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Research Article
Effect of Avocado (*Persea americana* Mill.) Seed Oil on Lip Balm Characteristics

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Abstract

Background and Objective: Avocado seed oil is an oil derived from avocado seeds, which contains a lot of unsaturated fatty acids such as oleic and linoleic which can increase moisture in the product such as lip balm. The purpose of this study was to determine whether there was an effect of the concentration of added avocado seed oil on lip balm characteristics. **Materials and Methods:** This research used a completely randomized design (RCD) with 5 treatments and 3 replications. Avocado seed oil was used in a lip balm formula and this lip balm was applied to the lips. The treatment in this study was the concentration of avocado seed oil added by 0, 2.5, 5, 7.5 and 10%. The observation was performed for the oil and also for the produced lip balm. Data were analyzed statistically using ANOVA and continued with Duncan's New Multiple Range Test (DNMRT) at the 5% level. **Results:** The best concentration of avocado seed oil addition to the addition of 10% avocado seed oil concentration with homogeneity test results is homogeneous, melting point 61.33°C, texture 21.80 N cm^{-2}, pH value 5.3, loss on drying 1.00%, activity antioxidant 262.93 ppm, moisture effectiveness 27.01% hedonic test, color 3.90 (like), aroma 3.86 (like), texture 3.83 (like), moisture 3.79 (like) and ease of application 3.86 (like). For organoleptic observation, there was no irritation observed and the product was stable for storage. **Conclusion:** Results showed that, avocado seed oil can be used for developing lip balm. Based on the characteristics of developed lip balm, further research on upscale production needs to be considered.

Key words: Antioxidant, cosmetics, fatty acids, moisturizer, avocado seed oil

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Avocado contains oil in the flesh and seeds which have the potential as a source of vegetable oil[1]. In general, only avocado flesh is used and the seeds become waste. Whereas the seeds if processed can produce oil that is no less valuable than other vegetable oil sources. Avocado seed oil is one of the oils that can be used in the cosmetic industry, such as in skin moisturizing creams and lipsticks. This is because it contains a lot of fat and includes healthy fats because it is dominated by monounsaturated fatty acids which acts as a strong antioxidant[1]. The main content of fatty acids in avocado seed oil is oleic, linoleic, palmitic, palmitoleic and stearic[2].

High unsaturated fatty acids such as oleic and linoleic function to provide a flexibility effect, making the skin healthier. The composition of the main fatty acids in avocado seed oil is oleic at 67.80%, linoleic at 13.67%, palmitic at 11.62%, palmitoleic at 5.91% and a stearate at 0.12%. In addition, according to Segovia *et al.*[3], it also contains-carotene and vitamin E bioactive compounds as much as 4.82 and 0.966 ppm, respectively. The β-carotene and vitamin E are oil-soluble compounds that can function as natural antioxidants that can counteract free radicals[4]. Therefore, avocado seeds are expected to be used as a moisturizer in the cosmetic industry such as lip balm.

Lips are one of the outer parts of the body that use cosmetics. Anatomically and physiologically slightly different from the rest of the inner skin, the lips consist of a very thin layer of skin, which is about 3 to 5 cellular layers. For example, the stratum is generally very thin and the dermis contains neither sweat glands nor oil glands. There are no hair follicles in the skin layer of the lips. This has shown that the lips do not have a protective layer like other skin so the lips become dry and even cracked, especially in cold and dry air[5].

Chapped lips are caused by the cracking of the surface layer of the rust. The contributing factors are frequent lip licking, hydration and sunlight. However, the specific cause is damage caused by exposure to UV rays[6]. Oxidative activity on the lips that causes the lips to become dry and cracked can be prevented by using cosmetic products such as lip balm.

Lip balm is a formulation applied to the lips to prevent drying and protect against adverse environmental factors. Lipsticks and lip balms have similarities in the form of the main ingredients, namely fatty acids which are in the form of wax, oil and butter which provide consistency and function as emollients and formulations[7]. The function of using lip balm is more for lip care than for makeup purposes. Lip balm is designed to protect and maintain lip moisture. It contains moisturizing substances and vitamins for the lips[8]. In some lip balm formulations, of course, there are special ingredients that can keep the lips from drying out and not losing moisture as a moisturizer. In this study, a moisturizer was used comprised of avocado seed oil. Using avocado seed oil could increase the functionality of lip balm because of its fatty acid content. Apart from that, the use of avocado seeds as agricultural product waste will also be an added value from this research.

MATERIALS AND METHODS

Study area: The study was carried out from December, 2020 to May, 2022. The study was performed at the Laboratory of Phytonutrient, Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Andalas University.

Materials: The main ingredient used in this study was avocado seed oil which was obtained at Eteris Nusantara. The oil was local farmer production. The quality of the oil was similar to the quality of commercial ones. The ingredients used for making lip balms were beeswax, glycerin and cocoa butter which were obtained from the cocoa factory in Payakumbuh.

Lip balm processing: The cocoa butter was weighed and put into a measuring cup then melted on a hot plate while stirring until all the cocoa butter was completely melted, the cocoa butter was melted at a melting temperature of 31-34°C. Weighed the beeswax to be melted at a melting temperature of around 62-64°C, afterwards cocoa butter was added to the melted beeswax. The glycerin was weighed and then put into the melted cocoa butter mixture and beeswax while stirring with a magnetic stirrer, the avocado seed oil was added to the melted mixture of ingredients while stirring using a magnetic stirrer. After all the ingredients were mixed, put them in a lip balm package and keep it at room temperature until freezes.

Analysis of samples: Avocado seed oil analysis (*Persea americana* Mill).

