Manufacturing of fermented goat milk with a mixed starter culture of *Bifidobacterium animalis* and *Lactobacillus acidophilus* in a controlled bioreactor

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Abstract

Aims: This work was undertaken to study the feasibility and the characteristics of a fermented product made of goat milk, using a mixed starter culture of *Bifidobacterium animalis* and *Lactobacillus acidophilus* under controlled conditions, and to determine their survival in the fermented milk during refrigerated storage.

Methods and Results: Goat milk was inoculated with *Lact. acidophilus* and *Bif. animalis* mixed starter, fermented in a glass bioreactor with controlled temperature (37°C) and anaerobiosis, and monitored for growth and acidification. The fermented milk was then stored for 10 days under refrigeration, and monitored daily for starter microflora survival and pH changes. *Lact. acidophilus* viable counts reached a maximum of $7{\cdot}1 \times 10^8$ colony-forming units (CFU) ml^{-1}, and *Bif. animalis* a maximum of $6{\cdot}3 \times 10^7$ CFU ml^{-1} by 20 h of fermentation. During refrigerated storage, both strains exhibited a good survival, with viable numbers remaining essentially constant throughout the experiment, whereas the pH of the fermented milk dropped slightly.

Conclusions: Mixed cultures of *Bif. animalis* and *Lact. acidophilus* may be used to produce fermented goat milk with high counts of both probiotic strains.

Significance and Impact of the Study: Goat milk fermented with *Bif. animalis* and *Lact. acidophilus* can be manufactured as an alternative probiotic dairy product.

Introduction

Innovation and optimization are keywords in food applied research and food industry. In the dairy field, several new brands of fermented foods are introduced in the market every year (Ventling and Mistry 1993). Many of these products belong to the so-called 'functional foods' group, containing selected bacteria such as *Lactobacillus acidophilus* or *Bifidobacterium* spp. which are claimed to provide several prophylactic and therapeutic benefits (Colombel *et al.* 1987; Ishida *et al.* 2005). The consumption of these products is increasing as their health-fostering properties become scientifically established (Ishibashi and Shimamura 1993), and are now available as powder, capsules, tablets or, more commonly, fermented dairy products (Kurmann 1993). However, because it is difficult to cultivate these bacteria in milk, their successful use requires improvement in the area of processing or use of new growth media (Kurmann 1993; Gibson and Wang 1994; Gomes *et al.* 1998). The use of milk from small ruminants (goat and ewe) or the combination of specific strains, which may grow in synergy, may represent one direction of innovation in the manufacturing of new products (Gomes *et al.* 1998). It is known that goat milk can be a good substitute for cow milk in situations where bovine milk causes an allergic reaction; furthermore, some consumers may prefer goat milk for dietetic reasons or questions of taste. In the European Union (EU), goat milk products are considered to be the dairy product with the greatest marketing potential and, therefore, several

characteristics of goat milk are currently the focus of increased research interest (Casalta *et al.* 2005). The lower buffering capacity of goat milk when compared with that of cow milk (Casalta *et al.* 1995) may allow for a faster acidification of that media, thus avoiding contamination during fermentation undertaken with species that grow slowly, as is the case of *Bif. animalis*, previously *Bifidobacterium lactis* (Klein *et al.* 1998), which is a common isolate from probiotic commercial preparations (Roy *et al.* 1996).

One research focus in our laboratory has been the characterization and the technological improvement of products from goat and ewe milk (Gomes and Malcata 1998a). Studies concerning the growth characteristics of *Lact. acidophilus* and *Bif. animalis* in small ruminant milk media (Gomes and Malcata 1998a,b), in cow milk (Gomes *et al.* 1995, 1998) and in artificial medium (Kongo *et al.* 2003) have been undertaken. Other studies within our group (not published) have also shown a commensalistic growth relation in mixed cultures of *Lact. acidophilus* and *Bif. animalis*, resulting in a shorter lag phase of the latter when compared with the lag phase in single strain cultures. Gomes *et al.* (1995) concluded that the incorporation of *Bifidobacterium* spp. and *Lact. acidophilus* as starters in a Gouda-type cheese is feasible, and can offer an alternative and interesting route of administering them to human beings. However, probiotic bacteria are still mainly ingested in yogurt-type dairy products (Tannock 1997).

The aim of this study was to manufacture and characterize the features of a fermented yogurt-like product made of goat milk, using a mixed starter culture of *Bif. animalis* and *Lact. acidophilus* under controlled conditions in a bioreactor.

Material and methods

Cultivation medium

Goat milk from a selected, single-breed flock (Alpina) was purchased from Direcção Regional de Agricultura da Beira Interior (Portugal). The microbiological quality of fresh milk was assessed by determining the total mesophile counts through plating duplicates of decimal dilutions (in peptone water) in plate count agar (PCA) medium incubated at 30°C for 48 h. Fat, protein, lactose and non-fat solids in the partially skimmed milk were determined in a Milko-Scan (Foss, Denmark) using calibration curves previously prepared for goat milk. After the skimming operation, the milk was stored at −30°C in 1-l portions. Just before use, the milk was thawed in a microwave oven, poured into the fermentor which was then sterilized at 110°C for 15 min, and then cooled to 37°C.

