ISSN: 2347-467X, Vol. 08, No. (1) 2020, Pg. 107-121

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Effects of Frozen Storage on Viability of Probiotics and Antioxidant Capacities of Synbiotic Riceberry and Sesame-Riceberry Milk Ice Creams

VARONGSIRI KEMSAWASD[1] and PITTAYA CHAIKHAM[2]*

[1]Institute of Nutrition, Mahidol University, Nakorn Pathom campus, Nakorn Pathom 73170, Thailand.
[2]Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University,
Phranakhon Si Ayutthaya Rajabhat 13000, Thailand.

Abstract

According to many recent studies, ice cream was found to be an effective carrier of probiotics along the human gastrointestinal tract. While probiotics have long been known to improve gut health, prebiotic-supplemented ice creams have demonstrated properties that could be linked to various health benefits and improvement of the gut microbiota. In this study, riceberry and sesame-riceberry milk ice creams were supplemented with inulin, *Lactobacillus casei* 01 and *Lactobacillus acidophilus* LA5 to examine the changes of probiotic populations in different formulations of ice cream. The survivability of probiotics after 60 days of frozen storage and the level of viable cell tolerance towards the simulated gastrointestinal environment were also assessed, followed by sensory evaluation with 100 untrained panelists and determination of chemical qualities of ice cream samples. Findings revealed *L. casei* 01 to be more resistant to frozen storage compared to *L. acidophilus* LA5, whereas addition of sesame milk and inulin were shown to minimize levels of viable cell loss following environmental and mechanical stress, suggesting enhanced probiotic activity. Significant reductions in probiotic viability were observed for all ice cream samples, however higher survival rates were observed in prebiotic-supplemented samples prior to and after 60 days of frozen storage. Probiotic cell counts in all samples exceeded the minimum recommended value (6 log CFU/g). In simulated gastric and bile fluid, all samples illustrated a significant change in probiotic levels, which significantly decreased with increase time of exposure to acidic and basic conditions. Probiotic strains in samples containing riceberry, sesame and inulin demonstrated greatest

Article History

Received: 26 June 2019
Accepted: 23 September 2019

Keywords:

Inulin;
Lactobacillus casei 01;
Lactobacillus acidophilus LA5;
Riceberry Milk;
Sesame Milk;

CONTACT Pittaya Chaikham pittaya.chaikham@gmail.com Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University, Phranakhon Si Ayutthaya Rajabhat 13000, Thailand.

Doi: http://dx.doi.org/10.12944/CRNFSJ.8.1.10

survivability as observed by reduction in pH and increased total acidity, with increased antioxidant and phenolic contents. On the other hand, changes in physicochemical properties of ice cream lowered overall sensory scores in terms of color and flavor. This study contributes to future development and applications of riceberry and sesame for inducement of synbiotic effects in novel probiotic products. survivability as observed by reduction in pH and increased total acidity, with increased antioxidant and phenolic contents. On the other hand, changes in physicochemical properties of ice cream lowered overall sensory scores in terms of color and flavor. This study contributes to future development and applications of riceberry and sesame for inducement of synbiotic effects in novel probiotic products.

Introduction

In the recent years, probiotics have been applied to many health products within the food industry due to widely acclaimed health benefits. Ingestion of probiotics contributed to modification and prevention of pathogenic invasion of the gut microflora, inducing positive effects in terms of enhanced immunity, digestion, and metabolism.[1] The role of probiotics as protective barrier against the growth of gut pathogens was shown to enhance gut health.[2] Additionally, the alteration of host environments leads to mitigation of obesity risks. Probiotics induce anti-inflammatory effects, which are inflammatory diseases resulting from microbiota imbalance in the gut.[3] Some probiotic species, such as many lactic acid bacteria, have also demonstrated hypocholesterolemic effects.[4] Moreover, probiotic synthesis of interleukin-12 and interferon-γ, known to prevent allergic effects, have shown to reduce allergy onsets caused by immune diseases and disorders in past *in-vitro* studies.[5] Therefore, applications of beneficial probiotics in food products aim towards improved consumer health and overall properties of functional foods.

There are several species of beneficial probiotics which have been commonly used such as *Lactobacillus casei, Lactobacillus acidophilus*, and *Bifidobacterium longum*. However, the main focus in this research are *L. casei* 01 and *L. acidophilus* LA5 strains, due to their various uses in dairy based products as well as numerous health benefit claims. Although milk-based products have a major role in the field of functional foods due to nutritionally rich properties, the prevalence of lactose intolerance has drastically increased over the recent years. Cereal milk, *viz.* riceberry and sesame milks, based ice cream was selected as the most suitable carrier. The complex structure of ice cream consisting of dispersed air cells in a continuous aqueous phase, with polysaccharides and proteins as major components, have proven it an suitable probiotic vehicle.

Primary industrial processing of sesame and riceberry generates and discards a huge amount of solid waste, which are important sources of beneficial dietary fiber. Dietary fibers are not subject to enzymatic digestion, however are primary sources of prebiotics capable of stimulating and activating the growth of beneficial bacteria in the human gut. When administered with probiotics, synbiotic effects have contributed to reduce levels of blood glucose and cholesterol content. In Clark and Slavin's, increased consumption of fiber by healthy human participants along a treatment period of 24 h showed 22% of the subjects experienced significant decrease in overall energy intake.[6] Dietary fiber inhibits absorption of nutrients by resisting the convective effects of intestinal contractions, a mechanism linked to the lowering plasma cholesterol concentrations.[7] Textural properties of fiber, including bulkiness and fibrousness may improve textural characteristics in terms of chewiness, as well as over sensory testing scores.

Riceberry rice is an agricultural product commonly grown throughout Thailand and applied in the production of various functional foods due to many proclaimed health benefits. It contains high content of phenolics, including gallic acid and total monomeric anthocyanins with high free radical scavenging ability and ferric reducing antioxidant activity.[8] Many studies on animal gut models have associated prevention of memory impairment, neurodegeneration, and the treatment of Alzheimer's disease with riceberry ingestion due to high levels of antioxidants, in which was shown to reduce acetylcholine hydrolysis and oxidative stress.[9] Riceberry consumption has demonstrated the ability to prevent dysfunction of endothelial cells, anti-inflammatory, and anti-apoptotic effects in rat models.[10] Likewise, sesame consists of a high number of bioactive compounds such as lignans, tocopherol homologues, and phytosterols.[11] It also contains phytates, polyunsaturated fatty acids (PUFA), and bioactive peptides. Many *in vivo* and *in vitro* studies showed a positive correlation of sesame oil supplementation with increased levels of high-density lipoprotein and decreased levels of low-density lipoprotein, lowering the risks of atherosclerosis and cardiovascular diseases.[12] Likewise, the consumption of sesame has demonstrated anti-inflammatory effects and antioxidant property.

Thus, this experimental study was performed to examine the effect of prolonged frozen storage on probiotic levels, chemical properties, antioxidant capacity and sensory acceptance of riceberry and sesame-riceberry milk ice cream supplemented with probiotics (*L. casei* 01 and *L. acidophilus* LA5) and inulin. Moreover, the survival cells of probiotics tolerance towards the simulated gastrointestinal environment were also monitored.

