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THE BENEFITS OF FERMENTED GOAT'S MILK WHEY MASK WITH HONEY AND RED FRUIT (Pandanus conoideus) AS ANTIOXIDANT AGENT

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Abstract: Fermented goat's milk whey, combined with honey and red fruit extract, can be used to produce face masks to enhance their effectiveness. This study aimed to ascertain the optimal formulation of the fermented whey mask and its physical characteristics and antioxidant properties. The physical characteristics tested for the fermented whey masks included pH, water activity, spreading ability, drying time, and antioxidant properties to identify the formulation that yielded the most favorable results. The study employed a completely randomized factorial design for testing. The results indicated that masks with an addition of 10% honey were more effective in enhancing the quality of the masks, as they yielded superior values in terms of pH, water activity (aw), spreading ability, drying time, and antioxidant activity. This is substantiated by the pH value of the fermented whey mask with the addition of 10% honey, which was closest to the natural skin pH, specifically between 6.71 and 7.48. The water activity value of the fermented whey mask with the addition of 10% honey also fell within a range of 0.670 to 0.751, which is lower than that of the mask with 5% honey. The spreading ability test results of fermented whey masks with the addition of 10% honey demonstrated higher diameter values, ranging from 4.47 to 5.20 cm. Furthermore, the antioxidant inhibition capacity values of masks with the addition of 10% honey were higher than those with 5% honey, ranging from 33% to 47%. Organoleptic tests on masks with the addition of 5% and 10% honey did not yield different results, and the panelists' acceptance of the mask preparations was relatively high. The findings from this study could be further developed into shelf-life stability and dermatological testing, as they hold potential for commercial development.

Keywords: *Fermented whey, honey, mask, red fruit*

1. Introduction

The use of masks is a facial skin treatment method that falls under deep cleansing (Tyas, 2018). This process works intensively to remove dirt, germs, and dead skin cells from the face's surface. Whey, a liquid product produced during the creation of cheese, casein, and similar products, is obtained by separating the curd from the liquid throuhe coagulatiss (Codex, 1995). Edwards and Jameson (2020) suggest that the immunoglobulins and lactoferrin content in whey can serve as effective raw materials for face masks. Whey derived from goat's milk enhances the efficacy of the raw ingredients in face masks. This is because goat's milk has smaller fat globules than cow's milk, making the mask's nutrients more readily absorbed into the skin.

Honey is rich in vitamins B1, B2, B3, B5, C, A, E, and flavonoids. Flavonoids are known to have antibacterial mechanisms due to osmotic pressure, acidity, and the presence of inhibin chemicals, which collectively inhibit the growth of contaminating microbes (Rio *et al.*, 2012). The phenol concentration in honey can also influence viscosity and pH, making bacterial growth challenging (Nadhila, 2014). Honey possesses various skin benefits, including acting as a natural antioxidant that eliminates bacteria and germs causing acne, a natural antiseptic that cleans acne wounds, and an anti-inflammatory that reduces wound inflammation (Vallianou *et al.*, 2014). Honey also serves as an osmotic wound cleanser, absorbs water, moisturizes the skin, and contains natural probiotic substances (Sabry, 2009).

Red fruit extract contains tannins, vitamin C, flavonoids, and natural antioxidant components such as alpha and beta carotene, tocopherols, and minerals (Murtiningrum *et al.*, 2012). These are beneficial for reducing reactive oxygen species (ROS) and free radicals formed during cell metabolism (Ichihsai *et al.*, 2009). Tannins and flavonoids are known to act as tyrosinase inhibitors, suppressing the process of melanogenesis. Additionally, flavonoids can prevent skin pigmentation caused by ultraviolet (UV) radiation exposure (Chang, 2009).

Lactic acid bacteria are expected to enhance the mask's ability to inhibit pathogenic bacteria (Dewi and Rahmiati, 2019), produce antibacterial compounds (Desniar *et al.*, 2012), and act as a natural stabilizer and thickener of dairy products. They produce exopolysaccharides (EPS) that benefit human health due to their anticancer, antiulcer, anti-inflammatory, anti-infective, and immune-stimulant properties (Halim and Zubaidah, 2013). Given these beneficial functions of whey, honey, and red fruit extract,

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the experiment was designed. The research aimed to determine the optimal formulation of the fermented whey mask with added honey and red fruit extracts, focusing on its physical characteristics and antioxidant properties.

2. Materials and Methods

2.1 Time and Location

This research was carried out from August to December 2021 at the Integrated Laboratory of the Department of Animal Production Science and Technology, Faculty of Animal Science, IPB University, Indonesia.

