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Development of a rice incorporated synbiotic yogurt with low retrogradation properties

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Abstract

Yogurts with probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12 were prepared using cow's milk and boiled rice (13% wt/wt) was incorporated into yogurts at the end of the incubation period. Four rice varieties namely, At-309, At-405, MA-2 and Thai Jasmine (KDML 105) were used for the study. Microbial, physico-chemical and sensory properties of rice yogurts were evaluated during 21 days of storage at 4^{o}C. Incorporation of rice into yogurts significantly improved the crude fiber contents ($p<0.05$) of yogurts. All rice incorporated yogurts showed higher water holding capacity compared to that of plain yogurts (without rice) throughout the storage. Titratable acidity, viscosity and hardness of yogurts demonstrated the similar trends. *Bifidobacterium* counts of the rice incorporated yogurts were higher (>8 cfu.g^{-1}) than that of plain yogurts at the end of storage period, probably due to prebiotic effect of rice. There were significant differences among the yogurt samples for evaluated sensory characteristics and At-405 rice yogurt demonstrated the lowest overall sensory acceptability. Therefore, careful selection of suitable rice varieties is vital in producing symbiotic rice yogurt with high quality.

Introduction

Probiotics are live microorganisms when administered in adequate amounts confer health benefits on the host (FAO/WHO, 2002). The reported beneficial effects of probiotic consumption include improvement of intestinal health, amelioration of symptoms of lactose intolerance, enhancement of the immune response, reduction of serum cholesterol, control of urogenital and respiratory tract infections, and reduction of the risk of colon cancers (Salminen *et al*., 2005; Vasudha and Mishra, 2013; Prasanna *et al*., 2014). Therefore, probiotics such as lactobacilli and bifidobacteria incorporated food products are popular among health conscious consumers (Ranadheera *et al*., 2014). Yogurt is a fermented dairy product produced by fermentation of milk using starter culture bacteria *Streptococcous thermophiles* and *Lactobacillus bulgaricus* subsp. *delbrueckii.* In addition, probiotic bacteria are also included in certain yogurts, hence can be considered as one of the most common carrier foods of probiotics to humans.

Rice is a nutritious cereal and one of the cheapest and major sources of food energy and protein for many people in the world. Brown rice contains nutritional components, such as dietary fibers, essential amino acids, minerals, proteins, vitamins and other non-nutrient essential phytochemicals, concentrated in the germ and outer layers of the starchy endosperm (Kohama *et al.*, 1987). The bran layer of rice kernel contains high level of bioactive compounds such as γ-oryzanol, anthocyanins and phenolic compounds which may reduce low-density lipoprotein cholesterol, improve lipid profiles and have anti-inflammatory and anti-oxidative activities which may help to fight against heart diseases and prevent diabetes (Nontasan *et al*., 2012). Rice, especially the bran contains dietary fibers which can have beneficial effects against chronic diseases such as cardiovascular diseases, diverticulosis, diabetes and colon cancer (Abdul-Hamid, 2000). Amarakoon *et al.* (2013) demonstrated that cooked rice facilitates the growth and survival of probiotic bacteria such as bifidobacteria in yogurt. Therefore, beneficial health effects of probiotic yogurts can be further enhanced by incorporation of rice into yogurts. The nutritional quality of rice based foods can also be improved through fermentation by amyololytic lactic acid bacteria such as certain strains of lactobacilli and bifidobacteria which may increase the availability

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of lysine and improve the digestibility of starch in young children (Gobbetti *et al.*, 2005; Espirito-Santo *et al*., 2014). Nowadays, cereals alone or mixed with other ingredients are widely utilized in producing novel food products with enhanced health properties (Blandino *et al.*, 2003; Coda *et al*., 2012). Nontasan *et al*. (2012) recently developed a yogurt with good color stability and higher levels of phytochemicals using black waxy rice bran. However, Amarakoon *et al.* (2013) reported significantly higher hardness development in rice incorporated yogurts compared to plain yogurts during refrigerated storage possibly due to retrogradation of rice.

Rice starch is made up of two polysaccharide types called amylose and amylopectin (Fredriksson *et al*., 1998). During cooking, hot water enters the semi-crystalline regions of raw rice starch, breaks down the intermolecular hydrogen bonds, and binds to the hydroxyl hydrogen and oxygen hence the chains of starch lose their crystallinity into the amorphous form. This gelatinized starch is not in thermodynamic equilibrium. During cooling or being left at a lower temperature for long periods, the linear molecules, amylose, and the linear parts of amylopectin molecules expel water and rearrange themselves again to a more crystalline structure. This recrystallization is known as retrogradation, resulting in hard and amylase-inaccessible textures with poor sensory quality and low nutritional value in cooked rice (Chung *et al.*, 2006, Okuda *et al.*, 2006; Zhu *et al*., 2013; Li *et al*., 2014). Therefore, retrogradation process is highly likely to affect the organoleptic and nutritional qualities and reduces the consumer acceptability of rice incorporated yogurts. To the best of our knowledge, very few studies investigated the quality of rice incorporated probiotic yogurts. Our preliminary studies demonstrated that the use of low-amylose rice varieties can be successfully utilized in producing rice incorporated yogurt with low retrogradation properties. This study aimed to develop rice incorporated synbiotic yogurt with acceptable organoleptic properties by using low-amylose rice varieties.