Materials and Methods

Preparation of Probiotic Pellets

Freeze-dried *L. casei* 01 and *L. acidophilus* LA5 (Chr. Hansen, Denmark) were rehydrated, activated and incubated in MRS broth (Hi-media, Mumbai, India) at 37°C for 16 h and 18 h, respectively. The cells were separated using a Rotina 46 R Centrifuge at 4,000 rpm (Tuttlingen, Germany) for 20 min and washed twice by sterile saline water. The pellets of both cultures were diluted in sterile saline water with concentrations of around 10^{12} CFU/mL prior to addition into the ice cream mixture.

Extraction of Riceberry and Sesame-Riceberry Milks

Thai riceberry (*Oryza sativa* L.) and black sesame seeds (*Sesamum orientale* L.) were harvested from organic farms in Chiang Mai, Thailand. Both seeds were dried at 60°C to approximately 13% moisture content. The dried riceberry or sesame plus riceberry (ratio 1:5, w/w) were mixed with boiling water at a ratio of 1:5 (w/v), and extracted using a multifunction soybean milk extractor (model JYL-H2, Joyoung, Shandong, China). The extracted milk was pasteurized at 85°C for 5 min before cooling to 30°C

Table 1: Compositions of synbiotic ricebery and sesame-riceberry milk ice creams

Samples	Abbreviations
***Lactobacillus casei* 01 (LC)**	
Riceberry milk ice cream	LC-R
Riceberry milk ice cream containing inulin	LC-RI
Sesame-riceberry milk ice cream	LC-SR
Sesame-riceberry milk ice cream containing inulin	LC-SRI
***Lactobacillus acidophilus* LA5 (LA)**	
Riceberry milk ice cream	LA-R
Riceberry milk ice cream containing inulin	LA-RI
Sesame-riceberry milk ice cream	LA-SR
Sesame-riceberry milk ice cream containing inulin	LA-SRI

(above section) were mixed with 7% (w/w) butter (KGC Corporation Co., Ltd., Bangkok, Thailand) and heated at 50°C for 2 min. Subsequently, 5% (w/w) skim milk powder (Param Dairy Ltd., Delhi, India),

Production of Riceberry and Sesame-riceberry Milk Ice Creams

In the production of riceberry and sesame-riceberry milk ice creams, 75% (w/w) pasteurized seed milks

12% (w/w) sucrose (Mitr Phol, Bangkok, Thailand), 0.6% (w/w) gelatin (Ingredient Center Co., Ltd.) and 0.4% (w/w) corn starch (Knorr, Bangkok, Thailand) were added into the mixture and stirred at the same temperature for 5 min before homogenization with a blender for 2 min. The homogenized ice cream mixtures were then pasteurized at 80°C for 5 min using a LAUDA thermostatic water bath (Alpha RA 12, Lauda-Bringkmann, Lauda-Königshofen, Germany) before cooling down to 30°C. Each mixture was then mixed with 1% (v/w) diluted cell pellets and immediately frozen in a batch ice cream maker for 4 h. After that, the samples were placed into plastic cups and covered using the lids before being frozen at -25°C for hardening. Compositions of synbiotic riceberry and sesame-riceberry milk ice creams and sample abbreviations are shown in Table 1.

Survival of Probiotics in Gastric and Bile Fluids

Ten grams of samples were incubated in 90 mL of 0.1% (w/v) sterile peptone water (Hi-media, Mumbai, India) which had been adjusted to pH 2 using 37% hydrochloric acid (Marck) and incubated at 37°C under anaerobic conditions (Anaerogen Gas-packs, Oxoid, Basingstocke, UK) for 0, 1, and 2 h before enumeration via spread plating count using MRS agar (Hi-media, Mumbai, India). To monitor the survival cells in bile fluid, the samples (10 g) were mixed with 0.1% peptone water (90 ml) containing 2% (w/v) bile salts (Sigma-Aldrich) followed by anaerobic incubation at 37°C for 0, 2, and 4 h. The numbers of viable probiotic cells were determined using a similar protocol to gastric fluid.[13]

Enumerations of Survival Probiotic Cells during Frozen Storage

The samples were collected at intervals of 0, 10, 20, 30, 40, 50 and 60 days of frozen storage. 10 g of each sample was diluted with 90 mL of sterile 0.1% (w/v) peptone water (Himedia, Mumbai, India) and stomached (IUL Instruments, Barcelona, Spain) for 2 min. Decimal dilutions were made with 0.1% (w/v) sterile peptone water before plating on LC agar 14 and MRS agar containing clindamycin15 for growth facilitation of *L. casei* and *L. acidophilus*, respectively. All plates were then incubated at 37°C for 48-72 h in anaerobic jars containing Anaerogen Gas-packs (Oxoid, Basingstocke, UK) before colony counting.

Determination of pH and Total Titratable Acidity

The pH of samples was measured using a pH meter (model PB-20, Sartorius, Goettingen, Germany), which was previously calibrated with pH 7.0 and 4.0 standard buffers. Total titratable acidity (as % lactic acid) of samples was determined following the standard method of AOAC.[16]

Determination of Antioxidant Activities

DPPH Assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical inhibition values of samples were assessed following the modified method of Chaikham and Apichartsrangkoon.[17] The samples (5 g) were well-mixed with 20 mL of 100% methanol for 15 min before centrifuging at 3,000 rpm for 10 min, and subsequently filtered through the Whatman® No. 4 filter paper. The filtrates (1.6 mL) were then mixed with 0.4 mL of 1.5 µM DPPH in methanol. After 30 min of reaction, the absorbance values of blue-complex solutions were measured using a spectrophotometer (Perkin Elmer UV WINLAB; Perkin Elmer, Waltham, MA) at λ_{max} of 517 nm. Percentage inhibition of DPPH radical was calculated following the formula: DPPH radical scavenging activity = $[1 - (A_1/A_0)] \times 100$, where, A_0= absorbance of control (methanol) and A_1= absorbance of the sample.

FRAP Assay

According to the method of Benzie and Strain, 3 mL of FRAP (Ferric reducing antioxidant power) reagent (10:1:1 of 300 mM pH 3.6 sodium acetate buffer, 10 mM 2, 4, 6-tripyridyls-triazine solution, and 20 mM $FeCl_3.6H_2O$ solution) was poured into 10 mL of the filtrate (as prepared in above section), mixed and incubated at 37°C for 30 min. The absorbance of the mixture was measured at λ_{max} 593 nm and the FRAP value was expressed as mM $FeSO_4$/100 g sample.[18]

Sensory Evaluation

Sensory attributes of samples were evaluated by 100 untrained panelists. A 9-point hedonic scale testing; 9 = extremely like, 5 = neither like nor dislike and 1 = extremely dislike was applied. Triplicate sets of 25 g of each sample were served. Before evaluation, participants were instructed to rinse their mouths with warm water (~30°C) after tasting the samples to improve result accuracy.

Data Analysis

All data was generated from the means of triplicate determinations with standard deviations (S.D). Analysis of variance (ANOVA) was carried out using SPSS version 23 for Windows (SPSS Inc., Chicago, IL, USA), and the determination of significant differences among treatment means was conducted under Duncan's multiple range tests ($P \leq 0.05$).

