Study of antioxidant activities, acceptability, and shelf life prediction of Ciplukan (*Physalis angulata* L.) juice drinks

R Luthfiyanti¹⁺, A C Iwansyah¹⁺*, Y Rahayu² and N S Achyadi²

¹Research Center for Appropriate Technology, Indonesia Institute of Sciences. Jl. Aipda K.S. Tubun No 5, Subang 41213, West Java, Indonesia
²Department of Food Technology, Faculty of Engineering, Pasundan University, Bandung. Jl. Setiabudi No.9, Bandung, West Java 40153 Indonesia
⁺These authors contributed equally
*Corresponding author email: chandra.iwansyah@gmail.com

Abstract. Antioxidants are free radical scavenger compounds that can reduce or prevent the adverse effects of reactive oxygen species and reactive nitrogen species (ROS / RNS). This study aims to study the antioxidant activity, acceptability, and shelf life of fruit extract drinks and ciplukan buds (*Physalis angulata* L.). The experimental design used factorial completely randomized design (FCRD), with two factors, namely the ratio of juice extracts and ciplukan buds (a) and stabilizer concentrations (b), three replications. The organoleptic analyzed by hedonic test, antioxidant activity by DPPH method, and total phenolic contents, total flavonoid contents and alkaloid using each of the Folin-Ciocalteau, aluminum chloride and gravimetric methods. The estimated shelf life is calculated using the Arrhenius method at 28°C for 28 days. The results showed that the ratio of fruit extracts and buds (a) affected the preference for taste, aroma, and color attributes. The interaction between juice extract ratio with a bud (a) and stabilizer concentration, (b) influences antioxidant activity, color attributes, and viscosity. The best ciplukan drink samples based on organoleptic calculations are juice with fruit extracts with buds 15:1 and stabilizer concentration of 0.1% (a³b₁) and fruit extracts with buds 20:1 and stabilizer concentration of 0.1% (a⁴b₁). The highest total phenolic and flavonoid contents obtained sample code a³b₁, while the highest alkaloid content from code sample a⁴b₁. Based on the estimated shelf life of total microbes, ciplukan bud extract has a shelf life of 9 months at 28°C.

1. Introduction
Ciplukan (*Physalis angulata* L.) has been used for decades as alternatives to traditional medicine due to its functional properties, including antihyperglycemic, antibacterial, antiviral, immunomodulatory, anti-inflammatory, antioxidant and anti-analgesic [1]. Ciplukan contains of various active compounds such as saponins, flavonoids, polyphenols, alkaloids, physalin B, physalin D, physalin F, protein, and chlorogenic citric acid, withangulatin A, palmitic acid, acetate acid, protein, vitamin C, tannins, malic acid. Some of these contents are considered as antioxidant compounds [2].

Antioxidants are compounds that are able to ward off free radicals, with mechanisms as electron-giving compounds or reductants. In the food sector, antioxidants are compounds contained in food and when present in low concentrations compared to oxidized substrates significantly reduce or prevent the adverse effects of oxidative stress on normal physiological functions of humans [3]. Oxidative stress is...
a condition where reactive oxygen species (ROS), reactive nitrogen species (RNS) and endogenous antioxidants found in the human body are not in a balanced state. This imbalance can cause damage to cells and various degenerative diseases, one of which is diabetes mellitus. Components that are present in the human body when interacting with ROS or RNS will form free radical compounds or compounds capable of producing free radicals [4].

Secondary metabolites of flavonoids, polyphenols, and alkaloids contained in ciplukan are natural antioxidant compounds. Flavonoids and polyphenols are antioxidant compounds that are included in the phenolic group, the mechanism of action of phenolic antioxidants, namely potential radical scavengers. The phenolic compound of a biologically active component is a substance that can contribute hydrogen to free radicals. It will break the lipid oxidation reaction chain at the initial initiation stage [5]. Alkaloids are compounds that have a basic hydrogen atom and are part of a cyclic system that can function as an antioxidant compound [6]. With the presence of antioxidants in ciplukan plants, it is expected to be able to prevent and reduce non-communicable diseases such as diabetes mellitus, coronary heart disease, and so on, by inhibiting damage to body cells due to the process of free radical oxidation.

