Shelf-Life Determination of "Hiwan Tahu" Using Accelerated Shelf-Life Testing (ASLT) with *Arrhenius* Model

Yoyok Budi Pramono [a,*], Sri Mulyani [a], Setya Budi Muhammad Abduh [a], Rafli Zulfa Kamil [a], Anang Mohamad Legowo [a], Tiara Yunistha Lestari [a], Ayu Tri Astuti [a], Deshinta Ramadhani Bastian Putri [a], Diyan Ayu Puspita [a], Farhandian Rezky Bramantyo [a]

[a] *Food Technology Department, Faculty of Animal and Agricultural Sciences, Diponegoro University, Prof. Soedarto, SH Road, Tembalang, Semarang, Indonesia*

*Corresponding author: *yoyokbudipramono@lecturer.undip.ac.id*

Abstract*—This study aims to determine the shelf life of "hiwan tahu" based on changes in Total Plate Count (TPC), pH, water content, and a_w. This research was carried out by storing the samples at three different temperatures, including 5°C, 27°C, and 40°C. The initial characteristic and quality degradation of "Hiwan Tahu" during storage were determined by TPC, pH, water content, and a_w tests. Observations were carried out every 3 hours until the 12th hour for storage at 40°C, every 24 hours until the 96th hour at 27°C, and every 48 hours until the 192nd hour for storage at 5°C. Shelf life was determined using the ASLT method with the *Arrhenius* model. Data were analyzed by Microsoft Excel 2016 by presenting data in a scatter diagram, selecting the reaction order based on the higher R^2, determining the *Arrhenius* equation using a chart with a trendline, and calculating shelf life using Excel formulas and functions. The results showed that quality decrease of "hiwan tahu" during storage, including an increase in TPC, pH, water content, and a_w value. The shelf life of "hiwan tahu" was determined based on the critical parameter of TPC with the smallest Energy Activation (Ea). The shelf life of "hiwan tahu," according to the TPC parameter, is 68.54 days if stored at −18°C, 16.27 days if stored at 5°C, and 5.05 days if stored at 27°C. The rate of change in "hiwan tahu" quality increases with high temperature and storage time.

***Keywords*—*Arrhenius*; ASLT; "hiwan tahu"; shelf-life; temperature.**

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I. INTRODUCTION

Tofu meatballs are traditional food from Ungaran made from tofu filled with meat. CV. Maju Jaya Indonesia is one of the medium food industries in Ungaran, which can produce tofu meatballs up to ±15,000 pieces per day. The production of tofu meatballs in this company has implemented quality control through a sorting process to separate tofu that is qualified and doesn't qualify for physical quality standards. This quality control produces by-products of tofu meatballs in the form of tofu that doesn't qualify for physical quality standards, for example, torn, having a size or thickness that is different from the standard, having a dull and uneven color, and having a texture that is too hard or too soft [1]. These by-products can be used as filling for "hiwan tahu" to increase their economic value.

"Hiwan" is a popular food among Chinese people in Indonesia. "Hiwan" is a fish meatball product with pork filling, so it is haram consumed by Muslims. It is necessary to develop a halal version of "hiwan" to expand consumers. "Hiwan tahu" is the halal version of "hiwan". "Hiwan tahu" is made from chicken meat, tapioca flour as the outer part, and tofu as the filling material. "Hiwan tahu" is easily damaged during storage because it contains high water content, high protein, and a neutral pH suitable for bacterial growth. Therefore, shelf-life testing is essential to ensure the quality and safety of "hiwan tahu" until it reaches consumers. Industries use two methods to determine shelf life: the conventional method and the acceleration method. The conventional method is carried out by periodically observing product quality until the product is damaged at the end of its shelf life. The acceleration method is carried out by observing the quality of the product, which is accelerated by storing it in extreme conditions [2]. Using the ASLT method, the product's shelf life can be predicted by extrapolating the equation under normal storage conditions. The acceleration method can be done quickly and accurately [3]. This method calculates the shelf life of “hiwan tahu”.

