

Shelf-life prediction of blondo-based probiotic ice cream using accelerated shelf-life testing method

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ABSTRACT

Blondo is a by-product of VCO production, commonly used for feed despite its rich nutrition and physiological properties. To reutilize blondo and take advantage of its nutrition, blondo probiotic ice cream is developed as a plant-based functional food. Determining the shelf-life of food products is very important because it reflects the product's durability during the time interval between production and consumption. This study aims to determine the shelf-life of blondo-based probiotic ice cream using the Accelerated Shelf-Life Testing (ASLT) method with the Arrhenius model. Blondo-based probiotic ice cream was stored for 28 days at temperatures of -6°C, -11°C, and -18°C. The parameters observed were total Lactic Acid Bacteria (LAB), total acid, pH value, and total solids. The results of the calculation of the Arrhenius model selected the total LAB parameter as a critical parameter. Therefore, we considered using the term "best before" to refer to probiotic efficacy instead of shelf-life. The blondo-based probiotic ice cream is best consumed before if stored at -6°C is 206.80 days or 6 months 27 days; at -11°C is 186.47 days or six months 6 days; and at -18°C is 160.22 days or 5 months 10 days.



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INTRODUCTION

Blondo is a plant-based protein that contains essential amino acids and can be used as an additional ingredient or an alternative to highly nutritious food at a low price (Figure 1). Blondo is produced from the coconut oil extraction process, and the coconut milk produced from the extraction process will be allowed to stand so that it forms a layer of skim at the bottom and cream at the top. Blondo is generally used as food with low economic value, such as chili products or other food additives (Tahir et al. 2018). Blondo contains high nutritional content, so it has great potential and can be an alternative food ingredient to replace non-fat solids in making ice cream.



Figure 1 Dried-blondo used in this research

Ice cream is a frozen dessert usually made from dairy products, such as milk, often combined with other ingredients and flavors. In addition to dairy products, ice cream contains sugar, stabilizers and emulsifiers, flavoring materials, water, and air. Ice cream can also be made from non-dairy ingredients such as blondo and coconut milk. One of the constituent components of ice cream is fat derived from animal fat or vegetable fat. The function of fat in the formation of ice cream is to contribute to melt resistance, crystallization, cold sensation, creamy mouth-feel, desirable flavor, or acting as a flavor carrier, which contributes largely to the quality of ice cream through the formation of a partially agglomerated continuous network of homogenized fat globules (Liu et al. 2018).

Commercial ice cream generally has a fat content of 10-12%, while according to SNI 01-3713-1995 specified for ice cream, the fat content is at least 5.0% w/w (BSN 1995). The composition of ice cream varies greatly depending on the type.

Ice cream consists of various kinds distinguished by its fat content. Economy ice cream has a fat content of 8% (Aziz et al. 2018). Most ice cream is made from animal ingredients, so vegetarians and people allergic to dairy tend to avoid ice cream. However, this can be overcome by replacing animal milk's essential ice cream ingredients with plant-based ingredients. Plant-based ice cream is a frozen dessert with almost the same characteristics as ice cream but does not use animal milk, so the fat content is lower. Producing plant-based ice cream is a technological challenge due to milk and dairy ingredients' unique flavor and structure. Still, it has promising nutritional and functional features, technological properties, and overall sensory appeal (Pontonio et al. 2022).

Consumers place great importance on eating healthy, saving time and energy, and consuming pleasurable food. This has increased public awareness of consuming functional food (Nystrand and Olsen 2021). The increased consumption of functional food comes not only from its nutritional value but also from the health benefits of these foods. Functional food promotes optimal health and reduces the risk of noncommunicable diseases. One of the functional food products is plant-based probiotic ice cream. Probiotic ice cream is a modification with probiotics as a constituent component. A Probiotic is a live microorganism that, when ingested or locally applied in sufficient numbers, provides the consumer with one or more proven health benefits (Kamil et al. 2021). Probiotic foods should have viable microorganisms at levels 10^6 - 10^7 CFU/ml at ingestion (Udayarajan et al. 2022).

As mentioned above, plant-based ice cream using blondo and supplemented with probiotics is an innovation of functional food. As a new functional food product, blondo-based probiotic ice cream must offer good quality. Since the incorporation of blondo as a by-product of VCO production can affect the ice cream quality, such as easily becoming rancid and further changing the acidity. In addition, concerning the health benefits of probiotics, viability during storage is important, whether the probiotic can survive and produce its metabolite at a slow growth rate or die due to the extreme condition. Therefore, shelf-life prediction is relatable when applied to blondo-based probiotic ice cream.

