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Evaluation of efficacy of Arrowroot (Marantaarundinacea) extract incorporated synbiotic ice-cream

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Abstract--*Arrowroot (Marantaarundinacea) is astarchy root which stated to comprisesignificantquantity of Fructooligosaccahrides in their extract. Due to the high production costs of commercial prebiotics, arrowroot extract has a potential to develop as a low cost, reliable, sustainable prebiotic source for the functional food industry. Therefore, this study was done to assesstheprebiotic activity of arrowroot extract and to assesstheLactobacillus cell viability in arrowroot extract incorporated synbiotic ice-cream during frozen storage. Based on the results, the highest prebiotic activity was given by the arrowroot extract compared to inulin and glucose. The prebiotic activity of arrowroot extract and inulin were significantly higher than the prebiotic activity of glucose ($p<0.05$), but the prebiotic activity of arrowroot extract and inulin were not significantly different from each other ($p>0.05$). The probiotic cell viability is more or less same in sample (arrowroot) and reference (inulin) ice-creams, the lowest probiotic cell viability was recorded in the control ice-cream, but there is no any significant difference in the probiotic cell viabilities in all three ice-cream types during frozen storage ($p>0.05$). According to the physiochemical results, there is a significant difference in total soluble solids (TSS) and titratable acidity (TA) between control and reference ice creams ($p<0.05$), but there is no significant difference in TSS and TA between sample and reference ice-creams ($p>0.05$). Therefore, the prebiotic activity results concluded that there is no significant difference in prebiotic activities of arrowroot extract and inulin. It shows that arrowroot extract has a potential to replace commercial prebiotics like inulin. Probiotic viability results concluded that the prebiotics incorporated ice-creams are having higher cell viabilities than control ice-cream. It indicates that the prebiotic substances had helped probiotics to survive during harsh frozen storage conditions.*

Keywords-- *Efficacy, arrowroot, synbiotic Ice-cream*

I INTRODUCTION

The term probiotic is defined as “live microorganism which when administered in adequate amounts confer a health benefit on the host (Durand, 2010) and prebiotic is specified as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of the gastrointestinal micro-flora and thus improves the host health” (Roberfroid, 2007). The probiotic bacteria cannot thrive well in digestive tract without prebiotics (Cruz *et al.,* 2009). The combination of probiotics with prebiotics has a synergistic effect on the host health (Wang, 2009).The therapeutic properties of probiotic microorganisms have

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been stated in numerous studies in the resulting terms: (a) controlling the intestinal flora; (b) improving lactose tolerance and breakdown; (c) dropping cholesterol heights; (d) production of B complex vitamins; (d) captivation of calcium; (e) modifying the immunological structure (Gibson, 2004).

Lactobacilli and Bifidobacteria are the intestinal microorganisms that are encouraged by prebiotics and some strains of these microorganisms have been incorporated to foods as probiotic microbes. In addition, prebiotics are gradually incorporated to yogurt and other fermented dairy foodstuffs, as well as a vast range of other products. Ultimately, it is the capability of prebiotics to survive food processing environments that permits for these prebiotic carbohydrates to reach the colon undamaged, causing the selective enhancement of colonic bacteria by giving these bacteria with a competitive advantage (Wang, 2009). Consequently, prebiotics alone or joined with probiotic bacteria in the arrangement of synbiotics are known to have the ability to influence and develop the gastrointestinal wellbeing of humans (Gibson, 2004).As stated by Huebner *et al.,* (2008), the prebiotic activity reveals the capability of a given substrate to aid the development of an organism comparative to other organisms and comparative to development on a non-prebiotic substrate, such as glucose (Viana *et al.,* 2008).In food preparations, they can considerably increase organoleptic properties, upgrading both taste and mouthfeel (Viana *et al.,* 2008). For prebiotics to work as functional food constituents, they must be chemically stable to food processing actions, such as heat, low pH, and Maillard reactions (Huebner *et al.,* 2008).

Most prebiotics and prebiotic derivatives identified today are non-digestible oligosaccharides. They are acquired either by extraction from plants (e.g., chicory inulin), or by production (by trans-glycosylation reactions) from mono- or disaccharides such as sucrose(fructooligosaccharides) or lactose (galactooligosaccharides) (Roberfroid, 2007). Within these prebiotics, inulin and oligosaccharides are the most studied prebiotics and have been familiar as dietary fibers in most countries (Roberfroid, 2007). The use of inulin and non-digestible oligosaccharides as fiber constituents is direct and frequently leads to better taste and texture. These precise types of dietary fiber are freely fermentable by specific colonic bacteria, such as Bifidobacteria and Lactobacilli species, developing their cell densities with the parallel formation of short-chain fatty acids (Roberfroid, 2000).

The tropical belt, inhabited a number of starch containing plants that include cereals, fruits, and vegetable crops, and most important the root crops (Viana *et al.,* 2008). Starchy roots and tuber crops are vital sources of animal feed and processed foodstuffs for human consumption and industrial use (Wang, 2009). The comparative importance of these crops is obvious through their annual worldwide harvest which is approximately 836 million tonnes. Asia is the key producer. Asian and African areas created 43% and 33%, respectively, of the worldwide production of roots and tubers (Chandrasekara & Josheph, 2016).

