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Research Article

Shelf Life Determination of Instant Granules Combination of Broccoli (*Brassica oleracea* L.) and Pegagan (*Centella asiatica* L.) Herb Extracts Using Arrhenius Model Accelerated Shelf Life Method

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ABSTRACT

Dementia is one of the diseases whose number of sufferers has increased over the past 10 years. Some natural ingredients that can be used to improve memory are broccoli (*Brassica oleracea* L.) and pegagan (*Centella asiatica* L.) Urban). The high interest of Indonesian people in using herbs in the prevention of diseases is an opportunity to develop granule preparations of broccoli and pegagan extracts. Granules preparations are packaged in a sachet to make it easier to consume them. Physical quality testing and determination of its shelf life are carried out to ensure the quality of instant granules preparations. This study aims to evaluate the physical quality and determine the shelf life of instant granules using the Accelerated Shelf Life Testing (ASLT) method of the Arrhenius model. Preparation was carried out by wet granulation and then packaged using aluminum foil sachets. Furthermore, physical quality parameters and stability tests were evaluated with storage conditions at 40 °C, 45 °C, and 60 °C for 4 weeks. Parameters include: organoleptic, loss on drying (LOD), dispersion time, pH, and flavonoid content. The results of the evaluation of the physical quality of instant herbal granules combined with broccoli and pegagan extracts are LOD 4.4%, ash content 1.5%, flow rate 8.19 g/sec, angle of repose 25-30^0, dispersion time 1 minute 24 seconds, pH 4.84, and flavonoid content 0.5 mg QE/g. The results of the stability test obtained data on the shelf life of instant granules for 13.2 weeks or 92 days at room temperature (25°C).

Keywords: Accelerated Shelf Life Testing; broccoli; instant granule; pegagan; shelf life

INTRODUCTION

Dementia is a term for several diseases that affect memory, thinking, and the ability to perform daily activities. The illness gets worse over time, it mainly affects older people, but not all people will get it as they age. In 2021, 57 million people had dementia worldwide, over 60 % whom live in low-and middle-income countries. Every year, there are nearly 10 million new cases (WHO, 2019). Dementia is a disease whose clinical symptoms affect memory, thinking ability, and social ability, characterized by a decrease in memory or memory and cognitive function (Alzobaidi et al., 2021).

Broccoli (*Brassica oleracea* L) contains secondary metabolite compounds such as glucosinolates, phenolics, especially flavonoids. In addition, broccoli contains fiber, vitamin C, vitamin E, and minerals such as calcium, magnesium, selenium, and potassium, which have the potential to help the transmission of amino acids to the brain. Thus, broccoli can be used to improve cognitive function in patients (Nagraj et al., 2020).

Based on a population study on the correlation between flavonoid intake and dementia in 23 developed countries, it was found that increased consumption of flavonoids, especially flavonols,

influenced the low level of dementia in the country (Beking & Vieira, 2010). Flavonoids can prevent neurotransmission by inhibiting enzymes necessary for neurotransmitter metabolism, thus playing an essential role in preventing neurodegenerative disorders such as Alzheimer's disease. Plants that can be developed into herbal products as antidementia are a combination of broccoli and pegagan herb extracts which are proven to improve memory in mice at a dose of 0.069 g/kg BW whose effect is comparable to prostigmin (Nihaya, 2016). Pegagan herb contains more than 70 types of secondary metabolites, including flavonoids and asiaticosides (Sabaragamuwa et al., 2018). The content of asiaticosides can reduce free radical levels and inhibit nerve cell death, thus playing a role in the prevention and treatment of Alzheimer's type dementia. As a nervine adaptogen, constituents of pegagan are capable of increasing intelligence, longevity, and memory. (Farooqui et al., 2018).

The combination of broccoli extract and pegagan herb was developed into an instant granule preparation, containing 25 mg of broccoli dry extract and 190 mg of pegagan. This combination is made into instant granules because it is more practical, easier to consume, and tastes good, so that it can be consumed by the elderly. When compared to tablets that often leave a bitter taste, instant granules that taste sweet are preferred. In terms of making this instant granule form, it has a better flow rate than ordinary powder, so that the weight between packages becomes more uniform.