Shelf life is when a food product remains safe and complies with physical and microbiological characteristics when stored under the

recommended conditions. There are various methods for estimating the shelf-life of food products. One is the ASLT method, which has been applied to predict the shelf-life of gummy candy probiotics (Kamil et al. 2021). The ASLT procedure is thus frequently proposed to speed up the shelf-life assessment process by using accelerated storage conditions, so the ASLT procedure allows the estimation of product shelf-life under storage conditions (Calligaris et al. 2019). Therefore, this study aims to determine the shelf-life of blondo-based probiotic ice cream using the ASLT method with the Arrhenius model.

METHOD

Materials

The ingredients used were blondo (Bibit Rejeki, Indonesia) egg yolk, yellow sweet potato, honey (La Tahzan, Indonesia), sugar (Rose brand, Indonesia), Carboxy Methyl Cellulose (CMC) (Koepoe Koepoe, Indonesia), de Man Rogosa Sharpe (MRS) medium (Merck, USA), *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* culture starter (Yoghurtmet, Canada), NaOH 0.1 N (Merck, Canada), and phenolphthalein indicator (PP) (Merck, Canada).

Raw Material Composition

Blondo contains 13.96% water content, 11.38% protein, and 38.01% fat. The raw material for milk substitute in the product is blondo, homogenized with coconut milk. The addition of blondo and coconut milk contains a water content of 69.10%, 2.71% protein, and 14.33% fat.

Storage Conditions

Samples were stored in the freezer at -6°C, -11°C, and -18°C. Samples were observed every 7 days until the 28th day. During storage, one cup of ice cream from each temperature is analyzed for total lactic acid bacteria (LAB), acid, pH, and solids.

Analysis of Total LAB

LAB was quantified using the pour plate method and MRS (De Man – Rogosa – Sharpe) agar medium. In brief, 1 g of samples was diluted into 9 ml of 0.85% saline water. At the appropriate dilution series, 1 ml of the suspension was inoculated on Petri and poured with MRS medium. The sample was then incubated for 48 hours at 37°C.

Analysis of Total Acid

Total acid was quantified according to (Ningsih et al., 2018). Ten milliliters of the sample were added with aquadest 100 ml and homogenized. After that, 10 ml of the mixture was analyzed for total acid using the PP (phenolphthalein) indicator titration method.

Analysis of pH

The pH of ice cream was quantified according to (Tumober et al. 2021). Twenty grams of ice cream was left to melt for 15-30 minutes at 25°C. The sample was then analyzed using a calibrated pH meter.

Analysis of Total Solids

Total solid analysis was done using gravimetry (Purdi et al. 2020). Briefly, 5 g of the sample was oven-dried to evaporate all the water content for 12 hours. The total solid was calculated as 100% subtracted from the sample's moisture content.

Shelf-life Prediction

The shelf-life prediction analysis was conducted according to Kamil et al. (2021). The first step in predicting shelf-life using the Arrhenius approach is to plot the quality change (N: total LAB, total acid, pH, total solid) versus time (t) for zero order, also the ln (N: total LAB, total acid, pH, total solid) versus (t) for first order, to obtain linear equations for each parameter as shown in the Equation (1) and (2)

$$N = N_0 - k_t \quad (1)$$

$$\ln N = \ln N_0 - k_t \quad (2)$$

N_0 is the initial parameter, N is the change of each parameter in everyday storages, t is storage time, and k is the inactivation rate constant (day^{-1}). The order reaction is selected from the curve equation, which has an R^2 close to 1. The slope, which represents the (k) value from the selected order reaction, was further used to obtain the Arrhenius equation as shown in the equation (3).

$$\ln k = \ln k_0 + \left(-\frac{E_a}{R} \cdot \frac{1}{T} \right) \quad (3)$$

E_a is the activation energy (kcal/mol), R and T are the universal gas constant ($R: 1.987 \text{ cal} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$), and the absolute temperature ($^{\circ}\text{K}$), respectively. Shelf-life prediction is obtained from the difference in quality change after storage and

before storage and divided by the value of k . The following equation (4) and (5) were used for the shelf-life prediction.

$$ts \text{ (for zero order)} = \frac{(N_0 - N_t)}{k} \quad (4)$$

$$ts \text{ (for first order)} = \frac{(\ln N_0 - \ln N_t)}{k} \quad (5)$$

ts is the predicted shelf-life, and N_t is the limit value of each parameter at the end of storage.

