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Estimation of the Shelf-Life of Corn Yoghurt Packaged in Polyethene Terephthalate Using the Accelerated Shelf-Life Method

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Abstract— **Corn yoghurt is a new product that is being developed, so it needs to determine its shelf life based on its packaging. Packaging that can be used for yoghurt is a bottle of polyethene terephthalate. The purpose of this study was to identify the kinetics of quality decrease of corn yoghurt packed in polyethene terephthalate bottles and estimate its shelf-life. The stages of establishing shelf-life included determining the quality change rate during storage, selecting reaction order, calculating activation energy, determining critical points, and calculating shelf-life. Corn yoghurt was packed in a polyethene terephthalate bottle, then stored at 25°C, 30°C, and 35°C. The analysis was done every seven days, from day-0 to day-21. Parameters analyzed were lactic acid bacteria content, pH, total acid, total dissolved solids, protein, and viscosity. The results showed that corn yoghurt is still acceptable by more than 50% panelists in 21 days of storage at a temperature of 35°C. Lactic acid bacteria, total dissolved solids, and protein content decreased during storage, while the pH, total acid, and viscosity increased. Corn yoghurt packed in polyethene terephthalate and stored at 5°C, 10°C, 15°C, and 20°C have 41, 40, 39, and 38 days of shelf-life, respectively. The implication is that yoghurt-corn packed with polyethene terephthalate has a better shelf life than existing yoghurt to be applied further.**

Keywords— **Accelerated shelf-life test (ASLT); arrhenius; corn yoghurt; polyethene terephthalate; shelf-life.**

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I. INTRODUCTION

Today's communities' trends on functional food requirements have developed food products that benefit health [1]. One type of functional food is probiotics which contain lactic acid bacteria that benefit digestion. One of the new products developed as a functional food is yoghurt from corn extract [2]. The shelf life must be examined using the appropriate packaging to develop new products to be accepted by consumers.

The packaging of food products is essential to prevent changes in product quality [3]. The packaging used for yoghurt can usually be a glass or polymer package (polystyrene, polypropylene) [4]–[6]. Although it is still controversial, polymers are still widely used as packaging because they are easy to make and inexpensive. The polymer is suitable for milk products and their derivatives, and polyethene terephthalate is widely used for yoghurt drinks.

The shelf life of food determined the type and composition, also affected by the type of packaging [7], [8]. The shelf-life information of food is required for many users, namely producers, consumers, sellers, and distributors [9]. The shelf life of corn yoghurt can be determined using the Accelerated Shelf-Life Test (ASLT) method with Arrhenius approach. The principle of ASLT method is to accelerate the physicochemical damage of products by temperature, and then, the actual shelf-life is determined by mathematical calculation [10]. Temperature is an essential key parameter determining food deterioration in the ASLT method because it is more easily damaged at a high temperature. The relationship between temperature and velocity of degradation quality can be determined by the Arrhenius equation [11]. This study aims to assign the kinetics of quality deterioration of corn yoghurt packed in polyethene terephthalate and estimate the shelf-life of corn yoghurt packed in polyethene terephthalate.

II. MATERIAL AND METHOD

A. Production of Corn Yoghurt

The production method of the corn yoghurt following Aini et al. study [2], namely, sweet corn extract was added to 15%

red sweet potato extract, 10% mung bean bean extract, sugar (15% b/v), and skim milk powder (10% w/v). The next stage is pasteurized for 15 min at 70°C and then left until 40-43°C. Next was the addition of the yoghurt culture and incubation for 8 hours at 37°C.

B. Packaging and Storage of Corn Yoghurt

Bottles of polyethene terephthalate are used for packaging corn yoghurt. Furthermore, corn yoghurt was stored at 35.30 and 25ºC. Storage is done for 21 days, and every 7 days, corn yoghurt was analyzed

C. Analysis of Corn Yoghurt during Storage

Corn yoghurt was analyzed by the number of lactic acid bacteria using the total plate count (TPC) method [12]. Potentiometric methods are used to measure the pH of corn yoghurt, and a refractometer is used to measure dissolved solids in corn yoghurt. The acid levels are analyzed by titration with a base solution using Mann acid test method, and protein content analysis was performed using Micro Kjeldahl method. Sensory testing was carried out using twenty trained panelists. The organoleptic analysis uses a scoring test with twenty trained panelists. The test was conducted using a scoring method of scale 1 to 5. Variables tested include acid flavour, colour, aroma and preference on a scale of 1 to 5.

