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The Effect of Concentration of Peanut Kefir: Physical Stability and Antioxidant Peel-Off Kefir Mask Arachi (*Arachis Hypogaea* L)

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ABSTRACT

The fermented product sector can produce kefir for use in cosmetic preparations. Kefir has weak storage stability and strong antioxidant properties, making it a suitable raw material for cosmetic formulations. The purpose of this study is to determine the best concentration of peanut kefir in Peel-off mask Arachi through antioxidant activity and physical stability during seven cycles of room temperature storage and freeze-thaw (4±2ºC and 40±2ºC). Using the DPPH method based on the IC_{50} value, the antioxidant activity test was conducted. It was retested after 7 cycles or 28 days. At ambient temperature and freeze-thaw temperature, organoleptic tests, pH, adhesion, and spreadability were conducted in cycles ranging from 0 to 7. The peel-off kefir mask preparations with F1 (0.5%), F2 (1%), and F3 (2%), concentrations created a homogeneous, viscous preparation that was stable at room temperature and freeze-thaw stable for seven cycles, according to the results. In terms of the pH test, the sample showed a drop in pH following storage, stable adhesion for up to seven cycles. However, for F1, the adhesion was not stable in either scenario. With a significant activity category, the formula's antioxidant activity also declines in IC_{50} values. Therefore, it can be said that even though the peel-off mask preparation changed after storage in a few different ways, it still falls short of the threshold in terms of the physical characteristics of the cosmetic preparation.

1. Introduction

This The development of legume-based food products is still very limited. Fermentation produces various kinds of products that are prominent for the customer (Gaffar & Suryani, 2023; Risna & Sri-Harimurti, 2022; Widowati & Misgiyarta, 2002). One of the fermented products that need to be developed and known to the public is kefir which is a type of fermented milk using lactic acid bacteria (LAB) and yeast that has a taste, color, and consistency like yogurt and has a distinctive tape aroma (Maris Stella, 2019). Kefir is generally made from cow's or goat's milk, fermented for a certain time. However, kefir can also be processed from vegetable milk such as nuts, one of which is peanuts (Rizqiati et al., 2020). Peanut (*Arachis hypogae* L) is included in a family that has a total acid content of 6.43%, 2.84% alcohol, and 2.80% protein content and contains vitamin E as an antioxidant (Utami et al., 2017). So it can be used as raw material variations in the manufacture of kefir. According to Talib et al., (2019) said that kefir shows antioxidant activity. Peanut extract kefir has been tested for antioxidants using DPPH and scavenging effect method with the results of antioxidant activity increasing along with the fermentation time of 8-32 hours with an increase ranging from 48-65% compared to peanut essence which has not undergone fermentation to become kefir (Chen et al., 2009; Liu et al., 2005).

Kefir products can be made from animal or vegetable milk. However, in fermented kefir using vegetable milk, the carbohydrate content is different from animal milk where animal milk, for example, cow's milk contains lactose, while vegetable milk (legumes) contains oligosaccharides and polysaccharides. In addition, the sugar content in nut milk that can be used by microorganisms in making kefir is very limited, so sugar is needed as a carbon source (Supriyono et al., 2014). Research conducted by Supriyono et al., (2014) on the manufacture of mung bean milk kefir added glucose concentration of 15%. Khairunnisa et al., (2022) the manufacture of peanut milk kefir added glucose with an optimal concentration of 3%. Kefir's ability to stop liver damage from paracetamol intoxication. The thiobarbituric acid reactive substance (TBARS) method can be used to measure malondialdehyde (MDA), a marker of oxidative stress. The findings demonstrated that MDA hepar levels might be lowered by administering 1.15 × 108 CFU/mL of kefir in 1 mL. Antioxidant elements and vitamins C and E included in kefir can prevent or counteract the production of free radicals (Susanti & Rahayu, 2023). The antioxidant content of peanuts can be developed into anti-aging cosmetic preparations such as peel-off masks. However, antioxidant compounds are usually unstable, especially those derived from natural ingredients, so stability tests are needed in cosmetic preparations Thus, it is necessary to test the stability for all temperature as well as test the antioxidant activity after being stored for 28 days so that it can be developed into other dosage forms.

