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The Effect of Arrowroot (*Maranta arundinacea*) Extract on the Survival of Probiotic Bacteria in Set Yoghurt

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***Abstract*-** The objective of this study was to determine the effect of Arrowroot carbohydrates on the survival of lactobacilli in bio-yoghurts. There were four treatments; probiotic yoghurt (control), probiotic yoghurt with 3% Arrowroot extract, probiotic yoghurt with 1.65% Raftilose® and yoghurt without probiotics or prebiotics. Lactobacilli population of control was 4.82 log CFU/mL at 11st day of refrigerated storage whereas Arrowroot carbohydrates and Raftilose® increased ($P<0.05$) it by 1.44 log CFU/mL and 1.17 log CFU/mL respectively compared to the control. These results support the conclusion that Arrowroot carbohydrates can be used to enhance the Lactobacilli population in bio-yoghurt during refrigerated storage.

***Index Terms*-** arrowroot, bio-yoghurt, prebiotics, probiotics

I. INTRODUCTION

The therapeutic properties of fermented milk are widely known and the contribution of yoghurt bacteria to the improvement of intestinal microflora has been widely recognized. Lactobacillus bulgaricus and Streptococcus thermophilus (yoghurt starter culture) are not bile acid resistant and do not survive the passage through the intestinal tract. However, L. acidophilus and B. bifidum (probiotic bacteria) incorporated into a yoghurt starter culture results in a milk product "of excellent therapeutic value" due to the ability of these species establish themselves amongst gut microflora (Tamime and Robinson, 2007).

In recent years, there has been growing interest in using probiotic microorganisms as dietary adjuncts in the dairy industry. To produce the desired benefits, probiotic bacteria should be presented in the product in sufficient quantities during its whole shelf-life. Shah (2000) recommended that the minimum dose able to assure therapeutic effect should 6 log CFU mL-1. However, the survival of bacteria in yoghurt is quite low because the pH of yoghurt ranges from 4.2 to 4.6. Lankaputhra et al. (1996) reported the survival of 3 out of 9 bacterial strains in the pH range of 3.7 to 4.3. Further, he found that 14 out of 17 strains lost their viability in fermented milk in the first week of storage. Therefore, the strict strain dependence and poor survival of probiotics under adverse processing conditions including low pH, oxygen tension and nutrient depletion are some of the problems faced by the fermented milk industry.

Many recent studies have shown that incorporation of prebiotic ingredients in probiotic yoghurt would probably leads to enhancement of the survival of those microorganisms. Prebiotics are selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota which confer benefits upon host well-being and health (Gibson et al., 2004). A range of oligosaccharides has been tested so far, with inulin and oligofructoses most frequently assessed and commercially incorporated in different products.

Arrowroot (Maranta arundinacea) is a locally available rhizomatous herbaceous plant. Arrowroot rhizomes have high level of fructo-oligosaccharides which may possess prebiotic properties and may be useful in manufacturing bio-yoghurt. This study was carried out to assess the effect of incorporating water-soluble carbohydrate extracted from Arrowroot rhizomes on the sensory properties and the survival of lactobacilli and lactic acid bacteria in set-type yoghurt during refrigerated storage.

II. MATERIALS AND METHODS

Extraction of Arrowroot (*Maranta arundinacea*) carbohydrates

Arrowroot carbohydrates were extracted as outlined in Figure 1.