Starter preparation

Bifidobacterium animalis and *Lact. acidophilus* cultures were obtained from CSK (Leeuwarden, The Netherlands) in the form of pure starter concentrates. Inoculum was aseptically prepared by adding 10 ml of each starter concentrate to a flask containing 200 ml of sterile goat milk, followed by anaerobic incubation for 8–10 h in BBL Gas-Pak culture system (Cockeysville, MD, USA) at 37°C.

Fermentation was started after pumping 25 ml of the preculture into the bioreactor and homogenization of the medium by automatic stirring for 5 min.

Controlled fermentation

A 2-l BIOSTAT B fermentor (B Braun Instruments, Melsungen, Germany) was used to half of its capacity, with the temperature maintained at 37°C and anaerobic conditions assured via continuous sparging with oxygen-free nitrogen. The data presented are the results of two experiments.

Growth assessment and acidification

For each batch, growth in the bioreactor was assessed by aseptically collecting 10-ml samples at preset time intervals; from each sample 1 ml was diluted (with vigorous vortexing to break down any bacteria clusters) in saline peptone water (Sigma, St Louis, MO, USA) at 0·1% (v/v). For total viable counts of *Bif. animalis*, spread plating was performed in duplicates on MRS (DeMan, Rogosa and Sharpe) agar (Merck, Darmstadt, Germany) containing 0·5 g l^{-1} cystein–HCl (Merck), 2 g l^{-1} bile salts (Difco, Detroit, MI, USA) and 50 ml l^{-1} sheep blood as selective agents (Klaver *et al.* 1993). For *Lact. acidophilus*, it was performed on TGV (Tryptone Glucose Meat extract) agar medium supplemented with 20 g l^{-1} NaCl. Plates were incubated anaerobically for 48 h at 37°C in BBL Gas-Pak culture system (BBL). Individual species were confirmed by visual inspection of colony morphology and microscopic observation. What remained of each sample was used for high-performance liquid chromatography (HPLC) analysis. Acidification (as pH change) was monitored and registered online, through an automatic data acquisition device attached to a computer.

Survival

By 20 h of fermentation, the bioreactor was stopped. Sample portions of 100 ml were aseptically transferred into sterile flasks (100 ml each), which were stored at refrigerating temperature (5–7°C) for 10 days. For each

run, three of the flasks were sampled at preset times for monitoring the survival of both species by total viable counts as previously indicated, and for pH changes by transferring samples to universal tubes where the pH was monitored with a Metrohom-pH meter (Switzerland) equipped with a combined electrode (Ingold, UK).

Sensorial tasting

Basic sensorial tests (taste and general aroma) were performed on the refrigerated samples after 2, 6 and 10 days of storage (by five panelists).

HPLC analysis

Lactose concentration and the major types of acids were determined by HPLC as follows: 1-ml aliquots were taken from the samples previously indicated, and were added to 0·10 ml of 35% (v/v) perchloric acid, vortexed vigorously and immediately cooled in ice; 55 μl of 7·0 mol l^{-1} KOH were added, the mixture was vortexed again and the resulting supernatant was filtered through 0·2 μm pore size Syrfil filters (Costar, Cambridge, MA, USA). The filtrate was finally injected into an HPLC system (Merck_LaChrom, Darmstadt, Germany) equipped with sequential UV and refractive index detectors and an Aminex anion exchange column (Phenomenex, Richmond, VA, USA), maintained at 60°C, and using 0·01 mol l^{-1} H_2SO_4 as eluant at a flow rate of 0·50 ml min^{-1}. Calculation of the concentration of lactose was based on calibration curves previously prepared with pure (chromatographic grade) standards.

Results

Microbiological and chemical analysis of the fresh milk used in this work showed average mesophile counts, protein and lactose content of 10^6 CFU ml^{-1}, and 4·07 and 4·91% (v/v) respectively.

The average counts of *Bif. animalis* and *Lact. acidophilus* at the start of fermentation were $2·5 \times 10^6$ and $6·7 \times 10^6$ CFU ml^{-1}, respectively, and by 20 h of fermentation, *Bif. animalis* reached a maximum of $6·3 \times 10^7$ CFU ml^{-1} and *Lact. acidophilus* a maximum of $7·1 \times 10^8$ CFU ml^{-1} (Fig. 1).

The pH dropped from the initial 6·4 to 5·1 by 20 h of fermentation, and a fine gel (clotting) could be detected in the medium *c.* 15 h after inoculation.

The HPLC results indicate that in general *c.* 70% of lactose was consumed by 20 h of fermentation, and that lactic acid and acetic acid were the major metabolites present in the medium (not shown).