RESULT AND DISCUSSION

Chemical characteristics of ice cream

According to Zang et al. (2023), data on the initial quality of the product before storage is needed to determine the shelf-life prediction model and its validation. The initial characteristics of ice cream will help determine changes in the quality of ice cream after being stored at different temperature conditions. The initial quality characteristics of blondo-based probiotic ice cream can be seen in Table 1.

The initial value of the total LAB of ice cream is 8×10^9 CFU/ml. This value follows the minimal viable cell of probiotic products, 10^6 CFU/ml or /gr (Kamil et al. 2021). The initial value of total acid is 0.90%, and the pH value is 4.20. The total acid is still in the range of the standard acid of yogurt, which is 0.5 – 2.0%, as regulated in SNI 2981:2009 (BSN 2009). The initial total solids value of plant-based probiotic ice cream was 30.50%. The total solid value for ice cream products is under the SNI 01-3713-1995, which is at least 34% (Badan Standardisasi Nasional 1995).

Table 1 Initial Quality Characteristics of Plant-Based Probiotic Ice Cream

Parameter	Value
Total LAB	8×10^9 CFU/ml
Total Acid	0.90%
pH Value	4.20
Total Solids	30.50%

The Changes in Total LAB During Storage

The initial total LAB value of plant-based probiotic ice cream was 8×10^9 CFU/ml. This value is higher than the minimum requirement for probiotic products, 10^6 CFU/ml, and therefore meets the standards as a probiotic product. During storage, the total LAB decreased consistently. The

fastest decrease in total LAB occurred at -18°C , whereas at -6°C , the rate of decline was slower.

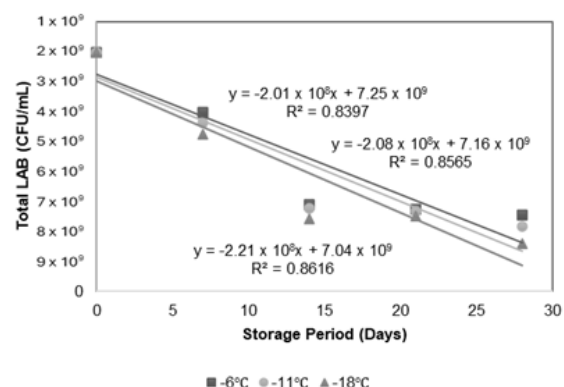


Figure 2 The Changes in Total LAB During Storage

Figure 2 shows the results of the total LAB analysis of blondo-based probiotic ice cream during 28 days of storage. The total LAB of ice cream at the end of the 28th day of storage at -6°C , -11°C , and -18°C , respectively, were 2.5×10^9 ; 2.2×10^9 ; and 1.6×10^9 CFU/ml. The decrease in total LAB becomes faster, along with lower storage temperature. Freezing temperature storage causes lactic acid bacteria to experience osmotic injury to cell proteins, such as membrane injury, protein and enzyme denaturation, and DNA damage, resulting in cell death (Kandil and Soda 2015). Extreme temperatures cause the inactivation of enzymes and the function of cell structures, such as cell membranes. Decreasing temperature causes a decrease in the fluidity of the phospholipid bilayer that makes up the cell membrane, causing an increase in the concentration of dissolved compounds in bacterial cells, which can encourage osmotic injury to cell proteins.

The Changes in Total Acid During Storage

Figure 3 shows the decrease in the total acidity of blondo-based probiotic ice cream during 28 days of storage. On the 28th day of testing, the total ice cream acid at -6°C , -11°C , and -18°C decreased to 0.80%, 0.76%, and 0.76%. At -18°C , the reduction in total acid occurs faster when compared to -6°C and -11°C storage, so the lower the storage temperature, the faster the damage reaction rate. The decrease in total acid was in line with the reduction of total LAB.

The total acid reduction was because lactic acid bacteria were dormant during frozen storage, and some cells died (Evivie et al. 2017). Bacterial cell death is triggered by environmental osmotic

pressure. Freezing causes a change in the internal osmolarity of cells, resulting in cell death. A decrease in the concentration of H^+ ions in the microbial growth medium at freezing temperatures can disrupt the balance of the selective permeability properties of the cell membrane to the electrolytes contained in the medium, as well as the concentration gradient between cells and their environment. This changes cells internal osmolarity, which will impact cell death.

