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Short communication

Co-culturing of probiotics influences the microbial and physico-chemical properties but not sensory quality of fermented dairy drink made from goats' milk

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ABSTRACT

Seven different types of fermented drinking milk were made from goats' milk using various culture compositions of *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 and novel putative probiotic *Propionibacterium jensenii* 702. Probiotic viability, physico-chemical and sensory properties of fermented milk were measured during 3 weeks of storage at 4 °C. All three probiotics were able to maintain high viability (>10^7 cfu mL^{-1}) during fermentation and subsequent storage regardless of the culture composition in goats' milk without major antagonistic effects. Acidity of all fermented milk samples increased during storage, however there were no significant differences among preparations for organoleptic properties. Generally, lower sensory acceptability was recorded for the samples stored for 3 weeks than the respective fresh products.

1. Introduction

Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefits on the host" (FAO/WHO, 2002). Probiotic dairy drinks are consumed in larger quantities than other non-dairy probiotic beverages (Ozer and Kirmaci, 2010) and provide advantages such as convenience, portability and the ability to deliver all the health and nutritional benefits of set yogurts (Allgeyer et al., 2010; Castro et al., 2013a,b). Another recent trend in the manufacturing of probiotic products is to combine two or more strains since each probiotic strain may offer different and specific health benefits (Collado et al., 2007). Although cows' milk is widely utilized in manufacture of fermented dairy products (Cruz et al., 2012, 2013a), goats' milk may also impart certain therapeutic and nutritional value compared to cows' milk such as better digestibility and desirable immunological properties (Slacanac et al., 2010), however, the production of fermented goats' milk through probiotic fermentation only (without yogurt starter culture microorganisms), has not yet been well developed (Slacanac et al., 2010; Uysal-Pala et al., 2006).

According to a previous study, co-culturing of probiotics in goat's milk had an influence on changing their functional properties such as gastrointestinal survival and intestinal adhesion in vitro (Ranadheera et al., 2014). While the varying beneficial effects of different probiotics points to co-cultivation as an obvious strategy for providing a broad range of health benefits to the host, the identification of possible symbiotic or antagonistic interactions among probiotics with respect to microbial, physico-chemical and sensorial properties, clearly deserves equal consideration in the development of novel functional probiotic foods, because probiotic combinations may also influence the probiotic viability, and physico-chemical and sensory characteristics of the final product. *Propionibacterium jensenii* 702 is a novel strain of dairy propionibacteria and previously has demonstrated a number of probiotic traits such as relatively satisfactory gastrointestinal survival in vitro (Ranadheera et al., 2012a) and in vivo (Huang et al., 2003), ability to adhere to a human gut epithelial cell line (Ranadheera et al., 2012a) and good technological properties including high survivability during food manufacturing and storage (Ranadheera et al., 2012b, 2013). Microbial, physico-chemical and sensory properties of *P. jensenii* 702 in different culture combinations in fermented dairy drinks has not previously been studied. This study was designed to produce fermented dairy drink with *L. acidophilus* LA-5, *B. animalis* subsp. *lactis* BB-12 and *P. jensenii* 702, using monocultures and in various co-culture combinations in goats' milk, and to exam-

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ine the microbial, physico-chemical, and sensorial properties in the product over 3 weeks of storage at 4 °C.

2. Materials and methods

2.1. Probiotic bacteria and growth conditions

Pure freeze dried probiotic cultures of *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12 (CHR Hansen Pty Ltd., Bayswater, VIC, Australia) and *P. jensenii* 702 (University of Newcastle, Callaghan, NSW, Australia) were grown in appropriate media: lactobacilli in de Man–Rogosa–Sharpe (MRS) broth (37 °C for 72 h), propionibacteria in sodium lactate broth (30 °C for 3–5 days) and bifidobacteria (37 °C for 72 h) in reinforced clostridial medium (Oxoid Australia Ltd., Adelaide, Australia). Bacteria cells were harvested by centrifugation in their stationary phases, washed three times with 0.1% sterile saline solution, and re-suspended in pasteurized goat milk as the inoculum and used for the experiment as explained in Ranadheera et al. (2014).

2.2. Production of fermented goats' milk

Fermented dairy drink was prepared as previously described by Ranadheera et al. (2014) using reconstituted and heat treated (85 °C for 30 min) goats' milk samples (12% total solids, Healtheries of New Zealand Ltd., Auckland, New Zealand) through fermentation for 10 h at 37 °C after inoculation (in order to achieve 10^7–10^8 cfu/mL each probiotic in each respective sample regardless of the combinations). Seven different types of fermented milks (2 L each) were produced simultaneously based on the different combinations of the probiotic bacteria (without yogurt starter culture bacteria: *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) as follows: *L. acidophilus* LA-5 (L), *P. jensenii* 702 (P), *B. animalis* subsp. *lactis* BB-12 (B), *L. acidophilus* LA-5 + *P. jensenii* 702 (L + P), *L. acidophilus* LA-5 + *B. animalis* subsp. *lactis* BB-12 (L + B), *P. jensenii* 702 + *B. animalis* subsp. *lactis* BB 12 (P + B) and *L. acidophilus* LA-5 + *P. jensenii* 702 + *B. animalis* subsp. *lactis* BB-12 (L + P + B). Milk samples were stored in sterile glass containers at 4 °C. Microbial, physico-chemical and sensory properties of the samples were analyzed on weekly basis for 3 weeks. Treatments were arranged according to the Completely Randomized Design. Each experiment has performed at least twice.

