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Shelf-life prediction of freeze-dried strawberries using accelerated shelf-life testing method in a tropical environment

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Abstract The result showed that the quality parameters of freeze-dried strawberries that changed the fastest during storage were redness (a*), yellowness (b*), color change (ΔE), water content, vitamin C, total acid, and total phenolic, while the slowest were lightness (L*), texture, and antioxidant activity by DPPH. The shelf-life prediction of freeze-dried strawberries based on the Arrhenius approach with water content parameters reached 62 days (rounded down) at -18°C, 23 days at 25°C, 19 days at 35°C, and 16 days at 45°C. Meanwhile, based on the yellowness (b*) parameter, the shelf life of freeze-dried strawberries reached 94 days (rounded down) at -18°C, 38 days at 25°C, 32 days at 35°C, and 27 days at 45°C.

Keywords: Freeze-drying, Packaging, Quality, Shelf-life testing, Strawberries

Introduction

Diversification of dry products is an important issue. Their area of use has increased in various types of food, such as freeze-dried fruit (Önal *et al*., 2019; Fauster *et al*., 2020). Dried fruit allows consumers to consume it all the time. The COVID-19 pandemic, which has occurred in the last two years, has been changing consumers' diets quite substantially. The COVID-19 pandemic is projected to significantly impact the fruit and vegetable processing market regarding the importance of safe, healthy, and nutritious food. There is limited access to grocery shopping in supermarkets, causing consumers to consume fewer fresh food products, especially fruits, vegetables, and seafood (Anonim, 2023). Changes in consumer behaviour and the increasing demand for healthy products with high quality make it necessary for the market or producers to maintain the nutritional and sensory properties of fresh products (Asioli *et al*., 2019).

Strawberries (*Fragaria x ananassa*) are a non-climacteric fruit with a high respiration rate, harvested at various maturity levels, depending on cultivars and

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market preferences (Rahman *et al*., 2016; Yue *et al*., 2020). Strawberries contain bioactive compounds, like antioxidant activity, phenolics, flavonoids, anthocyanins, and vitamin C (Kowalska *et al*., 2018; Yue *et al*., 2020). However, strawberries have perishable properties with a short shelf life and are prone to spoilage (Yue *et al*., 2020). Poor postharvest handling can be a severe problem as strawberry spoilage occurs faster and results in substantial losses of fresh fruit products. Therefore, good postharvest treatment of strawberries needs to be the concern of farmers or producers.

Strawberries are processed using various technologies, like freezing and drying processes, like freeze drying, osmosis, microwave, and hot drying (Muzaffar *et al*., 2018). Dried fruit is a concentrated form of fresh fruit with a lower water content because most has been removed through the drying method (Chang *et al*., 2016). Drying is a universal procedure for obtaining products with low water content, better preservation of nutritional compounds of food products, extending shelf life, and reducing costs for packaging, storage, handling, and transportation (Gamboa-Santos *et al*., 2014). Processing effects exert many possibilities on the overall quality of fruit products. Still, the most important are the loss or increase of natural components, the formation of new constituents with increased antioxidant potential or pro-oxidant activity, and interactions between different compounds (Figiel and Michalska, 2017).

Freeze-dried strawberries are an alternative to increase the shelf life and added value of fresh strawberries using a process based on freezing the product followed by sublimation of ice at reduced pressure. The freeze-drying method produces products with smaller changes in colour, taste, chemical composition, texture, and product quality that are better than other dehydration processes (Zhang *et al*., 2017) but has the disadvantages of high energy costs and long processing times (Prosapio and Norton, 2017). Freeze-dried fruit products provide another benefit to consumers, such as products that can be eaten directly or used as food ingredients, like breakfast cereals, bread, desserts, and confectionary products (Muzaffar *et al*., 2018).

