Shelf-Life Estimation of Cabe Jamu (Piper retrofractum Vahl) Herbal Drink with the Addition of Benzoate Using Accelerated Shelf-Life Testing (ASLT) Method

Pendugaan Umur Simpan Minuman Herbal Cabe Jamu (Piper retrofractum Vahl) dengan Penambahan Benzoat
Menggunakan Metode Accelerated Shelf-Life Testing (ASLT)

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Abstract
This study aims to determine the cabe jamu (Piper retrofractum Vahl) herbal drink shelf-life with the addition of sodium benzoate concentration and determine the sodium benzoate addition effect on the cabe jamu herbal drink shelf-life. This research used Accelerated Shelf-Life Testing (ASLT) method. Cabe jamu herbal drink was stored at 35 °C and 45 °C and then tested every week for 28 days. The test parameters used were pH, total dissolved solids (TDS), color, total microbes, and total phenolics. The results showed that the cabe jamu herbal drink without sodium benzoate addition stored at a lower temperature had a longer shelf-life. Cabe jamu herbal drink with 400 ppm sodium benzoate addition and stored at 35 °C has the most extended shelf-life, which was 201.21 days. Sodium benzoate addition had a significant effect on the cabe jamu herbal drink shelf-life at a temperature of 35 °C and 45 °C.

Keywords: Accelerated Shelf-life Testing, cabe jamu herbal drink, shelf-life

Abstrak
Penelitian ini bertujuan untuk mengetahui umur simpan minuman herbal cabe jamu dengan penambahan konsentrasi natrium benzoat dan mengetahui pengaruh penambahan natrium benzoat terhadap umur simpan minuman herbal cabe jamu. Metode yang digunakan dalam penelitian ini adalah Accelerated Shelf-Life Testing (ASLT). Minuman cabe jamu disimpan pada suhu 35 °C dan 45 °C kemudian diuji setiap minggu selama 28 hari. Parameter yang digunakan adalah pH, total padatan terlarut (TPT), warna, total mikroba, dan total fenolik. Hasil penelitian menunjukkan bahwa minuman herbal cabe jamu tanpa penambahan natrium benzoat memiliki umur simpan yang lebih lama jika disimpan pada suhu yang lebih rendah. Minuman herbal cabe jamu dengan penambahan natrium benzoat 400 ppm dan disimpan pada suhu 35 °C mempunyai umur simpan terlama, yaitu 201,21 hari. Penambahan natrium benzoat berpengaruh signifikan terhadap umur simpan minuman herbal cabe jamu pada suhu 35 °C dan 45 °C.

Kata kunci: Accelerated Shelf-life Testing, minuman herbal cabe jamu, umur simpan

INTRODUCTION

Cabe jamu (Piper retrofractum Vahl) is a spice and phytopharmaca plant originating from Indonesia. These plants are widely grown in East Java and Lampung (Evizal, 2013). The cabe jamu plant has benefits as a stimulant, tonic, carminative, maternity care, asthma, stomach cramps, fever, colds, influenza, low blood pressure, headache, phlegm remover, anti-fungal, cholesterol-lowering, and appetite generator (Evizal, 2013). Cabe jamu plants are widely used as herbal medicinal plants or traditional herbs, especially in Madura, East Java, because they contain many valuable substances. Technological developments have made several manufacturers begin to develop cabe jamu products into cabe jamu herbal drinks.

According to Law of The Republic Indonesia No. 7 of 1996 concerning Food (Government of the Republic of Indonesia, 1996), Government
Regulation No. 69 of 1999 concerning Labels and Advertising (Government of the Republic of Indonesia, 1999), and Government Regulation No. 28 of 2004 concerning Food Safety, Quality, and Nutrition (Government of the Republic of Indonesia, 2004) all food products must have and include an expiration date or best before date. Many new food product innovations do not have an expiration date. **Cabe jamu** herbal drink is a new innovative product for herbal drinks that do not have an expiration date, so a study on estimating its shelf-life should be done.