Materials and Methods

Yogurt production

The experiment was conducted at the Dairy Science Laboratory, University of Peradeniya, Sri Lanka. Fat content of cow's milk (Livestock Field Station, University of Peradeniya, Sri Lanka) was adjusted to contain 3.2% by adding skimmed milk (provided by the Livestock Field Station, University of Peradeniya, Sri Lanka). Sugar (20% wt/wt) and gelatin (0.5% wt/wt) (Central Essense Pvt. Ltd, Kandy, Sri Lanka) were also added before the yogurt mix was homogenized at 200 bar pressure for 30 min using a homogenizer (Tessa JJ05125, Israel). The yogurt mix was pasteurized at 91°C for 5 min using a double jacketed heating vat (Tessa NMRV063, Israel). The pasteurized mix was cooled to 42°C using a plate heat exchanger (Tessa plate 11+11, Israel). STI 12 culture granules containing yogurt starter culture *Streptococcus thermophiles* (CHR Hansen, Denmark) and probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12 (CHR Hansen, Denmark) were inoculated as a direct vat set (DVS) into the yogurt mix as per the manufactures recommendations. Then the mix was incubated at 42°C for 3-4 hrs until reaching ~ 4.6 pH.

Through preliminary trials four low amylose rice varieties (*Oryza sativa* L.) were selected to be incorporated into yogurt mixture (Thai Jasmine rice-KDML 105 with 12%-17% amylose content and three local verities MA-2, At-309 and At-405 with < 22% amylose contents). Thai Jasmine rice was purchased from Arpico Supermarket, Kandy, Sri Lanka while local rice varieties were obtained from Rice Research Institutes (RRI) of Sri Lanka (MA-2 from RRI, Bathalagoda, Sri Lanka and At-309 and At-405 from RRI, Ambalantota, Sri Lanka). After incubation, the yogurt mixture was divided into four batches aseptically and respective pressure cooked rice varieties were added as 13% (wt/wt) into the incubated yogurt and stirred well approximately for 3 min. After mixing yogurt were filled into the plastic cartons (80 g) and stored at 4°C.

Physico-chemical properties

Total solid content (oven dry method), fat content (Gerber method) pH (by using a digital pH meter, eutEch Instruments Pvt.Ltd, Singappore) and titratable acidity (by titraing against 0.1 N NaOH in the presence of phenolphthalein) of raw cow's milk were measured prior to yogurt production (AOAC, 2005).

Total solids (oven drying method), ash content (ignition of solid materials at 600°C in an electric muffle furnace, Carbolite Co., England), protein (Kjeldhal method), fat content (Soxhlet extraction method) and crude fiber contents of the yogurt samples were analyzed during the storage (AOAC, 2005). The titratable acidity and pH of yogurt samples were also measured during the storage as previously described. In here, yogurt without any added rice was also used to evaluate the nutritional changes that may be caused by addition of rice into yogurt.

Water holding capacity (WHC) of rice yogurt samples was measured by a method reported earlier (Isanga and Zhang, 2012) with slight modifications. Ten grams of yogurt was measured into an oven dried centrifuge tube and centrifuged at 4600 rpm for 30 min and the WHC was calculated as follows:

$$WHC\ (\%) = (1 - W1 / W2) \times 100$$

Where: W1= Weight of whey after centrifugation, W2 = Initial weight of yogurt sample.

Viscosity of rice yogurts was measured as described by Ranadheera *et al.* (2012) with small modifications using a viscometer (BL model Tokimec, Japan). Viscosity was determined based on measuring resistance to a rotating spindle (Spindle no. 4 at 12 rpm) at constant temperature (4°C) for 5 min in a yogurt filled 6.36 cm diameter 6 cm height cup.

Texture hardness in rice yogurts was measured as described by Alfaro *et al.* (2015) with small modifications using an Instron machine texture analyser (Instron 4465, Japan) with a 2 KN launch cell. Yogurt samples produced in a cylindrical container (6.36 cm diameter 6 cm height) and force needed to destruct the gel (force per area) was measured in N. A 38 mm in diameter flat base cylinder with a constant speed of 150 mm/min was thrust into the cylindrical container in order to measure the texture hardness of yogurt.

Sensory properties

Sensory evaluations were conducted to evaluate the color, odor, texture, flavor and overall acceptability of rice yogurt samples at 7 and 14 days of the storage as described by Stone *et al.* (2012) with slight modifications. Sensory attributes were evaluated through a 1-4 scale (4- most preferred to 1- least preferred) with 30 untrained panellists. The tasting panel was randomly selected among the students and staff members of the Faculty of Agriculture, University of Peradeniya, Sri Lanka, based on their willingness to participate the study. The samples (80 g each) were served in white color cups with randomly selected three digits numbers for the evaluations. The sensory evaluation procedure was approved by the Department of Animal and Food Sciences, Rajarata University of Sri Lanka.

