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Shelf Life Estimation of Probiotic Peanut Chocolate Jam by Accelerated Shelf Life Testing (ASLT) Method

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Abstract

Probiotics are beneficial bacteria found in probiotic products that can maintain the balance of microflora in the gut. Peanut chocolate jam has the potential to be developed into a probiotic food product. However, the shelf life of this product has not been determined so it is necessary to determine the shelf life using the Accelerated Shelf Life Testing method. This method uses the Arrhenius approach. Determination of shelf life through the calculation of total lactic acid bacteria, a_w value, pH value, and total acid during storage. The storage of probiotic peanut chocolate jam used three temperature treatments: 4 ℃, 30 ℃, and 37 ℃. Observations of samples stored at 4 ℃ were made every 5 days until day 25, while samples at 30 ℃ and 37 ℃ were made every 3 days until day 15. The data were analyzed using Microsoft Excel 2016 through the presentation of data in the form of a scatter diagram accompanied by a trendline and then described descriptively. The shelf life of probiotic peanut chocolate jam based on best before stored at 4 ℃, 30 ℃, and 37 ℃ were 49.52 days; 10.98 days; and 7.64 days, respectively. The jam can still be consumed up to 255.29 days; 86.73; and 66.89 days but no longer has the benefits of the probiotics.

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Introduction

Consumer interest in health and fitness is on the rise. This will also influence nutritional habits and food choices. Consumers' nutritional understanding has changed from simply meeting energy needs to providing a healthy and balanced nutritional profile. Functional foods are becoming one of the most sought-after by consumers today. In addition, consumers are also looking for products that are quite practical due to high mobility in this era of globalization. The market for probiotic functional foods is on the rise as information regarding good gut health is a major factor in the proper functioning of the body and makes consumers interested in healthy food choices (Gkitsaki et al., 2024).

Probiotics have attracted special attention due to their functional and nutritional properties, and various studies have been developed to determine their durability in probiotic products. According to Soares et al. (2023) probiotics, especially lactic acid bacteria (LAB) strains, can be damaged or lose viability due to adverse conditions in the manufacture of certain foods or due to their intrinsic characteristics. Probiotics used include *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* bacteria. According to Wu et al. (2021) there are three potential probiotic strains from different genera, namely *Lactobacillus*, *Streptococcus*, and *Bifidobacterium*. Products with these microorganisms can be considered as probiotics because they can have beneficial effects on health when consumed in certain amounts.

One of these practical daily food products is jam as a complementary food ingredient. According to Merda and Novitasari (2017) jam is a food product processed from various kinds of fruit and has a thick texture, then in general jam has a solid or semi-solid form, and the selection of fruit for making jam is usually ripe fruit. Groundnut (*Arachis hypogaea*) is added as an additive to chocolate jam to improve texture, flavor, aroma, and nutrition in probiotic chocolate jam. According to Alim et al. (2024) peanut is a local product with nutrients needed for humans and contains important micronutrients such

as vitamins and minerals for development and growth, and can be considered as a source of protein that is cheap, rich, distinctive, and superior among other vegetable proteins so it is suitable to be used as a jam that has high nutritional content.

The shelf life of a food product is very important to know. In addition to knowing the expiration date as it relates to consumer safety and health, it can also be a benchmark for the best way to store the product. According to Purwanto and Weliana (2018) to determine the best storage conditions for food products, it is important to know the characteristics of these products at various storage temperature conditions. Determination of shelf life can be done by the Accelerated Shelf Life Testing method which is commonly called the ASLT method. The Accelerated Shelf Life Test (ASLT) method is used to estimate the shelf life of food products whose damage is greatly influenced by changes in temperature by triggering chemical reactions that contribute to the damage of the product (Djarkasi et al., 2017).