The estimation of product shelf-life can be determined using two methods: the Real-time Shelf-life Testing (RST) method, which is also called the conventional method, and the Accelerated Shelf-Life Testing (ASLT) method (Calligaris et al., 2019). Shelf-life determination using conventional methods is carried out by storing the product in daily storage conditions and observing its quality decline until it reaches the expired quality. The ASLT method is a product shelf-life test accelerated by storing the product in extreme conditions, i.e. high temperature or humidity. Expired quality is a condition when the observed product has decreased in quality and is inedible.

The ASLT method has been widely used to determine the shelf-life of food products, including determining the shelf-life of fried rice (Kurniadi et al., 2017), *cingur* salad (Yuwono et al., 2019), apple pie (Pulungan et al., 2016) and apple brownies (Pulungan et al., 2018). The ASLT method has also been widely used to determine the shelf-life of beverage products, including *Dialium indum* syrup (Rifkowaty & Muttaqin, 2016), functional drinks from *Imperata cylindrica* root extract (Anagari et al., 2020), and soursop juice drinks (Arif et al., 2021). Shelf-life is also influenced by the addition of chemical preservatives (Asiah et al., 2018), including sodium benzoate. Sodium benzoate is the first chemical preservative approved for food. Research related to the chemical preservatives effect on shelf-life has been carried out by several researchers, including the effect of sodium benzoate on canned tomatoes shelf-life (Rahayu et al., 2014) and the effect of a combination of preservatives (including sodium benzoate) on the apple drinks shelf-life (Moazzem et al., 2019).

Natural sources of sodium benzoate (**C₆H₅NaO₂**) can come from berries, prunes, plums, cinnamon, and cloves. Sodium benzoate is in a white granule or crystal powder form, odorless and stable in the air. This material is easily soluble in water but slightly soluble in 90% ethanol. The solubility of sodium benzoate in water at a temperature of 25 °C is 660 gr/L with an active ingredient form as a preservative of 84.7% in the pH range of 4.8. The maximum limit for the use of sodium benzoate in soft drinks is 600 mg/kg, while in other foods is 1 g/kg (Cahyadi, 2013).

Benzoates are most effective against mold, yeast, and bacteria in acidic environments. The benzoates mechanism is changed the cell membranes, energy generation elimination, intracellular pH reduction, and inhibition of specific enzymes (Chipley, 2020). Sodium benzoate is usually used in salt forms, such as sodium or ammonium salt. Salt form sodium benzoate is more effective than acid form sodium benzoate because salt is more soluble. The solubility of benzoic acid in water is 0.35%, while the solubility of benzoate in sodium salt form is 50% (Khurniyati & Estiasih, 2015). The activity of benzoate affects the pH. Benzoates are used for preservatives in acidic food products at pH 4-4.5 or lower and are effective at pH 2.5-4.0. Benzoates are used to preserve fruit juices, syrups, soft drinks, and margarine (Estiasih et al., 2015). Ulya et al. (2020) have investigated the effect of adding sodium benzoate to *cabe jamu* herbal drink products but have not analyzed the shelf-life aspect. This study aims to determine the shelf-life of *cabe jamu* herbal drink products using the ASLT method and determine the effect of sodium benzoate concentration on the shelf-life of *cabe jamu* herbal drinks.

**METHODS**

The method used to determine the product shelf-life was Accelerated Shelf-Life Testing (ASLT). This method used extreme storage temperatures to determine *cabe jamu* herbal drink shelf-life with sodium benzoate concentration addition. This study’s best quality resistance results can be used as a reference to determine the shelf-life of *cabe jamu* herbal drink products. The materials used in this study were *cabe jamu* (from Jember Regency, East Java, Indonesia), water, sugar, and sodium benzoate. Materials for analytical of shelf-life were distilled water, Plate Count Agar (PCA), aluminum foil, tissue, cotton,
Folin reagent, and Na2CO3. The equipments used in the *cabe jamu* herbal drink production were an autoclave, stove, filter cloth, and digital scales. The tools used in the analysis and shelf-life testing were Petri dish, test tube, test tube rack, volume pipette, pipette pump, Samson craft paper, incubator, hot plate, Erlenmeyer, oven, stove, pH meter, refractometer, colony counter, bunsen fire, beaker glass, graduated cylinder, color reader, spectrophotometer and rotary evaporator.