Bifidobacterium *viability in yogurt and yeast, mold and coliform counts*

Microbial analyses of the yogurt samples were conducted on day 1, 7, 14 and 21 during the storage. *Bifidobacterium* agar (Himedia laboratories, India) was used to enumerate *Bifidobacterium animalis* subsp. *lactis* BB-12. One gram of yogurt sample was serially diluted in sterile peptone water and enumeration was done using the pour plate technique. The plates were incubated at 37°C for 72 hrs in anaerobic jars (Oxoid Ltd, Hampshire, UK) with an anaerobic environment (<1% O_2 and 9-13% CO_2), generated using anaerobic AnaeroGen® sachets (AN0025A, Oxoid Ltd, Hampshire, UK). Enumeration of yeast and mold was conducted in Potato Dextrose Agar (PDA) using spread plate technique. The plates were incubated at 25°C for 5 days. Enumeration of Coliform was conducted in Violet Red Bile Agar using spread plate techniques following aerobic incubation at 35°C for 5 days. Viable bacteria counts were expressed as log cfu.g^{-1} of yogurt sample.

Statistical analyses

The data from the sensory evaluations were analyzed using non parametric Friedman test and physico-chemical and microbiological results in triplicates were analyzed by one way ANOVA for Completely Randomized Design using MINITAB software. The means were separated by Tukey test. A p value <0.05 was considered statistically significant for all analysis.

Results and Discussion

Physico-chemical properties of yogurt

Titratable acidity, pH, total solid and fat contents of cow's milk used to produce rice incorporated yogurts were 0.16±0.01%, 6.24±0.02, 13.65±0.43% and 3.70±0.05%, respectively. Physico-chemical properties of different rice incorporated yogurts are shown in Table1. As expected, the total solids content was lowest in plain yogurt, because no added rice in plain yogurt. The average fat and protein contents were also lowest in plain yogurt. Incorporation of At-309 and At-405 rice have significantly increased ($p<0.05$) the protein content of yogurts. The crude fiber content of rice yogurts were within the range of 1.12-1.49% whereas no fiber was present in plain yogurt. Incorporation of rice into the yogurt had significantly increased ($p<0.05$) the fiber content of yogurt compared to that of plain yogurt.

Figure 1 shows the variations of physico-chemical properties of plain and rice yogurts during the storage period. pH value of all yogurts decreased with the time up to 16th day and started to slightly increase thereafter. Present trend agrees with Vahedi *et al.* (2008) and Ranadheera *et al.* (2012) who reported that pH values of fruit yogurt decreased during storage

Table 1. Physico-chemical properties of plain and rice incorporated yogurts

Thai rice yogurt	MA-2 rice yogurt	At-309 rice yogurt	At-405 rice yogurt	Plain yogurt
22.45 ± 4.63^{ab}	22.85 ± 0.48^{ab}	23.23 ± 0.26^{ab}	24.28 ± 0.32^{a}	16.58 ± 2.99^{b}
2.75 ± 4.39^{a}	2.84 ± 0.58^{a}	2.59 ± 0.23^{a}	2.85 ± 0.38^{a}	0.75 ± 0.04^{b}
3.26 ± 0.26^{a}	3.27 ± 0.16^{a}	3.65 ± 0.34^{a}	3.51 ± 0.58^{a}	3.14 ± 0.22^{a}
3.63 ± 0.27^{ab}	3.28 ± 0.11^{b}	3.75 ± 0.08^{a}	3.73 ± 0.15^{a}	3.24 ± 0.07^{b}
1.12 ± 0.21^{a}	1.36 ± 0.13^{a}	1.46 ± 0.28^{a}	1.49 ± 0.25^{a}	0.00 ± 0.00^{b}

Mean±SD ^{a,b}Values in the same rows having different superscripts are significantly different ($p<0.05$)

