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ORIGINAL ARTICLE

Survival of *Bifidobacterium bifidum* in cow- and camel-milk yogurts enriched with *Cinnamomum verum* and *Allium sativum*

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Abstract The effects of *Allium sativum* and *Cinnamomum verum* water extracts on the survival of *Bifidobacterium bifidum* during 21 days of refrigerated storage and after simulated gastrointestinal digestion (SGD) were investigated. Two types of yogurt (cow- and camel-milk yogurts) were prepared in the presence of *A. sativum* or *C. verum*. The viable cell counts (VCC) of *B. bifidum* in fresh *A. sativum*- or *C. verum*-cow milk yogurt (1 day) were higher (8.1×10^9 cfu/ml and 6.6×10^9 cfu/ml, respectively; $p < 0.05$) than plain-yogurt (1.9×10^9 cfu/ml). In contrast, *B. bifidum* VCC in fresh plain-camel milk yogurt was 1.99×109 cfu/ml whereas the presence of *A. sativum* or *C. verum* in yogurt increased ($p < 0.05$) VCC to 19.61×109 cfu/ml and 25.55×109 cfu/ml, respectively. The VCC of *B. bifidum* in both herbal-yogurts decreased ($p < 0.05$) during refrigerated storage for both types of yogurt. The VCC of *B. bifidum* was $\sim 1.3 \times 10^9$ cfu/ml in all fresh cow milk yogurts after 1 h gastric digestion. Intestinal digestion (1 h) increased VCC of *B. bifidum* in all fresh yogurts but not in 7 day old yogurts (plain- and *A. sativum*-yogurts). However, prolonged digestion to another 1 h in intestine reduced ($p < 0.05$) VCC of *B. bifidum* in all fresh and storage yogurts. In contrast, all fresh camel milk yogurts showed VCC of *B. bifidum* $\leqslant 1 \times 10^9$ cfu/ml after SGD. Seven day old *A. sativum* – camel milk yogurt showed the lowest survival of *B. bifidum* after gastric digestion compared to plain- and *C. verum*-yogurt. The VCC reduced ($p < 0.05$) in all camel milk-yogurts after 2 h intestinal digestion.

1. Introduction

Nowadays, there has been a worldwide increasing interest about the survival of probiotic bacteria in yogurt. Probiotics are live microorganisms that provide health benefits on the host when administered in sufficient amounts (Wang et al., 2012). Yogurts containing probiotics are claimed to provide

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several health benefits such as improve lactose utilisation (De Vrese et al., 2001), prevent cancer (Rafter, 2003), maintain intestinal microflora balance (Mainville et al., 2005) and reduce serum cholesterol level (Baroutkoub et al., 2010). Moreover, yogurt containing *Bifidobacterium bifidum* Bb-12 improved immunoglobulin A (IgA) production in the intestine that enhances local immunity against gastrointestinal infection (Kabeerdoss et al., 2011). It also has inhibitory effects on commonly known food borne pathogens (Goderska and Czarnecki, 2007) and ability to control intestinal infections by producing inhibitory/antimicrobial substances such as organic acids, hydrogen peroxide, deconjugated bile acids, antibiotics and bacteriocins (Schiffrin and Blum, 2001).

Viable numbers of probiotics in the final product suggested being at least 10^6–10^7 cfu/g to be accepted as the therapeutic minimum (Madureira et al., 2011). Several studies have investigated the survival ability of probiotic cultures during refrigerated storage (Donkor et al., 2007; Ramchandran and Shah, 2010).

The ability of probiotic bacteria to survive through the gastrointestinal tract varies according to species and even strain-dependent (Wattiaux and Howard, 2000). In addition, functional properties of this probiotic can be affected by the food matrix used in delivery (Lahtinen et al., 2007; Ranadheera et al., 2012) because the buffering capacity of food would help to enhance the viability of probiotics during gastric transit (Kailasapathy and Chin, 2000Kailasapathy and Chin, 2000; Mainville et al., 2005). Ranadheera et al. (2012) reported that the addition of certain ingredients such as cocoa powder and stabilizers guar gum and dextrose in the ice cream enhanced the viability of probiotics by providing some protection. Other study showed that the presence of *Allium sativum* or *Cinnamomum verum* in yogurt enhanced the growth of lactic acids' bacteria (Shori and Baba, 2012). The objective of this work is to evaluate the viability of *B. bifidum* in *C. verum*- or *A. sativum*-yogurt during 21 days of refrigerated storage and the survival of these bacteria after simulated gastrointestinal digestion.