The acceleration method has several approaches, including a semi-empirical approach using the *Arrhenius* model and the critical water content approach using the *Labuza* model [4]. The *Arrhenius* method is usually used for products sensitive to temperature changes, such as “hiwan tahu” [5]. Shelf-life testing of “hiwan tahu” in this study involved parameters of Total Plate Count (TPC), pH, water content, and water activity (a_w). During product storage at extreme temperatures, an increase in microbial growth can occur which also causes an increase in pH, moisture content, and a_w of “hiwan tahu”.

II. MATERIALS AND METHOD

A. The Process of Making “Hiwan Tahu”

“Hiwan Tahu” was made using a predetermined formulation summarized in Table 1. Making “hiwan tahu” begins with the chicken breast being ground in a chopper and the mustard greens being mashed in a blender. Seasonings such as garlic, candlenut, pepper, salt, and Monosodium Glutamate (MSG) were mashed. Tapioca flour, eggs, mustard greens, and ground spices were mixed with the chicken meat. The dough was mixed until evenly distributed. The by-product of tofu meatballs was cut into small squares weighing ± 6 g. Next, the dough was formed into a round shape, and then the middle of the dough was filled with tofu. After that, “hiwan tahu” was boiled in boiling water for five minutes, drained, and cooled.

TABLE I
FORMULATION OF “HIWAN TAHU”

Material	Composition (g)
Chicken breast	900 g
Tapioca flour	100 g
Tofu (a by-product of tofu meatballs)	300 g
Mustard greens	10 g
Garlic	14.2 g
Egg	125 g
Pepper	3 g
Salt	20.8 g
Candlenut	7.1 g
Monosodium Glutamate (MSG)	25 g
Water	10 mL

B. Product Storage and Observation of Quality Changes

“Hiwan tahu” were packed in vacuum packaging and stored at three different storage temperatures, including 5°C, 27°C, and 40°C. Furthermore, the initial characteristics and quality degradation of “hiwan tahu” during storage were determined by testing the total plate count (TPC) [6], pH, water content, and water activity (a_w) [7]. Observations were carried out every 3 hours until the 12th hour for storage at 40°C, every 24 hours until the 96th hour at 27°C, and every 48 hours until the 192nd hour for storage at 5°C. The pH was determined [8] with a pH meter that had previously been calibrated using a standard buffer solution of pH 4 and pH 7. Furthermore, 5 g of samples were mashed and dissolved into 45 mL of distilled water, and then the suspension was stirred until homogeneous. The electrode was dipped into the suspension, and the pH value can be seen on the monitor screen.

C. Shelf-Life Determination of “Hiwan Tahu”

Determination of the shelf life of “hiwan tahu” using the ASLT method with the Arrhenius model begins with the data obtained from observation during storage are plotted with storage time to obtain a regression equation for zero order graphic, while a regression equation for the first order graphic is obtained by change the data into ln and then plotted with storage time. In the graphic, three regression equations y = a + bx will appear to represent quality changes in each of the three storage temperatures, where y is the change in product quality, x is the storage time (hours), a is the initial quality value of “hiwan tahu”, and b obtained from the slope is the rate of change in quality (k value). The reaction order is determined by comparing the R^2 value of each equation. The reaction order chosen is the reaction order that has the largest R^2 value or is closest to 1. The next step is making the graphic correlation between the ln k value and 1/T (K). The quality degradation constant (k value) obtained from the regression equation on the reaction order graph is changed to ln k for each storage temperature. The ln k value is then plotted with 1/T, where T is the storage temperature in Kelvin units [9]. The equation obtained from this graphic is equal to this equation:

$$\ln k = \ln k_0 - \left(\frac{Ea}{R}\right)\left(\frac{1}{T}\right) \quad (1)$$