Free fatty acids (FFA)[9]: The sample was weighed as much as 2.5 g in an Erlenmeyer. Add 50 mL of hot neutral alcohol and 2 mL of phenolphthalein (PP) indicator. Titrate with standardized 0.1 N NaOH solution until a pink color is reached and does not disappear for 30 sec. The percentage of free fatty acids is expressed as FFA (%) or acid number:

$$\text{FFA (\%)} = \frac{\text{NaOH (mL)} \times \text{N} \times \text{Fatty acid molecules (as oleic acid)}}{\text{Sample weight}} \times 100$$

where, the fatty acid molecule (as oleic fatty acid) has a value of 282.

Color analysis[9]: Color analysis with a colorimeter was based on direct measurement of the L*, a* and b* values of the sample. The tool that has been used is the HunterLab colorimeter. Avocado seed oil was put on a transparent glass plate. Before the colorimeter is used to measure the sample, the instrument needs to be calibrated first with a white standard plate. This standard plate has a value of L*, a* and b* or X, Y and Z. The optical head was attached to the white plate so that the white part of the plate faces the light source. Then the menu was selected for the use of the L*, a* and b* reading scale. A calibrated tool will show the same reading value as the standard plate value. The sample was measured in the same way as the standard, where the optical head was attached to the sample, then press the start button. From the results of this measurement, the L*, a* and b* values of the sample will be read.

Iod number: Weigh 0.5 g of oil in a closed Erlenmeyer. Add 10 mL of chloroform and 25 mL of Wijs solution (solution for determination of iodine number) and leave in a dark place for 30 min with occasional shaking. Then add 10 mL of 15% KI solution and 100 mL of boiled distilled water and immediately titrate with Sodium Thiosulfate solution ($Na_2S_2O_3$ 0.1 N) until the solution turns pale yellow, then add 2 mL of starch solution. The titration was continued until the blue colour disappeared. The blank solution made from 25 mL of Wijs solution was added to 10 mL of 15 mL of KI solution, diluted in 100 mL of boiled distilled water and titrated with sodium thiosulfate solution:

$$\text{Iod number} = \frac{\text{Titration (mL) (blank-sample)}}{\text{Sample weight (g)}} \times \text{N thio} \times 12{,}691$$

Density[10]: The pycnometer was cleaned and dried in an oven at 105°C for 15 min, then weighed the empty pycnometer was then filled with oil. Oil is filled into the pycnometer until no air bubbles form. Afterward, it was weighed.

The density formula is as follows:

$$\rho = \frac{m}{v}$$

Where:

ρ = Density (g mL^{-1})
m = Mass (pycnometer and oil-pycnometer empty) (g)
v = Volume (mL)

Lip balm analysis

Homogeneity of lip balm[11]: As much as 5 g of lip balm was applied to a piece of glass or other flat transparent material, the preparation must show a homogeneous arrangement and no visible coarse grains.

It was put in an oven with an initial temperature of 50°C for 15 min and observed whether it melted or not, after that it was increased by 1°C every 15 min and observed at what temperature the lip balm began to melt.

pH analysis: Measurement of the pH of the preparation was carried out using litmus paper or a pH indicator. One gram of sample was dissolved in 10 mL of distilled water, then litmus paper was dipped in the solution. Leave it for a while until the litmus paper or pH indicator changes color, after the color is stable, match the color obtained by the litmus paper or pH indicator with the color chart instructions. Measurement of pH 3 times, then the average value was taken.

Stability analysis: Five grams of the sample was put into a container and then stored at room temperature. Lip balm preparations were evaluated on days 7, 14, 21 and 28 which included organoleptic observations (color, odor and texture) and whether there was a change during storage at room temperature.

Antioxidant activity[12,13]: One gram of lip balm was added to 10 mL of methanol and then vortexed, then ultrasonically for 10-15 min, the solution was pipetted 0.5, 1 and 1.5 mL, respectively and then the volume was made up to 10 mL with methanol to obtain a solution with concentrations of 50, 100 and 150 ppm. Then it was homogenized by vortexing (IKA, Vortex-3, Germany), after that 2 mL samples were taken from each dilution and 1 mL of DPPH was then vortexed. Then it was left for 15 min in a dark room. Absorption was measured by UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) at a wavelength of 517 nm. Then the percentage of inhibition against DPPH radicals was calculated from each sample solution and the antioxidant activity was determined which was expressed by the IC_{50} value. The blank solution consisted of 2 mL of methanol and 1 mL of DPPH.

The percentage of inhibition of DPPH radicals from each concentration of sample solution was calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Blank absorbance-Sample absorbance}}{\text{Blank absorbance}} \times 100$$

After obtaining the percentage of inhibition from each concentration, the equation y = bx+a was determined by inhibition concentration 50% or IC_{50}, namely the concentration of the sample that can reduce DPPH radicals by 50%. The IC_{50} value was obtained from the x value after replacing it with 50.