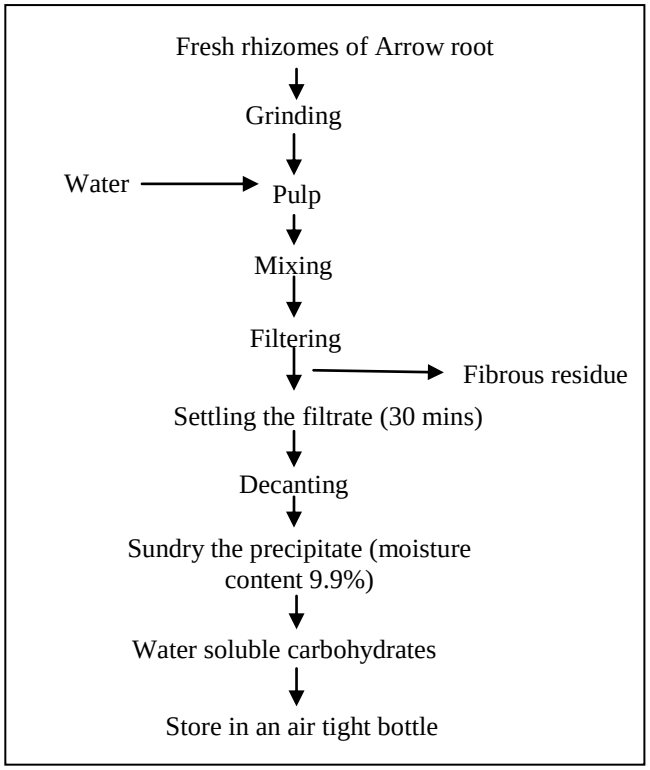

Figure 1: Extraction of carbohydrates from Arrowroot rhizomes

Preparation of Cultures

Probiotic cultures were prepared using a freeze dried lactic culture (ABT-3, Hanson, Denmark). This culture contained *Lactobacillus acidophilus, Bifidobacterium bifidum and Streptococcus thermophilus*. Standardized and pasteurized (90 ^{o}C, 5 min) cow milk was inoculated and incubated at 42 ^{o}C for 5 h. The prepared probiotic culture was stored at 4 ^{o}C. Yoghurt starter culture (Rich®), which contain *Streptococcus thermophilus* and *Lactobacillus delbrueckii sub sp. Bulgaricus* was used to produce non-probiotic yoghurt.

Preparation of Yoghurt

Set-yoghurts were manufactured at the Dairy Technology Laboratory, Department of Animal Science, Faculty of Agriculture, University of Peradeniya using standardized, low fat, cow milk (fat 1.4%, SNF 8.03) with slight modifications to the yoghurt composition. Table I shows the formula for different treatments.

Table I: Formula for different yoghurt treatments

Ingredients /1 L of yoghurt mix	Treatments			
	Yoghurt without probiotics (T_1)	Control (T_2)	Probiotic yoghurt with Raftilose® (T_3)	Probiotic yoghurt with Arrowroot (T_4)
Cow milk (L)	1.0	1.0	1.0	1.0
Sugar (g)	100.0	100.0	100.0	100.0
Gelatin (g)	7.0	7.0	7.0	7.0
ABT-3 Probiotic culture (g)	*	20.0	20.0	20.0
Rich® Yoghurt culture (g)	20.0	*	*	*
Arrowroot (g)	*	*	*	30.0
Raftilose® (g)	*	*	16.5	*

*Ingredients do not include in the respective treatments.

Three replicate trials were conducted in the manufacturing of yoghurt. There were four treatments; probiotic yoghurt without prebiotics (control), probiotic yoghurt with 3% Arrowroot, probiotic yoghurt with 1.65% inulin (Raftilose®, BENEO-Orafti, Morris Plains, USA) and yoghurt without probiotics or prebiotics. Raftilose® contains 98% inulin whilst water soluble carbohydrates in Arrowroot contain 55% fructo-oligosaccharides. To match the quantity of prebiotics in Arrowroot and Raftilose® 3% and 1.65% levels of incorporations were selected respectively for the experiment. The yoghurt mixtures were heated at 90 ^{o}C for 5 min after addition of sugar. Then they were cooled to 45 ^{o}C and inoculated with 2% (w/v) of probiotic cultures. The yoghurt mixtures used for non-probiotic yoghurt were inoculated with 2% (w/v) of yoghurt starter culture. They were dispensed into 80 mL polyethylene cups and incubated at 42 ^{o}C for 3.5 h. Then samples were stored at 4 ^{o}C. They were withdrawn at 1, 6, 11 and 16 days of storage for objective quality evaluation.

