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The Rate of Change in the Quality of Herbal Skin Creams Using Gelatin Extracted From Tuna Bone as Stabilizer Agent

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Abstract. Changes that lead to product damage can be caused by intrinsic or extrinsic factors which will then react in the product in the form of chemical reactions or other physical processes such as air or gas absorption from the environment. The purpose of this study was to assay the rate of change in the quality of herbal skin creams using gelatin extracted from Tuna bones through physical, chemical and microbiological parameters during storage. The herbal skin creams product tested was formula using 7% gelatin. Data analyzed were vitamin C, viscosity, antioxidant activity, sensory values, and total bacteria during storage at room temperature, temperature 35 ° C, and 45 ° C. The method used was experimental methods which are Linear Regression and Arrhenius Equations to compile the rate of change in the quality of the product. The results show that the longer the storage, vitamin C increases but the sensory values, antioxidant activity and viscosity decrease. The rate of change in antioxidant activity, vitamin C, viscosity, and sensory values , respectively, ie, 104.8 / day, 0.87 / day, 2044 / day, and 7.04 / day.
Keywords: Fishbone, gelatin, stability of product quality

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1. Introduction

Skin creams is one type of cosmetics that has been used since time immemorial. According to popular term, cosmetics are materials that are polished, sprayed or rubbed on the skin to provide freshness, smoothness, softness, cleanliness and fragrance for the consumer. In short, cosmetics are preparation that can modify aspects of the skin, so they can be as skin care and also as beauty ingredient.

Various internal and external factors can affect the structure and function of the skin, especially environmental influences such as sunlight, UV radiation, and air pollution. The skin can become dry and wrinkled so it needs protection and care. One way to treat and protect the skin from the effects of aging is with skin creams.

Along with science and technology development, various creams appear on the market. However, not all of these products meet pharmaceutical principles, which are safe, efficacious and qualified. Therefore it is necessary to develop skin creams using natural ingredients and it can be called herbal skin creams. Some natural ingredients that can be used in formulas either as active substances which are cucumber, yam and orange extracts (active substances and fragrances) and gelatin as stabilizers or emulsifiers. Santoso (2005) states that cucumber (*Cucumis sativus*) has antioxidant activity that can protect cell walls from free radicals effects that can be formed through air pollution. While kaffir lime (*Citrus hystrix*) is efficacious as a stimulant and freshener for skin, besides its essential oil content is high enough so that it can be used as an aromatherapy ingredient (Roni 2008). Lukitaningsih *et al*. (2013) reported that there were 6 compounds in yam which had anti-oxidant activity and could whiten the skin, including *daidzein, daidzin, genistin, (8,9) -furanyl-*

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1. Introduction

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Along with science and technology development, various creams appear on the market. However, not all of these products meet pharmaceutical principles, which are safe, efficacious and qualified. Therefore it is necessary to develop skin creams using natural ingredients and it can be called herbal skin creams. Some natural ingredients that can be used in formulas either as active substances which are cucumber, yam and orange extracts (active substances and fragrances) and gelatin as stabilizers or emulsifiers. Santoso (2005) states that cucumber (*Cucumis sativus*) has antioxidant activity that can protect cell walls from free radicals effects that can be formed through air pollution. While kaffir lime (*Citrus hystrix*) is efficacious as a stimulant and freshener for skin, besides its essential oil content is high enough so that it can be used as an aromatherapy ingredient (Roni 2008). Lukitaningsih *et al.* (2013) reported that there were 6 compounds in yam which had anti-oxidant activity and could whiten the skin, including *daidzein*, *daidzin*, *genistin*, *(8,9) -furanyl-*

pteropcarpan-3-ol. A natural compound that can be as stabilizer and emulsifier in the skin creams formula is gelatin which is a protein derivative of collagen fiber that is present in the skin, bones and cartilage.

Previous study has carried out active ingredients and gelatin formulations, as well as characterizing the physicochemical properties of herbal skin creams with various concentrations of gelatin, i.e., 3%, 5%, and 7%, and resulting herbal skin creams with 7% gelatin as the best formulas based on viscosity, vitamin C levels, antioxidant activity, and pH (Naiu and Yusuf, 2018). Based on the results, this research was to assay the rate of change in the quality of herbal skin creams containing 7% gelatin for 28 days storage at three different temperature levels, namely room temperature, temperature 35 ° C and 45 ° C as well.

2. Materials and method

2.1. Material
Tuna bones (waste disposal from fillet and loin industry) and palm vinegar (fermentation product of palm sap) were used for the synthesis of gelatin. Cucumber, orange, vaseline, sterile distillate water, also gelatin were used at the concentrations mentioned in the respective sections for formula herbal skin creams.