2. Materials and Methods

Glassware (Iwaki pyrex), mortar and stamper, analytical balance (Ohauss Pioneer PA123), adhesion test kit, spreadability test kit, hotplate stirrer (Stuart CB 302), pH meter, stopwatch, thermometer, and UV-Vis spectrophotometry (Genesys 10 UV-Vis). Peanut (*Arachis hypogaea* L) which has been determined at the Banua Botanical Garden Regional Research and Development Agency (050/492-LIT/KRB), yeast Brand "Fermipan", D-glucose (Prida Lab) and (MRS) Merck (Nitrakimia, Yogyakarta).

2.1. Formulation peel-ff mask Arachi

The preparations were made by dissolving nipagin in CO_2-free aquadest (1:30) at 80 °C and stirring continuously using a magnetic stirrer. The nipagin solution was then added with glycerin and stirred until homogeneous. HPMC powder was dissolved in CO_2-free aquadest at 80 °C, then stirred using a magnetic stirrer until a gel base was formed. A mixture of nipagin and glycerin solutions was added to the gel base, and then stirred until homogeneous. Then added kefir peanut essence that has been filtered into the mixture, TEA, ethanol, and CO_2-free aquadest until the weight reaches 100%. The formulation of Peel-of Mask Arachi can be seen at Table 1.

Table 1. Formulation of Peel-off Mask Arachi

Materials	Function	(%w/w)		
		F1	F2	F3
Kefir	Active agent	0.5	1	2
HPMC	Gelling Agent	4	4	4
Glycerin	Humectant	8	8	8
TEA	Alkalizing Agent	1	1	1
Nipagin	Preservatives	0.2	0.2	0.2
Ethanol 96%		5	5	5
CO_2-free water	Solvent	Ad 100	Ad 100	Ad 100

2.2. Organoleptic Test

Tests are carried out by looking at changes in the preparation's shape, smell, and consistency (Lestari, 2021).

2.3. pH Test

The preparations were tested using a pH meter to determine the suitability between the pH of the preparation and the skin. The pH of a good topical preparation is by the pH of the skin, namely 4.5-6.5 (Setiawati & Sukmawati, 2018).

2.4.Stickiness Test

0.25 grams of the preparation is placed on the object glass and covered with another glass object. Then the glass object is given a load of 1 kilogram for 5 minutes. After 5 minutes the weight was lifted, and the slide was mounted on the test kit. An 80-gram load was attached to the test equipment on each side and the time needed was calculated until the two slides came off (Wijayanti et al., 2015).

2.5.Spreadability Test

An amount of 1 gram of sample is placed on a glass plate and covered with another glass plate. Then given a load of 100 grams for 1 minute and calculated the diameter of the preparation was. Good spreading power ranges from 5-7 cm (Marwarni & Adriani, 2020).

2.6.Determination of Antioxidant Activity of Arachi Peel-off Mask

Antioxidant tests were carried out on days 0 and 28. The tests were carried out on each formulation (Gajić et al., 2024; Januarti et al., 2024).

2.6.1. Determination of IC50 value of quercetin reference solution

Quercetin standard solutions were prepared with various concentrations of 2, 4, 6, 8, and 10 ppm. 4 mL of each solution was taken, and 1 mL of 0.4 mM DPPH solution was added, then the solution was vortexed for 1 minute. The solution is stored in a dark room for a predetermined operational time. The absorbance of each solution was read with a UV-Vis spectrophotometer at the maximum wavelength that was obtained (Mardiah et al., 2017). The reason quercetin as a reference solution because quercetin is a natural flavonoid with high antioxidant activity (IC_{50}= 1.511 ppm). It can effectively neutralize free radicals, making it a reliable standard for comparing the antioxidant potential of other compounds. The chemical structure of quercetin, including its multiple hydroxyl groups, contributes to its strong free radical scavenging ability. Its mechanism of action in antioxidant activity is well-documented, providing a benchmark for comparison.