There have been few efforts to develop freeze-dried strawberry products in Indonesia. Imported products still dominate freeze-dried strawberry products circulating in local markets and e-commerce. Fresh strawberry production in Indonesia, which reached 9,860 tons in 2021 (Anonim, 2022), can be a driving factor for the development of freeze-dried strawberry products and can be an alternative in suppressing imports of freeze-dried strawberry products from China, Thailand, Singapore, and Korea currently circulating in the Indonesian market.

Based on Clause Two (2) of the Food and Drug Monitoring Agency Regulation No. 31/2018, food labels must be present on processed food packaging. One of the information that must be included in the food packaging label is expiration information related to the shelf life of the product. The shelf life of food products is essential information for consumers because it is related to product safety and provides quality assurance to consumers that the product is in good condition and safe for consumption (Wulandari *et al*., 2020).

Accelerated Shelf-Life Testing (ASLT) is a method of calculating shelf life with the advantages of considerable accuracy and precision, shorter testing time, and more analytical parameters (Asiah *et al*., 2018). The shelf-life prediction of freeze-dried strawberry products using the Arrhenius model is essential because dried fruit has a high susceptibility to changes in storage temperature, which can affect the degradation gradient of the product, such as water content, an increase in the number of naturally occurring microorganisms in the product, or organoleptic changes in the product due to biochemical reactions (Wulandari *et al*., 2020). Indonesia, being a tropical country, has unfavorable implications, where the decline in quality of dried processed fruits occurs faster. Therefore, the study aimed to determine the shelf life of freeze-dried strawberry products based on physical and chemical quality parameters using accelerated shelf-life testing.

Materials and methods

Freeze-dried strawberries production

Fresh strawberries obtained from Inggit Strawberry Farm, Sawangan, Magelang, Central Java, Indonesia, were harvested when fully ripe with perfect fruit shape and >80% of the fruit surface showing dark red colour (Rahman *et al*., 2016). Fresh strawberries of uniform size and without defects were cleaned from dirty leaves and washed under running water. Next, fresh strawberries were drained and cut into two halves vertically, stored in 750 mL thin wall boxes with a weight range of ±80-90 and ±50-60 grams. All boxes were stored in a refrigerator (*Modena MD0303S Chest Freezer Box 300 L*) at -18±2°C for four days.

Fresh strawberries were freeze-dried (*Benchtop Pro with Omnitronics, model btp.3xl.oox, genevac ltd, ipswich England*) for 24 hours. The initial temperature on the freeze dryer is -3°C and will continue to decrease until the temperature is -70°C. In contrast, the initial pressure is 914 Torr and will continue to decrease until the pressure is 94 mTorr. Freeze-dried strawberries products were packaged without vacuum using a primary packaging of 75-micron nylon monolayer plastic with a sealer tool (*Sayota Sinbo, DZ-280/2SD, China*) and a secondary packaging of aluminium foil standing pouch equipped with window packaging.

Experimental design

The experiment used a completely randomized design (CRD) with storage temperature treatment factors. Primary and secondary packaging as part of a component product for a tool to help the shelf-life prediction of freeze-dried strawberry products and the effect of using primary and secondary packaging in this study were not discussed in detail. The choice of different test time intervals at each storage temperature of -18, 25, 35, and 45°C, respectively, was shown in Table 1 and is based on the initial research.

Table 1. Experimental design

Temperature Storage	Primery Packaging	Testing Point (Days-)
-18°C	T_1K_1	0, 15, 30, 45, 60
25°C	T_2K_1	0, 10, 20, 30, 40
35°C	T_3K_1	0, 7, 14, 21, 28
45°C	T_4K_1	0, 3, 6, 9, 12

Quality parameter of freeze-dried strawberries

The measurement of quality parameters of freeze-dried strawberries was determined using standard methods. The physical quality tested was colour using a Chromameter (*Konica Minolta CR-400, Japan*) with test results in the form of the CIELAB colour space and texture using the Fruit Hardness Tester (*FHT 200, Extech Instrument A Flir Company, Taiwan*). Difference of color (ΔE) were calculated by $\Delta \mathrm{E} = \sqrt{\Delta \mathrm{a}^2 + \Delta \mathrm{b}^2 + \Delta \mathrm{L}^2}$, where $\Delta a = (a_\mathrm{f}^* - a_0^*)$, $\Delta b = (b_\mathrm{f}^* - b_0^*)$, $\Delta L = (L_\mathrm{f}^* - L_0^*)$. The subscripts *0* and *f* denote the fresh and freeze-dried states, respectively (de Bruijn *et al*., 2015).