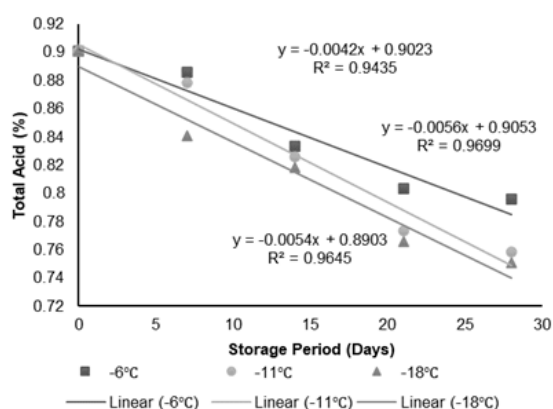


Figure 3 The Changes in Total Acid During Storage

The Changes in pH Value During Storage

The pH value of ice cream continues to increase every week. Figure 4 shows the increase in the pH of ice cream during 28 days of storage. On the 28th day, the pH of ice cream at -6°C , -11°C , and -18°C is 4.39, 4.42, and 4.49. The increase in pH was in line with the decrease in total LAB and total acid in ice cream. During frozen storage, lactic acid bacteria will experience dormancy and death due to osmotic pressure; LAB will be unable to carry out metabolic activities and produce lactic acid during frozen storage.

Blondo, one of the essential ingredients for making ice cream, can experience oxidation due to more extended storage and contact of oil with oxygen during storage. The primary oil damage due to oxidation events results in the formation of peroxides and aldehydes. Fat oxidation produces primary oxidation products such as hydroperoxides. It can be decomposed into secondary oxidation products, including aldehydes, aliphatic alcohols, formic acid, formate ester, shorter chain fatty acids, and species with higher molecular weights (Kumar 2016). Oil oxidation produces oxygen-derived free radicals and hydroxylation products, becoming FFAs, aldehydes, and ketones. The $C=O$ functional

group of ketone or aldehyde compounds has basic properties (Monika et al. 2021). The alkaline nature produced by this aldehyde will affect the acidity of the ice cream.

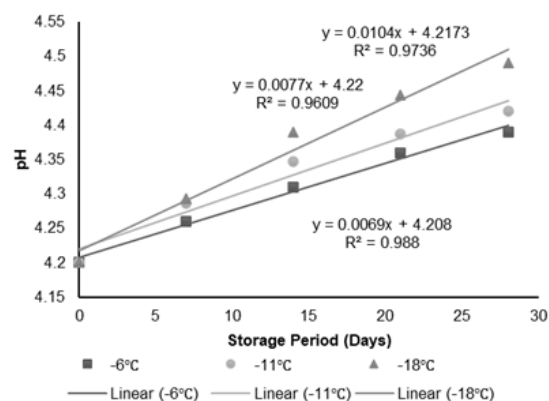


Figure 4 The Changes in pH Value During Storage

The Change of Total Solids During Storage

The total solids value of ice cream decreased the longer the storage time. The fastest decrease occurred at the highest storage temperature, which was -6°C , whereas the slowest decrease occurred at -18°C . Figure 5 shows the decrease in total solids during 28 days of storage. On the 28th day of testing, the total ice cream solids at -6°C , -11°C , and -18°C decreased to 29.55%, 29.83%, and 30.14%. The increase in water content aligns with the decrease in total solids.

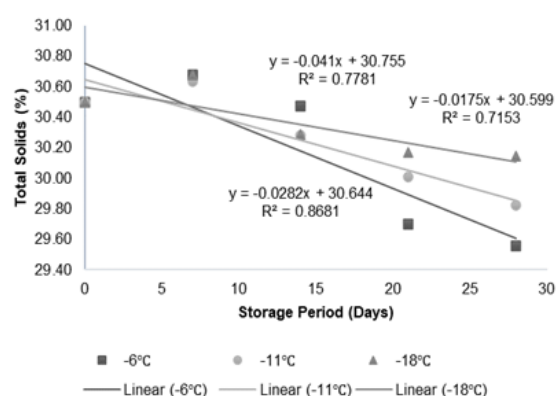


Figure 5 The Changes in Total Solids During Storage

In ice cream products, an increase in temperature causes recrystallization, which can affect the quality of the product, both the physical and sensory quality. Lowering the freezing point of liquids can reduce crystallization during freezing to produce smoother products with smaller ice crystals. Ice recrystallization causes cell death (Deller et al. 2014). The longer storage, the more ice crystals form, and many bacterial

cells are damaged (Dewi et al. 2015). Freezing will form ice crystals; the larger the ice crystals form, the harder the texture of the ice cream will be. The higher water content during cold temperature storage in ice cream products causes larger crystals to form so that the texture of the ice cream becomes rough. During the storage period, syneresis can occur, namely the separation of solids and liquids in probiotic beverage products. Syneresis in ice cream can occur during freezing and storage (Abd Al-Maqsoud et al. 2022).

