Enhancing the Survival of *Lactobacillus acidophilus* in Acidophilus Milk by Incorporating *Maranta arundinacea* (Arrowroot) Extract

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Introduction

Acidophilus milk is a traditional fermented beverage produced using *Lactobacillus acidophilus* as the starter culture. It has a mild sour taste (Amiri *et al*., 2010). *Lactobacillus acidophilus* is one of the probiotic bacteria used in the food industry. The potential health benefits associated with fermented products containing *Lactobacillus acidophilus* as dietary adjuncts are improved digestion of lactose, control of serum cholesterol, antagonistic action towards pathogens and control of certain intestinal cancers (Maria, 2007; Lampert, 1975). To produce desired benefits, there should be minimum of 10^{8} - 10^{9} CFU of probiotic bacteria /gram or mL of product at the time of consumption (Lourens-Hattingh *et al*., 2001). However, many studies have shown that the number of viable *Lactobacillus acidophilus* colonies decline with the storage time. This is one of the major limitations faced in the functional food industry. Incorporation of prebiotics such as fructooligosaccharide (FOS) can be used to enhance the survival of probiotic bacteria (Gibson and Roberfroid, 1995). Arrowroot (*Maranta arundinacea*) is a locally available rhizomatous herbaceous plant which contains 29.1 mg/g of fructooligosaccharides (Kaligayahan, 2009). Therefore, the extract of Arrowroot rhizomes may be used to enhance the survival of probiotic bacteria in dairy products. The objective of this study was to assess the effect of water soluble extract of Arrowroot on the survival of *Lactobacillus acidophilus* in acidophilus milk during refrigerated conditions.

Methodology

This study was conducted at Uva Wellassa University, Badulla, Sri Lanka. Matured arrowroot rhizomes were collected from home gardens of Badulla District. Within a day of harvest, yams were thoroughly washed with running water and cut into cubes (20-30 g). According to Maria *et al.* (2002), Extract of prebiotic compounds using water extraction under 40 ^{o}C and 70 ^{o}C. Arrowroot samples were grounded with water (1:2) at 27 °C in to a pulp. One sample was continuously stirred (100 rpm) for 20 minutes under 40 °C and the other sample was maintained under 70 °C for another 20 minutes. Then, they were filtered through three layered cheese cloth to remove suspended particles to obtain a clear extract. Similar to Ogunlakin *et al*. (2012), arrowroot powder was prepared by oven dry method using cleaned and washed Arrowroot cubes at 70°C for 4 hours. Then, they were ground into a powder and sieved.

The acidophilus milk was prepared using the method of Yildiz (2010) with some modifications. Standardized cow milk (fat 2.5 %, SNF 8.25%) was used to prepare acidophilus milk. Probiotic culture was prepared using a freeze dried lactic culture (La-5 , Chr. Hansen®, Denmark) which contained *Lactobacillus acidophilus* strain LA-5. There were five treatments according to the type of prebiotics; acidophilus milk with 3% (w/v) powdered Arrowroot rhizomes (T_1), acidophilus milk with 45% (v/v) Arrowroot extract (extracted at 70 °C) (T_2), acidophilus milk with 45% (v/v) of Arrowroot extract (extracted at 40 °C) (T_3), 2% (w/v) of inulin (Raftilose®, Sigma Aldrich, USA) (T_4) and the control sample without any prebiotics.

The best incorporation levels of prebiotics without affecting sensory properties of acidophilus milk were identified by preliminary trials. Arrowroot extract and inulin incorporated cow milk were heated to 95 °C for 8 minutes separately. Then they were cooled to 43 °C and inoculated with 10% (w/v) LA-5 probiotic culture which contains *Lactobacillus acidophilus*.

Mixture was dispensed into sterilized glass bottles and incubated at 37 °C for 18 h. Then the product was stored in a refrigerator at 4 °C for 24h. Viable counts of *Lactobacillus acidophilus*, pH and titratable acidity (TA) were measured at day 0, 7 and 14 of refrigerated storage (4 °C).

