Carbohydrate Polymers 117 (2015) 255–261

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Bifidogenic characteristic and protective effect of saba starch on survival of *Lactobacillus plantarum* CIF17AN2 during vacuum-drying and storage

Tipparat Hongpattarakere*, Supansa Uraipan

Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, 15 Kanchanavanich Rd., Hat Yai, Songkhla 90112, Thailand

ARTICLE INFO

Article history:
Received 9 September 2013
Received in revised form 2 June 2014
Accepted 18 September 2014
Available online 7 October 2014

Keywords:
Probiotic viability
Banana resistant starch
Lactobacillus plantarum
Vacuum-drying
Bifidogenic
Fecal microflora

ABSTRACT

Resistant starch (RS) from unripe saba banana (*Musa sapientum* (Linn)) (Kluai Hin) exhibited high resistance to gastric acid and intestinal amylases. Its bifidogenic effect under competition of human fecal microflora was determined in the simulated proximal region of human colon. In addition, saba RS effectively protected *Lactobacillus plantarum* CIF17AN2 during drying process. The maximum survival of 85.81% was achieved under vacuum drying operated at 37 °C when saba RS was added. The addition of saba RS to formulate a synbiotic product was able to retain high viability of the vacuum-dried *L. plantarum* during 8-week storage at ambient temperature. This is because saba RS can stabilize the moisture content of the synbiotic product. In contrast, the dramatic increase of moisture content in the vacuum-dried *L. plantarum* without saba RS led to significant decrease in cell survival. Moreover, saba RS could potentially protect the vacuum-dried *L. plantarum* from gastric acid and bile exposures.

1. Introduction

The increasing consumption of foods containing probiotic bacteria has led to the development of probiotic preservation technology (Ying et al., 2010). In order to achieve the therapeutic benefits, a probiotic product has been suggested to contain live probiotic bacteria in minimum level of 10^5–10^7 viable cell mL^{-1} or g^{-1} until the date of the product expiration (Dave & Shah, 1997; Sultana et al., 2000; Ying et al., 2010). Therefore, it is important that bacterial viability must be maintained during manufacture, storage and delivery to the target site in the gastrointestinal tract. However, many observations noted that the number of probiotic bacteria in these products does not always meet the level claimed in the product label. The probiotic supplement product from UK and continental Europe often contained markedly reduce numbers and/or had extraneous strains and/or strains specified on the label were missing (Hamilton, Shah, & Winkler, 1999). Two out of eight commercial lactic acid bacteria (LAB) products in Taiwan contained no viable LAB as indicated in their labels on the packages (Lin, Hwang, Chen, & Tsen, 2006).

The preservation of bacteria in frozen or dried form relatively gives high levels of bacterial survival. A frozen culture requires high storage and transportation costs in maintaining at very low temperatures such as −20 to −40 °C (Santivarangkna, Kulozik, & Foerst, 2006). Therefore, drying generally used to remove moisture from a substance is an alternative choice for the preservation of probiotic bacteria (Ratti, 2001). However, a drying disadvantage of thermal and dehydration inactivation can lead to a significant loss of bacterial viability (Santivarangkna et al., 2006). The excessive heat results to a denaturation of macromolecule structure or destroys the chemical bonds between monomeric units. The cell stress occurred from dehydration targets directly to cytoplasmic membrane leading to an alteration of membrane fluidity and physical property. Lipid peroxidation is also triggered by the dehydration of the membrane (Fu & Chen, 2011). An introduction of certain additives such as carbohydrates and plant fibers showed high potential to prevent membrane damage during freezing and dehydration processes (Linders, de Jong, Meerdink, & van't Riet, 1997; Hongpattarakere, Rattanaaubon, & Buntin, 2013). Moreover, survival of microorganisms during drying processes and subsequent storage depends on many factors, such as species and strains, drying condition, inoculums, culture media, additives and protectants (Otero, Espeche, & Nader-Macías, 2007; Hongpattarakere et al., 2013).

Prebiotic fibers are non-digestible polysaccharides which can specifically enhance growth of beneficial bacteria in the host colon (Gibson & Roberfroid, 1995). They must survive the digestion in stomach and small intestine. It is well established that starch

* Corresponding author. Tel.: +66 74 286371; fax: +66 74 558866.
E-mail address: tipparat.h@psu.ac.th (T. Hongpattarakere).

http://dx.doi.org/10.1016/j.carbpol.2014.09.065
0144-8617/

from unripe banana can escape digestion in human small intestine and then pass into the colon. This fraction is referred to as resistant starch (RS) (Bello-Pérez, Agama-Acevedo, Sánchez-Hernández, & Paredes-López, 1999). Saba (*M. sapientum* Linn) or *Musa* ABB group called Kluai Hin (in Thai) is a domestic cooking banana available only in the three southernmost provinces of Thailand. It contained high content of resistant starch up to 68.1%, much higher than other tropical banana cultivars (Vatanasuchart, Niyomwit, & Wongkrajang, 2012). However, the bifidogenic effect of RS from this banana in the presence of human fecal microflora has never been evaluated. In addition, the protective effect of plant prebiotic polysaccharides on the viability and stability of the vacuum-dried probiotic LAB during drying and storage is rarely studied.

In this study, prebiotic functionality of saba RS was investigated under condition mimic proximal colon condition in the presence of human fecal microflora. Its prebiotic potentials in term of gastric acid and gut enzyme resistance were also determined. The protective effect of saba RS on the viability of *Lactobacillus plantarum* CIF17AN2 from various vacuum-drying processes at low temperature was investigated. Such protective effect of saba RS was also determined when the dried LAB were exposed sequentially to gastric acid and bile and when they were stored at refrigerated and unrefrigerated temperatures.

2. Materials and methods

2.1. Preparation of saba RS

Banana starch was prepared according to Aht-Ong and Charoenkongthum (2002) with some modifications. Unripe saba banana (*M. sapientum* (Linn)) (Kluai Hin) was peeled and sliced into small pieces and then dried at 55 °C for 7 h. The dried saba was soaked in 0.05 N NaOH overnight before it was washed 3 times with water. The washed saba was mixed with water in the ratio of 1:1 before being ground using a high speed Waring blender. The slurry was filtered through a sieve in the mesh size series of 80, 120, and 170. The starch sediment remaining on the sieves was rinsed several times with the excess amount of distilled water until the water was substantially free of color. Starch sediment was dried in a hot air oven (Memmert BE500, Germany) at 65 °C for 15 h. The dried starch powder was then blended and sieved through the mesh size of 230.