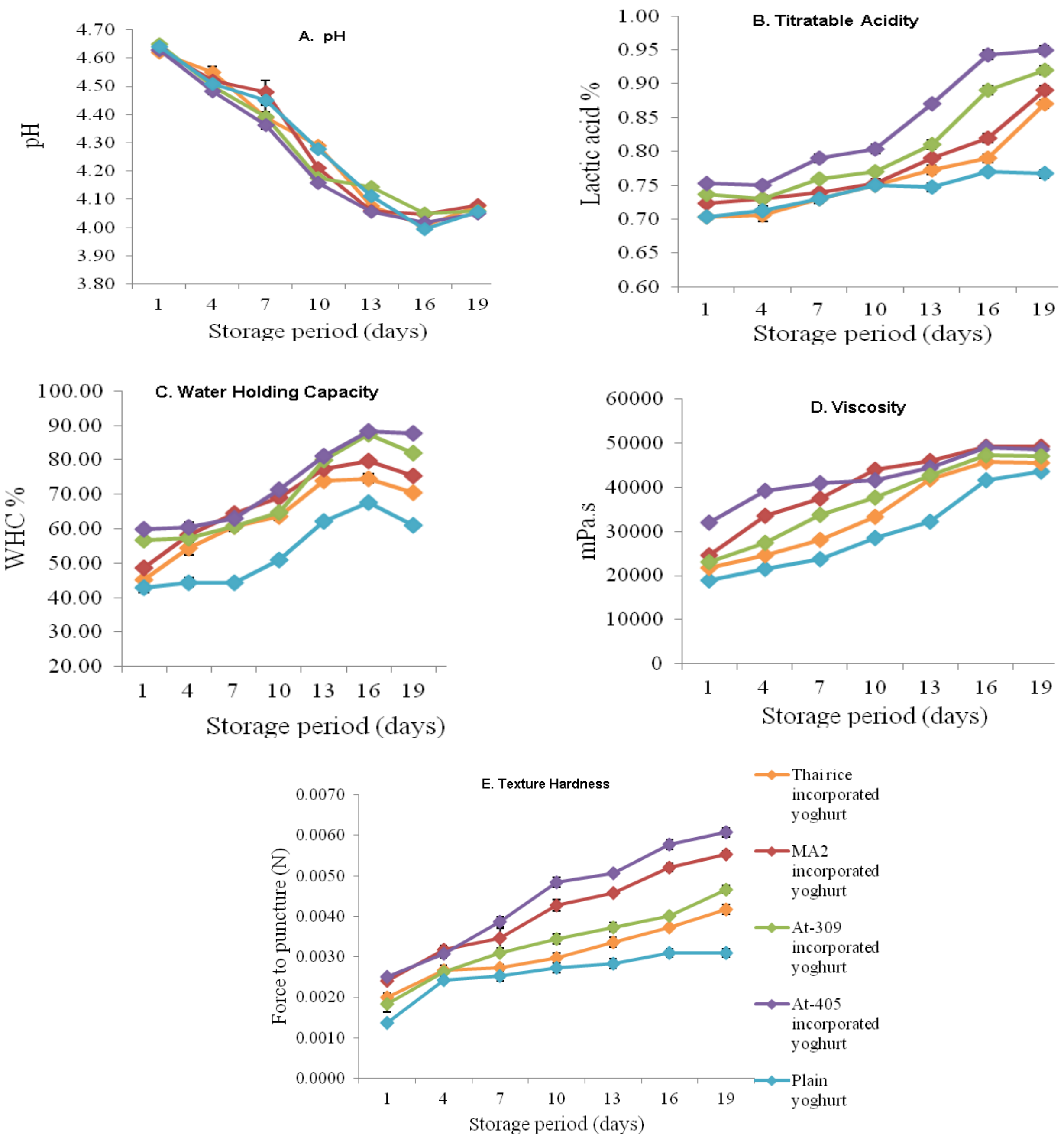

Figure 1. Variations of some physico-chemical properties of plain and rice yogurts during storage: (A) pH, (B) Titratable Acidity, (C) Water Holding Capacity, (D) Viscosity and (E) Texture Hardness

Table 2. Mean scores for sensory properties of rice incorporated yogurts after 7 and 14 days of storage at 4°C

Characteristic	Storage time (days)	Thai rice yogurt	MA-2 rice yogurt	At-309 rice yogurt	At-405 rice yogurt
Color	7	2.63±1.05^{a}	2.87±1.16^{a}	3.13±1.26^{a}	1.88±1.04^{a}
	14	2.38±0.95^{a}	1.88±1.22^{a}	3.13±1.07^{a}	2.13±1.15^{a}
Texture	7	3.38±1.10^{a}	2.75±1.12^{a}	1.63±1.22^{a}	2.25±0.98^{a}
	14	2.25±1.07^{a}	3.25±1.19^{a}	2.75±1.04^{a}	1.75±1.13^{a}
Flavor	7	2.25±1.05^{a}	3.25±1.24^{a}	2.75±1.14^{a}	1.75±1.03^{a}
	14	2.88±1.11^{a}	2.25±0.99^{a}	3.25±1.02^{a}	1.63±1.22^{a}
Odor	7	3.25±1.13^{a}	2.75±1.14^{a}	2.25±1.11^{a}	1.75±1.08^{b}
	14	2.75±0.92^{a}	1.75±1.21^{b}	2.75±1.13^{a}	2.25±1.14^{a}
Overall acceptability	7	2.75±1.10^{a}	3.25±1.22^{a}	2.25±1.19^{ab}	1.75±0.99^{b}
	14	2.75±0.88^{a}	2.25±1.17^{ab}	3.25±1.03^{a}	1.75±1.11^{b}

Mean±SD ^{a,b}Values in the same rows having different superscripts are significantly different ($p<0.05$)

