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Predict the Shelf Life of Instant Chocolate in Vacuum Packing by Using Accelerated Shelf Life Test (ASLT)

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ABSTRACT

To determine the shelf life of a product, an appropriate method is needed. This research aims to predict the shelf life of instant chocolate by using accelerated shelf life test (ASLT) with the Arrhenius equation. Instant chocolate was stored at 30°C, 40°C, and 50°C in polypropylene plastic packaging for 42 days under vacuum conditions. Changes in water content, free fatty acids, flavour, clumping and total microbial number were detected. Results showed that an increase in temperature and shelf life duration can increase the water content and free fatty acid levels and reduce the score on the flavour reception of instant chocolate. Shelf life was estimated based on free fatty acid levels and first-order reactions. The shelf life of instant chocolate stored at 30°C, 40°C, and 50°C is 281.58, 240.99 and 113 days, respectively.

1. INTRODUCTION

Instant chocolate is one of the instant beverage products made from chocolate powder. The product is in the form of dry powder added with milk, emulsifiers, flavourings and sweeteners to increase citasara and nutrient content [1] or added with hydrocolloids to prevent chocolate sedimentation in solution [2]. This combination determines the colour and taste of the product [3]. Cacao contains polyphenols, which are good for health [4]. Chocolate drinks are thought to reduce the risk of heart diseases [5-7] increase systolic blood pressure and promote cardiovascular protective properties, such as vasorelaxation (vasodilation) [8-10] and reduce high-density lipoproteins (HDL), insulin resistance indices and depressive states, increase the cognitive capacity and act as an anti-inflammatory [11]. Antioxidants in cocoa beans include catechins, epicatechins and procyanidins, which are similar to those of tea [12].

Cocoa is a rich source of polyphenols, and defatted unfermented cocoa beans contain about 120-180 g of polyphenolic compounds per kilogram; fat content in cocoa powder ranges from 10% to 22% (depending on the type of cocoa powder desired). Proteins make up 10%-15% of the dry weight of cocoa seeds. Primary carbohydrates in fermented dried cocoa beans are starch (6%) and cellulose (9%) [4]. Instant powder drinks are consumed by brewing hot water or cold water. Instant drink is more practical in terms of packaging and presentation and has extended shelf life because of its low water content, which slows down the work of microorganisms [13]. In addition, powder has low volume, thereby facilitating packaging and distribution. Chocolate powder should have water content of 3.0% to 4.3% and can be categorised into three groups based on fat content: low fat content (10%-12%), medium fat content (13%-17%) and high fat content (17%-22%) [14]. The disadvantage of instant chocolate is that it is hygroscopic or easily absorbs water; in this regard, packaging materials, such as polypropylene (PP) plastics, which can prevent the ingress of moisture or have low moisture permeability, are needed.

The advantages of PP plastics are light weight, transparency, clarity, low water vapour permeability, resistance to high temperatures of up to 150°C and resistance to acids, bases and oils. PP has a melting point of 190°C-200°C and a crystallisation point between 130°C-135°C. PP has high chemical resistance but low impact strength. Oxygen in packaging negatively affects the quality and shelf life of some foods because it leads to product oxidation [15] or promotes the growth of aerobic microorganisms [16], resulting in colour modification [17], sensory changes [18], or nutritional losses [19]. Vacuum packaging can delay lipid oxidation (auto oxidation) because it limits the number of oxygen molecules [20]. Vacuum packaging is conducted by removing air in a container followed by hermetic sealing [21]; in vacuum packages, oxygen supply is limited, and the gas phase is determined by the rate of gas permeation through the film and the rate of oxygen consumption. Vacuum packaging inhibits microbial growth [22]. The study of vacuum packaging is expected to overcome some of its problems and thus maintains the quality of the product for a relatively longer period [23].