Results and Discussion

Effects of Ice Cream Processing on Probiotic Viability

In this study, ice cream mixture was blended with active live culture pellets of *L. casei* 01 and *L. acidophilus* LA5 prior to freezing for 4 h. Obtained results as shown in Table 2 can be explained by the application of mechanical stress from ice cream mixing and freezing processes contributing to disruption of probiotic cell membrane, and cell injuries hindering functionality and metabolic activities.[19] However, the percentage yield of probiotic survivability after frozen storage for both *Lactobacillus* species exceeded 99% for all ice cream samples, indicating insignificant reductions in cell viability and the tendency of probiotic strains to be capable of adapting to low temperature conditions. Furthermore, decline in probiotic levels can be attributed to the sudden incorporation of oxygen within a short time frame, which limits survivability of poorly adaptive cultures.

Table 2 : Cell numbers of probiotic before and after ice cream production

Samples	Cell numbers (CFU/g)		% Yield ($\log N_1 \times 100/N_0$)
	Before (N_0)	After (N_1)	
	***Lactobacillus casei* 01**		
LC-R	$4.75 \pm 0.68 \times 10^{10}$	$4.30 \pm 1.10 \times 10^{10}$	99.60^{B}
LC-RI	$5.34 \pm 1.03 \times 10^{10}$	$4.96 \pm 0.92 \times 10^{10}$	99.70^{AB}
LC-SR	$4.60 \pm 1.45 \times 10^{10}$	$4.30 \pm 0.84 \times 10^{10}$	99.73^{A}
LC-SRI	$4.89 \pm 0.91 \times 10^{10}$	$4.66 \pm 0.82 \times 10^{10}$	99.80^{A}
	***Lactobacillus acidophilus* LA5**		
LA-R	$6.41 \pm 1.23 \times 10^{10}$	$5.67 \pm 0.78 \times 10^{10}$	99.51^{C}
LA-RI	$6.02 \pm 0.93 \times 10^{10}$	$5.43 \pm 1.07 \times 10^{10}$	99.58^{BC}
LA-SR	$6.83 \pm 1.39 \times 10^{10}$	$6.20 \pm 0.96 \times 10^{10}$	99.61^{B}
LA-SRI	$6.78 \pm 1.15 \times 10^{10}$	$6.25 \pm 1.01 \times 10^{10}$	99.67^{B}

Means were the determination of six replications ($n = 6$). Means in the same columns with the same capital letters indicate no significant difference ($P > 0.05$).

Samples LC-R, LC-RI, LC-SR, and LC-SRI have shown slightly higher percentage yields of survivability from initial cell populations, compared to samples containing *L. acidophilus* LA5 (Table 2). The results indicated insignificant differences between all samples containing inulin for both strains. There was also no significant difference between sesame-riceberry milk ice cream samples and those containing inulin, while LC-R and LA-R samples exhibited significantly lower survivability yields compared to other samples. The results suggested the addition of inulin to enhance levels of probiotic survivability, as demonstrated by higher viability yields in inulin-containing samples than samples without. The findings of Balthazar *et al.,* have demonstrated that the prebiotic characteristics of inulin serve as a protective barrier for probiotics, in which minimizes the severity of cell damage caused by environmental stress.[20] Such results correlate with this study's findings of greater viable probiotic yields in inulin-supplemented ice cream samples.

Table 3 : Survival of probiotics along with different ice cream types during incubation in simulated gastric (2 h) and bile fluids at 37°C

Samples	Survival of probiotics under simulated gastric and bile fluids (CFU/ml)							
	Simulated gastric fluid				Simulated bile fluid			
	0 h	1 h	2 h	Reduction cells ($\log N_2$ $\log N_0$)	0 h	2 h	4 h	Reduction cells ($\log N_4$ $\log N_0$)
				Lactobacillus casei 01				
LC-R	$5.59 \pm 1.05^a \times 10^8$	$1.82 \pm 0.42^b \times 10^6$	$6.03 \pm 2.25c \times 10^4$	3.97^A	$5.50 \pm 2.08a \times 10^8$	$3.19 \pm 0.41^b \times 10^7$	$5.11 \pm 1.23c \times 10^5$	3.03^A
LC-RI	$5.17 \pm 1.27^a \times 10^8$	$4.47 \pm 0.67^b \times 10^6$	$2.11 \pm 0.61c \times 10^5$	3.39^C	$5.27 \pm 1.59a \times 10^8$	$4.70 \pm 0.71^b \times 10^7$	$8.64 \pm 1.65c \times 10^5$	2.79^C
LC-SR	$5.42 \pm 0.93^a \times 10^8$	$2.20 \pm 0.31^b \times 10^6$	$8.15 \pm 1.84c \times 1^{04}$	3.82^{AB}	$5.64 \pm 1.42a \times 10^8$	$4.16 \pm 0.64^b \times 10^7$	$5.23 \pm 1.12c \times 10^5$	3.03^A
LC-SRI	$5.31 \pm 1.30^a \times 10^8$	$5.08 \pm 0.82^b \times 10^6$	$3.87 \pm 0.73c \times 10^5$	3.14^D	$5.60 \pm 0.91a \times 10^8$	$5.02 \pm 0.80^b \times 10^7$	$9.15 \pm 0.67c \times 10^5$	2.79^C
				Lactobacillus acidophilus LA5				
LA-R	$5.84 \pm 1.40^a \times 10^8$	$2.50 \pm 0.60^b \times 10^6$	$8.81 \pm 1.40c \times 10^4$	3.82^{AB}	$5.60 \pm 0.99a \times 10^8$	$3.36 \pm 0.65^b \times 10^7$	$5.41 \pm 1.06c \times 10^5$	3.01^A
LA-RI	$6.04 \pm 1.36^a \times 10^8$	$5.73 \pm 1.02^b \times 10^6$	$3.97 \pm 0.50c \times 10^5$	3.18^D	$5.56 \pm 1.07a \times 10^8$	$4.63 \pm 0.49^b \times 10^7$	$9.06 \pm 2.19c \times 10^5$	2.79^C
LA-SR	$5.77 \pm 1.00^a \times 10^8$	$3.16 \pm 0.44^b \times 10^6$	$1.02 \pm 0.39c \times 10^5$	3.75^B	$6.71 \pm 0.54a \times 10^8$	$4.30 \pm 0.40^b \times 10^7$	$6.01 \pm 1.53c \times 10^5$	3.05^A
LA-SRI	$5.83 \pm 0.85^a \times 10^8$	$5.82 \pm 0.75^b \times 10^6$	$5.41 \pm 0.84c \times 10^5$	3.03^E	$6.65 \pm 1.48a \times 10^8$	$4.98 \pm 0.77^b \times 10^7$	$9.17 \pm 1.81c \times 10^5$	2.86^B

Means were the determination of six replications (n = 6). Means in the same rows with the same lowercase letters, and means in the same columns with the same capital letters indicate no significant difference (P>0.05).