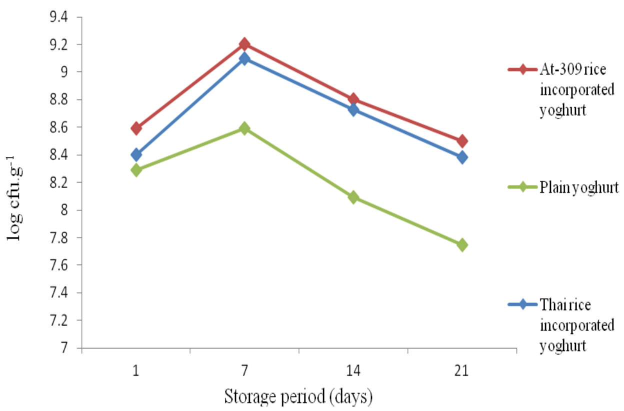

Figure 2. Viability of *Bifidobacterium animalis* subsp. *lactis* BB-12 in plain and rice yogurts during storage

and slightly increased at the latter part of storage. Decrease of pH may be due to increase of lactic acid production by lactic acid bacteria. As sugar sources finish, microorganisms begin to consume proteins and producing some products such as peptides by microorganisms as a result of biochemical metabolism, may cause slight pH increase at the later part of storage (Vahedi *et al.*, 2008). The pH values of all the rice yogurts were within generally acceptable level for yogurt during the storage.

Titratable acidity of all the yogurt samples were within the recommendations (0.8-1.25%) given by Sri Lanka Standards Institute (1989) and increased during storage period. Similar trend was observed by many authors for fermented dairy foods (Vahedi *et al.*, 2008; Güler and Gürsoy-Balcı, 2011; Ranadheera *et al.*, 2012). The increase in titratable acidity during storage could be attributed to the activity of *S. thermophiles* and *Bifidobacterium* cultures used in the present study. It is widely accepted that lactic acid bacteria usually convert lactose into lactic acid and thereby increase the acidity in fermented dairy foods. Higher titratable acidity values were observed for rice yogurts compared to plain yogurt throughout the storage. Sandra *et al.*, (2008) previously demonstrated the positive effect of fiber extracted from lemon and orange juice by-products on the growth and survival of *L. acidophilus* and *B. bifidum* in fermented milk during incubation and refrigerated storage. Similarly, fiber in rice might have improved the growth and activity of lactic acid bacteria in the rice yogurt and thereby higher lactic acid production in rice yogurts than in plain yogurt might be possible. This hypothesis is further supported by the higher survivability of bifidobacteria in rice yogurts compared to plain yogurt in the present study (Figure 2). The highest titratable acidity was observed in the At-405 rice yogurt throughout the storage period. Among all the rice yogurts, the highest crude fiber content (Table 1) was also observed in At-405 rice yogurt, demonstrating the effect of fiber towards increasing titratable acidity in probiotic dairy foods possibly through promoting the growth of lactic acid bacteria during storage.

Usually higher acidity stimulates the whey separation in yogurts (Tamime and Robinson, 1999). Although rice yogurts demonstrated higher acidity, less whey separation or higher WHC in rice yogurt compared to plain yogurt was observed during the storage in the present study. This may be probably due to high total solids (Remeuf *et al.*, 2003) and fiber content in the rice yogurt (Table 1) which could have strengthened the yogurt gel structure in rice yogurts and thereby making less susceptible to loose whey under centrifugal force. Fiber components can improve the texture, gelling, thickening and stabilizing properties in food products (Abdul-Hamid, 2000). Furthermore, Shori *et al.* (2014) have suggested that exopolysaccharides produced by bacteria in the yogurts, which act as hydrocolloids help to increase WHC in yogurts. *B. animalis* subsp.

lactis is known to produce exopolysaccharides (Prasanna *et al*., 2014) and higher viable numbers of Bifidobacterium was observed in rice yogurt than in plain yogurt in the present study (Figure 2). Therefore, the amount of exopolysaccharides production by bifidobacteria might have been high in rice yogurts than in plain yogurts. Similar to the findings from the current study, increasing trend in WHC during storage of control and calcium fortified fruit yogurts has previously been reported by Singh and Muthukumarappan (2008). Increase in WHC as the storage time progresses could also be due to re-absorption of whey back into the yogurt gel (Cassarotti, 2014). In contrast, Küçükçetin *et al.* (2011) reported the decreasing trend in WHC of yogurt gel during 15 days of storage at 4°C. Previous studies also have reported that the effective immobilization of the aqueous phase by the gelatin in the yogurt could significantly reduce the whey separation from yogurt and gelatin may induce the formation of a gel network with the sodium casemate in the yogurt mixture (Lal *et al*., 2006; Supavititpatana *et al*., 2008). In the present study gelatin was added into the yogurt as a stabilizer during manufacturing and this might also have been a reason to observe increasing WHC in yogurts during the storage.

As shown in Figure 1 there was an increasing trend in viscosity of yogurts with time. All the rice incorporated yogurts exhibited higher ($p< 0.05$) viscosity values throughout the storage period. High total solid contents in rice yogurts could be suggested as a reason for increased viscosity in rice yogurts in the present study. According to Tamime and Robinson (1999), increasing total solid content in yogurt could result higher consistency and viscosity values. In the present study total solids in rice yogurts were within the range of 22.45±4.63 - 24.28±0.31% while total solid content in plain yogurt was 16.58±2.99% (Table 1). Other than the total solid content, some researchers claim that viscosity and texture of yogurt is positively affected by the microbial exopolysaccharide produced by lactic acid bacteria in yogurt since exopolysaccharides may act as texturizers and stabilizers (Duboc and Mollet, 2001). Therefore, exopolysaccharides may increase the viscosity of the final product as well as the rigidity of the casein network by binding hydration water and interacting with other milk constituents such as proteins and micelles. Thus, higher viability of *B. animalis* subsp. *lactis* BB-12 in rice yogurts compared to plain yogurt in the present study (Figure 2) might have been responsible for the increasing viscosity values in rice yogurts through production of exopolysaccharides. Retrogradation process of rice may also have influenced the viscosity of rice yogurt.