2.6.2. Determination of the IC_{50} value of peel-off mask preparations

A total of 5 grams, 2.5 grams, and 1.25 grams of formula 1, 2, and 3 were dissolved with methanol p.a in a 25 mL volumetric flask then the solution was stirred until homogeneous, 100 ppm stock solution was obtained. Then a series of concentrations of solutions were made with concentrations of 10, 15, 20, 25, and 30 ppm. Each concentration series solution was taken as much as 4 mL and then 1 mL of 0.4 mM DPPH solution was added (Sadi & Ferfera-Harrar, 2023). The solution was vortexed for 1 minute and then stored in a dark room during operational time. The absorbance of each solution was measured using a UV-Vis spectrophotometer at the wavelength obtained (Khalid et al., 2024). The % inhibition value is calculated for each sample by the formula (1):

$$\% \text{ inhibition} = \frac{A_0 - A_S}{A_0} \; x \; 100 \; \% \qquad (1)$$

Information:

A_o: Standard absorbance

A_s: Sample absorbance

The IC_{50} value is the sample concentration that can inhibit free radicals by 50%, calculated from the linear regression equation y = bx + a

3. Results and Discussion

The peel-off mask that has been made is based on an optimized formula and the good physical characteristics of the gel preparation (Khairunnisa et al., 2022). The results obtained were in the form of gel peel-off mask preparations with concentrations of F1 (0.5%), F2 (1%), and F3 (2%) with a thick consistency and clear color. The peel-off mask was then tested for stability at cycles of 0 to 7 cycles (28 days) and stored at room temperature and freeze-thaw. The result of Peel-off mask Arachi can be seen in (Figure 1) and the characteristic formulation Peel-off mask Arachi can be seen in Table 2.

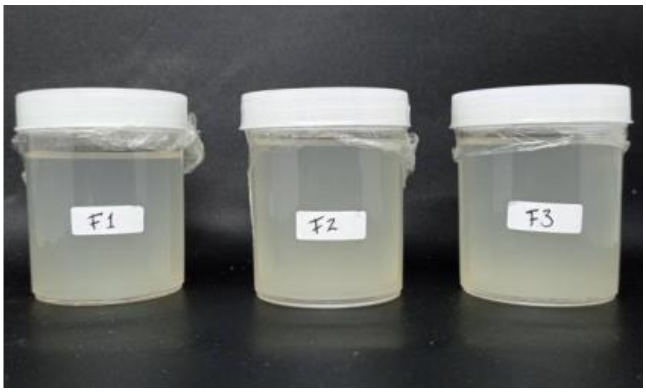


Fig 1. Results of Peel-off mask Arachi (F1:F2:F3)

Table 2. Characteristic formulation Peel-off mask Arachi

Formula	pH	Spreadability (cm)	Stickiness (sec)
F1	5.21±0.049*	3.75±0.153	97.00±2.6*
F2	4.92±0.066	3.80±0.397	73.00±7.638
F3	4.88±0.015	4.38±0.147	63.33±7.638

Note: there is a significant difference in those marked with (*)