Shelf life is defined as the duration when food can still be consumed. The criteria used to determine shelf life are changes in physical, chemical, biological or sensory characteristics [24]. Determination of shelf life can be done by several methods. The first method is by storing the product until the product is damaged or unacceptable so that the shelf life of the product can be known. This method can be done for products that have a short shelf life. For products that have a long shelf life, this is not practical and requires a long time [25] so another method was developed by accelerating the deterioration of product quality through changes in storage conditions known as the Accelerated Shelf-life Testing method. (ASLT) so that the product's shelf life in a relatively short time can be predicted without having to wait for the product to spoil at room temperature. Manzocco et al. [26] developed an accelerated shelf life assay using light and temperature to promote oil oxidation over a suitable time period. One of the approaches taken in the ASLT method is the Arrhenius Equation, namely the kinetic theory which generally uses zero or one order for food products. Zero-order degradation is a constant degradation. The Arrhenius model approach is used to estimate the shelf life of products that are easily damaged due to chemical reactions by simulating the acceleration of product damage at extreme/high temperatures. In this regard, other methods have been developed by accelerating the decline of product and investigating changes under known storage conditions.

2. RESEARCH METHODOLOGY

Instant chocolate powder was obtained from the Melati Berbakti Farmer Group Sungai Langka Village, Pesawaran District, Lampung Province and has a composition of 15% cocoa powder, 70% sugar, 7% milk powder, 7% powdered creamer and 1% flavour. PP plastic with a thickness of 0.8 mm was used as packaging. Chemical and microbiological analyses used 70% alcohol, aquades, plate count agar and buffered peptone water (BPW), 0.1 N NaOH, phenolphthalein (PP), 95% alcohol and hexane.

Instant chocolate samples packed using 0.8 mm-thick PP were stored at three storage temperatures, namely, 30°C, 40°C and 50°C for 6 weeks (42 days). On day 0, water, fat, protein, free fatty acid (FFA), and ash content were analysed. Water content, FFA levels and instant chocolate flavour were analysed once a week on days 0, 7, 14, 21, 28, 35 and 42. Data on water content, FFA levels and sensory profiles (flavour, colour and lumping) were used to determine the shelf life of instant chocolate. Acceleration method (accelerated storage) with the Arrhenius method (kinetic reaction) was used to estimate the shelf life. About 30 g of instant chocolate was packed using PP plastics (18 packets) under non-vacuum conditions and stored at 50°C to determine the critical point. Organoleptic tests were carried out every week to evaluate clumping, colour and rancidity. Instant chocolate that was rejected by 75% of the panelists was regarded as damaged. Analysis of water content and FFA was then carried out. Water content and FFA were expressed as critical water content and FFA.

The shelf life of instant chocolate was calculated by acceleration method. A linear regression chart was prepared from the results of observations of the quality parameters (water content, FFA levels and sensory tests including rancidity, colour and instant chocolate clumping) of the samples stored at 30°C, 40°C and 50°C for 42 days.

(i) A linear regression chart of data observations of quality parameters is made by finding the value of k.
(ii) The value of k is converted into the value of Ln k, which is plotted as coordinate y (ordinate). I/T is a unit of temperature in Kelvin and is plotted to coordinate x (abscissa).
(iii) The slope of the straight line is Ea/R value in the Arrhenius equation, and the intercept is a value of k0. The shelf-life value obtained is then converted into room temperature (25°C) to indicate the actual shelf life of the product.
(iv) After obtaining the value of Ln intercept and -Ea/R., it is then entered into the formula:

where k: constant rate of decline in quality, ko: constant (frequency factor that is not temperature dependent), Ea: activation energy (cal/mol), T: absolute temperature (K = C + 273) and R: ideal gas constant (1,986 cal/mol K).

(v) The shelf life of instant chocolate is estimated using the equation zero and order one as follows:

$$t = \frac{(At - A0)}{k} \tag{1}$$

$$t = \frac{(Ln\ At - Ln\ A0)}{k} \tag{2}$$

Instant chocolate samples packed with PP plastic and stored at 30°C, 40°C and 50°C were evaluated in terms of water content, Free Fatty Acid, ash content, fat level (Soxhlet method) [27] and total plate count [28]. Sensory analysis was conducted by scoring using questionnaires to test for flavour, colour and clumping. Fifteen students were employed as panelists and asked to evaluate the samples one by one (storage temperatures of 30°C, 40°C and 50°C) in terms of flavour (rancidity) and clumping and compare them with the controls. The instant chocolate sample that was not stored was used as control. The flavour of the samples was scored as follows: 7 = normal (same as control), 6 = normal (presumably has off flavour but has not yet been smelled), 5 = normal (off flavour starts but is very weak), 4 = off flavour weakly smelled, 3 = off clear smell, 2 = off strong or rancid smell, and 1 = off strong smell or very rancid. Clumping was scored as follows: 7 = normal or equal to control (no clumping), 5 = normal with little clumping, 3 = more clumping and 1 = mostly clumping.