2.2. Resistance of saba RS to digestibility under simulated gastrointestinal conditions

RS from saba banana was tested for the digestibility under the simulated stomach condition (0.14 M HCL buffer pH 1, 2 and 3 + 0.3% pepsin) at 37 °C for 4 h according to Hongpattarakere, Cherntong, Wichienchot, Kolida and Rastall (2012). After that, the acid-digested aliquot was adjusted to pH 6.9 using 5 M NaOH and then incubated at 37 °C for 6 h in the presence of 1 unit mL^{-1} of porcine pancreatic α-amylase (Sigma, Saint Louis, Missouri 63103, USA). Total carbohydrate (expressed in glucose equivalents) and reducing sugar were examined before and after digestion using phenol sulfuric acid and dinitrosalicylic acid methods (Fox & Robyt, 1991), respectively. Percentage of hydrolysis was calculated from reducing sugar released from the digestion divided by initial total carbohydrate content.

2.3. Probiotic bacteria

The probiotic *L. plantarum* CIF17AN2 isolated from 5 month old healthy infant feces was used in this study. This strain was intensively examined for various probiotic characteristics. These included their acid and bile tolerances, adhesion to mucin and HT-29. *L. plantarum* CIF17AN2 inhibited many food-borne pathogenic bacteria effectively through various mechanisms, including antibacterial secretion and competitive exclusion to mucin adhesion. *L. plantarum* CIF17AN2 was routinely cultivated in MRS medium (Himedia, Mumbai, India) at 37 °C for 24 h. The bacterium was stored as a stock culture in 30% glycerol (0.5 mL of 60% sterile glycerol and 0.5 mL of culture broth) at −196 °C under liquid nitrogen.

2.4. Fermentation of RS saba by fecal microflora in the simulated human proximal colon condition

The prebiotic effect of saba RS was conducted in a simulated colon model with pH controlled at 5.5 to mimic proximal region of human colon. A water-jacketed fermenter equipped with a pH controller (BEMT MCl-6C, B.E. Marubishi, Thailand) to maintain pH at 5.5 throughout fermentation period. Each fermenter vessel was filled with 135 mL of sterilized basal medium. The medium contained, per liter, 2 g of peptone water, 2 g of yeast extract, 0.1 g of NaCl, 0.04 g of K_2HPO_4, 0.01 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $CaCl_2 \cdot 6H_2O$, 2 g of $NaHCO_3$, 0.05 g of hemin, 0.5 g of L-cysteine hydrochloride, 0.5 g of bile salts, 2 mL of tween 80, 10 μL of vitamin K and 4 mL of 0.025% (w/v) resazurin solution (Macfarlane, Macfarlane, & Gibson, 1998). All vessels were magnetically stirred and the temperature was controlled at 37 °C using a circulating water bath. Culture pH was automatically controlled at 5.5 by an addition of either 0.1 N HCl or NaOH as appropriate. The anaerobic condition was maintained by continuous nitrogen flushing into the vessels at a rate of 15 mL min^{-1}. Fecal slurry (10% w/v) freshly prepared using mixture of fresh stool samples from 3 healthy adult volunteers (without antibiotic treatment for 6 months) was used as an inoculum. The fecal slurry and 1% saba RS were added into the vessel to begin the fermentation. The vessel inoculated with only fecal slurry was used as control treatment. Samples (5 mL) were taken from each vessel at 0, 3, 6, 9, 12, 18, 24 and 48 h for enumeration of dominant fecal microflora using fluorescent in situ hybridization technique (FISH).

2.5. Quantification of dominant fecal microflora by FISH technique

FISH technique was performed as described by Hongpattarakere et al. (2012). The genus-specific 16S rRNA target oligonucleotide probes labeled with the fluorescent dye Cy3 used were Bif 164 (5′-CATCCGGCATTACCACCC-3′), Bac 303 (5′-CCAATGTGGGGGACCTT-3′), Lab 158 (5′-GGTATTAGCAYCTTCCA-3′), Chis 150 (5′-TTATGCGG TATTAATAT(C/T) CCTTT-3′), Eub 338 (5′-GCTGCCTCCCGTAGGAGT-3′) specific for *Bifidobacterium*, *Bacteroides*, *Lactobacillus/Enterococcus* spp., *Clostridium histolyticum* group, and *Eubacterium* group, respectively (Rycroft, Jones, Gibson, & Rastall, 2001; Rochet, Rigottier-Gois, Rabot, & Dore, 2004; Al-Tamimi, Palframan, Cooper, Gibson, & Rastall, 2006; Mandalari et al., 2007). The nucleotide target 4′,6-diamidino-2-phenylindole (DAPI) (Sigma, Singapore) dye was used for total bacteria count. Samples taken from batch culture (375 μL) were added to 1.125 mL of 4% (w/v) filtered-sterile paraformaldehyde (chilled), mixed and stored at 4 °C overnight to fix the bacterial cells. The fixed cells were then centrifuged at 10,000 rpm (Eppendorf, Germany) for 5 min and washed twice with cool filtered sterile PBS (0.1 mol L^{-1}), pH 7.2 and resuspended in 150 μL of PBS. After that, ethanol (150 μL) was added then mixed thoroughly. The fixed samples were stored at −20 °C at least 1 h or until further analysis (within 3 months). The fixed cells were diluted to obtain appropriate dilution and then 20 μL of suitable dilutions were placed onto the well of teflon- and poly-L-lysine coated slides. The slides were placed on a slide

