Physical characteristic of probiotic ice cream substituted by encapsulated lactic acid bacteria (LAB) with variety of aging time

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Abstract. Probiotic ice cream is one of methods in improving benefit and functional value of common ice cream. This research studies about the effect of probiotic added in form of capsules and maturation (aging) time on the physical quality and sensory acceptability. The article presents data of overrun, melting time, viscosity, and panelists’ preferences in terms of probiotic ice cream characteristics. The data is calculated statistically by using ANOVA. The results show that aging time determines most of evaluated physical characteristics, while inserting probiotic capsules tends to affect overrun specifically. Combination between a moderate percentage of probiotic capsules and aging time, 5% and 18 hours respectively, generates good and preferred quality of probiotic ice cream in all evaluated characteristics.

1. Introduction

Ice cream is a milk-based frozen food product that has semi-solid consistency, contains nutritional value but does not have functional properties in maintaining health [1], so that it needs probiotic fortification to add its value and beneficial properties.

Probiotic ice cream is an ice cream containing probiotic bacteria or lactic acid bacteria (LAB) that can balance the micro flora in the digestive tract. The obstacle in producing probiotic ice cream is that a series of processing stages, especially stirring and freezing steps. Stirring on ice cream causes oxygen to diffuse into the mixture and form toxic compounds such as superoxide and hydroxide which cause damaging of probiotic cells. In addition, the viability of probiotic bacteria decreases due to the freezing process and formation of ice crystals inside and outside the cell causing cell death [2] [3] [4]. The application of probiotic encapsulation methods is a proper technique to address this problem and maintain the living probiotic cells.

The encapsulation method of probiotic bacteria is the right way to protect cells, improve the viability of LAB, and maintain its functional properties until the product is consumed [5]. The addition of probiotic capsules is completed after the homogenization process or before the freezing and hardening stage [6][6]. The percentages of probiotic capsules added to ice cream are varies, depending on the effectiveness of the capsules. The production of probiotic ice cream can add 2% until 5% probiotic cultures into the ice cream mixture [5] [7].
The quality of probiotic ice cream may also depend on the maturation or aging process in forming stability of macromolecules. Aging is a storing step of ice cream mixture at 4°C for 4 hours to 24 hours. It aims to crystallize fat, hydrate proteins and carbohydrates, formulate membranes, interact between protein and emulsifier, and increase coalitions of components. This condition can improve the development and texture of ice cream. Aging time affects the quality and acceptance of consumers of ice cream produced [8]. This study aims to examine the physical properties and consumer preferences of probiotic ice cream which is made from addition of probiotic capsule and variety of aging time.

2. Methodology
The materials used in this research were strains of Lactobacillus bulgaricus, carrageenan, alginate, MRS agar and broth (Merck). The tools used in this research were digital scales, laminar flow cabinet (Bassaire Model 04HB; Astecair 3000L), incubator (Eyela Personal Incubator SLI-170D; Eyela Soft Incubator SLI-450N), waterbath (Memmert), thermometer, centrifuge (HettichZentrifugen EBA 20), hot plate (DIAB MS-H280-Pro) and glassware.

This study used two factors, namely percentage of probiotic capsule (2.5%, 5.0%, and 7.5%) and aging time (12 hr, 18 hr, and 24 hr). The data was analyzed using ANOVA statistically. Probiotic capsule was made prior to the ice cream. Extrusion method was used for encapsulation of L. bulgaricus. L. bulgaricusin pellet form was dissolved into sterile distilled water for a culture solution. Carrageenan as encapsulating agent was mixed with 3% of tofu waste flour. The culture solution and the encapsulating solution were mixed with a ratio of 1:4. This mixture was dripped into the hardener solution. The finished capsules were stored in the refrigerator [9] [10].

The ice cream formula consisted of cornstarch (3.4%), granulated sugar (17%), powdered skim milk (10.8%), full cream milk (25.2%) mixed and heated at 40-50°C by adding the ratio of coconut milk and water (3:1). The used formulation was a modification between laboratorial test and some references [6] [11]. Furthermore, this mixture was pasteurized at a temperature of 67-70°C for 20 minutes and cooled at 4°C (aging) in accordance with the treatment of aging time (12, 18, 24 hours). After that, the ice cream mixture (velva) was mixed with emulsifier/sucrose ester (6.68%) and homogenized for 10 minutes, and probiotic capsules were added (2.5%, 5.0% and 7.5%) according to treatment. The ice cream mixture was frozen and stored at range from -10°C to -18°C.

Analyses in this research were physical characteristics of ice cream (overrun, melting time, and viscosity) and organoleptic test. The percentage of ice cream volume increasing over the mixture volume was calculated by comparing the gap volume with the mixture volume [12] [13] [14]. Melting time of ice cream was examined by placing 3 grams ice cream on a wire screen (54 holes/inch²) on the top of a cup. It was left in room temperature about ±2°C and the time of first drip and the dripped weight was recorded until 30 minutes [15]. Viscosity was measured by using Brookfield Viscometer DV-II Pro instrument. Organoleptic test was conducted by using 15 panelists to assess probiotic ice cream based on preference parameters [16].