3. RESULT AND DISCUSSION

3.1 Determination of parameters and critical points

Powdered instant chocolate samples were prepared using raw materials with high fat content so they can cause damages in the form of lumps and rancidity during storage. The critical parameters were water content and FFA level. The critical point was determined through organoleptic testing. Instant chocolate that was rejected by 75% of the panelists was declared to be damaged. Water content and FFA were known as critical water and FFA. Data were obtained from the samples during storage and from organoleptic testing of lumps and rancidity every week. After 9 weeks of storage, the panelists rejected the instant chocolate products. The critical point occurred at a water content of 5.41% and FFA of 7.8.

3.2 Declining quality of instant chocolate

Water content is an important characteristic of powdered instant chocolate products [29]. Water content in instant chocolate will increase with prolonged storage. Increased moisture content in dry products can cause damage characterised by product clumping. Increased water content in powder products is due to the permeability of product packaging materials to water vapour. The product has hygroscopic contents and thus absorbs water vapour from the air. The water vapour first meets the surface of a solid body and then potentially (depending on the structure of the solid body) moves inward. When water vapour becomes in contact with solids, water-solid interactions occur through five mechanisms: adsorption onto the surface of solid particles, capillary condensation, deliquescence, formation of crystalline hydrates and absorption of steam into amorphous solids [30]. Water vapour tends to absorb more polar or ionic surfaces than nonpolar surfaces at ambient temperatures. Reducing the moisture content makes the products more prone to microbial spoilage and may cause alterations in texture and appearance, resulting in decreased shelf-life [31]. Figure 1 shows the water content in instant chocolate products vacuum packaged by using 0.8 mm-thick PP plastic during storage at 30°C, 40°C and 50°C for 6 weeks in order 0.

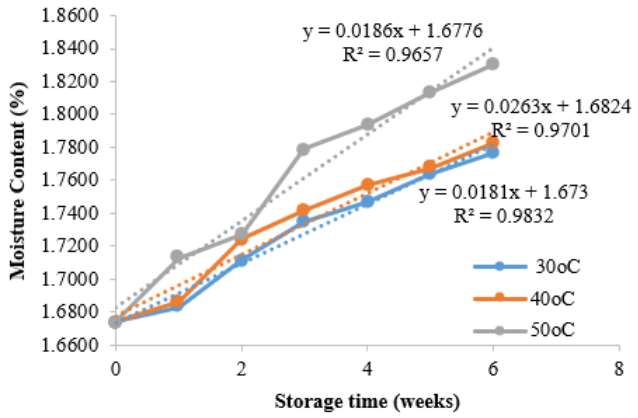

Figure 1. Linear regression of order 0 of moisture content in instant chocolate vacuum packaged using PP plastic

The average water content (% wb) of instant chocolate vacuum packed with 0.8 mm-thick PP plastic during 6 weeks of storage was changed in the form of ln to determine the value of slope (k), intercept (b) and correlation (R^2) in order one in the linear regression graph and the water content line equation (Figure 2 and Table 1). The highest correlation value is in order 0 compared with that in order 1, so the former was used for the Arrhenius equation (Figure 3).

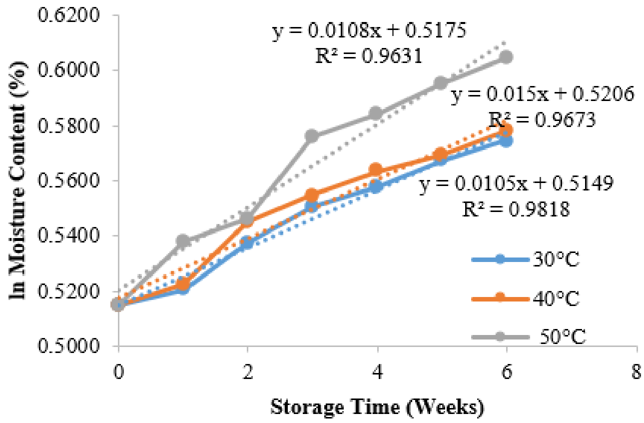

Figure 2. Order 1 linear regression of water content in instant chocolate vacuum packaged using PP plastic

