ISSN: 2347-467X, Vol. 11, No. (3) 2023, Pg. 1363-1373

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Microbiological, Physicochemical, and Sensory Characters of Synbiotic Ice Cream from Fermented Milk using *Lactiplantibacillus Plantarum* Subsp. *Plantarum* Dad-13 Combined with Inulin

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Abstract

Ice cream, which includes dairy product, is a good carrier of addition probiotics and prebiotics. This study was designed to assess the microbiological, physicochemical, and sensory properties of the ice cream. The characteristics of ice cream made from fermented milk using *L. plantarum* Dad-13 combined with inulin (0%, 1%, and 2%) were evaluated such as cell viability, pH, titratable acidity, overrun, melting rate, sensory evaluation with hedonic test, microstructure using scanning electron microscopy, and volatile organic compounds using HS-GC-MS. The results showed that cell viability in synbiotic ice cream with 2% inulin decreased by 1 log cycle, which showed the most stable value during storage until the 12th week was still 1.03 x 107 CFU/g. Synbiotic ice cream with 2% inulin showed the highest overrun value of 35.72% and the slowest melting rate of 40.71% of ice cream melted in 20 minutes. Overall attributes in the hedonic test of synbiotic ice cream with 2% inulin showed a value of 4, which means the most preferred by panelists. Hence, this research showed that ice cream containing 2% inulin, in particular, has a better microstructure due to the prevention of ice recrystallization, which results in smaller ice crystals. The ketone volatile organic compound only found in ice cream with 1% inulin was cyclopentadecanone, 2-hydroxy- with percentage of area 10.25% while for ice cream that contains 2% inulin, it was oxacyclotetradecan-2-

Article History
Received: 22 November 2023
Accepted: 19 December 2023

Keywords
Ice cream;
Inulin;
L. Plantarum Dad-13;
Prebiotic;
Probiotic.

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Doi: https://dx.doi.org/10.12944/CRNFSJ.11.3.35

one with percentage of area 9.31%. Furthermore, several volatile organic compounds, such as 2-trifluoroacetoxydodecane, 4-propionyloxytridecane, and anthracene, 9-butyltetradecahydro, were only found in the synbiotic ice cream. This study has the potential to be a novel functional food containing probiotic indigenous *L. plantarum* Dad-13 and prebiotic inulin.

Introduction

Ice cream is included in frozen food products made from milk, which is very popular with various groups, from children to adults. The process of making ice cream is done by freezing the pasteurized mixture while stirring it for air penetration and ensuring a uniform consistency.[1] As the primary sources of probiotics and prebiotics in functional foods, dairy products like ice cream have emerged as pioneers in this space. Synbiotics are combinations of live microbes and substrates that the host uses specifically to promote their own health.[2] Consuming prebiotic meals, foods containing probiotic microbes, or a mix of the two can improve the health of the human digestive system.[3]

Live bacteria known as probiotics can help their hosts' health when taken in sufficient amounts.[4] A microorganism can be categorized or act as a probiotic through a predetermined probiotic criteria screening process. Probiotics in sufficient quantities must be resistant to extreme conditions in the digestive system, such as low pH, stomach acid, bile salts, digestive enzymes such as amylase, lysozyme, and pepsin, as well as changes in body temperature.[5]

L. plantarum Dad-13 is an indigenous isolate that can act as a probiotic. The probiotic has been used by the community for a long time as a traditional food, namely 'dadih'. 'Dadih' is fermented buffalo milk originating from the people of Minang, West Sumatra.[6] *L. plantarum* Dad-13 was proven to survive in the digestive tract of healthy Indonesian volunteers. After ingestion was stopped, *L. plantarum* was still found in feces even though the amount had decreased. The existence of *L. plantarum* also reduces the number of *Enterobacteriaceae* and *E. coli* in feces.[7]

Some fruits and vegetables naturally contain inulin, a soluble fiber-containing substance that can ferment and be nearly fully digested in the colon.[8] Isolated carbohydrate inulin is a prebiotic that has bifidogenic properties.[9] For many years, inulin has been utilized as a fat substitute or texture enhancer in dairy-based goods, particularly in low fat dishes like yogurt ice cream,[10] ice cream,[11,12] and starch based dairy desserts.[13] Constipation can be avoided, the composition of the intestinal microbiota is improved, proteolytic metabolism is suppressed (primary indicator organisms are lactic acid bacteria and *bifidobacteria*), calcium absorption from food is stimulated, lipid metabolism is modulated, and cancer is prevented by inulin. It is now known that inulin is a significant prebiotic that influences the microbial content of the gastrointestinal tract and the health of the host.[14]