Preparations derived from natural ingredients tend to have low stability due to the diverse content of active ingredients and high water content (Oktami et al., 2021). However, herbal preparations are often challenged by stability issues due to their complex composition and susceptibility to environmental conditions. Therefore, stability testing is critical to ensure the safety, efficacy, and quality of the final product during its shelf life. One effective approach is the Accelerated Shelf-Life Testing (ASLT) method based on the Arrhenius model, which simulates long-term storage conditions by exposing the product to elevated temperatures and humidity, enabling faster and accurate shelf-life predictions (Chaudhary & Kumari, 2022; Różyło et al., 2023).

To ensure the quality of instant granule products remains of good quality during the storage period and safe for consumption, a stability test must be conducted. This study aims to evaluate the physical and chemical stability of instant granule formulations containing broccoli and pegagan extracts using the ASLT method. The results are expected to support the development of stable, effective, and elderly-friendly herbal anti-dementia products

METHODS

Material

The materials used inthis study included fresh broccoli (*Brassica oleracea* L.) (Plantation located in Gunung Putri Village Sukatani, Pacet District, Cianjur Regency, West Java), dried extract of *Centella asiatica* (L.) herb (PT Pytochemindo Reksa), distilled water, 96% ethanol (Merck®), methanol (Merck®), $AlCl_3$ (Merck®), sodium acetate (Merck®), magnesium (Merck®), HCl (Merck®), bouchardat (Merck®), mayer (Merck®), $FeCl_3$, ethyl acetate (Merck®), anhydrous acetic acid (Merck®), sulfuric acid (Merck®), PVP K-30 (Anhui), sucralose, mocha coffee essence (RedBell®), chocolate essence (Toffieco®), mannitol (Shizajuang), lactose (Koliba).

Equipment

The tools used in this study included of glassware (Pyrex®), oven Memmer UN30® (Schwabach, Germany), flow tester Intralab Trading Mandiri® (Bandung, Indonesia), Moisture Balance Bel ®(Monza, Italy), pH meter Ohaus® ST3100 (Jersey, USA), aluminum foil sachet packaging, thermo-hygrometer, UV-Vis Spectrophotometer Jasco® V730 (Tokyo, Japan)

Material Collection and Plants Determination

Brocoli are obtained from Cianjur, West Java, Indonesia. The determination of Brocoli was cunducted at the Herbarium Depokensis Departemen of Biology FMIPA University Indonesia (UIDEP), Depok, Indonesia. The dry extract of the pegagan herb is obtained from PT Pytochemindo Reksak which contains 15% pure pegagan.

Physical Characteristic Test

Evaluating physical characteristic including organoleptic (visual inspection of color, smell, and taste); determining water content and ash content was perfomed according to Indonesia Herbal Pharmacopeia (Kementrian Kesehatan , 2017).

Phytochemical Screening

Phytochemical screening was carried out to identify alkaloids, flavonoids, , triterpenoids, saponins, and tannins. Flavonoid testing was carried out using concentrated HCl 2N and Mg powder, positive results were indicated by the presence of a dark red color. Alkaloids were tested using Mayer reagent, Wagner reagent and Dragendorff reagent. Formed White precipitate on Mayer's reagent, brown precipitate on Wagner's reagent, and orange precipitate on Dragendorff's reagent in each test result showed positive results containing alkaloids. Testing for triterpenoids was carried out using concentrated sulfuric acid and anhydrous acetic acid, if is red or orange, it is positive for triterpenoids. The saponin

examination was carried out by vigorously shaking the extract solution, if the foam was formed, it was positive for saponins. The procedure carried out refers to the WHO guidelines and Depkes (WHO, 1998; Depkes, 1980).

Determination of Total Flavonoid

The total flavonoid content was determined using quercetin as a reference standard and analyzed with a UV-Visible Spectrophotometer (Jasco V-730®). A standard quercetin solution at a concentration of 10 ppm was prepared, followed by the addition of methanol, 10% aluminum chloride ($AlCl_3$), 1 M sodium acetate, and distilled water. The absorbance was then measured within the wavelength range of 400–450 nm (Chang et al., 2002). To determine the optimum incubation time, absorbance measurements of the 10 ppm quercetin solution were taken at intervals of 5, 10, 15, 20, 25, and 30 minutes until a stable and consistent absorbance value was achieved (Chang et al., 2002).