3. Results and Discussion
3.1. Physical Characteristics
3.1.1 Overrun. Overrun is percentage increase in volume of ice cream caused by the formation of air bubbles in the ice cream mixture during homogenization in which they are surrounded by fat globules in an emulsion system and maintained until frozen condition. Overrun is one of the important physical properties of ice cream related to softness of the texture due to air trapping condition. Ice cream will have good quality if it has an overrun value between 60-100% [17].
Based on Figure 1, it can be seen that, as overall, overrun of all samples tested meet the requirements of good ice cream quality, with an average overrun of 67%. The two factors tested in the study showed that the longer aging time, the lower percentage of overrun produced, while the higher percentage of probiotic capsules added, the slightly higher percentage of overrun. A higher overrun improves the softness and creaminess of ice cream [14]. The longest aging time (24 hours) might cause a decrease in the ability of milk protein to bind free water, so that the amount of free water increases [11]. The increase of free water in ice cream mixture reduces its viscosity which is linearly related to overrun. Factors influencing overrun are viscosity, fat, emulsifiers, stabilizers, and processing conditions [18]. Aging time for 18 hours indicates more stable value in all combinations compare to 12 and 24 hours.

The optimal percentage of probiotic capsules up to 5% can increase overrun. It might occur because the capsules along with the fat globules also preserve the trapped air in ice cream mixture, so that the volume becomes elevate. However, adding probiotic capsules in the highest number (7.5%) can hinder the dispersion of fat globules and inhibit the formation of air bubbles in the ice cream mixture. Therefore, it may reduce overrun of the ice cream.

3.1.2. Melting time. Melting time is the amount of time needs for ice crystals to melt perfectly at room temperature. A long melting time is one of preferred characteristics of ice cream.

![Figure 2. The effect of aging time on melting time of probiotic ice cream](image-url)
Figure 2 shows that aging time affects melting period, which is the longer aging time; the longer time is needed for ice crystal to be melted. Aging is the maturation stage of ice cream dough/mixture stored at 4°C for about 3 to 24 hours. It aims to optimize the ability of stabilizer and protein to bind water to the dough so that it forms an ice cream mixture that has more stable viscosity [19]. Based on the graph, aging time of 12 hours and 18 hours do not show a significant difference in melting time, compared to 24 hours. Related to slightly low overrun of 24 hours aging treatment, a mildly longer melting time in 24 hours aging treatment is not necessarily caused by water binding, but perhaps, it might occur due to ice crystal formation of the free water.

3.1.3 Viscosity. Viscosity measurement was carried out on ice cream mixture after aging time. This analysis was conducted to assess the level of viscosity of ice cream mixture and its effect on other physical characteristics, such as overrun.

![Figure 3. The effect of aging time on viscosity of probiotic ice cream](image)

Based on Figure 3, it can be comprehended that the longer the aging time, the lower viscosity of the ice cream mixture. High or low viscosity of ice cream dough affects the percentage of ice cream overrun (Fig 1). The highest viscosity can be achieved by applying aging for 12 hours, but the overrun value (Fig 1) is not stable enough for all treatment combinations. The lowest viscosity on 24 hours aging time (5889 Cp) is also not desirable because it causes a more like-liquid ice cream in terms of its texture. Aging time for 18 hours has a fairly good thickness, preferred consistency and texture to produce a stable ice cream in all combinations.

3.2 Sensory Evaluations
There are five parameters used in sensory evaluation to assess the quality of probiotic ice cream based on panelists’ preferences, which are texture, taste, aroma, appearance and acceptance (Table 1). Additional probiotic capsules did not affect to the organolectic parameters, while aging time cause the difference of consumers’ perception, especially about taste and acceptance of the ice cream. Panelists prefer probiotic ice cream with a shorter aging time rather than the longer ones with 4.06 or like for taste and 4.15 or like for acceptance. A very long aging time can cause components in ice cream absorb volatile compounds around it and affect its taste. In addition, a long aging time also causes the tendency of releasing protein bonds and stabilizers to free water, so that the thickness of the ice cream mixture decreases and becomes more watery. This condition also influences the taste formed by the diffusion of flavor compounds in a large number of water components. The decrease in panelists’ preference of taste may affect the overall panelists' acceptance of probiotic ice cream.
Table 1. Preference Level of Probiotic Ice Cream Characteristics

| Aging Time | Texture | Taste | Aroma | Appearance | Acceptance |
|------------|---------|-------|-------|------------|------------|
| 12 Hours (W₁) | 4.01<sup>a</sup> | 4.06<sup>b</sup> | 3.75<sup>a</sup> | 3.91<sup>a</sup> | 4.15<sup>b</sup> |
| 18 Hours (W₂) | 3.86<sup>a</sup> | 3.81<sup>a</sup> | 3.65<sup>a</sup> | 3.86<sup>a</sup> | 3.87<sup>a</sup> |
| 24 Hours (W₃) | 4.02<sup>a</sup> | 3.86<sup>a</sup> | 3.64<sup>a</sup> | 3.80<sup>a</sup> | 3.90<sup>a</sup> |

Scale: 1=very dislike; 2=dislike; 3=neutral; 4=like; 5=very like
<sup>a-b</sup>Means in the same column followed by different letters were significantly different (p< 0.01)

However, overall, all of samples were evaluated in “like” scale (3.64 – 4.15) in which all combinations of probiotic ice cream had good quality and sensory characteristics (Table 1).

4. Conclusion
Addition of encapsulated probiotic bacteria and using of different aging time affect the physical quality of probiotic ice cream. A proper percentage of probiotic capsules could improve overrun of ice cream (68.8 – 90.6%), but using capsules higher than 5% might reduce the overrun until 45.6%. Aging time occupies dominant effects to melting time, viscosity and most of organoleptic parameters. An extended aging time (24 hours) is required to prolong melting time (9.91 minutes), while it might produce low viscosity of ice cream mixture (5889 Cp) and decrease the preference level of panelists in terms of taste and acceptance (3.86 and 3.90 respectively). Based on the data, it may conclude that 5% of probiotic capsule with 18 hours of aging time are good combination to produce high quality of probiotic ice cream.

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