



Determination of Shelf Life of Herbal Products from the Combination of *Stevia rebaudiana*, *Curcuma zanthorrhiza* and Honey (Stekurmin MD) through the Accelerated Shelf Life Test (ASLT) Method

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Abstract

Stekurmin MD is a syrup preparation formulated from the extract of *Stevia rebaudiana* leaves, temu lawak (*Curcuma zanthorrhiza*), and honey. One of the active compounds in Stekurmin MD products is phenolic and flavonoid compounds in which the active phenolic and flavonoid compounds can be degraded during the storage period. This study aimed to determine the shelf life of Stekurmin MD products based on the degradation of phenolic compounds and flavonoids. The Accelerated Shelf Life Test (ASLT) method was used in determining the shelf life kinetics of this product. Phenolic and flavonoid concentrations were determined using the UV-Vis spectrophotometric method with the gallic acid standard for phenolic and quercetin standard for flavonoids. Degradation of phenolic and flavonoid compounds was determined every 7 days during 35 days of storage at 25, 35, 45, and 55°C ± 2°C. In natural ingredients, there is a multi-mechanism reaction. The shelf life of Stekurmin MD products based on the degradation of phenolic and flavonoid active ingredients at room temperature were 39.909 days and 23.53 hours, respectively.

1. Introduction

One of the main aspects of the development of pharmaceutical ingredients for active pharmaceutical ingredients (API) is an accurate estimate of chemical stability. The expiry or shelf life of a medicinal product, according to the definitions of the USA Food Drug Administration (FDA) stability guidelines, is the time from the date of manufacture to the date on which the chemical activity of the drug decreases or when the product degrades to predetermined levels, under recommended storage conditions [1]. Inspection specifications or storage time or product life specifications must be met throughout the shelf life with API stability of 90% -110% [2]. If a pharmaceutical product exceeds or less than the specified limit, the product is declared to have no impact on health. Therefore, pharmaceutical or health products must be stable for some time after manufacture. Shelf life must be

determined quickly and accurately to improve the formulation development process.

Martono and Muninggar [3] makes a health product from a combination of herbs from *Stevia rebaudiana*, temu lawak (*Curcuma zanthorrhiza*), and honey is named Stekurmin MD. Stekurmin MD product was proven to have antidiabetic activity in streptozotocin (ZTZ) induced mice. The active compounds or APIs contained in Stekurmin MD products include total phenolic compounds and flavonoids. One method that can be used to determine Stekurmin MD products' shelf life is the Accelerated Shelf Life Test (ASLT) with the Arrhenius approach [4]. Where k (reaction rate constant), k₀ (pre-exponential constant), E_a (activation energy (cal/mol)), R (gas constant 1.986 (cal/mol)) and T (temperature (K)) with the equation as following:

$$\ln k = \ln k_0 - \frac{E_a}{R} \times \frac{1}{T} \quad (1)$$

The literature search shows that the use of the ASLT method has been widely reported. Putri and Yuniastri [5] conducted an estimation of the shelf life using the Arrhenius Model (ASLT) method applied to Jamu "Sari Rapet Super." The parameters observed were moisture content with storage treatment at 25°C, 35°C, 45°C and observed on days 0, 1, 5, 10, 15, 20 for one month. The results showed that storage with packaging at 25°C was 34,826 days, while without packaging at 25 °C was 10,989 days. Swadana and Yuwono [6] conducted an estimation of apple-flavored drinks' shelf life using the ASLT method with the Arrhenius Approach, which was simulated at three storage temperature conditions in the incubator (30°C, 35°C, and 45°C). During the storage process, the parameters observed were pH, color, total acid, vitamin C levels, total dissolved solids, and organoleptic. The results showed that the smallest activation energy value was used to determine the product's shelf life, namely the total acid parameter (first-order reaction) with linear regression $y = -2127.944x - 0.244$. The apple-flavored beverage's shelf life is one year two months at 25°C [6]. While Arif *et al.* [4] estimated the shelf life of soursop fruit juice (*Annona muricata* L.), based on physical and chemical damage parameters using the ASLT method, including analysis of vitamin C, brightness, total acid, and pH and observed every five days for one month in an incubator at a temperature of 30°C, 35°C and 40°C. The results showed that the soursop juice had a shelf life at a temperature of 30°C, 35°C and 40°C, for 3.8, 2.8, and 2.1 months, respectively. Prchalová *et al.* [7] determined the shelf life of baby food fruit, which was determined based on the degradation of active phenolic compounds at 20 and 30°C with a shelf life of 692 and 318 days, respectively. However, the estimation of natural herbal products' shelf life based on the content of phenolic compounds and flavonoids is still limited.

