Jayampathi and Jayatilake, J Prob Health 2018, 6:1
DOI: 10.4172/2329-8901.1000199

Journal of Probiotics & Health

Research Article Open Access

Arrowroot (*Maranta arundinacea*) Extract Increases the Survival of Probiotic *Lactobacillus acidophilus*

Jayampathi T*and Jayatilake S

Department of Food Science and Technology, Wayamba University of Sri Lanka, Sri Lanka

***Corresponding author:** Jayampathi T, Department of Food Science and Technology, Wayamba University of Sri Lanka, Makandura, Gonawila, 60171, Sri Lanka, Tel: 0094766404873; E-mail: tjayampathi@wyb.ac.lk

Received date: May 10, 2018; **Accepted date:** May 24, 2018; **Published date:** May 31, 2018

Abstract

Probiotics are live microorganisms that benefit a host by sustaining healthy micro flora in the gut. However several factors including pH, metabolites and dissolved oxygen limit the growth and survival of probiotic organisms. Prebiotics are selectively fermented ingredients that may increase the survival and activity of probiotics. Arrowroot is rich in prebiotics and may increase the biomass of probiotics. This study was aimed at determining the prebiotic potential of water extractable carbohydrates of local yam arrowroot. Arrowroot carbohydrates were extracted by wet milling and recovery was 14.32% (w/w). The results of the proximate analysis revealed that the percentages of moisture, ash, protein and fat were 10.15%, 4.91%, 0.13% and 0.32% respectively. The contents of starch and low molecular weight carbohydrates were 79.65% and 1.93% respectively. No reducing sugars were detected. 12% (w/v) of arrowroot carbohydrates were incorporated into 12% skim milk and distilled water along with an inoculum of 10^9 colony forming units (CFU)/g of *Lactobacillus acidophilus* in order to test the growth performances. 12% skim milk was used as a control. Treatments were incubated at 37°C anaerobically for 12 h followed by storage at 4°C for 14 days. The pH, titrable acidity and colony counts of *L. acidophilus* were determined throughout the storage period. Media added with arrowroot gave a significantly ($p<0.05$) higher pH compared to the control although there was no significant difference ($p>0.05$) among the two media where arrowroot was added. Similar results were observed for titrable acidity as well. Results showed that arrowroot carbohydrates significantly ($p<0.05$) increased the CFU (log 10)/g of *L. acidophilus* compared to the control (6.71) in both distilled water (9.34) and skim milk (9.01) media. It was revealed that water extractable arrowroot carbohydrates shows significant prebiotic effect by maintaining a higher viability of probiotics.

Keywords: Prebiotic; Probiotic; Fermentation; *Lactobacillus acidophilus*; Arrowroot; Colony forming units; Fructooligosaccharides

Introduction

Probiotics are defined as live microorganisms which can beneficially affect an animal or human host by improving the indigenous microbiota [1]. They are claimed to provide health benefits such as anti-carcinogenic activity, improved nutritional value of foods, reduction of serum cholesterol levels and lactose intolerance, improving immune system and maintaining the balance of normal human intestinal microflora [2]. To be a probiotic organism it should be an inhabitant of normal human microflora and survive passage through the upper gastrointestinal tract [3]. For this the organism should be resistant to low gastric pH, bile salts, enzymes and metabolites produced during digestion [4].

There are several factors that affect the viability of probiotics of *Lactobacillus* sp. in dairy products such as probiotic strain used, pH, presence of dissolved oxygen, presence of hydrogen peroxide, concentration of metabolites such as lactic and acetic acids, medium buffering capacity, storage temperature, and nature of ingredients which added to the milk [5-8].

It is important to maintain the viability of probiotics to obtain health benefits. The viability of probiotic bacteria can be improved by using containers which are impermeable to oxygen, micro-encapsulation, selection of acid and bile resistant strains, stress adaptation, sonication of bacteria added to fermented products like yoghurt and incorporation of prebiotics and micronutrients such as peptides [9].

A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora like *Lactobacilli* and *Bifidobacteria* that confers benefits upon host well-being and health [10]. They are food ingredients that pass to the large intestine without being digested in the small intestine and act as substrates for growth of probiotics [11]. Prebiotics such as short chain carbohydrates [12] resist digestion by pancreatic and gut enzymes and passes to the large intestine where they are fermented to produce shortchain fatty acids [13].