2.3. Microbiological analysis

Serial dilution and spread plating were performed to determine total viable counts for all microorganisms in fermented milk samples and expressed as cfu mL^{-1} by using appropriate media and conditions as previously explained (Ranadheera et al., 2012b, 2014). In brief, anaerobic incubation on MRS-sorbitol agar (37 °C for 72 h), MRS-NNLP agar (37 °C for 72 h) and sodium lactate agar (30 °C, 5–7 days) were performed to enumerate lactobacilli, bifidobacteria and propionibacteria respectively ($n = 4$).

2.4. Analysis of physico-chemical properties

Titratable acidity (by titration using 0.1 N NaOH solution), pH (Cyberscan 510 digital pH meter, EUTEOH Instruments, Singapore), total solids by oven dried method (using an air oven, Thermoline Scientific, Wetherill Park, NSW, Australia), ash content (using an electric muffle furnace, Labec Laboratory Pty Ltd., Marrickville, NSW, Australia) and fat content (Gerber method) of samples were determined according to AOAC (2005) during storage. The syneresis was determined as described in Ranadheera et al. (2012b). To determine the lactic acid content, 1.5 g of each fermented milk sample was diluted with 0.5 mL of 2.5 m Mmethanesulfonic acid (Sigma–Aldrich Pty., Ltd., Castle Hill, NSW, Australia), centrifuged at 2500 × *g* for 30 min and the supernatant was filtered through 0.22 μm membrane filters (Millipore Corporation, Bedford, MA, USA). The quantification of lactic acid was achieved by High Performance Liquid Chromatography on a Hewlett Packard (Series 1100) instrument (Santa Clara, CA, USA) fitted with a pyrospher RP-18 (125 × 4 mm, 5 μm) column (maintained at 30 °C) and a UV detector, using 2.5 mM methanesulfonic acid with a flow rate of 1 mL min^{-1} as the mobile phase. All physico-chemical properties were analyzed in duplicates.

2.5. Evaluation of sensory characteristics

Sensory evaluation of the fermented goat's milk preparations over storage was conducted by 7 semi-trained taste panellists (students of the University of Newcastle, Australia, 5 male and 2 female in the age range of 20–45 years) with previous experience in sensory evaluation of dairy foods, based on a 9 point hedonic scale (like extremely = 9, dislike extremely = 1), as described by Harry and Hildegarde (2010) with small modifications. Initially, panellists were trained in a 1/2 h session prior to evaluation in order to be familiar with sensory attributes and scaling procedures of fermented milk samples. During the sensory evaluation procedure, all the samples were presented in uniform plastic cups coded with random three digit numbers (80 mL each). Orders of serving were completely randomized. The panellists were asked to evaluate the samples (color and appearance, aroma, body and texture, taste and overall acceptability) and comment on sensory characteristics. All sessions were conducted in individually arranged booths in a test room with no disturbances at the room temperature of 25 ± 2 °C, under normal white fluorescent illumination. Water was provided between samples to cleanse the palate. The sensory evaluation was conducted following approval by the Human Research Ethics Committee of the University of Newcastle, Australia (Ethics approval number: 2008–0212).

2.6. Statistical analysis

Data analyses were performed using SPSS/PASW statistical software version 17 (SPSS Inc., Chicago, IL, USA). Microbial viability and physico-chemical properties were analysed using repeated measure ANOVA while Bonferroni post hoc test was performed for means comparison. Where appropriate, T-tests were performed for comparison of two means. Sensory data were statistically tested using nonparametric tests. A p value <0.05 was considered statistically significant for all analysis.

3. Results and discussion

3.1. Microbial growth and viability

In this experiment both *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12 have demonstrated a relatively higher growth after fermentation in the presence of each other as well as in the presence of *P. jensenii* 702 except *Bifidobacterium* monoculture, perhaps indicative of their natural symbiosis (Table 1). Normally *Bifidobacterium* strains are weakly proteolytic and higher proteolytic activities of *L. acidophilus* compared to bifidobacteria (Lourens-Hattingh and Viljoen, 2001) may support the growth of *Bifidobacterium* in these preparations. However, lactobacilli demonstrated a significant growth in each preparation despite the culture composition. After fermentation, a growth reduction was observed for *P. jensenii* 702 for all preparations, possibly due to competition for nutrients.

In the present study, *L. acidophilus* LA-5 demonstrated better resistant at refrigeration storage when in co-culture with *P. jensenii* 702 probably due to synergistic effect. *B. animalis* subsp. *lactis*