The addition of Lacto-b probiotic powder to peanut chocolate jam is expected to have an effect on increasing the functional nutritional value of peanut chocolate jam products. In addition, food products can be categorized as probiotic food if they contain a certain amount of live probiotics in order to have a beneficial effect on the health of the human body. According to (Kamil et al., 2021) probiotic bacteria in a food product must have an amount of 6 - 7 log CFU/g or ml to be able to provide health effects and be able to colonize. During storage, the number of probiotic bacteria can be affected by the ability of bacteria to grow under certain environmental conditions. Therefore, the total lactic acid bacteria (LAB) needs to be considered to determine the best before of the probiotic peanut chocolate jam product. The a_w value, pH value, and total acid are also used to estimate the shelf life of the probiotic peanut butter product.

Materials and Methods

Materials

The materials used were probiotic powder (Lacto-b, initial cell count ≥ 1×10^9 CFU/g) with a mixture of lactic acid bacteria (*Lactobacillus acidophilus*, *Bifidobacterium longum*, *Streptococcus thermophillus*), cocoa powder, peanut, oil, sugar, salt, and lecithin. The chemicals used in this research were analytical reagent grade.

Methods

This research was conducted in January–February, 2024 at the Laboratory of Chemistry and Food Nutrition and the Laboratory of Food Engineering and Agricultural Products, Faculty of Animal and Agriculture Sciences, Diponegoro University, Semarang.

The process of making peanut chocolate jam refers to Fatmawati et al. (2023). All ingredients are weighed including probiotic powder (3 g), peanuts (10 g), cocoa powder (20,7), refined sugar, oil, vanila, lecithin, and salt. Peanuts are then roasted and mashed using a blender. Cocoa powder, refined sugar, oil, and crushed peanuts are put into a heat-resistant bowl to be heated over boiling water (double pan method) at a heating temperature 80 ℃ while stirring evenly for 5 minutes. Vanila and lecithin were added into the bowl then stirred until the mixture is homogeneous. Turn off the stove and wait until the peanut chocolate jam mixture has the temperature of 30 ℃, then add the probiotic powder and mix until evenly distributed. Next, transfer the probiotic peanut chocolate jam into each container and can be tested for day 0 data. After testing for day 0, the containers containing the probiotic peanut chocolate jam with 4 ℃ storage temperature treatment were stored in a refrigerator with the appropriate temperature. Testing of the four parameters on the jam at 4 ℃ storage temperature was carried out for 25 days every 5 days. Containers containing probiotic peanut chocolate jam with storage temperature of 30 ℃ and 37 ℃ were stored in a cabinet dryer with the temperature set to 30 ℃ and 37 ℃ respectively. Testing of the four parameters on the jam at storage temperatures of 30 ℃ and 37 ℃ was carried out for 15 days every 3 days.

Total Lactic Acid Bacteria (LAB) was identified using de Man Rogosa Sharpe Agar (MRSA) medium with Total Plate Count (TPC) method (Nugroho et al., 2023), a_w value of jam was identified using an a_w meter tool (Ramadhani et al., 2017), pH value was measured using pH meter (Rasbawati et al., 2019), and total acids was analyzed using the titration method (Usman et al., 2018).

The obtained data of probiotic peanut chocolate jam including total lactic acid bacteria, a_w value, pH value, and total acid were analyzed through the Arrhenius Equation displayed using Microsoft Excel 2016 in the form of a scatter diagram accompanied by a trendline.

Results and Discussion

The Initial Quality Characteristics of Probiotic Peanut Chocolate Jam.

Table 1 shows the preliminary quality characteristics of probiotic peanut chocolate jam based on total lactic acid bacteria, a_w value, pH value, and total acid. The result showed that the initial value of total LAB of probiotic peanut chocolate jam is 8.44 log CFU/g. This value is still in accordance with SNI for probiotic products. National Standard SNI 7552:2009 states that the minimum requirement for a good total LAB value is 6 log CFU/mL or /g. A product is said to be a probiotic product if the product contains total LAB that is still alive at the time of consumption ≥ 10^6 CFU/mL The amount of LAB produced needs to refer to the probiotic target for food products until the end of their shelf life in order to compete with pathogenic microbes and to ensure the probiotic effect can survive in the target digestive tract (Usman et al., 2018).