Local yams like Arrowroot (*Maranta arundinacea*) are rich in soluble dietary fibre which acts as prebiotics [14]. It has been found that Arrowroot contains fructo-oligosaccharides [15], which can be used to obtain higher biomasses of probiotics and maintain their viability in fermented products [16]. Arrowroot carbohydrates extracts have also been shown to enhance the survival of probiotics in yoghurts [15]. Therefore this study is aimed at extraction of water soluble carbohydrates of arrowroot and investigation of its prebiotic effect on probiotic *Lactobacillus acidophilus*.

Materials and Methods

Extraction of arrowroot (*Maranta arundinacea*) carbohydrates

Water soluble carbohydrates of arrowroot were extracted by a wet milling process as shown in the Figure 1.

The same procedure was repeated with a single washing step of the ground arrowroot slurry. The recovery percentages of single washing and washing three times were compared and the method with the highest recovery percentage was used for the extraction of arrowroot carbohydrates.

Determination of composition of arrowroot extract

Moisture, ash, crude protein, crude fat and crude fiber contents of arrowroot carbohydrate extract were determined [17]. All experiments were done at least in triplicate and analytical results were expressed on a dry matter basis.

The starch content of extract was determined by measuring absorbance value of blue colored starch-iodine complex using a UV/Vis spectrophotometer (JP Selecta V-1100) at 680 nm [18]. The reducing sugar content of extracted arrowroot carbohydrates was determined using Lane and Eynon method [17]. Low molecular carbohydrates content was determined using a gravimetric method [17].

Preparation of cultures

The prebiotic organism *L. acidophilus* was isolated from freeze dried *Lactobacilli* culture (CHR Hansen ABT3 BB12) started in sterile milk by streak plating on *Lactobacillus* MRS agar (Oxoid LTD, UK) and incubating for 48 hours under anaerobic conditions. The Gram's stain [19], motility test [20], catalase test [21] and carbohydrate fermentation test [22] were used to confirm that the isolated organism was *L. acidophilus*.

Sample preparation and growth of *Lactobacilli*

12% (w/v) of arrowroot carbohydrates were incorporated into 12% skim milk (ASM) and distilled water (ADM) and 12% skim milk (SM) was used as a control. Media were homogenized at 85°C for 10 minutes followed by sterilization at 121°C for 15 minutes [23]. An inoculum of 1 g containing 10^9 CFU/g of *Lactobacillus acidophilus* was inoculated in three prepared broth media and incubated at 37°C for 12 hours. Fermented media were stored at 4°C and samples were drawn for analysis was withdrawn at 0, 3, 7, 10 and 14 days of storage.

Microbiological analysis

Colony counts for each medium were obtained at 12 hours of incubation after serial dilution and plating in triplicates using MRS agar incubated anaerobically at 37°C for 48 hours.

