THE EFFECT OF LAVENDER OIL CONCENTRATION ON PREFERENCE AND SKIN IRRITATION OF NIGHT BODY LOTION CONTAINING NANOSTRUCTURED LIPID CARRIERS-UBIQUINONE

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ABSTRACT

Lavender Oil (LO) has a calming scent and antioxidant activity. Therefore, this oil has potential as a fragrance and second active ingredient in Night Body Lotion (NBL) that contained Coenzyme Q10 (CoQ10) loaded Nanostructured Lipid Carrier (NLC) systems. This study aims to observe the effect of LO's concentration on the physical characteristics, consumer preference level, and skin irritation of NBL that contained NLC-CoQ10. There are three formulas observed in this study, namely F0 (0% LO), F1 (2% LO), and F2 (4% LO). The physical characteristics assessed included organoleptic, homogeneity, pH, spreadability, and viscosity. In vivo skin irritation tests observe erythema and edema on the skin of a white rat. In a consumer preference test, the panelists assess the organoleptic properties of F0, F1, and F3 using a 7-point hedonic scale. Based on the physical characteristic test, it is known that the three formulas have the same color, consistency, homogeneity, and spreadability value. The difference between the three formulas lies in their aroma. F0 is unscented; F1 and F2 have a lavender scent. All formulas have a pH in the range of 5.89-6.06. Statistically, increasing the concentration of LO decreases the pH of NBL. The three formulas have a viscosity in the range of 2.140–2.890 cP. Statistically, the viscosity of NBL increased with the increase in LO concentration. Based on the skin irritation test, it is known that all formulas are non-irritating. Based on preference level analysis, the most preferred formulas based on their scent are F1 and F2.

Keywords: Body Lotion, Lavender Oil, Nanostructured Lipid Carriers, Preference Level, Skin Irritation

INTRODUCTION

Cosmetics are substances that come into direct contact with various parts of the human body. Cosmetics can help improve or change the external appearance of the human body (Forestryana *et al*., 2021). Body lotion is an example of a cosmetic product used for skin care. Also known as hand and body lotion, it helps reduce water evaporation from the skin and attracts moisture from the air into the dehydrated stratum corneum, thereby moisturizing the skin (Sumbayak & Diana, 2019).

In this study, a body lotion was developed for use at night; therefore, it is called a night body lotion. According to Alexander *et al.* (2018), the efficacy of topical preparations used at night is higher than that of topical preparations. The preparations were used in the morning or afternoon. This is because skin permeability is higher at night than in the morning or afternoon. In addition, DNA repair and cell proliferation at night are higher. To be effective as an antiaging agent, the night body lotion is added with active substances containing antioxidant effects such as ubiquinone.

Ubiquinone is an endogenous antioxidant whose levels in the body decrease with age; therefore, ubiquinone intake is added from outside the body. Ubiquinone has a low penetration rate because of its high lipophilicity. In addition, ubiquinone is unstable to light and high temperatures, so it is easily degraded (Montenegro, 2014). To overcome this, ubiquinone was formulated in a Nanostructured Lipid Carrier (NLC) system.

NLC is a delivery system suitable for ubiquinone administration. Because of its small size, NLC can increase the penetration of active ingredients by passing through the stratum corneum and can increase the solubility of drugs in lipids (Ghasemiyeh & Samani, 2020). NLC can also increase the stability of active ingredients because it can protect them in the lipid matrix (Deepak *et al.*, 2019).

In this study, a night body lotion with ubiquinone as the active ingredient was formulated using the NLC system. Lavender oil was also added to the preparation. This oil functions as a secondary active ingredient and fragrance. Lavender oil contains terpenes with antioxidant activity (Bhavaniramya *et al.*, 2019). It is hoped that adding lavender oil can increase the antioxidant activity of the night body lotion. Lavender oil contains linalyl acetate (21.57%) and linalool (32.52%), which have a distinctive pleasant aroma (Ramadhan & Zettira, 2017). The aroma of lavender oil is also effective in reducing sleep-related problems (Tessema *et al.*, 2021). However, linalyl acetate and linalool can irritate the skin (Sindle & Martin, 2021). A study conducted by (Rai *et al.*, 2020) showed that in vivo tests with a concentration of 2% lavender oil showed almost no visible skin irritation, while 10% lavender oil slightly irritated the skin.