Stekurmin MD stability research is significant to determine its shelf life. This study aims to determine Stekurmin MD herbal products' shelf life based on the content of total phenolic compounds and flavonoids, which provide kinetic parameters for reaction rates. The results can be used to characterize the relationship between degradation and storage conditions. Reaction kinetics include simple and complex reaction kinetics. So far, the analysis has only been carried out to the simple reaction analysis with the reaction order's determination. No statistical analysis has been carried out on the possibility of various reaction orders, which are still started with a simple reaction. Determination of the reaction order is done by matching the experimental graph to a rate law in order to obtain a result that satisfies the correlation coefficient and standard deviation parameters. Then proceed with reviewing the prediction of the mechanism that occurs.

2. Methodology

2.1. Research venue and time

This research was conducted at the Chemistry Laboratory, Chemistry Study Program, Faculty of Science and Mathematics, Satya Wacana Christian University

Jalan Diponegoro 52-60 Salatiga, Central Java, Indonesia. This research was conducted in September–November 2019.

2.2. Equipment and Materials

The samples used were the leaves of *Stevia rebaudiana* (Bert.) (Tawangmangu Plantation, Central Java). The chemicals used are distilled water, NaCl (Merck CAS 7647-14-5), aluminum plate, Folin–Ciocâlțeu (pro-analysis degree, Merck, Germany), Na₂CO₃, gallic acid standard with a purity of 98% (Merck, Germany), NaNO₂, Al(NO₃)₃, NaOH, quercetin standards (pro analysis degree, Sigma, Germany), honey, temu lawak, CMC and glycerin.

The equipment used was cabinet drying, grinder (AIRLUX), 60 mesh sieve, hotplate stirrer (model L-81), analytical balance 0.1 mg (Ohaus, TAJ602), power supply (Goldstar), glassware (Pyrex), spectrophotometer. UV-Vis (UV Mini 1240 Shimadzu).

2.3. Sample Preparation

Samples that have been cleaned from the ground are dried in a drying cabinet for 24 hours at a temperature of ± 50°C and then crushed using a grinder. The fine samples were sieved using a 60 mesh sieve [8].

2.4. Sample Extraction Preparation

Samples of sieved stevia leaves were weighed and extracted by maceration at 60°C using distilled water with a sample: distilled water ratio of 1:40 w/v. Maceration was carried out for 20 minutes for four cycles of re-extraction [9].

2.5. Dechlorophylation [10]

To 100 mL of extracted filtrate, 1% salt (NaCl) was added to the total volume. Then electrolysis was carried out for 26 minutes using an aluminum plate (measuring 3 × 15 cm) as the electrode. The current used in the power supply was 0.8 A. Then, the filtrate was filtered and prepared for further treatment.

2.6. Stekurmin MD Preparation

Stevia syrup was prepared by formulating the water extract of *S. rebaudiana*, temu lawak, and honey. The syrup was made by adding CMC and glycerin.

2.7. Accelerated Shelf Life Test (ASLT)

A total of 20 mL of Stekurmin MD syrup was put into sample bottles and stored at various temperatures (25°C, 35°C, 45°C and 55°C ± 2°C) for 35 days. The measurement of phenolic and flavonoid content was carried out every seven days. Furthermore, the degradation reaction order was determined based on the highest coefficient of determination (R²). Then the value of ln k for each temperature was curved following equation 1. Determination of shelf life using the chosen reaction order was at the critical limit of the degraded active ingredient content of 10% or t₉₀ [4].

2.8. Determination of Total Phenolic Content

A total of 1.0 mL of sample was added with 2.0 mL of 10% Folin–Ciocâlțeu and 2.5 mL of 7.5% Na₂CO₃. Then,

the solution's absorbance was measured with a UV-Vis spectrometer at a wavelength of 718 nm. Total phenolic content was measured based on the standard curve between the standard concentration of gallic acid (μg GAE/mL sample) and the absorbance at a wavelength of 718 nm. The total phenolic content is expressed as gallic acid equivalent/mL sample (μg GAE/mL sample) [11].