Arrhenius-Based Acceleration Method

The acceleration method based on a semi-empirical approach is carried out with the help of the Arrhenius equation with kinetic theory using a zero-order or first-order reaction (Setyadjit et al. 2017). The data is plotted on a graph of the relationship between storage time (days) and the average decline in quality/day (k); the x-axis represents storage time, and the y-axis represents the average decline in quality/day (k). Data plotting was carried out to obtain a regression equation to determine the value of the reaction rate constant at each storage temperature and its R^2 value.

The order reaction with a more excellent R^2 value or closer to 1 is the order reaction used by that parameter. Table 2 shows that the total LAB, total acid, and total solids use a first-order reaction, while the pH value uses a zero-order reaction. Each linear regression equation for each temperature will get the value of k (slope), a constant x. The k value of each linear regression equation for each temperature is converted into the $\ln k$ form and plotted against $1/T$ ($^{\circ}\text{K}$) to form a

linear regression equation graph equivalent to the Arrhenius equation.

Shelf-life Prediction

The determination of shelf-life begins with the determination of critical quality parameters, which are determined from changes in quality with the lowest activation energy. The parameter that has the highest R^2 value is the total LAB parameter. In addition, the total LAB parameter also has the lowest activation energy value and slope (k), so the total LAB parameter is used as a critical parameter in determining the shelf-life of plant-based probiotic ice cream. The parameter that has the most excellent R^2 value is the total LAB (Table 3). The value of R^2 or the coefficient of determination is a value that measures how far the model's ability to explain the dependent variable, which in this case is the effect of temperature (x-axis) on the rate of change of total LAB (y-axis).

The shelf life of blondo-based probiotic ice cream according to total LAB parameters at -6°C , -11°C , and -18°C were 206.80 days, 186.47 days, and 160.22 days (Table 4). The shelf-life of blondo-based probiotic ice cream is still relatively short compared to the shelf-life of ice cream in general, up to 24 months (Park et al. 2018). The shelf-life of blondo-based probiotic ice cream depends on the total LAB parameter. It is due to freezing temperatures, which are unsuitable for the growth of lactic acid bacteria. Therefore, we consider using the term "best before" for probiotic efficacy, which means blondo-based probiotic ice cream can offer the health benefit of probiotics if consumed before the date with the other acceptable parameters.

Table 2 Determination of the order reaction for total LAB, total acid, pH value, and total solids

Quality Parameter	Temperature ($^{\circ}\text{C}$)	R^2		Selected Order
		Zero-Order	First-Order	
Total LAB	-6°C	0,8397	0,865	1
	-11°C	0,8565	0,9039	
	-18°C	0,8616	0,9222	
Total Acid	-6°C	0,9435	0,9458	1
	-11°C	0,9699	0,97	
	-18°C	0,9645	0,97	
pH Value	-6°C	0,988	0,9867	0
	-11°C	0,9609	0,958	
	-18°C	0,9736	0,9709	
Total Solids	-6°C	0,7781	0,7788	1
	-11°C	0,8681	0,869	
	-18°C	0,7153	0,7152	

Table 3 Arrhenius equation and activation energy (Ea)

Parameter	Arrhenius Equation	R ²	Ea (cal/mol)	Slope
Total LAB	$y = 1448,1x - 8,56$	0,9954	2875,9266	1448,1
Total Acid	$y = 1463,2x - 10,721$	0,565	2905,9152	1463,2
pH Value	$y = 2367,9x - 13,867$	0,9765	4702,6494	2367,9
Total Solids	$y = -4738x + 11,136$	0,9823	9409,668	4738

Table 2 Shelf life of plant-based probiotic ice cream

	Temperature (°C)	Shelf Life
Total Lactic Acid Bacteria	-6°C	206,80 days
	-11°C	186,47 days
	-18°C	160,22 days

CONCLUSION

Based on the research conducted, it can be concluded that blondo-based probiotic ice cream stored at three different temperatures experienced a decrease in quality during storage, including a reduction of total lactic acid bacteria, bacterial cells damaged by freezing temperatures, a decrease in total acid and an increase in pH value due to reduced total LAB in the product and decreased total solids due to the formation of ice crystals due to increased water content. The critical parameter is the total LAB because it has the smallest Ea and depends on temperature. The blondo-based probiotic ice cream is best consumed when stored at -6°C, -11°C, and -18°C, respectively, were 206.80 days, 186.47 days, and 160.22 days.

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