Texture analysis: Texture analysis was concerned with the hardness, softness and crispness of a product. Lip balm texture measurement can be done with the Texture Analyzer tool (Brookfield, CT3, USA). The working principle of the texture analyzer is the durability of the product by the compressive force of the tool or the ability of a product to return to its initial condition after the compressive load is removed. The texture testing procedure is a sample needle (probe) is installed and positioned then the tool is turned on and it is ensured that the monitored value is zero. Select the start test menu so that the probe moves to pierce the sample, the test is complete when the probe returns to its original position[14]. The test results can be seen in the form of graphs and values (numbers). The hardness measurement is calculated by the following formula:

$$\text{Hardness value: } \frac{B}{C}$$

Where:

B = Sample weight × 9.8 m sec^{-1}

C = Surface area of the sample

Drying loss analysis[15]: Weighed 1 g sample of lip balm and Petri dish. The Petri dish containing the sample was placed in an oven (Memmert, UN110, Germany) at 105°C for 3 hrs. The heated sample was reweighed. The weight lost during heating is calculated by the equation:

$$\text{Drying loss (\%)} = \frac{\text{A-B (g)}}{\text{A (g)}} \times 100$$

Where:

A = Initial sample weight

B = Weight of sample after drying

Irritation test: The irritation test was carried out using the open patch test method. The test was carried out on the inner forearm of 15 researchers who were willing and wrote a statement. The open patch test was carried out by applying lip balm on the forearm, leaving it open and observing the reaction. This test was carried out 3 times a day for two consecutive days. The reaction observed was the occurrence of erythema, populae, vasculature or edema. There were signs to note patch test reactions[16]:

No reaction	-
Erythema	+
Erythema and papules	++
Erythema, papules and vasculature	+++
Edema and vasculature	++++

Erythema is a red macule, as in lupus dermatitis, erythematosus. Papules are solid protrusions above the skin surface, well-defined, measuring less than 1/2 cm. Vesicula are vesicles filled with serous fluid with a diameter of less than 1 cm.

Statistical analysis: Using the SPSS package program version 11.5 (SPSS Inc., Chicago, Illinois, USA), statistical analysis was carried out. One-way Analysis of Variance (ANOVA) was used to assess the data and Duncan's Multiple Ranges *post hoc* Test was then performed. The Mean ± SD of the triple samples was used to express the results. The $p<0.05$ was used to indicate the significance of differences.

RESULTS AND DISCUSSION

Raw material analysis (avocado seed oil): Analysis of raw materials was carried out to determine the content of the raw materials that will be used as the basic ingredients for making products. Analysis of raw materials in avocado seed oil which includes iodine number, peroxide number, free fatty acid, color and density tests. The results of the analysis of raw materials was shown in Table 1.

The free fatty acid (FFA) analysis was used to determine the content of free fatty acids in the oil. The increase in the FFA value indicates the oil was damaged due to hydrolysis, the higher the FFA value in the oil, the lower the oil quality and the lower the FFA value in the oil, meaning that the quality of the oil was getting better[17]. In the free fatty acid analysis of the avocado seed oil that will be used, an FFA value of 1.05% was obtained. This result is in the range of FFA values reported in previous research, namely 0.96-1.61%[18]. The difference in percentage of FFA obtained caused by the oil extraction process, type of raw material and geographical conditions of plants and avocado seeds obtained from different places[19-22].

Table 1: Quality information of avocado seed oil analysis

Analysis	Value ± SD
Free fatty acid (FFA) (%)	1.05 ± 0.48
Density (g mL^{-1})	0.91 ± 0.85
Iodine number (g iod g^{-1})	40.55 ± 1.34
Peroxide number (meqO_2 g^{-1})	2.32 ± 0.82
Color analysis (°Hue)	94.32 (purplish yellow)

SD: Standard deviation

Density is one of the important criteria in determining the quality and purity of oil. Oil density value was defined as the ratio between the weight of a volume of oil or fat sample at a temperature of 25°C with the weight of water at the same volume[23]. In the density analysis, the results obtained were 0.91 g mL^{-1}. This result was in the slightly lower than reported data that of the density result was 0.915-0.916 g mL^{-1}. The lower density value indicated the purity of an oil is increasing and if the density value is higher, the purity of an oil will decrease, this was as stated by Aktar and Adal[24] that the density value of a liquid depends on the components in the liquid. The more components present in the liquid, the higher the weight fraction so that the specific gravity was also greater.

In the analysis of the iodine number, the results obtained were 40.55 mg iodine g^{-1}. The results showed that avocado seed oil contains high unsaturated fatty acids because the value of the iodine number is high. The results obtained are quite high when compared to VCO oil which had high saturated fatty acids (lauric acid) where the results of the iodine number are 4.1-11.0 g iodine/100 g. It can be concluded that oil that has high unsaturated fatty acids will have a high iodine number and oil that has low unsaturated fatty acids will produce a low iodine number. The iodine number test is carried out to find out how much the degree of unsaturation of the oil is. The higher the iodine number, the more the number of double bonds in the oil[25].

In the results of the peroxide number, 2.32 meqO_2 g^{-1} was obtained. Peroxide number is an important value to determine the degree of oil damage due to oxidation. Peroxide number test is used to determine how much oxidation level in unsaturated oil is caused by air. The higher the value of the peroxide number in the oil, the lower the quality of the oil and the lower the value of the peroxide number, the better the quality of the oil.

Moreover, the peroxide number might also be affected on the degradation of carotene. The purplish yellow color of avocado seed oil comes from beta carotene contained in avocado seed oil, beta carotene is one of about 500 carotenoids that exist in nature. Carotenoids are a group of orange, red or yellow pigments[26].

Analysis of lip balm

Lip balm homogeneity test: Lip balm with the addition of avocado seed oil with various concentrations was checked for homogeneity by applying it on a flat transparent surface. A homogeneity test was carried out to find out if each ingredient used could mix well in the process of forming lip balm. The homogeneity test results showed that lip balm made without the addition of avocado seed oil and the addition of 2.5% avocado seed oil, 5, 7.5 and 10% has a homogeneous composition, this is indicated by the absence of coarse grains when the lip balm was applied to transparent glass.

In the processing of lip balm, the ingredients that have been used are cocoa butter, beeswax and avocado seed oil, all of which are non-polar so they will easily dissolve in the mixing process and the ingredients are mixed using a magnetic stirrer at the same speed of 600 ppm which produces a homogeneous lip balm.