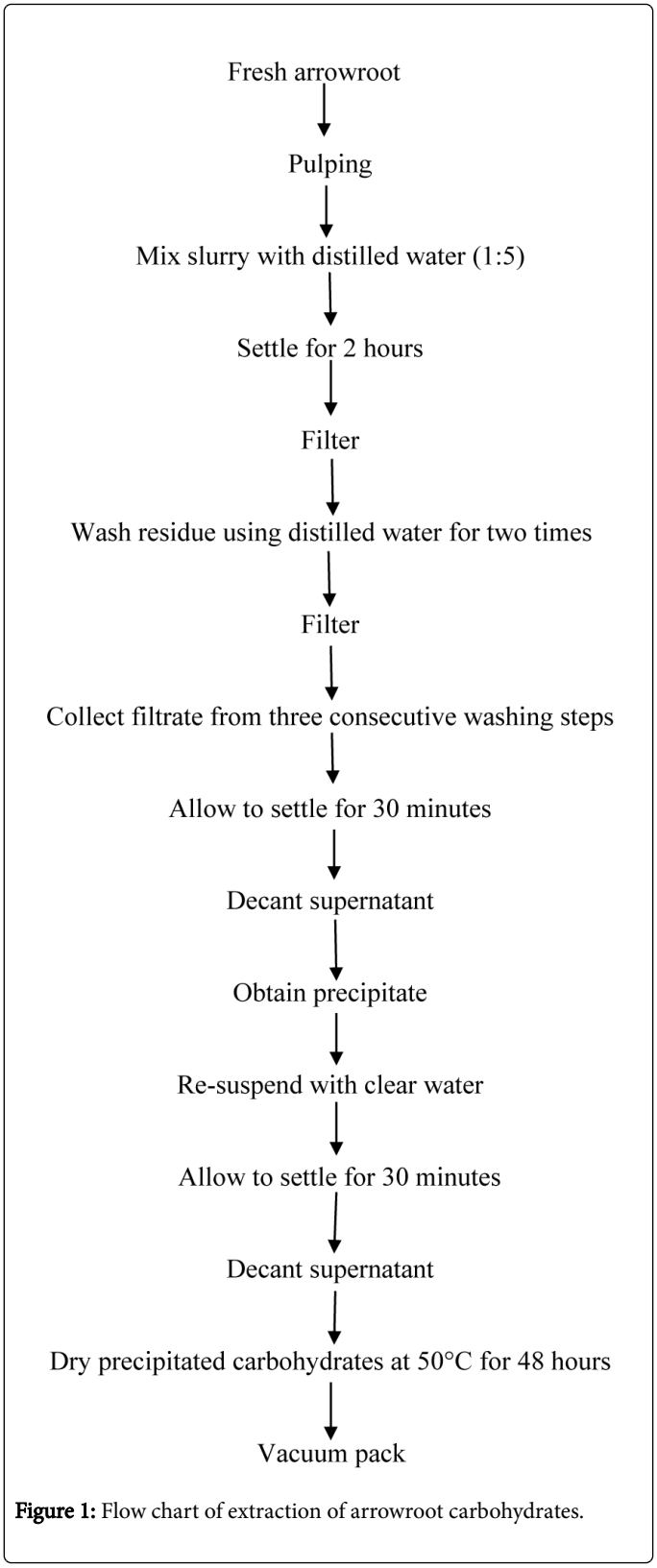

Figure 1: Flow chart of extraction of arrowroot carbohydrates.

pH and titratable acidity

The pH was determined using electronic pH meter (Thermo scientific, Orion star AIII) and titrable acidity was determined by titrating with 0.1N NaOH in the presence of 1% Phenolphthalein indicator [17].

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 at 0.05 level of significance using SPSS 16.0 software.

Results and Discussion

Arrowroot carbohydrates were extracted by two methods, one by washing ground arrowroot slurry for three times and other by washing for one time. The recovery percentages when two different methods were used are given in Table 1.

Treatment	Recovery %
Washing once	9.95 ± 0.1
Washing thrice	14.32 ± 0.2

Table 1: Recovery percentages of arrowroot carbohydrates. Values are mean ± standard deviation of triplicate analysis expressed in wet weight basis. Means were significantly different at $p<0.05$.

The recovery percentage was significantly higher ($p<0.05$) when washing step was repeated three times. Therefore it is considered to give a higher yield of water extractable arrowroot carbohydrates. This may be due to higher extraction of carbohydrates trapped in the fibre when washing for higher number of times. Similarly according to [15] Abesinghe et al. The recovery percentage of arrowroot carbohydrates was 9.6%. A study in Tifton and St. Vincent in West Indies showed that the extraction rates were 9.4% and 8-16% respectively [24].

The Tables 2 and 3 shows the composition of the arrowroot extract prepared by following three washing steps during the wet milling process.

Moisture%	Ash%	Protein%	Fat%
10.15 ± 0.1	4.91 ± 0.55	0.13 ± 0.01a	0.32 ± 0.03

Table 2: Non carbohydrate components of arrowroot extract. Values are mean ± standard deviation of triplicate analysis expressed in dry weight basis. Means were significantly different at $p<0.05$. [a]Nitrogen*6.25.

Starch %	Low molecular weight carbohydrate %	Reducing sugar %
79.56 ± 0.2	1.93 ± 0.03	nd

Table 3: Carbohydrate components in arrowroot extract. Values are mean ± standard deviation of triplicate analysis expressed in dry weight basis. Means were significantly different at $p<0.05$. nd=not detected.

The results of the proximate analysis showed the percentages of moisture, ash, protein and fat as 10.15%, 4.91%, 0.13%, and 0.32% respectively. The contents of starch and low molecular weight carbohydrates were 79.65% and 1.93% respectively. No reducing sugars were detected. However [15] Abesinghe et al. states that 78.7% (w/w) starch, 9.9% (w/w) moisture, 7.8% (w/w) polysaccharides and 3.6% (w/w) reducing sugars are found in extracted carbohydrates. [24] Erdman mentions that the content of protein, fat and ash in arrowroot carbohydrates extracted from arrowroot from Tifton were 0.12, 0.36 and 5.2 respectively and from St. Vincent were 0.27, 0.28 and 2.41 respectively. These differences may be due to the geographic differences, processing techniques and differences in variety maybe a cause for the different composition of extracted carbohydrates.