Different plant species naturally contain the soluble fiber known as inulin as a reserve polysaccharide. It can especially encourage the growth of gut microbial to maintain health.[15] Various dairy products have been enriched with inulin to improve their texture and rheological properties.[16] A reduction in water molecule mobility and a decrease in ice recrystallization are the effects of these polysaccharides, which have previously been connected to their strong gelling power, viscosity, or interaction with the ice cream's protein component.[17] However, some studies suggested polysaccharides may attach to the ice surface and prevent ice recrystallization.18 Ice recrystallization, which results in a reduction in the product quality of frozen foods like ice cream, is the enlargement of ice crystals that have previously formed without altering the ice mass.[19]

The fact that ice cream ingredients can protect lactic acid bacteria, it is thought that ice cream serves as an appropriate carrier for the growth of lactic acid bacteria during storage.[20] But during processing and storage, osmotic pressure, mechanical cutting, and oxygen stress could pose a threat to the lactic acid bacteria in ice cream.[21] Viability of multiple strains of *Lactobacillus delbrueckii*,[22] *L. acidophilus*,[23,24] and *bifidobacteria*[25] were found to decrease after

the process of making ice cream. Because of its unpredictable impact on the quality and preservation of bioactivity in this food matrix, the detrimental effect of lactic acid bacteria strains' survival is a barrier to their employment in the food sector.[26] Hence, synbiotic ice creams that use prebiotics such as inulin are expected to protect the viability of probiotics during storage. Therefore, this study was designed to assess the the character of synbiotic ice cream made from fermented milk using indigenous probiotic *L. plantarum* Dad-13 combined with inulin.

Materials and Methods

Materials

The probiotic powder *L. plantarum* Dad-13 from Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. The prebiotic was organic inulin from Blue agave (Now Foods, Bloomingdale, USA). Other ingredients from local markets such as skim milk (Lactona), gelatin (PIS), super polymer emulsifier (Koepoe Koepoe), whipped cream (Ellenka), milk (Diamond), and sugar (Gulaku). Microbiological media from Merck (Darmstadt, Germany) and others chemical was obtained from Sigma Aldrich (St. Louis, Missouri, USA).

Preparation of Ice Cream

Cow milk mixed with 2% skim milk and 8% sugar was heated at 80 °C for 30 minutes. Pasteurized cow milk was fermented using 1% of culture *L. plantarum* Dad-13 with variations of 0% (IC0), 1% (SIC1), and 2% (SIC2) of inulin then incubated for 24 hours at 37 °C. To make ice cream, dry components were mixed together such as skim milk and gelatin into milk and then mixing to homogenize the ingredients. Next, heat it over low heat and add the sugar to the liquid mixture. The purpose of adding sugar at the heating stage to make the sugar can be completely dissolved. After the temperature was 60 °C, it was cooled and mixed with fermented milk and whipped cream into the mixture. The mixture was stored for twenty to twenty-four hours at 4 °C in a refrigerator. The next stage was adding an emulsifier to the ice cream mixture. After that, churn the mixture with an ice cream maker and put it in a 100 mL cup, then store it in the freezer at −18 °C.

Microbiological Analysis

Every two weeks, a microbiological study with viability assessment were performed on the samples. *L. plantarum* Dad-13 probiotic cell viability was the components used in the viability evaluation of this study. De Man, Rogosa, and Sharpe agar containing CaCO3 was utilized to determine the total lactic acid bacteria, which were then incubated for 48 hours at 37 °C, in which lactic acid bacteria formed colonies with a clear zone around each colony.

pH and Titratable Acidity

A pH meter (Zen Test PH60S-Z Smart Spear pH Tester, Europe) was used to determine the pH of the samples. Using oxalic acid solution as a standard, 0.1 N NaOH was used for the titratable acidity analysis. Using the indicator phenolphthalein, the titration method with 0.1 N NaOH was used to determine the titratable acidity to a pink color.