Diabetes mellitus is caused by a disruption in the β cells of the islets of Langerhans, which causes reduced insulin secretion and the development of hyperglycemia [7]. Hyperglycemia is a condition where fasting glucose levels in the blood are higher than 110 mg/dL due to interference with insulin secretion, insulin action or both [8]. The content of alkaloids, polyphenols, and flavonoids in ciplukan leaf and fruit extracts tested was instrumental in the anti-hyperglycemia activity in pancreatic β cells [9-10]. Our previous study, regarding the characteristics of each part of the ciplukan plant, showed the highest antioxidant activity in leaves, fruit and buds (fruit peels) [11]. Utilizing the antioxidant content in ciplukan carried out the development of functional food-based products, namely fruit juice drinks from fruit extracts and ciplukan buds. In this study estimating the shelf life at room temperature (28°C) was also carried out. This is because the antioxidant content is susceptible to microbiological, chemical and physical damage, causing a decrease in the quality of beverage products that affect the shelf life.

2. Materials and Methods

2.1. Materials
The materials used for making ciplukan drinks were fresh fruit and bud ciplukan (Physalis angulata L.) obtained from the village area of Dawuan, Dawuan District, Subang Regency. Ciplukan plants were collected from January until June 2018. The botanical identification of these ciplukan plants has been carried out at Herbarium Bogoriense, Center for Biological Research-LIPI (No. 886/IPH.1.01/F.07/IV/2018). Citric acid, non-calorie sugar (stevia), and CMC were obtained from Setia Guna Chemical Store, Bogor; while honey is bought at CV. Nutrima, Bogor. The materials used for the analysis were methanol, DPPH, distilled water, quercetin, 10% AlCl₃, 1M sodium acetate, gallic acid, Folin-Ciocalteu, 7.5% Na₂CO₃, and ammonium hydroxide were purchased from Sigma-Aldrich, Singapore.

2.2. Preparation samples
The Fresh ciplukan fruits and buds were sorted, weighed, and then washed with running water. The washed ciplukan fruits and buds, were blended at 70°C for two minutes. The extraction process of ciplukan juice and buds was carried out by weighing the ingredients and extracting them using a water solvent, each ratio of 1:2 and 1:20 (w/v). Each extract was filtered using filter paper, then collected for the next process (11). The production of ciplukan juice drinks was processed by mixing ciplukan juice extract, and bud ciplukan bud extracts with a ratio, namely: 5:1, 10:1, 15:1, and 20:1 (v/v). Then, the extract was mixed and added with honey, non-calorie sweetener, and stabilizer. Further, pasteurization process was done at the temperature of 70°C for 15 minutes. After that, citric acid was added, homogenized, and packaged in the sterilized bottles [12].
2.3. Experimental design
This current study uses a factorial completely randomized design (FCRD), with 2 factors, namely (1) the ratio of juice extracts and buds of ciplukan plants (a), including: ratio of 5: 1 (a1), 10: 1 (a2), 15: 1 (a3), and 20: 1 (a4), and (2) stabilizing concentrations (b), including: 0.1% (b1), 0.2% (b2), and 0.3% (b3), with three replications, 36 trial units were obtained. The parameters tested were: sensory quality (hedonic test) and antioxidant activity. Analysis of total phenols, total flavonoids, and alkaloids was carried out on the two best samples based on sensory test results and antioxidant activity. Ciplukan juice drinks with the best formula were analyzed for estimating shelf life by Arrhenius for 28 days, every seven days observing parameters of vitamin C levels, pH, and total microbial count (total plate count).

2.4. Procedure analysis

2.4.1 Organoleptic test. The sensory quality of ciplukan juice drinks was analyzed using the preferred method (hedonic) with the parameters: color, aroma, taste and viscosity [13]. The preferred test was conducted at the Organoleptic Laboratory, Center for Appropriate Technology Research - LIPI, using 30 semi-trained panelists, with a rating scale of 1 (very dislike) - 6 (very like).