dryer (46–50 °C, 15 min). They were subsequently soaked in alcohol series (50, 80 and 90% ethanol) for 3 min at each concentration to allow permeabilization and penetration of DNA probe, before they were finally dried on the slide dryer. A lysozyme treatment was required for *Lactobacillus*/*Enterococcus* cells before alcohol dehydration. A pre-warmed hybridization buffer was mixed with 5 μL of genus-specific probe (50 ng μL^{-1}). The probe solution (50 μL) was added onto each well. The probe-target hybridization was then allowed in a hybridization oven (Boekel, USA) for 4 h at 46 °C for Eub 338 and Bac 303 probes; 50 °C for Lac 158, Bif 164 and Chis 150 probes. Once the hybridization completed, the slides were washed by soaking into a pre-warmed washing buffer, (0.9 mmol L^{-1} NaCl, 20 mmol L^{-1} Tris–HCl, pH 7.2) containing 20 μL DAPI solution (50 ng μL^{-1}) for 15 min at 48 or 50 °C depending on the bacterial group determined. The slides were then dipped into cold distilled water before subjected to a quick air-blown drying. Five microliters of Antifade (Fluka, Singapore) were then added onto each slide well and a cover slip was placed over the slide. The DAPI stained and the hybridized cells were examined and counted using a fluorescence microscope (Nikon Eclipse 80i, USA) under UV light and Cy3 filter, respectively. A minimum of 15 fields, each containing 10–100 cells, was counted for each well.

2.6. Effects of saba starch and drying methods on survivals of L. plantarum CIF17AN2

The overnight culture of *L. plantarum* CIF17AN2 was centrifuged at 10,000 rpm. The bacterial cells were washed twice with phosphate buffer saline (PBS) pH 7.2 and then resuspended and adjusted to obtain 1×10^{10} CFU mL^{-1} (N_0) with the same buffer. Cell suspension of *L. plantarum* CIF17AN2 was added to saba RS at the ratio of 1:1 (v/w). After homogenization, the mixture was dried at the following conditions; (1) at 37 °C for 4 days in incubator (Memmert BE 500, Germany), (2) at 45 °C for 3 days in incubator (Memmert BE 500, Germany), (3) at the ambient temperature (~27 °C) for 5 days, (4) vacuum drying in desiccator connected to suction pump (400 mbar) for 4 days and (5) at 37 °C in a vacuum oven (Napco 5831, United States) with 30 mmHg for 12 h. The probiotic LAB without saba RS addition was used as control. The synbiotic mixture was dried until its moisture content reached approximately 5.6% (Zayed & Roos, 2004). For cell recovery, the dried sample was brought to its original volume with PBS to obtain bacterial suspension, which was then serially diluted and transferred to the appropriate medium for viable counts on MRS agar (N_1). The results were expressed as % survival which was calculated from the following equation: % survival = $(\log N_1/\log N_0) \times 100$.

2.7. Effect of saba RS on shelf life of the synbiotic product

The drying method with the highest cell survival was chosen to prepare the synbiotic product (saba starch + *L. plantarum* CIF17AN2). The cell suspension of probiotic bacteria was added to saba starch at the ratio of 1:1 (v/w) and dried under low pressure (30 mmHg) at temperature of 37 °C for 12 h in a vacuum oven. The dried synbiotic powder was vacuum-packed and hermetically sealed in an aluminum foil-laminated polyethylene sachet. The product was kept at 4 °C and ambient temperature for 8 weeks. Every 7 days, a new package was opened and analyzed for probiotic viability by plating the appropriate dilution onto MRS agar. The moisture content of the products was determined according to AOAC (1999) method. The percentage of survival was calculated from the viable cell counts before and after storages at each sampling period according to the equation described in Section 2.6.

2.8. Sequential exposure to acid and bile salt of the synbiotic product

The dried synbiotic product was firstly exposed to acidic condition (0.85% NaCl containing 3 g L^{-1} pepsin (Sigma, Geramany) and adjusted to pH 2 with 1 N HCl) and bile (0.3% ox-gall bile (Merge, Darmstadt, Germany) and 3 mg mL^{-1} pancreatin (Sigma, USA)). The exposures were carried on at 37 °C for 3 h and 6 h with gentle agitation (by shaker) in the presence of acid and bile, respectively. The overnight fresh culture of *L. plantarum* CIF17AN2 and vacuum-dried *L. plantarum* without saba starch addition were carried out in parallel as the control treatments. The viable cells before (N_0) and after (N_1) the exposures were enumerated by spreading the appropriate dilution onto MRS agar. The percentage of survival was calculated according to the equation described in Section 2.6.

2.9. Statistical analysis

The data were analyzed by using SPSS software version 15 for Windows. Statistical significance was evaluated using one way analysis of variance and Duncan's multiple range tests. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Resistance of saba RS to degradation under simulated gastrointestinal conditions

Saba RS showed high resistance to the simulated gastric acid and pancreatic amylases in the intestine. It was digested at the respective rates of 1.38, 1.10 and 1.17% hydrolysis in the first hour of gastric acid exposures at pH 1, 2 and 3, respectively. The hydrolysis percentage remained quite stable after 2–4 h of digestion periods. Moreover, only 2.49–3.4% hydrolysis (Fig. 1) was observed after the additional 6 h exposure to the simulated intestinal juice. This indicated that the major component of saba RS was very stable to gastric acid hydrolysis and remained almost intact during exposure to the pancreatic amylase under the simulated intestinal condition for 6 h.

3.2. Bifidogenic effect of saba RS in the presence of human fecal microflora under simulated human proximal colon condition

The effect of saba RS on dominant gut microflora in the system mimic proximal region of human gastrointestinal tract (pH 5.5) was investigated. FISH analysis was performed to quantitatively determine the dominant members of human fecal microflora. Saba starch showed prebiotic effect on dominant gut microflora by selectively enhancing growth of beneficial fecal bacteria. The supplementation of saba RS significantly enhanced bifidobacterial population hence the increases of total fecal bacteria and *Eubacterium* group (Fig. 2). Similar trend was observed in *Bacteriodes* group in the presence of saba RS. *Lactobacillus*/*Enterococcus* population remained stable throughout the fermentation period, whereas significant decline was shown in the absence of saba RS. With saba RS supplement, clostridia significantly reduced even more in comparison to the control. The results indicate that saba RS was greatly specific to enhance growth of *Bifidobacterium* and maintain *Lactobacillus*/*Enterococcus* population. Therefore it is considered to be a potential bifidogenic prebiotic ingredient.