Regular consumption of nutritious food such as starchy vegetables, non-starchy vegetables, fruits, legumes, nuts, fish, etc. reduces the risk of diet related non-communicable diseases like obesity, type 2 diabetes, cardiovascular diseases, hypertension, strokes and cancers which are rapidly increasing around the world due to increased consumption of fast foods, empty calorie foods and sweetened beverages (Huebner *et al.,* 2008). Therefore modern society tends to seek more healthy eating patterns and also functional foods play an exciting role in modern food industries and nutrition field (Cruz *et al.,* 2009).

Arrowroot (*Marantaarundinaceae* L.) belongs to Marantaceae family and is a big perennial herb found in tropical woodlands. The plant is adapted in Florida, but is cultivated mostly in the West Indies, Australia,

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Southeast Asia and South and East Africa (Nogueira *et al.,* 2018). Economically, arrowroot has been used specially for starch production, due to the great starch amount in its rhizomes (Nogueira *et al.,* 2018). Even though the arrowroots are locally available, they are underutilized. Astuti *et al.,* (2018) stated that the arrowroot is a rich source of fructooligosaccarides, but only few studies have been conducted to evaluate the potential use of arrowroot extract as a prebiotic ingredient in the modern functional food industry. Therefore, the main objective of this study is to evaluate the prebiotic activity of arrowroot extract in order to reveal its prebiotic properties to the development of various functional foods through modern functional food industry. Additionally, this study may have focused in the evaluation of probiotic cell viability during frozen storage of arrowroot extract incorporated synbiotic ice cream.

II MATERIALS AND METHODS

This research was carried out in the preservation laboratory and microbiology laboratory in the Department of Food Science and Technology of Wayamba University of Sri Lanka (Manakdura, Gonawila, Sri Lanka). Kothmale fresh milk, Sera real coconut milk, Anchor whipping cream, Motha gelatin, Delmage vanilla essence, Bonlac skim milk powder and White sugar were purchased from super market at Pannala, Kurunegala District, North Western Province, Sri Lanka. The ice-cream stabilizer Cremadon 30 was purchased from local market shop at Pettah, Colombo District, Western Province, Sri Lanka. The probiotic yogurt culture (ABY-3) was purchased from J.L. Morison Son & Jones (Ceylon) PLC, 620, Biyagama Road, Pethiyagoda, Kelaniya, Gampaha District, Western Province, Sri Lanka. The arrowroots were purchased from local farmer's market at Batuwatta, Gampaha District, Western Province, Sri Lanka. Commercial Inulin (Orafti90), MRS agar (Himedia M641) and MRS broth (Himedia M369) were provided by the microbiology laboratory, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka.

2.1. Extraction of prebiotics from raw arrowroots

Prebiotics were extracted from raw arrowroots according to a previously established method with some slight modifications (Abesinghe *et al.,* 2012).

The purchased raw arrowroots were sorted out to remove any spoiled arrowroots. The remaining arrowroots were washed thoroughly using ordinary tap water to remove mud, soil and any other unwanted materials. Then the washed arrowroots were cut into small pieces. Then those small arrowroot pieces were grounded into a mash using ordinary kitchen blender. After that, the raw arrowroot mash was mixed with distilled water in a ratio of 1:5 (arrowroot mash: distilled water). Then the mixture was allowed to settle for 30 minutes. Then the mixture was filtered out to remove the remaining particles of arrowroots and allowed the filtrate to settle for another 1 hour. After 1 hour, supernatant was removed and precipitate was washed slowly using a slow current of clean water without disturbing it. Finally, the white color precipitate was separated from the container and dried at 50 ^{o}C for 24-36 hours. Then The dried precipitate was blended usingto obtain a fine powder. Finally, the fine powder was vacuum packed and stored at refrigerated conditions (4 ^{o}C).

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2.2. Incubation of probiotics

Conical flask (250 mL) was filled with 100 mL of water and sterilized. Then Skim milk powder 13 g was added and dissolved well to prepare 13% Skim milk solution. Probiotic yogurt culture (0.1 g) was accurately weighed and incorporated into 13% Skim milk solution and conical flask was sealed with the cotton plug. Prepared probiotic solution was incubated for 16 hours at 37 ^{o}C(Huebner *et al.,* 2007).

2.3. Preparation of coconut milk substituted synbioticice-creams

Coconut milk substituted synbiotic ice-creams were prepared according to a previous study (unpublished). A control ice-cream (without any addition of prebiotics), sample ice-cream (by addition of Arrowroot extract) and reference ice-cream (by addition of commercial prebiotic inulin) were prepared and stored under freezing conditions for further analysis.

Fresh milk and coconut milk were mixed according to 60:40 ratios respectively. This was the initial mixture. All the ingredients were measured for 100 ml of initial mixture. White sugar (23 g) was added into the initial mixture and dissolved well by beating. Then vanilla (2 drops) was added and beat for 2 minutes. After that whipping cream (16 mL) was added and continuously beat. While beating gelatin (1g) was dissolved in initial mixture (2 mL initial mixture/1 g gelatin) at80 ^{o}C. The dissolved gelatin was added into the ice-cream mixture and beat for 2 minutes. After that Cremadon 30 (0.5 g) was added into the ice-cream mixture and beat for another 2 minutes. Then the final mixture was kept in the freezing temperature (-18 ^{o}C) for 30 minutes. After 30 minutes' mixture was taken out and beat for 10 minutes. This same step was carried out for five times with intermittent freezing. Finally, ice-cream mixture was allowed to freeze (-18 ^{o}C) for 12 hours and stored at same temperature for the further analysis.