Survivability of Probiotics during Incubation in Simulated Gastric and Bile Fluid

Survival tendencies of *L. casei* 01 and *L. acidophilus* LA5 were assessed following *in vitro* conditions stimulating the anaerobic human gastrointestinal tract. Ice cream samples were exposed to gastric and bile fluids, and evaluated in terms of probiotic survivability following mimicked conditions of the normal digestion process. From Table 3, initial probiotic cells of all ice cream samples were of an estimated value exceeding ~8 log CFU/g. Following immersion in pH 2 hydrochloric acid solution, results demonstrated decreased levels of viable cells with increased time of exposure along a 2-h time frame. However, the survival rates were still high, suggesting the capability of selective strains in tolerating exposure to gastric conditions, bile salts, enzymes, and toxic metabolites involved in the digestion process.[21] Also, the resistance of probiotics towards bile salt and acidic conditions was improved with the use of cereal milk (i.e. soy milk) as a medium and carrier.[22] Furthermore, observed results demonstrated that among all samples containing *L. casei* 01, LC-SRI exhibited highest resistance to gastric conditions, followed by LC-RI, LC-SR and LC-R as portrayed by the rate of reduction in probiotic cells. A similar trend was illustrated in samples containing *L. acidophilus* LA5.

To mimic the conditions of the small intestine, probiotic ice creams were immersed and incubated in bile salt solution for 4 h. Similar to when exposed to gastric conditions, there was a significant reduction in viable cell counts of probiotics over time, and after 2 h decreased to the range of 5 log CFU/g (Table 3). On the other hand, the rate of reduction of viable cells was lower compared to when samples were exposed to the simulated gastric fluid. It can be noticed that the addition of inulin maintained slightly higher probiotic cell concentrations in ice cream samples compared to samples without. Many studies have shown that the combination of probiotics and prebiotics in ice cream has enhanced growth and survivability of probiotics in the digestive tract.[23] From findings as observed in Table 3, ice cream samples with inulin resulted in significantly higher survivability rates ($P \leq 0.05$) of probiotic cells than samples without. Synergistic effects can be observed from inulin supplementation, suggesting enhanced probiotic protection against simulated digestion conditions. Rastall revealed enhanced mineral absorption in the gut with inulin treatment.[24] Inulin was used as energy source by probiotics, stimulating growth and thus increased probiotic population. Previous studies have also indicated that inulin addition has improved the growth of various probiotic bacteria in fermented skim milk, frozen yoghurt, and soy products.[25-27] This suggested the supplementation of inulin to contribute to the growth rate of probiotics exceeding the rate of viable cell reduction.

Survivability of Probiotics in Different Types of Ice Creams during Frozen Storage

The survivability of the *L. casei* 01 and *L. acidophilus* LA5 was monitored over an interval of 10 days for 60 days frozen storage at -25°C. Reduction in probiotic counts was observed following frozen storage of all ice cream samples. Results from Table 4 evidently indicated a significant difference in the rate of decrease in bacterial cell numbers with increased time of storage along each time interval. The *L. casei* 01 strain also exhibited higher endurance to freezing conditions over the storage duration. This can be examined by significantly lower reduction rates in cell numbers of *L. casei* 01 ($P \leq 0.05$) compared to *L. acidophilus* LA5 in riceberry milk ice cream. A research study by Di Criscio et al. demonstrated that cold storage at -20°C did not interfere with the viability of *L. casei* in ice creams, and cell populations remained above 7 log CFU/g.[28] Therefore, the difference in strains can be taken into account as one of the main factors determining probiotic survivability rate under low temperature conditions.

Inulin supplementation to riceberry milk ice cream was shown to minimize the reduction of probiotic populations for both strains. The number of viable cell loss for LC-R and LA-R were 2.81 and 3.05 log CFU/g, while LC-RI and LA-RI samples reduced in cell numbers by 2.78 and 3.04 log CFU/g. It can be seen that inulin considerably reduces the death rate of cells. According to Closa-Monasterolo *et al.,* inulin enhances the growth rate and sustainability of *Lactobacillus* spp. in the infant formula milk.[29] As a result, adding prebiotic components improved the survivability of probiotics in unfavorable conditions.

Table 4 : Survival cells of probiotics in difference types of ice creams during frozen storage

Samples	Viable probiotic cells during storage at 4ºC for 60 days							
	Initial stage	Day 10	Day 20	Day 30	Day 40	Day 50	Day 60	Reduction cells ($\log N_{60}$ $\log N_0$)
				Lactobacillus casei 01				
LC-R	$5.12 \pm 0.90^a \times 10^{10}$	$3.47 \pm 0.62^b \times 10^{10}$	$8.84 \pm 1.67^c \times 10^9$	$4.12 \pm 1.19^d \times 10^9$	$8.77 \pm 0.94^e \times 10^8$	$3.76 \pm 0.73^f \times 10^8$	$7.94 \pm 1.50^g \times 10^7$	2.81^C
LC-RI	$5.07 \pm 1.03^a \times 10^{10}$	$3.32 \pm 0.21^b \times 10^{10}$	$9.15 \pm 1.52^c \times 10^9$	$4.47 \pm 1.39^d \times 10^9$	$9.19 \pm 1.72^e \times 10^8$	$4.07 \pm 0.90^f \times 10^8$	$8.35 \pm 1.36^g \times 10^7$	2.78^{CD}
LC-SR	$5.35 \pm 0.75^a \times 10^{10}$	$3.64 \pm 0.40^b \times 10^{10}$	$9.54 \pm 1.22^c \times 10^9$	$4.73 \pm 0.80^d \times 10^9$	$9.59 \pm 1.01^e \times 10^8$	$4.49 \pm 1.05^f \times 10^8$	$8.26 \pm 0.89^g \times 10^7$	2.81^C
LC-SRI	$5.25 \pm 0.92^a \times 10^{10}$	$3.60 \pm 0.55^b \times 10^{10}$	$9.53 \pm 0.98^c \times 10^9$	$5.02 \pm 1.04^d \times 10^9$	$9.60 \pm 0.80^e \times 10^8$	$4.69 \pm 0.90^f \times 10^8$	$8.93 \pm 0.91^g \times 10^7$	2.77^D
				Lactobacillus acidophilus LA5				
LA-R	$5.40 \pm 1.16^a \times 10^{10}$	$2.18 \pm 0.91^b \times 10^{10}$	$6.14 \pm 1.02^c \times 10^9$	$1.71 \pm 0.33^d \times 10^9$	$6.19 \pm 0.85^e \times 10^8$	$9.00 \pm 0.78^f \times 10^7$	$4.80 \pm 0.41^g \times 10^7$	3.05^A
LA-RI	$5.58 \pm 0.80^a \times 10^{10}$	$2.83 \pm 0.59^b \times 10^{10}$	$7.28 \pm 0.96^c \times 10^9$	$2.03 \pm 0.45^d \times 10^9$	$6.93 \pm 1.24^e \times 10^8$	$9.52 \pm 1.40^f \times 10^7$	$5.12 \pm 0.77^g \times 10^7$	3.04^A
LA-SR	$5.36 \pm 0.98^a \times 10^{10}$	$2.75 \pm 0.50^b \times 10^{10}$	$7.35 \pm 0.78^c \times 10^9$	$2.40 \pm 0.41^d \times 10^9$	$6.81 \pm 1.30^e \times 10^8$	$9.45 \pm 1.12^f \times 10^7$	$5.47 \pm 0.62^g \times 10^7$	2.99^{AB}
LA-SRI	$5.41 \pm 1.23^a \times 10^{10}$	$3.01 \pm 0.42^b \times 10^{10}$	$7.60 \pm 1.14^c \times 10^9$	$2.81 \pm 0.39^d \times 10^9$	$7.14 \pm 0.95^e \times 10^8$	$9.90 \pm 0.88^f \times 10^7$	$5.98 \pm 0.95^g \times 10^7$	2.96^{BC}

Means were the determination of six replications (n = 6). Means in the same rows with the same lowercase letters, and means in the same columns with the same capital letters indicate no significant difference (P>0.05).