Table 1 Initial Quality Characteristics of Probiotic Peanut Chocolate Jam

Parameters	Value
Total Lactic Acid Bacteria	8.44 log CFU/g
a_w Value	0.436
pH Value	7.67
Total Acid	0.0675%

The initial value in the a_w value data results is 0.436. The aw value can be caused by the raw materials for making probiotic peanut chocolate jam. One of the raw materials is peanut which has a low a_w value. The a_w

value contained in peanuts is 0.2-0.33 (Kusuma et al., 2017). Chocolate jams whose raw materials do not contain peanuts tend to have a higher a_w value than this probiotic peanut chocolate jam product. According to (Tirgarian et al., 2023) chocolate jam can actually be classified as a product with a high a_w content because it has a value of 0.80-0.87.

The initial pH value of probiotic peanut chocolate jam was 7.67. This pH value can be produced because the probiotic bacteria added have not adapted to the environment so they have not metabolized and produced lactic acid. In addition, the raw materials in probiotic chocolate jam use chocolate powder that has passed the alkalization process which has a pH range of 6-8. According to Puchol-Miquel et al. (2021) during the alkalization process, the pH of the cocoa powder will increase in the range of 6 to 8.

The initial value of total acid is 0.072%. The total acid was influenced by the pH value of the probiotic peanut chocolate jam. This value is also low because LAB has not metabolized to produce lactic acid. In addition, the pH value of the jam did not fall into the acidic category so that the total acid produced was also not so high. The pH value of a product is related to the acid content produced so that there is an inversely proportional relationship with the value of total titratable acid (Prastujati et al., 2018).

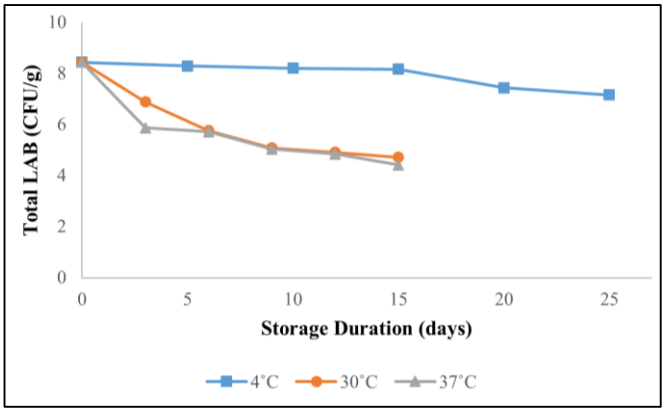

Figure 1 Total LAB Change During Storage

Quality Deterioration of Probiotic Peanut Chocolate Jam

Total Lactic Acid Bacteria

Figure 1 shows that the total LAB decreased during storage with the highest decrease at 37 ℃. On the first day of testing the total LAB of probiotic peanut chocolate jam was 8.43696 log CFU/g. On the 15th day calculation, the total LAB of peanut chocolate jam stored at 30 ℃ and 37 ℃ changed to 4.72016 and 4.41497 log CFU/g, while the calculation on the 25th day for the total LAB of peanut chocolate jam stored at 4 ℃ also decreased to 7.15987 log CFU/g. Temperature is one of the key factors influencing probiotic stability, along with pH, water activity, chemical composition, and oxygen levels. When bacteria are exposed to non-optimal temperatures, their growth is typically inhibited, leading to a decrease in the viability of lactic acid bacteria (LAB) at various incubation temperatures (Kamil et al., 2021). According to Aini et al. (2017), the amount of probiotics needed to maintain the benefits of probiotic food is 10^7 - 10^8 CFU/g or 7 log CFU/g–8 log CFU/g. This shows that the total LAB at the end of storage with various storage temperatures, only the temperature of 4 ℃ meets the standards of probiotic products.

a_w Value

Figure 2 shows that the a_w value in the three storage temperatures experienced different trends. Storage temperatures of 4 ℃ and 37 ℃ experienced a downward trend, while at 30 ℃ storage experienced an upward trend. However, this value can be said to be stable because it is still in the value range of 0.4.