The equation above comes from this Arrhenius equation:

$$k = k_0 . e^{-\frac{Ea}{RT}} \quad (2)$$

where k is quality degradation constant, k0 is pre-exponential constant, Ea is activation energy, T is absolute temperature (K), and R is gas constant (1986 cal/mol K)

The next step is determining the critical parameters based on the lowest activation energy. The slope in the graphic correlation of ln k value and 1/T is equivalent to the value (Ea/R), so the activation energy can be obtained by substituting the R-value (ideal gas constant = 1.986 cal/mol K) into the equation. The equation on the graphic correlation of the ln k value with 1/T can also be used to find the k value. The k value is the *Arrhenius* constant which indicates the quality degradation rate at each storage temperature. The k value is obtained by substituting the storage temperature into the equation. The k value obtained will be used in calculating the shelf life of “hiwan tahu” using the following formula:
for 0 order reaction rate:

$$ts = \frac{N_t - N_0}{k} \quad (3)$$

for 1st order reaction rate:

$$ts = \frac{\ln(N_t - N_0)}{k} \quad (4)$$

where ts is shelf life, N_0 is initial quality value of the product, N_t is critical value, and k is quality degradation constant.

III. RESULTS AND DISCUSSION

A. Initial Characteristics of “Hiwan Tahu”

Based on the observations, “hiwan tahu” has a fresh light green color, a springy, elastic, and not mushy texture, a distinctive chicken aroma, and a savory chicken meat taste. The initial characteristics of “hiwan tahu” are summarized in

Table 2. The initial Total Plate Count (TPC) value of "hiwan tahu" was 7.4×10^3 CFU/g. The initial value of TPC still qualified the maximum TPC value for meatballs which is 1×10^5 CFU/g. An increase in total microbes indicates a decrease in the microbiological quality of processed meat products, such as "hiwan tahu" during storage. The initial pH of "hiwan tahu" is 5.31. "Hiwan tahu" is classified as a chicken meat product. The pH of chicken meat is 5.8 [10]. High pH will accelerate the growth of microbes' growth, resulting in meat products deteriorating more quickly. The initial water content of "hiwan tahu" is 67.3116%. The initial water content still qualified the maximum water content limit for meatballs which is 70%. High water content can accelerate the deterioration of meat products [11]. The initial water activity (a_w) of "hiwan tahu" is 0.740. "Hiwan tahu" is classified as a processed meat product. Meat products twithan a_w around 0.91 tend to have a long shelf life because the pathogens can't grow [12]. The high of a_w will accelerate microbial growth in chicken meat products [13].

TABLE II
INITIAL CHARACTERISTICS OF "HIWAN TAHU"

Characteristic	Value
Total Plate Count (CFU/g)	7.4×10^3
pH	5.31
Water content (%)	67.3116
Water activity (a_w)	0,740

B. Total Plate Count (TPC) Changes During Storage

The results of the Total Plate Count (TPC) analysis of "hiwan tahu" during storage are summarized in Figure 1.