Combination of sesame and riceberry milks in ice cream was shown to minimize the loss of probiotic populations of *L. acidophilus* LA5, however, the same trend was not observed among *L. casei* 01. Both riceberry and sesame milks contain many active compounds with antioxidant properties capable of protecting probiotic cells from oxidative damage and toxicity, resulting in higher viability rate of cells.[30] The synbiotic effect contributing to increase rate of probiotic survival may have risen from combined synergism of prebiotics, namely riceberry and sesame, and selective probiotic species.[31] Additionally, the lowest values of decline in probiotic populations was observed in LC-SRI and LA-SRI, with values of 2.77 and 2.96 log CFU/g, respectively. Similar trends observed from previous results indicate the addition of prebiotics has enhanced probiotic survival rate.

It is widely known that the minimum acceptable number of probiotic cells added into food products for the purpose of inducing health benefits is 6 log CFU/g.[32] In this study, probiotic populations in all ice cream samples remained at an estimated number of ~7 log CFU/g after 60 days of frozen storage, in which they can be claimed as probiotic food. *Lactobacillus* spp. bacterium are capable of surviving and adapting to the low temperature and cold shock conditions,[33] thus explaining insignificant reductions in viable cell numbers following cold storage treatment. Furthermore, many studies have reported extensive growth of probiotics in soymilk due to their ability to metabolize oligosaccharides.[34] The availability of ammonia groups and peptides in cereal milk improves the viability and stability of probiotics,3[5] thus demonstrates the capability of probiotics to grow and maintain viability in cereal based ice cream products.

Chemical Changes of Different Types of Ice Creams during Frozen Storage

Results from chemical assessments of all ice cream samples, as shown in Table 5, demonstrated similar trends of reduction in pH and increase in titratable acidity following 60 days of frozen storage. The initial pH of samples ranged from 5.31 to 5.43, and subsequently decreased to pH along the range of 5.27 to 5.39. Comparing between probiotic strains, ice cream inoculated with *L. casei* 01 underwent greater reduction in pH along with greater increase in total acidity values. This was observed to correlate with the lower reductions in *L. casei* 01 populations compared to *L. acidophilus* LA5 towards the end of frozen storage (Table 4). To elaborate, the findings suggest higher populations can promote the production of organic acids such as lactic acid, acetic acids, and propionic acids, which are byproducts of lactic acid bacteria metabolism.[36] Probiotic-riceberry and sesame milk ice creams supplemented with inulin at the 60th day of frozen storage presented the lowest pH values of 5.27 and 5.33, with total acidity values of approximately 0.38 and 0.35 for LC-SRI and LA-SRI, respectively. The combination of probiotics and prebiotics in ice cream samples provided a synergistic effect to improve probiotic activity and metabolism. Therefore, the decline in pH and increase in total acidity would indicate extensive growth of probiotic cells.

Antioxidant Activity of Ice Cream Samples during Frozen Storage

The antioxidant potential of ice cream samples was assessed via DPPH and FRAP assays. It can be noticed that sesame-riceberry milk ice creams obtained the highest DPPH inhibition values of 56.83 ± 3.05% for LC-SR and 55.80 ± 2.71% for LA-SR, while similar inhibition values of insignificant difference (P>0.05) were also obtained in samples with the addition of inulin (LC-SRI and LA-SRI). On the other hand, after 60 days of storage, there was a significant reduction of DPPH inhibition in all ice cream samples. Free radical scavenging ability followed a decreasing trend in the order of SRI, SR, RI and R containing samples for both probiotic strains. Both strains showed similar levels of DPPH inhibition and ferric reducing power, as can be observed from initial FRAP values of 27.31 ± 0.63, 27.09 ± 1.15, 26.93 ± 0.81, and 27.49 ± 0.80 mM $FeSO_4$/100 g for LC-SR, LC-SRI, LA-SR, and LA-SRI, respectively. At the 60th day of frozen storage, sesame-riceberry milk ice creams supplemented with inulin demonstrated stronger, more pronounced redox potential compared to the rest of the samples. The same trend was also observed in percentage values of DPPH inhibition. A study by Min *et al.,* showed that the rice bran and husk contain high levels of antioxidant activity, in which the highest contents of γ-oryzanol and tocopherols were found in the bran, while greater phenolic acid concentrations, namely ferulic, vanillic, and *p*-coumaric acids, were majorly sourced from the husk.[37] In addition, sesame seeds consisted of abundant lignans, including sesamin, sesamolin, and lignan glycosides.[38] According to Xu *et al.,* brown pigment extracts from black sesame seeds were shown to possess excellent antioxidant activity.[39] Such findings support the results of study, by which indicated the supplementation of riceberry and inulin to enhance levels of DPPH inhibition and FRAP values in probiotic ice creams.

Table 5 : Changes of acidity in difference types of ice creams during frozen storage

Samples	Changes of acidity during storage at 4ºC for 30 days							
	pH				Total titratable acidity (%, lactic acid)			
	Initial stage	Day 10	Day 30	Day 60	Initial stage	Day 10	Day 30	Day 60
				Lactobacillus casei 01				
LC-R	5.40 ± 0.02^{ABa}	5.38 ± 0.01^{Ba}	5.37 ± 0.03^{ABa}	5.37 ± 0.04^{ABa}	0.24 ± 0.01^{Cb}	0.27 ± 0.01^{Ca}	0.28 ± 0.01^{Da}	0.28 ± 0.02^{Ca}
LC-RI	5.37 ± 0.03^{Ba}	5.35 ±0.02^{Ca}	5.34 ± 0.04^{BCa}	5.32 ± 0.03^{Ba}	0.26 ± 0.01^{Bc}	0.30 ± 0.02^{Bb}	0.32 ± 0.01^{Cab}	0.34 ± 0.01^{Ba}
LC-SR	5.32 ± 0.01^{Ca}	5.30 ±0.03^{Dab}	5.31 ± 0.02^{Cb}	5.29 ± 0.01^{Cb}	0.31 ± 0.02^{Ab}	0.34 ± 0.02^{Aab}	0.37 ± 0.01^{Aa}	0.37 ± 0.03^{ABa}
LC-SRI	5.31 ± 0.02^{Ca}	5.29 ±0.03^{Dab}	5.29 ± 0.03^{Cab}	5.27 ± 0.01^{Cb}	0.31 ± 0.01^{Ab}	0.35 ± 0.02^{Aa}	0.36 ± 0.02^{ABa}	0.38 ± 0.01^{Aa}
				Lactobacillus acidophilus LA5				
LA-R	5.43 ± 0.01^{Aa}	5.42 ± 0.02^{Aab}	5.40 ± 0.03^{Aab}	5.39 ± 0.02^{Ab}	0.23 ± 0.03^{Cb}	0.25 ± 0.02^{Db}	0.27 ± 0.03^{Dab}	0.30 ± 0.01^{Ca}
LA-RI	5.42 ± 0.02^{Aa}	5.40 ± 0.02^{ABa}	5.40 ± 0.01^{Aa}	5.38 ± 0.03^{ABa}	0.26 ± 0.02^{BCb}	0.29 ± 0.02^{BCab}	0.31 ± 0.01^{Ca}	0.31 ± 0.02^{Ca}
LA-SR	5.38 ± 0.02^{Ba}	5.37 ± 0.04^{BCa}	5.36 ± 0.03^{Ba}	5.36 ± 0.01^{ABa}	0.28 ± 0.02^{ABb}	0.32 ± 0.01^{ABa}	0.33 ± 0.02^{BCa}	0.35 ± 0.02^{ABa}
LA-SRI	5.37 ± 0.02^{Ba}	5.36 ± 0.01^{Ca}	5.35 ± 0.02^{Bab}	5.33 ± 0.02^{Bb}	0.29 ± 0.01^{Ab}	0.33 ± 0.03^{ABab}	0.34 ± 0.01^{Ba}	0.35 ± 0.01^{Ba}