The force needed to puncture the yogurt gel (hardness value) was increased with time in the present study. The rice incorporated yogurts demonstrated higher texture hardness compared to plain yogurt throughout the storage period. High total solid and fiber contents of rice yogurts (Table 1) might have been affected to texture hardness similar to viscosity and WHC in the present study. Higher total solid contents decrease the moisture content of food products. Moisture content and the total solids affected on the texture, whereas low moisture content and high total solid may increase the texture hardness and consistency of food products such as yogurts. Moreover, from 7th day onwards, At-309 and Thai rice yogurts showed significantly lower ($p<0.05$) force to disturb the yogurt gel compared to that of other two local rice incorporated yogurts. The reason might be the soft texture of the At-309 and Thai rice yogurts compared to other local rice incorporated yogurts possibly due to low retrogradation rate of At-309 and Thai rice compared to other local rice varieties.

Sensory properties of yogurt

There were significant differences among yogurts for sensory parameter during the storage (Table 2). At-309 rice yogurt scored the highest average rank for the color during the storage while At-405 rice yogurt demonstrated the lowest overall acceptability for tested sensory characteristics probably due to its natural brownish color. When consider texture, both Thai jasmine rice and MA-2 rice yogurts scored the highest average rank, while At-309 rice yogurt scored the highest overall acceptability at the day 14th of storage.

As reported by Amarakoon *et al*. (2013), the sensory characteristics of cooked rice varieties directly affect the overall sensory properties of rice incorporated yogurt. Sensory properties of rice can be influenced by many factors such as contents of amylose and protein contents, charlky grain rate, pasting properties, springiness and stickiness (Yang *et al*., 2013), hardness, storage duration and conditions. Clearly, little is known concerning the effect of physico-chemical and sensory properties of various rice varieties on the consumer acceptability of rice incorporated yogurts, suggesting necessity of more scientific research towards rice incorporated synbiotic yogurts.

Viability of bifidobacteria in rice yogurt

It is reported that the yogurts containing live probiotics confer health benefits to the host when they are consumed in appropriate quantity (Mckinley, 2005). Thus, it is necessary for probiotic cultures to survive in yogurts during their shelf life prior to being consumed. It is generally accepted that a concentration of approximately 10^6-10^7 viable cells per g or mL of product at the time of consumption is beneficial to confer health effects in humans.

Since At-405 rice variety showed the least consumer acceptability for most sensory attributes, At-405 rice incorporated yogurts did not include in assessing bifidobacteria viability. Only the control, and two other rice yogurt samples representing one local rice variety (At-309) and the imported rice variety (Thai Jasmine rice) which demonstrated an appropriate sensory acceptability were included in this study. B. animalis subsp. lactis BB-12 counts in yogurts slightly increased up to 7th day of storage and gradually decreased thereafter in the present study (Figure 2). In At-309 rice yogurt, initial Bifidibacterium count (8.59±0.69 log cfu.g^{-1}) was decreased to 8.50±0.51 log cfu.g^{-1} while in Thai rice yogurt, initial Bifidibacterium count was decreased from 8.40±0.57 log cfu.g^{-1} to 8.38±0.45 log cfu.g^{-1} at the end of storage. Highest viability loss for bifidobacteria was observed in plain yogurt. Initial Bifidibacterium count in rice yogurt (8.29±0.58 log cfu.g^{-1}) was decreased to 7.26±0.47 log cfu.g^{-1} at the end of storage. Parmijit *et al*. (2011) suggested that the reduction of *Bifidibacterium* viability during storage might be attributed to hydrogen peroxide produced by starter culture *Lactobacilli.* On the other hand Bari *et al*. (2009) reported that the dissolved oxygen content might directly affect the survival of probiotic cultures. According to Champagne *et al.* (2005) oxygen affects the probiotic cultures in two ways. The first is direct toxicity to cells. Certain probiotic cultures are very sensitive to oxygen and die in its presence, presumably due to the intracellular production of hydrogen peroxide. When oxygen is in the medium, certain cultures, particularly, yogurt starter culture *L. delbrueckii*, excrete peroxide in the medium and thereby indirect inhibition of probiotics such as *Bifidibacterium* may also occur.

In the present study, despite the decrease of viable cell count with storage time, *Bifidibacterium* count in both rice and plain yogurts were able to maintain the minimum recommended therapeutic level. There were no significant differences ($p>0.05$) in *Bifidibacterium* counts among yogurts at the day of production. However, rice yogurts recorded significantly higher ($p<0.05$) *Bifidibacterium* counts during the refrigerated storage compared to that of plain yogurt demonstrating certain prebiotic effect of rice towards bifidobacteria.