2.9. Determination of Total Flavonoid Content

A total of 2 mL of sample was added with 0.2 mL of 5% NaNO_2 then allowed to stand for 6 minutes, then added with 0.2 mL of $\text{Al}(\text{NO}_3)_3$ 10% and left for 6 minutes, followed by the addition of 2 mL of 4% NaOH and let stand 15 minutes. The absorbance of the solution was measured using a UV-Vis spectrometer at a wavelength of 501 nm. Total flavonoid levels were measured based on a standard curve between the standard concentration of quercetin and the absorbance at a wavelength of 501 nm. The total flavonoid level was expressed as a quercetin equivalent/mL sample (μg QAE/mL sample) [12].

2.10. Data analysis

The determination of shelf life was carried out based on data obtained from phenolic and flavonoid analysis, using regression between the concentration of the active ingredient and the length of storage time (days) at each temperature. The k values obtained from linear regression are then plotted as $\ln k$ against $1/T$ to determine the activation energy (Joule).

3. Results and Discussion

3.1. Phenolic

In this study, total phenolic measurements were carried out by the Folin–Ciocalteu method in an alkaline atmosphere. This method's principle is the formation of a complex blue compound whose absorbance can be measured at a wavelength of 718 nm (A_{718}) by UV-Vis spectrophotometry. Folin–Ciocalteu reagent oxidizes phenolate (alkaline salt) or phenolic-hydroxy groups to reduce heteropoly acid (phosphomolybdate-phosphotungstate), which is present in Folin–Ciocalteu reagent, to become a molybdenum-tungsten complex which is bluish-green [13].

In the measurement, Na_2CO_3 was added because phenolic compounds can only react with Folin–Ciocalteu reagent in alkaline conditions so that protons in phenolic compounds dissociate into phenolic ions [13, 14]. The occurrence of a reaction was indicated by the appearance of blue color in the solution. The blue color formed was increasingly concentrated equally to the concentration of phenolic ions formed so that A_{718} increased. The greater the concentration of phenolic compounds, the more phenolic ions reduce heteropoly acids [15].

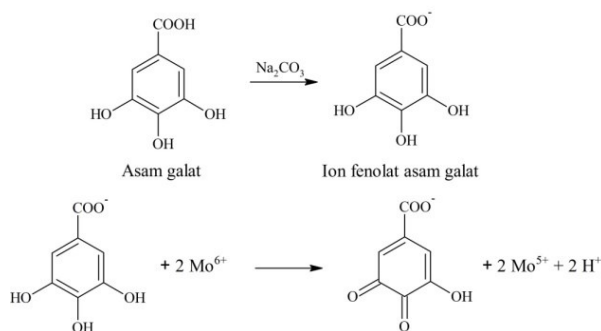


Figure 1. The reaction of gallic acid in the Folin–Ciocalteu reagent [16].

Table 1 and Figure 3 present the average value of phenolic content at various storage periods. It can be seen that the phenolic concentration value increased during initial storage. This can happen because phenolic compounds are more soluble in the Stekurmin MD product system. In addition, flavonoid compounds are degraded into phenolic compounds with smaller molecular weights [17]. The product kinetics of Stekurmin MD was determined based on the kinetics of the increase in phenolic active ingredients using the Arrhenius equation. In this study, an analysis of phenolic and flavonoid compounds was carried out because flavonoid compounds are phenolic compounds, but not all phenolic compounds are included in flavonoid compounds. Therefore, there may be phenolic compounds that are not flavonoid compounds that can influence the Stekurmin MD system in determining shelf life.