The Food and Drug Supervisory Agency Regulation (2021) states that cosmetics in circulation must be safe, functional, and high quality. To determine the safety and quality of the antiaging night body lotion, an irritation test was performed on the skin in vivo using the Draize test method.

Based on the above description, research is necessary on the effect of different concentrations of lavender oil (0%, 2%, and 4%) on the physical characteristics, level of preference, and skin irritation of antiaging night body lotion containing NLC-ubiquinone.

RESEARCH METHODS

Tools and materials

The tools used in the research included analytical scales (Durascale DAB-E223), magnetic stirrer, Zetasizer nano (Malvern Instrument), glassware (pyrex), glass plates, High Shear Homogenizer (Fluko FM30D), Brookfield Digital Viscometer (Ametek DV -I+ viscometer), RV Spindle Set (Brookfield ametek), Hot Plate (Thermo Fisher scientific), Stopwatch.

The materials used in this research included Coenzyme Q10 (Brataco Chemical), beeswax (Brataco Chemical), lavender oil (Rumah Atsiri Indonesia), illipe butter (PT. Gunung Hijau Masaran), Jojoba oil (jojoba desert), Tween 80 (Brataco Chemical), Span 80 (Nirvana Kimia), Propylene glycol (Nirvana Kimia), Aquades, BHT, Glycerin (Nirvana Kimia), Na EDTA (Nirvana Chemical), Carbopol 940 (Nirvana Chemical), TEA, Phenoxyethanol, Sodium Dihydrogen Phosphate, Di-Sodium Hydrogen Phosphate.

Procedures

Test Formula

The test formula in this research can be perceived in **Error! Reference source not found.** below:

Table I. Antiaging body lotion formula containing NLC-Ubiquinone and lavender oil

	Percentage		
	F0	**F1**	**F2**
NLC-Ubikuinon[1)]	50	50	50
Basic gel[2)]	Ad 100	Ad 100	Ad 100

Lavender oil	-	2	4

Information :

1) NLC-ubiquinone contained ubiquinone (2%) as an active ingredient, beeswax (0.990%) and illipe butter (2.970%) as solid lipids, jojoba oil (2.640%) as a liquid lipid, Tween 80 (13.604%) and Span 80 (6.896%) as surfactant/emulsifiers, propylene glycol (3.5%) as a co-surfactant, and aquademineral as the water phase.
2) The gel base consisted of glycerin (10%) as an emollient and humectant, disodium EDTA (0.3%) as a chelating agent, carbopol 940 (1.5%) as a gelling agent, triethanolamine (2.5%) as an alkalizing agent, phenoxyethanol (0.5%) as a preservative, and aquamineral (up to 100%) as the water phase.

Preparation of NLC-Ubiquinone

The preparation process began with an oil phase, which included oil, beeswax, jojoba oil, and illipe butter. Illipe butter and beeswax were melted, and jojoba oil was added. Tween 80 and Span 80, which had been previously heated to 70°C, were then incorporated.

The water phase was prepared by mixing aquamineral and propylene glycol in a beaker, stirring the mixture until it became homogeneous, and heating it to 70°C. The water phase was then added to the oil phase drop by drop while stirring with NLC-Ubiquinone, which was created using high shear homogenization with a FLUKO FM30D at 5,000 rpm for 13 minutes, divided into five cycles. The first cycle involved 5 minutes of stirring, while the 2nd to 4th cycles each consisted of 2 minutes of stirring with a 30-second break between cycles.

Preparation of gel base

The gel base was prepared using a mortar and a pestle. First, glycerin was dissolved in heated, distilled water. Carbopol was then sprinkled until it swelled. Next, disodium EDTA was added and the mixture was ground until homogeneous. TEA was incorporated to adjust the pH, followed by the addition of phenoxyethanol.

Organoleptic Test

The organoleptic examinations included examination of color, odor, and consistency (Iskandar & Leny, 2021).

pH check

The samples were diluted with distilled water at a 1:9 ratio. The pH of the test solution was measured using a pH meter (Mayangsari *et al.*, 2021).

Homogeneity Test

A homogeneity test was conducted using 1 gram of lotion from each formula. Each sample was smeared on a glass surface, which was then touched and rubbed. The lotion mass should exhibit a homogeneous composition, meaning that no solid material should be felt on the glass (Dominica *et al.*, 2019).