Analysis of Yoghurt

Microbiological analysis

Yoghurt samples (1 mL) were mixed for 2 minutes using a VORTEX-GENIE™ machine (Model K-550-GE, Scientific Industries, Bohemia, USA) and serially diluted 10 fold with sterilized Ringer solution (BR 52, Oxoid Ltd, Hampshire, UK). An aliquot (100 µL) was plated on the following agar media using spread plate technique. Lactobacilli were enumerated on Rogosa agar (CM 0627, Oxoid Ltd, Hampshire, UK). Bacterial population was counted after anaerobic incubation at 39 deg C for 48 h in anaerobic jars (Oxoid Ltd, Hampshire, UK) with anaeroGen™ sachets (AN 0025A, Oxoid Ltd, Hampshire, UK). The numbers of total lactic acid bacteria (*Lactobacilus sp*., *S. thermophilus* and *Bifidobacterium sp*.) were determined on MRS agar (CM 361, Oxoid Ltd, Hampshire, UK) after anaerobic incubation at 37 deg C for 48 h. Bacterial populations were expressed as $\log_{10}$ CFU/mL of yoghurt samples.

Titratable acidity

Titratable acidity (TA) as percent lactic acid was measured for all the treatments using 0.1N NaOH and 1% phenolphthalein (Sigma-Aldrich Co, USA) solution as endpoint indicator according to the technique of Marshall (1992).

pH

The pH was determined for all the samples with a Microprocessor pH meter (Model 211, TOA Electronics Ltd, Tokyo, Japan).

Sensory analysis

Sensory characteristics of the yoghurt samples were evaluated using 30 untrained panelists. Colour, odor, taste, texture and overall acceptability were evaluated using a five-point hedonic scale from 1-like extremely to 5-dislike extremely.

Statistical analysis

A Completely Randomized Design (CRD) was used for the experiment. Analysis of variance was followed by a mean separation procedure using Turkey's test. All analyses were performed using procedures for the General Linear Model (PROC GLM) of SAS (SAS institute Inc., Cary, NC, USA). Each experiment was replicated 3 times. The data obtained were analyzed at 0.05 level of significance. Data from sensory analysis was analyzed by Friedman non-parametric test using a Minitab 14 software package.

III. RESULTS AND DISCUSSION

Recovery percentage of Arrowroot carbohydrate was 9.6%. There were 78.7% (w/w) starch, 9.9% (w/w) moisture, 7.8% (w/w) polysaccharides and 3.6% (w/w) reducing sugars in the extracted Arrowroot carbohydrates and 55.1% (w/w) of starch in Arrowroot carbohydrates was fructo-oligosaccharides. Probiotic lactobacilli survived well in bio-yoghurts with prebiotics for up to 11 days of storage under refrigerated conditions (4 ^{o}C) and thereafter the counts decreased ($P<0.05$) below 10^6 CFU mL^{-1} (Fig. 2), the minimum population for an acceptable probiotic product. However, bio-yoghurt without prebiotics reached this value at 8th day of storage.

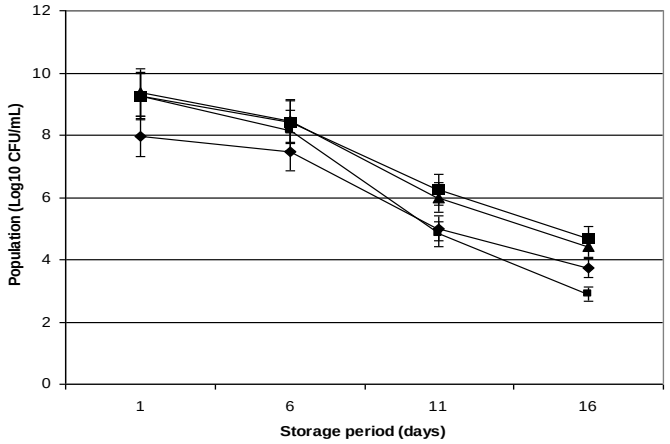

Figure 2: Changes of lactobacilli population in Yoghurts [◆] probiotic yoghurt [■], Probiotic yoghurt with inulin [▲] and probiotic yoghurt with Arrow root carbohydrates [■] with time at 4 ^{o}C

Lactic acid bacteria survived in prebiotics (Arrowroot carbohydrate and Raftilose®) incorporated yoghurt up to 15 days of storage and thereafter the counts decreased ($P<0.05$) below an acceptable level (Fig. 3).