2.2. Method

2.2.1. Gelatin extraction.
Gelatin extraction refers to Naiu and Yusuf (2014). The raw material of fish bones was cleaned from the remnants of meat that was still attached to fish bones, cut into small parts ± 1-1.5 cm, soaked in hot water for 30 minutes, then soaked in vinegar solution with a comparison of fish bones: sugar palm vinegar was 1: 3 until ossein was formed. The bones that have become ossein were washed until neutral pH (6-7) then extracted using distillate water at 80°C for 6 hours. The extraction results were filtered and dried using a dryer with a temperature of 55°C, then ground into gelatin.

2.2.2 Herbal skin creams formulation.
The stages of formulation: the raw material of cucumber was extracted by shredding all parts of the cucumber except the seeds, then filtered to get cucumber's sap, kaffir lime was squeezed and filtered to get the sap, yam was mashed, squeezed and filtered to get the sap. All active ingredients and gelatin dissolved in distilled water were used as the liquid phase, while the oil phase was vaselin. The liquid phase and the oil phase were mixed at the same hot temperature and stirred over the bath to form a smooth cream. Formulas for herbal skin creams constituents can be seen in Table 1.

Table 1. Ingredients of *herbal skin cream*

Bahan	Jumlah (%)
Gelatin	7
Ekstract of keffir lime	10
Ekstract of yam	10
Ekstract of cucumber	10
Vaselin	50
Aquadest	Add 100

2.2.3. Sampling technique.
Assessing the rate of change in the quality of skin creams was carried out during the storage period. The product is stored for 28 days at 3 (three) different temperature levels, namely room temperature, 35 ° C and 45 ° C in white plastic bottles. Sampling for assaying was done 5 times with different time periods according to the storage temperature. The product stability tests include vitamin C levels, antioxidant activity, viscosity, and sensory values (appearance, aroma, and moist impression). Data were analyzed using linear analysis and assisted by Arrhenius equations.

Viscosity was measured using Viscometer Brookfield. The spindle is heated at a temperature of 75°C and then attached to the device. The spindle is right adjusted in the hot solution, then the viscometer is turned on and the temperature is measured. When the temperature of the solution reaches 75°C, the viscosity value can be known

by reading the scale of 1 to 100. The reading is done after one minute of full rotation using spindle no 7 with a value of 400. The tool speed used is 50 rpm. The FSR value is 80,000.

2.2.5.Vitamin C assay. Preparation of standard vitamin C solution: 0.05 g standard crystalline ascorbic acid was dissolved in 100 mL of distilled water to prepare a 500 ppm standard stock solution. Preparation of sample: 10 g blended sample was homogenized with about 50 mL of 5% metaphosphoric acid-10% acetic acid solution. Then it was quantitatively transferred into a 100 mL volumetric flask and was shaken gently until an h*2.2.4. Viscosity assay.* homogeneous dispersion was obtained. Then it was diluted up to the mark by the 5% metaphosphoric acid-10% acetic acid solution. Then the solution was filtered and the clear filtrate was collected for the determination of vitamin C in that sample (Kahn *et al*. 2006).

2.2.6. Antioxidant activity. 0.25 gram of sample dissolved in 50 ml of methanol. The formed solution was diluted to get a concentration of 25, 50, 100, 200, and 400ppm. DPPH solution was made by dissolving 0.0197 grams of DPPH in 50 ml of methanol p.a to obtain a concentration of 1 mM. The solution was maintained at low temperatures and protected from light. A total of 4 ml of the test solution was reacted with 1 ml of DPPH solution in a test tube. The mixture was incubated at 37 ^{o}C for 30 minutes. Then the absorbance was measured using a spectrophotometer at a wavelength of 517 nm (Molyneux 2004).

3. Results and discussion

Skin creams stability is influenced by the rate of change in quality obtained from the calculation of parameter data during storage at different temperatures.

3.1 Antioxidant activity
Antioxidant activity is shown by 50% concentration inhibition against free radicals (IC_{50}). The inhibitory concentration (IC_{50}) is higher, the antioxidant activity gets lower. The data from the test results of the antioxidant activity described by the concentration of free radical (IC_{50}) inhibition of skin creams during storage at various temperature levels can be seen in Figure 1.
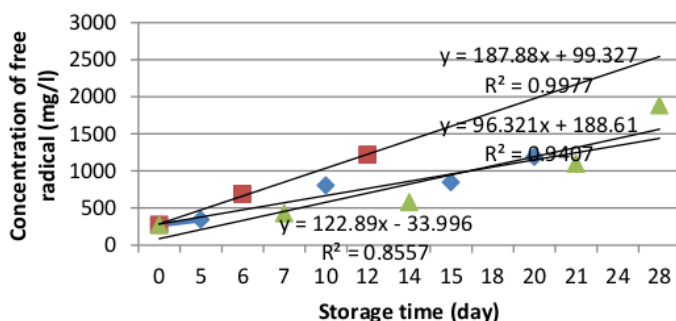

Figure 1. Skin creams free radicals (IC $_{50}$) concentration during storage at various temperature levels.