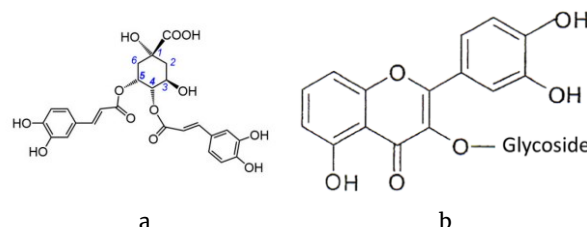


Figure 2. (a) Structure of phenolic compounds (b) Structure of flavonoid compounds

Table 1. Mean values of phenolic content (μg GAE/mL sample) at various storage periods

Time (day)/	Temperature ($^{\circ}\text{C}$)			
	25 $^{\circ}\text{C}$	35 $^{\circ}\text{C}$	45 $^{\circ}\text{C}$	55 $^{\circ}\text{C}$
0	30.578	30.578	30.578	30.578
7	181.313 ± 0.989	178.646 \pm 1.469	189.728 \pm 0.514	220.424 \pm 1.141
14	192.05 6 ± 0.42 4	194.741 \pm 0.723	195.667 \pm 0.278	285.250 \pm 0.139
21	175.667 ± 1.002	183.444 \pm 1.273	188.259 \pm 0.404	310.111 $\pm 3.$ 056
28	177.148 ± 0.404	169.972 \pm 1.250	193.167 \pm 0.321	342.333 \pm 1.398
35	181.870 ± 0.723	175.019 \pm 0.912	202.519 \pm 1.092	379.833 \pm 0.278

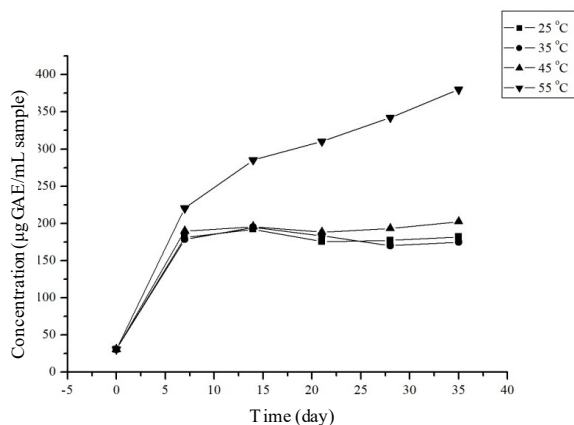


Figure 3. Mean Phenolic Content (µg GAE/mL sample) vs. Storage Time at various temperatures (days)

Based on Table 2, the kinetics of the increase in Stekurmin MD products' increase in phenolic compounds follows the order 0 reaction. The determination of this reaction order is because it provides the highest determination coefficient value (R²).

From the linear regression equation obtained from the graph of the effect of storage on the phenolic value at each storage temperature, it can be obtained that the k value (constant reaction rate of increasing the value of phenolic content) which is the slope of the curve (gradient). The increase in phenolic values at each storage temperature follows the linear regression curve equation shown in Table 3.

Table 2. Determination of the Phenolic Reaction Order based on the Value of the Coefficient of Determination (R²)

Temperature (°C)	Phenolic		
	Order 0	Order 1	Order 2
25	0.8802	0.8682	0.8612
35	0.8196	0.8408	0.8515
45	0.9028	0.8789	0.8648
55	0.9573	0.9015	0.8683

Table 3. Equations of linear regression curves; concentration versus time relationship

Temperature (°C)	Kinetics parameters		
	Equation	R ²	k (µg GAE/mL sample/day)
25	y = 4.5832x + 45.068	0.8802	4.5832
35	y = 4.3702x + 47.98	0.8196	4.3702
45	y = 5.1681x + 45.101	0.9028	5.1681
55	y = 10.309x + 49.228	0.9573	10.309

Based on the Arrhenius equation, the k value is expressed as ln k plotted against storage temperature (1/T). The curve relating ln k to storage temperature (1/T) is presented in Figure 4.

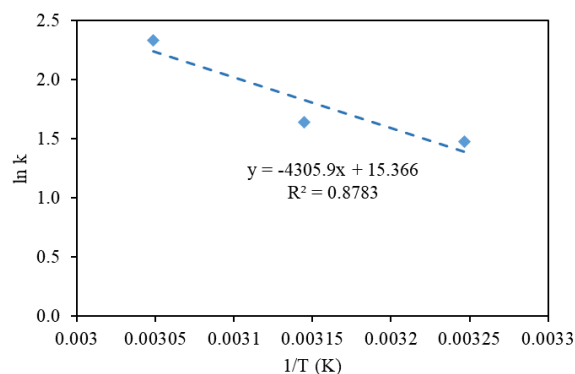


Figure 4. Relationship between ln k and storage temperature (1/T) of phenolics

From the relationship between storage temperature (1/T) and ln k, the equation $y = -4305.9x + 15.366$ with the coefficient of determination (R²) is 0.878. The curve slope is the activation energy divided by the gas constant (Ea/R). R has a value of 8.314 J/mol K. The amount of activation energy is obtained by multiplying R and Ea so that the activation energy value is 35799 J.

