J. Dairy Sci. 98:5905–5916
http://dx.doi.org/10.3168/jds.2015-9450

Effect of refrigerated storage on probiotic viability and the production and stability of antimutagenic and antioxidant peptides in yogurt supplemented with pineapple peel

B. N. P. Sah,* T. Vasiljevic,* S. McKechnie,† and O. N. Donkor*[1]
*College of Health and Biomedicine, and
†College of Engineering and Science, Advanced Food Systems Research Unit, Victoria University, Werribee Campus, PO Box 14428, Melbourne, Victoria 8001, Australia

ABSTRACT

Fruit by-products are good resources of carbohydrates, proteins, vitamins, and minerals, which may function as growth nutrients for probiotic bacteria. This research aimed at evaluating effects of pineapple peel powder addition on the viability and activity of *Lactobacillus acidophilus* (ATCC 4356), *Lactobacillus casei* (ATCC 393), and *Lactobacillus paracasei* ssp. *paracasei* (ATCC BAA52) in yogurts throughout storage at 4°C for 28 d. Plain and probiotic yogurts supplemented with or without pineapple peel powder or inulin were prepared. The probiotic counts in supplemented yogurts at 28 d of storage ranged from 7.68 and 8.03 log cfu/g, one log cycle higher compared with nonsupplemented control yogurt. Degree of proteolysis in synbiotic yogurts was significantly higher than plain yogurts and increased substantially during storage. Crude water-soluble peptide extract of the probiotic yogurt with peel possessed stronger antimutagenic and antioxidant activities [evaluated measuring reducing power and scavenging capacity of 1,1-diphenyl-2-picrylhydrazyl; 2,2'-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid), and hydroxyl radicals] than control and maintained during storage. Pineapple peel, a by-product of juice production, could be proposed as a prebiotic ingredient in the manufacture of yogurts with enhanced nutrition, and functionality.
Key words: pineapple, probiotic, peptide, antioxidative activity, and antimutagenic activity

INTRODUCTION

Demand for development of healthy foods is increasing rapidly due to growing interest of consumers to maintain their health and well-being. Day et al. (2009) defined functional foods as "foods or ingredients of foods that provide additional physiological benefit beyond their basic nutrition." Milk is considered a source of functional ingredients, such as bioactive peptides, which are encrypted in the primary structure of milk proteins and could modulate physiology of consumers only after their proteolytic release (Bhat and Bhat, 2011). Several possible ways exist to obtain these bioactive peptides to functionalize foods. One way could be through direct release of peptides from proteins by action of proteolytic systems of bacteria commonly used in manufacturing of fermented food products (Choi et al., 2012). Therefore, yogurt appears to be a suitable matrix for production of such functional ingredients.

Yogurt is an excellent vehicle to deliver probiotics to consumers; however, to be beneficial for health, the product should contain the suggested minimum number of 10^6 cfu/g at the time of consumption (Shiby and Mishra, 2013; Mani-López et al., 2014). The viability of probiotic organisms is thus considered a key parameter for developing probiotic food products. The major factors for achieving and maintaining this minimal level in yogurt include nutrients, pH, water activity, oxygen tension of the product, storage conditions (e.g., temperature, humidity, and light), the interactions with the starter cultures, as well as strain types (Vasiljevic and Shah, 2008). To minimize their adverse effects, different approaches have been suggested, including microencapsulation of probiotics (Corona-Hernandez et al., 2013), addition of enzymes (Cruz et al., 2013), and prebiotics (Al-Sheraji et al., 2013).

A prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" (Gibson et al., 2004). Common prebiotics are inulin, fructooligosaccharides, galactooligosaccharides, and other oligosaccharides, such as resistant starch and lactulose (Thammarutwasik et al., 2009). Inulin represents a group of plant polysaccharides having linear fructans

Received February 10, 2015.
Accepted May 18, 2015.
[1]Corresponding author: Osaana.Donkor@vu.edu.au

with β-(2←1) fructosyl-fructose glycosidic linkages and usually prepared by aqueous extraction of chicory roots. However, human digestive enzymes are specific for the hydrolysis of α-glycosidic bonds. Consequently, they are indigestible and only fermented by colonic microflora (Roberfroid, 2007). A high-performance type of inulin is a long-chain inulin with degree of polymerization of 10 to 60, average being 25. In addition to inulin, pineapple peel powder appeared a good source of dietary fiber and has been reported to show prebiotic potential (Diaz-Vela et al., 2013).

