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Combined effects of low-fat ice cream supplemented with probiotics on colon microfloral communities and their metabolites during fermentation in a human gut reactor

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ABSTRACT

This study aimed to investigate the combined effects of low-fat ice cream supplemented with *Lactobacillus casei* 01 and *Lactobacillus acidophilus* LA5 on modulating of colon microbiome using an *in vitro* gut model. The microbial metabolite contents (i.e. short-chain fatty acids, lactic acid and ammonia) and colon microbes (i.e. lactobacilli, bifidobacteria, clostridia, fecal coliforms and total anaerobes) during experiment run were assessed. After administration, when compared to pure low-fat ice cream, the probiotic-low-fat ice creams, especially those with added *L. casei* 01 cells, were found to have significantly enhanced production of beneficial microbial metabolites viz. acetate, propionate, butyrate and lactic acid in proximal and distal colon vessels, whereas the toxic ammonia levels apparently decreased. Accordingly, based on the profiles of colon microflora, most treatments were shown to stimulate the maximal counts of colon lactobacilli and bifidobacteria, while other harmful microbes such as clostridia and fecal coliforms gradually declined. Therefore, this study may suggest that low-fat ice cream supplemented with both probiotics could provide a beneficial impact on human health.

1. Introduction

Health-promoting effects of probiotic foods have been described to include: antimicrobial activity, prevention and treatment of diarrhea, relief of symptoms caused by lactose intolerance, anti-mutagenic/anti-carcinogenic activities, lowering blood cholesterol level and stimulation of the immune system (Chaikham, 2015; Sanders et al., 2013). Essentially, the probiotics are required to be able to tolerate processing and storage conditions to be viable in foods for more than 6–7 log CFU/g or log CFU/ml (Chaikham & Apichartsrangkoon, 2012; Nualkaekul, Lenton, Cook, Khutoryanskiy, & Charalampopoulos, 2012). Also, they should be capable of enduring the undesirable environments of the gastrointestinal tract and competition with the intestinal microflora (Chaikham, Apichartsrangkoon, Jirarattanarangsri, & Van de Wiele, 2012; Cruz, Antunes, Sousa, Faria, & Saad, 2009; Fooks & Gibson, 2002). Kailasapathy and Sultana (2003) and Cruz et al. (2009) reported that ice cream was found to have promising potential to be used as a probiotic vehicle.

Therefore, addition of such potential probiotics in ice cream can be further explored. Nowadays, since the intake of such products was found to decrease the risk of obesity and cardiovascular diseases (Akbari, Eskandari, Niakosari, & Bedeltavana, 2016), a number of consumers have become interested in consumption of the low-fat products, especially low-fat ice cream (Akalin, Karagözlü, & Ünal, 2008).

Importantly, numerous researchers have investigated the addition of probiotics to food products using various dynamic models of the gastrointestinal system. In fact, these models consist of stomach, small intestinal and colon compartments and they have been successfully used for investigating viable colon bacteria and their metabolites (Apichartsrangkoon, Chaikham, Pankasemsuk, & Baipong, 2015; Chaikham et al., 2012; Mainville, Arcand, & Farnworth, 2005; Mäkivuokko, Forssten, Saarinen, Ouwehand, & Rautonen, 2010; Uriot et al., 2016; Van de Wiele, Boon, Possemiers, Jacobs, & Verstraete, 2004). Possemiers, Marzorati, Verstraete, and Van de Wiele (2010) fed *Lactobacillus helveticus* CNCM I-1722 and *Bifidobacterium longum* CNCM I-3470 into the simulator of the human gastrointestinal microbial ecosystem (SHIME reactor) and found increases of beneficial bacteria and short-chain fatty acids (SCFA) in the colon compartments. Gmeiner et al. (2000) also elucidated that, after oral administration of synbiotic milk-based product into the dynamic gut model, the viable lactobacilli and bifidobacteria were found to increase, whereas the number of *Escherichia coli* declined. Macfarlane and Gibson (1997) showed that