Melting point lip balm: The results of the examination of the melting point of the lip balm showed that the lip balm preparation of avocado seed oil ranged from 61-63°C. The acceptable melting temperature of the lip balm is 50-70°C. This shows that the lip balm with the addition of avocado seed oil concentration has met the melting point requirements. The results of the melting point examination can be seen in Fig. 1.

The proper melting point for lip balm was between 36 and 38°C, which was similar to the warmth of human lips. However, the lip balm's melting temperature was raised to between 55 and 75°C in order to prevent it from melting at room temperature and to preserve its shape stability during the distribution, storage and use processes. This was necessary because it was important to consider the environmental factors in the immediate vicinity, particularly the tropical temperature. According to the variance analysis, there was no significant difference in the lip balm's melting point at the 5% level when avocado seed oil was added to the formula. The decrease in the melting point of lip balm was caused by the low melting point of avocado seed oil, which was 10.5°C, so the melting temperature will decrease with increasing concentration of avocado seed oil.

Hardness of lip balm: Hardness tests have been carried out to maintain the lip balm against pressure or impact, so that it will retain its shape during distribution, storage and use. The results of the hardness test were shown in Fig. 2.

From the results of the tests carried out, the highest value in treatment A without the addition of avocado seed oil was 38.87 N cm^{-2} and the lowest value in treatment E with the addition of 10% avocado seed oil was 21.80 N cm^{-2}, the hardness test value obtained still higher than commercial lip balm which was 10.46 N cm^{-2}. The results of the variance showed that the lip balm hardness value was significantly different at the level of $\alpha = 5\%$, where the higher the concentration of avocado seed oil added, the lower the lip balm hardness value obtained, this was because avocado seed oil has a low melting point, thus lowering the level of hardness. The hardness value was directly proportional to the melting point value where the lower the melting point value obtained, the lower the hardness value.

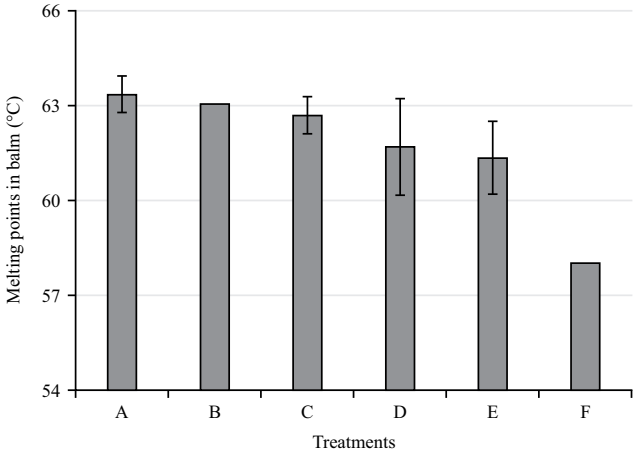

Fig. 1: Melting point of lip balm form

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil, E: Addition of 10% avocado seed oil and F: Commercial lip balm

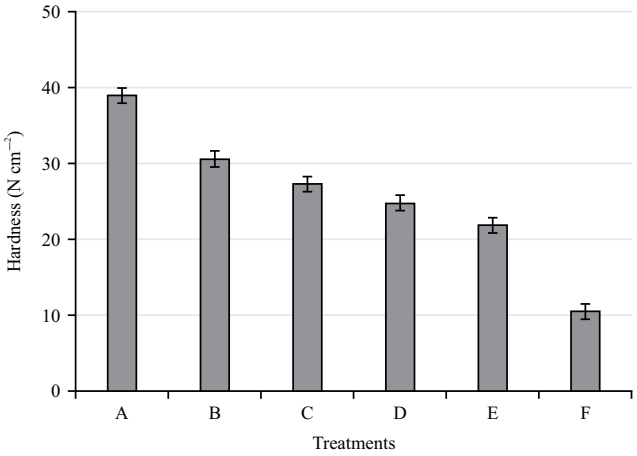

Fig. 2: Hardness of lip balm

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil, E: Addition of 10% avocado seed oil and F: Commercial lip balm

Lip balm pH measurement: The results of pH measurements showed that avocado seed oil lip balm preparations ranged from 5.3 to 5.7. The difference in pH in lip balm preparations was due to the addition of avocado seed oil. The pH value of the lip balm produced has met the cosmetic pH requirements, which was in the physiological pH range of 4.5-6.5[5]. The results of pH measurements can be seen in Fig. 3.

In the results of the lip balm pH in treatment A without the addition of avocado seed oil, it was obtained 5.7 and the addition of 2.5, 5, 7.5 and 10% avocado seed oil was the same, namely 5.3 on the variance of the results obtained not significantly different at the level of $\alpha = 5\%$. Increasing the concentration of avocado seed oil added can reduce the pH value of the lip balm because the increase in the concentration of avocado seed oil will be directly proportional to the increase in fatty acids contained in the lip balm so it will cause a decrease in the pH value of the lip balm, the decrease in pH is caused by hydrolysis of triglycerides. which becomes free fatty acids, causing the pH to become acidic. Generally, the more acidic the material on the skin, the more difficult it was for the skin to neutralize it and the skin becomes dry, cracked, sensitive and susceptible to infection[21], therefore the pH of cosmetics was sought to be the same or as close as possible to the physiological pH of the skin, which was 4.5-6.5.