Activation energy is the minimum energy used for phenolic compounds, an increase of 35.799 kJ. The value of activation energy at increasing the value of phenolic concentrations was lower than the value of activation energy for phenolic degradation in herbal products from *Syzygium cumini* L., agar, pectin, and polyextrose, which was 39.22 kJ/mol [18]. Increasing the phenolic content is more possible in this case, because in the herbal product system Stekurmin MD, there are compounds that can be degraded into phenolic compounds.

3.2. Determination of shelf-life based on active phenolic compounds using polynomial regression

The kinetics of phenolic increase with the Ea value obtained could not determine phenolic active compounds' degradation value for Stekurmin MD products. This is because there has not been a degradation reaction of phenolic compounds during 35 days of storage, as evidenced by the increased kinetics of phenolic compounds. Therefore, it is assumed that a multi-component reaction occurs, which may be due to complex intermediates in the reaction [19]. An oxidation reaction may occur in the herbal product Stekurmin MD, which results in degradation of the phenolic compounds [20]. Oxidation by enzymatic and non-enzymatic mechanisms can occur. Enzymatic oxidation by the oxidation mechanism of phenolic compounds includes hydroxylation at ortho positions adjacent to existing phenolic substrate groups and oxidation of ortho-dihydroxybenzenes on ortho-benzoquinones (Figure 5).

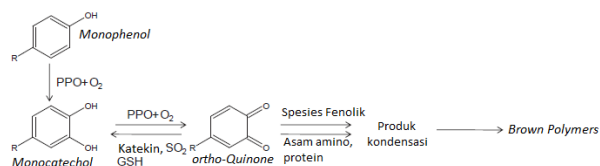


Figure 5. Enzymatic oxidation process [21]

In this study, a calculation using polynomial equations was tried to provide more accurate modeling with actual data and provide a better coefficient of determination (close to 1). Polynomial regression is a nonlinear regression model formed by adding up the effect of each predictor variable (X), which is elevated to the k-order. In general, the polynomial equation is as follows:

$$y = b_0 + b_1X + b_2X^2 + \dots + b_kX^k + \varepsilon \quad (2)$$

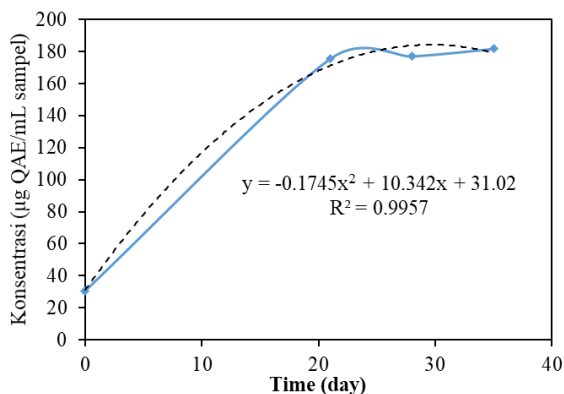


Figure 6. The curve of Phenolic Polynomials at 25°C

Table 5. Polynomial equations and phenolic determination coefficient

Temperature (°C)	Phenolic	
	Polynomial Line Equations	R ²
25	$y = -0.1745x^2 + 10.342x + 31.02$	0.9957
35	$y = -0.2057x^2 + 11.159x + 31.419$	0.984
45	$y = -0.1746x^2 + 10.93x + 31.047$	0.9961
55	$y = -0.2243x^2 + 17.711x + 31.171$	0.9983

In the line equation obtained from the concentration-time relationship curve, there is a peak point at a certain concentration and time. The determination of the degradation of t₉₀ is calculated from the peak point of the polynomial regression. The vertex is defined as the first derivative of the polynomial curve equation presented in Table 6. Time (days) is the time required to reach the phenolic concentration at the peak point (A [µg GAE/mL sample]). The 90% degradation of active phenolic compounds is calculated from the peak point entered into the polynomial line equation using the abc formula in Equation 3. So that the shelf life value is obtained based on the active phenolic compounds presented in Table 6.