Viscosity Measurement

The viscosity was determined using a Brookfield Digital Viscometer DV-I+ (LV and RV series) at a speed of 100 rpm (Ulfa *et al.*, 2019).

Spreadability

Weigh 0.5 grams of the test preparation and place it at the center of a round glass plate. The samples were covered with transparent glass and a 125 g weight was applied. After 1 minute, the diameter of the spread was measured. (Kurnianto *et al.*, 2017).

Testing Particle size, polydispersity index (IP)

The first stage involved diluting the preparation. Weigh 50 mg of the sample using an analytical balance, and add aquamineral to achieve a total volume of 50 ml. The mixture was stirred with a magnetic stirrer at 500 rpm for 10 minutes. Next, 2 ml of this solution and 8 ml of aquamineral were added. The mixture was stirred again at 100 rpm for 10 minutes. In the second stage, the particle size and polydispersity index were determined. The diluted test sample was placed in a cuvette, and the particle size and polydispersity index were measured using a Malvern Zetaseizer Nano Series (Mayangsari *et al.*, 2021).

Skin Irritation Test

The skin irritation test method used in this study received "Ethical Approval" from the Chairman of the Ethics Commission at Ahmad Dahlan University, under No. 022309118. The

antiaging night body lotion irritation test was conducted in vivo on white rat, with three rats per group. The inclusion criteria required the rats to be male, weigh between 200-250 grams, and be healthy, without disabilities, skin diseases, or serious injuries. The irritation test involved five treatments: 1) test animals were administered 0.5 ml of 3% formalin as a negative control, 2) test animals received no treatment as normal controls, 3) test animals were smeared with F0 (night body lotion without lavender oil), 4) test animals were smeared with F1 (night body lotion with 2% lavender oil), and 5) test animals were smeared with F2 (night body lotion with 4% lavender oil).

The test procedure involved shaving the animal's fur and dividing the back into five sections, each measuring 2 × 3 cm. After 24 hours, 0.5 grams of each test material was applied to the shaved areas. The exposure sites were then covered with gauze and secured with a non-irritant plaster, which was kept loose with a semi-occlusive dressing throughout the exposure period. The test animals were observed for erythema and edema, with assessments made at 1 hour and at 4, 7, 24, 48, and 72 hours after removing the patch (for test preparations that were not corrosive or irritating). Erythema (redness) and edema (swelling) are scored on a scale of 0 to 4, where 0 is no reaction and 4 indicates severe redness or swelling. After that, The Primary Irritation Index (PII) is calculated using the following formula (Aryantini *et al*., 2020).:

$$PII = \frac{Total\ erythema\ and\ edema\ score}{number\ of\ treatment\ \times number\ of\ observations}$$

Hedonic Test / Consumer Preference Test

The hedonic test is an organoleptic sensory analysis used to determine the magnitude of the difference in quality between several similar products by providing an assessment or score on the specific properties of a product and determining the preference level of a product (Suryono *et al*., 2018). The inclusion criteria were 15-40 years old, willingness to be a research subject and sign an informed consent form, no skin disease or serious injuries, and physical and mental health (not experiencing smell disorders such as colds, psychological disorders, or color blindness).

The test procedure involved providing three samples placed in tubes, each coded as F0, F1, and F2. Each panelist was asked to individually assess the antiaging night body lotion preparations and fill out the organoleptic test form according to their responses. The panelists provided responses regarding color, aroma, and texture on (scale of 1-7): 1: really do not like, 2 = do not like, 3 = slightly dislike, 4 = neutral, 5 = somewhat like, 6 = like, and 7 = really like.

Data analysis

Organoleptic and homogeneity were analyzed descriptively, while pH, viscosity, and spreadability were analyzed statistically using SPSS One Way Annova. If the data obtained had significant differences, it was continued with Post Hoc Tukey. However, if the data obtained are abnormal, the Kruskal-Wallis test will be used. Data from the consumer preference level test (hedonic test) were analyzed using the Kruskal–Wallis method; if the significance value was <0.05, it was continued with Post Hoc Dunn. In the skin irritation test, the presence or absence of erythema and edema was observed visually, and the primary skin irritation index value was analyzed from the erythema and edema scores. The PII value was then calculated.