Overrun

The mass of the mix (M1) and mass of the ice cream (M2) was measured in order to determine the overrun of ice cream. The percentage of overrun was determined using the improved equation.[27]

$$\text{Overrun (\%)} = (M1-M2)/M2$$

M1 = mass of mixture with the same volume
M2 = mass of ice cream with the same volume

Melting Rate

The technique was considerably modified in order to determine the rate at which each batch of ice cream melted.28 After being stored for a week at −18 °C, about 20 g of ice cream samples were put through a 1.0 mm sieve metal and set up above a collection container at 25 °C. Following a 20-minute period, it was determined how much of the ice cream melted by measuring the amount of each melted sample. The analyses were done in triplicate.

Sensory Evaluation

With protocol number KE/FK/ 0285/EC/2023, this study was accepted in February 2023 by Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing at Universitas Gadjah Mada, Indonesia. Samples of ice cream were put into a 20 mL plastic cup and distributed at random to 111 untrained panelists for hedonic analysis. To neutralize the taste buds, crackers and mineral water were available during the test. The panelists were given five scale with the options 1 (strongly disliked), 2 (disliked), 3 (neutral), 4 (liked), and 5 (strongly

liked) to score the color, texture, aroma, sourness, sweetness, and overall acceptance.

Microstructure Ice Cream

Microstructure was analyzed using scanning electron microscopy (JEOL 6510 LA, Tokyo, Japan). For analysis, the 30 g of ice cream was measured the mass and put in a plastic cup with a cover punctured with tiny holes. A day of freezing at -18 °C was spent on ice cream samples. Then, the ice cream was dried for about 24 hours using a freeze-dryer. The dried ice cream was cut into thin slices and sputter-coated with gold. Then, SEM was used to examine the gold-plated ice cream samples. The holes seen in the image of the ice cream sample were then measured using a 500x–5000x magnification.[29]

Analysis Volatile Organic Compounds

Analysis was done on the seventh day of storage. Using a continuous magnetic stirrer, a 5 g sample was first combined with internal standard (5 µL of 1,2-dichlorobenzene + 1 g of NaCl) in 15 mL vials. A silicone septum is used to seal the bottle tightly. The mixture was given 20 minutes to equilibrate at 40°C in a water bath.

The GC (Trace 1310, Thermo Scientific, Waltham, MA, USA) and MS detector (ISQ LT, Thermo Scientific, Waltham, MA, USA) used for the analysis. Using an HP-5MS UI column (30 m, 0.25 mm, and 0.25 µm), the volatiles were separated. The oven's programming called for a temperature of 40 °C for three minutes, followed by a ramp up to 210 °C (5 °C/min) and an elevation to 230 °C (15 °C/min). As a carrier gas, helium was utilized, and these temperatures were sustained for three minutes. Splitless injection mode was used, and the desorption process took 5 min at 250 °C. The mass scan range for the MS detector was 33-450 amu, and the detector was operated in electron ionization 70 eV. Both the transmission line and the source of ion were had their temperatures set to 250 °C.[28]

Statistical Analysis

Every experiment's data was collected in triplicate. IBM SPSS Statistic 26 was used for the data analysis. Analysis of variance was utilized for the group study of findings with a normal distribution for parametric test, and the Duncan multiple range test was employed at the 5% probability level. In the case of non-normal distribution results for non-parametric test, all groups were compared with a 5% probability using the Kruskal Wallis test. Following a Kruskal Wallis test, the results were compared using the Mann-Whitney test.