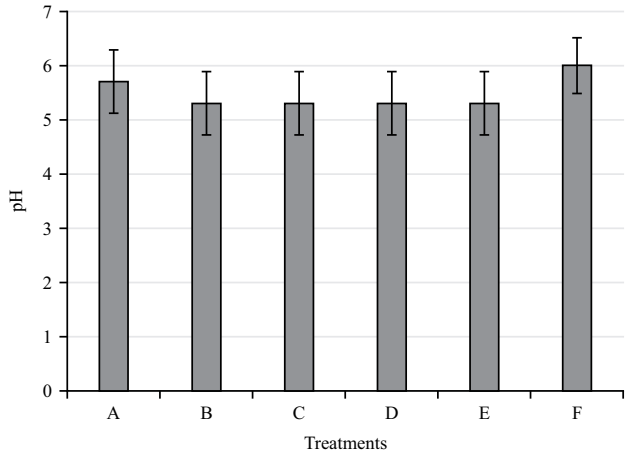

Fig. 3: pH of lip balm

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil, E: Addition of 10% avocado seed oil and F: Commercial lip balm

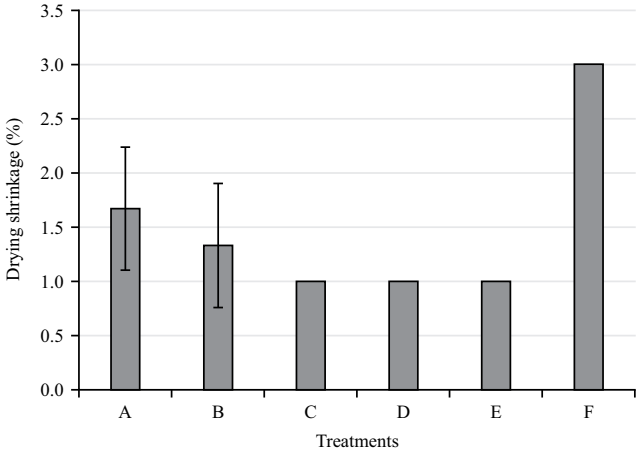

Fig. 4: Lip balm drying shrinkage

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil, E: Addition of 10% avocado seed oil and F: Commercial lip balm

Drying shrinkage analysis: Drying shrinkage is the weight of the material lost during drying. Measurement of drying loss was carried out to determine the ability of lip balm to reduce evaporation of the material. The results of drying shrinkage obtained in Fig. 4.

In the research conducted, the highest drying loss value was found in treatment A, which was 1.67% and the lowest was in treatment C, D and E, which was worth 1.00%, these results can state that the higher the concentration of avocado seed oil added, the lower drying shrinkage value of lip balm. The drying shrinkage value obtained was lower than that of commercial lip balm, which is 3.00%, so that the lip balm made has a lower evaporation rate of the material. The results of the variance showed that the addition of avocado seed oil to lip balm was not significantly different at the level of $\alpha = 5\%$ on drying shrinkage. The decrease in drying shrinkage occurs with increasing the concentration of avocado seed oil so that it will increase the fat content in the lip balm. Fats are molecules that are hydrophobic; hydrophobic is a property of compounds that repel water. The lipid component forms a strong hydrophobic membrane to retain water[26], so the more hydrophobic groups, the less water was absorbed by the lip balm and the lower the evaporation process in the lip balm, so the weight of the lost material will be lower.

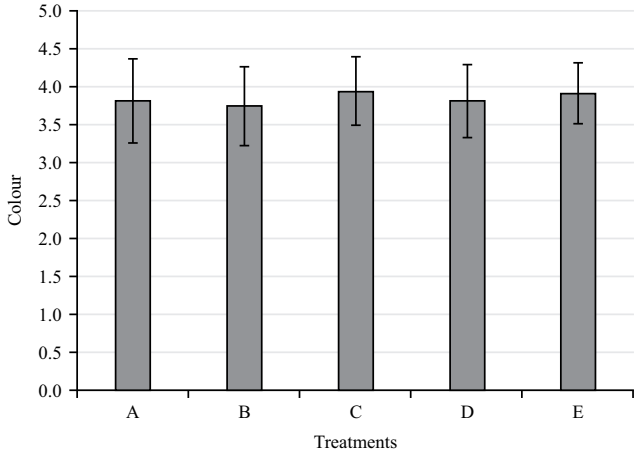

Fig. 5: Colour acceptance test on lip balm

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil and E: Addition of 10% avocado seed oil

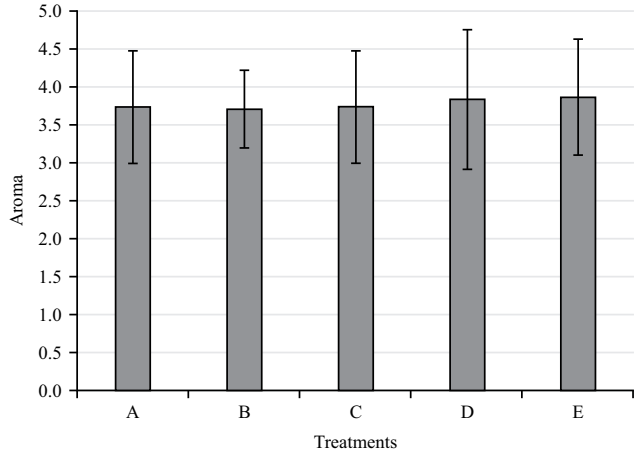

Fig. 6: Aroma acceptance test on lip balm

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil, E: Addition of 10% avocado seed oil

Sensory analysis of lip balm

Colour: The results of the analysis of the lip balm colour acceptance test ranged from 3.73 to 3.93, which can be seen in Fig. 5.

In the colour test, the highest value of lip balm was obtained with treatment C adding 5% avocado seed oil formula, which was 3.93 (likes) and the lowest in treatment B adding 2.5% avocado seed oil with a value of 3.73 (likes). Based on the variance was not significantly different at the level of $\alpha = 5\%$, this happened because the lip balm product with the addition of avocado seed oil produced did not have a colour difference so the panellists made almost the same assessment in each treatment. The colour of avocado seed oil was influenced by the content of beta carotene which has orange, red or yellow pigments[26].