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the ecological diversities of the intestinal tract could be enhanced by probiotic interactions which promoted the degradation of food components by gut microflora. In fact, probiotics can break down undigested polysaccharides and oligosaccharides, including prebiotics and resistant starch, and produce SCFA (i.e. acetate, propionate, butyrate and lactate). The beneficial functions of SCFA were previously reported by Chaikham et al. (2012), Tuohy, Rouzaud, Brück, and Gibson (2005) and Wong, de Souza, Kendall, Emam, and Jenkins (2006), including inhibition of colon pathogens, reduction of cancer risk, prevention of cardiovascular diseases and stimulation of immune system.

However, there is no research on the combined impacts of low-fat ice cream supplemented with probiotics on modulation of the colon microbiome. Therefore, this study aimed to monitor the diversity of colon microorganisms, including lactobacilli, bifidobacteia, clostridia, fecal coliforms and total anaerobic bacteria, in an *in vitro* gut model after feeding and subsequent fermentation with different treatments of low-fat ice cream supplemented with probiotic bacteria (*Lactobacillus casei* 01 and *Lactobacillus acidophilus* LA5). The microfloral metabolic products including SCFA, total lactic acid and toxic ammonia were also determined.

2. Materials and methods

2.1. Activation of probiotic cultures

Freeze dried *L. casei* 01 and *L. acidophilus* LA5 (Chr. Hansen, Hørsholm, Denmark), were activated according to the methods described by Chaikham et al. (2013). Briefly, the dried cultures of *L. acidophilus* LA5 and *L. casei* 01 were aseptically inoculated into MRS broth (Hi-Media, Mumbai, India) and anaerobically incubated at 37 °C for 18 and 16 h, respectively. The cell pellets of both cultures were harvested by centrifugation at 4000×*g* for 20 min at 4 °C (model Rotina 46 R, Tuttlingen, Germany), washed twice with 0.85% (w/v) sterile saline water (Merck, Darmstadt, Germany), then diluted in sterile distilled water (RCI Labscan, Bangkok, Thailand) at the concentration of approximately 10^{11} CFU/ml, and finally mixed with the ice cream.

2.2. Preparation of probiotic-low-fat ice cream

Low-fat and probiotic-low-fat ice creams were produced according to the process described by Aboulfazli, Shori, and Baba (2016) with some modifications. In brief, 76% (w/w) ultra-high temperature (UHT) treated skim milk (CP Meiji, Saraburi, Thailand) and 2% (w/w) butter (KGC Corporation Co., Ltd., Bangkok, Thailand) were heated to 50 °C before mixing with 7% (w/w) skim milk powder (Param Dairy Ltd., Delhi, India), 12% (w/w) sucrose (Mitr Phol, Bangkok, Thailand), 0.2% (w/w) guar gum (Ingredient Center Co., Ltd., Bangkok, Thailand), 0.6% (w/w) gelatin type B from bovine skin (Ingredient Center Co., Ltd.) and 0.4% (w/w) corn starch (Knorr, Bangkok, Thailand). One l of mixture was thoroughly homogenized using a blender (National, Bangkok, Thailand) for 2 min, then pasteurized at 80 °C for 2 min using a LAUDA thermostatic water bath (Alpha RA 12, Lauda-Bringkmann, Lauda-Königshofen, Germany) before cooling down to 20 °C. The pasteurized mixture was refrigerated at 4 °C for 12 h. Each mixture was then mixed with or without 2% (v/w) diluted cell pellets (Section 2.1) and immediately frozen in a batch ice cream maker (National) for 4 h. The final concentrations of the probiotic cells in ice cream mixtures were 7.5×10^8 and 8.1×10^8 CFU/g for *L. acidophilus* LA5 and *L. casei* 01, respectively. After that, the samples were placed into 50 ml plastic cups and covered using the lids before being frozen at −40 °C for hardening and storage up to 2 months (The Cool Prima VU-710.2, SNR Cooling & Trading Co., Ltd., Bangkok, Thailand).