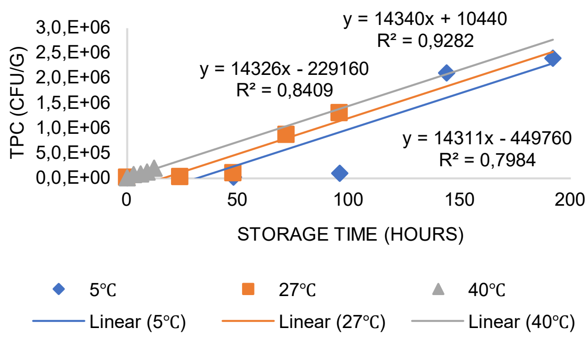

Fig. 1 Changes in Total Plate Count (TPC) during storage

The TPC value of "hiwan tahu" increases during storage. The total microbes of "hiwan tahu" at the beginning of storage is 7.4×10^3 CFU/g, while the total microbes at the end of storage at 5°C (192nd hour), 27°C (96th hour), and 40°C (12th hour) are 2.4×10^6 CFU/g, 1.3×10^6 CFU/g, and 2×10^5 CFU/g. The maximum limit for the TPC value of meatballs is 1×10^5 CFU/g [14]. The TPC value of "hiwan tahu" at the beginning of storage still qualified for SNI standard, but the TPC value at the end no longer qualifies for the requirement. The reaction rate of changes in TPC at each storage temperature can be seen from the graph's k value (slope). The k values at 5°C, 27°C, and 40°C are 14.311, 14.326, and 14.340. The higher the k value (slope), the higher the rate of change in the TPC value, so the increase in TPC at a storage temperature of 40°C occurs faster than at 5°C and 27°C.

Storage temperature can affect the growth of spoilage microorganisms in meat products. Based on growth temperature, microbes are divided into psychrophilic, psychotropic, mesophilic, and thermophilic microbes. Psychrophilic microbes grow optimally at 12°C–15°C, psychotropic microbes grow optimally at 20°C–30°C, mesophilic microbes grow optimally at 30°C–40°C, and thermophilic microbes grow optimally at 55°C–65°C [15]. Bacteria that cause spoilage in meat products grow optimally at 37°C [16].

C. The pH Changes During Storage

The results of the pH analysis of "hiwan tahu" during storage are summarized in Figure 2. The pH of "hiwan tahu" increased during storage. The pH of "hiwan tahu" at the beginning of storage is 5.31 while pH at the end of storage at 5°C (192nd hour), 27°C (96th hour), and 40°C (12th hour) are 6.02, 6.30, and 6.42. "Hiwan tahu" is made from chicken meat which has a pH standard of 5.8. A high pH value (6.1–7.2) causes the structure of chicken meat to become closed and facilitates microbial growth [17]. The reaction rate of changes in pH at each temperature can be seen from the k value (slope) on the graph. The k value at 5°C, 27°C, and 40°C are 0.0041, 0.0085, and 0.078. The higher the k value (slope), the higher the rate of change in pH, so that the increase in pH at a storage temperature of 40°C occurs faster than at 5°C and 27°C.

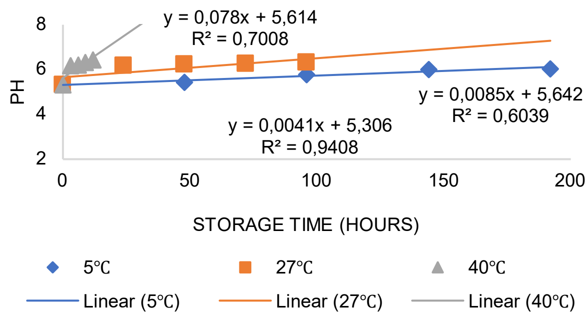

Fig. 2 Changes in pH during storage

Storage temperature can affect pH because it is closely related to microbial growth, affecting the pH of meat products. Spoilage bacteria generally grow optimally at temperatures of 35°C–47°C and pH 6.5–7.5 [18]. Besides temperature, storage time also affects the pH of "hiwan tahu". The longer the storage, the higher the pH of meat products increases due to the accumulation of metabolite compounds resulting from microbial activity through protein deamination reactions [19].

D. Water Content Changes During Storage

The results of the water content analysis of "hiwan tahu" during storage are summarized in Figure 3—the water content of "hiwan tahu" increases during storage. The water content of "hiwan tahu" at the beginning of storage is 67.3116%, while the water content at the end of storage at 5°C (192nd hour), 27°C (96th hour), and 40°C (12th hour) are 70.34%; 70.28%; and 70.04%. The maximum water content for meatballs is 70% [14], so the water content of "hiwan tahu" at the beginning of storage still qualified for the SNI requirement, but at the end of storage, it doesn't qualify for the requirement. The reaction rate of changes in water content at each storage temperature can be seen from the k value (slope) on the graph. The k value at 5°C, 27°C, and 40°C are

0.0137, 0.0296, and 0.2168. The higher the k value (slope), the higher the reaction rate of changes in water content, so the increase in water content at a storage temperature of 40°C occurs faster than at 5°C and 27°C.