A calibration curve was constructed using quercetin standard solutions at concentrations of 2, 4, 6, 8, 10, 12, and 14 ppm. The relationship between concentration and absorbance was plotted to generate a linear regression equation of the form y = bx + a (Chang et al., 2002). To quantify the total flavonoid content in the broccoli extract and pegagan extract, each190 mg of the dry extract was weighed and dissolved in methanol, followed by the addition of 10% $AlCl_3$, 1 M sodium acetate, and distilled water. The mixture was homogenated and incubated for the previously determined optimum time. Absorbance was then measured at the maximum wavelength. flavonoid content was calculated using equation 1.

$$flavonoid\ (mEq/g) = \frac{C\ sample\ x\ volume\ x\ Fp}{w} \quad \text{.....(1)}$$

Description
C sample = Concentration of sample (ppm)
Volume = Volume sample (ml)
Fp = dilution ratio
w = Sample weight (g)

Preparation of Instant Granules

The instant granules were prepared using the wet granulation method. A binder solution of PVP K-30 was first prepared in water at 60 °C and left to stand overnight. Broccoli dry extract, pegagan dry extract, sucralose, mannitol, and lactose were each passed through a 30-mesh sieve and weighed according to the formula (Table 1). All dry ingredients were mixed until homogeneous, after which the PVP K-30 binder solution, along with mocha coffee and chocolate essences, was added gradually and mixed until a moist, cohesive mass was formed.

The moist mass was then passed through a 12-mesh sieve and dried in an oven at 60 °C for 2 hours. When the granules reached a semi-dry state, they were removed from the oven, passed through a 16-mesh sieve, and returned to the oven for final drying until completely dry.

Evaluation of Granules

Loss on Drying

Approximately 10 grams of granules were accurately weighed and placed into a moisture content analyzer. The analysis was initiated by activating the instrument, and after 10 minutes, the percentage of moisture loss (Loss on Drying) was automatically displayed by the device flow. (Putriana et al., 2021).

Flow Ability and Angle of Repose

For flowability testing, 20 grams of granules were weighed and placed into a funnel with a closed orifice. During the test, the orifice was opened, and the time required for the granules to completely flow through the funnel was recorded using a stopwatch. The flow rate was calculated using equation 2 (Voight, 1994, Husni 2021). The angle of repose was determined by allowing the granules to flow freely onto a flat surface, forming a conical pile. The height (h) and radius (r) of the cone were measured, and the angle of repose (α) was calculated using equation 3.

$$\boldsymbol{Flowability} = \frac{w}{t} \quad \text{.....(2)}$$

$$\propto = \boldsymbol{tan^{-1}}\ \frac{h}{r} \quad \text{.....(3)}$$

Description :
w = weight of the granules (g)
t = time required for the granules to flow (s)
α = angle of repose (°)
h = heap height (cm)
r = heap radius (cm)

Table 1. Composition of Instant Granule Formula

Ingredient	Function	Amount (w/b)
Brocoli dry extracts	Active substance	0.5
Pegagan dry extracts	Active substance	3.8
PVP K-30	Binder	4
Sucralose	Sweetener	0.3
Mocha coffee Essence	Flavoring	6
Chocolate Essence	Flavoring	2
Mannitol and Lactose (1:1)	Filler	Ad 100

Table 2. Stability testing parameters

Temperature	Week				
	0	1	2	3	4
25^{0}C	**	*	**	*	**
40^{0}C	**	*	**	*	**
45 ^{0}C	**	*	**	*	**
60^{0}C	**	*	**	*	**

(40ºC, 45ºC, 60ºC)

Notes:

* Organoleptic, moisture content, dispersion time, sediment height, pH solution

** Organoleptic, moisture content, dispersion time, sediment height, pH solution, and flavonoid content

Dispersibility

A total of 5 grams of instant granules were dissolved into 100 mL aquadest at 60 ^{0}C. The solution was stirred until it was dispersed. The time until the formation of a precipitate is observed and recorded (Putriana, et al., 2021).

pH

The pH measurement was carried out using a pH meter. A total of 5 grams of instant granules was dissolved in 150 mL of water at 60 °C. The pH meter electrode was immersed into the pH was recorded once a stable reading was displayed on the instrument (Rahmawati & Luliana, 2022).

