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Probiotic Survival in Yogurt Made from Ultrafiltered Skim Milk During Refrigeration Storage

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Abstract: Chemical composition, growth and survival of *Lactobacillus acidophilus* and *Bifidobacterium Lactis* as probiotic bacteria in yoghurt made from ultrafiltered milk were studied and compared with control yoghurt with 2% (w/v) skim milk powder in triplicate. The results showed that, increasing the protein content and total solid concentration of milk by ultrafiltration, increased buffering capacity, acidity and the survival of probiotics. However, the survival of probiotics decreased throughout the storage period at 2°C. This study indicates the importance of protein content on survival of probiotic strains because of more available peptides and amino acids and also its effect on buffering capacity that reduce the lethal effect of lactic acid on yoghurt bacteria.

Key words: Probiotic, survival, ultrafiltration, yoghurt

INTRODUCTION

Probiotic bacteria, defined as iving micro-organisms exert health benefits such as anti-mutagenic, anti-carcinogenic and anti-infection properties, immune system stimulation, serum cholesterol reduction, alleviation of lactose intolerance and nutritional enhancement. Species of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantice* and *Bifidobacterium lactis* are the most popular bacteria applied for food probiotic products. *Lactobacillus acidophilus*, having mentioned characteristics is an important health promoting probiotic strain (Kailasapathy and Rybka, 1997). Although, there is no standard concerning the population of the probiotic bacteria in yoghurt at the end of product shelf life, it is recommended that between 10^5 and 10^8 CFU/g is an acceptable level for live probiotic population in final product (Shah *et al*., 1995).

Yoghurt is considered to be healthy and its consumption has been significantly increased because of its health benefits. The formulation of products such as yoghurt with optimum consistency and stability to syneresis is a main concern to dairy industry. The solid content of milk can be increased by applying evaporation of treated milk, addition of Skim Milk Powder (SMP) or protein concentrates and concentrating the skim milk by ultrafiltration (UF) or Reverse Osmosis (RO). Addition of milk powder or whey is a general method for increasing the total solids of yoghurt. However, the thermal degradation of proteins and vitamins through heat treatment of milk can be very important in these cases, reducing the nutritional value of yoghurt. Also milk powder itself is a product witch has undergone extremely harsh thermal processing that alters some of its constituents. An alternative to obtain yoghurt with higher nutritional value such as protein content is to increase the total solids in milk by means of membrane technology (Tamime and Robinson, 1999; Walstra and Geurts, 1999).

The present study was carried out to examine the effect of increasing protein content of milk by ultrafiltration on survival of *L. acidophilus* and *B. lactis* as probiotics during refrigeration storage.

MATERIALS AND METHODS

Experiments were done in triplicate in dairy pilot plant of Department of Food Science and Technology, Faculty of Biosystem Engineering, Campus of Agriculture and Natural Resources, University of Tehran, Tehran, Iran.

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Ultrafiltration: Pasteurized milk was ultrafiltered with a poly ether sulphone spiral wound membrane (nominal molecular weight cut-off 20,000 D) at 50°C. Retentates were obtained form the system at three steps with attention to their TS contents. Samples were named A, B and C, respectively.

Fermentation step: When the necessary concentration was reached by ultrafiltration, the samples were heated at 78°C for 1 min in stainless steel buckets with stirring continued during cooling to 42°C. These samples were inoculated with the starter culture, code ABY-1 from CHR-Hansen (Denmark), including *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *L. acidophilus* and *B. Lactis*, at a level of 0.4% v/v (Nu-trish, 2004, Hansen) and dispensed into yoghurt pots of 200 mL. The containers were then incubated at 42°C until acidity reached 0.9%. After incubation, the samples were transferred to a refrigerator at 2°C that was recommended for probiotics (Nu-trish, 2004, Hansen). A control sample was prepared with pasteurized milk (with 3.23% fat content) and addition of 2% SMP (w/v) with the same inoculation and named as D sample.

Analysis: Total solid, fat, protein and lactose content of retentates were determined by a Milko-Scan (133 B N. FOSS Electric, Denmark). The pH of yoghurt samples were measured using a digital pH-meter (Metrohm 691, Swiss), while acidity was titrated by M/10 NaOH solution.

For microbiological analysis, samples (1.0 mL) of yoghurts were decimally diluted in sterile peptone water (0.1%) (Merck, Germany) and 0.1 mL aliquot dilutions were spread over the surface of plates of MRS bile agar (Merck, Germany) and incubated in 37°C for 3 days. After this period Colony Forming Units (CFU) were counted by a colony counter.

Statistical analysis: Statistical analysis of obtained data was assessed by SAS software version 8. The mean differences were analyzed using LSD test at $p<0.05$.