2.4.2 Total phenolic, flavonoids, alkaloids contents and antioxidant activity. Total phenolic content of ciplukan juice drinks was determined by Folin-Ciocalteau method [14]. The total flavonoids content of ciplukan juice drinks were analyzed using the method [15]. Total alkaloids from ciplukan drinks were analyzed using gravimetric methods [16]. Analysis of antioxidant activity of ciplukan juice drinks was measured by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [17].

2.4.3 Vitamin C. Vitamin C was analyzed using the titration method [18]. A total of ten mL of the ciplukan juice drinks sample was pipetted into a clonal measuring flask and KI (5 mL; 10%) added with H₂SO₄ (1 mL; 0.3 M) into the measuring flask. After that, KIO₃ (10 mL; 0.01 M) was added to the measuring flask, and homogenized. The excess iodine was titrated using Na₂S₂O₃ (0.01 M) with a blank solution in the form of titration of distilled water (10 mL). The vitamin C content can be calculated by the following equation (1):

\[
\text{Vitamin C} = \text{meq KIO}_3 - \text{meq Na}_2\text{S}_2\text{O}_3
\]  

2.4.4 pH value. The pH value was measured by using a pH meter (Metler Toledo Pro2Go)[19]. pH meter before use, first turned on and stabilized for 15-30 minutes. Calibration is carried out using a buffer solution at pH 4 and pH 7. Samples of ciplukan drinks are put into a beaker glass, then the pH meter electrode is dipped into the drink sample and left to show a stable number. Samples were measured triplicates.

2.4.5 Total microbes (total plate count). The total microbes in ciplukan juice drinks were counted using the total plate number (TPC) method [20]. A serial ten-fold dilution was performed until 10⁻⁴. Furthermore, 1 mL of each dilution was taken and put into a petri dish which contained 15 mL of PCA media in duplicate, then homogenized and covered, then incubated at 37°C for 48 hours. The calculation was done for dilution with the number of colonies of 30-300. The total plate number (TPC) is calculated by the following equation (2):

\[
\text{Number of colonies} \times \frac{1}{\text{dilution factor}}
\]  

2.5. Statistical analysis.
The data are presented in mean ± standard deviation (sd). Data were analyzed by analysis of variance (ANOVA), with a confidence interval of 95% and tested for normality of the data first. Duncan's comparison test was used to determine the average difference between each treatment. The data processing program used is Microsoft Excel 2013 and SPSS Statistics Software 24.00 for Windows.
Linear regression analysis is used to determine the relationship between the variables measured by storage time. The $k$ value obtained from the regression equation is applied to the Arrhenius equation to estimate the shelf life of ciplukan juice drinks [21].

3. Results and Discussion

3.1. Sensory parameter

The sensory quality of fruit juice extracts and ciplukan buds was analyzed using the preferred method (hedonic) with the test parameters: color, aroma, taste, and viscosity attributes [13]. Taste is one of the characteristics that can determine the level of consumer preference in response to the sense of taste and smell. Winarno [22] states that the factors that can affect the taste are interactions between taste components in food ingredients or products, concentration, temperature, and chemical compounds. Comparison of fruit juice extracts and ciplukan buds significantly affected the flavor attribute of ciplukan juice drinks ($P<0.05$). Duncan's multiple comparison tests showed that the extract ratio of 20:1 between juice and bud (a4) had the highest average value of preference (Figure 1). The more ciplukan juice extracts are added, the tendency for panelists to increase. This tendency is because, in ciplukan fruit, there is the sugar content that can add a sweet taste in drinks. In contrast, in ciplukan buds, there are alkaloids, tannin, and saponin compounds that have bitter taste characteristics [23].