3.3. Effects of saba RS and drying methods on survivals of L. plantarum CIF17AN2

L. plantarum CIF17AN2 survived in all of low temperature-drying methods as shown in Fig. 3. At 45 °C, a complete destruction of *L.*

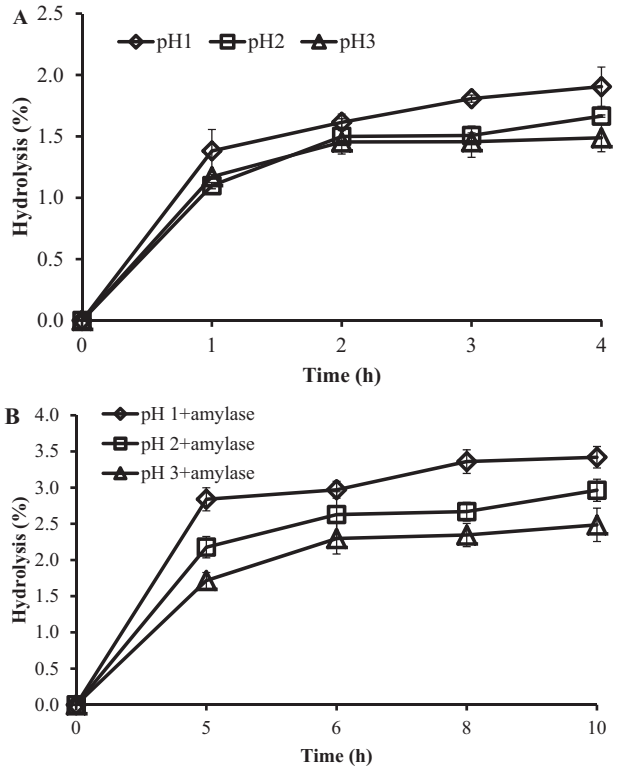

Fig. 1. Hydrolysis percentages of RS starch from green saba banana exposed to pH 1, 2 and 3 under simulated gastric acid and simulated intestinal conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

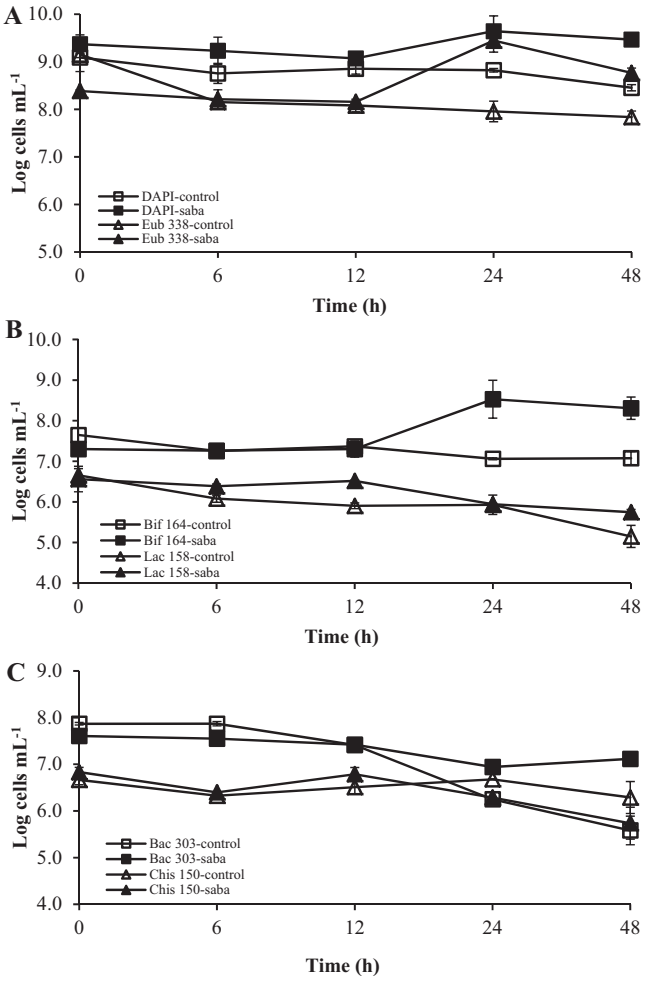

Fig. 2. Effect of saba RS on total fecal bacteria (DAPI), *Eubacterium* (Eub 338), *Bifidobacterium* (Bif 164), *Lactobacillus*/*Enterococcus* spp. (Lac 158), *Bacteriodes* (Bac 303) and *Clostridium* (Chis 150) in *in vitro* pH-controlled (5.5) batch culture fermentation under simulated human proximal colon condition.

plantarum CFI17AN2 was observed. However, a maximum survival was resulted when the vacuum drying was carried out at 37 °C for 12 h. The drying process conducted in a vacuum oven showed the highest survival of 85.81% in the presence of saba RS. Without the starch, the LAB survival reduced dramatically to 51.75% when it was dried under the same condition. In the presence of the starch, all drying methods conducted at 37 °C for 4 days, at the ambient temperature (27–30 °C) for 5 days and in a vacuum-desiccator for 4 days provided 55.45, 66.76 and 63.99% survival, respectively. Meanwhile, LAB survival reduced to 20.30, 44.40 and 37.75%, respectively without the starch addition. The results confirmed that saba RS can effectively protect *L. plantarum* from dehydration but cannot prevent them from heat destruction. The longer drying process, the lower survival rate was resulted.

3.4. Shelf-life of synbiotic product

The dried synbiotic product vacuum-packed in an aluminum foil laminated polyethylene sachet was evaluated for its microbiological and physiological characteristics during 8 weeks of storage at 4 °C and at ambient temperature (27–30 °C). The slight viability loss of the probiotic LAB was observed in the presence of saba RS throughout 8 week storage at both temperatures (Fig. 4A). At 4 °C storage, *L. plantarum* CIF17AN2 was maintained its viable number at the same level from the beginning to 6 weeks of storage in the saba RS supplemented treatment. At the end of storage, the log reduction of *L. plantarum* CIF17AN2 combined with saba RS was only 0.5 log CFU mL^{-1} and 1 log CFU mL^{-1} when the products were stored at temperatures of 4 °C and 27–30 °C, respectively. In contrast, a sharp decrease of viable cells was detected at both temperatures in the absence of saba RS with log reductions of 1.36 and 2.13 log CFU mL^{-1}, respectively. The moisture content of this synbiotic product stored at both storage temperatures remained stable throughout the whole storage period (Fig. 4B). On the contrary, the significant increase of the moisture content was observed in the dried *L. plantarum* CIF17AN2 stored at non-refrigerated temperature without saba RS addition.