Yeast and mold and coliform counts in yogurts

Undesirable levels of yeast and molds (>10^2 cfu.g^{-1}) (SLSI, 1989) were not recorded in any yogurt sample in the present study. Although it was not significantly difference ($p>0.05$) yeast and mold growth was slightly higher in rice yogurts than in plain yogurts, probably due to contamination during rice incorporation into yogurt. No coliform bacteria were detected in any of the yogurt samples due to employment of good hygienic practices during the manufacturing of yogurt in this study.

Conclusions

Incorporation of rice into probiotic yogurts seemed beneficial in improving the fibre content of yogurts. Generally, rice incorporated yogurts demonstrated higher total solids, fat, protein content, titratable acidity, water holding capacity, viscosity and texture hardness compared to plain yogurts without rice. Rice incorporated yogurts were within the acceptable pH range and their sensory properties were varied based on the rice varieties used. Rice yogurts demonstrated the ability in maintaining higher viability of probiotic *B. animalis* subsp. *lactis* BB-12 than plain yogurts during 21 days of storage at 4°C suggesting favorable prebiotic properties in rice. Therefore, it is possible to produce high quality synbiotic yogurts through incorporation of cooked rice into yogurts. However, careful selection of suitable rice varieties is necessary in order to maintain the satisfactory quality characteristics during storage.

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References

Abdul-Hamid, A. and Luan, Y. S. 2000. Functional properties of dietary fibre prepared from defatted rice bran. Food Chemistry 68(1): 15-19.

Alfaro, L., Hayes, D., Boeneke, C., Xu, Z., Bankston, D., Bechtel, P. J. and Sathivel, S. 2015. Physical properties of a frozen yogurt fortified with a nano-emulsion containing purple rice bran oil. LWT-Food Science and Technology – In Press.

Amarakoon, A. M. S. B. K., Jayawardane, I. N. T., Senaviratne, N. D., Ranadheera C. S., Silva, K. F. S. T. and Vidanarachchi, J. K. 2013. Development of a rice incorporated symbiotic yogurt. In Vidanarachchi,

J. K. and Himali, S. M. C. (Eds). Proceedings of the Twenty Third Annual Student Research Sessions, p. 5. Peradeniya: Department of Animal Science, University of Peradeniya, Sri Lanka.

Association of Official Analytical Chemists (AOAC). 2005. Official Methods of Analysis, Gaithursburg, MD, USA.

Bari, M.R., Ashra, R., Alizade, M. and Rofehgarineghad, L. 2009. Effect of different contents of yogurt starter/probiotic bacteria, storage time and different concentration of cysteine on the microflora characteristics of bio-yogurt. Research Journal of Biological Science 4: 137-142.

Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D. and Webb, C. 2003. Cereal-based fermented foods and beverages. Food Research International 36(6): 527-543.

Casarotti, S. N., Monteiro, D. A., Moretti, M. and Penna, A. L. B. 2014. Influence of the combination of probiotic cultures during fermentation and storage of fermented milk. Food Research International 59: 67-75.

Champagne, C.P., Gardner, N.J. and Roy, D. 2005. Challenges in the addition of probiotic cultures to foods. Citrical Reviews in Food Science and Nutrition 45: 61-84.

Chung, H. J., Lim, H. S. and Lim, S. T. 2006. Effect of partial gelatinization and retrogradation on the enzymatic digestion of waxy rice starch. Journal of Cereal Science 43(3): 353-359.

Coda, R., Lanera, A., Trani, A., Gobbetti, M. and Di Cagno, R. 2012. Yogurt-like beverages made of a mixture of cereals, soy and grape must: microbiology, texture, nutritional and sensory properties. International Journal of Food Microbiology 155(3): 120-127.

Duboc, P. and Mollet, B. 2001. Applications of exopolysaccharides in the dairy industry. International Dairy Journal 11: 759–768.

Espirito-Santo, A. P. D., Mouquet-Rivier, C., Humblot, C., Cazevieille, C., Icard-Vernière, C., Soccol, C. R. and Guyot, J. P. 2014. Influence of co-fermentation by amylolytic Lactobacillus strains and probiotic bacteria on the fermentation process, viscosity and microstructure of gruels made of rice, soy milk and passion fruit fiber. Food Research International 57: 104-113.

FAO/ WHO. 2002. Joint Food and Agriculture Organization /World Health Organization Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada.

Fredriksson, H., Silverio. J., Andersson, R., Eliasson, A. C. and Aman, P. 1998. The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starch. Carbohydrate Polymers 35: 119-134.

Gobbetti, M., De Angelis, M., Corsetti, A. and Di Cagno, R. 2005. Biochemistry and physiology of sourdough lactic acid bacteria. Trends in Food Science and Technology 16(1): 57-69.

Güler, Z. and Gürsoy-Balci, A. C. 2011. Evaluation of volatile compounds and free fatty acids in set types yogurts made of ewes', goats' milk and their mixture using two different commercial starter cultures during refrigerated storage. Food Chemistry 127(3): 1065-1071.

Isanga, J. and Zhang, G. 2009. Production and evaluation of some physicochemical parameters of peanut milk yogurt. LWT-Food Science and Technology 42(6): 1132-1138.