For the preparation of control ice-cream 10 mL of previously incubated (16 hours in 13% skim milk solution) probiotic culture per 100 mL of initial mixture was added to ice-cream mixture at the last beating during intermittent freezing.For the preparation of sample ice-cream 10 mL of previously incubated (16 hours in 13% skim milk solution) probiotic culture per 100 mL of initial mixture and 7% (w/v) Arrowroot extract were added to ice-cream mixture during the beating with intermittent freezing.

For the preparation of reference ice-cream 10 mL of previously incubated (16 hours in 13% skim milk solution) probiotic culture per 100 mL of initial mixture and calculated amount of commercial prebiotic (with the equivalent amount of prebiotic matter as Arrowroot extract) were added to ice-cream mixture during the beating with intermittent freezing.

2.4. Overrun

Overrun was calculated using following equation

$$overrun = \frac{(final\ volume - initial\ volume)}{initial\ volume} \times 100$$

2.5. Determination of total soluble solids (TSS)

The total soluble solids were measured as Brix using Brix meter (ATAGO – Japan) (Ministry of Health and Family Welfare Government of India, 2015).

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2.6. Determination of titrable acidity (TA)

Acidity of samples were determined according to titrationmethod and basedon lactic acid concentration. Two grams of ice cream sample was diluted with 25 mL of distilled water and titrated against 0.1247M NaOH in presence of phenolphthalein indicator (Ministry of Health and Family Welfare Government of India, 2015).

2.7. Determination of pH

The pH of ice-cream samples was measured by using pH meter (OHAUS-USA) which has been calibrated to pH 4.0 and 7.0 using standard solutions (Ministry of Health and Family Welfare Government of India, 2015).

2.8.Prebiotic activity assay

The prebiotic activity assay was performed according to a previously describe method with some slight modifications (Huebner *et al.,* 2007).

For the prebiotic activity assay freeze dried probiotic culture (0.1-1.0 g) was incubated in MRS broth for 24 hours at 37 ^{o}C. After the incubation streak plate was prepared in MRS agar and incubate for 48 hours at 37 ^{o}C to isolate *Lactobacillus* pure colonies. After obtaining *Lactobacillus* pure colonies, small inoculum was inoculated into 10 mL MRS broth and incubate for overnight at 37 ^{o}C.After that culture tubes were prepared by adding 1% (wt/v) Glucose, 1% (wt/v) Arrowroot extract and 1% (wt/v) commercial prebiotic (Inulin) into 10 mL MRS broth. Then 1% (v/v) overnight incubated *Lactobacillus* culture was added into those same culture tubes. After preparation, culture tubes were incubated for 0 hr/ 24 hr/ 48 hr and 72 hours at 37 ^{o}C. The colony forming units (CFU) were counted and recorded by preparing spread plates (triplicates) for all three culture tubes in MRS agar for each time period. (CFU were counted using a colony counter).

2.9.Probiotic viability assay during frozen storage of ice-creams

Probiotic viability assay was performed according to a previously describe method with some slight modifications (Cagetti *et al.,* 2013)).

Samples (1.0 mL) from all three previously prepared ice-creams (control/sample/reference) were added separately to 9.0 mL of sterilized distilled water containing dilution tubes. Then appropriate dilution series were prepared separately (from 10^{-1} to 10^{-8}). After that CFU were counted and recorded for each three samples by preparing spread plates (triplicates) (from relevant dilutions) in MRS agar (incubate at 37 ^{o}C for 48 hours). This assay was performed during frozen storage period with two weeks' intervals. The CFU were counted using a colony counter.

2.10 Invitro simulated gastrointestinal tolerance assay for Lactobacillus sp. in ice-cream samples

Ice creams were tested for possible effect of prebiotic (Arrowroot extract) on the cell viability of *Lactobacillus sp.* under simulated gastrointestinal conditions. Ice-cream samples were analyzed after 1,13,25,41 and 55 days of frozen storage at -18 ^{o}C. The tolerance of *Lactobacillus sp.*to *invitro* simulated gastric and enteric conditions was performed according to the method described by (F. C A Buriti, Castro, & Saad, 2010) with minor modifications.

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2.11.Statistical analysis

The experimental results were expressed as mean ± standard deviation of at least three replicates. Data were analyzed by factorial one-way ANOVA with multiple mean comparisons (LSD) using SPSS16 software at a level of significance ofp< .05.

III RESULTS AND DISCUSSION

For the sample plan three ice-creams were prepared, control (without addition of prebiotics), sample (with addition of arrowroot extract) and reference (with additionof inulin). Physiochemical and microbiological analysis were done throughout the frozen storage period of 55 days.

3.1.Evaluation of physiochemical parameters

3.1.1.Change of overrun in ice-creams

Table 1: Overrun values of ice-cream samples (mean±standard deviation)

Ice-cream type	Overrun value
Control	30%±0.57[a]
Sample (arrowroot extract incorporated)	35%±0.43[a]
Reference (inulin incorporated)	47%±0.23[b]

Means within a column superscripted by same letter are not significantly different at $p>0.05$.