Stability test for Instan granules

The stability study was conducted using the Accelerated Shelf-Life Testing (ASLT) method based on the Arrhenius model. Samples were stored in aluminum foil packaging under controlled conditions at room temperature (25 °C) and at elevated temperatures of 40 °C, 45 °C, and 60 °C for a period of four weeks. A thermohygrometer was used to monitor and ensure the accuracy and consistency of temperature and humidity inside the storage oven throughout the stability testing period. The testing intervals and parameters assessed are summarized in Table 2.

Estimation of Shelf Life

The analysis data for each parameter is plotted against time (in days), resulting in a linear equation (equation 4) (Syska, et all, 2023). To determine of the reaction order for a specific parameter, the regression value R^2 of each equation at the same temperature is compared. The reaction order with the highest R^2 value is considered the appropriate order for that parameter. After obtaining the linear equation for each storage temperature, the slope value (from equation 4), which reflects the change in the flavonoid, is calculated as ln a for use in the Arrhenius equation (equation 5).

$$y = ax + b \quad \text{.....(4)}$$

$$ln\, k = ln\, k_0 - \frac{(\frac{Ea}{R})}{(\frac{1}{T})} \quad \text{.....(5)}$$

$$k = k_0 \, . e^{Ea/RT} \quad \text{.....(6)}$$

Description: y = represent the flavonoid
x = denotes storage time (in days)
a = indicated the rate of change of flavonoid
b = represent the initial value of the flavonoid (k)
Ko = intercept
Ea/R = represent the slope
Ea = denotes the activation energy
R = the ideal gas constant (1.986 cal/mol)

From the equation, the constant k indicates the exponential factor associated with quality reduction at normal storage temperatures, while the activation energy (Ea) signifies the energy barrier for changes in the flavonoid. Additionally, the model for the reaction rate equation concerning temperature is established, allowing for the calculation of k, which reflects the quality reduction of the products, using Equation 6. Using the Arrhenius equation and the calculation the shelf life of instant granules can be estimated through the reaction order equations 7 or 8.

$$t_{zero\ order} = \frac{\Delta A}{k} \quad \text{.....(7)}$$

$$t_{first\ order} = \frac{ln(\frac{A_0}{A})}{k} \quad \text{.....(8)}$$

Description :
t = represent the predicted shelf life (in days)
ΔA = denotes the change in flavonoid
A_0 = initial flavonoid value
A = Remaining flavonoid value at time t
k = constant for quality reduction at normal temperature

Figure 1. Dry extract of broccoli (A) and pegagan herb (B)

RESULTS AND DISCUSSION

Preparation of Dry Extracts

Broccoli was extracted using a juicer with water as the solvent. This method was chosen due to its relatively quick extraction time and the absence of heating, which allows for a higher yield of extracted flavonoids. The resulting liquid extract was subsequently dried using a vacuum dryer for 20 minutes at a temperature of 70 °C. This method was selected because it enables a higher drying rate, as moisture can evaporate at low temperatures, and the microstructure of the product expands to enhance heat and mass transfer. The dried extracts of broccoli and pegagan herb can be seen in Figure 1. Additionally, this process effectively retains the active ingredients and the quality of the product under oxygen-deficient conditions (Xu et al., 2021). The yield from the liquid extraction process was 71.42%, while the yield of the dried extract was 2.07%.

Evaluation of Dry Extract

Physical characteristic

The dried broccoli extract produced a powdery consistency with a green-brown color, a specific flavor and a bitter taste. Similarly, the dried extract of the pegagan herb exhibited a powdery texture, light green color, characteristic flavor and bitter taste. The water content in the dry extract significantly influences the stability and quality of the extract, as high moistire levels can create a conductive environmental for microbial growth, leading to a shoeter shelf life. The results of the water content analysis for both broccoli and pegagan extracts comply with established requirement of less than 10 % (Depkes RI, 2008) indicating that the extract are suitable for use, as shown in Table 3.

Determining ash content of the extracts serves assess their mineral contnt. The data obtained indicated that the ash contes=nt is less than 10 %. This data presented in Tabel 3 (Depkes RI, 2008). The measurement if ash content is crucial for evaluating the purity and quality of the extracted materials, which in turn affects the quality of preparation derived from natural sources.

Phytochemical Screening

Phytochemical testing was carried out to determine the content of secondary metabolites in broccoli and pegagan herb extracts. Broccoli extract showed positive results on alkaloid compounds, flavonoids, saponins, tannins, and terpenoids. The results of secondary metabolites in broccoli extracts are in accordance with the literature (Lutfiyati et al., 2017) and the pegagan herb extracts show the presence of alkaloid, flavonoid, and terpenoid compounds. Saponins and tannins in the pegagan herb extracted with water solvent showed no detectable saponin and tannin content. The results of the phytochemical test result as in Table 3.