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad (3)$$

This result is different from the shelf-life value of the herbal product *Syzygium cumini* L., agar, pectin, and polyextrose. The degradation of active phenolic compounds at 10°C has a shelf life of 186 days [18]. This very significant difference is due to different matrices in the sample. The herbal products in this study have a more complex matrix with various natural ingredients in the product. The estimated shelf life of herbal products such as the herbal medicine “Sari Rapet Super” under storage conditions at 25°C is 34,826 days [5]. These results

indicate that Stekurmin MD herbal products' shelf life, based on the content of phenolic compounds, is not much different from the herbal product “Jamu Sari Rapet Super.”

Table 6. Determination of 10% degradable Phenolic Content

Temp. (°C)	Phenolic			Shelf life (day)
	Phenolic Peak Point - Order 2 Polynomial		A _{90%} (µg GAE/mL sample)	
	Time (day)	A (µg GAE/mL sample)		
25	29.633	184.253	165.828	39.909
35	27.124	182.760	164.484	36.550
45	31.300	202.102	181.892	42.059
55	39.481	380.792	342.712	52.510

3.3. Flavonoid

Total flavonoids were measured by the AlCl₃ colorimetric method. The principle of determining the flavonoids' levels in the aluminum chloride method is the formation of complexes between aluminum chloride and the keto group on the C-4 atom and the hydroxy group on the neighboring C-3 or C-5 atoms of the flavone and flavonol groups [21]. This complex's formation will shift the wavelength towards the visible where the solution changes color to yellow [22]. The use of NaNO₂ and NaOH will form a complex NaNO₂-AlCl₃-NaOH system, which shows a unique color based on the reaction of aluminum ions with flavonoids under alkaline conditions to form complex compounds [23].

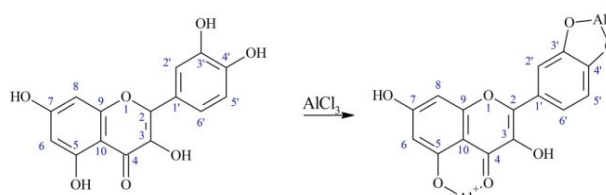


Figure 7. The reaction of quercetin with AlCl₃ reagent [21]

The compound used as a standard in determining the flavonoids' levels is quercetin because quercetin is a flavonoid in the flavonol group with a keto group on the C-4 atom and the hydroxyl group on the neighboring C-3 and C-5 atoms [21]. Measurement of total flavonoids refers to the standard quercetin curve that has been made. Standard curves were made at various concentrations of 10–90 µg/mL and the equation $y = 0.0017x - 0.0032$ with $R^2 = 0.999$ was obtained. The coefficient of determination, which is getting closer to 1, indicates that the analysis method is good. According to Harmita [23], the acceptance criteria are the correlation coefficient (R) close to 1 ($0.990 \leq R \leq 1$). Table 7 shows the average value of the flavonoid content at various storage times at different temperatures. Based on Table 7 and Figure 4, it can be seen that the value of the flavonoid content has decreased during storage. The product kinetics of Stekurmin MD was determined based on the derivation

kinetics of the flavonoid active ingredients using the Arrhenius equation (equation 1).

Table 7. Mean values of flavonoid content (µg QAE/mL sample) at various storage periods

Time (day)	25°C	35°C	45°C	55°C
0	354.118	354.118	354.118	354.118
7	317.782 ± 2.618	330.276 ± 2.676	338.441 ± 0	415.153 ± 0
14	157.059 ± 2.941	183.529 ± 0	198.235 ± 2.941	383.529 ± 0
21	124.118 ± 2.941	165.294 ± 0	150.588 ± 0	241.765 ± 0
28	97.647 ± 0	118.235 ± 2.941	130 ± 0	241.765 ± 0
35	144.706 ± 0	138.824 ± 0	149.118 ± 1.471	297.647 ± 2.941