Aroma determines whether or not consumers accept a product. Comparison of fruit juice extracts and buds significantly affected the aroma attributes in fruit juice drinks and ciplukan buds ($P<0.05$). At the same time, the concentration of stabilizers and their interactions were not significantly different ($P>0.05$). Duncan's multiple comparison test showed that fruit extract drinks and constipation buds of 10:1 (a2) had the highest average value of flavor attributes (Figure 1). The aroma associated with volatile compounds in food, the more volatile compounds, and the form of aroma will be sharper [24]. The aroma of functional drinks of fruit juice and ciplukan buds is estimated to be influenced by additional ingredients such as honey. Honey has high volatility and hygroscopic properties, hydrocolloid can reduce volatile compounds [25].

![Figure 1. Effect of fruit extract ratio + bud and stabilizer concentration on taste, color, aroma and viscosity attributes of ciplukan functional drinks (n=3).](image)

Color has produced the stimulation of a beam of radiation energy that falls into the retina of the eye. It is associated as a factor that describes the level of maturity, freshness, purchasing power, and safety of a product or foodstuff [26]. The interaction between the juice extract ratio with the ciplukan buds and stabilizer concentration significantly affected the color attributes ($P<0.05$). Duncan's multiple
comparison test showed that extracts of fruit juice and buds with a ratio of 15:1 and 20:1 with a stabilizer concentration of 0.1%, namely a3b1 and a4b1, had the highest preference for the color attribute of the ciplukan bud extract juice (Figure 1). This result is thought to be due to differences in the ratio of fruit extract and ciplukan bud extract. The higher the percentage of fruit juice extracts added to the buds, the brighter the colors produced. The brownish-yellow color of this functional drink is derived from the antoxantin flavonoid pigment, but it also comes from tannins and alkaloids, owned by ciplukan fruits and buds [24].

Viscosity is very influential on the level of consumer preference on the product. Factors that affect viscosity include temperature, the concentration of the solution, molecular weight of solution, presence of other substances, and pressure of the material used. The interaction between the juice extract ratio and the ciplukan buds and stabilizer concentration significantly affected the viscosity attribute (P<0.05). Duncan's multiple comparison test showed that the treatment with a ratio of 15:1 with a stabilizer concentration of 0.1% (a3b1) had the highest preference value on the ciplukan bud extract's color attribute. According to Kumalasari et al. [27] the higher the stabilizer concentration, the higher the viscosity that the drink will be thicker. The viscosity of the fruit juice and ciplukan buds is influenced by the amount of liquid added, the more liquid added, the lower the viscosity, and conversely, the more solids added, the higher the viscosity. The use of stabilizers will increase the continuous phase's viscosity into suspended particles so that they do not settle quickly [25].

3.2. Antioxidant activity
Antioxidant activity was tested by the DPPH method, which was classified according to IC50 values. The IC50 value is a large amount of concentration that can inhibit DPPH by as much as 50%. The smaller the IC50 value, the greater the antioxidant activity. The presence of antioxidant activity in the sample is marked by the fading of the color which results in a decrease in the absorbance value of the visible light from the spectrophotometer, so the fade the color the lower the absorbance value which shows, the more free radicals that react with antioxidants contained in functional drinks [28]. Comparison of fruit extracts with buds, stabilizer concentrations, and their interactions significantly affected antioxidant activity (P<0.05) (Figure 2).