Total Flavonoid Content

Determination of flavonoid content of dry extracts of broccoli and pegagan herb using UV-Vis Spectrophotometer with flavonoid standard quercetin. Measurement of the maximum wavelength of standard quercetin was carried out in the λ range. From the test results, the maximum wavelength data obtained was 429 nm. The calibration curve obtained has a linearity equation y = 0.0807x - 0.0016 with a correlation coefficient (r) = 0.9985.

The results of flavonoid quercetin content in broccoli extract and pegagan herb extract obtained were an average of 4.88 mg QE/g ± 0.09 and 1.58 mg QE/g ± 0.02, respectively. Broccoli extract had higher flavonoid levels than the pegagan herb. The flavonoid content of the mixed extract of broccoli and pegagan herb gave an average result of 1.17 mg QE/g ± 0.01. The combined flavonoid levels obtained are lower because when plants that have flavonoid content are combined there will be interactions that can affect antioxidant content and the possibility of hydrogen bond interactions with flavonoids so that flavonoid levels in combined extracts decrease (Hidalgo et al., 2010). The results of flavonoid content can be seen in Table 3.

Table 3. Evaluation Results of Broccoli Extract and Pegagan Extract

Parameter Test	Broccoli extract (EB)	Pegagan extract (EP)	Combination of Brocoli and Pegagan Extract
Water content	8.2% ± 0.0718	5.53%± 0.0016.	-
Ash content	7.98% ± 0.2095	0.89%± 0.5184	-
Phytochemical Screening			-
- Alkaloid	+	+	
- Flavonoid	+	+	
- Saponin	+	-	
- Tanin	+	-	
- Terpenoid	+	+	
Flavonoid Total	4.88 mg QE/g ± 0.09	1.58 mg QE/g ± 0.02.	1.17 mg QE/g ± 0.01

Evaluation of Instant Granules

Here is the produced instant granules are characterized by a pale green powdery appearance, a sweet taste, and a coffee aroma. The image of the granules can be seen in Figure 2.

Figure 2: Instant Granules

The evaluation of the instant granules is presented in Table 4, where the moisture content of the instant granules is measured at 4.4%, which meets the requirement of being below 5% (Putriana et al, 2021). The ash content test shows a result of 0.21%, which complies with the standards for Traditional Beverage Powder according to SNI 01-4320-1996, where it should not exceed 1.5% (BSN 1996).

The flow rate assessment indicates that the instant granules demonstrate a favorable flow rate of 8.19 g/s ± 0.21, with a repose angle of 25.690 ± 0.33 (Voight, 1994). Granules that exhibit good flow characteristics are those that can move easily. The flow properties are influenced by factors such as particle shape, particle size, and the cohesiveness among particles. The angle of repose represents the maximum angle that the surface of the granules can form relative to the horizontal plane. This angle is affected by particle size, the strength of the attractive forces, and the friction between the particles.

The dispersion time is intended to assess the duration required for the granules to fully disperse in water. The dispersion time of the granules is influenced by their size distribution, with smaller granules dispersing more rapidly than larger ones. Additionally, uniform particle size facilitates easier penetration of the water medium into the granule particles, thereby enhancing the granule dispersion rate. The requirement for dispersion time is set at less than 5 minutes. The instant granules are capable of complete dispersion within 1 minute and 24 seconds, satisfying the criteria for high-quality instant granules, which should dissolve within 5 minutes (Rani et al., 2021).

The pH of the solution was assessed to determine the precise pH of the granules after dispersion in water, with the formulations indicating a pH range of approximately 4 (Putriana, 2021). The acidity observed in the instant granules is attributed to the essences utilized in the formulation. This formulation incorporates two essences: mocha coffee and chocolate, both of which exhibit an inclination towards acidic pH levels, specifically with chocolate essence at 5.0 and coffee essence at 4.2. The flavonoid content in the instant granules was found to be lower compared to the flavonoid content in the mixture of broccoli extract and pegagan extract prior to granulation. The decrease in flavonoid content is attributed to the heating involved in the granulation process using the wet granulation method (Syafrida et al., 2018).