2.2 Materials

The equipment utilized in this study included a hot plate with a magnetic stirrer, a porcelain dish, a thermometer, a UV-Vis 01601 spectrophotometer, a water bath, an analytical balance, a pH meter, an aw meter, a watch glass, and a beaker glass. The materials used in the study were categorized into three parts. For the production of fermented whey, the materials included fresh Etawa crossbreed goat's milk, vegetable rennet, citric acid, and lactic acid bacteria (*L. plantarum* IIA-1A5 and *L. bulgaricus*). The active ingredients used in the study were honey and red fruit extract. The ingredients for the mask preparations included rice flour, hydroxypropyl methylcellulose (HPMC), carbomer, polyvinyl alcohol propylene glycol, ethanol, glycerin, and triethanolamine. Other materials used for the antioxidant analysis were methanol, ascorbic acid, 2.2-*diphenyl-1-picrylhydrazyl* (DPPH), and distilled water.

2.3 Research Procedure

2.3.1 The Manufacture of Fermented Whey

The goat's milk was pasteurized at a temperature of 62°C-65°C for 30 minutes and then allowed to cool to a temperature of 30°C-35°C. Rennet was subsequently inoculated into the milk at a concentration of 0.25 g L-1, followed by 100 mL of citric acid. The milk was then left to coagulate for 60-120 minutes, forming a curd and producing a whey liquid. The whey was separated and stored at a temperature of 4°C-18°C for later inoculation with lactic acid bacteria. The whey was inoculated with a 2.5% starter culture of *L. bulgaricus* and *L. plantarum*, then homogenized and incubated at 37°C for 24 hours to produce fermented whey (Rahman *et al.*, 2014). The fermented whey was subsequently stored at a temperature of 4°C-10°C for further use in the mask formulation.

2.3.2 Mask Formulation

Fatmawati *et al.* (2020) introduced a novel method for the production of face masks. The mask is composed of a blend of whey, honey, red fruit extract, rice flour, carbomer, hydroxypropyl methylcellulose, polyvinyl alcohol, propylene glycol and ethanol, glycerin, and triethanolamine. As shown in Table 1, the mask formulation was divided into eight treatments. These included F1, which served as the control, F2 which consisted of whey and *L. plantarum*, F3 which was made up of whey and *L. bulgaricus*, and F4 which included whey, *L. plantarum*, and *L. bulgaricus*.

Table 1. Fermented whey mask formulation

Materials (%)	Treatments (%)							
	Honey 5%				Honey 10%			
	F1	F2	F3	F4	F1	F2	F3	F4
Fermented whey	32	32	32	32	27	27	27	27
Honey	5	5	5	5	10	10	10	10
Redfruit extract	2	2	2	2	2	2	2	2
Rice powder	35	35	35	35	35	35	35	35
PG+ethanol	6	6	6	6	6	6	6	6
Glycerin	5	5	5	5	5	5	5	5
HPMC	5	5	5	5	5	5	5	5
Karbomer	1	1	1	1	1	1	1	1
PVA	2	2	2	2	2	2	2	2
TEA	1	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100	100

2.3.3 pH value

A total of 5 grams of the sample was placed in a petri dish and measured with the digital pH meter Ionix Instruments pH5, calibrated at pH 4 and 7 according to SNI (1995) guidelines. The test was conducted three times (Trenggono and Latifah, 2013).

2.3.4 Water Activity

The determination of water activity was carried out using the Novasina LabSwift water activity tool. A 5g sample was inserted into the available container and left for approximately 2 minutes until the aw value was displayed. The test was conducted in a room with a controlled temperature of 25°C, maintained by an air conditioner, for three repetitions (Kingwatee *et al.*, 2015).

2.3.5 Dry Test Time

Approximately 1 gram of the mask sample was evenly applied to the back of the hand. The mask was then left to dry, and the time taken for the mask to dry and be ready for rinsing was observed. A suitable mask drying time of 10-20 minutes was used (Sunnah *et al.*, 2019).

2.3.6 Spreadability Test

A total of 5 grams of the sample was placed between two watch glasses and then subjected to a 100-gram load. The spreading power was calculated using the diameter of the spread after 1 minute of loading (Sunnah *et al.*, 2019).