RESULTS AND DISCUSSION

The aim of this research are to determine the effect of lavender oil concentration on the physical characteristics, level of preference, and skin irritation of antiaging night body lotion preparations containing NLC-ubiquinone.

Lavender oil contains linalyl acetate and linalool, which can be used in aromatherapy (Ramadhan & Zettira, 2017). This oil can stimulate the olfactory nerve, which sends impulses to the brain via the olfactory bulb, which is related to brain structures/limbic systems such as

the amygdala, which is the emotional center, and the hippocampus, which is related to memory (including smells), so that inhaling lavender can have a calming effect. Reticular Activating System (RAS) activation decreases when the body relaxes, and the Brainstem Reticular Formation (BSR) takes over, causing sleep. Therefore, lavender aromatherapy improves sleep quality (Desbats *et al*., 2014).

The initial stage in this research was to make NLC-Ubiquinone using the High Shear Homogenization method. Then test the physical characteristics of NLC-Ubiquinone to ensure that the NLC-Ubiquinone used meets the desired specifications. Physical characteristic tests carried out for NLC-Ubiquinone, namely organoleptic tests, pH, particle size, and polydispersity index.

The organoleptic test of NLC-ubiquinone indicated that the preparation was yellow, odorless, and had a liquid consistency. Particle size testing revealed that NLC-Ubiquinone had a particle size of 146 ± 1.8 nm. The NLC particle size requirement is <1.000 nm (Chauhan *et al*., 2020). Based on these results, it can be said that the small particle size in NLC preparations can increase absorption down to the stratum corneum and can increase the rate of drug release, which can be controlled (Annisa *et al*., 2016). The polydispersity index test results for NLC-Ubiquinone showed a value of 0.580 ± 0.000. The PDI value obtained meets the requirements in the range of 0-0.7, where the PDI value closest to 0 indicates a homogeneous particle size and a narrow particle distribution, meaning that the particle size is uniform but has a different shape and a different particle distribution. wide (Danaei *et al*., 2018). The pH test results for NLC-Ubiquinone showed a value of 6.16 ± 0.01. The pH value obtained is considered safe because it meets the desired normal skin pH specifications of 4.5–6.5 according to SNI 16-4399-1996 (Rahayu *et al*., 2016). The viscosity test results for NLC-Ubiquinone showed a value of 68.80 ± 0,23 cP.

After the preparation of NLC-Ubiquinone, the next step is the preparation of gel base. Then test the physical characteristics of gel base to ensure that the gel base used meets the desired specifications. Physical characteristic tests carried out for gel base, namely organoleptic, pH and viscosity. The organoleptic test of gel base indicated that the preparation was translucent, odorless, and had a semisolid consistency. The pH test results for gel base showed a value of 6.02 ± 0.02. The pH value obtained is considered safe because it meets the desired normal skin pH specifications of 4.5–6.5 (Rahayu *et al*., 2016). The pH test results for gel base showed a value of 32,160 ± 333.07 cP. The viscosity value obtained meets the requirements in the range of 2,000-50,000 cP with a torque value between 10-100% (Mayangsari *et al*., 2021).

After the preparation of NLC-Ubiquinone and gel base, the next step is the preparation of F0 (0%), F1 (2%), and F2 (4%), then physical characteristics were observed. The results of the observations are summarized in **Table II**. The organoleptic test revealed that all formulas exhibited the same yellow color and had a semisolid consistency. However, there were differences in aroma: F0 odorless, whereas F1 and F2 had the aroma of lavender oil. The organoleptic testing demonstrated that added of lavender oil in the preparation affect the aroma.

The homogeneity test results showed that F0, F1, and F2 had good homogeneity. This was proven because there were no coarse grains in the preparations. In the homogeneity test, the results for the three formulas showed that the preparation was homogeneous, marked by the absence of coarse grains when observed through transparent glass (Agustina *et al*., 2019).