3.5. Probiotic survival of the vacuum-dried synbiotic product after sequential exposures of simulated gastric acid and intestinal fluid

The survival of *L. plantarum* CIF17AN2 in vacuum-dried synbiotic product was determined after sequential exposures to gastric acid for 3 h and intestinal fluid for 6 h. The vacuum-dried *L. plantarum* CIF17AN2 both with and without saba RS addition were highly sensitive to these transit conditions (Fig. 5). Meanwhile, fresh cells showed high tolerant to the exposure compared to dried cells. This indicates that drying process can enhance LAB susceptibility to the stresses caused by simulated gastric acid and intestinal fluid. Without addition of saba RS, loss of LAB viability (72.46% reduction) was much higher than the synbiotic product, which 40.83% reduction was observed. The saba RS therefore effectively protect the probiotic from such exposures.

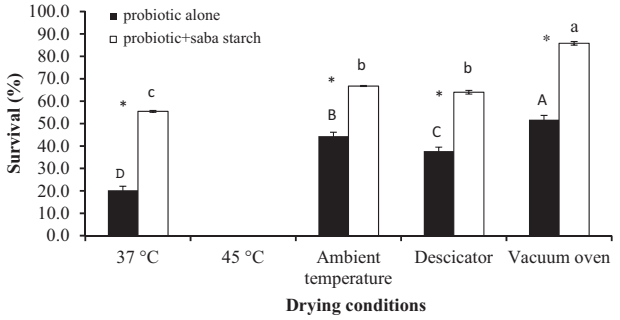

Fig. 3. Effect of saba RS addition on survival of *L. plantarum* CIF17AN2 from various drying conditions.

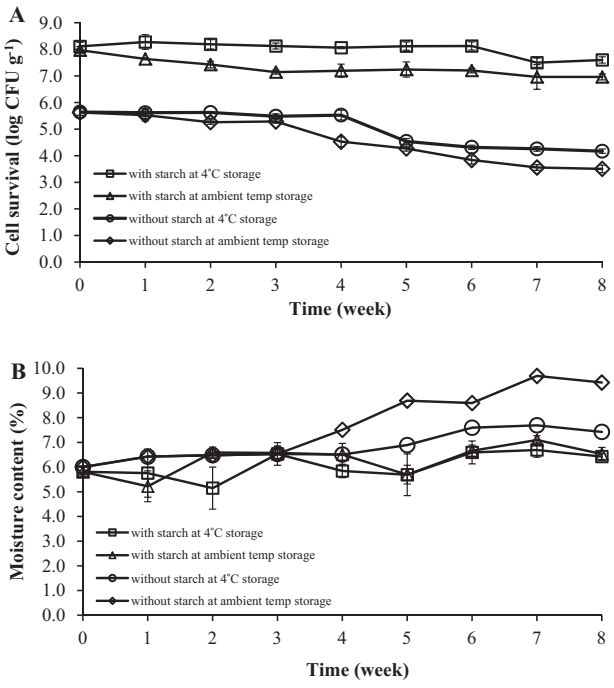

Fig. 4. Effect of saba RS on the survival and moisture content of the vacuum-dried *L. plantarum* CIF17AN2 during 8-week storage at 4 °C and ambient temperature (27–30 °C).

4. Discussion

RS starch from green saba banana showed significant prebiotic characteristics of high resistances to gastric acid in stomach and intestinal amylases. Saba RS was more resistant than other banana starch which was previously observed (Englyst & Cummings, 1986; Faisant et al., 1995; Bello-Pérez et al., 1999). It tolerated to gastric acid hydrolysis at much higher level than cereal fructans (fructo-oligosaccharides) reported by Nilsson, Öste, Jägerstad and Birkhed (1988). At pH 1.05, the cereal fructan was hydrolyzed up to 15% in the exposure of the simulated gastric juice at 37 °C for 1 h (Nilsson et al., 1988). Banana starch is previously claimed for its high resistance to amylases both *in vitro* and *in vivo* (Faisant et al., 1995). A large amount (up to 78–83.7%) of α-glucans from banana survived the digestion in the distal ileum of ileostomy subjects (Englyst & Cummings, 1986; Faisant et al., 1995). This study strongly emphasized the low susceptibility of saba RS to harsh conditions in the upper gastrointestinal transit.

In addition, its selective enhancement of beneficial gut bacteria particularly bifidobacteria was obvious. These characteristics indicate great prebiotic potential of saba RS. Furthermore, prebiotic characteristic of saba RS was determined for its efficiency to support the growth of beneficial human gut microflora in the system mimicking proximal region of human colon. It showed high potential of bifidogenic characteristic which was considered to be the most important criteria of prebiotic ingredient (Lavermicocca et al., 2005). Interestingly, the addition of saba starch significantly lowered detrimental bacteria like *Clostridium* group, and maintained stability of *Lactobacillus/Enterococcus* and *Bacteriodes* group. The latter group belongs to the Bacteroidia class formerly called Bacteriodetes. The significant increase of Bacteriodetes was related to loss of body weight in 12 obese adults under the treatment of the restricted diet. Moreover the obese volunteers had fewer Bacteriodetes than the lean ones before the diet therapy (Ley, Turnbaugh, Klein, & Gordon, 2006). Meanwhile, only a few number of resistant starch claimed to be prebiotics have been introduced and launched to the market. The resistant starch 'CrystalLean', which is a retrograded, amylose starch, displayed the 10–100 fold increases of lactobacilli and bifidobacteria numbers in the germ-free rats colonized with the human fecal microflora from UK and Italian adults in comparison with the sucrose-fed group. But no significant reduction of clostridia was observed (Silvi, Rumney, Cresei,