Acidophilus milk with highest viable cell count of *Lactobacillus acidophilus*, lowest TA and highest pH during storage was used to develop sweetened, flavored acidophilus milk. Several flavors were tested in preliminary trials and almond and lemon flavor were selected as the best to incorporate in Acidophilus milk. Paired-preference test was conducted using 20 untrained panelists to select the most preferred sample. Proximate composition was analyzed for crude fat, crude protein and dry matter and cost analysis was conducted for selected sweetened, flavored lactobacillus milk sample. Complete Randomized Design (CRD) was used for the experiment. Each treatment was triplicated. Data were analyzed by analysis of variance (ANOVA) followed by mean separation with Turkey's test using SAS (SAS institute Inc., Cary, NC, USA) at 0.05 level of significance. Non-parametric data were analyzed using two tailed binomial table.

Result and Discussion

According to the table 1, incorporation of Arrowroot extract (40 °C) showed highest population of *L. acidophilus* during day 0 of storage at 4 °C whereas Arrowroot extract (70 °C) showed least population of *L. acidophilus* at day 0 ($p<0.05$). Further, incorporation of arrowroot extract (extracted at 40 °C) increased ($p<0.05$) the viable cell count of *L. acidophilus* than the control at day 14 of refrigerated storage. This may be due to the higher solubility of Arrowroot FOS in water at 40 °C compared to 70 °C.

Table 1. Effect of prebiotics on the survival of *L. acidophilus* of storage condition (4 °C)

	Population (log CFU/mL)		
Treatments	Day 0	Day 7	Day 14
Control	6.81^{d}	6.00^{ab}	5.37^{bc}
Powdered Arrowroot rhizome (T_1)	8.01^{b}	7.65^{ab}	7.32^{a}
Arrowroot extract (70 °C) (T_2)	5.37^{c}	5.11^{b}	4.84^{c}
Arrowroot extract (40 °C) (T_3)	8.36^{a}	7.79^{a}	7.31^{a}
Inulin (Raftilose ®) (T_4)	7.45^{b}	6.57^{ab}	6.47^{ab}

^{a,b,c,d} Mean values in the same column with the different superscript letters are statistically different ($p<0.05$)

In general, minimum number of probiotic bacteria in dairy products at the time of consumption should be 7 log CFU/mL (Adhikari *et al*., 2000). Both powdered Arrowroot and its extract (40 °C) incorporated acidophilus milk showed higher levels of live probiotic bacteria than the recommended level until 14 th day of refrigerated storage and may exert a therapeutic effect on the host. The variation of TA in acidophilus milk is shown in Figure 1.

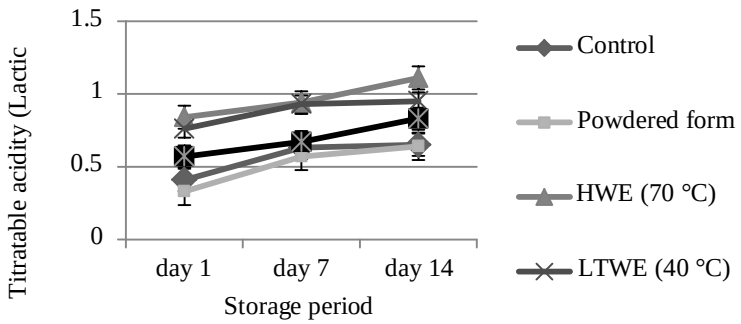

Figure 1. Changes of titratable acidity (TA) over storage period of 14 days at 4 °C

As shown in figure 1 TA of all five treatments increased with the storage period ($p<0.05$). According to Lampert (1975) *Lactobacillus acidophilus* does not multiply rapidly in milk and showed low rate of acid production. According to Codex Standards (2003) minimum TA of acidophilus milk should be 0.6 lactic acid % (w/w) and the TA of T_1 and control is lower than this. According to the Sri Lanka Standards specifications about set-yoghurts the maximum level of TA should be below 1.25 lactic acid % (w/w) and all five treatments showed lower TA recommended, during 14 days of storage. According to the results of sensory evaluation, panelist prefer lemon incorporated acidophilus milk over almond flavored acidophilus milk ($p<0.05$). The cost for 1 L of lemon flavored, sweetened acidophilus milk was RS 110.00.

Conclusions

According to the results, most desirable method to enhance the survival of *L. acidophilus* in acidophilus milk during refrigerated storage is the incorporation of 45% (v/v) Arrowroot extract obtained by water extraction method under 40 C. Lemon flavored acidophilus showed good consumer acceptance.

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