D. Determination of Shelf Life

According to Labuza and Fu [13], the determination of shelf-life was done in three stages. The first stage was determining the rate of quality change; 500 ml of corn yoghurt was packed in a polyethene terephthalate bottle and stored at 35°C, 30°C, and 235°C. The analysis was done once every seven days from day-0 to day-21. Parameters analyzed were lactic acid bacteria content, pH, total acid, total dissolved solids, protein, and viscosity. Data of each parameter is plotted on the y-axis, and as the x-axis is the day, thus obtained linear regression curve. Each of the parameters obtained three curves at storage temperatures 25, 30 and 35°C. Linear regression curves have the formula y = a + bx; where x is storage day, y is the product characteristic value, a is the initial characteristic value of the product, and b is the rate of quality loss, which can be seen from the slope.

The second step is determining the reaction order and activation energy for each parameter. The reaction order for each parameter and temperature is selected based on the correlation coefficient (R2) value. The values of ln k and 1/T(K-1), which are the Arrhenius parameters, were tabulated. The next step is plotting ln k on the x-axis and 1/T(K-1) on the y-axis. The plotting obtained linear regression ln k = ln k0 - (Ea/R) (1/T). R shows the ideal gas constant (1,986 cal/mol), while Ea shows the activation energy. From the equation, the constant value was k_0, and the reaction rate was k, and changes in product character can be calculated using k = k_0.e-E/RT.

The last stage was the determination of critical parameters and the calculation of shelf-life. The parameter that has the lowest positive activation energy is a key parameter in determining shelf life. Calculation of shelf life by the reaction kinetics equation based on the reaction order based on the formula:

$$\mathbf{t_s} = \frac{\mathbf{A0 - At}}{\mathbf{k.t}} \text{ (Orde 0), and } \mathbf{ts} = \frac{\mathbf{ln}\,(\frac{\mathbf{A0}}{\mathbf{At}})}{\mathbf{kt}} \text{ (Orde 1)} \quad (1)$$

III. Results and Discussion

A. Quality Change During Storage

Determination of the critical quality characteristics of corn yoghurt was done by storing the product at a temperature higher than its normal storage temperature. Yoghurt is usually stored at 15°C-18°C on sale. Determining the critical quality of corn yoghurt was done by the storage at 35°C to accelerate product damage.

A sensory test showed that corn yoghurt is still acceptable by more than 50% of the panelists at 35°C for 21 days (Table 1). The approval of the yoghurt is influenced by all sensory factors, including odor, flavor, and color. Typical yoghurt odor is obtained by the formation of lactic acid, acetaldehyde, diacetyl, and acetic acid. According to Mani-López et al. [14], lactic acid bacteria and volatile substances produce components that give sour taste and yoghurt flavor. During storage at high temperatures, volatile compounds in the yoghurt evaporate and are replaced with other compounds that cause off odor, or the loss of the distinct odor, of yoghurt so that the odor decreases. According to Sah et al. [15], the decrease of odor parameters occurs due to a lack of packaging strength in retaining gas. The loss of volatile compounds in the odor and flavors follows the gas and steam permeation of plastic materials.

TABLE I
SENSORY CHARACTERISTICS OF CORN YOGHURT DURING STORAGE AT 35ºC

Days	Odor	Flavor	Color	Preference	Rejection
0	3.45	4.23	4.97	4.28	0
7	2.95	4.05	4.15	3.9	25
14	3.05	4	3.95	3.8	30
21	3.05	3.55	3.2	3.85	40

The panelists assessed the color parameter at the initial storage, where it is light yellow, until the end of storage, where it turned to a whitish-yellow. Corn yoghurt is yellow due to the presence of carotenoid compounds in corn. During storage, oxidation occurs, which results in a decrease in the color and flavor of corn yoghurt [16].