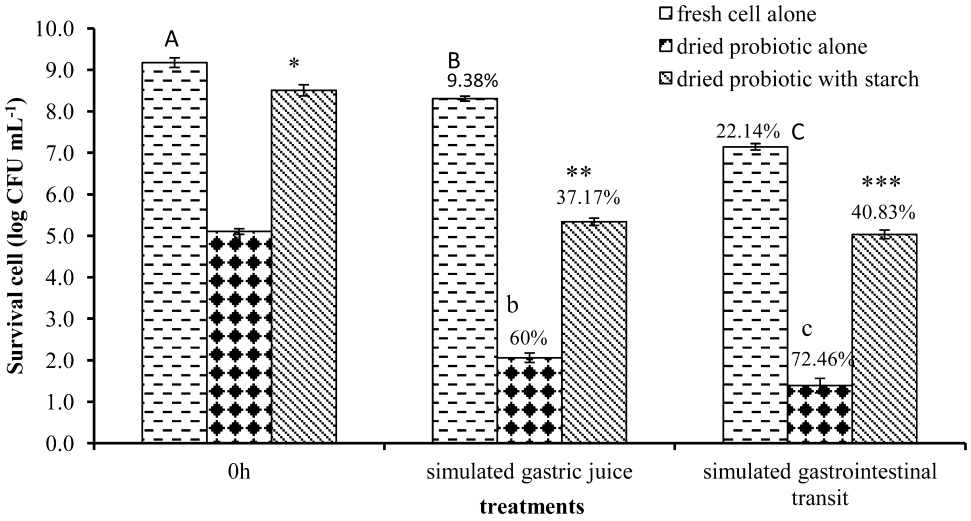

Fig. 5. Survival of the vacuum-dried synbiotic product (*L. plantarum* CIF17AN2 + saba RS) and vacuum-dried *L. plantarum* CIF17AN2 alone after sequential exposure to simulated gastric and bile salt fluid.

& Rowland, 1999). Rodríguez-Cabezas et al. (2010) combined the resistant starch (Fibersol® −2) with well-known prebiotic frutoologosaccharides (FOS) (Beneo® −95) for synergistic effect on changes in the intestinal microbiota by increasing lactobacilli and bifidobacteria in caecum and colonic contents of healthy rats. Apart from the enhancement of the beneficial gut bifidobacteria, saba RS could possibly benefit for the body weight control.

Most commercial starter cultures are preserved in frozen or freeze dried form because of relative high levels of survival and maintenance of activity. Frozen cultures bear high storage and transportation costs by keeping cultures at very low temperatures such as −20 to −40 °C (Santivarangkna et al., 2006). Freeze drying has high production costs because the energy requirement and the investment are about double of those needed for vacuum-drying (Bauera, Schneider, Behr, Kulozik, & Foerst, 2012). On the contrary, vacuum-drying is one of the promising processes, of which the moisture evaporation rate is higher. Besides, the application of low pressure allows the avoidance of the severe thermal inactivation (Santivarangkna et al., 2006; Guergoletto, Magnani, Martin, Andrade, & Garci, 2010). In this study, the dehydration at 37 °C using the vacuum oven was chosen as the most effective and economical method. It also shortens the drying period, meanwhile provides the anaerobic atmosphere favorable to all human probiotic bacteria. The addition of the prebiotic saba RS in combination with this type of drying to formulate the synbiotic product could effectively reduce loss of probiotic viability at the low expenses.

However, the addition of this prebiotic starch could not protect *L. plantarum* CFI17AN2 during drying in a hot-air oven at the temperature of 45 °C for 3 days. Too long exposure to high temperature and uneven evaporation of water could be the major cause. Similar observation on *Lactobacillus helveticus* was reported when the vacuum drying was conducted at 43 °C for 24 h. The damage of cell envelop during vacuum drying was revealed only when the cell lost free water (Santivarangkna et al., 2006). Generally bacterial cells consist of 70–95% water. The water removal could alter the physical property of membrane phospholipids, leading to change of membrane fluidity and increase of lipid peroxidation (Crowe, Crowe, Carpenter, & Wistrom, 1987). In addition, the heat itself could directly cause denaturation of various cellular macromolecules such as DNA, RNA and heat susceptible proteins (Linders et al., 1997). Bauera et al. (2012) noted about the occurrence of thermal damage during vacuum-drying at low temperatures of 15, 25 and 30 °C. Changes in membrane phase transition leading to the inactivation of the membrane proteins was suspected to occur when low temperature was applied in drying. The major changes of bacterial cell envelop and DNA destruction of vacuum-dried *L. helveticus* were confirmed by using atomic force microscopy and Fourier transform infrared spectroscopy (Santivarangkna et al., 2006).

Certain dehydration protectants were investigated to prevent probiotic destruction during drying. The non-prebiotics such as sorbitol, trehalose, sucrose, skim milk, maltose and lactose were reported to protect viability of dried probiotic cells. Foerst, Kulozik, Schmitt, Bauer and Santivarangkna (2012) found no significant loss of *Lactobacillus paracasei* F19 vacuum-dried with the presence of sorbitol when stored at room temperature and 4 °C. Zayed and Roos (2004) reported the high survival of *Lactobacillus salivarius* subsp. *salivarius* (UCC 500) after freeze–drying in the presence of trehalose + sucrose + skim milk. Mono and disaccharides are commonly use as the cryoprotectants when freeze–drying is applied. However, they were ineffective in the protection of probiotic subjected to the vacuum-drying (Guergoletto et al., 2010). Thus, the combination of the prebiotic saba RS with vacuum-drying at 37 °C, at which *L. plantarum* CFI17AN2 was cultivated, was the effective strategy to retain high probiotic viability under the dehydration stress.

Only a few of information is available regarding application of prebiotics for probiotic protection during vacuum drying. Guergoletto et al. (2010) introduced the exposure of *Lactobacillus casei* (LC-1) to allow adhesion to prebiotic inulin and various vegetal fibers for 1 h to improve the probiotic survival before vacuum-drying at 45 °C. The adherence of *L. casei* (LC-1) to oat bran and green banana flour showed the survival of 79% and 76%, respectively after drying. However, the lowest viability (55%) was observed in the presence of inulin. In contrast, the presence of saba starch did effectively enhance survival of *L. plantarum* CFI17AN2 (85.81%) during vacuum drying process meanwhile functioned as the strong bifidogenic prebiotic.

