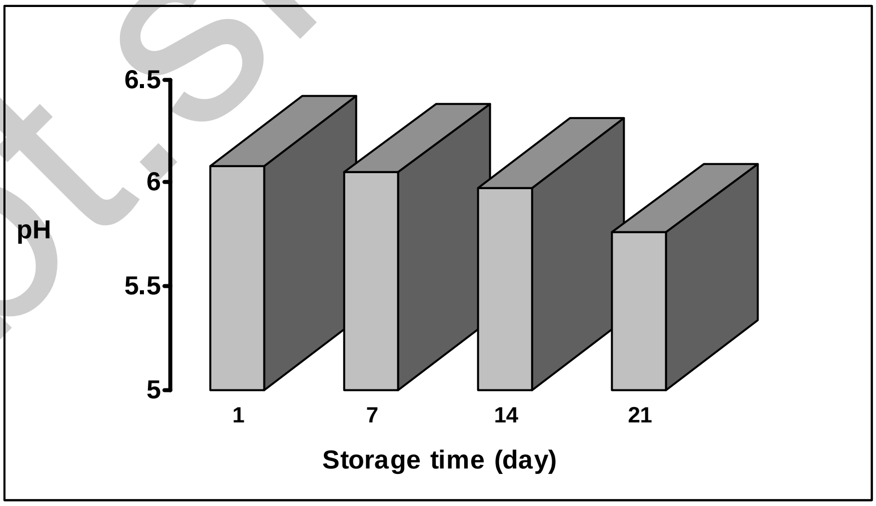

Fig. 2. Variation of the pH value in Acidophilus milk during 21 days at refrigerator

Probiotic viability: The viable counts of *Lb. acidophilus* significantly decreased till the 14 days of storage period and this trend intensified at the second assessment interval between 7 and 14 days of storage (Fig. 3).

This falling rate style of viable count of *Lb. acidophilus* might be ascribed to the entered cold shock which bacteria encounter at their entrance to refrigerator and then the increase of acidity during the refrigerated storage period which results in acid injury of microorganisms. These are two convincing reasons causing the reduction of cell viability in fermented milks after their refrigerated storage (Martinez-Villaluenge *et al*., 2006), but as it shown in Fig. 3, the viable count of *Lb. acidophilus* had a sudden and significant ($P \geq 0.05$) increase at the last day of storage period.

The cause of this strange shift, which was observed in all replicates, might probably be attributed to the proteoletic activity of *Lb. acidophilus* (Donkor *et al*., 2006). As Donkor *et al* 2007 reported the storage time plays an important role in the extent of overall proteoletic activity, consequently increases the amount of liberated amino acids and might cause higher growth rate of probiotic bacteria even in acidic environment.

The changes of viable count of *Lb. acidophilus* in the samples after passing 21 days in refrigerated storage reached 7.93×10^{7} cfu/ml, therefore this count is still in the range (10^{6}-10^{7} cfu/ml) allocated for probiotic products (IDF, 1992).

Conclusion

Acidophilus milk is a probiotic milk drink that contains *Lb. acidophilus* as probiotic agent. Therefore the definition of suitable cold storage conditions to minimize its undesirable changes is a notable case. Some of the obtained results from the assessments on these shifts revealed that although *Lb. acidophilus* as a thermophilic microorganism can not grow in low temperatures but like lactobacilli of yoghurt starter culture has post acidification ability and the buffering capacity of milk suppresses the drop of the samples pH up to the 14 days of storage period. The viable counts of *Lb. acidophilus* fell down after 14 days of storage period, presumably for the entered cold shock of refrigerator and also for acid injury of bacteria. Therefore due to the proteoletic property of lactobacilli strains, the counts of viable *Lb. acidophilus* increased in the last microbial assessment at the 21 day of refrigerated storage. It should also be considered that the counts of *Lb. acidophilus* in the samples remained in the range allocated for probiotic products, after passing 21day refrigeration storage period.

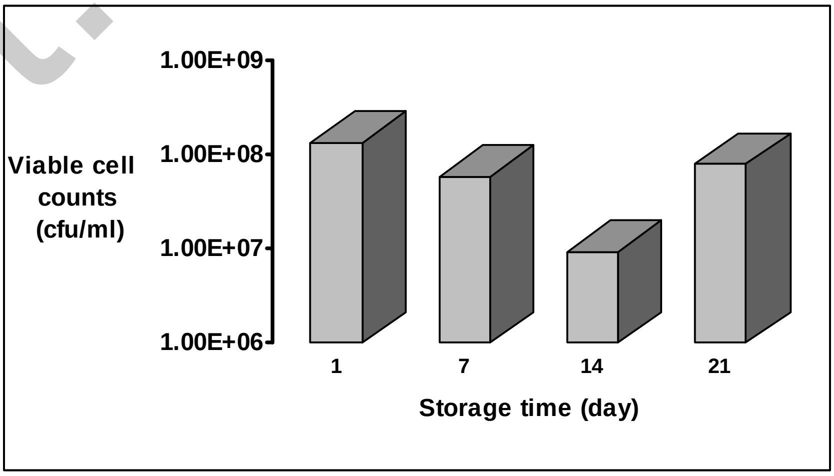

Fig 3. Variation of viable count of *Lb. acidophilus* during 21 days at refrigerator

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