Overrun is the process where air incorporation takes place during ice-cream processing. These results increase in volume, creamy airy texture of a particular ice-cream. Commercial ice-creams tend to have 75% - 100% overrun during processing (Akalin & Erişir, 2008). Due to the hand beating method, the resulted overrun values are comparatively lesser than normal commercial ice-creams (Table 1). Control ice-cream was having the least overrun value compared to prebiotics incorporated ice-creams. Inulin incorporated ice-cream was having the highest overrun value among all three ice-creams. Akin, (2005) stated that the overrun value was double, when inulin was incorporated to ice-cream mix compared to other prebiotics. And also, the same study was found that there is no any significant effect from probiotics to the overrun of a particular ice-cream. According to the statistical analysis, there is no any significant difference between mean overrun values of control and sample ice-creams ($p>0.05$), but there is a significant difference in mean overrun value of reference ice-cream with other two ($p<0.05$).

Due to the higher overrun values, the air incorporation also high in reference ice-cream. This will directly affect to the probiotic (*Lactobacillus*) cellsurvivability in reference ice-cream compared to sample ice-cream during frozen storage (Figure 5). The high air concentration may create a toxicity to the probiotics which are facultative anaerobes or micro-aerophiles. This high air concentration will lead probiotic cells towards their death resulting less cell viability.

3.1.2.Change of total soluble solids (TSS) in ice-cream samples during frozen storage period

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Index of refraction used to determine the total soluble solids (TSS) in a solution (food) and the value would be given as degrees of Brix. Refractometer is the equipment used in the TSS calculation (Sun-Waterhouse *et al.,* 2013).Soluble sugars are the major contributors to the Brix value of a respective solution (food). Brix value measures the TSS percentage in a solution (food) per 100 grams (El-Nagar *et al.,* 2002).

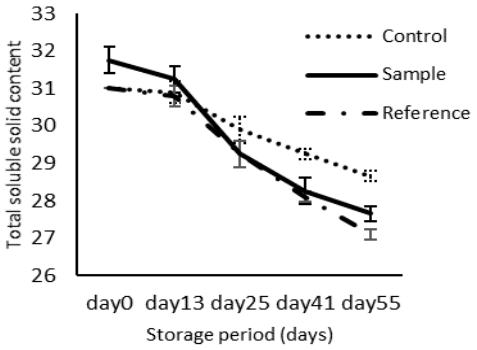

Figure 1: Change of total soluble solid content in prepared ice-creams during frozenstorage of

According to the graph (Figure 1), there is a gradual decrement of TSS in all three types of ice-cream during frozen storage period. At the beginning of the graph (0^{th} and 13^{th} day), the arrowroot extract incorporated ice-cream (sample) indicated the highest mean (31.75±0.35/31.25±0.35) for the TSS. This may be due to the incorporation of arrowroot starch and soluble sugar fractions with the arrowroot extract (fructooligosaccharide fraction of arrowroot extract usually would be 55% (Abesinghe *et al.,* 2012), other fraction would be impurities like arrowroot starch, soluble proteins, soluble sugars etc.), but at the end of the graph (55^{th} day), control ice-cream indicated the highest mean (28.65±0.21) value for the TSS. Reason for the gradual decrement of TSS during frozen storage would be the probiotic microbial activities. Even though the ice-creams are stored under frozen conditions, microbial metabolic activities can be happened. During their metabolic activities probiotic microorganisms may utilize available soluble sugars as their energy sources. In control ice- cream, there is a less probiotic cell survivability and activity due to lack of prebiotics, this may be the reason to observe high TSS value at the end of the storage time period. In arrowroot extract incorporated (sample) and inulin (commercialprebiotic/Orafti90) incorporated (reference) ice creams, probiotic cell survivability and activity tend to high due the effect of prebiotics. Therefore, it would be the reason to low TSS values in sample and reference ice-creams at the end of the storage time period.

Considering the statistical analysis, there is a significant difference in the change of TSS of ice- creams during frozen storage period ($p<0.05$). When considering multiple mean comparisons, control and sample ice-creams would not show any significant difference in change of TSS during storage period ($p>0.05$). The reference and sample ice creams show the same statistical results as control and sample ice-creams, but control and reference ice creams show statistically significant difference in change in TSS during frozen storage period ($p<0.05$).

3.1.3.Change of titratable acidity (TA) in ice-cream samples during frozen storage period

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Titratable acidity (TA) is the total amount of acid or an approximation of total acidity in a solution which is

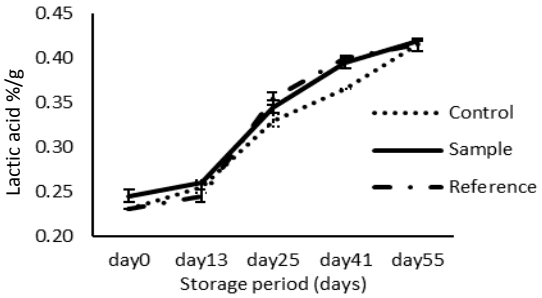

Figure 2: Change of titratable acidity of prepared ice-cream samples during frozen storage of 55 days

determined by a titration using a standard solution (titrant). Typically this titrant would be sodium hydroxide (NaOH) (Akin, 2007). During determination of TA in a food sample, the acids get neutralized by the standard base. The end point of the titration would be either a target pH or a color change of a pH sensitive dye known as pH indicator, typically this indicator would be phenolphthalein (Homayouni, 2008)**.** In dairy base products, especially dairy based probiotic food products, the prominent organic acid would be lactic acid due to lactose present in the milk and lactic acid bacteria present in the probiotic cultures (Homayouni *et al.,* 2008).