Generally, all dried probiotic bacteria remain stable when stored at 4–8 °C. Nevertheless the dried ones which can retain their viability at room temperature are in great demands. This can significantly reduce the costs of storage facility and transportation. Saba RS not only protected probiotic bacteria during vacuum-drying process but also stabilized probiotic viability during the subsequent storage at room temperature. In the presence of saba RS, the moisture content of the synbiotic product was quite stable throughout the storage period at both ambient and low temperatures. On the contrary, the dramatic reduction of *L. casei* (LC-1) adhered to banana flour was observed in the subsequent 4 week storage at 25 °C correlating to the significant increase of the moisture content (Guergoletto et al., 2010). The correlation between loss of viability with the increase of moisture content in dried probiotics were noted by several researchers (Guergoletto et al., 2010; Jagannath, Raju, & Bawa, 2010; Kurtmann, Carlsen, Risbo, & Skibsted, 2009; Bauera et al., 2012; Zayed & Roos, 2004).

Moreover, the addition saba RS provided better protection of the vacuum-dried *L. plantarum* CIF17AN2 from simulated gastric and intestinal conditions than the control treatment. The significant decrease of cell survival was observed when compared with the beginning. This may be due to bacterial structure was damaged during drying process. The cytoplasmic membrane is generally considered to be the main site of dehydration inactivation (Gardiner et al., 2000). The damaged cell cytoplasmic membrane caused to increase the sensitivity to chemicals such as NaCl, bile salt, lysozyme, penicillin G and acidic condition (Teixeira, Castro, & Kirby, 1995; Golowczyc, Silva, Teixeira, Antoni, & Abraham, 2011). Because the rate of water outflow of cell is limited by the hydraulic membrane permeability therefore high water flow from the cell may cause cell injury (Santivarangkna et al., 2006). In contrast with our result, the cell adhesion of *L. casei* (LC-1) to oat bran was effective in protecting of this probiotic strain during 4 week storage and exposures of the simulated gastrointestinal conditions. No morphological change was observed through scanning electron micrograph after drying (Guergoletto et al., 2010). Combination of fiber adhesion with saba starch will be further investigated to develop the synbiotic formulation to maximize the probiotic viability and stability with low cost of drying method.

5. Conclusion

Saba RS showed great resistance to digestibility caused by gastric acid and intestinal amylases. Beside, it specifically enhanced growth of bifidobacteria and stabilized lactobacilli/enterococci population in the highly competitive environment of human fecal microflora. Interestingly, the significantly reduced number of clostridia group was also observed. The combination of *L. plantarum* CFI17AN2 with saba RS significantly reduced probiotic destruction due to drying process, subsequent dry storage and gastrointestinal exposures. Its protective effect toward *L. plantarum* CIF17AN2 during vacuum-drying conducted at 37 °C was dominant. Saba RS therefore is a high potential prebiotic ingredient containing the

additional function to extend probiotic stability during vacuum-drying process as well as long storage period of dehydration.

Acknowledgments

This research was funded by Annual Government Expenditure 2014–2015 (Grant No. AGR570227S), Prince of Songkla University, Thailand for financial support.

References

Aht-Ong, D., & Charoenkongthum, K. (2002). Thermal properties and moisture absorption of LDPE/banana starch biocomposite films. *Journal of Metals, Materials and Minerals*, *12*, 1–10.

Al-Tamimi, M. A. H. M., Palframan, R. J., Cooper, J. M., Gibson, G. R., & Rastall, R. A. (2006). *In vitro* fermentation of sugar beet arabinan and arabinooligosaccharides by the human gut microflora. *Journal of Applied Microbiology*, *100*, 407–414.

AOAC. (1999). *Official methods of analysis* (16th ed.). Washington, DC: Association of Official Agricultural Chemists.

Bauera, S. A. W., Schneider, S., Behr, J., Kulozik, U., & Foerst, P. (2012). Combined influence of fermentation and drying conditions on survival and metabolic activity of starter and probiotic cultures after low-temperature vacuum drying. *Journal of Biotechnology*, *159*, 351–357.

Bello-Pérez, L. A., Agama-Acevedo, E., Sánchez-Hernández, L., & Paredes-López, O. (1999). Isolation and partial characterization of banana starches. *Journal of Agricultural and Food Chemistry*, *47*, 854–857.

Crowe, J. H., Crowe, L. M., Carpenter, J. F., & Wistrom, C. A. (1987). Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochemical Journal*, *242*, 1–10.

Dave, R., & Shah, N. P. (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal*, *7*, 31–41.

Englyst, H. N., & Cummings, J. H. (1986). Digestion of the carbohydrates of banana (*Musa paradisiaca sapientum*) in the human small intestine. *American Journal of Clinical Nutrition*, *44*, 42–50.

Faisant, B. N., Buleon, A., Colonna, P., Molis, C., Lartigue, S., Galmiche, J. P., et al. (1995). Digestion of raw banana starch in the small intestine of healthy human: Structural features of resistant starch. *British Journal of Nutrition*, *73*, 111–113.

Foerst, P., Kulozik, U., Schmitt, M., Bauer, S., & Santivarangkna, C. (2012). Storage stability of vacuum-dried probiotic bacterium *Lactobacillus paracasei* F19. *Food and Bioproducts Processing*, *90*, 295–300.

Fox, J. D., & Robyt, J. F. (1991). Miniaturization of three carbohydrate analyses using a microsample plate reader. *Analytical Biochemistry*, *95*, 93–96.

Fu, N., & Chen, X. D. (2011). Towards a maximal cell survival in convective thermal drying processes. *Food Research International*, *44*, 1127–1149.

Gardiner, G. E., O'sullivan, E., Kelly, J., Auty, M. A. E., Fitzgerald, G. F., Collins, J. K., et al. (2000). Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. *Applied and Environmental Microbiology*, *66*, 2605–2612.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, *125*, 1401–1412.

Golowczyc, M. A., Silva, J., Teixeira, P., Antoni, G. L. D., & Abraham, A. G. (2011). Cellular injuries of spray-dried *Lactobacillus* spp. isolated from kefir and their impact on probiotic properties. *International Journal of Food Microbiology*, *144*, 556–560.