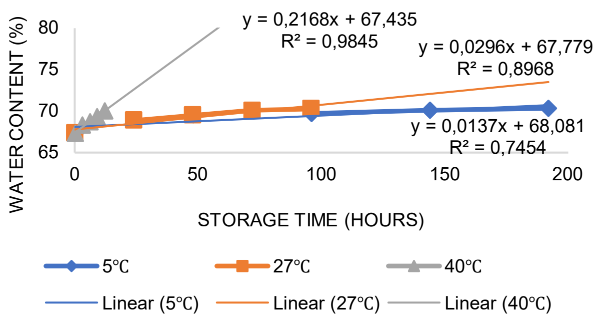

Fig. 3 Changes in water content during storage

Storage temperature can affect the water content. The water content of "hiwan tahu" increases at higher temperature storage. During the storage of meatballs, microbes carry out metabolism by breaking down glucose into water and CO_2 as well as releasing energy [20]. The higher number of microbes, the more water is produced from metabolism which can contribute to the water content of the product.

E. Water Activity (a_w) Changes During Storage

The results of the water activity (a_w) analysis of "hiwan tahu" during storage are summarized in Figure 4.

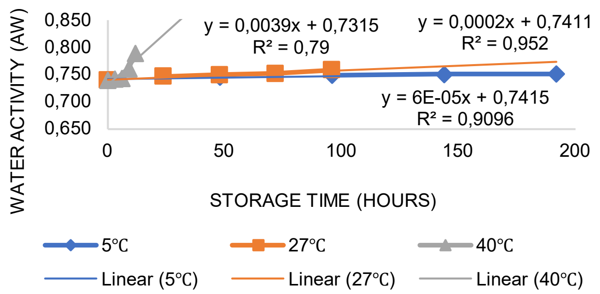

Fig. 4 Changes in water activity during storage

Water activity (a_w) of "hiwan tahu" increases during storage. Water activity (a_w) of "hiwan tahu" at the beginning of storage is 0.740; while a_w at the end of storage at 5°C (192nd hour), 27°C (96th hour), and 40°C (12th hour) are 0.7515, 0.758, and 0.7885. Microbes generally can grow in food products with a minimum a_w value of 0.60 [21]. The rate change of a_w at each storage temperature can be seen from the k value (slope). The k value at 5°C, 27°C, and 40°C are 0.00006, 0.0002, and 0.0039. The higher the k value (slope), the higher the reaction rate of changes in the a_w value so that the increase in a_w value at a storage temperature of 40°C occurs faster than at 5°C and 27°C.

Storage temperature can affect water activity (a_w) because it is related to the growth and activity of microbes. The higher tthe temperature and the longer the torage of "hiwan tahu", the higher the rate of increase in the water activity (a_w). Storage at higher temperatures causes microbes to grow rapidly in a shorter time. During storage, the degradation of material molecules by microbes occurs by releasing bound water to form free water [22].

F. Determination of Reaction Order

The Arrhenius model can simulate the rate of food product deterioration accelerated under extreme storage temperature conditions. The rate of quality degradation in food products generally follows the zero-order or first-order reaction. Zero-order reactions occur when the quality degradation follows a linear line, while first-order responses occur when the quality degradation follows an exponential line [23]. The reaction order of each quality parameter must be determined to determine the degradation of "hiwan tahu" quality during storage based on these parameters, following a zero or first-order reaction. Determining the correct reaction order will result in better accuracy in calculating the shelf life of "hiwan tahu". The determination of the reaction order of each parameter is summarized in Table 3.