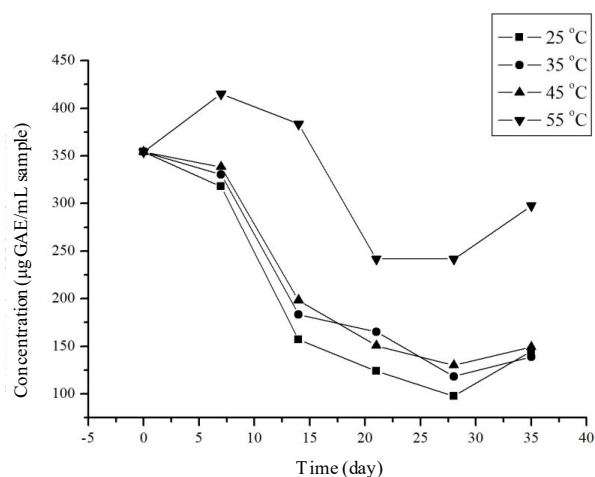


Figure 8. Mean values of flavonoid content (µg QAE/mL sample) vs. storage time (days) at various temperatures

Based on Table 8, the kinetics of the degradation of flavonoids in Stekurmin MD products follow a second-order reaction. Determination of this reaction order because it provides the highest coefficient of determination (R^2). From the linear regression equation obtained from the graph of the effect of storage on the value of flavonoids at each temperature, it can be obtained that the value of k (constant rate of reaction to decrease the value of flavonoid content) is the slope of the curve (gradient). The decrease in flavonoid content at each temperature follows the linear regression curve equation shown in Table 9.

Table 8. Determination of the Flavonoid Reaction Order based on the Value of the Coefficient of Determination (R^2)

Temperature (°C)	Flavonoid		
	Order 0	Order 1	Order 2
25	0.9047	0.945	0.9626
35	0.9153	0.9429	0.9387
45	0.9115	0.9412	0.9555
55	0.6042	0.6346	0.6601

Table 9. Equations of linear regression curves relationship [1/concentration] to time

Temp. (°C)	Kinetics parameters		
	Equation	R2	k (µg QAE/mL sample ⁻¹ day ⁻¹)
25	$y = 0.0003x + 0.0022$	0.9626	3×10^{-4}
35	$y = 0.0002x + 0.0023$	0.9387	2×10^{-4}
45	$y = 0.0002x + 0.0023$	0.9555	2×10^{-4}
55	$y = 6E-05x + 0.0024$	0.6601	6×10^{-5}

This result is different from the research of Wang and Zhao [24], who obtained that the rate constant for quercetin degradation based on order 1 was greater, namely 4.37×10^{-2} to $5.97 \times 10^{-2} \text{ h}^{-1}$. A larger reaction rate constant indicates the compound is easier to degrade. This may be due to the complexity of the different sample matrices. Based on the Arrhenius equation above, the k value expressed as $\ln k$ is plotted against storage temperature ($1/T$). The curve connecting $\ln k$ to storage temperature ($1/T$) can be seen in Figure 9.

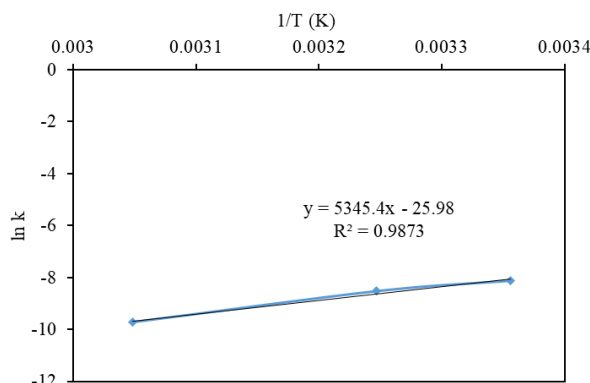


Figure 9. Relationship between $\ln k$ and storage temperature ($1/T$) of flavonoids

From the relationship between storage temperature ($1/T$) and $\ln k$, the equation $y = 5345.4x - 25.98$ with a coefficient of determination (R^2) 0.9873 is obtained. The curve slope is the activation energy divided by the gas constant (E_a/R), R has a value of 8.314 J/molK. The amount of activation energy is obtained by multiplying R and E_a so that the activation energy value is 44441 J.