Means were the determination of six replications (n = 6). Means in the same rows with the same lowercase letters, and means in the same columns with the same capital letters indicate no significant difference (P>0.05).

Sensory Evaluation of Different Types of Ice Creams

The ice cream samples were evaluated by 100 untrained panelists following a 9-point hedonic scale in order to assess the differences in sensory elements of ice cream samples between the initial and 60th day of frozen storage. Sensory parameters, including appearance, color, mouth-feel, flavor, and overall perceived quality, were tested with results as demonstrated in Table 7. Based on a 9-point hedonic scale, all ice creams samples at day 0 were shown to rank higher than 7 points in terms of overall

Table 6 : Changes of acidity in difference types of ice creams during frozen storage

Samples	Changes of antioxidant activities during frozen storage for 60 days							
	DPPH inhibition (%)				FRAP value (mM $FeSO_4$/100 g)			
	Initial stage	Day 10	Day 30	Day 60	Initial stage	Day 10	Day 30	Day 60
	Lactobacillus casei 01							
LC-R	43.62 ± 2.35^{Ba}	40.09 ± 0.82^{Bb}	35.96 ± 1.13^{Dc}	30.60 ± 2.30^{CDd}	20.92 ± 1.03^{Ba}	18.64 ± 0.84^{Bb}	15.93 ± 1.17^{Cc}	12.18 ± 0.39^{Cd}
LC-RI	44.07 ± 1.90^{Ba}	41.64 ± 3.81^{Ba}	37.12 ± 0.93^{Cb}	33.15 ± 1.72^{Cc}	21.14 ± 0.45^{Ba}	17.92 ± 0.60^{Bb}	16.15 ± 1.08^{Cc}	13.03 ± 0.80^{Cd}
LC-SR	56.83 ± 3.05^{Aa}	52.27 ± 2.65^{Aa}	46.65 ± 2.14^{ABb}	40.91 ± 0.54^{Bc}	27.31 ± 0.63^{Aa}	24.60 ± 0.49^{Ab}	20.96 ± 0.87^{ABc}	17.29 ± 1.11^{Bd}
LC-SRI	55.94 ± 2.61^{Aa}	53.66 ± 1.62^{Aa}	47.50 ± 2.71^{ABb}	43.64 ± 1.34^{Ac}	27.09 ± 1.15^{Aa}	24.81 ± 0.76^{Ab}	22.08 ± 0.65^{Ac}	19.13 ± 0.91^{ABd}
	Lactobacillus acidophilus LA5							
LA-R	44.15 ± 3.47^{Ba}	41.24 ± 1.89^{Ba}	34.02 ± 2.65^{Db}	29.93 ± 1.20^{Dc}	21.85 ± 0.74^{Ba}	17.02 ± 1.02^{Bb}	15.09 ± 0.76^{Cc}	12.50 ± 0.70^{Cd}
LA-RI	45.68 ± 2.33^{Ba}	40.92 ± 0.95^{Ba}	35.64 ± 1.38^{Dc}	33.61 ± 1.17^{Cc}	22.05 ± 1.55^{Ba}	18.39 ± 0.83^{Bb}	15.40 ± 1.83^{Cc}	13.46 ± 1.32^{Cc}
LA-SR	55.80 ± 2.71^{Aa}	51.85 ± 1.62^{Aa}	45.67 ± 0.95^{Bb}	41.55 ± 0.85^{Ac}	26.93 ± 0.81^{Aa}	25.17 ± 0.62^{Ab}	19.65 ± 1.25^{Bc}	18.02 ± 0.65^{Bc}
LA-SRI	55.67 ± 3.80^{Aa}	52.04 ± 2.04^{Aa}	48.01 ± 1.48^{Ab}	44.13 ± 2.16^{Ac}	27.49 ± 0.80^{Aa}	24.90 ± 1.14^{Ab}	21.84 ± 0.95^{Ac}	19.90 ± 0.73^{Ad}

Means were the determination of six replications ($n = 6$). Means in the same rows with the same lowercase letters, and means in the same columns with the same capital letters indicate no significant difference (P>0.05).

quality, in which the only significant differences were observed in terms of flavor. Ice cream samples containing both sesame and riceberry (LC-SR and LA-SR), and samples containing sesame, riceberry, and inulin (LC-SRI and LA-SRI) scored similarly and among the highest compared to the rest of the samples regardless of probiotic strain on the intial day of storage (P>0.05). Similar flavor scores were obtained comparing between samples containing riceberry (LC-R and LA-R), and those containing both riceberry and inulin (LC-RI and LA-RI). Overall lower scores were obtained for all ice cream samples following 60 days of frozen storage among all sensory parameters, in which there was a significant difference (P>0.05) among the samples in terms of color and flavor. Results have shown the difference in flavors to be most pronounced. Ice creams without the addition of sesame seemed to score lowest in terms of overall acceptability by the panelists compared to other samples. Fazilah *et al.,*have reported that the interaction of probiotics during milk fermentation induced major changes in product flavor.[40] Furthermore, the presence of organic acids as byproducts of the fermentation process contribute to perceived sourness, which in turn could result in lower sensory and likeability scores. However, there is currently insufficient research information on the effects of the addition of prebiotics to probiotic foods and their influence on overall sensory scores and consumer acceptability. With regards to the findings of this study, it was found that LA-SRI and LA-SR achieved the high overall liking scores of 7.33 ± 0.43 and 7.29 ± 0.42, respectively, while LC-R and LA-R scored lowest. This suggested the addition of riceberry milk to influence the lowering of overall consumer acceptability scores of ice cream samples.