Figure 1 shows the change in antioxidant activity with the same tendency, namely the longer storage time at each temperature level, the less antioxidant activity. The graph of concentration of free radical inhibition, as shown in Figure 1, indicates an increase which means that antioxidant activity declines. Calculations using linear regression techniques obtained a high coefficient of determination between storage time and antioxidant activity ($R> 85\%$). The rate of decreasing in antioxidant activity of the product was obtained from the results of linear regression at three temperature levels, namely $k = 104.89^{(-0.103\,(1|T)}$ where k is the decreasing in antioxidant activity, and T is °K (temperature (°C) + 273). Based on the equation, the rate of

decrease in antioxidant activity can be calculated if the product is stored at various temperatures. At lower storage temperatures, for example, at 20 °C, the rate of decrease in antioxidant activity is 104.85/day, lower than room temperature.

Antioxidant activity is not only influenced by the structural framework which will chemically react with *peroxyls* and other active species but also influenced by other factors, such as concentration, temperature, light, substrate type, physical status in the system, also by a number of micro components acting as prooxides or synergistic. Yanishlieva-Maslarova (2001) states that the initial concentration of polymer antioxidant products, namely hydroperoxide, greatly reduces the efficiency and effectiveness of antioxidants added to the system because as a chain initiator, hydroperoxide reduces the time for inhibitors to inhibit the termination process. The difference in temperature and light can also change the mechanism of action of several antioxidants so that it influences the effectiveness of antioxidants.

3.2 Vitamin C

Vitamin C is a compound that can help brighten skin especially for those who have dark skin and has a skin problem/hyperpigmentation because it can prevent the formation of melamine by inhibiting the formation of dopa to dopa quinone (Kembuan et al. 2012). The creams skin formula in this study used ingredients that were known to contain vitamin C. The stability of vitamin C was tested during storage at various temperature levels and can be seen in Figure 2.
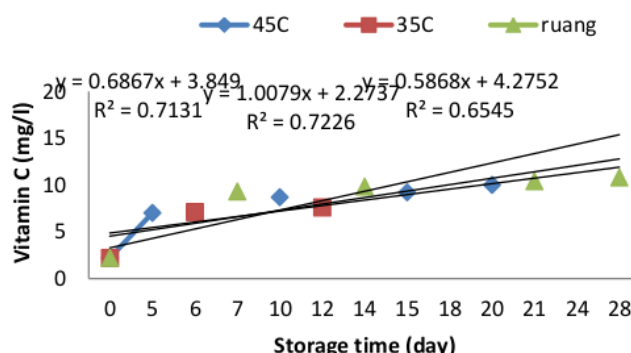

Figure 2. Vitamin C levels during storage at various temperature levels.

Based on the results of the linear regression test, the relationship between storage time and vitamin C levels was only 65.4% to 72.2%. This means that the presence of vitamin C in the product is quite stable (not much changed) during storage at various temperatures tested. The rate of change in vitamin C in different storage temperatures is calculated using the Arrhenius model. The equation obtained from the linear regression at the three temperature levels is plotted into the Arrhenius model. Based on these calculations, the rate of change of vitamin C in skin creams products is obtained, namely

$k = 0.867e^{(0.079\,(1\,|\,T))}$ where k is the rate of change in vitamin C, and T is °K (temperature (°C) + 273)

Based on the equation, the rate of change in vitamin C can be calculated if the product is stored at various temperatures. At lower temperature storage, for example, at 20 °C, the rate of increase in vitamin C is 0.867/day.