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2.3.7 Antioxidant

The free radical inhibitor technique of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol was used to test the antioxidant activity. A total of 1 mL of previously centrifuged sample liquid was reacted into a test solution of 0.5 mL DPPH with a concentration of 6 x 10^{-6} in a 2 mL vial. The mixture was then homogenized and incubated in a water bath for 30 minutes at 37°C. The absorbance wave length (λ) at 517 nm of the solution was then determined using spectrophotometry. The following formula was utilized to calculate the percentage of antioxidant activity:

$$\% \text{ inhibition} = [\frac{Control - absorbance\ value}{Control}] \times 100$$

The antioxidant capacity was calculated by converting the inhibitory value to a standard curve. The antioxidant capacity was measured in milligrams of vitamin C equivalent (VCE) per 100 mL. Vitamin C standard curves were created by adjusting % antioxidant activity against DPPH inhibition for the following vitamin C concentrations: 0, 0.5, 1, 1.5, and 2 mg/100 mL distilled water (Shori and Baba, 2013).

2.4 Data Analysis Method

The statistical analysis employed in this study was a Completely Randomized Design (CRD) with one factor with Minitab as a statistical tool, namely, the concentration of honey addition with two levels: 5%, and 10%. This study comprised threedata replicationsd, namely, the pH, a_W, dispersion, dry time, and antioxidant activity values. The following mathematical model was employed (Mattjik and Sumertajaya 2002):

$$Y_{ijk} = u + A_i + B_j + ABij + \varepsilon_{ijk}$$

where:

Yijk : Observation of physical and antioxidant properties of masks observed with the ith honey level (i = F1, F2, F3, and F4)

U : General average

Ai : Effect of physical and antioxidant properties of masks at 5% level of honey

Bj : Effect of physical and antioxidant properties of masks at 10% level of honey

ABij : Interaction on the addition of 5% honey, the addition of 10% honey, and the k^{th} repetition

Eijk : Error on the addition of 5% honey, the addition of 10% honey, and the k^{th} repetition

3. Results and Discussions

3.1 Fermented Whey Mask pH Value

Based on the results obtained, the fermented goat's milk mask with honey and red fruit has the pH values shown in Table 2. According to SNI 16-4399-1996, the pH value of a facial skin product ranges from 4.5 to 7.5.

Table 2. Results of pH mask test

Treatment	Treatment Level	
	5%	10%
F1	7.40 ± 0.05^{a}	7.48 ± 0.07^{a}
F2	5.73 ± 0.26^{d}	6.71 ± 0.24^{cd}
F3	7.05 ± 0.23^{b}	6.93 ± 0.02^{c}
F4	7.17 ± 0.02^{ab}	7.29 ± 0.02^{ab}

(F1; control, F2; fermented whey+*L. plantarum*, F3; fermented whey+*L. bulgaricus*, F4; fermented whey+*L. plantarum*+*L. bulgaricus*)

[a] means with different superscript are significantly different (P<0.05)

According to SNI 16-4399-1996 (BSN, 1996), the acceptable pH range for a facial skin product is between 4.5 and 7.5. The pH test results in Table 3 show values ranging from 5.3 to 7.4, indicating that fermented whey masks could be safe for use on facial skin. The pH value in the test, with either the addition of 5% or 10% honey, produced significantly different values. Based on the data obtained, the control treatment (F1) had the highest pH value compared to the other treatments. This pH value is still suitable for external skin use because a pH that is too high can cause the skin to become dry and scaly, while a pH that is too low can lead to skin irritation (Rahmawanty *et al.*, 2015).

The concentration of honey added to the fermented whey mask influences the pH value because honey contains oligosaccharide carbohydrates. Lactic acid bacteria utilize carbohydratescteria during the fermentation process. The lactic acid produced from this metabolism can reduce the pH value and increase acidity. This finding aligns with research by Baguna *et al.* (2020), which suggests that using more than 5% honey will yield a product with a pH value below 6. The addition of another ingredient, carbomer, which is presumed to be a gelling agent with a neutral pH value, results in a product with a neutral pH value (Dewi and Saptarini, 2016).

3.2 Fermented Whey Mask Water Activity Value

The higher the water activity, the more microorganisms can grow, which can impact the shelf life of a product (Herawati, 2008). The minimum value of a product that has the potential to support the growth of gram-positive bacteria is 0.9 (Setyawardani and Sumarmono, 2015). The results of the water activity test on the face mask, as shown in Table 3, indicate that the control mask and the masks with varying levels of honey did not produce a water activity value that had a significant effect.

The water content in products containing honey is typically around 20-30%. Lactic acid bacteria also contribute to the

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production of metabolites such as organic acids, hydrogen peroxide, diacetyl, and bacteriocins. Bacteriocins have the ability to maintain the water content of the environment, making them suitable for use as natural preservatives in food products (Arifin *et al.*, 2021). Phenolic compounds in honey have a positive correlation with other compounds from lactic acid bacteria, resulting in low water activity (Angioi *et al.*, 2021).