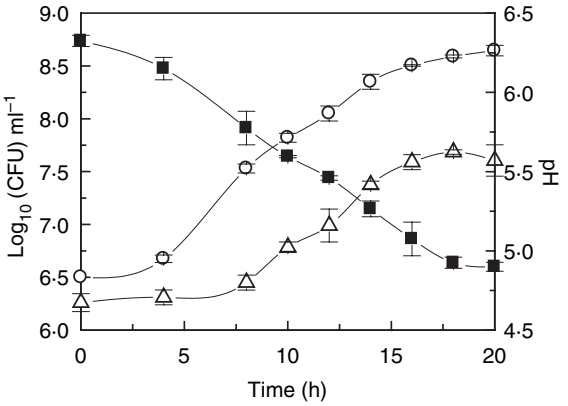

Figure 1 Growth of *Lactobacillus acidophilus* (○) and *Bifidobacterium animalis* (△), and pH changes (■) during fermentation of goat milk for 20 h. Each symbol represents the mean and standard deviation (as error bars) of two experiments.

The survival of both strains and the pH changes in fermented milk stored at 5°C, for 10 days are shown in Fig. 2. The highest decrease in total viable counts occurred on the first storage day: from $7·1 \times 10^8$ to $7·9 \times 10^7$ CFU g^{-1} for *Lact. acidophilus*, and from $6·3 \times 10^7$ to $3·1 \times 10^7$ CFU g^{-1} for *Bif. animalis*. On the subsequent days, the counts remained essentially constant for both species. Correspondingly, a faster pH drop was observed in the fermented milk on the first day (5·1–4·8) changing only slightly toward the end of the experiment. However, samples stored at 22°C showed a sharp decrease in viable counts and pH (not shown).

The fermented milk was considered, in general, by the panelists as having a strong acid taste and a pleasant aroma.

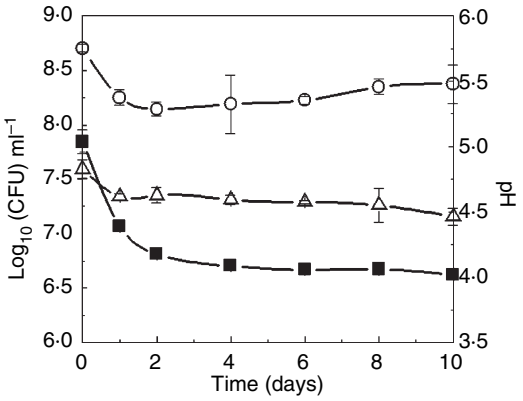

Figure 2 Survival of *Lactobacillus acidophilus* (○) and *Bifidobacterium animalis* (△), and pH changes (■) in fermented goat milk during refrigerated storage for 10 days. Each symbol represents the mean and standard deviation (as error bars) of two experiments.

Discussion

The total counts in the fresh milk indicate that the fresh whole milk had an acceptable microbiological quality (Brazis 1990).

Inoculum preparation is an important factor when fast acidification and reproducibility of batches are required, especially to avoid contamination which occurs easily in milk cultures with these slow-growing strains. Hughes and Hoover (1995) suggested the addition of lactose to the preculture medium (for boosting the beta-galactosidase enzyme system); this requirement was met in our study by preparing the precultures in milk. Gomes *et al.* (1995), in turn, suggested the addition of a higher inoculum (2–5%); the results presented here are those resulting from an inoculation level of 2·5%, although other inoculations levels were also tried (not shown).

In general, goat milk is less rich in lactose (St-Gelais *et al.* 2005). This feature and the low level of lactose obtained at the end of fermentation during this study may be thought of as an interesting characteristic to provide a probiotic fermented milk for lactose-intolerant consumers.

To offer the expected beneficial health effects, probiotic bacteria should still be alive by the time of consumption, and at a concentration no lower than 10^6 CFU g^{-1} (Kurmann and Rasic 1991); these requirements were met by both strains in the manufactured fermented goat milk, even after storage for 10 days.

Survival, as expected, was higher in refrigerated samples, and there was a correspondence between the decrease of pH and the decrease of bacterial counts. This agrees with previous reports, which indicate that high acidity is the main reason for the low survival of bifidobacteria in fermented products with very low pH values (Laroia and Martin 1991; Hughes and Hoover 1995; Shah *et al.* 1995).

At room temperatures, there is a lower survival as a result of 'over-acidification' (Wang *et al.* 2002). Thus, refrigeration and pH are key factors for keeping high levels of probiotic bacteria in the fermented milk. This was the reason for stopping the fermentation process with *Bif. bifidum* when pH reaches 4·9, or coupling the fermentation process with a filtering device in a continuous culture, thus allowing a higher survival of the starter in the growth medium maintained at pH 6·5 (Corre *et al.* 1992).

In conclusion, after 20 h of fermentation in the bioreactor, an acidic yogurt-like fermented milk with a pleasant aroma was obtained.

Manufacture of the aforementioned food on a regular basis requires a careful control of important processing steps, such as inoculum preparation, low oxygen levels (in the medium) during fermentation, and control of temperature during refrigerated storage.

The low levels of lactose concentration found at the end may be considered an important characteristic to yield a probiotic product for lactose-intolerant consumers.

The high survival of the starter microflora during the first 10 days of storage at refrigerating temperatures may provide the fermented goat milk with the expected beneficial health effects based on a high concentration of probiotic bacteria.

Therefore, goat milk fermented with *Bif. animalis* and *Lact. acidophilus* is feasible.

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