RESULTS AND DISCUSSION

The UF experiments were aimed at providing membrane retentates with solid levels at 11.93, 13.68 and 16.18% for subsequent production of yoghurt. The apparent protein content of retentates increased from 3.07% in pasteurized milk to 6.80% in the most concentrated retentate having total solids of 16%. Lactose was slightly decreased, fat content increased from 1.80-3.74% and also total solid increased from 10.24-16.18% in the final received sample (C) (Table 1). Acidity was detected for all the samples during storage (Fig. 1).

Table 1: Compositions of pasteurized milk, UF retentates, permeate and control sample

sample	Fat	Protein	Lactose	TS	SNF
Pasteurized milk	1.80±0.01	3.07±0.01	4.78±0.02	10.24±0.03	8.44±0.03
A (Cf = 1.5)	2.46±0.01	4.28±0.02	4.62±0.01	11.93±0.01	9.46±0.02
B (Cf = 1.8)	2.91±0.07	5.26±0.01	4.55±0.06	13.68±0.02	10.31±0.09
C (Cf = 2.2)	3.74±0.01	6.80±0.11	4.47±0.01	16.18±0.03	12.48±0.02
D (Control)	3.23±0.03	3.06±0.01	4.92±0.05	11.80±0.03	8.57±0.07
permeate	0.90±0.01	0.13±0.01	4.07±0.01	4.83±0.02	3.77±0.02

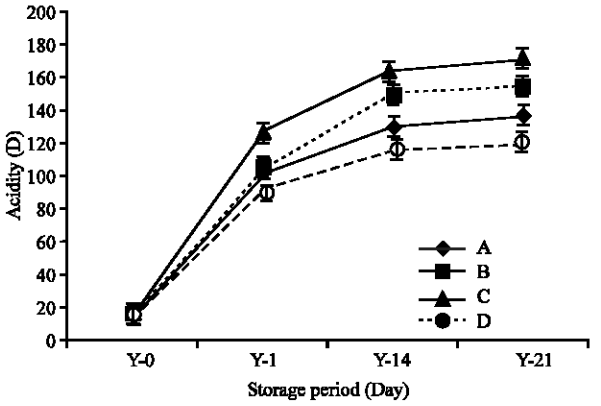

Fig. 1: Acidity changes during storage period (1, 14 and 21 days) of ultrafiltered samples and control. Error bars indicate the standard deviation. A, B, C: retentates with concentration factors of 1.5, 1.8 and 2.2, respectively, D: control

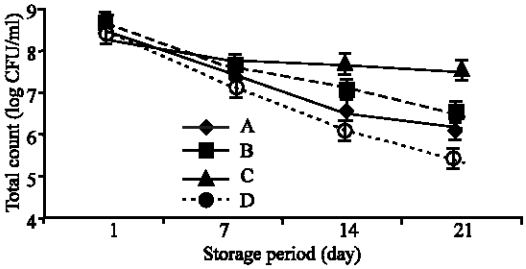

Fig. 2: Total counts of probiotic bacteria with different protein content of yoghurt samples within 21 days. A, B, C: retentates with concentration factors of 1.5, 1.8 and 2.2, respectively, D: control

As was shown in Fig. 1 the acidity increased in all samples at the day one of storage during fermentation and followed within storage period. Also the acidity of UF yoghurts are always more than control (D) which is because of more protein content of these samples and accordingly more buffering capacity that permit more acid production (Biliaderis and Khan, 1992; Mistry and Hassan, 1992).

During refrigeration period, the survival of probiotics, *L. acidophilus* and *B. lactis* decreased significantly ($p<0.05$) (Fig. 2). The number of viable probiotics were

increased by increasing the protein content of yoghurt. It could be inferred from this result that protein enriched yoghurt can provide a better media for probiotics. Generally the rate of fermentation of probiotics are slow and accordingly inoculations take more time (Ozer and Robinson, 1999). Although, milk has all the required nutrients for probiotics growth but some of them are not in the form of absorbable or accessible or are less than required. The non Protein Nitrogen (NPN) is the most important of these nutrients. Debility of probiotics for proteolytic activity leads to the lack of NPN. On the other hand acidity and low pH of yoghurt is one of the most important factors which can reduce the survival of probiotics. Ultrafiltration of milk increases protein concentration that leads to more buffering capacity and accordingly more survival of probiotics, because protein prevents from pH fall with absorb of hydrogen ions and leads to more ionized lactic acid that results less lethal effect on the yoghurt bacteria (Shah *et al*., 1995; Ozer and Robinson, 1999).

CONCLUSION

The results of chemical analysis showed that the increase of the protein content in ultrafiltered samples causes more but slow acid production because of the more buffering capacity of these samples. Also, more protein content showed more survival of probiotics than control. On the whole, it is possible to increase the number of viable probiotics in final yoghurt with increasing protein content in order to reach the minimum survival level recommended by International Dairy Federation (10^7) that is necessary for its beneficial effects.

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