2.3. In vitro *gut model setting*

Carbohydrate-based medium was prepared by mixing 1 g of arabinogalactan (Sigma-Aldrich, St. Louis, MO, USA), 2 g of pectin (Ingredient Center Co., Ltd.), 1 g of xylan (Sigma-Aldrich), 3 g of potato starch (Thai Thum, Chonburi, Thailand), 0.4 g of *D*(+)-glucose (Merck), 3 g of yeast extract (Hi-Media), 1 g of peptone water (Hi-Media), 4 g of mucin from bovine submaxillary gland (CAS NO. 84195-52-8, 5 U/mg: Merck) and 0.5 g of *L*-cysteine (Sigma-Aldrich) into 1 l of distill water and then sterilized (Autoclave GI36T, Zealway Instrument Inc., Fujian, China) at 121 °C for 15 min (Chaikham et al., 2012).

Fecal fluid was prepared from fecal samples collected from five adult volunteers, aged between 27 and 35 years, who had no history of antibiotic treatments in the last 6 months (Chaikham et al., 2013). Briefly, 30 g of mixed matter were mixed with 150 ml of 0.7 M potassium phosphate buffer (pH 7: Merck) and 1.5 g of sodium thioglycolate (Sigma-Aldrich) as a reducing agent, and then homogenized for 10 min using a stomacher (IUL Instruments, Barcelona, Spain) followed by centrifugation at 3000×*g* for 5 min. The supernatant was separated to be used as fecal fluid and then transferred to the proximal and distal colon vessels. Both proximal and distal colon vessels were adjusted to 600 and 800 ml, respectively, using sterilized carbohydrate-based medium.

An *in vitro* gut model which consisted of stomach, small intestine, proximal colon and distal colon was set up following the protocol for a dynamic gut simulator described by Woramethachanon et al. (2014) with some modifications. The system temperature was controlled at 37 °C using a circulating thermostatic water bath and flushed with nitrogen gas (> 99% purity: Dynaweld Trading, Co., Ltd., Pathum Thani, Thailand) to maintain the anaerobic condition. The experimental periods and compositions are listed in Table 1. After the reactor was operating, 20 ml of fluid samples were withdrawn daily from both colon compartments for determination of colon bacteria and their metabolites.

2.4. Determination of SCFA

SCFA were extracted as described by Chaikham et al. (2012) with some modifications. Briefly, 2 ml of sample were mixed with 0.4 g of sodium chloride (Merck) and acidified by adding 0.5 ml of sulfuric acid (Sigma-Aldrich). Then, 0.1 ml of 2-methylhexanoic acid (internal standard) (2.8 ml in 1 l deionized water: Sigma-Aldrich) and 2 ml of diethylether (Merck) were added to the mixture, which was subsequently extracted for SCFA by agitation using a shaker for 10 min and centrifugation at 1000×*g* for 5 min. A 1-µl aliquot of the diethyl ether layer (top layer) was injected into a capillary free fatty acid-packed column (25 m×0.53 mm, film thickness 1.2 µm: Superchrom S.R.L, Milano, Italy) and analyzed using a Shimadzu GCMS-QP2010 gas chromatography (Shimadzu Cooperation Analytical & Measuring Instruments Division, Kyoto, Japan) equipped with a flame ionization

Table 1
Treatment conditions for gut model experiment (total experimental duration =90 days).

Experimental period	Treatment composition
Basal (18 days)	150 ml carbohydrate-based medium
Treatment I (12 days)	150 ml carbohydrate-based medium plus 50 g low-fat ice cream
Washout I (18 days)	150 ml carbohydrate-based medium
Treatment II (12 days)	150 ml carbohydrate-based medium plus 50 g low-fat ice cream containing 1.5% (w/w) cell pellets of *L. casei* 01
Washout II (18 days)	150 ml carbohydrate-based medium
Treatment III (12 days)	150 ml carbohydrate-based medium plus 50 g low-fat ice cream containing 1.5% (w/w) cell pellets of *L. acidophilus* LA5

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