The chemical qualities tested were water content by thermogravimetric method, vitamin C by titration, total phenolic by Folin-Ciocalteu, total acid by titration method, and antioxidant activity by DPPH. All quality measurements were performed in double replication and reported as mean and standard deviation. All post-harvest treatment of freeze-dried strawberries samples was carried out at the Laboratory of the Department of Agroindustrial Technology, Faculty of Agricultural Technology, and freeze-drying processes were conducted in the Faculty of Pharmacy, Universitas Gadjah Mada. The research was conducted from July – December 2022.

Shelf-life prediction

Based on Asiah *et al*. (2018), the steps for predicting shelf-life with the ASLT method are as follows. The general regression linear equation is $y = a +$

bx, where y is the change in product quality, x is the storage time, a is the initial product quality value, and b is the slope, which can be called k is the rate of change in product quality. The rate of chemical reactions that decrease the qualities of food from the type of reaction order followed. The zero-order graph is a relationship between the k value and storage time (equation 1), and the first order is between *ln k* and storage time (equation 2).

$$A_t - A_0 = -k_t \quad [1]$$

$$\ln(A_t) - \ln(A_0) = -k_t \quad [2]$$

The order of the reaction with the more significant value of R^2 (determination) is chosen from the two equations. After determining the reaction order, the slope value (b) is the reaction rate for changes in product characteristics or degradation rate (k). Using the Arrhenius approach, the *ln k* value is plotted against 1/T(K^{-1}), resulting in equation 3.

$$\ln(k) = \ln(k_0) - \left(\frac{E_A}{R}\right)\left(\frac{1}{T}\right) \quad [3]$$

The k_0 value is from the intercept value in equation 3, and the slope value represents the Ea/R value. Then, the reaction rate equation model (k) changes in quality parameters at each storage temperature are determined by equation 4. The value of the activation energy for each quality parameter can be found by multiplying the slope value with the ideal gas constant (R), which is 1.986 cal/mol K. The change in quality with the lowest activation energy (E_A) is the critical quality parameter.

$$k = k_o.(e)^{-E_A/RT} \quad [4]$$

The shelf life of freeze-dried strawberries was estimated by calculating the difference in scores when the product was not liked and the initial score divided by the rate of deterioration (k) at the storage temperature of the alleged distribution expressed through equations 5 and 6.

$$t_s = \frac{\ln(N_o - N_t)}{k}, \text{ for a first order reaction rate} \quad [5]$$

$$t_s = \frac{(N_o - N_t)}{k}, \text{ for a zero order reaction rate} \quad [6]$$

The final step is to determine the temperature at which you want to know the shelf life using the Q_{10} method by equation 7 and the estimation of the shelf life at various storage temperatures by equation 8.

$$Q_{10} = (e)^{\frac{E_A(T_2 - T_1)}{R \cdot T_1 \cdot T_2}} \quad [7]$$

$$t_{s\,(T_2)} = \frac{t_{s\,(T_1)}}{(Q_{10})^{\left(\frac{T_2 - T_1}{10}\right)}} \quad [8]$$

Results

Determination of reaction order

The speed of change in the quality of each parameter of a food product is different. The physical quality parameters that changed the fastest during storage were redness (a*), yellowness (b*), and color change (ΔE), while the slowest were lightness (L*) and texture. The chemical quality parameters that changed the fastest during storage were water content, vitamin C, total acid, and total phenolic, while the slowest was the antioxidant activity by DPPH. As the storage temperature increased, the quality changes occurred faster.