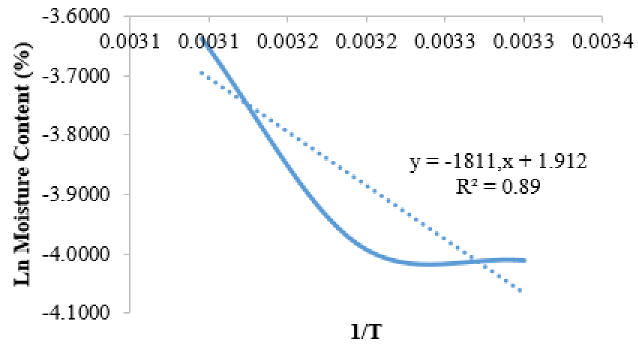

Figure 3. Graph of the relationship between 1/T value with ln k of moisture content in instant chocolate vacuum packaged with PP plastic

Table 1. Slope (k), intercepts (b), and correlations (R^2) of instant chocolate water content during storage at different temperatures

Temperature (°C)	Order 0			Order 1		
	Slope (k)	Intercept (b)	correlations (R^2)	Slope (k)	Intercept (b)	correlations (R^2)
30	0.0181	1.6730	0.9832	0.0105	0.5149	0.9818
40	0.0186	1.6776	0.9657	0.0108	0.5175	0.9631
50	0.0263	1.6824	0.9701	0.0150	0.5206	0.9673

Table 2. Slope (k), intercept (b), and correlation (R^2) values of the parameters of instant chocolate FFA during storage at different temperatures

Temperature (°C)	Order 0			Order 1		
	Slope (k)	Intercept (b)	correlations (R^2)	Slope (k)	Intercept (b)	correlations (R^2)
30	0.0261	6.7361	0.9927	0.0038	1.9075	0.9932
40	0.0304	6.7389	0.9924	0.0044	1.9080	0.9928
50	0.0354	6.7368	0.996	0.0052	1.9077	0.9963

3.3 Free Fatty Acid (FFA)

Figures 4 and 5 show the FFA levels of instant chocolate products vacuum packaged using 0.8 mm-thick PP plastic during storage at 30°C, 40°C and 50°C for 6 weeks in order 0 and order 1 forms. Correlations between Order 1 and 2 were compared to determine the suitable order for determining the Arrhenius equation (Table 2).

Increased temperature and duration of storage can accelerate the rate of chemical reactions, thereby accelerating the decline in product quality [32]. A gradual increase in FFA formation in all samples was observed due to hydrolysis of phospholipids and triglycerides [33]. FFA are known to undergo further oxidation to produce low molecular weight compounds that are responsible for off-flavour and undesirable taste [34]. Walton et al. [35] analysed nuts for changes in peroxide values and FFA during storage to indicate the development of rancidity. Increasing FFA levels at ambient temperatures indicate caution about longer storage time at ambient temperatures. The moisture content in the stored kernels could increase FFA because hydrolytic cleavage of triglycerides can occur when the moisture content is above the critical monolayer level at which enzymes are activated [36]. Temperature is also a factor; hydrolysis of oils increases as time elapses under ambient conditions but is insufficient to significantly increase FFA under refrigeration. Hydrolysis of nuts appears to be related to storage at excessively high ambient temperatures [36].