TABLE III
DETERMINATION OF REACTION ORDER

Parameter	Temperature (°C)	R^2 Zero-order	R^2 First-order	Selected reaction order
Total Plate Count (TPC)	5°C	0,7984	0,9356	
	27°C	0,8409	0,9629	1
	40°C	0,9282	0,7769	
pH	5°C	0,9408	0,9394	
	27°C	0,6039	0,5961	0
	40°C	0,7008	0,6861	
Water content	5°C	0,7454	0,7414	
	27°C	0,8968	0,8932	0
	40°C	0,9845	0,9838	
Water acitivity (a_w)	5°C	0,9096	0,9086	
	27°C	0,952	0,9517	0
	40°C	0,79	0,794	

The reaction order is determined based on the highest R^2 value. The higher of R^2 value, the more accurate the equation [24]. If the value of zero-order R^2 is higher than that of first order R^2, then the rate of quality degradation will follow zero-order. If the value of first order R^2 is higher than that of zero-order R^2, then the rate of quality degradation will follow the first-order. Based on Table 3, the deterioration reaction of "hiwan tahu" based on the TPC parameter has an R^2 value of first order that is higher than the R^2 value of zero-order. Hence, the rate of change in the TPC value follows the first order. In contrast to the pH, water content, and a_w parameters which have R^2 values of zero-order that are higher than the value of R^2 of the first order so the rate of change in pH, water content, and a_w follow zero-order.

G. Determination of Critical Parameter

After determining the reaction order, the next step is plotting the ln k value with 1/T (Kelvin). The k value (slope) or the quality degradation rate constant obtained from the regression equation on the reaction order graph is changed to the ln k and plotted with 1/T (Kelvin) to form a linear regression equation graph which is equivalent to the *Arrhenius* equation ln k = ln k0 – Ea/RT so that the activation energy (Ea) of each parameter can be calculated to select one critical parameter [25]. The results of plotting the ln k and 1/T for each parameter are summarized in Table 4.

TABLE IV
ARRHENIUS EQUATION AND ACTIVATION ENERGY

Parameter	*Arrhenius* Equation	R^2	Ea	*Slope*
TPC	y = -4433.1x + 12.416	0.8335	2232.18	4433.1
pH	y = -6721.7x + 18.415	0.8016	3384.54	6721.7
Water content	y = -6345x + 18.302	0.8278	3194.86	6345
a_w	y = -9610.7x + 24.509	0.8355	4839.22	9610.7

H. Determination of Shelf-Life

The next step after determining the critical parameter is calculating the k value or the degradation rate at each storage temperature based on the selected critical parameter. The *Arrhenius* equation in the correlation graph between the ln k value and 1/T (Kelvin) can be used to find the k value. The k value is obtained by substituting the storage temperature into the equation. The k value can be obtained through the calculation below:

1) Freezer temperature (-18°C or 255 K)

$$\ln k = \ln k0 - Ea/RT$$
$$\ln k = 12.416 - 4433.1\ (1/T)$$
$$\ln k = 12.416 - 4433.1\ (1/255)$$
$$\ln k = -4.968705882$$
$$k = 0.006952139$$

2) Refrigeration temperature (5°C or 278 K)

$$\ln k = \ln k0 - Ea/RT$$
$$\ln k = 12.416 - 4433.1\ (1/T)$$
$$\ln k = 12.416 - 4433.1\ (1/278)$$
$$\ln k = -3.530402878$$
$$k = 0.029293112$$

3) Room temperature (27°C or 300 K)

$$\ln k = \ln k0 - Ea/RT$$
$$\ln k = 12{,}416 - 4433.1(1/T)$$
$$\ln k = 12{,}416 - 4433.1(1/300)$$
$$\ln k = -2.361$$
$$k = 0.09432585$$