Determination of the reaction order is a way to predict quality degradation in estimating shelf life. If the damage rate is constant or linear, it follows zero reaction order. However, if the damage rate is not constant, logarithmic, or exponential, it follows the first reaction order**.** The choice of reaction order is carried out by plotting the degradation data following order zero or order one based on physical quality parameters as shown in Table 2 and chemical quality parameters as shown in Table 3. The order of reaction chosen was the order of reaction with the most significant R^2 value.

Table 2. The rate of change in product quality (*k*) and R^2 value of reaction order for physical quality parameters

Parameter	**T (°C)**	**K**		**R^2**	
		Order 0	Order 1	Order 0	Order 1
Lightness (L*)	-18	0.0684	0.0015	0.6706	0.6788
	25	0.194	0.0043	0.8773	0.8777
	35	0.7967	0.0165	0.8982	0.8997
	45	0.8708	0.0184	0.3819	0.3902
Redness (a*)	-18	0.0294	0.0011	0.7362	0.7292
	25	0.4214	0.0178	0.8782	0.8739
	35	0.7247	0.018	0.7427	0.7425
	45	4.0576	0.2292	0.8314	0.8047
Yellowness (b*)	-18	0.0736	0.0045	0.9714	0.9671
	25	0.0761	0.0048	0.9443	0.9437
	35	0.1137	0.0071	0.9507	0.9444
	45	0.4559	0.0274	0.912	0.901
Change of Color (ΔE)	-18	0.0925	0.0126	0.9083	0.9335
	25	0.2546	0.0326	0.9535	0.9412
	35	0.7644	0.0845	0.8424	0.8544
	45	2.5744	0.2462	0.888	0.8787
Texture	-18	0.1121	0.0288	0.9928	0.9825
	25	0.4907	0.0851	0.9494	0.9825
	35	0.7309	0.1275	0.8313	0.9555
	45	0.2995	0.119	0.6464	0.6474

Table 3. The rate of change in product quality (*k*) and R^2 value of reaction order for chemical quality parameters

Parameter	T (°C)	k		R^2	
		Order 0	Order 1	Order 0	Order 1
Water Content	-18	0.1391	0.0113	0.9659	0.9595
	25	0.326	0.0238	0.9988	0.9965
	35	0.3535	0.0278	0.993	0.9899
	45	0.658	0.0522	0.9894	0.9885
Vitamin C	-18	0.5196	0.0022	0.987	0.9878
	25	0.9584	0.0041	0.9816	0.9814
	35	2.7851	0.0112	0.9854	0.9841
	45	6.526	0.028	0.9791	0.9815
Total Acidity	-18	0.9062	0.013	0.6902	0.6769
	25	2.3445	0.0284	0.6998	0.7397
	35	2.5346	0.0316	0.9036	0.938
	45	1.4987	0.0159	0.947	0.9572
Total Phenolics	-18	0.145	0.0946	0.8389	0.7946
	25	0.2165	0.1403	0.8502	0.837
	35	0.302	0.1889	0.8996	0.9646
	45	0.6668	0.3789	0.8205	0.7599
Antioxidant Activity-DPPH	-18	1.243	0.0176	0.8577	0.825
	25	2.2095	0.0336	0.8383	0.7527
	35	2.2072	0.0293	0.6866	0.6448
	45	4.356	0.0432	0.6076	0.5981

Determination of critical parameters

Based on the quantitative assessment, the higher the R^2 values indicate, the more appropriate the test use as a parameter for determining shelf life. Another criterion in selecting critical parameters for product shelf life is the parameter most sensitive to temperature changes, as seen from the lowest activation energy because it shows the most significant contribution to product damage. If the gradient value is converted to ln and plotted with 1/T (units of degrees Kelvin) or one per absolute temperature, the Arrhenius equation is formed, as shown in Table 4.