Changes of pH in growth medium

The highest pH of 4.65 ± 0.01 and least pH of 4.45 ± 0.01 were given by skim milk (control) after 14 days of storage at 4°C. Media added with arrowroot gave a significantly ($p<0.05$) higher pH compared to the control although there was no significant difference ($p>0.05$) among the two media where arrowroot was added. Table 4 shows that there has been a rapid decrease in the pH during the first three days followed by a gradual decrease till the 14th day in all three treatments.

		pH	
Number of days	**Arrowroot in Distilled Water (ADM)**	**Arrowroot in Skim Milk (ASM)**	**Control (SM)**
0	5.25 ± 0.02	5.32 ± 0.01	5.01 ± 0.02
3	4.72 ± 0.02	4.79 ± 0.01	4.54 ± 0.03
7	4.69 ± 0.01	4.67 ± 0.01	4.6 ± 0.02
10	4.67 ± 0.03	4.65 ± 0.02	4.52 ± 0.01
14	4.65 ± 0.01	4.58 ± 0.01	4.45 ± 0.01

Table 4: Change of pH in media during 4°C storage period.

Changes of titrable acidity (TA) in growth medium

The highest (1.77 ± 0.01%) and least TA (0.22 ± 0.01%) were recorded for skim milk (control) and 12% (w/v) arrowroot in distilled water respectively, after 14 days of storage at 4°C. There was a significant difference ($p<0.05$) between the TA of three treatments. However there was no significant difference between the TA of 12% (w/v) Arrowroot in Skim milk and 12% (w/v) skim milk as shown in Table 5. Similarly [16] Jayatilake et al. (2008) found that a significantly lower pH was maintained in media with arrowroot powder. In addition

[15] Abesinghe et al. also states that probiotic yoghurts added with arrowroot carbohydrates had a higher pH and lower titrable acidity.

		Titrable activity%	
Number of days	**Arrowroot in Distilled Water (ADM)**	**Arrowroot in Skim Milk (ASM)**	**Control (SM)**
0	0.09 ± 0.01	0.65 ± 0.01	0.72 ± 0.01
3	0.11 ± 0.01	1.37 ± 0.01	1.44 ± 0.02
7	0.14 ± 0.01	1.42 ± 0.02	1.64 ± 0.02
10	0.20 ± 0.01	1.44 ± 0.01	1.68 ± 0.01
14	0.22 ± 0.01	1.75 ± 0.01	1.77 ± 0.01

Table 5: Change of titrable acidity in media during 4°C storage period.

Changes of lactic acid bacteria (LAB) counts in growth medium

There was a significantly higher ($p<0.05$) count of *Lactobacillus acidophilus* in both media where arrowroot carbohydrates were added. Table 6 shows that there has been a decrease in the counts of *Lactobacillus acidophilus* in all three treatments during the storage The LAB count of 12% (w/v) Arrowroot in distilled water varied from 10.0^8 CFU/g to 9.34 CFU/g during the 14 days storage period while 12% (w/v) Arrowroot in skim milk had counts varying from 9.99 CFU/g to 9.01 CFU/g. on the other hand there was rather lower count of LAB ranging from 8.88 CFU/g to 6.71 CFU/g in the12% (w/v) skim milk. This shows that arrowroot carbohydrates show a significant prebiotic effect and increase the survival of LAB in fermented products. Similarly [16] Jayatilake et al. has shown that arrowroot powder increases the survival of *Lactobacillus acidophilus*. In addition [14] Mary et al. found that arrowroot powder increases the survival of *Lactobacillus curvatus* in fermented products. Also arrowroot carbohydrates increased the survival of probiotic bacteria in bio-yoghurts [15]. However, the test results showed a gradual reduction of the LAB during the storage period. This is due to lactic acid fermentation where colonies may be destroyed during storage due to hydrogen peroxide production, post acidification and permeation of oxygen [16].

Lactobacillus acidophilus counts (log 10) CFU/g			
Number of days	**Arrowroot in Distilled Water (ADM)**	**Arrowroot in Skim Milk (ASM)**	**Control (SM)**
0	10.08	9.99	8.88
3	9.86	9.88	8.75
7	9.69	9.67	8.11
10	9.51	9.32	7.23
14	9.34	9.01	6.71

Table 6: Change of *L. acidophilus* in media during 4°C storage period.

Conclusion

The results of this study support that the incorporation of arrowroot carbohydrate extracts reduce the titrable acidity, increase the pH and gives higher biomass of probiotics. Thus it can be concluded that arrowroot extracts possess prebiotic potential and could be applied in maintaining greater than 10^6 CFU/g for *Lactobacillus acidophilus* during storage of fermented products.

Conflicts of Interest

All authors declare that there are no conflicts of interest regarding the publication of this paper.

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