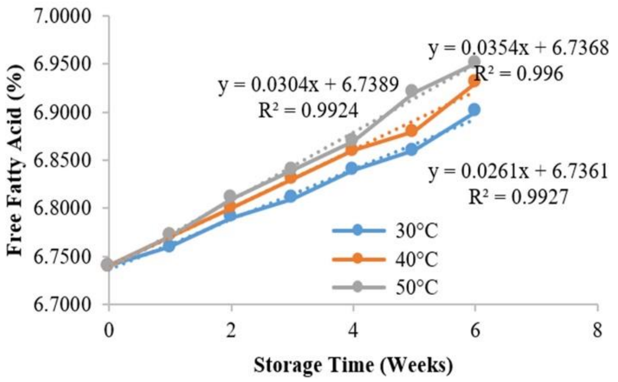

Figure 4. Linear regression of FFA levels in instant chocolate vacuum packaged using PP plastics (zero order)

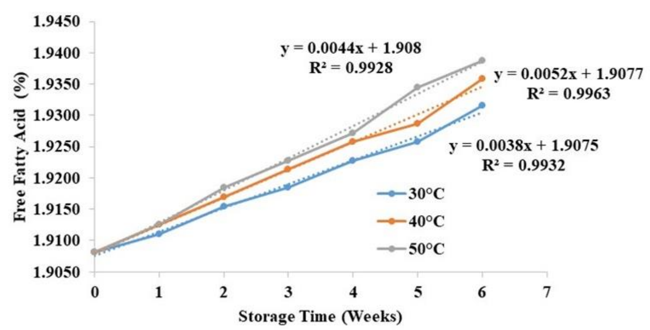

Figure 5. Linear regression and line equation for free male acid levels in first order in PP plastic packaging with a thickness of 0.8 mm under vacuum

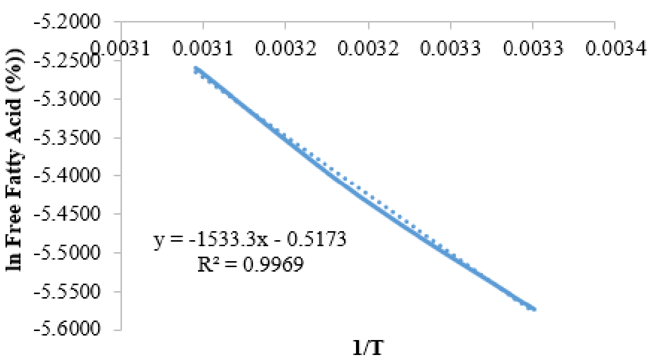

Figure 6. Graph of the relationship between 1 / T value with ln k instant chocolate FFA levels in PP vacuum packaging

Correlation value (R^2) is greater in order 1, which means that the reaction follows order 1. The value of k at the third storage temperature in order 1 was then applied to the Arrhenius equation. Each value of ln k and 1/T at each storage temperature was plotted as ordinate and abscissa in the graph (Figure 6).

3.4 Flavours

Figures 7 and 8 show the average score for the flavour of instant chocolate vacuum packed with 0.8 mm-thick PP plastic during storage (6 mg) on order 0 and order 1. During storage (6 weeks) instant chocolate experienced a change in flavour from normal or equal to the control (score 7) to normal when rancidity began to smell very weak (score 4,325). Changes in the flavour of instant chocolate allegedly due to an increase in FFA levels and the occurrence of further reactions. Fatty products such as cocoa if stored for a long time will experience rancidity. Rancidity in fat stored for a long time due to oxidation, hydrolysis and enzymes. Lipid oxidation and hydrolysis during storage are the most common causes of deterioration in the sensory and nutritional quality of nuts [36]. One of the ingredients used in instant chocolate is milk powder. The taste and shelf life of milk powder is influenced by initial milk quality, processing variables, air quality, water content, packaging, oxygen exposure, addition of antioxidants, exposure to light, and storage temperature [37]. Flavour is a major factor influencing consumer acceptance of milk powder [38] studied milk chocolate made with various milk ingredients, anhydrous milk fatty acids, and whey protein concentrate, and found that consumer acceptance decreased with the presence of malty or off-notes donated by stored milk ingredients. Hall and Andersson [39], who found that taste changes were mostly caused by lipid oxidation products, especially straight chain aldehydes. Whetstine and Drake [40] report that straight chain aldehydes are active compounds that increase the WMP that is stored for up to 2 years. Increased levels of oxidised taste, as determined by trained panels, resulted in decreased consumer acceptance. FFA are very important for nuts, as they contribute off-flavours. Changes in the flavour of instant chocolate allegedly due to an increase in FFA levels and water content. Rancidity intensity is used [41] to estimate the sensory shelf life of dark chocolate containing hazelnut paste.