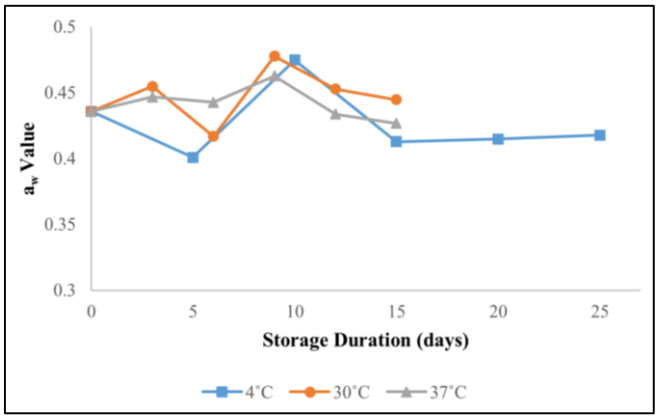

Figure 2 Changes in a_w Value During Storage

The initial a_w value of probiotic peanut chocolate jam was 0.436. Storage at 4 ℃ for 25 days and calculation every 5 days resulted in a_w values are 0.436; 0.401; 0.475; 0.413; 0.415; and 0.418, respectively. Storage at 30 ℃ for 15 days and calculation every 3 days resulted in a_w values are 0.436; 0.455; 0.417; 0.478; 0.453; and 0.445, respectively. Storage at 37 ℃ for 15 days and calculation every 3 days resulted in a_w values are 0.436; 0.447; 0.443; 0.463; 0.434; and 0.427, respectively. The a_w value of probiotic peanut chocolate jam shows a value that is not too influential during storage because there is no significant change. It can be influenced by the composition of probiotic peanut chocolate jam. One of the compositions of the product is peanuts. Peanuts have an a_w value of 0.418 (Aulia et al., 2018) so that it also affects the a_w value of the probiotic peanut chocolate jam product in the range of 0.4.

pH Value

Figure 3 shows that the pH value at the three storage temperatures experienced a decreasing trend as the storage time progressed. The initial pH value of probiotic peanut chocolate jam was 7.67. Storage at 4 ℃ for 25 days and calculation every 5 days resulted in pH values are 7.67; 7.39; 7.2; 7.45; 7.42; and 7.37, respectively. Storage at 30 ℃ for 15 days and calculation every 3 days resulted in pH values are 7.67; 7.17; 7.29; 7.44; 7.16; and 7.26, respectively. Storage at 37 ℃ for 15 days and calculation every 3 days resulted in pH values are 7.67; 7.59; 7.58; 7.55; 7.31; and 7.3, respectively. The high initial pH value of probiotic peanut chocolate jam can also be caused by the composition of the ingredients, namely chocolate powder that undergoes an alkalization process. According to Greño et al. (2022) mildly alkalized cocoa has a pH range of 6.0–7.2, moderately alkalized cocoa has a pH range of 7.2–7.6, and strongly alkalized cocoa has a pH value higher than 7.6. The decrease in pH during the growth of

lactic acid bacteria (LAB) is primarily due to the production of organic acids, mainly lactic acid, as a result of carbohydrate fermentation. LAB metabolize sugars like glucose through pathways such as homofermentative or heterofermentative fermentation, leading to the accumulation of lactic acid. This buildup of acid in the environment lowers the pH. Additionally, some LAB strains may produce other organic acids, such as acetic acid and formic acid, further contributing to the pH decline. As LAB growth and metabolic activity increase, more acids are generated, causing a continuous drop in the medium's pH (Prastujati et al., 2018).