Figure 2 shows a better treatment of antioxidant activity (IC50) demonstrated by a sample with a 5:1 juice and bud ratio and a CMC concentration of 0.3% (a1b3) 73.45 µg/mL. According to Iwansyah et al. [11], reported that ethanol extracts from ciplukan fruits and buds (fruit peels) had antioxidant activity, which was 133.76 µg/mL and 134.53 µg/mL, respectively. The results of this study indicate that the greater extract of fruit juice added, and the higher the concentration of the stabilizer mixed, the higher the antioxidant activity (Figure 2). These results agreement with Farikha et al. [29], who reported that the higher the stabilizer concentration, the higher the antioxidant activity of the dragon fruit juice drink.
A significant decrease in antioxidant activity in ciplukan juice drink can be caused by processing. According to Burda and Oleszek [30] states that materials that have the potential for an antioxidant activity that is processed with heat and are directly exposed to air will be easily oxidized and degraded so that it will affect its antioxidant activity. Antioxidants in food can eliminate ROS/RNS to stop radical chain reactions, which are called free radical scavengers (FRS) antioxidants. In contrast, those that can inhibit reactive oxidants from their formation are called preventive or secondary antioxidants. Several mechanisms of antioxidant reactions can function as: (1) protecting the body by preventing the formation of ROS and its responses in important parts of the body; (2) capturing or eliminating chemical compounds that absorb energy and electrons; (3) with catalytic systems that neutralize or reduce ROS, for example, the antioxidant enzyme SOD (superoxide dismutase), catalase, and glutathione peroxidase; (4) binding or inactivation of metal ions to prevent the formation of ROS, for example, ferritin, ceruloplasmin, catechins; and (5) antioxidants as chain breakers and damage ROS bonds, for example, ascorbic acid (vitamin C), tocopherol (vitamin E), glutathione, flavonoids [3].

3.3. Total phenolic contents, total flavonoids and alkaloids
Determination of the best sample is chosen based on the highest number of organoleptic test results from all of the attributes assessed and antioxidant activity. Based on this, two samples of the best ciplukan drinks were obtained, namely: (1) the ratio of juice extract with buds 15: 1 and the stabilizer concentration of 0.1% (a3b1), and (2) the ratio of juice extracts with buds 20:1 and stabilizer concentration of 0.1% (a4b1). Subsequent samples were tested for total phenolic, flavonoid, and alkaloid. The results of the analysis of the best samples of functional fruit drinks and ciplukan buds can be seen in Table 1.

Table 1. Total phenolic, flavonoid and alkaloid from the best formulations of Ciplukan juice drinks.

| Sample | Total flavonoid (mg QE/L) | Total fenolink (mg GAE/L) | Alkaloid (%) |
|--------|---------------------------|---------------------------|--------------|
| a3b1   | 0.25±0.04"               | 18.74±0.67"              | 5.80±0.51"   |
| a4b1   | 0.19±0.05"               | 15.57±0.71"              | 6.61±0.28"   |

| Parameter |
|-----------|
| Total flavonoid (mg QE/L) |
| Total fenolink (mg GAE/L) |
| Alkaloid (%) |

Data are presented as means ± standard deviation (sd) (n = 3). QE: Equivalent to quercetin. GAE: Gallic acid equivalent. a3b1: extract ratio of 15:1 with a CMC concentration of 0.1%. A4B1: Extract ratio of 20:1 with CMC concentration of 0.1%. Independent t-test, different alphabetic in same colomn was significantly, α = 5%.

Table 1 shows that the highest total phenolic and flavonoids were obtained from beverage samples with a juice extract ratio and a bud of 15: 1 and a CMC concentration of 0.1% (a3b1), i.e., 18.74 mg GAE per liter and 0.25 mg QE per liter, respectively. Beverage samples obtained the highest levels of alkaloids with an extract ratio of juice and buds 20: 1 and CMC concentrations of 0.1% (a4b1), amounting to 6.61% (P <0.05). These results are in line with Julianti et al. [23], which reported higher flavonoid and phenolic contents in the ciplukan buds while higher alkaloids contained in the ciplukan fruit.

Flavonoids play a role in giving flavor and color to various fruits and vegetables. The high content of flavonoids is also related to the high phenolic content, where flavonoids are a subset of phenolic compounds. Therefore, the high phenolic content in a material indicates the high content of flavonoids in the material [31]. As a natural antioxidant, flavonoids can capture free radicals. These antioxidant activities allow flavonoids to capture free radicals (such as ROS or RNS) associated with phenolic -OH groups to improve the condition of damaged tissue [32]. Flavonoids are reported to have an antidiabetic activity that can regenerate cells in the islets of Langerhans by clearing excessive free radicals, binding metal ions (chelating), breaking the chain of free radical reactions and blocking the polyol pathway by inhibiting the enzyme aldose reductase [33–34].