**Experimental Design**

This study used a factorial Completely Randomized Design (CRD) experimental design with two factors studied, i.e. sodium benzoate concentration and storage temperature. The concentration levels of sodium benzoate were 0 ppm, 200 ppm, and 400 ppm, while the storage temperature levels were 35 °C and 45 °C. The experimental units were 12 from two replications. Table 1 shows the overall experimental design.

| Sodium Benzoate Concentration (ppm) | Temperature 35 °C (T1) | Temperature 45 °C (T2) |
|-------------------------------------|-------------------------|-------------------------|
| 0 (A1)                              | A1T1                    | A1T2                    |
| 200 (A2)                            | A2T1                    | A2T2                    |
| 400 (A3)                            | A3T1                    | A3T2                    |

**Production Process of Cabe Jamu Herbal Drink**

Production Process of Cabe Jamu Herbal Drink were based on research by Ulya et al. (2020), *cabe jamu* herbal drink was made from the extraction of dried *cabe jamu* using water. The mixture ratio of dry *cabe jamu* and water was 100 grams of *cabe jamu* to 1 liter of water. Heating process using an autoclave at a pressure of 15 lbs and a temperature of 121 °C for 30 minutes. Temperature and pressure sensors have been equipped in the autoclave. The *cabe jamu* extraction process was completed in a single autoclave for 12 samples. Sugar with a concentration of 100 g/L was then added to the chilled *cabe jamu* herbal drink. The sugar and *cabe jamu* herbal drink mixture was heated again to dissolve the sugar to *cabe jamu* herbal drink. Sodium benzoate was then added to each treatment according to Table 1. The final process of *cabe jamu* herbal drink production was packing the product using aluminum foil packaging with 20 mL per package.

**Test Parameters**

The test parameters used in the study were:

1. pH (AOAC, 1982) pH value tests were carried out in duplicate using a pH meter on the *cabe jamu* herbal drink.
2. Total Dissolved Solids (TDS) (AOAC, 1980) TDS test was carried out using a refractometer with cleaned glass. Cleaning was done by dripping distilled water to the refractometer glass surface, then dried with a tissue. Samples were dropped on the refractometer's windshield, 2-3 then the scale was read.
3. Color (Hutchings, 2012) The color analysis was done using a color reader. Calibration is carried out before measurement by using a plate that matches the sample color. Then measurements were carried out by placing the instrument on the top of the sample.
4. Total microbial (Total Plate Count) (Maturin & Peeler, 2001) Total microbial analysis was carried out by diluting 1 mL sample in 9 mL of distilled water at dilution levels of 10¹, 10², 10³, 10⁴, and 10⁵ in the test tubes. Plate Count Agar (PCA) of 12-15 mL was poured into a petri dish, then 1 mL of the sample from the last three dilutions was planted on PCA that has hardened in a petri dish using a triangle to spread the microbial cells evenly. The petri dish was then wrapped in paper and stored at 35 °C for 2x24 hours (48 hours). The number of colonies that grew was counted after incubation.

**Estimation of Cabe Jamu Herbal Drinks Shelf-Life**

*Cabe jamu* herbal drink shelf-life estimation using the ASLT method was done by plotting the test results data on a graph of the relationship between storage time (days) and average quality loss/day (k) (Faridah et al., 2013). The x-axis represents the storage time (days), and the y-axis represents average quality loss/day (k). Linear regression analysis was then performed to obtain a linear regression equation at each storage temperature. The Arrhenius equation curve was then drawn with the x-axis representing 1/T and the y-axis representing ln k. The k value shows the gradient of the linear regression obtained from the storage temperature, while T is the storage temperature. The results of linear regression analysis obtained from the Arrhenius curve are the basis for predicting the product's shelf-life.
Determination of Cabe Jamu Herbal Drinks Critical Characteristics

The criteria for determining shelf-life are based on the product quality degradation parameters, which are the highest value of correlation coefficient ($R^2$) and the lowest activation energy value ($E_a$). $E_a$ is used as the parameter because this value is the most sensitive parameter to temperature changes.