3.3 Viscosity

Viscosity test was carried out to see the thickness and flow rate of particles in herbal skin creams. The higher the viscosity value will cause better product stability, but it will be difficult to apply to the skin. The low viscosity of the product will increase the flow of power to the skin. Product thickness is influenced by the amount of material that dissolves both in water and in oil in the formula. The greater the water-soluble material

used, the smaller the level of viscosity of the creams. Romanowski and Schueller (2001) state that product stability is influenced by the amount of material that dissolves, both in water and in oil. The more dissolved materials used, the smaller the viscosity level. Viscosity stability is tested during storage at various temperature levels and can be seen in Figure 3.
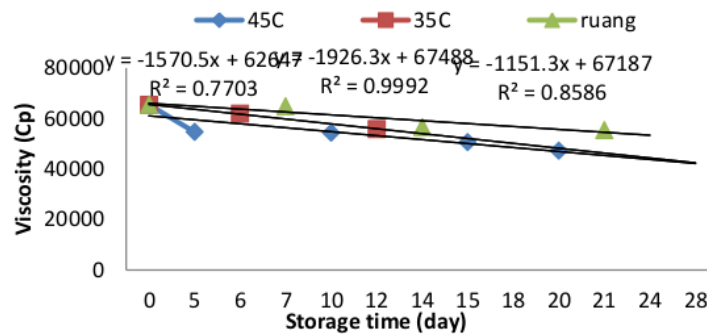

Figure 3. Values of viscosity during storage at various temperature levels.

Based on the linear regression test, the relationship between storage time and viscosity value is high, which ranges from 77.0% to 99.9%. This means that the viscosity stability in the product is influenced by the storage time at the various temperatures tested. The rate of change in the value of viscosity at different storage temperatures is calculated using the Arrhenius model. The equation obtained from the linear regression results at the three temperature levels is plotted into the Arrhenius model. Based on these calculations, the viscosity change rate equation in skin creams is obtained, namely $k = 2065\ e^{(-0.155\ (1|T))}$ where k is the rate of change in viscosity, and T is $^\circ$K (temperature ($^\circ$C) + 273).

Based on the equation, the rate of change in viscosity can be calculated if the product is stored at various temperatures. At lower storage temperatures, for example, at 20 $^\circ$C, the rate of decrease in viscosity is 2044.5/day.

3.4 Sensory value
Sensory values include visibility, aroma, moist impression, and consistency of skin creams. Panelists for testing the products are semi-trained panelists consisting of 25 people. Figure 4 shows changes in overall sensory quality during storage at three different temperature levels.
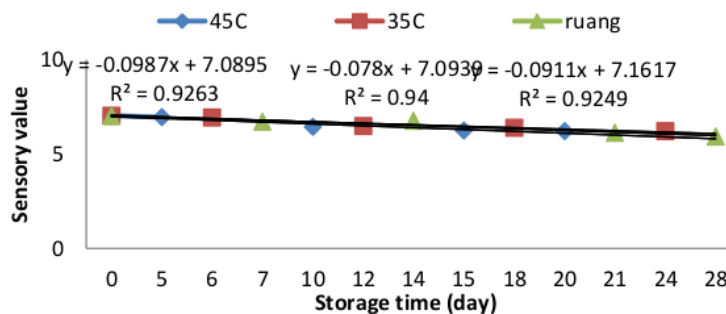

Figure 4. Sensory skin creams values during storage at three temperature levels

Figure 4 shows an increase in sensory values during storage at all temperature levels tested. Based on the results of the linear regression test, the relationship between storage time and sensory value is very high, which is above 92%. This means that storage conditions are very influential in decreasing sensory values. Decreasing

changes in sensory values during storage can be influenced by external factors, such as temperature and humidity. The skin creams studied are not enriched with antimicrobial or antioxidant additives and only contain natural ingredients that are easily oxidized and degraded due to their naturalness.

The rate of change in sensory values at different storage temperatures is calculated using the Arrhenius model. The equation obtained from the linear regression results at the three temperature levels is plotted into the Arrhenius model. Based on these calculations, the rate of change in sensory value in skin creams products is obtained, namely $k = 7.036\ e^{(0.005\ (1\,|\,T))}$ where k is the rate of change in sensory value, and T is ^{o} K (temperature (^{o} C) + 273).

Based on the equation, the rate of change in sensory value can be calculated if the product is stored at various temperatures. At lower storage temperatures, for example, at 20 ^{o} C, the rate of decrease in sensory value is 7.04/day.

4. Conclusion

Results show that the sensory quality stability and physicochemical quality characteristics of skin creams are closely related to the storage time which is indicated by a high coefficient of determination (R^2) for all parameters assayed. R^2 values of antioxidant activity, vitamin C, viscosity, and sensory values respectively are 85.5 - 99.7%, 65.4 - 72.2%, 77.0 - 99.9%, and 92.4 - 94%. The rate of change in the quality of the antioxidant activity, vitamin C, viscosity, and sensory values , respectively, is 104.8/day,   0.87/ day, 2044/day, and 7.04/day.

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