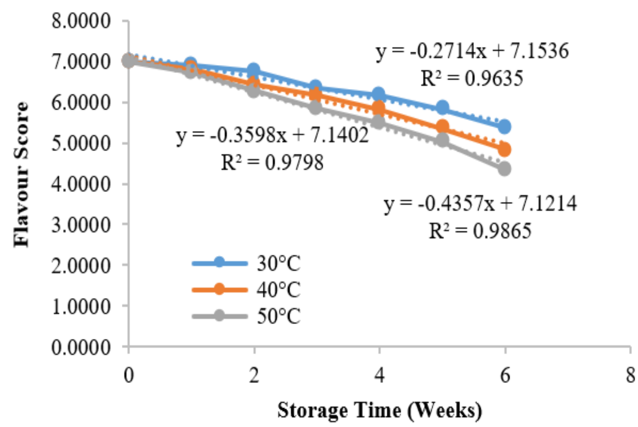

Figure 7. Linear regression of instant chocolate flavour on zero order PP vacuum plastic packaging

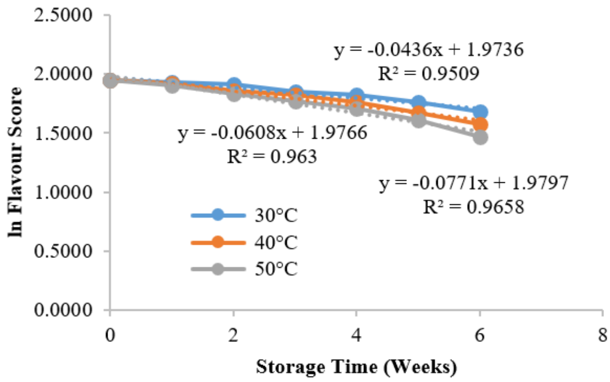

Figure 8. Linear regression of instant chocolate flavour on PP vacuum packaging order

Referring to Figure 8, the graph of linear regression equations (zero and one orders) the relative decrease of instant chocolate flavour obtained slope (k), intercept (b), and correlation (R^2) values at each storage temperature are presented in Table 3. Correlation value (R^2) is greater in order 0 which means the reaction follows order 0. The value of k at the third storage temperature in order 0 is then applied to the Arrhenius equation. Each value of ln k and 1/T (unit of temperature in degrees Kelvin) at each storage temperature is plotted as ordinate and abscissa in the graph shown in Figure 9.

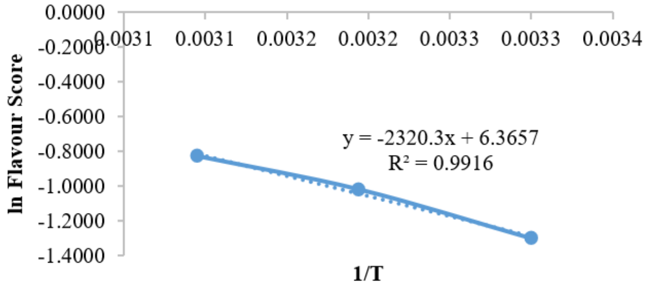

Figure 9. Graphic of the relationship between 1 / T value and instant chocolate aroma in vacuum PP plastic packaging

Table 3. Slope (k), intercept (b), and correlation (R^2) values of instant chocolate flavour parameters during storage at different temperatures

Temperature (°C)	Order 0			Order 1		
	Slope (k)	Intercept (b)	correlations (R^2)	Slope (k)	Intercept (b)	correlations (R^2)
30	-0.2714	7.1536	0.9635	-0.0436	1.9736	0.9509
40	-0.3598	7.1402	0.9798	-0.0608	1.9766	0.963
50	-0.4357	7.1214	0.9865	-0.0771	1.9797	0.9658

Table 4. Slope values (k), intercepts (b), and correlations (R^2) parameters for instant chocolate caking during storage at different temperatures

Temperature (°C)	Order 0			Order 1		
	Slope (k)	Intercept (b)	correlations (R^2)	Slope (k)	Intercept (b)	correlations (R^2)
30	-0.3059	7.0848	0.9858	-0.0502	1.9647	0.9802
40	-0.3729	7.1205	0.9754	-0.0635	1.9747	0.9656
50	-0.4339	7.0839	0.9848	-0.0773	1.9746	0.9617