The correlation coefficient value ($R^2$), which is close to 1 is chosen. The color and total microbial parameters use the zero-order in all treatments. Parameters of pH, TDS and total phenolics used first-order in all treatments. The highest correlation coefficient ($R^2$) value in each parameter is then used to find plots of $\ln k$ and $1/T$ for each parameter. $T$ is the temperature used which is first converted in Kelvin temperature units so that each parameter Arrhenius plot equation can be obtained.

The Arrhenius plot equation on critical parameters or characteristics is used to find the value of $k$ using the following equation:

$$\frac{E_a}{R} = B \quad (1)$$

The $B$ slope value is generated from the linear regression equation between $\ln k$ and $1/T$; and the value of $k_0$ is obtained as follows:

$$\ln k_0 = A \quad (2)$$

The regression results obtained on the Arrhenius curve are used as the basis for predicting the product shelf-life based on the rate of deterioration equation as follows:

$$k = k_0 e^{-E_a/RRT} \text{ or } \ln k = \ln k_0 - \frac{E_a}{R} \frac{1}{T} \quad (3)$$

where,

$E_a$ : activation energy (kJ/mol)
$R$ : gas constant (1.986 kal/mol)
$\ln k_0$ : intercept
$k$ : deterioration rate
$k_0$ : deterioration (does not depend on temperature)
$T$ : absolute temperature (K)

The values of $\ln k_0$ and -$Ea/RT$ are constant numbers so that equation (3) can be written as follows:

$$\ln k = A - B\left(\frac{1}{T}\right) \text{ or } \ln k = \ln k_0 - \frac{E_a}{R} \left(\frac{1}{T}\right) \quad (4)$$

The remaining reaction rate can be found by inserting the calculated value into the following equation:

$$t_s = \frac{(A_0 - A_f)}{k} (\text{zero-order})$$

or

$$t_s = \frac{(\ln A_0 - \ln A_f)}{k} (\text{first-order}) \quad (5)$$

where,

$t_s$ : shelf-life
$A_0$ : initial quality characteristic value
$A_f$ : final quality characteristic value
$k$ : reaction constant

Analysis of the Sodium Benzoate Addition Effect on Shelf-life

This study also analyzed the effect of adding sodium benzoate as a preservative on cabe jamu herbal drink shelf-life. Meanwhile, the effect of benzoate concentration and storage temperature on each parameter was not reported in this study. Data analysis was performed using the Analysis of Variance (ANOVA) method with a 95% significance level. If the $p$-value < 5%, the addition of benzoate has a significant effect on the cabe jamu herbal drink shelf-life. This analysis was performed using SPSS 21.0 software.

RESULTS AND DISCUSSION

Total Microbes

Figure 1 shows that the average yield of total microbes in cabe jamu herbal drink increases along the storage time. This total microbe increase follows the results of research by Talasila, Veehalapu, & Shaik (2012) on the stability of apple juice storage with preservation. The highest increase in total microbes occurred in cabe jamu herbal drink without sodium benzoate addition and storage temperature of 35 °C. The total microbes on the 28th day of storage were $35 \times 10^5$ CFU/mL. The lowest total microbial increased in cabe jamu herbal drink was treatment with 400 ppm sodium benzoate addition and a storage temperature of 45 °C. The number of microbes on the 28th day of storage was 19.5x$10^5$ CFU/mL. This study also shows that sodium benzoate addition concentration can suppress the cabe jamu herbal drink microorganisms’ growth.

During storage, the total microbes increase was more significant in control samples (samples without preservatives or antimicrobials) (Nwachukwu & Ezeigbo, 2013). In fact, Cabe
*jamu* contains terpenes compounds as antimicrobial (Panphut, Budisabun, & Sangsuriya, 2020) and can uses as a natural preservative. However, because this product is manufactured at high temperatures, the terpenes become unstable and lose their antimicrobial function. Garcia-Sotelo et al. (2019) stated that terpene loss occurred at temperatures commonly used in food processing. Pandey & Upadhyay (2012) stated that antimicrobials could effectively extend the product's shelf-life. Talasila, Vechalapu, & Shaik (2012) stated that a higher concentration of sodium benzoate indicates a more significant inhibition of microorganisms' growth. Sodium benzoate is better at suppressing the total microbes increase during storage than adding lime, pasteurization, and the combination of pasteurization and lime in fruit juice products (Nwachukwu & Ezeigbo, 2013).