Panelists' assessment of the yoghurt flavor at the initial storage was 4.23, which was not acidic, while at the end of storage, it was 3.55 (acidic) (Table 1). Sour is the distinctive flavor of yoghurt. The increased acid flavor is due to an increase in organic acids present in the yoghurt after fermentation and after storage. After fermentation, lactose will be converted into lactic acid so that the environmental conditions also become acidic [17]. After storage, the lactic acid content increased and, along with it, the sour taste of yoghurt at the end of storage was also improved.

The flavor of yoghurt is developed by combining lactic acid derived from the breakdown of sugar, the presence of aroma components, the content of fatty acids from sweet corn, and the sweet aroma of sweet corn [18],. During the fermentation, the resulting flavor components, such as diacetyl, acetyl methyl carbonyl, 2, 3 butylene glycols, and acetaldehyde provide a distinctive taste in the yoghurt. Lactic acid bacteria form lactic acid so that it gives a sour taste to yoghurt

Table 2 shows the physical and chemical properties of the product change storage. During 21 days of storage, lactic acid bacteria, dissolved solids, and protein content decreased, while the total acid, pH, and viscosity increased. The longer the storage and the more optimum the storage temperature, there will be more lactic acid as lactic acid bacteria optimally produce organic acids at 10ºC-40ºC. The storage used to determine the product's final quality was 35°C, which is close to the optimal temperature of lactic acid bacteria activity [19]. *Lactobacillus bulgaricus* is a lactic acid bacterium that has an optimal growth temperature of 10-40ºC. Total acid will increase if lactic acid bacteria work at optimum conditions.

At the beginning of storage, the number of lactic acid bacteria was 7.57 log cfu/ml, while at the end of storage was 6.5-6.7 log cfu/ml (Figure 1). At the end of storage, corn yoghurt still fulfilled the criteria as a probiotic food. According to Donkor et al [20], food can be categorized as a prebiotic food if the amount of lactic acid bacteria is at least 6 log cfu/ml.

TABLE II
CHARACTERISTICS OF INITIAL AND FINAL QUALITY OF CORN YOGHURT PACKED IN POLYETHENE TEREPHTHALATE AT 35ºC STORAGE

Quality Parameter	Initial Value	End Value
Lactic acid bacteria (log cfu/ml)	7.57	6.7
Dissolved solids (ºBrix)	22.7	22
pH	4.4	4
Total acid	0.77	1.01
Viscosity (cP)	160	175
Total protein (% db)	2.18	2