Table 4. Evaluation Results of Instant Granules

Parameter Test	Result
LOD	4.4 % ± 0.014
Ash content	0.21 % ± 0.05
Flow properties	8.19 g/s ± 0.21 (free flowing)
Angle of repose	25.69^0 ± 0.33
Dispersion time	1' 24"
pH	4.83 ± 0.006
Flavonoid	0.50 mg QE/g ± 0.003

Table 5. Stability Testing Results

Temperature/ Parameter	Week 1	Week 2	Week 3	Week 4
Organoleptic				
25°	Pale green, sweet, weak coffee flavor	Pale green, sweet, weak coffee flavor	Pale green, sweet, weak coffee flavor	Pale green, sweet, weak coffee flavor
40°	Pale green, sweet, weak coffee flavor	Pale green, sweet, weak coffee flavor	Pale green, sweet, weak coffee flavor	Brownish green, sweet, weak coffee flavor
45°	Pale green, sweet, weak coffee flavor	Pale green, sweet, weak coffee flavor	Brownish green, sweet, weak coffee flavor	Brownish green, sweet, weak coffee flavor
60°	Pale green, sweet, weak coffee flavor	Pale green, sweet, weak coffee flavor	Brown, sweet, weak coffee flavor	Brown, sweet, weak coffee flavor
Loss on drying (%)				
25°	4.5	4.7	4.8	5.0
40°	4.3	4.1	3.9	3.7
45°	4.3	4.0	3.9	3.6
60°	4.0	3.7	3.6	3.5
Dispersion time				
25°	1' 34''	1' 42''	1' 52''	2' 10''
40°	1' 37''	1' 41''	1' 48''	1' 55''
45°	1' 30''	1' 35''	1' 42''	1' 50''
60°	1' 25''	1' 33''	1' 40''	1' 48''
pH solution				
25°	4.851	4.824	4.813	4.806
40°	4.783	4.762	4.746	4.737
45°	4.773	4.731	4.722	4.702
60°	4.696	4.669	4.640	4.634
Flavonoid content (mg QE/g)				
25°	0.50	0.46	-	0.44
40°	0.50	0.44	-	0.37
45°	0.50	0.41	-	0.36
60°	0.50	0.36	-	0.33

Stability Test and Estimation of Shelf Life

The stability test was conducted for 4 weeks under four different storage temperature conditions: 25℃, 40℃, 45℃, and 60 ℃ (Monita, 2023). The evaluations included organoleptic properties, loss on drying (LOD), dispersion time, pH, and flavonoid content. The shelf life prediction was performed using the Accelerated Stability Testing (ASLT) method, which determines the product's shelf life by accelerating quality changes in critical parameters. This method employs environmental conditions that expedite the degradation reactions of the product. The products were stored under extreme temperature conditions, leading to a decline in critical parameters due to heat exposure. In the ASLT method, storage conditions are set outside of normal parameters to facilitate faster degradation, allowing for the determination of shelf life (Arif, 2016). In this study, flavonoid content was utilized as the critical parameter. The results of the stability test observations can be seen in Table 5.

The organoleptic evaluation indicated that the most pronounced changes occurred at a storage temperature of 60 ℃. The instant granules, which originally exhibited a green hue, rapidly changed to brown, developed a bitter flavor, and lost their coffee aroma, leaving only the distinct scent of broccoli extract. These changes began to appear from the second week. At storage temperatures of 40 ℃ and 45 ℃, noticeable alterations were first observed in the third week, with the initial change being the gradual loss of the coffee aroma. By the conclusion of the observation period, the coffee scent had completely vanished.

Table 6. R^2 value

Temperature (℃)	Coefficient of Determination (R^2)		
	0 orde	1st Orde	2nd Orde
40 ± 2	0.9980	0.9925	0.9836
45 ± 2	0.9735	0.9858	0.9945
60 ± 2	0.8775	0.8988	0.9203