The results of pH measurements showed a decrease with the increase in the amount of peanut milk kefir. This was due to the addition of the concentration of peanut milk kefir, the solution would become more acidic as the lactic acid content in the preparation increased, resulting in a decrease in pH (Khairunnisa et al., 2022). The standard pH range for gel preparations for topical use is by the pH of the skin, which is between 4.5 and 7.0 (Kusmita et al., 2020). The statistical test results using One-way ANOVA obtained a significance value of 0.000 ($p < 0.05$) which stated that there was a significant difference for each formula (Table 3). The pH stability test at room temperature and freeze-thaw (Figure 2) in cycles 0-7 showed that formulations 1, 2, and 3 had significant differences in the preparations since the first cycle but the resulting pH still met the pH range for topical preparations between 4.5-6 (Marwarni & Adriani, 2020). Higher kefir concentrations imply a higher microbial load or longer fermentation time, leading to increased metabolic activity and consequently, higher lactic acid production. Subsequently, acidic environments stabilize antioxidants like phenolic compounds, reducing degradation during storage. As fermentation progresses, the production of secondary metabolites helps maintain antioxidant activity over time, even during extended storage (Nurwantoro et al., 2020; Rizqiati et al., 2024).

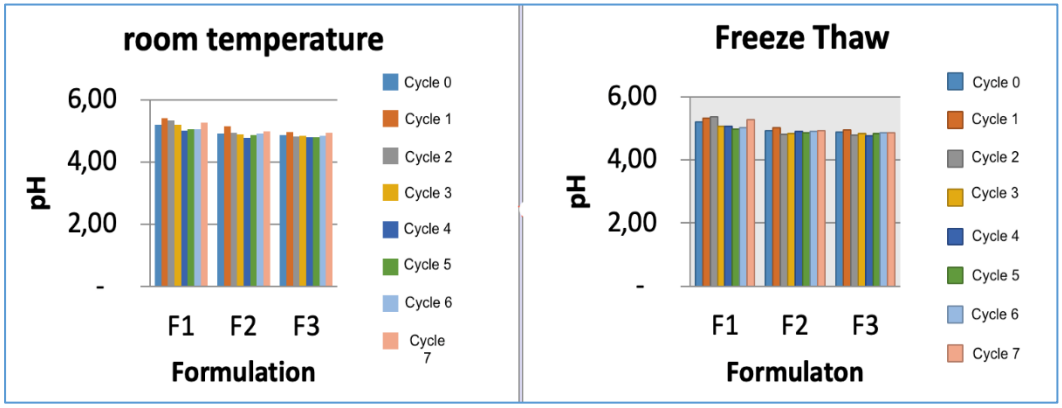


Fig 2. Graph of the cycle relationship with the pH of the Arachi peel-off gel mask preparation at room and freeze thaw temperature

Table 3. The statistical test results of pH in room temperature (a) and freeze-thaw (b)

Cycle	Sig.		
	F1	F2	F3
	0.050	0.050	0.046*
	0.050	0.513	0.046*
	0.268	0.513	0.072
	0.050	0.046*	0.050
	0.046*	0.275	0.050
	0.050	0.827	0.077
	0.077	0.268	0.050

(a)

Cycle	Sig.		
	F1	F2	F3
	0.050	0.050	0.003*
	0.050	0.046*	0.003*
	0.050	0.127	0.134
	0.046*	0.658	0.000*
	0.050	0.275	0.134
	0.046*	0.817	0.862
	0.050	0.827	0.862

(b)