Polyphenols are bioactive and can reduce free radicals or antioxidants [35]. The antioxidant role of polyphenols is thought to protect pancreatic β cells from the toxic effects of free radicals produced under chronic hyperglycemia [36]. According to Barbosa [37], antioxidants of green tea polyphenols can...
reduce oxidative stress by preventing a chain reaction of converting superoxide to hydrogen superoxide by donating hydrogen atoms from the aromatic hydroxyl (-OH) polyphenol group to bind free radicals and remove them from the body through excretion system.

Alkaloids include alkaline compounds that contain one or more nitrogen atoms, usually in combination as part of a cyclic system. Alkaloids are often toxic to humans, and many have prominent physiological activities, so they are widely used in processing [38]. Alkaloids are antioxidant compounds that can regenerate damaged pancreatic β cells [39]. Cell damage caused by free radicals can be overcome by the presence of antioxidants that function as a lowering agent and decrease oxidizing agents before damaging cells so that cell damage can be reduced [40].

3.4. The shelf life of ciplukan juice drinks
The shelf life is determined based on the parameters of vitamin C test, pH, and total microbes. One selected sample is the sample in a4b1 treatment (comparison of fruit extracts with buds 20:1 and stabilizer concentration of 0.1%). Determination of the shelf life of fruit drinks and ciplukan buds packed in plastic bottles is carried out at room temperature (28 °C), and observations are carried out regularly every seven days starting from day 0 to 28.

Vitamin C is a non-enzymatic antioxidant that can act as a reducing agent. A donor of hydrogen atoms because it effectively captures free radicals, especially ROS or reactive oxygen species, prevents the occurrence of oxidized Low Density Lipoprotein (LDL), absorbs metals in the digestive tract, and has biological activity so that it can help maintain metabolism in the body [41]. In countering free radicals, vitamin C can directly react with superoxide anions, singlet oxygen, lipid peroxides, and hydroxyl radicals. Ascorbic acid, which acts as a reducing agent, will donate one electron semi-dehydroascorbic, to form dehydroascorbate, and degraded to form oxalic acid and treonic acid. Thus, vitamin C's ability as an inhibitor of free radicals is essential in maintaining the integrity of cell membranes [42]. The graph of decreased vitamin C, pH and increased total plate count levels during storage can be seen in Figure 3, 4 and 5.

Figure 3 shows that during storage at room temperature, there is a decrease in vitamin C levels. Regression equation for vitamin C levels in ciplukan functional drink is \( Y = -0.4257x + 25.331 \) (\( R^2 = 0.973 \)) which means a constant decrease in quality (k) 0.4257. The activation energy (Ea) changes in Vitamins C levels of 0.8454 cal/mol, which means to initiate changes in vitamin C levels in functional drinks and require that much energy. Decreased levels of vitamin C during storage occur due to degradation can be caused by temperature, sugar concentration, pH, oxygen, and light or direct light. With the degradation of vitamin C, it will form compounds that are brown and not have vitamin C activity again. Temperature can cause loss of vitamin C, even in anaerobic conditions [43]. Ascorbic acid experiences quality degradation under anaerobic conditions to 2,3-dicogulonic acid, followed by lactone hydrolytic cleavage, and it produces deoxypentose, so it does not show vitamin C activity [44].