3.5 Clumping

Average scores of instant chocolate lumps packed in 0.8 mm thickness PP plastic containers under vacuum during storage (6 mg) on order 0 and order 1 are shown in Figures 10 and 11. During storage (6 weeks) instant chocolate experienced a change in clot from normal or equal to control (score 7) to normal with little clumping (score 4.275). Changes in instant chocolate clumping are thought to be due to an increase in water content. The average instant chocolate lump which is packed with 0.8 mm PP plastic packaging under vacuum during storage (6 mg) is made in the form of a relationship of clumping relationship with storage time (weeks). Condensed water has the potential to facilitate hydrolysis reactions, initiate mutual benefits, initiate crystalline hydrate formation and absorb into amorphous solids. Examples of the adverse effects of water absorption into amorphous powder are often related to the effects of water plasticisation, clumping, discolouration due to physical collapse, and crystallisation with various adverse effects such as poor reconstitution properties. This water can condense between particles, produce changes in flow behavior and create potential moisture-induced phase transformations (such as deliquescence and formation of crystalline hydrates) or the plasticisation of amorphous solids (potentially producing a transition from-to-rubber or possibly crystallisation from an amorphous state).

Referring to Figure 11, the graph of linear regression equations (zero and one orders) the relative decrease of instant chocolate aroma obtained slope (k), intercept (b), and correlation (R^2) values at each storage temperature are presented in Table 4.

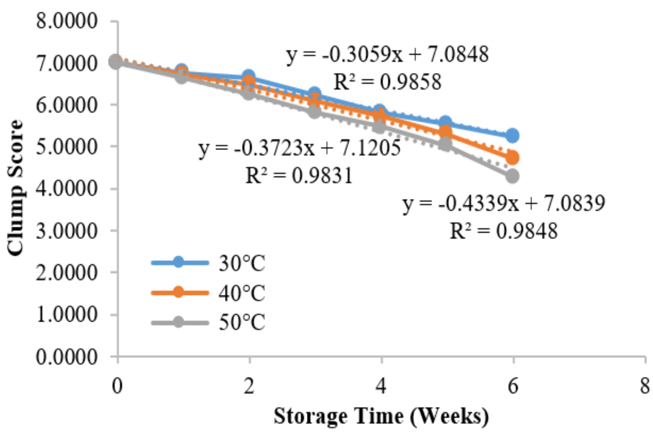

Figure 10. Linear regression of instant chocolate clump on zero order plastic PP vacuum packaging

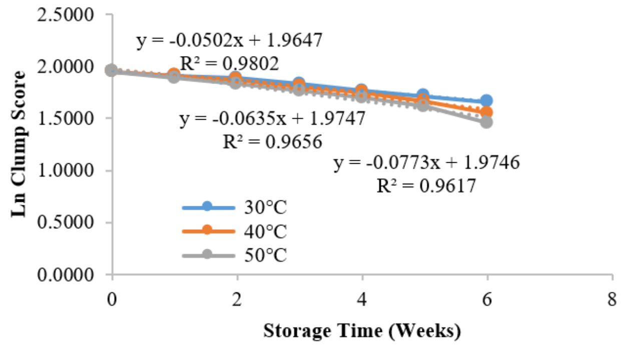

Figure 11. Linear regression image of instant chocolate clump in PP plastic packaging of first order

3.6 Number of microorganisms

Testing the number of microorganisms for instant chocolate products was carried out to determine the total number of microbes, both in the form of moulds, yeasts and bacteria in instant chocolate products (Table 5). Based on observations, the total number of microorganisms obtained in instant chocolate products increased during storage. The highest total number of microorganisms is 2.7 × 103 in instant chocolate stored at 40°C with non-vacuum conditions. The total number of microorganisms is still in accordance with SNI 3747-2009, which is a maximum of 5×10^3. Increasing the number of microorganisms that grow during storage can be caused by an increase in product water content during storage. Increasing the product moisture content will increase the product aw value and aw requirements for microbes to grow by 0.85 or less, although moulds usually require higher water activity. Reducing in moisture makes the products more prone to microbial spoilage and may cause alterations in texture and appearance, consequently reducing the shelf life [31, 42].