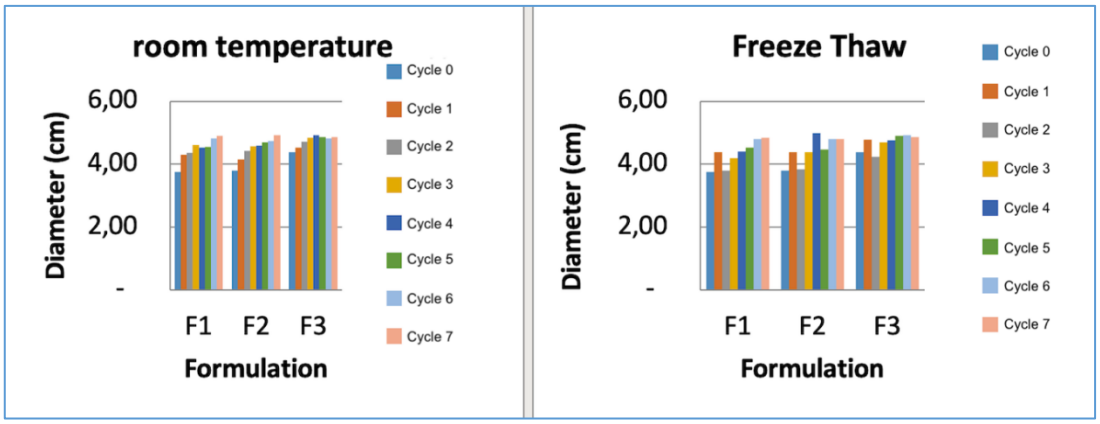


Fig 3. Graph of cycle relationship with spreading power of arachi peel-off gel mask preparations at room freeze thaw temperature

Statistical tests on the adhesion of formulations 1, 2, and 3 at room temperature and freeze-thaw showed no significant difference between the average adhesion and storage cycles. The adhesion of the preparations remained stable until the 7th cycle of storage. All the adhesion times obtained for this peel-off mask preparation were still consistent with the good adhesion time of preparations, which was not less than 4 seconds (Yati K et al., 2018). Evaluation of the average adhesion of formulations 1, 2, and 3 to the length and difference in storage temperature can be concluded that the gel preparations obtained were quite stable in the physical test of the adhesive properties of the preparations because there were no significant changes that occurred after storage. Adhesion effects are critical in antioxidant masks because they ensure the mask's efficacy in delivering active compounds, removing impurities, and enhancing user satisfaction. Formulators must strike a balance between adhesion strength, user comfort, and antioxidant stability to maximize the mask's benefits (Suhery & Anggraini, 2016).

Statistical test of spreadability at room temperature storage showed that Formula 1 showed a significant difference in the first cycle, while Formula 2 and 3 did not show a significant difference until the 7th cycle, because a significant value was obtained ≥ 0.05. In freeze-thaw, formulations 1 and 2 showed a significant difference in cycles 2 and 3, while formula 3 did not show a significant difference between the average spreadability values. The spreadability test on the peanut essence kefir peel-off mask preparation for 7 cycles showed a change with increasing time and differences in storage temperature. The spreadability test can be seen in Figure 3.

Determining the antioxidant activity of the Arachi peel-off mask preparation waas carried out at a wavelength of 519 nm. Determination of the IC_{50} value used quercetin as a comparison where the IC_{50} value of quercetin was 5.88 ± 0.826 and classified as having a very strong antioxidant activity (Table 4). The antioxidant activity of peel-off masks with the best $IC5_0$ value at a concentration of 2% peanut milk kefir was in the active category (Table 5). Based on the IC_{50} value obtained (Table 5), the antioxidant activity of the preparation is directly proportional to the addition of the concentration of

peanut kefir essence to the preparation, that is, the greater the concentration of kefir, the stronger the antioxidant activity.

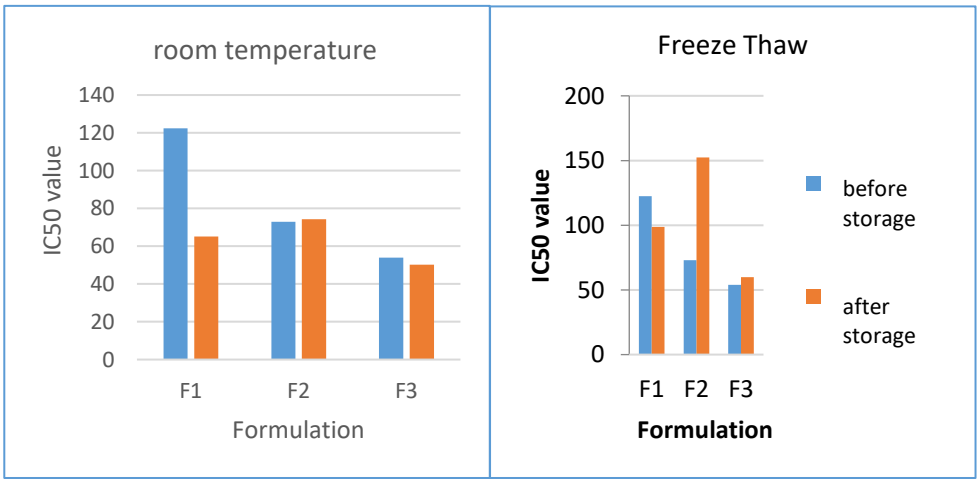
Table 4. Results of Determining the IC_{50} Value of Quercetin standard solution