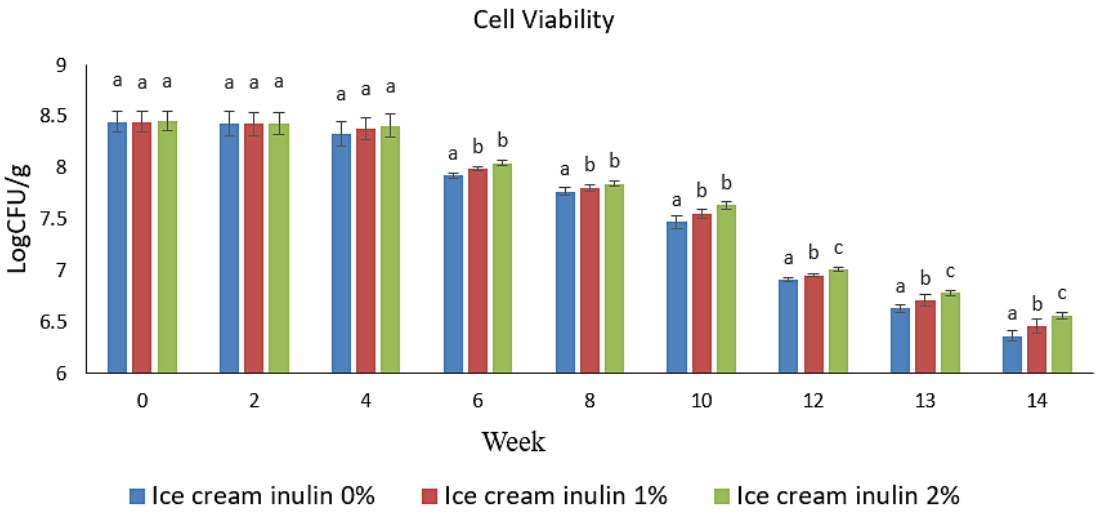

Fig. 1: Viability of *L. plantarum* Dad-13 in ice cream during storage. Different letters indicate the significant differences ($p < 0.05$)

Results

Viability of *L. Plantarum* Dad-13 In Ice Cream during Storage

While there was a trend toward a decrease in the viable counts of *L. plantarum* Dad-13 following storage, levels exceeding 6 log CFU/g were noted for every sample. Guidelines for probiotic products from the World Health Organization (WHO) specify that viable probiotic counts at least 6 log CFU/g. According to Figure 1, at the beginning of week 0, the cell viability of each sample, IC0 was 2.81 x 10^8 CFU/g, SIC1 was 2.80 x 10^8 CFU/g, and SIC2 was 2.84 x 10^8 CFU/g. In weeks 2 and 4 of the testing period, the three samples did not significantly differ. After the 6th week, significant differences began in samples that used and did not use inulin. This happened until the 10th week, SIC1 or SIC2 was found to have higher cell viability than IC0. After the 12th week, the highest cell viability was best for SIC2 (1.03 x 10^7 CFU/g), followed by SIC1 (9.01 x 10^6 CFU/g), and IC0 being the lowest (8.14 x 10^6 CFU/g). The decreases continued until the 14th week. The results showed that the three samples experienced a decrease of 1 log cycle until the 12th week. SIC2 showed the most stable cell viability until the 12th week of 1.03 x 10^7 CFU/g.

Physicochemical

According to Table 1, fermented milk samples with variations in 0%, 1%, and 2% inulin had pH values of 4.81, 4.75, and 4.75, respectively, which were not statistically different. Likewise, the titratable acidity value was around 1.05%. The pH rises after fermented milk was made into ice cream. There was no statistical difference seen between the pH values of fermented milk and ice cream, IC0 had a pH of 6.39, while SIC1 and SIC2 was around 6.38. The titratable acidity value of the samples with variations in 0%, 1%, and 2% inulin was 0.80%, 0.80%, and 0.79%.

Table 1. pH and titratable acidity with variation of inulin

Inulin	Fermented Milk		Ice Cream	
	pH	Acidity (%)	pH	Acidity (%)
0%	4.81 ± 0.05^a	1.05 ± 0.02^a	6.39 ± 0.05^b	0.80 ± 0.01^b
1%	4.75 ± 0.05^a	1.05 ± 0.02^a	6.38 ± 0.05^b	0.80 ± 0.00^b
2%	4.75 ± 0.02^a	1.05 ± 0.01^a	6.38 ± 0.05^b	0.79 ± 0.00^b

Different letters indicate the significant differences ($p < 0.05$).

Table 2: Overrun and melting rate

Samples	Overrun (%)	Melting Rate (%/20min)
IC0	33.99 ± 0.21^a	44.86 ± 0.33^a
SIC1	33.95 ± 0.27^a	44.15 ± 0.11^b
SIC2	35.72 ± 0.16^b	40.71 ± 0.06^c

Different letters indicate the significant differences ($p < 0.05$)

According to Table 2, IC0 and SIC1 had overrun values of 33.99% and 33.95%, respectively, which were not statistically different. Meanwhile, SIC2 had an overrun value of 35.72%, which shows the highest overrun value among the third samples. IC0, SIC1, SIC2 had statistically different melting levels of 44.86%, 44.15%, and 40.71% of the ice cream that melted in 20 minutes, respectively. In this study, SIC2 showed the slowest melting rate value, with 40.71% of the ice cream that melted in 20 minutes.