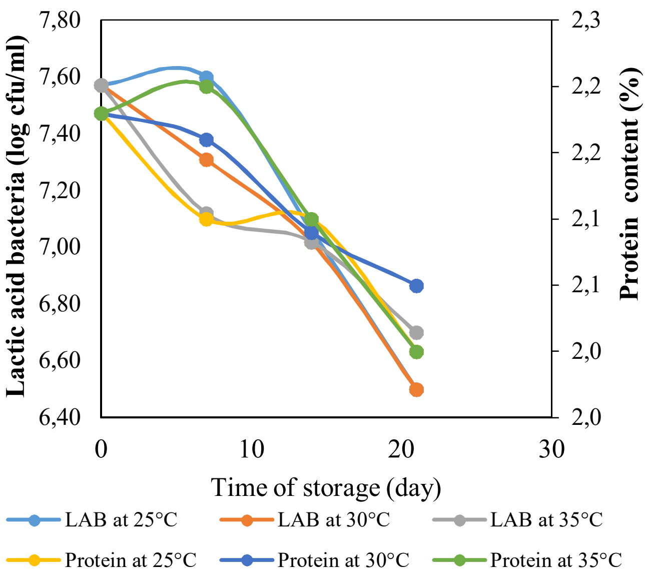

Fig. 1 Lactic acid bacteria and protein content during storage

According to Salvucci et al.,[21], the longer the storage time, the more lactic acid is produced, which causes lactic acid bacteria to lack nutrients and experience a death phase. According to Sah et al. [22], bacterial activity decreases as the resulting acidity hampers it. Also, with increasingly rapid growth, there will be more sugar reduction that is used both for its growth and to form lactic acid; therefore, the sugar content decreases. The decreasing sugar results in a much-needed substrate for bacterial growth and decreases so that the substrate will be used up. In the mortality phase, rate of the dead probiotic bacteria continues to increase, and the cell division rate is zero. The amount of lactic acid bacteria was nearly the same as in the study by Saccaro et al [23], which stated that storage of yoghurt up to 28 days can maintain lactic acid bacteria of 4.72 to 7.1 log cfu/ml.

The total acids in the yoghurt are calculated as lactic acid. Lactic acid (C_3H_6O) is the largest acid component formed from fermented milk to yoghurt. Lactic acid is formed from lactose changes that can be used by *Lactobacillus acidophilus, Streptococcus thermophilus* and *Lactobacillus bulgaricus,* as a source of carbon and energy for their growth. Lactose is hydrolyzed by both lactic acid bacteria into lactic acid and other organic acids [24], [25].

The total acid of corn yoghurt was initially 0.77% and increased to 0.98%-1.01% during storage (Figure 2). The increase in total acid is supported by [23], which showed that the acidity level of probiotic yoghurt and fermented milk increased after five days of storage at 4°C, namely, from 0.68% and 0.77% to 0.82% and 0.84%, respectively. It then had a constant acidity value after 21 days of storage. The production of lactic acid produced in milk fermented by *L. acidophilus* and *L. casei* would interact with yoghurt bacteria for 28 days of cold storage [19].

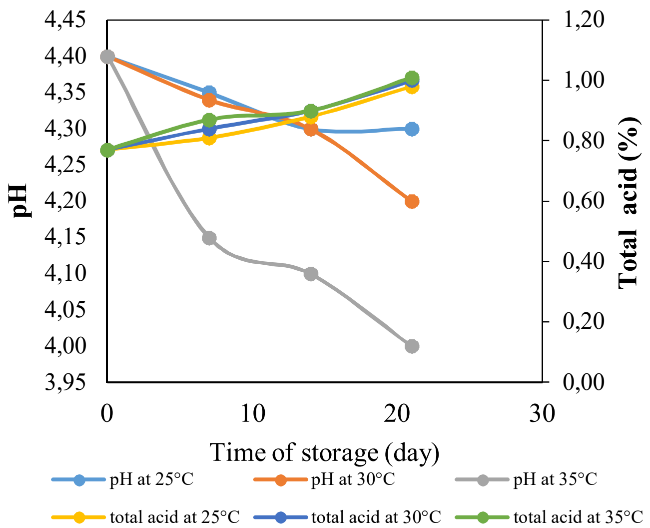

Fig. 2 Changes of pH and total acid during storage

In general, when the pH value decreases, the total acid will increase, or vice versa, although that relationship is not always the case. Total acid consists of undissociated and dissociated acids, while pH only consists of dissociated acid components in the form of H^+ ion [23]. The acid component produced in corn yoghurt is dominated by an undissociated acid so that the pH and total acid tend to be directly proportional to the decreasing pH yoghurt during storage. In this research, the yoghurt corn's initial pH was 4.4 and increased to 4.6-4.8 at the end of storage (Figure 2). During storage, there is a decrease in pH due to the increasing amount of lactic acid formed. According to Shori and Baba [26], an increase in lactic acid population cause the acidity of the product raised, and the pH decreased.