The k value that has been obtained is then used to calculate the shelf life of "hiwan tahu" using the formula $t = \frac{\ln(N_t - N_0)}{k}$ for the first order reaction rate, where N_0 is the initial quality value while N_t is the critical quality limit, and k is the degradation rate constant at temperature T (K). Shelf life of "hiwan tahu" at storage temperature T(K):

1) Freezer temperature (-18°C or 255 K)

$$t = \frac{\ln(N_t - N_0)}{k}$$
$$t = \frac{\ln(10^5 - 7{,}4 \times 10^3)}{0.006952139}$$
$$t = 1644.9677 \text{ hours}$$
$$t = 68.54 \text{ days}$$

2) Refrigeration temperature (5°C or 278 K)

$$t = \frac{\ln(10^5 - 7{,}4 \times 10^3)}{0.029293112}$$
$$t = 390.4005 \text{ hours}$$
$$t = 16.27 \text{ days}$$

3) Room temperature (27°C or 300 K)

$$t = \frac{\ln(10^5 - 7.4 \times 10^3)}{0.09432585}$$
$$t = 121.2398 \text{ hours}$$
$$t = 5.05 \text{ days}$$

The higher the storage temperature, the shorter the "hiwan tahu" shelf life. The shelf life of "hiwan tahu" based on ASLT calculations according to the Total Plate Count (TPC) parameter at freezer temperature (-18°C), refrigerator temperature (5°C), and room temperature (27°C) are 68.54 days, 16.27 days, and 5.05 days. The shelf life of "hiwan tahu" in this study is similar to that of other processed meat products such as meatballs. Based on the research [26], the shelf-life of chicken meatballs stored at freezer temperature (-20°C) is 2 months. Other research [27] states that beef meatballs have a shelf life of 9 days if stored at ±5°C while chicken meatballs with edible coating control treatment at ±5°C temperature storage are no longer suitable for consumption on the 10th day because TPC value is 5.92 logs CFU/mL and exceeds the SNI standard, which is 5 logs CFU/mL [28]. When stored at room temperature (25°C), the shelf-life of vacuum-packed beef meatballs is 4.81 days [29].

Processed meat products such as "hiwan tahu" are easily damaged and have a short shelf life because they contain relatively high-water content, high nutrients, especially protein, and a pH close to neutral (5.5 – 6.5) which supports microbial growth [30]. Spoilage bacteria can decompose proteins, carbohydrates, and fats which causes off-flavors, a sticky or slimy texture, and discoloration of meat. The breakdown of protein into peptides or amino acids causes off-odors and off-flavors in the meat products due to the formation of ammonia, hydrogen sulfide, methyl sulfide, amines, and other volatile compounds [31]. "Hiwan tahu" is easily damaged, especially if stored at high temperatures. The higher temperature of storage, the faster the rate of change in the "hiwan tahu" quality. Therefore, the recommended storage to extend the shelf life of "hiwan tahu" is frozen storage at -18°C in the freezer. During frozen storage, some of the product's water content undergoes crystallization, inhibiting water mobility so that water activity decreases and microbial growth can be inhibited due to dehydration [32].

Dehydration effectively inhibits microbial growth and can also affect the texture, flavor, and nutritional content of the meat. Additionally, proper hygiene, storage conditions, and packaging are critical to maintaining the quality and safety of dehydrated meat products. Following food safety regulations and guidelines is important to ensure that the dehydration process meets industry food safety standards [33].

IV. Conclusion

Based on the research, it can be concluded that the quality of "hiwan tahu" stored at three different storage temperatures decreased during storage. During storage, the Total Plate Count (TPC), pH, water content, and a_w value of "hiwan tahu" decreased. The higher the temperature of storage, the faster the degradation rate. The critical parameter is the Total Plate Count (TPC) because it has the lowest activation energy. The shelf life of "hiwan tahu" stored at -18°C, 5°C, and 27°C was 68.54 days, 16.27 days, and 5.05 days.

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