Hedonic Evaluation

According to Figure 2 IC0, SIC1, SIC2 showed results that were not statistically different in the color, sweetness, and acidity attributes based on the panelists. This indicates that, from a sensory standpoint, adding inulin to ice cream had no effect on its color, sweetness, and sourness attributes. Regarding texture, aroma, and overall attributes, the panelists assessed no significant difference between

SIC1 and IC0. Likewise, SIC1 was not statistically different from SIC2. Meanwhile, IC0 was statistically different from SIC2. Panelists preferred SIC2 based on aroma, texture, and overall attributes.

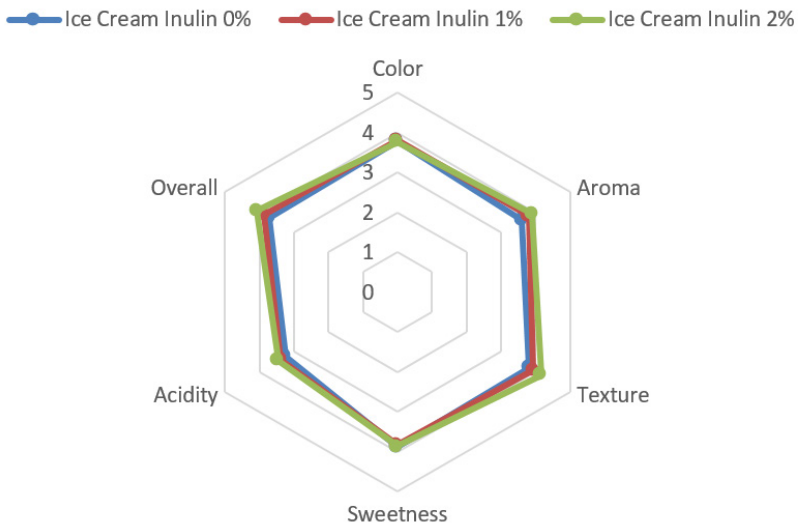

Fig. 2: Hedonic analysis of ice cream inulin 0%, ice cream inulin 1%, and ice cream inulin 2%

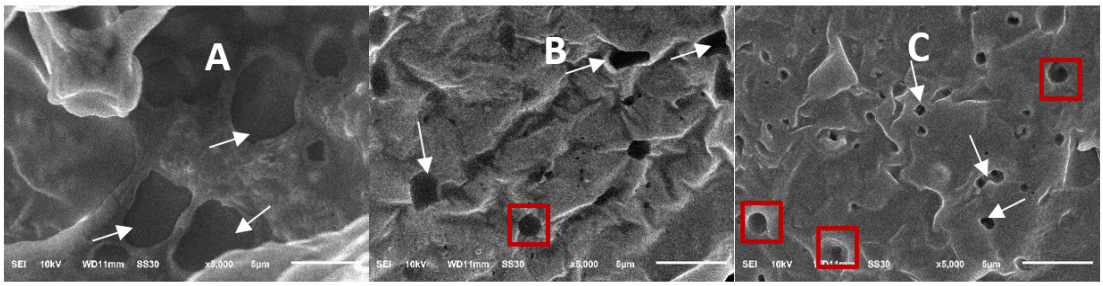

Fig. 3: Microstructure of freeze-dried ice cream using scanning electron microscopy (SEM): A (0% inulin), B (1% inulin), and C (2% inulin) with x5000 magnification. White arrow = ice crystal, red square = air bubble

Microstucture

Figure 3A showed the analysis results of IC0 with several larger ice crystals on the surface and no air bubbles found. Figure 3B shows the surface analysis results of SIC1. The ice crystals were smaller than SIC2 (Figure 3C). Figure 3C also showed that many ice crystals were tiny in size and also found some air bubbles. When compared between the three samples, it was clear that the ice crystals showed differences in size based on the addition of inulin. Based on the surface analysis, the sample with the highest inulin had smaller ice crystals and found some air bubbles.