The basis of milk fermentation or yoghurt making is fermenting the components of the sugars present in milk, mainly, lactose to lactic acid and other acids. The lactic acid produced during fermentation can enhance flavour and acidity, or reduce pH. The lower the pH, or the degree of acidity of milk after fermentation, will cause fewer microbes that can survive and inhibit the process of pathogenic microbial growth and milk-damaging microbes, so the shelf-life of milk may become longer. Low storage temperatures could lead to an inhibition of lactase enzyme activity or lactic acid that has been formed to the maximum [20]. Thus, there is no increase in total titrated acids and decreased pH, resulting in bacterial growth inhibition. The standard of total acid of yoghurt based on Indonesian standard number SNI 2981:2009 is 0.5%-2.0%. Therefore, the value of yoghurt's titratable acidity for 21 days at various temperatures still fulfil that standard, which is between 0.87%-0.96%. According to Allgeyer et al. [27], the yoghurt titrated acids concentration reach 0.9%-1.2% when a pH of 4.4 is achieved. Few commercial yoghurts qualify for the Mexican Ministry Health, total titrated acids between 0.85% to 1.8%.

The corn yoghurt's initial protein level was 2.18% and slightly decreased to 2%-2.05% after three weeks of storage (Figure 1). According to Widyastuti and Febrisiantosa [25], during the making of yoghurt, *Streptococcus thermophilus* plays a significant role in early fermentation by utilizing free amino acids formed due to heating processes in yoghurt production, or due to protein degradation by the *Lactobacillus bulgaricus* to a pH of 5.5, and this results in a decrease in protein levels.

During storage, the viscosity of corn yoghurt was rise (Figure 3). According to Muniandy et al. [6], the rearrangement of protein molecules causes yoghurt's viscosity to increase during storage. Viscosity values are closely related to proteins and the formation of lactic acid. Generally, yoghurt shapes like porridge, not too thin and not too dense, but relatively thick. This is because lactic acid bacteria produce lactic acid, which can agglomerate protein in milk, mung bean, and corn. Lactic acid results in the transformation of colloidal casein/phosphate complexes into a soluble calcium/phosphate fraction so that the casein destabilizes. The casein's solubility is lost at a pH near 4.6 so that the casein will undergo hydrophobic interactions. This increases yoghurt's consistency, which will also change the water holding capacity, viscosity, and syneresis.

During storage, the soluble solids tend to decrease (Figure 3). According to Gänzle [28], during storage, the hydrolyzed substrate is reduced, and the hydrolysis process decreases, and ultimately, leads to the decrease in the soluble solids. The longer the fermentation process, the soluble solids in the yoghurt tend to decrease due to the reduced dissolved sugar in the product. Reduced sugar is caused by bacteria that use sugar as a carbon source for metabolism.

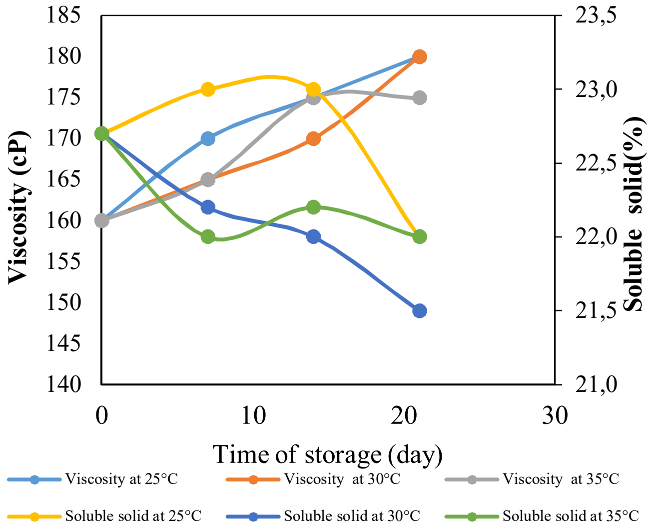

Fig. 3. Changes in viscosity and soluble solids during storage

B. *Determination of Reaction Order and Energy of Activation*

The reaction order of each parameter is determined based on the highest correlation coefficient. Table 3 shows the correlation coefficient calculation of each parameter. The lactic acid bacteria of corn yoghurt increases following the reaction of zero-order that occurs continuously. The linear regression equation of the plot of ln k and 1/T on the change in the number of lactic acid bacteria in corn yoghurt is y = 2963, 1x - 12.836 with $R^2 = 0.895$.