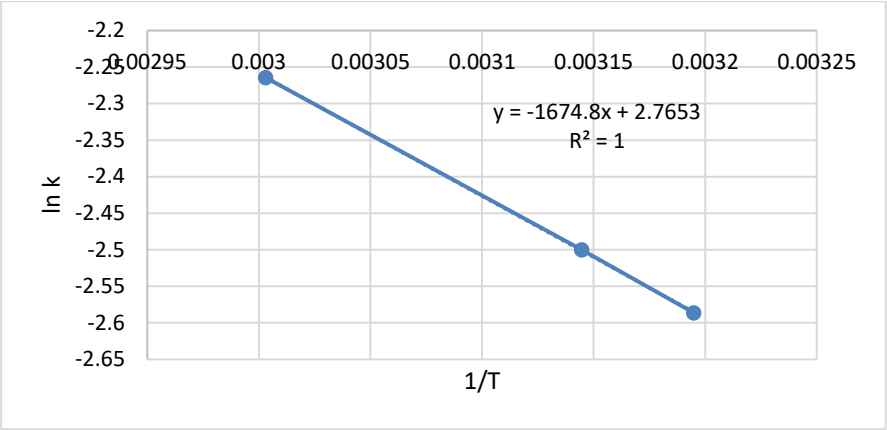

Figure 4. Plot 1/t vs ln k

Color changes were first observed in the third week, with the granules exhibiting a brownish-green tint by the conclusion of the observation period. In contrast, samples stored at 25 °C showed no alterations in either color or odor. These findings suggest that elevated storage temperatures significantly impact the stability of aroma and color. The loss of coffee aroma can be attributed to the instability of the essence used, which is prone to volatilization during prolonged storage. Higher drying temperatures exacerbate this effect, leading to a reduction in volatile compounds. Additionally, during storage, a noticeable change in flavor was detected, characterized by a slight bitterness. This alteration may result from enzymatic activity, either from microbial sources or naturally occurring enzymes present in the raw materials (Sulistiawati, 2016). Overall, the data indicate that as storage temperature increases, the rate of changes in aroma and color accelerates.

The moisture content of the instant granules during the stability testing remained within acceptable limits, below 5%. The data indicate that as the storage temperature increases, the moisture content of the instant granules decreases. This is attributed to the high storage temperatures, which can evaporate the moisture present in the granules. The dispersion time parameter shows that as the storage temperature increases, the dispersion time of the instant granules decreases. This is related to the moisture content within the granules; higher moisture content results in longer dispersion times. An increase in moisture content in food materials can create bonds that lead to clumping, requiring more time to break the bonds between particles (Marlina et al., 2021). The pH of the instant granule solution indicates that with higher storage temperatures and longer storage durations, the pH value decreases, yet it remains within the acceptable range of approximately pH 4.

Based on the results of the total flavonoid content during the 4-week storage period, a decrease was observed each week. The instant granules stored at an accelerated temperature of 60 ± 2 °C exhibited the lowest total flavonoid content throughout the storage process. This may be attributed to the fact that at high temperatures, heat continues to flow, leading to the breaking of conjugated carbon double bonds through oxidation reactions (Susiani et al., 2017, as cited in Hohakay et al., 2019). Therefore, prolonged storage at elevated temperatures results in a further reduction of the flavonoid content in the formulation.

Determination of Shelf Life

Stability testing was conducted using the Arrhenius Accelerated Stability Testing (ASLT) method, with shelf life predictions based on flavonoid content data at storage temperatures of 40 °C, 45 °C, and 60 °C. Flavonoid levels were measured at the second and fourth weeks, with all tests performed in a single replicate. From the obtained data, the R^2 values were calculated, identifying the value closest to 1, indicating that the degradation of flavonoids in this instant granules formulation follows first-order kinetics. The predicted shelf life using the Arrhenius ASLT method yielded an activation energy (Ea) of 13.92 kJ/mol, with a rate constant (k) at 25 °C of 0.05761/week, resulting in a shelf life of 13.2 weeks or 92 days as displayed in Table 6. The R^2 values for each time point and the graph of 1/T versus ln(k) are plotted in the equation as follows: Y = 1674.8 x + 2.7653 (Figure 4).

CONCLUSION

The instant granules of broccoli (*Brassica oleracea* L.) and pegagan *Centella asiatica* (L.) have met the required formulation criteria, including organoleptic properties, loss on drying (LOD), dispersion time, and pH. However, a significant reduction in flavonoid content was observed. The estimated shelf life, determined using the Arrhenius Accelerated Stability Testing (ASLT) method, is 13.2 weeks or 92 days. Further research is necessary to develop strategies to maintain optimal flavonoid levels in the instant granules, with the goal of extending the shelf life to a minimum of six months.

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