Volatile Organic Compounds

The result of this study showed that 20 volatile organic compounds were identified. The compounds included aldehyde (1 compound), ketone (2 compound), carboxylic acid (1 compound), esters (7 compounds), alcohol (1 compound), and hydrocarbons (8 compounds). The compounds were classified into chemical groups based on literature and National Institute of Standars and Technology (NIST) library. Volatile organic compounds found between 1 and 40 minutes retention time did not all have Chemical Abstract Services (CAS) based on the NIST library. Furthermore, three volatile organic

compounds, such as 2-trifluoroacetoxydodecane, 4-propionyloxytridecane, and anthracene, 9-butyltetradecahydro-, were only found in the synbiotic ice cream samples.

Table 3: Volatile organic compounds in ice cream

Retention time (min)	Chemical Abstracts Service (CAS)	Volatile Organic Compound	Area (%)		
			Ice cream inulin 0%	Ice cream inulin 1%	Ice cream inulin 2%
13.91	1894-68-4	2-Trifluoroacetoxydodecane	-	27.78	15.03
16.67	72845-33-1	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-	9.03	-	-
16.79	948-60-7	Pterin-6-carboxylic acid	5.37	-	-
17.24	-	5-Cyclopropylcarbonyloxypentadecane	-	-	11.33
17.67	-	2-Trifluoroacetoxytridecane	-	-	11.35
21.00	40607-48-5	2-Octen-1-ol, 3,7 -dimethyl-	2.02	-	-
28.51	-	2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a, 5,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde	11.65	43.09	-
28.52	-	(7a-Isopropenyl-4,5 dimethyloctahy droinden-4-yl) methanol	-	-	36.79
28.65	55133-89-6	Anthracene, 9-butyltetradecahydro-	-	13.38	9.78
29.73	-	4-Propionyloxytridecane	-	5.50	6.40
32.07	56687-68-4	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	3.75	-	-
32.07	4727-18-8	Cyclopentadecanone, 2-hydroxy-	-	10.25	-
32.08	1725-04-8	Oxacyclotetradecan-2-one	-	-	9.31
34.05	112-39-0	Hexadecanoic acid, methyl ester	11.54	-	-
37.20	-	Methyl 9-cis,11-trans-octadecadienoate	3.99	-	-
37.31	13481-95-3	10-Octadecenoic acid, methyl ester	28.70	-	-
37.73	2490-25-7	Heptadecanoic acid, 10-methyl-, methyl ester	6.28	-	-
38.66	10544-96-4	Octadecane, 6-methyl-	2.67	-	-
39.96	110-94-1	Glutaric acid, di(2-propylpentyl) ester	8.72	-	-
40.06	-	2-Myristynoyl pantetheine	6.28	-	-

Discussion

Inulin supplementation could maintain cell viability while being stored, especially in SIC2. This characteristic can be linked to the inulin's gelling qualities and the increased water binding, which raise the mixture's viscosity and alter its rheology.[20,12] The character of water binding ability made free water bound with inulin. So, large ice crystal formation can be minimized by reducing free water. By reducing ice crystals size, cell damage able to be reduced during frozen storage so that cell viability can be better maintained.

Inulin added to synbiotic ice cream was also proven to increase probiotic cell survivability during storage. The probiotic viability was greater than 10^7 CFU/g (150-day storage), indicating the synbiotic ice cream's potential.[30] In the other study, it was demonstrated that all of the tested synbiotic apple ice cream formulations had viable counts of B. animalis Bb-12 and *L. acidophilus* La-5 (84-day storage). These ranged from 7.5 to 8.5 log CFU/g, indicating that the ice cream compositions serve as appropriate matrices for the probiotic distribution.31 In this study, the viability of *L. plantarum* Dad-13 in SIC2 decreased by 1 log cycle, which showed the most stable value during storage until the 12th week was still 1.03 x 107 CFU/g.

The fermented milk had pH values between 4.75 and 4.81, in synbiotic ice cream ranged from 6.38-6.39 not statistically different. The addition of inulin unaffected to the pH of fermented milk as well as the titratable acidity values ranged from 0.79 to 0.80 %. Ice cream made with fermented milk has an increased pH value and a decreased titratable acidity. This is caused by mixing with other ingredients, such as skim milk and whipped cream, which have a pH above the fermented milk.