Table 7: Liking scores of difference types of ice creams

Samples	Sensory attributes				
	Appearance	Color	Mouth feel	Flavor	Overall
			Frozen at Day 0		
LC-R	7.62 ± 0.61^{A}	7.42 ± 0.38^{A}	7.42 ± 0.57^{A}	7.31 ± 0.42^{BC}	7.30 ± 0.35^{B}
LC-RI	7.58 ± 0.47^{A}	7.58 ± 0.42^{A}	7.60 ± 0.49^{A}	7.30 ± 0.37^{BC}	7.36 ± 0.42^{B}
LC-SR	7.83 ± 0.53^{A}	7.51 ± 0.43^{A}	7.56 ± 0.50^{A}	7.78 ± 0.40^{A}	7.73 ± 0.39^{A}
LC-SRI	7.95 ± 0.50^{A}	7.45 ± 0.48^{A}	7.53 ± 0.41^{A}	7.81 ± 0.43^{A}	7.85 ± 0.41^{A}
LA-R	7.59 ± 0.55^{A}	7.60 ± 0.51^{A}	7.54 ± 0.43^{A}	7.10 ± 0.41^{BC}	7.25 ± 0.46^{B}
LA-RI	7.73 ± 0.56^{A}	7.63 ± 0.50^{A}	7.62 ± 0.38^{A}	7.23 ± 0.40^{BC}	7.30 ± 0.52^{B}
LA-SR	7.80 ± 0.48^{A}	7.54 ± 0.45^{A}	7.55 ± 0.54^{A}	7.82 ± 0.47^{A}	7.81 ± 0.47^{A}
LA-SRI	7.71 ± 0.60^{A}	7.49 ± 0.60^{A}	7.60 ± 0.47^{A}	7.80 ± 0.39^{A}	7.80 ± 0.34^{A}
			Frozen at Day 60		
LC-R	6.83 ± 0.45^{B}	6.95 ± 0.41^{B}	6.52 ± 0.42^{B}	6.72 ± 0.36^{C}	6.55 ± 0.47^{C}
LC-RI	6.96 ± 0.38^{B}	7.03 ± 0.42^{AB}	6.47 ± 0.36^{B}	6.80 ± 0.42^{C}	6.66 ± 0.36^{C}
LC-SR	6.80 ± 0.42^{B}	6.97 ± 0.35^{B}	6.63 ± 0.38^{B}	7.34 ± 0.40^{AB}	7.24 ± 0.45^{B}
LC-SRI	6.79 ± 0.45^{B}	6.90 ± 0.37^{B}	6.68 ± 0.40^{B}	7.25 ± 0.41^{BC}	7.19 ± 0.43^{B}
LA-R	6.85 ± 0.41^{B}	7.11 ± 0.36^{AB}	6.73 ± 0.37^{B}	6.57 ± 0.43^{C}	6.50 ± 0.40^{C}
LA-RI	6.77 ± 0.47^{B}	6.99 ± 0.40^{B}	6.51 ± 0.38^{B}	6.73 ± 0.41^{C}	6.63 ± 0.38^{C}
LA-SR	7.02 ± 0.39^{B}	6.83 ± 0.43^{B}	6.70 ± 0.43^{B}	7.45 ± 0.32^{AB}	7.29 ± 0.42^{B}
LA-SRI	6.87 ± 0.40^{B}	7.08 ± 0.35^{AB}	6.64 ± 0.41^{B}	7.23 ± 0.40^{BC}	7.33 ± 0.43^{B}

Values are expressed as mean values ± standard deviation (n = 50). Means in the same columns with the same capital letters indicate no significant difference (P>0.05).

Conclusions

In this study, greater percentage yields of viable *L. casei* 01 compared to *L. acidophilus* LA5 were obtained following ice cream production. There were no significant differences among ice cream samples supplemented with both riceberry and sesame, while samples supplemented with riceberry alone resulted in significantly lowest percentage yields of survivability. Obtained results suggest the addition of inulin to induce protective effects towards probiotic growth and metabolism, thus resulting in greater population yields. Both probiotic strains in ice cream samples containing a combination of riceberry, sesame, and inulin demonstrated higher resistance to simulated human gastric conditions, as shown by lowest levels of cell reduction. Following the simulated digestion process, probiotic populations in all ice cream samples remained above 5 log CFU/g, thereby deducing that probiotic cultures were able to carry out metabolic activities and achieve growth within the gastrointestinal tract. Similar results were obtained following frozen storage conditions, in which *L. casei* 01 maintained higher levels of survivability compared to the other strain. Furthermore, the reduction of probiotic cells was minimized with the addition of riceberry, sesame, and inulin, which can be considered as an effective combination of prebiotics in enhancing probiotic ice cream quality. Over prolonged frozen storage, prominent reductions in pH and increase in total acidity were shown in all samples, in which correlated with the decline in overall acceptability and significantly lower sensory scores in terms of color and flavor. As mentioned early, many research studies have proven riceberry and sesame as rich sources of antioxidants and phenolic compounds, thus the samples illustrated the highest percentages of DPPH inhibition and FRAP values. However, increased storage time was taken into account for the decline in antioxidant levels.

Acknowledgements

We would like to thank Mahidol University and Phranakhon Si Ayutthaya Rajabhat University for providing the facilities to support our research.

Funding

This research was financially supported from the Thailand Research Fund (Grant number TRG5880002).

Conflict of Interest

The authors do not have any conflict of interest.

References

1. Kerry, R.G., Patra, J. K., Gouda, S., Park, Y., Shin, H.-S., Das, G. Benefaction of probiotics for human health: A review. *Journal of Food and Drug Analysis*. 2018; 26(3): 927–939.
2. Pérez-Cobas, A. E., Moya, A., Gosalbes, M. J., Latorre, A. Colonization resistance of the gut microbiota against *Clostridium difficile. Antibiotics (Basel, Switzerland).* 2015; 4(3): 337–357.
3. Iqbal, M. Z., Qadir, M. I., Hussain, T., Janbaz, K. H., Khan, Y. H., Ahmad, B. Review: probiotics and their beneficial effects against various diseases. *Pakistan Journal of Pharmaceutical Sciences*. 2014; 27(2): 405–415.
4. Larsen, N., Vogensen, F. K., van den Berg, F. W. J., Nielsen, D. S., Andreasen, A. S., Pedersen, B. K., Jakobsen, M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PloS One.* 2010; 5(2): e9085.
5. Song, S., Lee, S-J., Park, D-J., Oh, S., Lim, K-T. The anti-allergic activity of *Lactobacillus* plantarum L67 and its application to yogurt. *Journal of Dairy Science*. 2016; 99(12): 9372–9382.
6. Clark, M. J., Slavin, J. L. The effect of fiber on satiety and food intake: a systematic review. *Journal of the American College of Nutrition.* 2013; 32(3): 200–211.
7. Grundy, M. M. L., Edwards, C. H., Mackie, A. R., Gidley, M. J., Butterworth, P. J., Ellis, P. R. Re-evaluation of the mechanisms of dietary fibre and implications for macronutrient bioaccessibility, digestion and postprandial metabolism. *British Journal of Nutrition*. 2016; 116: 816–833.
8. Chinprahast, N., Tungsomboon, T., Nagao, P. Antioxidant activities of Thai pigmented rice cultivars and application in sunflower oil. *International Journal of Food Science & Technology.* 2016; 51(1): 46–53.
9. Pannangrong, W., Wattanathorn, J.,

Muchimapura, S., Tiamkao, S., Tong-Un, T. Purple rice berry is neuroprotective and enhances cognition in a rat model of Alzheimer's disease. *Journal of Medicinal Food.* 2011; 14(7–8): 688–694.