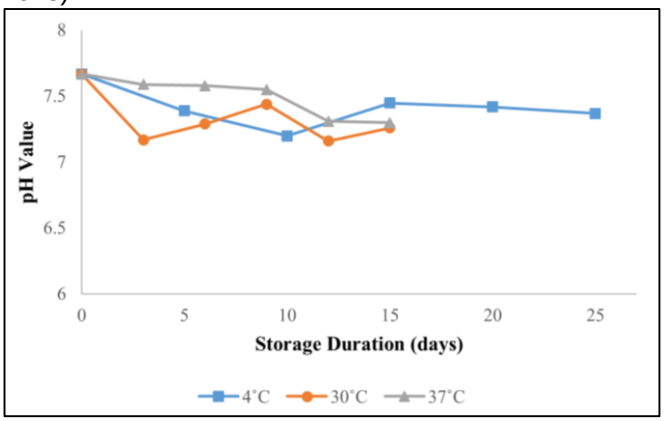

Figure 3 Changes in pH Value During Storage

Total Acid

Figure 4 shows that the total acid at the three storage temperatures showed different trends. The initial total acid of probiotic peanut chocolate jam was 0.0675%. Storage at 4 ℃ for 25 days and calculation every 5 days resulted in total acid are 0.0675; 0.0945; 0.0945; 0.072; 0.072; and 0.0945, respectively. Storage at 30 ℃ for 15 days and calculation every 3 days resulted in total acid are 0.0675; 0.1035; 0.09; 0.072; 0.1035; and 0.09, respectively. Storage at 37 ℃ for 15 days and calculation every 3 days resulted in total acid are 0.0675; 0.0675; 0.0765; 0.072; 0.09; and 0.09, respectively. The total acid produced is in line with the pH of the probiotic peanut chocolate jam. The value belongs to the small total acid. According to Camelo-Silva et al. (2024) pH values above 6 make the product non-acidic. The total acid produced is due to the presence of lactic acid bacteria added to the product. The activity of LAB in breaking down lactose will produce lactic acid as the final product of LAB metabolism (Rizal et al., 2016). Therefore, LAB metabolism will affect the final result of total acid in probiotic peanut chocolate jam products. According to Rohman et al. (2019) the total acid yield can be influenced by whether or not lactic acid is formed by lactic acid bacteria.

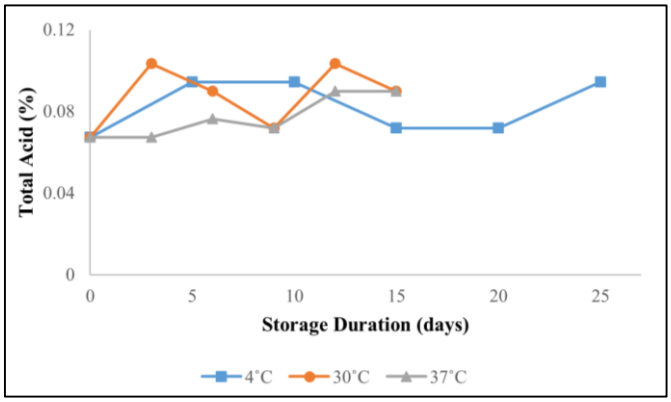

Figure 4 Total Acid During Storage

Determination of Reaction Orde

The selection of reaction order kinetics is done by comparing the correlation coefficient (R^2) value of each linear regression equation on one parameter at the same temperature of the zero order reaction (A plotted against time) and the first ord reaction (ln A plotted against time) (Bagja et al., 2015). The deterioration reaction of probiotic peanut chocolate jam based on the pH value parameter has an R^2 value at order 0 which is greater than R^2 at order 1. Therefore, the rate of decrease in pH value follows order 0. As for the total LAB parameter, the R^2 value at order 1 is greater than R^2 at order 0 so that the rate of decrease in total LAB follows order 1. Each linear regression equation for each temperature will obtain the value of k (slope) which is the constant x. The value of k from each linear regression equation for each temperature is converted to ln k and plotted against 1/T (Kelvin) to form a linear regression equation graph equivalent to the Arrhenius equation. The value of k is plotted against 1/T and ln K which is the intercept and slope of the linear regression equation $\ln k = \ln k_0 - Ea/RT$ (Pertiwi et al., 2020).