Aroma: The aroma of a product can be assessed by inhaling the aroma or odor released from the product. The aroma acceptance test with the addition of avocado seed oil ranged from 3.70 to 3.86. The results of the analysis have been shown in Fig. 6.

The results of the aroma test obtained the highest value on lip balm with the addition of 10% avocado seed oil with a value of 3.86 (likes) and the lowest was obtained with the addition of 2.5% avocado seed oil with a value of 3.70 (likes). The results of the variance of lip balm with the addition of avocado seed oil were not significantly different at the level of $\alpha = 5\%$, this was because the resulting product does not have a different aroma because the aroma of the lip balm was dominated by the aroma of cocoa fat so that the aroma of avocado seed oil was covered by cocoa butter aroma[16].

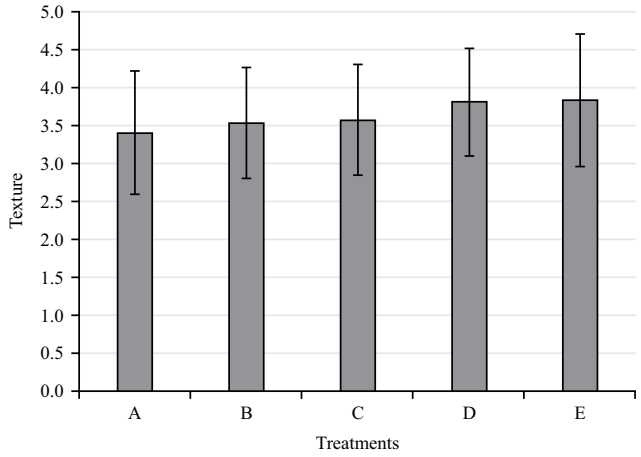

Fig. 7: Texture acceptance test on lip balm

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil and E: Addition of 10% avocado seed oil

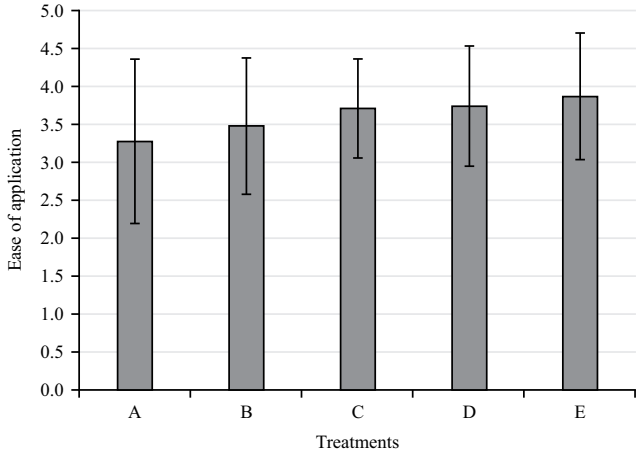

Fig. 8: Ease of application acceptance test on lip balm

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil and E: Addition of 10% avocado seed oil

Texture: An acceptance test of the texture was carried out to see the texture that was preferred by the panellists. The results of the lip balm texture preference test with the addition of avocado seed oil ranged from 3.40 to 3.83. The results of the texture preference test can be seen in Fig. 7.

In the texture test results, the lip balm with the addition of avocado seed oil has the highest value in treatment E with the addition of 10% avocado seed oil, which was 3.83 (likes) and the lowest value in treatment A without the addition of avocado seed oil with a value of 3.4 (normal). The results of the variance were not significantly different at the $\alpha = 5\%$ level, this was because the textures obtained are almost the same, namely solid.

Ease of application: The ease of application was assessed by applying lip balm on the back of the panelist's hand and observing how many times the lip balm was applied so that the results were evenly distributed. The preference scale is 1 (dislike very much) if the lip balm was applied five times or more to get an even result, the preference scale is 2 (disliked) if the lip balm was applied four times to get even results, the preference scale was 3 (normal) if the application of lip balm three times shows even results, the preference scale was worth 4 (likes) if the application of lip balm twice shows even results and the scale was worth 5 (very like) if the application of lip balm 1 time has shown even results. The results of the preference test obtained ranged from 3.27-3.86, which can be seen in Fig. 8.

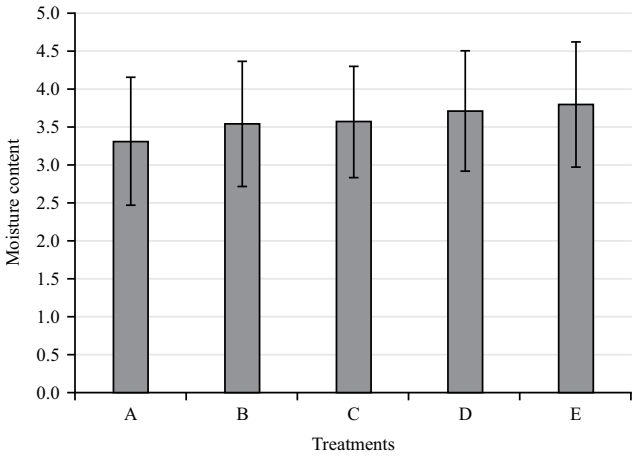

Fig. 9: Test of Acceptance for adding avocado seed oil to lip balm moisture

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil and E: Addition of 10% avocado seed oil