Kohama, Y., Matsumoto, S., Mimura, T., Tanabe, N., Inada, A. and Nakanishi, T. 1987. Isolation and identification of hypotensive principles in red-mold rice. Chemical and Pharmaceutical Bulletin 35: 2484-2489.

Küçükçetin, A., Demir, M., Aşci, A. and Çomak, E. M. 2011. Graininess and roughness of stirred yogurt made with goat's, cow's or a mixture of goat's and cow's milk. Small Ruminant Research 96(2): 173-177.

Lal, S. N., O'Connor, C. J. and Eyres, L. 2006. Application of emulsifiers/stabilizers in dairy products of high rheology. Advances in Colloid and Interface Science 123: 433-437.

Li, X., Li, D., Tian, H. and Park, K. H. 2014. Reducing retrogradation of gelatinized rice starch and rice meal under low temperature storage by addition of extremely thermostable maltogenic amylase during their cooking. Food Research International 62: 1134-1140.

Mckinley, M. 2005. The nutrition and health benefits of yogurt. International Journal of Dairy Technology 58(1): 1-12.

Nontasan, S., Moongngarm, A., and Deeseenthum, S. 2012. Application of functional colorant prepared from black rice bran in yogurt. APCBEE Procedia 2: 62-67.

Okuda, M., Aramaki, I., Koseki, T., Inouchi, N. and Hashizume, K. 2006. Structural and retrogradation properties of rice endosperm starch affect enzyme digestibility of steamed milled-rice grains used in sake production. Cereal Chemistry 83(2): 143-151.

Parmijit, S.P. and Shinde, C. 2011. Effect of storage on syneresis, pH, *Lactobacillus acidophillus* count of Aloe vera fortified probiotic yogurt. Current Research in Dairy Sciences 4(1): 17–23.

Prasanna, P. H. P., Grandison, A. S. and Charalampopoulos, D. 2014. Bifidobacteria in milk products: An overview of physiological and biochemical properties, exopolysaccharide production, selection criteria of milk products and health benefits. Food Research International 55: 247-262.

Ranadheera, C. S., Evans, C. A., Adams, M. C. and Baines, S. K. 2014. Effect of dairy probiotic combinations on in vitro gastrointestinal tolerance, intestinal epithelial cell adhesion and cytokine secretion. Journal of Functional Foods 8: 18-25.

Ranadheera, C. S., Evans, C. A., Adams, M. C. and Baines, S. K. 2012. Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. Food Chemistry 135(3): 1411-1418.

Remeuf, F., Mohammed, S., Sodini, I. and Tissier, J. P. 2003. Preliminary observations on the effects of milk fortification and heating on microstructure and physical properties of stirred yogurt. International Dairy Journal 13(9): 773-782.

Salminen, S.J., Gueimonde, M. and Isolauri, E. 2005. Probiotics that modify disease risk. Journal of Nutrition 135: 1294–1298

Sendra, E., Fayos, P., Lario, Y., Fernández-López, J., Sayas-Barberá, E. and Pérez-Alvarez, J. A. 2008. Incorporation of citrus fibers in fermented milk containing probiotic bacteria. Food Microbiology 25(1): 13-21.

Shori, A.B., Baba, A.S., Misran, M. and Tan, H.W. 2014. Influence of *Allium sativum* or *Cinnamomum verum* on physicochemical characteristics of yogurt. International Journal of Dairy Science 9(2): 45-55.

Singh, G. and Muthukumarappan, K. 2008. Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. LWT-Food Science and Technology 41(7): 1145-1152.

SLSI. 1989. Specification for fermented milk products - part 2 - Yogurt. Amendment No.01 to SLS 824. Sri Lanka Standards Institute, Colombo.

Stone, H., Bleibaum, R. and Thomas, H. A. 2012. Sensory evaluation practices. 4th edn. London: Academic press.

Supavititpatana, P., Wirjantoro, T. I., Apichartsrangkoon, A. and Raviyan, P. 2008. Addition of gelatin enhanced gelation of corn–milk yogurt. Food Chemistry 106(1): 211-216.

Tamime, A.Y. and Robinson, R.K. 1999. Yoghurt: Science and Technology. 2nd edn. Boca Raton, FL: CRC Press.

Vahedi, N., Tehrani M.M. and Shahidi, F. 2008. Optimizing of fruit yogurt formulation and evaluating its quality during storage. American-Eurasian Journal of Agricultural and Environmental Scence 3(6): 922–927.

Vasudha, S. and Mishra, H. N. 2013. Non-dairy probiotic beverages. International Food Research Journal 20: 7-15.

Yang, X., Lin, Z., Liu, Z., Alim, M. A., Bi, J., Li, G., Wang, Q., Wang, S. and Ding, Y. 2013. Physicochemical and Sensory Properties of japonica Rice Varied with Production Areas in China, Journal of Integrative Agriculture 12: 1748-1756.

Zhu, F., Wang, S. and Wang, Y. J. 2013. Physical properties and enzyme susceptibility of rice and high-amylose maize starch mixtures. Journal of the Science of Food and Agriculture 93(12): 3100-3106.