The overrun, which represents the amount of air absorbed, is the proportion of the mixture's volume that is increased to the ice cream.[32] In this study, SIC2 showed the slowest melting rate value, with 40.71% of the ice cream melting in 20 minutes. This characteristic is linked by the enhanced binding of water and the gelling qualities of inulin, thereby increasing the viscosity and changing the rheology.[20,12] This property also affected to the melting rate. But because of its ability to retain water, inulin increases the rate at which ice cream drips and melts at first, preventing water molecules from moving and lowering the rate of water crystallization.[33,34] Therefore, the physicochemical factors, like melting rate and overrun, that are important in ice cream product, should be the same or even better to increase the acceptance.[31] In this study SIC2 got a score of 4, which means it was the most preferred by panelists based on aroma, texture, and overall attributes.

Scanning electron microscopy using x5000 magnification was done to analyze the microstructure. The results showed that IC0 had a larger ice crystal size. This is due to the process of ice recrystallization. Ice recrystallization is the formation of ice crystals that have previously formed without changing the mass of the ice.19 Meanwhile, SIC2 formed a smaller ice crystal size because the ice recrystallization process was prevented. The prevention of recrystallization is caused by inulin's capacity to bind water, raising the mixture's viscosity and altering its rheology.[20,12] A reduction in water molecule mobility and a reduction in ice recrystallization are the effects of inulin. However, some studies suggested polysaccharides might attach itself to the ice's surface and prevent the ice from recrystallizing.[18] Hence, this research showed that SIC2, in particular, has a better microstructure due to the prevention of ice recrystallization, which results in smaller ice crystals.

Volatile organic compounds analysis needs to be implemented to evaluate consumer acceptance of dairy products especially fermented milk. Probiotics may be used as starter cultures to produce fermented dairy products with improved flavor and/or aroma because they can produce distinctive flavor components. It is sufficient to say that strain affects the synthesis of flavor components in fermented dairy products.[35] It was thought that aldehydes and ketones had a significant role in the compositions of volatile organic compounds in fermented milk that contains probiotic.[36] Samples of ice cream without inulin and no ketone volatile organic compound were found. The ketone volatile organic compound only found SIC1 was cyclopentadecanone, 2-hydroxy- with percentage of area 10.25% while in SIC2, it was oxacyclotetradecan-2-one with percentage of area 9.31%. In IC0, the volatile organic compound that detected dominantly based on the percentage of area was 10-Octadecenoic acid, methyl ester with a percentage of 28.70%. Volatile organic compound in SIC1 was 2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a -octahydro-naphthalen-2-yl) -propionaldehyde with percentage area 43.09% while SIC2 was (7a-Isopropenyl-4,5 -dimethyloctahydroinden-4-yl) methanol with a percentage of 36.79%. Furthermore, several volatile organic compounds, such as 2-trifluoroacetoxydodecane, 4-propionyloxytridecane, and anthracene, 9-butyltetradecahydro, were only found in the synbiotic ice cream.

Conclusion

Cell viability in SIC2 decreased by 1 log cycle, which showed the most stable value during storage until the 12th week, which was still 1.03 x 10^{7} CFU/g. SIC2 showed the highest overrun value of 35.72% ± 0.16, and the slowest melting rate was 40.71% ± 0.06 portions of ice cream melted in 20 minutes. Overall attributes in the hedonic test of SIC2 showed a value of 4, which means it was the most preferred by panelists. The presence of inulin also improves the microstructure of ice cream, especially SIC2, which formed smaller ice crystals because the ice recrystallization process was prevented. The ketone volatile organic compound only found in SIC1 was cyclopentadecanone, 2-hydroxy- with

percentage of area 10.25% while in SIC2, it was oxacyclotetradecan-2-one with percentage of area 9.31%. Furthermore, several volatile organic compounds, such as 2-trifluoroacetoxydodecane, 4-propionyloxytridecane, and anthracene, 9-butyltetradecahydro, were only found in the synbiotic ice cream. The synbiotic ice cream in this study has the potential to be a functional food containing probiotic indigenous *L. plantarum* Dad-13 and prebiotic inulin.

Acknowledgements

The authors are grateful to Educational Fund Management Institution LPDP, Ministry of Finance of the Republic of Indonesia for the scholarship. We are also thankful to the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia through the University Center of Excellence for Research and Application on Integrated Probiotic Industry, Gadjah Mada University, Yogyakarta, Indonesia, for the provision of probiotic cultures.

Funding Sources

This study received funding from Educational Fund Management Institution LPDP, Ministry of Finance of the Republic of Indonesia (Contract Numbers KEP-301/LPDP/LPDP.3/2022).

Conflict of Interest

The authors declare no conflict of interest.

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