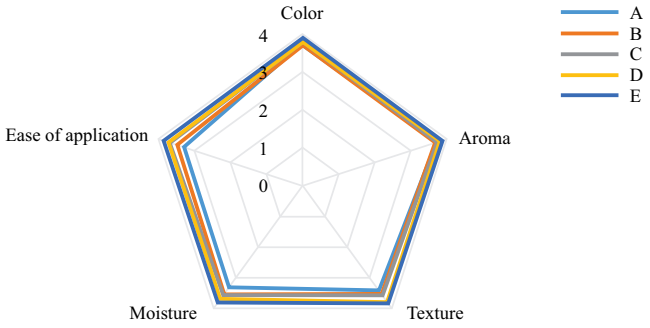

Fig. 10: Acceptance test results

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil and E: Addition of 10% avocado seed oil

In the results of the preference test for ease of application, the highest value was found in the addition of 10% avocado seed oil, namely 3.86 (like) and the lowest value for lip balm without the addition of avocado seed oil with a value of 3.27 (ordinary). The results of the variance showed a significant difference at the level of $\alpha = 5\%$, this was because the texture of the lip balm had different hardness so when applying it had a slightly different ease, but overall, when applying it was easier and evenly distributed on the skin.

Moisture content: Moisture assessment was carried out to determine the panelists' preference for the humidity felt when using lip balm, the moisture of the lip balm was assessed by applying lip balm on the back of the panelist's hand and feeling the level of moisture produced by the lip balm[18]. The results obtained in the analysis of the humidity preference test ranged from 3.30 to 3.79, which can be seen in Fig. 9.

In the results of the lip balm moisture preference test with the addition of avocado seed oil, the highest value was obtained in lip balm with treatment E with the addition of 10% avocado seed oil with a value of 3.79 (like) and the lowest in treatment A without the addition of avocado seed oil with a value of 3.30 (ordinary), the variance was significantly different at the level of $\alpha = 5\%$. This was because the easier it was to apply lip balm, the moister the lip balm was used.

The results of the average value of the overall organoleptic analysis can be seen in Fig. 10.

Based on the radar acceptance test analysis and the average value of the panelists' acceptance rate for color, aroma, texture, moisture and ease of application, the best treatment obtained in sensory analysis was treatment E (Addition of 10% avocado seed oil) with an average color value. As 3.9 (like), aroma 3.86 (like), texture 3.83 (like), moisture 3.79 (like) and ease of application 3.86 (like).

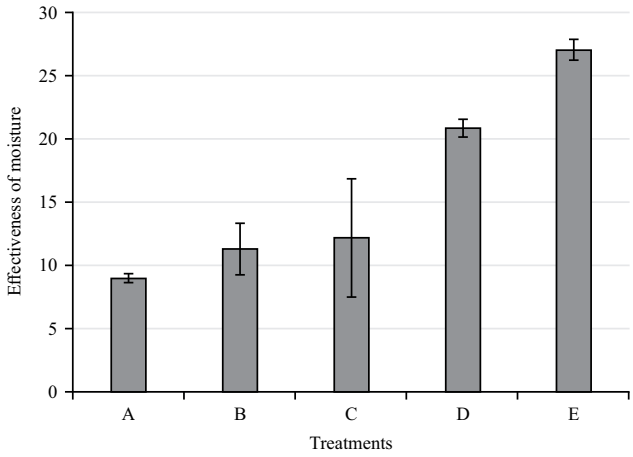

Fig. 11: Average value of the effectiveness of moisture

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil and E: Addition of 10% avocado seed oil

Lip balm irritation test: Based on irritation test conducted by 15 panelists in which every 3 panelists apply lip balm with the same treatment (without the addition of avocado seed oil and with the addition of avocado seed oil 2.5, 5, 7.5 and 10%) on the skin of the forearm for 2 consecutive days, the results obtained that all panelists did not show a reaction to the irritation parameters observed, namely the presence of erythema, papules, edema or the presence of vesicles. From the results of the irritation test, it can be concluded that the lip balm made did not cause irritation. Irritation to the skin did not occur because the ingredients used in making lip balm are natural ingredients and there was no addition of synthetic ingredients, it can reduce the factors that cause irritation to the skin[24].

Lip balm stability test: Lip balm stability tests were carried out by observing changes in the texture, color and aroma of the lip balm without the addition of avocado seed oil and lip balm with the addition of 2.5% avocado seed oil, 5, 7.5 and 10% during storage at room temperature, which were observed once every 7 days (on days 7, 14, 21 and 28 days). The results of the stability examination of lip balm preparations obtained stable texture, color and aroma from each observation, in which the texture obtained from each treatment remained solid, the color remained light yellow and the aroma produced remained the aroma of cocoa.

The purpose of testing lip balm stability was to determine the occurrence of changes in color, aroma and texture. From the results of observations for 28 days, the texture of the lip balm was still solid and not liquid during the storage process at room temperature, this was due to the high melting point of the lip balm, which was 61-63°C, so that at room temperature (20-25°C) the lip balm was not as liquid. The resulting color was still stable and the aroma did not change.

Moisture effectiveness lip balm: Assessment of the effectiveness of lip balm moisture on the lips was carried out on 15 panelists. The panelists first measured the moisture of their lips using a skin analyzer, then measured again after 7, 14, 21 and 28 days of using lip balm. The results of the effectiveness of lip balm moisture on the lips was shown in Fig. 11.

In the results of the effectiveness of moisture in lip balm, the highest value was found in treatment E with the addition of 10% avocado seed oil, which was 27.01% and the lowest value in treatment A without the addition of avocado seed oil, which was 8.99 level $\alpha = 5\%$. The increase in moisture levels on the lips was in line with the increase in the concentration of avocado seed oil added. The higher the avocado seed oil concentration, the greater the percentage increase in water content, which means increasing moisture on the lips. Lips that have high moisture will be more difficult to experience damage such as dryness and chaps even in extreme environments. The ability of lip balm to moisturize the lips was supported by the ingredients[25].