When observing the graph (Figure 2) a gradual increment of TA (lactic acid amount) in all three types of ice-creams (control/sample/reference) could be seen during frozen storage period.The variation in TA between sample and reference ice-creams tend to be more or less same, but control ice-cream shows relatively low variation than other two types. At the beginning of the graph (0^{th} day), the highest mean value (0.25±0.01%) was obtained by the sample ice-cream. This may be due the non-homogenization during addition of overnight incubated probiotic bacterial cultures (subcultures). This subculture might already contain some amount of lactic acid due to lactic acid fermentation during incubation. This initial lactic acid may not homogenize throughout whole sample ice-cream due to hand mixing. At the end of the graph (55^{th} day), mean TA values became more or less same in all three types of ice-creams.

According to the statistical analysis, there is a significant difference in change in TA between three types of ice-creams during frozen storage period ($p<0.05$). When considering the multiple mean comparisons, control/sample and control/reference ice-creams indicate significant difference in TA between each pair ($p<0.05$). The control and sample ice-creams show the highest significant difference in TA according to the statistical analysis, but sample and reference ice-creams did not show significant difference in TA according to the multiple mean comparisons results ($p>0.05$).

3.1.4.Change of pH in ice-cream samples during frozen storage period

pH is a measurement of Hydrogen ion (H^+) concentration in a solution. In other hand pH is determined by the available free H^+ ions in a solution. Solutions (food) containing high amount of H^+ ions will have a low pH and solutions containing less amount of H^+ ions will have a high pH value. Therefore, the relationship between pH and H^+ concentration seem to have a negative relationship. Other than that, pH is normally affected by the relevant temperature of the solution (food). The following equation is used to calculate the pH value of a given solution,

$$pH = -\log[H+]\,concentration$$

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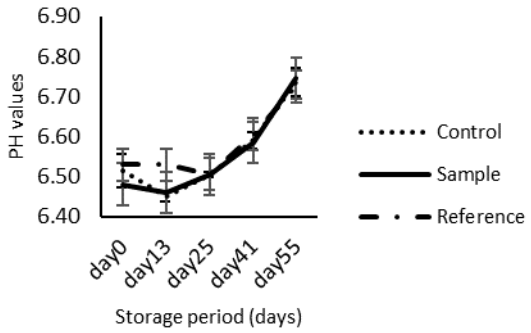

Figure 3: Change of pH values in prepared ice-cream

As shown in the graph (Figure 3), there is a gradual increment of pH in all three types of ice-creams (control/sample/reference) during frozen storage period, but the increment is in a very low rage (from about pH 6.5 to 6.75). The total increment difference is about 0.25 points in pH scale. Initially (0^{th} day), the control and reference ice-creams have more or less same mean pH values (6.51±0.00/6.53±0.04) which are higher than the sample mean pH value (6.48±0.04). At end of the storage period (55^{th} day) mean pH value of all three ice-creams reach more or less same value (about pH 6.72). Variation in mean pH values from 13^{th} day to 55^{th} day tend to have a same pattern in all three types of ice creams.

According to the statistical analysis, there is no significant difference in change in pH of all three types of ice-creams during frozen storage period ($p>0.05$).

3.2.Prebiotic activity assay

According to the definition, prebiotics (prebiotic carbohydrates/ food materials) can only be metabolized by a selected number of gastrointestinal microorganisms (Marcel Roberfroid, 2007)and also these carbohydrates have an ability to affect the microbial population with in the gastrointestinal tract due to their selective utilization by specific microbial strains (Huebner *et al.*,2008**).** Therefore, the prebiotic activity can be elaborated as an ability of a prebiotic substrate to support the probiotic microorganisms in their survivability, growth and development with compared to a non-prebiotic substrate like glucose (Fooks & Gibson, 2002).

Table 2: Increase in cell density of probiotics (*Lactobacillus sp.*) during 0 - 72 hours of incubation reported as $\log_{10}$cfu mL^{-1} ±sd

Incubation time (hours)	Treatment		
	Glucose	Arrowroot extract	Inulin
0hr	3.68±0.30	3.68±0.33	3.63±0.25
24hr	4.61±0.23	5.47±0.28	5.62±0.27
48hr	5.45±0.37	6.67±0.29	6.45±0.27
72hr	5.69±0.33	8.41±0.24	7.55±0.25

sd: standard deviation

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Therefore, in this study, the prebiotic activity assay was designed to evaluate the prebiotic activity of arrowroot extract (which was rich in fructooligosaccharides) with compared to inulin and glucose. Various strategies and measurements can be used to determine the prebiotic activity of a given substrate. Changes in bacterial populations (cell densities), substrate assimilation, growth rates, byproducts formation (short chain fatty acid) would be some of the measurements in prebiotic activity evaluation (Vulevic, 2004). Compared to other methods, this study method was comparatively simple, because in this study, change in cell population of *Lactobacillus* sp. were measured by preparing spread plates on MRS agar. The variations in cell population on prebiotics were measured compared to non-prebiotic glucose. This would be a quick way to evaluate the prebiotic ability of a given substrate due to low incubation periods.