Replication	Regression Linier	r values	IC_{50} (ppm)	IC_{50} ± SD (ppm)
1	y = 7.1358x + 11.268	0.995	5.428	
2	y = 7.4717x + 9.811	0.989	5.379	5.88 ± 0.826
3	y = 7.2x + 0.8	0.995	6.833	

Table 5. Results of determining the IC_{50} value of the Arachi peel-off mask preparation

Antioxidant Activity IC_{50} (ppm)		
F1 (0,5%)	**F2 (1%)**	**F3 (2%)**
122,458 ± **0.240**(medium)	72.905±0.161(active)	53.968±0.112(active)

For antioxidant stability tests on Arachi peel-off mask preparations (Figure 4), statistically analyzed using the Paired Samples Test method on formulas 1 and 2 showed that in F1 there was a significant difference between antioxidant activity before and after storage at room temperature where it decreased in value. IC_{50} which means an increase in antioxidant activity. For F2 and F3, there was no significant difference in antioxidant activity before and after storage. The IC_{50} values obtained from freeze-thaw storage in F1 and F2 showed significant differences in antioxidant activity before and after storage, while in F3 there were no significant differences in the preparations before and after storage.



(a) **(b)**

Fig 4. Graph of Cycle Relationship with Antioxidant Activity Stability of Arachi Peel-off Mask Preparations at (a) Room Temperature (b) Freeze-Thaw after being stored for 28 days

The increase in antioxidant activity that occurred in formula 1 both at room temperature and freeze-thaw could be due to the nature of kefir essence which is a fermented product so when added to the preparation, kefir continues to ferment during storage. Where the longer the fermentation process, the more lactic acid is formed, the production of this lactic acid will produce H^+ and $CH_3CHOHCOO^-$ ions which result in a decrease in absorbance due to the presence of compounds from kefir extract that donate H^+ to DPPH, thereby changing the purple DPPH free radicals to Hydrazine DPP non-radical compound which has a pale yellow or purple color disappears (Husni & Dewi, 2019). Meanwhile, in formula 2, the antioxidant activity decreased significantly during freeze-thaw storage. This was possible because of extreme temperature treatment on the preparation, which could affect the oxidation process in preparations containing antioxidants (Kim et al., 2017).

Kefir concentration significantly affects its physical characteristics during storage, such as texture, viscosity, pH, and stability. These changes are primarily influenced by the microbial activity and biochemical composition of kefir. A higher concentration of kefir increases the amount proteins, polysaccharides (e.g., exopolysaccharides from lactic acid bacteria), and fat content, leading to a thicker and creamier texture. However, at the during storage, the continued activity of microbes and interactions between proteins and polysaccharides may cause further changes in viscosity, either stabilizing or slightly increasing it. In terms of pH, higher kefir concentrations result in greater microbial activity, leading to more lactic acid production during storage. This accelerates the decrease in pH, creating a more acidic product (Nurwantoro et al., 2020).

4. Conclusion

Variations in the concentration of peanut kefir extract influenced the antioxidant activity of the preparation. Formula 3 with 2% kefir extract has the highest antioxidant activity and is classified as active. Furthermore, variations in the concentration of kefir extract from peanuts influence the stability of the preparation, especially for pH and antioxidant activity in peel-off mask preparations.

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Competing Interests

"The authors declare no conflict of interest."

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