pH is a value that indicates the level of acidity in a solution. The size of the pH value is determined by the H + ions in the solution. Figure 4 shows that during storage, the room temperature decreased in pH. The regression equation for the pH value of functional drinks of fruit juice and ciplukan buds is \( Y = -0.0229x + 6.09 \) (\( R^2 = 0.9531 \)), which means a constant decrease in quality (k) 0.0229. The activation energy (Ea) changes in pH value of 0.0455 cal/mol, which mean to initiate changes in pH in functional drinks requires that amount of energy. The decrease in pH value in functional drinks possibly due to the ability of microbial activity that can break down organic acids in fruit juices and ciplukan buds into carbon dioxide by oxidative yeasts. The decrease caused by temperature causes an increase in total acid. Low pH will result in sucrose being converted into sugar. In a solution in an acidic atmosphere or the presence of the enzyme hydrolysis, invertase can occur to produce sugar invert from sucrose, namely α-D-Glucose and β-D-Fructose. Glucose that is broken down will produce pyruvic acid, which in anaerobic conditions, pyruvic acid will be converted into acetic acid and alcohol, causing acid levels to increase, and pH values tend to decrease [45].
The total microbes contained in an ingredient or food product is one indicator of the level of safety and damage to the product. Increased growth of undesirable microbes can indicate that inside the work, there has been contamination from outside or insufficient processing so that it can reduce product quality. Total microbes are tested by the TPC method (total plate count). This method is to determine the number of microbes in a product by counting the bacterial colonies that grow on agar media. According to the Indonesian National Standard [46], it states that the maximum total amount of microbes that are still permitted to be contained in fruit drinks is $1 \times 10^4$ colonies/mL. The increase in total microbes in fruit juice drinks and ciplukan buds until the end of storage is still below the maximum limit determined by SNI (Figure 5). It is estimated because the acidity or pH level decreases during the storage period. Acidic pH will cause the bacterial cell's internal pH to decrease so that it can interfere with bacterial cell activity, and bacterial growth is inhibited [47]. The pH value of the polluted environment also influences microorganisms' ability to carry out cellular functions, transport membrane cells, and balance reactions carried out by microorganisms. The pH value is inversely proportional to the acidity value, if the pH decreases, the acidity increases where the number of microorganisms during storage also affects the pH value [48].

Figure 5 shows that during room temperature storage, an increase in TPC value causes a decrease in product quality. The regression equation for the TPC value of the ciplukan functional drink is $Y = 55.571x + 824$ ($R^2 = 0.999$), which means a constant decrease in quality ($k$) 55.571. The $k$ and time ($t$) values of the TPC ciplukan functional drink at room temperature (28 °C) are entered into the Arrhenius
equation, which is \( k = k_0 e^{-E/RT} \) atau \( k = \ln k_0 - E/RT \). Because \( \ln k_0 \) and \(-E/R \) are constants, then the equation can be written \( \ln k = A + B \left( \frac{1}{T} \right) \), so that the equation \( \ln k = -824 - 55.571 \left( \frac{1}{T} \right) \) is obtained. With this equation, the activation energy of 110.363 cal/mol will be obtained. So the value of the constant (k) rate of decline in the quality of the TPC parameters obtained is 5.829/day. By using the equation \( A(t) = A_0 - k_0 T \), the storability of ciplukan juice drink based on the TPC value at room temperature (28 °C), ie, for nine months (Table 2).

Table 2. The shelf life of ciplukan juice drinks (P. angulata).

| Parameter | Arrhenius Equation | \( R^2 \) | Energy Activation (kal/mol °K) | k value | Temp. | Shelf Life (months) |
|-----------|--------------------|---------|---------------------------------|--------|------|---------------------|
| TPC       | \( \ln k = -824 - 55.571 \left( \frac{1}{T} \right) \) | 0.999   | 110.363                         | 5.5829 | 28°C | 9 months            |

TPC: total plate count

4. Conclusion

Based on the results, it can be concluded that the extract ratio of juice and bud (a) influences the liking of flavor, aroma, and color attributes. The interaction between juice extract ratio with a bud (a) and stabilizer concentration, (b) influences antioxidant activity, color attributes, and viscosity. The best Ciplukan drink sample is based on organoleptic calculation results and antioxidant activity, namely a3b1 and a4b1. The highest total phenolic and flavonoid obtained sample code a3b1, while the highest alkaloid content from code sample a4b1. The selected sample is suspected to be of shelf life at room temperature (28°C). In the estimation of shelf life, a decrease in vitamin C levels and pH values, as well as an increase in total microbes during storage. Based on the calculation of the Arrhenius equation for total microbes, the shelf life of the Ciplukan bud extract is nine months.

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