Several investigations (Donkor et al., 2007a; Al-Sheraji et al., 2012) have focused on probiotic viability in yogurt containing prebiotic supplements and during refrigerated storage. Whereas prebiotic supplementations may result into several functional benefits for probiotic organisms and ultimately consumers, this approach may influence the bioactivity of yogurt, as bacterial proteolytic enzymes may further hydrolyze milk proteins as well as bioactive peptides during storage (Donkor et al., 2007b). Notably, milk proteins emerge as a prolific source of peptides with anticarcinogenic potentials (Sah et al., 2015). However, studies are lacking regarding the effects of prebiotic addition on antimutagenic and antioxidant activities of the liberated peptides in yogurt during storage. Thus, our study aimed to assess the effect of pineapple peel powder (**PPP**) addition on viability and performance of *Lactobacillus acidophilus* (ATCC 4356), *Lactobacillus casei* (ATCC 393), and *Lactobacillus paracasei* ssp. *paracasei* (ATCC BAA52) in regard to the liberation of bioactive peptides with antioxidant and antimutagenic potentials in yogurts during 28 d of refrigerated storage.

MATERIALS AND METHODS

Substrates and Chemicals

Trichloroacetic acid, o-phthaldialdehyde (**OPA**), trifluoroacetic acid, 1,1-diphenyl-2-picrylhydrazyl (**DPPH**), 2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) (**ABTS**), salicylic acid, vancomycin, clindamycin, sodium azide, and serine were purchased from Sigma Chemical Company (St. Louis, MO), whereas acetonitrile was from Merck (Darmstadt, Germany). Hydrogen peroxide, ferrous sulfate, and potassium ferricyanide were obtained from Ajax Finechem (Seven Hills, NSW, Australia). Bacteriological agar, M17 medium, de Man Rogosa and Sharpe (**MRS**) medium, and peptone were supplied by Oxoid Australia (West Heidelberg, Victoria, Australia), whereas Davis minimal agar was purchased from Becton Dickinson Pty Ltd. (Sydney, NSW, Australia). Skim milk powder was procured from a local store (Woolworths Limited, Melbourne, Australia). Aqueous solutions were prepared in Milli-Q water (18.2 MΩ·cm) obtained from a Millipore water-purification system (Millipore, North Ryde, Australia). Whole pineapples were purchased from a local supermarket (Woolworths Limited).

Propagation of Cultures

Pure cultures of *Streptococcus thermophilus* ASCC 1275 and *Lactobacillus delbrueckii* ssp. *bulgaricus* Lb1466 were obtained from the Victoria University Culture Collection (Werribee, Australia). *Lactobacillus acidophilus* ATCC 4356, *L. casei* ATCC 393, and *L. paracasei* spp. *paracasei* ATCC BAA52 were procured from Cell Biosciences Pty Ltd. (Heidelberg, Victoria, Australia). All organisms were stored at −80°C in MRS broth containing 40% (vol/vol) glycerol. The resuscitated strains after 3 successive transfers were employed to prepare starters as described by Sah et al. (2014).

Preparation of PPP

Pineapple peel powder was prepared from the peel of pineapples [*Ananas comosus* (L.) Merrill], as described by do Espírito Santo et al. (2012) with some modifications. Briefly, crushed peel (~2 × 2 cm sizes) was washed by dipping in hot water (90°C) for 30 min to inactivate potential pathogens and proteolytic enzymes (Jutamongkon and Charoenrein, 2010). The peel was then freeze-dried using an Alpha 1–4 LSC Christ freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). The dried peel was milled to fine powder, standardized particle size less than 180 μm using wire mesh sieves (Endecotts Ltd., London, UK; Mesh Series BS410/1986) and sterilized with UV irradiation for 30 min (Coman et al., 2013).

Preparation of Yogurts Supplemented with Prebiotics

Set-type plain and probiotic yogurts with inulin or PPP supplementation or without supplementation (control) were prepared as described by Sah et al. (2014) with some modifications. Briefly, 3 batches of milk base were prepared by reconstituting skim milk powder in Milli-Q water at 140 g/L; 2 batches were separately supplemented with 1.0% (wt/vol) of commercial inulin Orafti HP (Beneo-Orafti Ltd., Tienen, Belgium) and PPP. All milk bases were heated for 30 min at 85°C, cooled to 45°C, and then inoculated with 1% (vol/vol) of each *S. thermophilus* and *L. bulgaricus* monocultures aseptically. The mixes were divided into 2 equal portions; one portion was further inoculated with 1% (vol/vol) of each probiotic monocultures. The final