According to graph **(**Figure 4) there is a gradual increment in cell population of *Lactobacillus sp.* during incubation time period. Arrowroot extract had recorded the highest mean (log_{10} cfu mL^{-1}) value 8.41±0.24 after 72 hours of incubation with compared to the mean values of inulin (7.55±0.25) and glucose (5.69±0.33). The

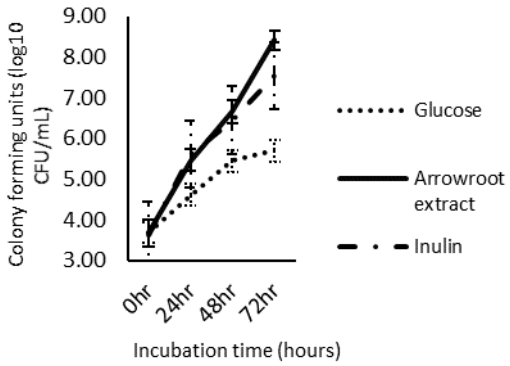

Figure 4: Change in cell populations of Lactobacillus sp. during 0-72 hours of incubation with relevant substrates

lowest mean value (5.69±0.33) for the cell population was recorded by the non-prebiotic glucose. According to

the previous studies, commercial prebiotics like purified inulin and purified FOS had shown greatest prebiotic activity values with compared to other substrates(Huebner *et al.,* 2007). And also some studies had proven that purified FOS are having even greater prebiotic activities than purified inulin (Olano-Martin, 2002). This study results also support for those studies by reporting the highest prebiotic activity value for the arrowroot extract which was rich in fructooligosaccharides. Arrowroot extract was not a purified form of fructooligosaccharides, but roughly it contains about 55% FOS in dry weight (Abesinghe *et al.,* 2012). The other 45% would be soluble starch materials, proteins, lipid components etc. Those residue materials might support probiotics (*lactobacillus sp.*) in their growth and development with compared to purified inulin and glucose. This would be the indirect reason for the recorded highest prebiotic activity mean values in arrowroot extract.

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Statistical analysis results show that there is a significant difference in prebiotic activities between glucose/ arrowroot extract and glucose/ inulin ($p<0.05$), but there is no any significant difference between arrowroot extract and inulin ($p>0.05$). Therefore, statistically proven that, the unpurified arrowroot extract and purified commercial inulin are having more or less same prebiotic activities compared to non-prebiotic glucose. Therefore, arrowroot extract is having a great potential to develop as a low cost, sustainable, reliable prebiotic source in the functional food and commercial prebiotics industry.

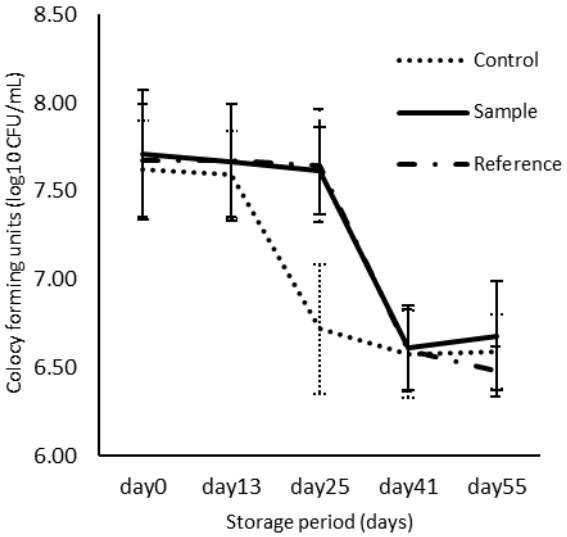

Figure 5: Survivability of probiotic (Lactobacillus sp.) populations in ice-creams during frozen storage of 55 days

However, it is better to understand, that some pathogenic commensal floral microorganisms like *E-coli* has an ability to ferment prebiotic compounds (Fooks & Gibson, 2002). Therefore, to get an elaborated prebiotic activity of arrowroot extract, it is required to observe the activity of pathogenic enteric bacteria like *E-coli* with the presence of arrowroot extract. And also, it would be better to expand this study towards other important probiotics like Bifidobacteria. With all these necessary observations, it would be easier to create a full elaborated picture of prebiotic activity of arrowroot extract which would be a precious finding for the functional food industry.

3.3.Probiotic viability assay

Table 3: Probiotic (*Lactobacillus sp.*) cell survivability during frozen storage of 55 days reported as $\log_{10}$cfu mL^{-1} ±sd

Storage period (days)	Treatment		
	Control	Sample	Reference
0^{th} day	7.62±0.28	7.71±0.37	7.68±0.32
13^{th} day	7.60±0.25	7.67±0.33	7.68±0.32
25^{th} day	6.72±0.37	7.62±0.25	7.65±0.32
41^{th} day	6.58±0.25	6.61±0.24	6.60±0.23
55^{th} day	6.69±0.21	6.68±0.31	6.48±0.14

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sd: standard deviation

Ice-cream is a novel product in relation to the probiotics and prebiotics. Generally probiotics and prebiotics involve in the functional dairy products like fermented probiotic drinks, probiotic and synbiotic yogurt etc. (Marcel Roberfroid, 2007). Synbiotics are the food products which contain both prebiotics and probiotics, in order to gain synergistic effect after consumption of the products (Homayouni *et al.,* 2008). Ice-cream would be a novel challenge in the case of survivability of probiotics and functional ability of the final product. In order to a product to be a functional, there must be at least 10^6-10^7cfu mL^{-1} probiotic viable counts at the time of consumption of a particular product (Di Criscio *et al.,* 2010). In this study, the initial viable counts of all three ice-creams (control/ sample/ reference) are in a range of 10^7cfu mL^{-1}. And also, after 55 days of frozen storage, still the viable counts are in a range of 10^6cfu mL^{-1} (Figure 5).Therefore, even after 55 days of frozestorage, these three ice-creams can be called as functional.