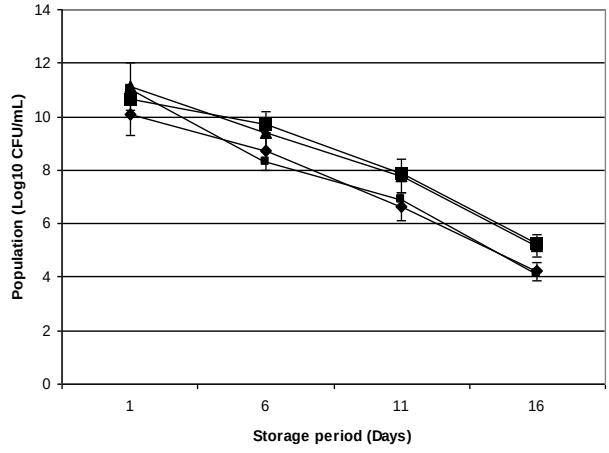

Figure 2: Changes of lactic acid bacteria population in Yoghurts [◆]probiotic yoghurt [■], Probiotic yoghurt with inulin [▲and probiotic yoghurt with Arrow root carbohydrates [■] with time at 4 ^{o}C

However, lactic acid bacteria can survive only 13 days of storage under 4 ^{o}C in bio-yoghurt without incorporation of prebiotics. These results are similar to the finding of Aryana *et al*. (2007). He reported that 1.5 % (w/v) inulin enhance the survival of Lactobacilli in fat free plain yoghurt during refrigerated storage. Oliveira *et al.* (2008) reported that fructo-oligosaccharides led to lower post acidification and lactic acid release. Survival of probiotic bacteria under low pH ranges from 4.2 to 4.6 is very low (Lankaputhra *et al*., 1996). Therefore, the incorporation of Arrowroot carbohydrates could reduce the post acidification than the control during refrigerated storage and thus improve the stability and survival of probiotic Lactobacilli and lactic acid bacteria.

Figure 4 and 5 show the variation in TA and pH of yoghurt samples during storage at 4 ^{o}C.

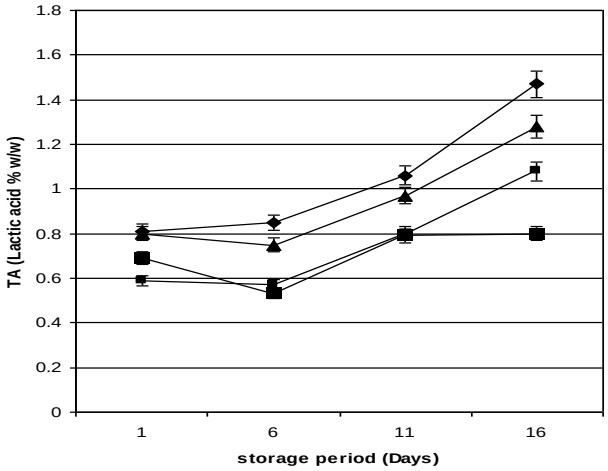

Figure 4: Changes in Titratable acidity in Yoghurts [◆] probiotic yoghurt [ ], Probiotic yoghurt with inulin [▲] and probiotic yoghurt with Arrow root carbohydrates [■] with time at 4 ^{o}C