Table 2 Determination of Reaction Order

Parameters	Temperature (℃)	R^2		Selected Order
		Order 0	Order 1	
Total LAB	4	0.8524	0.8541	1
	30	0.8691	0.9086	
	37	0.7867	0.8522	
a_w Value	4	0.0497	0.0486	1
	30	0.0685	0.0695	
	37	0.0739	0.0791	
pH Value	4	0.167	0.1612	0
	30	0.282	0.2777	
	37	0.8663	0.864	
Total Acid	4	0.033	0.0383	1
	30	0.1096	0.1297	
	37	0.8098	0.817	

Table 3 Arrhenius Equation and Activation Energy (Ea)

Parameters	Arrhenius Equation	R^2	Ea (kal/mol)	Slope
Total LAB	y = -4863.5x + 12.579	0.9617	9658.911	4863.5
pH Value	y = -3485.1x + 7.552	0.9965	6921.4086	3485.1

Shelf Life Determination

Table 3 shows the parameter that has the highest R^2 value is the pH value parameter. In addition, the pH value parameter also has the lowest activation energy value and the lowest slope (k) so that this parameter is used as a critical parameter in determining the shelf life of probiotic peanut chocolate jam. However, the total LAB parameter was used as the parameter used to determine the best before of the product because this chocolate jam is a probiotic product that is closely related to total LAB. The slope value (k) shows the relationship between the value of quality deterioration and the length of storage per day (Iswari, 2021).

The calculation of shelf life begins with finding the Arrhenius constant value obtained using the equation $k = k_0.e^{-Ea/RT}$ which is derived to $\ln k = \ln k_0 - Ea/RT$. The equation for the shelf life of probiotic peanut chocolate jam was carried out using the equation obtained from the correlation graph between ln k and 1/T pH value, namely y = -3485.1x + 7.552 which is equivalent to the Arrhenius equation $\ln k = -3485.1\ (1/T) + 7.552$, where $\ln k_0 = 7.552$ and Ea/R = 3485.1x. To obtain the value of k (rate of deterioration) at each storage temperature, the temperature (K) needs to be replaced with each storage temperature. In addition, the best before is determined by the total BAL calculated using the equation y = -4863.5x + 12.579 which is equivalent to the Arrhenius equation $\ln k = -4863.5\ (1/T) + 12.579$, where $\ln k_0 = 12.579$ and Ea/R = 4863.5x.

The “use by date” and “best before” of this product are different because the results of the ASLT calculation show that the pH parameter is a critical parameter, but this product also has a dependency on total LAB which certainly should not be ignored because this product is a probiotic product. Probiotic peanut chocolate jam past the best before date indicates that the total LAB in the jam has decreased to less than 10^6 or the minimum requirement of total LAB for probiotic products. If the jam has passed the best before date, then the jam cannot be considered as probiotic peanut chocolate jam. However, the jam product is still suitable for consumption according to the expiration date set from the pH parameter.

Table 4 Shelf Life and Best Before of Probiotic Peanut Chocolate Jam

Critical Parameters	Temperature (℃)	Shelf Life / Best before
pH Value	4	255.29 days
	30	86.73 days
	37	66.89 days
Total Lactic Acid Bacteria	4	49.52 days
	30	10.98 days
	37	7.64 days

Conclusion

Based on the research, it can be concluded that probiotic peanut chocolate jam stored at three different temperatures experienced a decrease in quality during storage including a decrease in total LAB, an increase in total acid, a decrease in pH value, and a stable a_w value. The critical parameters are pH value, and total LAB as the best before reference because this chocolate jam product is closely related to LAB. The shelf life of probiotic peanut chocolate jam based on best before stored at 4 ℃, 30 ℃, and 37 ℃ were 49.52 days, 10.98 days, and 7.64 days, respectively. The jam can still be consumed up to 255.29 days; 86.73; and 66.89 days but no longer has the benefits of the probiotics.

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