**pH**

Figure 2 shows the frequency of decreasing pH values in each treatment during four weeks of storage. All treatments on *cabe jamu* herbal drink decreased the pH value during storage. In the fourth week of storage, the lowest pH value occurred in the *cabe jamu* herbal drink without sodium benzoate addition, while the highest pH value occurred with 400 ppm sodium benzoate addition.
The pH of control sample (0 ppm benzoate) of cabe jamu herbal drink also decreased significantly during the storage period. Moazzem, Sikder, & Zzaman (2019) stated that the pH of juice control product decreased most significantly during storage. This decrease may also be due to citric acid and ascorbic acid on the product’s sugar and protein components (Singh et al., 2014). Increased acidity can also be caused by microbial metabolism over time (Cock & Cheesman, 2018). The pH decrease during storage also occurred in orange juice products (Yang et al., 2017) and sugarcane juice (Khare et al., 2012). The pH decrease occurred less in the product with more benzoate addition. The higher the benzoate concentration, the smaller the total microbe (Fig. 1), and thus the acid produced by microbial metabolism was also low. According to Nwachukwu & Ezeigbo (2013), sodium benzoate is better at lowering juice drink’s pH value during storage than preservation using lime, pasteurization, or a combination of both.

**Total Dissolved Solids (TDS)**

Figure 3 shows the total dissolved solids decreased during storage in the treatment without sodium benzoate (0 ppm sodium benzoate), 200 ppm sodium benzoate, and 400 ppm sodium benzoate. The highest decrease in TDS value occurred in the treatment without sodium benzoate (0 ppm), while the lowest decrease in TDS value occurred in the treatment with 400 ppm sodium benzoate. The decrease in TDS value indicates the level of sucrose in the drink has decreased. The increase in TDS value during storage may be due to the polysaccharides’ conversion into simple sugars and pectin degradation in soluble solids (Singh et al., 2016). This condition also occurred in the storage of bael fruit and aloe vera drinks (Tiwari & Deen, 2015).
**Total Phenolics**

Figure 4 shows the total phenolics during storage at 35 °C and 45 °C and the addition of various benzoates. These results indicate that the lowest decrease in total phenolics occurred in *cabe jamu* herbal drink without sodium benzoate with a storage temperature of 35 °C. The total phenolics value on the 28th day of storage was 0.537%. The highest decrease in total phenolics occurred with the addition of 400 ppm sodium benzoate with a storage temperature of 45 °C. The total value of phenolics on the 28th day of storage was 0.471%. A decreasing trend was also observed probably due to the degradation of polyphenolic compounds, following changes in the pattern of enzyme activity (Galani et al., 2017). The increase in total microbes during storage (Figure 1) could increase the enzyme activity of the microbes present, resulting in a lower amount of phenol. These phenolics decrease in accordance with the research results by Wisnu, Kawiji, & Atmaka (2015), which showed a decrease in total phenolics in *wedang uwuh* ready to drink during storage. Oxidized polyphenolics compounds will form quinone compounds that are not detected when the total phenolics test is carried out so that the total phenolics content decreases during storage.

**Color**

Figure 5 shows that the lightness (L) or the brightness value of *cabe jamu* herbal drink products decreases with the more extended storage time. A decrease in the L value indicates that the product color is getting darker. Figure 5 also shows that the color decrease is lower with the increase of sodium benzoate addition. Color changes during storage also occurred in functional drinks from a mixture of cucumber and melon (Kausar et al., 2012). Research conducted by Adeola & Aworh (2014) showed that consumer acceptance of the color aspect of the tamarind drink added with benzoate decreased with the longer storage time. The darker color change is strongly suspected because of enzymatic browning, strongly correlated with the total phenolics content (Persic et al., 2017). This enzymatic browning caused the darker color of *cabe jamu* herbal drink with extended storage time.