All variables indicated that the change during storage followed a zero-order reaction because the correlation coefficient is greater than the first-order correlation coefficient (Table 3). This result shows that the reaction of each parameter change happens continuously. Based on that, each parameter's activation energy calculation was done according to a zero-order reaction equation.

TABLE III
REGRESSION EQUATIONS AND CORRELATION COEFFICIENTS OF EACH VARIABLE ON THE ORDER OF ZERO AND FIRST ORDER

Parameter	Zero-Order Linear Regression	Zero-Order Correlation	One Order Linear Regression	One Order Correlation	Activation Energy (KJ/mol)
Lactic acid bacteria	y = 2963,1x -12,8	0,895	y = 3040,5x - 15,0	0,883	-24.64
Dissolved solids	y = 853,6x - 6,2	0,015	y = 863,2x - 9,3	0,013	-7.1
pH	y = -11679,4x + 20,3	0,998	y = -12006,1x + 33,5	0,997	97.1
Total acid	y = -636,7x - 2,5	0,758	y = - 463,4x - 2,9	0,668	5.29
Viscosity	y = 1524,7x - 5,2	0,742	y = 1407,8x - 9,9	0,731	-12.68
Protein	y = -1534,5x + 0,2	0,256	y = -1492,7x - 0,7	0,230	12.76

C. Determination of Critical Point and Shelf-life

Determination of the critical point was done based on the lowest positive activation energy value. Activation energy is the energy required to initiate the alteration in each of the parameters. For example, the activation energy of pH is 97.1 kJ/mol, meaning that the pH change needs up to 97.1 kJ of energy. The activation energy at pH changes occurs due to the growth of microorganisms that produce compounds that can increase the pH. Table 3 shows the activation energy of each variable.

Based on Table 3, the total acid is set as the critical point in determining the yoghurt shelf-life as it has the lowest positive value activation energy. This point corresponds to [29], who stated that the most rapid critical factor of damage for symbiotic yoghurt is the total of acids and coliform. Moreover, [30] showed that in beverage products containing milk, the critical parameter is pH. Therefore, in this study, the selection of critical parameters refers to total titrated acids that have the smallest activation energy.

The calculation results showed that corn yoghurt's shelf-life packaged in polyethene terephthalate PET bottles at a 5ºC to 20ºC range from 38 to 41 days. A suitable storage temperature for yoghurt is usually refrigeration of 5°C, which has a shelf life of 41 days. This result is better than Saccaro et al [23] that states yoghurt stored at 4°C still has good properties for up to 21 days. Yoghurt will be condensed and stable in the refrigerator for a week or more. Meanwhile, Supavititpatana et al [31] states that yoghurt from corn and cow's milk stored at 5ºC has a shelf life of 14 days.

Corn yoghurt packed in a PET bottle has a short shelf-life compared to corn yoghurt stored in glass bottle packaging, which has a shelf-life of 2.9 to 4.4 months [5]. This difference in shelf-life is due to differences in packaging properties. Polyethene terephthalate has a density of 1.4 g/m^3, whereas glass density is 2.49 g/m^3. Low-density packaging indicates a more open structure, meaning more easily penetrable by fluids, such as water, oxygen, or CO_2. In contrast, high-density packaging has closed structures or a more dense polymer chain arrangement, so their permeability to water and gas is lower [32].

This result means that the corn yoghurt packed with polyethene terephthalate has a better shelf life than existing yoghurt. However, glass packaging can maintain the shelf life of corn yoghurt longer than polyethene terephthalate packaging.

IV. Conclusion

Changes in corn yoghurt quality during storage at 35°C decreased the lactic acid bacteria, protein content and dissolved solids. Viscosity, pH, and total acid increase during storage. The yoghurt packed in polyethene terephthalate bottles and stored at 5ºC, 10ºC, 15ºC, and 20ºC have a shelf-life of 41, 40, 39, and 38 days, respectively.

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