Calculation of shelf-life prediction

The parameters of water content and yellowness (b*) have the lowest energy activation but have an R^2 close to one. In addition, based on SNI 3710:2018, one of the requirements for the quality of dried fruit tested is moisture content (maximum 20%). Therefore, the parameter of water content was chosen as a parameter for estimating shelf life. However, the yellowness parameter (b*) is still calculated for its shelf life as a supporting parameter.

Table 4. Results of activation energy and R^2 for each parameter

Parameter	Order	Arrhenius Equation	R^2	Activation Energy (cal/mol K)
Lightness (L*)	1	ln k = -3199.3 (1/T) + 5.9196	0.8667	6353.81
Redness (a*)	0	ln k = -5677.9 (1/T) + 18.574	0.9318	11276.31
Yellowness (b*)	0	ln k = -1585.4 (1/T) + 3.3816	0.4125	3148.60
Change Color (ΔE)	1	ln k = -3300.6 (1/T) + 8.3625	0.8089	6554.99
Texture	1	ln k = -1955.1 (1/T) + 4.1309	0.973	3882.83
Water Content	0	ln k = -1756 (1/T) + 4.8625	0.9107	3487.42
Vitamin C	1	ln k = -2790.6 (1/T) + 4.6152	0.7484	5542.13
Total Acidity	1	ln k = -703.38 (1/T) – 1.4715	0.3166	1396.91
Total Phenolics	0	ln k = -1564.2 (1/T) + 4.0792	0.7033	3106.50
Antioxidant Activity-DPPH	0	ln k = -1313.8 (1/T) + 5.308	0.7909	2609.21

After determining the critical quality parameters for the estimated shelf life, the value of k or the product degradation constant can be calculated from the regression equation between the ln value for each quality parameter and the storage time at four storage temperatures, as shown in Figure 1. The value of k from each temperature is then used to calculate the shelf-life estimation, as shown in Table 5.

Q_{10} equation

Equation Q_{10} (reaction acceleration factor) can calculate the shelf life of products at various storage or distribution temperatures. In the calculation of Q_{10}, a combination of higher temperatures (35°C and 45°C storage temperatures) is better used to estimate the minimum shelf life. The estimated shelf life is based on the Q_{10} value, as seen in Table 6.

Table 5. Calculation results of estimated shelf life

T(°C)	T (K)	ln k	k	Critical Point Value	Initial Score	Shelf-life (Days)
			Water Content			
-18	255	-2.0238	0.1322	20	11.70	62.8425
25	298	-1.0301	0.3570	20	11.70	23.2656
35	308	-0.8388	0.4322	20	11.70	19.2143
45	318	-0.6595	0.5171	20	11.70	16.0606
			Yellowness (b*)			
-18	255	-2.8357	0.0587	20	14.47	94.2611
25	298	-1.9385	0.1439	20	14.47	38.4342
35	308	-1.7658	0.1710	20	14.47	32.3371
45	318	-1.6039	0.2011	20	14.47	27.5045