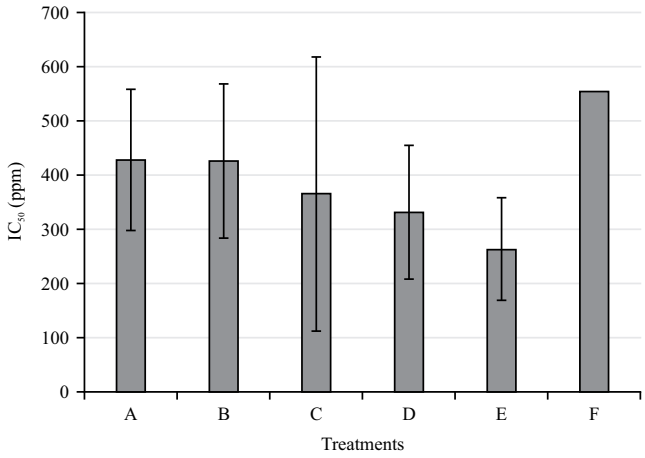

Fig. 12: Lip balm antioxidant activity

A: Without the addition of avocado seed oil, B: Addition of 2.5% avocado seed oil, C: Addition of 5% avocado seed oil, D: Addition of 7.5% avocado seed oil, E: Addition of 10% avocado seed oil and F: Commercial lip balm

The avocado seed oil contains beta carotene and vitamin E. Vitamin E (alpha tocopherol) plays an active role as an antioxidant by donating hydrogen ions so that it can convert peroxide radicals (products of lipid peroxide) into less reactive tocopherol radicals, it was unable to damage fatty acid chains[27]. Therefore, the oxidation process on the lips is inhibited so that the moisture of the lips is maintained. Moreover, the moisture effectiveness test of commercial lip balms was indicated by the value of 24.93%[28], it can be seen that the moisture effectiveness value of lip balms with avocado seed oil concentration is 10% higher than commercial lip balms.

Antioxidant activity of lip balm: Antioxidants are compounds that can function as a deterrent to the oxidation process that causes skin damage such as the appearance of wrinkles, scales, dryness and cracks[29]. The antioxidant activity of lip balm was tested using the DPPH method with UV-Vis spectrophotometry with a maximum wavelength of 517 nm. Then the magnitude of the antioxidant activity was indicated by using the IC_{50} value. The IC_{50} value of the antioxidant activity of lip balm can be seen in Fig. 12.

In the results obtained, the highest value was found in treatment A without the addition of avocado seed oil, which was 427.25 ppm and the lowest value was found in treatment E with the addition of 10% avocado seed oil, which was 262.93 ppm, which the results obtained were lower than lip balm commercial. The results of the variance showed that the addition of avocado seed oil in lip balm was not significantly different at the level of $\alpha = 5\%$ to the value of the antioxidant activity produced.

Based on the results, the higher the addition of avocado seed oil, the IC_{50} value decreased and the antioxidant activity increased. The increase in antioxidant activity was influenced by the content of antioxidants in avocado seed oil, namely; carotene, vitamin E (alpha tocopherol) and cocoa fatty acids which are strong antioxidants. The higher the concentration of antioxidants, the higher the antioxidant activity[30]. Vitamin E functions as an antioxidant in protecting cells from oxidation and free radical damage would maintain the integration of elastic fibers between the dermis and collagen so that cell flexibility can be maintained[31].

In the manufacture of lip balm without the addition of avocado seed oil, there was still antioxidant activity where this was due to the use of cocoa fat in the lip balm formula. The use of cocoa butter in the beauty sector is as a source of vitamin E which had many benefits for the skin, for example as a skin moisturizer, protects the skin from pollution and can also ward off free radicals because it contains tocopherols and polyphenols[32].

The commercial antioxidant activity obtained was lower than lip balm with the addition of avocado seed oil and without the addition of avocado seed oil. Commercial lip balm was added in the form of a synthetic antioxidant compound, namely Butylated Hydroxytoluene (BHT), the low antioxidant activity of commercial lip balms can be caused by temperature factors and product storage time before testing antioxidant activity. The storage temperature at low temperatures could not stop the oxidation process, therefore, even at low temperatures, the oxidation process still occurs which causes the antioxidant activity during storage to decrease[33,34].

CONCLUSION

The best concentration of avocado seed oil addition from this study was in the treatment with the addition of 10% avocado seed oil concentration, based on the lip balm quality parameters, namely the homogeneity test results were homogeneous, melting point 61.33°C, texture 21.80 N cm^{-2}, pH value 5.3, humidity effectiveness 27.01%, no irritation caused and stable product stability check, based on preference test, namely color 3.90 (like), aroma 3.86 (like), texture 3 .83 (like), moisture 3.79 (like) and ease of greasing 3.86 (like). Based on the weight loss test of 1.00%, the antioxidant activity was 262.93 ppm (IC_{50}). The highest utilization of avocado seed oil impressed that highest obtained quality of produced lip balm. Therefore, increment of using avocado seed oil still can be considered. How much of the optimum concentration of avocado seed oil can be used for production of good quality of lip balms need to be found further. Moreover, the upscale study for optimizing the formula also need to be performed for commercialization research result from the university.

SIGNIFICANCE STATEMENT

In this research, the use of avocado seed oil as an ingredient in making lip balm has been developed. Avocado seed oil contains many beneficial compounds which are suitable as a characteristic lip balm used to keep lips moist. The findings of this research are efforts to utilize agricultural product waste to provide more benefits to society.

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