Table II shows the observations of the Potential Hydrogen (pH) test. The observation results indicated that the three formulas had pH values that fell within the pH specifications for the final preparation in the normal skin pH range, namely 4.5-6.5 (Rahayu *et al*., 2016). If the pH of the preparation is too alkaline, it will cause dry and scaly skin, and if the pH is too acidic, it can cause skin irritation, such as redness (Sari, Lestari and Syamsurizal, 2021). Statistical analysis using One-way ANOVA revealed significant differences among the three formulas, with a significance value of 0.000 (<0.05). Tukey's post-hoc test confirmed that all preparations differed significantly, with a significance value of 0.000 (<0.05). Formula F2 had the lowest pH value, while F0 had the highest. The lower pH of F2 was attributed to the higher

concentration of essential oils compared to F0 and F1. Since the pH of lavender oil used in this study was 5, varying concentrations of lavender oil influenced the pH of the anti-aging night body lotion preparation.

The results of the spreading power test are presented in **Table II**. The spreadability results obtained within the lotion dosage range, namely 5-7 cm (Dominica *et al*., 2019). Based on One-Way ANOVA, a significance value of 1.000 (>0.05) was obtained, indicating no difference in the spreadability values among the three formulas. The varying concentrations of lavender oil in the anti-aging night body lotion containing NLC-Ubiquinone did not affect the spreadability of the preparation. Despite the differences in viscosity among the formulas, there were no variations in consistency.

The results of viscosity testing to determine the viscosity of the preparation are presented in **Table II**. The viscosity values of the three night body lotion formulas fell within the typical range for lotions, which is 2,000-50,000 cP with a torque value between 10-100% (Mayangsari *et al*., 2021). Statistical analysis using One-Way ANOVA revealed significant differences among the formulas, with a significance value of 0.000 (<0.05). Tukey's post-hoc test confirmed that all preparations were significantly different from each other. Formula F2 had the lowest viscosity, while F0 had the highest. This indicates that the addition of lavender oil at concentrations of 2% and 4% significantly reduced the viscosity of the anti-aging night body lotion containing NLC-Ubiquinone.

Table II. Results of Observation of Physical Characteristics

	F0	F1	F2
Organoleptic	Yellow; semisolid consistency; odorless	Yellow; semisolid consistency; lavender scented	Yellow; semisolid consistency; lavender scented
Homogeneity	Homogen	Homogen	Homogen
pH	6,06 ± 0,005	5,98 ± 0,008$^{(*)}$	5,89 ± 0,014$^{(*,\#)}$
Viscosity (cP)	2.890 ± 5,774	2.660 ± 5,774$^{(*)}$	2.140 ± 5,774$^{(*,\#)}$
Spreadability (cm)	5,19 ± 0,044	5,35 ± 0,055	5,40 ± 0,049

Information :
- The pH, viscosity, spreadability values displayed are the average values ± standard error of the mean
- The sign (*) is meaningfully different from F0
- The sign (#) is meaningfully different from F1

Irritation skin test was carried out using animals by assessing the safety of the preparation when exposed to the skin with erythema and edema score (Safitri *et al*., 2022). Observations were made at 1 hour; 7, 24, 48, and 72. The results showed that F0 (0% lavender oil), F1 (2% lavender oil), and F2 (4% lavender oil) did not cause skin irritation on the backs of mice after 72 hours of treatment. The primary irritation index for all three formulas was 0, placing them in the "non irritation - negligible irritation" category (0-0.4). In contrast, the positive control, which used 3% formalin, caused skin irritation, as evidenced by erythema and edema. The primary irritation index for 3% formalin was 1.6, indicating mild irritation (0.5-1.9).

Table III. Observation Results for Erythema and Edema After 72 Hours

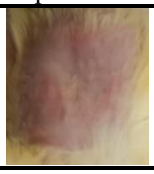
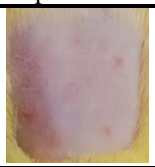
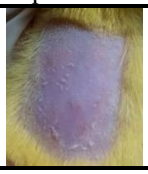
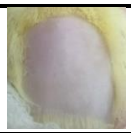
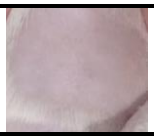
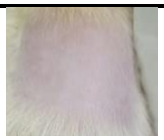
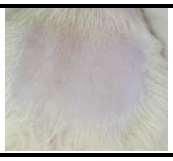
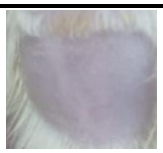
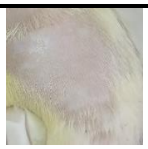
	Replication 1		Replication 2		Replication 3	
KP						
	Erythema: 3	Edema: 2	Erythema: 2	Edema: 2	Erythema: 2	Edema: 2
KN						
	Erythema: 0	Edema: 0	Erythema: 0	Edema: 0	Erythema: 0	Edema: 0
F0						
	Erythema: 0	Edema: 0	Erythema: 0	Edema:0	Erythema: 0	Edema: 0
F1						
	Erythema: 0	Edema: 0	Erythema: 0	Edema: 0	Erythema: 0	Edema: 0
F2						
	Erythema: 0	Edema: 0	Erythema: 0	Edema: 0	Erythema: 0	Edema: 0