2. Materials and methods

2.1. Plant water extraction

Commercially available dried *A. sativum* or *C. verum* powder was mixed with sterile dH_2O in the ratio of 1:10 in a 250 ml bottle. The final concentration of both herbal extracts was 0.1 g/ml. The mixture was left for 12 h (Shori and Baba, 2011a) in a water bath at 70 °C (Julabo, Model Sw-21c) followed by centrifugation (1000 rpm, 15 min at 4 °C). The supernatant was removed and used as herbal water extract in the making of herbal-yogurt.

2.2. Preparation of starter culture and bio-yogurt

Commercially available direct vat set (DVS) starter culture powder used in yogurt preparation consisting of a mixture of *Lactobacillus acidophilus* LA-5, *Bifidobacterium* Bb-12, *Lactobacillus casei* LC-01, *Streptococcus thermophilus* Th-4 and *Lactobacillus delbrueckii ssp. bulgaricus* (Chris-Hansen, Denmark) was in the ratio of 4:4:1:1:1. The preparation of starter culture from cow or camel milk was carried out using the method described by Shori and Baba (2011b). Two groups of bio-yogurt made from cow and camel milk and three types of yogurt (plain-, *A. sativum*- and *C. verum*-yogurts) were prepared in each group as described by Shori and Baba (2011b).

2.3. In vitro gastrointestinal model

2.3.1. Preparation of gastric and duodenum juices

The gastric and duodenum solutions were freshly prepared according to the protocols described by Huang and Adams (2004). To simulate the *in vivo* saliva, 100 ml of a sterile electrolyte solution (6.2 g/l NaCl, 2.2 g/l KCl, 0.22 g/l $CaCl_2$, 1.2 g/l $NaHCO_3$) was added to lysozyme (10 mg) to obtain a final concentration of 100 ppm. To simulate the stomach environment (gastric juice), the electrolyte solution was added to 0.3% pepsin and the pH was adjusted to 3 by adding 5 M HCl. To simulate the intestinal digestion (duodenum juice), the electrolyte solution (6.4 g/l $NaHCO_3$, 0.239 g/l KCl, 1.28 g/l NaCl) containing 0.3% bile salts and 0.1% pancreatin (v/w concentrations) was adjusted to pH 7.2 by using 5 M NaOH.

2.3.2. Simulation of gastrointestinal digestion (SGD)

Yogurt samples were mixed with the artificial saliva solution in the ratio of 1:1 followed by incubation at 37 °C for 5 min. Samples were then mixed with artificial gastric fluid solution in the ratio of 3:5 prior to a second incubation at 37 °C for 1 h. After 1 h, 30 ml of samples from the "stomach digestion" was taken out for analysis. The remaining solutions from "stomach digestion" were then mixed with artificial duodenal secretion in the ratio of 1:4 followed by a third incubation at 37 °C for 2 h. Samples (30 ml) were taken out for analysis after every hour interval of "intestinal digestion". All samples were manually agitated and stirred intermittently during the incubation time in order to ensure adequate enzymatic digestion to mimic gastrointestinal movement.

2.4. Viable cell counts (VCC) of B. bifidum

Cultures of *B. bifidum* were enumerated using MRS-LP agar. The formulation of MRS-LP was prepared according to Vinderola et al. (2000) where 0.2% (w/v) of lithium chloride (solid–powder) and 0.3% (w/v) of sodium propionate (solid–powder) were added to the MRS media (62 g/930 L dH_2O, 45 °C). Yogurt samples (1 ml) were mixed with 9 ml of 0.15% sterile buffered peptone water (20 g/L dH_2O). The mixture was thoroughly stirred and serial decimal dilutions were prepared by using buffered peptone water. One millilitre of diluted yogurt with buffered peptone water was mixed with 15 ml of autoclaved melted MRS–LP media using the pour plate method. The probiotic cultures were anaerobically incubated (GasPak System-OXOID) at 37 °C for 72 h. The viable *B. bifidum* counts were calculated (Sivakumar and Kalaiarasu, 2010) as follow:

$$\mathrm{CFU}*/\mathrm{ml} = \frac{\text{Number of colonies formed} \times \text{dilution factor of sample}}{\text{1 ml of sample}}$$

*CFU: colony forming unit.