The activation energy (E_a) of 44.441 kJ is the energy needed for the flavonoid active compound's degradation reaction in Stekurmin MD herbal products. The higher E_a value in Stekurmin MD explains that flavonoids are more difficult to degrade in this system. Based on the Arrhenius equation with an E_a value of 44441 J and the equation between $\ln k$ and $1/T$ is $y = 5345.4x - 25.98$, the shelf life value of Stekurmin MD products can be determined by determining the k value at 298 K/25°C (room temperature) and 277 K/4°C (refrigerator temperature) were $3.2 \times 10^{-4} \text{ µg QAE/mL sample}^{-1} \text{ day}^{-1}$ and $1.25 \times 10^{-3} \text{ µg QAE/mL sample}^{-1} \text{ day}^{-1}$ respectively. By using the 2nd order equation according to equation 4 below:

The activation energy (Ea) of 44.441 kJ is the energy needed for the flavonoid active compound's degradation reaction in Stekurmin MD herbal products. The higher Ea value in Stekurmin MD explains that flavonoids are more difficult to degrade in this system. Based on the Arrhenius equation with an Ea value of 44441 J and the equation between ln k and 1/T is $y = 5345.4x - 25.98$, the shelf life value of Stekurmin MD products can be determined by determining the k value at 298 K/25°C (room temperature) and 277 K/4°C (refrigerator temperature) were $3.2 \times 10^{-4} \mu\text{g QAE/mL sample}^{-1} \text{day}^{-1}$ and $1.25 \times 10^{-3} \mu\text{g QAE/mL sample}^{-1} \text{day}^{-1}$ respectively, using the 2nd order equation according to equation 4 below:

$$\frac{1}{[A]} = k \cdot t + \frac{1}{[A_0]} \quad (4)$$

Where [A] is the flavonoid concentration 90% of the initial flavonoid concentration, [A0] is the initial flavonoid concentration, k is the reaction rate constant at 298 K and 277 K, and t is the shelf life with a critical limit of flavonoid degradation up to 90%. From the initial concentration, the formula for determining the shelf life can be derived as in equation 5 below:

$$t_{90} = \frac{1}{9 \times k \times [A_0]} \quad (5)$$

Based on equation 5, the shelf life value can be determined, as shown in Table 10:

Table 10. Shelf life MD Stekurmin product based on Active Flavonoid Compounds

T (K)	Shelf Life (day)
298	0.981
277	0.251

Shelf life of Stekurmin MD products in Table 10 is determined based on the degradation of the flavonoid active compounds in Stekurmin MD products. The dominant flavonoid compounds found in the leaves of *Stevia rebaudiana* (Bert.) Include quercetin-O-glucoside, quercitrin, kaempferol-3-O-rhamnoside, centaureidin, and quercetin 3-O-arabinoside [25, 26]. From Table 10, it can be seen that at room temperature (298 K/25°C), Stekurmin MD products have a longer shelf life than refrigerator temperatures (277 K/4°C). This is because, at 4°C, the flavonoids experience degradation due to oxidation reactions. Quercetin at room temperature will undergo an oxidation reaction that can degrade the active compound [23]. The oxidation reaction involves two quercetin electrons. Quercetin loses one-electron to form semi-quinones, followed by losing a second one-electron to form o-quinones. Furthermore, oxygen is transformed into hydrogen peroxide, where the redox cycle Fe³⁺/Fe²⁺ and Cu²⁺/Cu⁺ is an intermediate cycle [21].

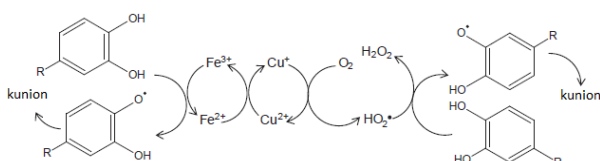


Figure 10. Oxidation reaction which produces kuning and hydrogen peroxide with catalyst Fe³⁺/Fe²⁺ and Cu²⁺/Cu⁺ [20].

4. Conclusion

The shelf life of Stekurmin MD products with the Accelerated Shelf Life Test (ASLT) method based on the degradation of the active ingredients of phenolic and flavonoids at room temperature were 39.909 days and 23.53 hours, respectively. In this study, the results were good enough for shelf life based on phenolic active ingredients and less satisfactory results based on active flavonoids.

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