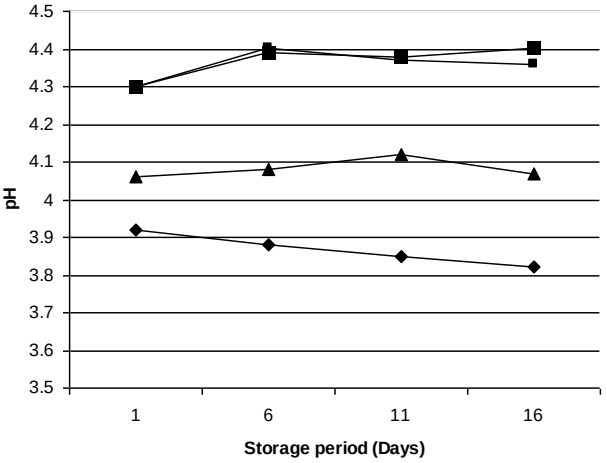

Figure 5: Changes in Titratable in Yoghurts [◆] probiotic yoghurt [■], Probiotic yoghurt with inulin [▲] and probiotic yoghurt with Arrow root carbohydrates [■] with time at 4 ^{o}C

Probiotic yoghurt with Arrowroot carbohydrate showed significantly lower ($P<0.05$) TA than the control at 16th day of storage whereas inclusion of Raftilose® ($P<0.05$) increased TA.

The effect of the duration of storage period on pH of yoghurt is not significant (P>0.05). This difference could be due to the degree of polymerization in fructo-oligosaccharides. It is possible that Arrowroot has higher degree of polymerization than Raftilose®. An increase of TA was found to be significant (P<0.05) after 6 days of storage for all four treatments. According to the SLS (1989) the levels of TA should lie between 0.8 and 1.25 lactic acid % (w/w) during their shelf-life. The, non-probiotic yoghurt samples reached maximum levels of SLS specification with respect to TA on the 13th day of refrigerated storage. Furthermore, all the samples that include probiotic bacteria showed lower (P<0.05) TA than non-probiotic yoghurt at 16th day of storage. According to the findings of Hekmat *et al*. (2008) most probiotic bacteria grow slowly in milk and the rate of acid production is usually too slow to support an adequate fermentation process in yoghurt. Standard yoghurt culture, *L. delbrukki ssp. bulgaricus* and *S. thermophilus* on the other hand, work together and cause an accelerated and efficient lactic acid production during fermentation process of yoghurt. However, incorporation of Arrowroot charbohydrate reduced (P<0.05) the development of TA compared to the control at 16th day of storage and may be used to extend the shelf-life of the bio-yoghurt.

Figure 6 shows the effect of incorporation of Arrowroot carbohydrates and Raftilose® on the sensory properties of yoghurt.

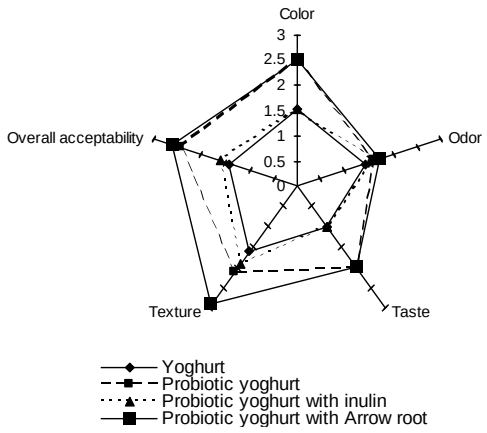

Figure 6: Sensory attributes of yoghurt samples

The sensory score presented in Figure 6 indicate that Raftilose® incorporated yoghurt was more acceptable than Arrowroot incorporated yoghurt. This is may be due to the nature of gelatinization in Arrowroot carbohydrates and it may lead to presence of granules in yoghurt. This result is similar to the findings of Shah (2007). He reported that yoghurt incorporating 2 % inulin had good overall acceptability. Panelists found that the samples tested to have similar odor. In terms of texture the highest scores (related to the presence of granules in the product) were given by the panelists to the yoghurt incorporating Arrowroot carbohydrate.

IV. CONCLUSION

The results of this study support that the incorporation of Arrowroot carbohydrate extract is a possible method for development of bio-yoghurt with enhanced survival of probiotic bacteria during prolonged cold storage. However, use of Arrowroot seems to negatively affect the smooth texture and taste of yoghurt possibly due to the presence of granules consisting of gelatinized carbohydrates and the low acidity of the product.

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