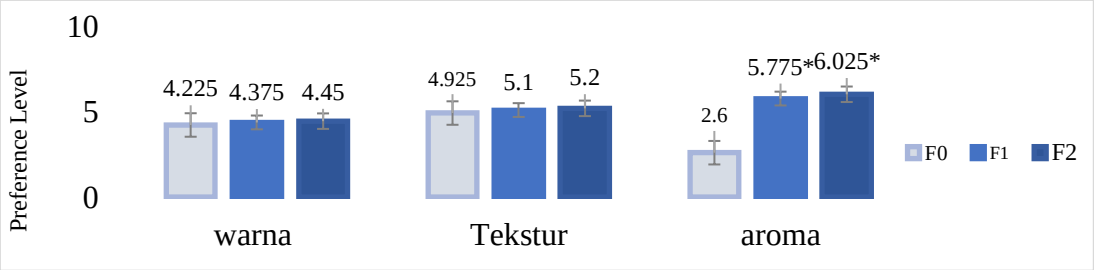
Table IV. Results of IIP Assessment of Antiaging Night Body Lotion in Mice

Treatment	Indeks Iritasi Primer	Irritant Category
KN (3% formalin)	1,6	Mildly irritating
KTP (without treatment)	0	Not irritating
F0 (without lavender oil)	0	Not irritating
F1 (2% lavender oil)	0	Not irritating
F2 (4% lavender oil)	0	Not irritating

KN: normal control
KTP: control without treatment
F0: Lavender add 0%
F1: Lavender add 2%
F2: Lavender add 4%

In testing the level of preference for antiaging night body lotion preparations, 3 aspects of assessment were observed: color, aroma, and texture. Based on the results of the hedonic test in terms of color, the average value obtained by F0 was 4.225 on a scale of 7, F1 the average value was 4.375 on a scale of 7, and F2 the average value was 4.45 on a scale of 7. In the Kruskal-Wallis test, the color parameters obtained a sig value of 0.691 (>0.05), which means there was no significant difference between F0, F1, and F2. Meanwhile, in terms of

texture, F0 obtained an average value of 4.925 from scale 7, F1 obtained an average value of 5.1 from scale 7, while F2 obtained an average value of 5.2 from scale 7. Based on statistical analysis using the Kruskal-Wallis test, a significance value of 0.707 (>0.05) was obtained, indicating no significant difference. However, significant differences were observed in aroma parameters, suggesting that the addition of lavender oil influenced the panelists' preference for aroma. The formula most favored by the panelists was F2, with an average score of 6.025, followed by F1, with an average of 5.775, and F0, which had no lavender oil, with an average score of 2.6. According to the Kruskal-Wallis' results, the significance value was 0.000 (<0.05), indicating a significant difference. Post-hoc Dunn's testing revealed that F0 was significantly different from F1 and F2, whereas F1 and F2 were not significantly different from each other. These findings indicate that adding lavender oil influences the panelists' preference for aroma.



Information :

- Aspects of color and texture are not significantly different, while aroma aspects have significantly different values (based on statistical analysis using Kruskal Wallis followed by Post Hoc Dunn
- The sign (*) is a significant difference from F0

Figure 1. Bar Diagram Of Average Values Of Hedonic Test Results

CONCLUSION

The tests on the physical characteristics of the anti-aging night body lotion containing NLC-Ubiquinone with added lavender oil (F0 at 0%, F1 at 2%, and F2 at 4%) indicated that lavender oil affected the aroma, viscosity, and pH of the preparation but did not influence the color, texture, spreadability, or homogeneity. In terms of skin irritation, all three formulas were classified as non-irritating. Regarding preference, different concentrations of lavender oil did not impact the texture or color, but there was a notable difference in aroma between F0 and F1 and F2 formulations. The most preferred formulas based on their scent are F1 and F2.

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