Guergoletto, K. B., Magnani, M., Martin, J. S., Andrade, C. G. T. J., & Garci, S. (2010). Survival of Lactobacillus casei (LC-1) adhered to prebiotic vegetal fibers. *Innovative Food Science & Emerging Technologies*, *11*, 415–421.

Hamilton-Miller, J. M. T., Shah, S., & Winkler, J. T. (1999). Public health issues arising from microbiological and labeling quality of foods and supplements containing probiotic microorganisms. *Public Health Nutrition*, *2*, 223–229.

Hongpattarakere, T., Cherntong, N., Wichienchot, S., Kolida, S., & Rastall, R. A. (2012). *In vitro* prebiotic evaluation of exopolysaccharides produced by marine isolated lactic acid bacteria. *Carbohydrate Polymers*, *87*, 846–852.

Hongpattarakere, T., Rattanaaubon, P., & Buntin, N. (2013). Improvement of freeze-dried *Lactobacillus plantarum* survival using water extracts and crude fibers from food crops. *Food and Bioprocess Technology*, *6*, 1885–1896.

Jagannath, A., Raju, P. S., & Bawa, A. S. (2010). Comparative evaluation of bacterial cellulose (nata) as a cryoprotectant and carrier support during the freeze drying process of probiotic lactic acid bacteria. *LWT—Food Science and Technology*, *43*, 1197–1203.

Kurtmann, L., Carlsen, C. U., Risbo, J., & Skibsted, L. H. (2009). Storage stability of freeze-dried *Lactobacillus acidophilus* (La-5) in relation to water activity and presence of oxygen and ascorbate. *Cryobiology*, *58*, 175–180.

Lavermicocca, P., Valerio, F., Lonigro, S. L., Angelis, M. D., Morelli, L., Callegari, M. L., et al. (2005). Study of adhesion and survival of lactobacilli and bifidobacteria on table olives with the aim of formulating a new probiotic food. *Applied and Environment Microbiology*, *71*, 4233–4240.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Human gut microbes associated with obesity. *Nature*, *444*, 1022–1023.

Lin, W. H., Hwang, C. F., Chen, L. W., & Tsen, H. Y. (2006). Viable counts, characteristic evaluation for commercial lactic acid bacteria products. *Food Microbiology*, *23*, 74–81.

Linders, L. J. M., de Jong, G. I. W., Meerdink, G., & van't Riet, K. (1997). Carbohydrates and the dehydration inactivation of *Lactobacillus plantarum*: The roles of moisture distribution and water activity. *Journal of Food Engineering*, *31*, 237–250.

Macfarlane, G. T., Macfarlane, S., & Gibson, G. R. (1998). Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the human colon. *Microbial Ecology*, *35*, 180–187.

Mandalari, G., Nueno, P. C., Tuohy, K., Gibson, G. R., Bennett, R. N., Waldron, K. W., et al. (2007). *In vitro* evaluation of the prebiotic activity of a pectic oligosaccharide-rich extract enzymatically derived from bergamot peel. *Applied Microbiology Biotechnology*, *73*, 1173–1179.

Nilsson, U., Öste, R., Jägerstad, M., & Birkhed, D. (1988). Cereal fructans: *In vitro* and *in vivo* studies on availability in rats and humans. *The Journal of Nutrition*, *118*, 1325–1330.

Otero, M. C., Espeche, M. C., & Nader-Macías, M. E. (2007). Optimization of the freeze–drying media and survival throughout storage of freeze-dried *Lactobacillus gasseri* and *Lactobacillus delbrueckii* subsp. *delbrueckii* for veterinarian probiotic applications. *Process Biochemistry*, *42*, 1406–1411.

Ratti, C. (2001). Hot air and freeze–drying of high-value foods: A review. *Journal of Food Engineering*, *49*, 311–319.

Rochet, V., Rigottier-Gois, L., Rabot, S., & Dore, J. (2004). Validation of fluorescent *in situ* hybridization combined with flow cytometry for assessing interindividual variation in the composition of human fecal microflora during long-term storage of samples. *Journal of Microbiological Methods*, *59*, 263–270.

Rodríguez-Cabezas, M. E., Camuesco, D., Arribas, B., Garrido-Mesa, N., Comalada, M., Bailòn, E., et al. (2010). The combination of frutoologosaccharides and resistant starch shows prebiotic additive effects in rats. *Clinical Nutrition*, *29*, 832–839.

Rycroft, C. E., Jones, M. R., Gibson, G. R., & Rastall, R. A. (2001). Fermentation properties of gentio-oligosaccharides. *Letters in Applied Microbiology*, *32*, 156–161.

Santivarangkna, C., Kulozik, U., & Foerst, P. (2006). Effect of carbohydrates on the survival of *Lactobacillus helveticus* during vacuum drying. *Letter in Applied Microbiology*, *42*, 271–276.

Silvi, S., Rumney, C. J., Cresci, A., & Rowland, I. R. (1999). Resistant starch modifies gut microflora and microbial metabolism in human flora-associated rats inoculated with faeces from Italian and UK donors. *Journal of Applied Microbiology*, *86*, 521–530.

Sultana, K., Godward, G., Reynold, N., Arumugaswamy, R., Peiris, P., & Kailasapathy, K. (2000). Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. *International Journal of Food Microbiology*, *62*, 47–55.

Teixeira, P., Castro, H., & Kirby, R. (1995). Spray drying as a method for preparing concentrated cultures of *Lactobacillus bulgaricus*. *The Journal of Applied Bacteriology*, *78*, 456–463.

Vatanasuchart, N., Niyomwit, B., & Wongkrajang, K. (2012). Resistant starch content, *in vitro* starch digestibility and physico-chemical properties of flour and starch from Thai bananas. *Maejo International Journal of Science and Technology*, *6*, 259–271.

Ying, D. Y., Phoon, M. C., Sanguansri, L., Weerakkody, R., Burgar, I., & Augustin, M. A. (2010). Microencapsulated *Lactobacillus rhamnosus* GG powders: Relationship of powder physical properties to probiotic survival during storage. *Journal of Food Science*, *75*, E588–E595.

Zayed, G., & Roos, Y. H. (2004). Influence of trehalose and moisture content on survival of *Lactobacillus salivarius* subjected to freeze–drying and storage. *Process Biochemistry*, *39*, 1081–1086.