According to Figure 5there is a gradual decrement in probiotics (*Lactobacillus sp.*) viable counts in all three ice-creams during frozen storage, but control ice-cream (only with probiotic culture) shows the highest decrement than prebiotics incorporated sample (arrowroot) and reference (inulin) ice-creams. Initial mean viable count in control ice-cream was 7.62±0.28 $\log_{10}$ cfu $mL^{-1}$and it was drastically reduced up to 6.72±0.37 $\log_{10}$ cfu mL^{-1} at about 25 days of frozen storage (Table 3).The highest probiotic viable count after 55 days of frozen storage was recorded in sample ice-cream (6.68±0.31 $\log_{10}$ cfu mL^{-1}), but the overall change in probiotics viable counts in both prebiotic incorporated ice-creams during frozen storage were more or less same (Figure 5). As mentioned earlier, ice-creams are a huge challenge in relation to the survivability of probiotics during frozen storage. It is because of the vast range of stresses that possess during production and storage of ice-creams. Mechanical stresses like beating, frozen storage stresses like cold shocks and freeze injuries, damages from ice-crystals tend to drastically reduce the viable probiotic counts in ice-creams. Beating will damage the probiotic cells and cold chocks will alter their metabolic activities(Akalin& Erişir, 2008).Intermittent freezing and beating would be the highest detrimental factor which affect the survivability of probiotics in ice-creams rather than the effect from frozen storage period.

During storage period ice-crystals tend to increase their sizes, normally double in size (30%-40% increment). The developing ice-crystals can directly damage probiotic cells which finally leads to cell death. Temperature fluctuations during frozen storage will enhance the melting and recrystallization of the ice-crystals which ultimately leads to larger ice-crystal formation (Gibson, 2004)**.** Therefore, temperature fluctuations during storage and transportation of probiotic /synbiotic ice-creams would be a negative factor in relation to the probiotic survivability.

In the control ice-cream, there is no any prebiotic compound. Therefore, probiotic cells did not have any sort of protection against above mentioned threats. As a result of that, viable probiotic counts in control ice-cream tend to drastically reduce during frozen storage compared to prebiotic incorporated ice-creams (Figure 5).In prebiotics incorporated ice-creams, the prebiotic compounds act as a stabilizer and tend to control the ice crystal formation (Di Criscio *et al.,* 2010)**.** And also, prebiotics aid the probiotics to withstand various stress conditions during processing and storage of ice-creams. Therefore, probiotic viable counts tend to higher in prebiotics incorporated synbiotc ice-creams than control.

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The reference ice-cream (inulin incorporated) had recorded the highest overrun during processing (47%±0.23) (Table 1). And also, the reference ice-cream shows lower probiotic cell survivability than sample ice-cream. Highest overrun means the highest incorporation of air into the ice-cream. This high level of air incorporation would be a death factor to the probiotics (*Lactobacillus sp.*) which are facultative anaerobes or microaerophiles. And also, inulin is a longer chained, high molecular weight molecule which leads to the production of inulin micro-crystals (Di Criscio *et al.,* 2010). Therefore, these two factors (highest overrun and inulin micro-crystal formation) would be the reason for the reduction of viable probiotic cell counts in reference ice-cream than the sample ice-cream.

In sample ice-cream (arrowroot extract incorporated), fructooligosaccharides and other residue materials (starch, soluble proteins, fat) from arrowroot extract would aid the survivability of probiotics than refence ice-cream. Fructooligosaccharides are smaller molecules compared to inulin. Their molecular weights, chain length and degree of polymerization are much lesser than inulin (Homayouni *et al.,* 2008). Due to their smaller nature, they tend to readily soluble in water which leads to less production of FOS micro-crystals during processing and storage of synbioitic ice-cream. And also, probiotic microbes tend to utilize FOS molecules as their carbon and energy sources than utilizing inulin. Due to these reasons, arrowroot extract incorporated (sample) ice-cream had shown the highest mean viable count (6.68 ± 0.31 $\log_{10}$ cfu mL^{-1}) even after 55 days of frozen storage (Table 3).

And also, during frozen storage, titratable acidity (TA) (Figure 2**)** and total soluble solids (TSS) (Figure 1**)** showed visible change in all three ice-creams. TA tend to gradually increase in all three ice-creams, but increment is more or less same in prebiotics incorporated ice-creams than control ice-cream. And also, there is a higher increment in TA in synbiotic ice-creams than control which shows high lactic acid production during storage. It shows that synbiotic ice-creams had more microbial activity than control ice-cream, which ultimately reveals that in synbiotic ice-creams the probiotic survivability is much higher than in control ice-cream. As well as, the TSS is visibly reducing in all three ice-creams which shows that all three ice-creams had microbial activities during storage period. One of the major compounds in ice-creams that responsible for the TSS is lactose. Due to probiotic activities, lactose tends to convert into lactic acid which leads to reduction in TSS and increment in TA. The reduction of TSS in synbiotc ice-creams are much higher than in the control ice-cream. It shows that survivability of probiotics in synbiotic ice-creams tend to higher than in control ice-creams. These physiochemical measurements (TA and TSS) in ice-creams indirectly related with the probiotic cell survivability. Therefore, these indirect measurements also reveal that there is a positive effect from prebiotics to the survivability and protection of probiotics in synbiotic ice-creams during processing and frozen storage.