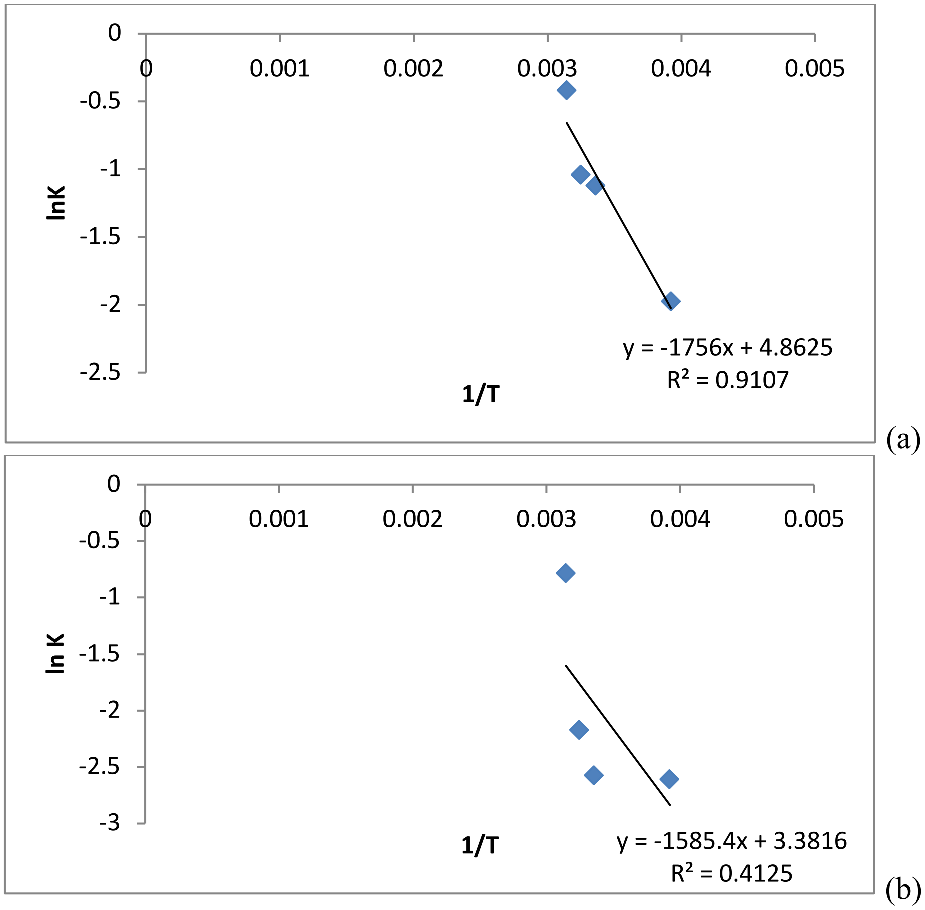

Figure 1. The relationship between 1/T and ln k on the values based on reaction order of (a) moisture content order 0, (b) yellowness (b*) order 0

Table 6. Estimation of shelf life for various storage temperatures

Temperature Storage (°C)	Shelf-life (Days)	
	Q_{10} Water Content = 1.1963	Q_{10} Yellowness (b*) = 1.1757
5	32.8996	52.5503
15	27.5002	44.6976
25	22.9869	38.0183
35	19.2143	32.3371
45	16.0609	27.5049
55	13.4205	23.3948

Discussion

In research conducted by Fallah *et al*. (2023), the final product was freeze-dried strawberries with pre-treatment. The pre-treatment was soaking fresh strawberries in $NaHCO_3$ solution for four hours and immersing fresh strawberries in 50°Brix concentrate solution for two hours at 25°C. Meanwhile, in research conducted by Putri *et al*. (2023), the final product was freeze-dried strawberries untreated. In another study, researchers conducted sensory tests in the form of hedonic tests on freeze-dried strawberries untreated, freeze-dried strawberries produced by local producers, and freeze-dried strawberries imported from Thailand. As a result, consumers liked the shape, color, and aroma attributes of untreated freeze-dried strawberries. While the attributes of taste, texture, and overall impression, consumers like freeze-dried strawberries imported from Thailand (Yuliani *et al*., 2023).

Physical parameter of freeze-dried strawberries

Color is one of the most significant parameters considered during product evaluation by consumers (Ansar *et al*., 2020; Yue *et al*., 2020). Color attributes can be lost or changed during processing, depending on the water content contained in food-dried products. Discoloration of food, both positive and negative, is a common phenomenon that occurs during processing and storage. This color changes the result of various chemical and biochemical reactions at the cellular level (Buvé *et al*., 2018). Thermal effects can destroy heat-sensitive substances in biological products and decrease product qualities, such as color, during the drying process and subsequent storage periods (Ciurzyńska *et al*., 2014). The color test results in the form of the CIELAB color space (L* a* b*), which is then also used to calculate the value of ΔE.