**Calculation of the Cabe Jamu Herbal Drinks Shelf-life**

The analysis results to obtain a linear regression equation for each parameter are shown in Table 2. The activation energy (Ea) value was then calculated using equations (1) and (2). The lowest Ea value was observed for all parameters to be used as a reference in determining the *cabe jamu* herbal drink shelf-life. Table 3 shows the activation energy values for each parameter.

Table 3 shows that the color parameter has the lowest Ea value so that the critical parameter used to calculate shelf-life was color. According to Winarno (2008), quality degradation generally influences several factors, i.e. taste, color, texture, nutritional value, and microbiology. The color factor comes first visually and is sometimes very decisive. The color parameter has an $R^2$ value close to 1 on the order of 0, so the *cabe jamu* herbal drink shelf-life with sodium benzoate addition can be calculated. Zero-order reactions were also used because the occurrence of a browning reaction. According to Anagari et al. (2011), the damage types that follow the zero-order are enzymatic damage reactions, non-enzymatic browning, and lipid oxidation. The Maillard reaction and caramelization can cause non-enzymatic browning.

![Figure 5. Graph of Cabe Jamu Herbal Drinks Color Changes during Storage](image-url)
The calculation of shelf-life was carried out with zero-order according to equation (5) which the results shown in Table 4. Table 4 shows that the shortest shelf-life of *cabe jamu* herbal drink product is without benzoate stored at 45 °C and 35 °C. The product shelf-life was longer if the concentration of benzoate added was higher. The product's shelf-life stored at 35 °C was also longer than products stored at 45 °C. The shelf-life dif-
ference in each treatment was caused by the addition of sodium benzoate and storage temperature. Sodium benzoate can inhibit the growth of microorganisms in food because sodium benzoate can interfere with the microbial metabolism (Rahasti et al., 2008). Sodium benzoate can also inhibit the Maillard reaction, a complex reaction that produces the brown pigment melanoidin in the final product. The Maillard reaction is also a significant cause of browning in foodstuffs during heating and long-term storage.

Effect of Sodium Benzoate Addition on Shelf-life

The analysis of variance results showed that the sodium benzoate addition, storage temperature, and the interaction between sodium benzoate concentration and storage temperature had a significant effect on the *cabe jamu* herbal drink shelf-life, with a significance value of 0.000, 0.000, and 0.018 (p<0.05) respectively.

Table 5 shows the average shelf-life of *cabe jamu* herbal drink at various benzoate concentrations and storage temperatures. The shortest average shelf-life was 133.50 days which was occurred in *cabe jamu* herbal drink without the sodium benzoate addition (0 ppm). The most extended shelf-life average was 201.50 days which was occurred in *cabe jamu* herbal drink with 400 ppm sodium benzoate. The shelf-life difference showed that *cabe jamu* herbal drink shelf-life was longer if the addition of sodium benzoate increased because sodium benzoate can inhibit the growth of microorganisms. A higher concentration of sodium benzoate indicates a more significant inhibition of microorganisms' growth (Talasila, Vechalapu, & Shaik, 2012). The product's shelf-life will be longer if preservatives are added, such as sodium benzoate (Moazzem et al., 2019).

The *cabe jamu* herbal drink shelf-life was also longer if the storage temperature was lower. The temperature of 45 °C was considered the optimum temperature for microbial growth compared to 35 °C, so the product is more durable if stored at 35 °C. Table 5 shows that the shelf-life of the *cabe jamu* herbal drink will be longer if the storage temperature is lower. Fermented beverages stored at lower temperatures will have a longer shelf-life (MinhThu & Son, 2019).

**CONCLUSION**

*Cabe jamu* herbal drink without sodium benzoate addition had a longer shelf-life if stored at a lower temperature. *Cabe jamu* herbal drink with 400 ppm sodium benzoate held at 35 °C had the most extended shelf-life of 201.21 days. The sodium benzoate addition, storage temperature, and the interaction between the sodium benzoate concentrations on the storage temperature significantly affect the *cabe jamu* herbal drink shelf-life. This study observed *cabe jamu* herbal drink production, starting from the product development stage, formulation, and estimating shelf-life on a laboratory scale. Scale-up from laboratory scale to pilot plant scale can be done for further research.

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