Even though there is a visible reduction in probiotic viable cell counts (Figure 5**)** in ice-creams, there is no any statistically significant difference in reduction of viable counts in all three ice-creams ($p>0.05$), but according to the multiple mean comparison, the difference between synbiotic ice-creams and control ice-creams are much higher than the difference between synbiotic (arrowroot and inulin) ice-creams. {*p* value (0.092/0.161) control/synbiotic<*p* value (0.757) synbiotics (arrowroot/inulin)}.

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3.4. Invitro simulated gastrointestinal tolerance assay for Lactobacillus sp. in ice-cream samples

Table 4: Survivability of Lactobacillus sp. (log cfu/g) of fresh probiotic culture and three types of ice-creamduring exposure toin vitrosimulated gastrointestinal assay

Time	Fresh probiotic culture	Control Ice-Cream	Arrowroot Ice-Cream	Reference Ice-Cream
0h	8.02±0.01[a]	7.62±0.28[a]	7.71±0.37[a]	7.68±0.32[a]
2h	3.04±0.06[a]	5.20±0.28[b]	5.42±0.17[b]	5.40±0.04[b]
4h	3.58±0.01[a]	5.31±0.14[b]	5.51±0.14[b]	5.55±0.06[b]
6h	4.06±0.04[a]	5.36±0.04[b]	5.82±0.13[c]	5.77±0.06[c]

Values are expressed as mean ± SD. [a,b,c] different superscript simple letters in a row denote significant differences between treatments ($p<0.05$) during exposure to *in vitro* simulated gastrointestinal conditions.

The average population of *Lactobacillus sp*. in fresh probiotic culture were decreased from 8.02 to 4.06 log cfu/g by the end of the *in vitro* simulated gastrointestinal tolerance assay. Also, the average population of *Lactobacillus sp.* were decreased from 7.62 to 5.36 log cfu/g (control ice-cream), 7.71 to 5.82 log cfu/g (Arrowroot ice-cream) and 7.68 to 5.77 log cfu/g (reference ice-cream) by the end of the *in vitro* simulated gastrointestinal tolerance assay on 1st day of storage. The highest decrease in *Lactobacillus sp.* was observed in fresh probiotic culture than three types of ice-cream by the end of the *in vitro* simulated gastrointestinal tolerance assay. Survivability of *Lactobacillus sp*. was significantly lower *($p<0.05$)* in fresh probiotic culture than three types of ice-cream by the end of the gastric phase (2 h) and enteric phases (4 h and 6 h) respectively. It suggested that ice-cream food matrix positively improved the viability of *Lactobacillus sp.* during exposure to *in vitro* simulated gastrointestinal conditions. Population of *Lactobacillus sp.*was significantly lower *($p<0.05$)* in control ice cream than arrowroot synbiotic ice-cream and reference ice-cream by the end of the in vitro assay (6 h), suggesting that the presence of arrowroot and inulin were positively affected to the survivability of *Lactobacillus sp.*during the *in vitro* simulated gastrointestinal conditions on 1st day of storage.

The average population of *Lactobacillus sp.* was decreased from 6.59 to 5.18 log cfu/g (control ice-cream), 6.68 to 5.60 log cfu/g (Arrowroot ice-cream) and 6.48 to 5.54 log cfu/g (reference ice-cream) by the end of the *in vitro* simulated gastrointestinal tolerance assay on 55thday of storage. The population of *Lactobacillus sp.*was significantly lower *($p<0.05$)* in control ice-cream than arrowroot synbiotic ice-cream and reference ice-cream by the end of the *in vitro* assay (6 h) on 55th day of storage.

When consider about the viability of *Lactobacillus sp*. for all storage periods evaluated, *Lactobacillus sp*. showed a considerable reduction from 0 to 6 h of assay for all three types of ice-cream. The highest decrease in *Lactobacillus sp*. was observed in the gastric phase (after 2 h of *in vitro*digestion), suggesting its high sensibility towards simulated gastric juice containing HCl and pepsin. This is in line with observations made by (Bedani, Rossi and Saad, 2013,), (Flávia C A Buriti et al., 2010) and (Diniz et al., 2014), who reported that the survivability of La-5 was significantly reduced from 0 to 2 h of *invitro* assay due to the adverse effects of gastric juice. Furthermore, no any significant differences*($p>0.05$*) were observed between control ice-cream and Arrowroot/reference ice-cream after 2 h of assay for all storage periods evaluated, suggesting that presence of

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Arrowroot and inulin in the ice cream did not affect to the viability of *Lactobacillus sp.* against the simulated gastric juice adverse effect.

IV CONCLUSIONS

In prebiotic activity there was no any significant difference between arrowroot extract and inulin. Therefore, arrowroot extract has a good potential to replace commercial prebiotics like inulin.

In probiotic cell viability, the viability is higher in Arrowroot extract (sample) and Inulin incorporated (reference) synbiotic ice-creams than control ice-cream. Therefore, there is a positive effect from prebiotics on cell survivability in synbiotic ice-creams during frozen storage.

The protective effect of Arrowroot was significant when compared to control ice-cream by the end of the *in vitro* simulated gastrointestinal conditions for all the storage periods evaluated. Arrowroot extract in ice-cream positively improved the viability of *Lactobacillus sp*. by the end of the *in vitro* assay. Therefore, the ice-cream food matrix may be considered as good vehicle for the probiotics tested and could play an important role in their protection against gastrointestinal juices.

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