The largest increase in the number of microorganisms in products stored at 30°C in non-vacuum conditions. The greatest increase in the number of microorganisms in products stored at 30°C shows that this temperature is suitable for the growth of microorganisms. The smallest increase in the number of microorganisms was observed in products stored at 50°C under vacuum. Kocatepe et al. [43], reported that vacuum packaging is an effective method to preserve chemical and microbiological quality of dry-salted bonito (lakerda) stored at 4°C during the 56th days. Microbiological analysis showed that treated vacuum packed Catla steaks had shelf life of up to 27 days, whereas untreated vacuum packed Catla steaks had shelf life of only 18 days [44]. Rezaabad et al. [34], suggested that chitosan coated and wrapped prior to vacuum packaging decreased the microbial count of the FV (treated with chitosan film, stored under vacuum packaging) and CV (treated with chitosan coating stored under vacuum packaging) during refrigerated storage. Vacuum packaging mainly contributes to prohibiting recontamination and exudation of ingredients from the packaging. Furthermore, oxidation mechanisms, colour degradation and aerobic microbial spoilage are prevented by removing oxygen from the packaging. Vacuum packaging is more effective in controlling fungal growth possibly due to the lack of oxygen at Brazil [45]. The absence of oxygen in the plastic packaging under vacuum is more effective in controlling the multiplication of these microorganisms; the absence of oxygen in the plastic packaging under vacuum is more effective in controlling the multiplication of these microorganisms.

Table 5. Number of microorganisms in instant chocolate products

Days-	Microbia Amount					
	Tem perature 30°C		Tem perature 40°C		Tem perature 50°C	
	Vacuum	Non Vacuum	Vacuum	Non Vacuum	Vacuum	Non Vacuum
1	5×10^2	5×10^2	5×10^2	5×10^2	5×10^2	5×10^2
2	2.5×10^3	2.9×10^3	2.3×10^3	2.7×10^3	2.1×10^3	2.2×10^3

3.7 Estimated shelf life

The parameters used in estimating shelf life are those that have the lowest activation energy (Ea). The activation energy value is obtained based on the Arrhenius equation in each order with the highest R^2 value. The lower the energy needed to start the damage reaction the lower so the damage reaction will last longer. The activation energy value (Ea) for each parameter is shown in Tables 6 and 7.

Shelf life was determined using the parameters with the lowest activation energy that is FFA in order one. Estimation was conducted using the equation Ln Ao = Ln At + Kt, where Ao is the initial parameter value before storage, t = 0 while At is the final parameter value after instant chocolate is rejected by the respondent, and t is the save time then a shelf life value is obtained (Table 7). The ASLT method can determine the shelf life of a product quickly compared to conventional methods or Extended Storage Studies (ESS), which is a method for estimating shelf life by storing in normal conditions and observing changes in quality until it reaches the unwanted quality. The ASLT method is carried out by accelerating quality changes in critical parameters. This method uses environmental conditions that can accelerate the reaction to a decrease in the quality of food products. Food products are stored in extreme temperature conditions, so that the critical parameters experience a decrease in quality due to the influence of heat.

Table 6. Activation energy (Ea) for each parameter

Parameter	Activation energy (Ea) (ka/mol)	k
Moisture content	3597.04	$6.7693e^{-1811.2\ (1/T)}$
Free fatty acid	2858.45	$0.44e^{-1439.3(1/T)}$
Flavour	4608.12	$581.55e^{-2320.3\ (1/T)}$
Clumb	3400.83	$87.58e^{-1712.4\ (1/T)}$

Table 7. Shelf life of instant chocolate stored under vacuum packaging conditions with 0.8 mm-thick PP plastic stored at 30°C, 40°C and 50°C

Temperature	Shelf life of instant chocolate (days)
	Vacuum
30°C	281.58
40°C	240.99
50°C	214

4. CONCLUSIONS

The method to determine shelf life can easily be done using the ASLT method. This method is carried out after knowing the critical point of the observed product quality. Shelf life of instant chocolate vacuum packed in PP plastics determined using ASLT method is 281.58, 240.99 and 113 days at 30℃, 40℃ and 50℃, respectively. This method can be further developed to determine the shelf life of processed agricultural products.

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