10. Arjinajarn, P., Chueakula, N., Pongchaidecha, A., Jaikumkao, K., Chatsudthipong, V., Mahatheeranont, S., Lungkaphin, A. Anthocyanin-rich riceberry bran extract attenuates gentamicin-induced hepatotoxicity by reducing oxidative stress, inflammation and apoptosis in rats. *Biomedicine & Pharmacotherapy*. 2017; 92: 412–420.
11. Pathak, N., Bhaduri, A., Rai, A. K. Sesame: Bioactive compounds and health benefits. In Bioactive Molecules in *Food. Springer, Cham*. 2018; 1–20.
12. Hsu, E., Parthasarathy, S. Anti-inflammatory and antioxidant effects of sesame oil on atherosclerosis: A descriptive literature review. *Cureus*. 2017; 9(7): e1438.
13. Chaikham, P., Apichartsrangkoon, A., Worametrachanon, S., Supraditareporn, W., Chokiatirote, E., Van der Wiele, T. Activities of free and encapsulated *Lactobacillus acidophilus* LA5 or *Lactobacillus casei* 01 in processed longan juices on exposure to simulated gastrointestinal tract. *Journal of the Science of Food and Agriculture*. 2013; 93(9): 2229–2238.
14. Ravula, R. R., Shah, N. P. Selective enumeration of *Lactobacillus casei* from yogurts and fermented milk drinks. *Biotechnology Techniques.* 1998; 12(11): 819–822.
15. Illupapalayam, V. V, Smith, S. C., Gamlath, S. Consumer acceptability and antioxidant potential of probiotic-yogurt with spices. *LWT - Food Science and Technology*. 2014; 55(1): 255–262.
16. A. O. A. C. Offiial methods of analysis of AOAC International, 18th edn. Association of Offiial Analytical Chemists; Maryland, MD. 2005.
17. Chaikham, P., Apichartsrangkoon, A. Comparison of dynamic viscoelastic and physicochemical properties of pressurised and pasteurised longan juices with xanthan addition. *Food Chemistry.* 2012; 134,: 2194–2200.
18. Benzie, I. F. F., Stain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power; the FRAP assay. *Analysis Biochemistry*. 1996; 239: 70–76.
19. Turgut, T., Cakmakci, S. Investigation of the possible use of probiotics in ice cream manufacture. *International Journal of Dairy Technology*. 2009; 62(3): 444–451.
20. Balthazar, C. F., Silva, H. L. A., Esmerino, E. A., Rocha, R. S., Moraes, J., Carmo, M. A. V., Cruz, A. G. The addition of inulin and *Lactobacillus casei* 01 in sheep milk ice cream. *Food Chemistry*. 2018; 246: 464–472.
21. 21 Lourens-Hattingh, A., Viljoen, B. C. Yogurt as probiotic carrier food. *International Dairy Journal.* 2001; 11(1): 1–17.
22. Nagpal, R., Kumar, A., Kumar, M., Behare, P. V., Jain, S., Yadav, H. Probiotics, their health benefits and applications for developing healthier foods: a review. *FEMS Microbiology Letters*. 2012; 334(1): 1–15.
23. Miremadi, F., Sherkat, F., Stojanovska, L. Hypocholesterolaemic effect and antihypertensive properties of probiotics and prebiotics: A review. *Journal of Functional Foods*, 2016; 25: 497–510.
24. Rastall, R. A. Functional oligosaccharides: application and manufacture. *Annual Review of Food Science and Technology.* 2010; 1: 305–339.
25. Oliveira, R. P. D. S., Perego, P., Oliveria, M. N. D., Converti, A. Effect of inulin as a prebiotic to improve growth and counts of a probiotic cocktail in fermented skim milk. *LWT–Food Science & Technology*. 2011; 44(2): 520–523.
26. Rezaei, R., Khomeiri, M., Aalami, M., Kashaninejad, M. Effect of inulin on the physicochemical properties, flow behavior and probiotic survival of frozen yogurt. *Journal of Food Science and Technology.* 2014; 51(10): 2809–2814.
27. Bedani, R., Rossi, E. A., Saad, S. M. I. Impact of inulin and okara on *Lactobacillus* acidophilus LA-5 and *Bifidobacterium animalis* Bb-12 viability in a fermented soy product and probiotic survival under *in vitro* simulated gastrointestinal conditions. *Food Microbiology.* 2013; 34(2): 382–389.
28. Di Criscio, T., Fratianni, A., Mignogna, R., Cinquanta, L., Coppola, R., Sorrentino, E., Panfili, G. Production of functional probiotic, prebiotic, and synbiotic ice creams. *Journal of*

Dairy Science. 2010; 93(10): 4555–4564.

29. Closa-Monasterolo, R., Gispert-Llaurado, M., Luque, V., Ferre, N., Rubio-Torrents, C., Zaragoza-Jordana, M., Escribano, J. Safety and efficacy of inulin and oligofructose supplementation in infant formula: results from a randomized clinical trial. *Clinical Nutrition (Edinburgh, Scotland).* 013; 32(6): 918–927.
30. Paseephol, T., Sherkat, F. Probiotic stability of yoghurts containing Jerusalem artichoke inulins during refrigerated storage. J*ournal of Functional Foods.* 2009; 1(3): 311–318.
31. Ayar, A., Siçramaz, H., Öztürk, S., Yilmaz, S. Ö. Probiotic properties of ice creams produced with dietary fibres from by-products of the food industry. *International Journal of Dairy Technology.* 2018; 71(1): 174–182.
32. Lee, Y.-K., Salminen, S. The coming of age of probiotics. *Trends in Food Science & Technology.* 1995; 6(7): 241–245.
33. Sauvageot, N., Beaufils, S., Mazé, A., Deutscher, J., Hartke, A. Cloning and characterization of a gene encoding a cold-shock protein in *Lactobacillus casei*. *FEMS Microbiology Letters*. 2008; 254(1): 55–62.
34. Champagne, C. P., Green-Johnson, J., Raymond, Y., Barrette, J., Buckley, N. Selection of probiotic bacteria for the fermentation of a soy beverage in combination with *Streptococcus thermophilus. Food Research International*. 2009; 42(5): 612–621.
35. Donkor, O. N., Nilmini, S. L. I., Stolic, P., Vasiljevic, T., Shah, N. P. Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. *International Dairy Journal.* 2007; 17(6): 657–665.
36. Millette, M., Luquet, F. M., Lacroix, M. *In vitro* growth control of selected pathogens by *Lactobacillus acidophilus*- and *Lactobacillus casei*-fermented milk. *Letters in Applied Microbiology.* 2007; 44(3): 314–319.
37. Min, B., McClung, A. M., Chen, M. -H. Phytochemicals and antioxidant capacities in rice brans of different color. *Journal of Food Science*. 2011; 76(1): C117–C126.
38. Rangkadilok, N., Pholphana, N., Mahidol, C., Wongyai, W., Saengsooksree, K., Nookabkaew, S., Satayavivad, J. Variation of sesamin, sesamolin and tocopherols in sesame (*Sesamum indicum* L.) seeds and oil products in Thailand. *Food Chemistry.* 2010; 122(3): 724–730.
39. Xu, J., Chen, S., Hu, Q. Antioxidant activity of brown pigment and extracts from black sesame seed (*Sesamum indicum* L.). *Food Chemistry.* 2005; 91(1): 79–83.
40. Fazilah, N. F., Ariff, A. B., Khayat, M. E., Rios-Solis, L., Halim, M. Influence of probiotics, prebiotics, synbiotics and bioactive phytochemicals on the formulation of functional yogurt. *Journal of Functional Foods.* 2018; 48: 387–399.