One of the color parameters, namely yellowness, is one of the critical parameters in estimating the shelf life of freeze-dried strawberry products. The yellowness value (b*) indicates the degree of color change from blue to yellow with a scale interval of -60 (the dominant color is blue) to +60 (the dominant color is yellow) (Wang *et al*., 2022). The b* value on freeze-dried strawberries indicates the yellow colors it has. In this study, the b* value decreased compared to the b* value of fresh fruit. In research conducted by Prosapio and Norton (2018), the freeze-drying process has the most significant effect on decreasing the b* value.

Based on the results, the longer the storage and the higher the storage temperature, the higher the b* value. At a storage temperature of -18°C and 25°C the b* value tends to be stable, while at a storage temperature of 35°C and 45°C, the b* value tends to increase. At higher storage temperatures, the color of freeze-dried strawberries quickly changes to a dull brown color due to enzymatic browning reactions (Buvé *et al*., 2018). The enzymatic browning process occurs due to a reaction between polyphenol oxidase and oxygen enzymes with phenolic substrates in strawberries (Ansar *et al*., 2020). In addition, the involvement of heat during the storage process also encourages the Maillard reaction (Oliveira *et al*., 2011; Önal *et al*., 2019). Ascorbic acid intermediates and sugar degradation, such as carbonyl compounds, can polymerize or react with amino acids to form brown pigments in the Maillard reaction (Buvé *et al*., 2018).

Chemical parameter of freeze-dried strawberries

One of the key quality parameters that affect changes in the characteristics of dry products is the water content. Water content can affect the texture, water activity, rate of damage reaction of materials, and product shelf life (de Bruijn *et al*., 2015). The transfer of moisture into the packaging is affected by storage temperature and humidity and the conditions with high temperature and high humidity used in shelf life tests. The increased water content of packaged food causes a decrease in physical, chemical, and sensory quality (Lee and Robertson, 2021). Water in food is also a suitable growth medium for microorganisms, especially for dry products, such as the growth of mold and yeast (Rozana *et al*., 2016). Therefore, what is expected is a low moisture content during storage because the lower the water content, the longer the shelf life and resistance to possible damage to the material by spoilage microorganisms (Rozana *et al*., 2016; Lee and Robertson, 2021).

Fresh strawberries have a moisture content of 88.81%. After the freeze-drying process, it has decreased to 11.70%. Based on the results of this study, the longer the storage and the higher the storage temperature, the water content of freeze-dried strawberries increases. During storage at -18°C, 25°C, 35°C, and 45°C, the water content of freeze-dried strawberries has tended to increase. The water content that increases during storage is still within the quality requirements of SNI 3710: 2018 dried fruit, which is 20%. Increased moisture content in packaged food products can be caused by several factors, such as the permeability of packaging materials to water vapor, the hygroscopic nature of packaged food materials, the humidity level of the environment for food products, and the temperature conditions of the storage environment (Kusnandar *et al*., 2010).

Shelf-life prediction

Based on the calculations that have been carried out using the parameters of moisture content and yellowness (b*), it can be concluded that storage temperature has a relationship with shelf life. Temperature affects the speed of damage or product quality degradation, as evidenced by the value of k in the linear regression equation. The higher the storage temperature, the greater the rate of deterioration or degradation (*k*), which causes a shorter shelf life. The same results in Arif (2016) showed that storage temperature affects the speed of deterioration or decrease in vitamin C content. The initial quality value is another factor that can affect the estimation of shelf life. A high initial quality value can result in a long or longer shelf life.

The shelf life of freeze-dried strawberries based on water content parameters reached 62 days (rounded down) at -18°C, 23 days at 25°C, 19 days at 35°C, and 16 days at 45°C. Meanwhile, based on the yellowness (b*) parameter, the shelf life of freeze-dried strawberries reached 94 days (rounded down) at -18°C, 38 days at 25°C, 32 days at 35°C, and 27 days at 45°C. Furthermore, it is necessary to carry out further research on pretreatment that can be applied before the process of freeze-drying and packaging of freeze-dried strawberries with the MAP system.

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