Table 3. Water activity test results (a_w)

Treatment	Treatment Level	
	5%	10%
F1	$0.757 \pm 0{,}027^{abc}$	0.751 ± 0.026^{abc}
F2	0.807 ± 0.022^{ab}	0.700 ± 0.052^{bc}
F3	0.739 ± 0.029^{bc}	0.725 ± 0.044^{bc}
F4	0.846 ± 0.011^{a}	0.670 ± 0.038^{c}

(F1; control, F2; whey+*L. plantarum*, F3; whey+*L. bulgaricus*, F4; whey+*L. plantarum*+*L. bulgaricus*)
[a] means with different superscript are significantly different (P<0.05)

3.3 Spreading Ability Test

The mask's ability to be easily smeared and spread on the skin surface is significantly influenced by the effectiveness of the raw materials used. The diameter obtained in the spreadability test impacts the ease of mask application (Puspitasari *et al.*, 2018). As shown in Table 4, the mask formulation with 5% honey produced a smaller diameter than the mask with 10% honey. This result is directly proportional to the outcomes of the water activity test, where the mask formulation with 10% honey content produced a smaller water activity value.

Table 4. Spreadability test results of the fermented whey masks

Treatment	Treatment Level	
	5%	10%
F1	4.23 ± 0.25^{b}	4.73 ± 0.15^{ab}
F2	4.53 ± 0.25^{b}	5.20 ± 0.10^{a}
F3	4.47 ± 0.15^{b}	5.17 ± 0.21^{a}
F4	4.60 ± 0.26^{ab}	4.47 ± 0.25^{b}

(F1; control, F2; fermented whey+*L. plantarum*, F3; fermented whey+*L. bulgaricus*, F4; fermented whey+*L. plantarum*+*L. bulgaricus*)
[a] means with different superscript are significantly different (P<0.05)

Honey is known to contain high levels of glucose and fructose, which can form a hydrogen bridge with water (Hadi *et al.*, 2016). Additionally, the polyphenols in honey contain hydroxyl groups that can enhance humectant properties. The interaction of other ingredients, such as glycerin and triethanolamine, also contributes to the hygroscopic nature of the mask. Furthermore, carbomers have gelling properties that help maintain viscosity (Sunnah *et al.*, 2019). The standard diameter for the mask spreadability test is between 5 and 7 cm (Garg *et al.*, 2002).

3.4 Dry Time Test

The dry time for mask preparation based on Budiman *et al.,* (2017) ranged from 15-30 minutes. This is following the observations of the length of time the fermented whey mask dries in Table 5.

Table 5. Drying time for fermented whey mask

Treatment	Treatment Level	
	5%	10%
F1	12.67 ± 0.83^{b}	15.40 ± 0.10^{a}
F2	15.30 ± 0.70^{a}	13.67 ± 0.76^{ab}
F3	13.23 ± 1.12^{b}	12.57 ± 0.57^{b}
F4	13.83 ± 0.40^{ab}	12.43 ± 0.71^{b}

(F1; control, F2; fermented whey+*L. plantarum*, F3; fermented whey+*L. bulgaricus*, F4; fermented whey+*L. plantarum*+*L. bulgaricus*)
[a] means with different superscript are significantly different (P<0.05)

Fermented whey masks with 5% and 10% honey treatment levels did not show significant differences in results. Both had a drying time range from 12 to 15 minutes. This is due to the interaction of honey with other ingredients, such as polyvinyl alcohol (PVA). Polyvinyl alcohol is a semi-crystalline and biodegradable synthetic polymer that functions to form an occlusive film, adhesive, and provides resistance to oil and solvents (Beringhs *et al.*, 2013). In small amounts, polyvinyl alcohol (PVA) can be used as an emulsifier for cosmetics, especially masks (Rowe *et al.*, 2009). The use of PVA in fermented whey masks amounted to 2% of the total preparation, allowing the mask to have a longer drying time than PVA above 10% (Sunnah *et al.*, 2019). However, this is still within the effective and expected drying time range, and can provide sufficient time to moisturize the skin.

3.5 Antioxidant Activity Value

Antioxidant activity was measured to assess fermented whey's ability with honey and red fruit to ward off free radicals. In this study, natural ingredients were used in the manufacture of masks to produce antioxidant values, which are available in Table 6.

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Table 6. Value of antioxidant activity

Treatment Level	Treatment	% Value of DPPH Inhibition	Antioxidant Capacity
5%	F1	28.348 ± 0.179[f]	44.814 ± 0.005[f]
	F2	40.094 ± 0.326[d]	62.700 ± 0.008[d]
	F3	45.860 ± 0.503[b]	71.478 ± 0.013[b]
	F4	44.157 ± 0.505[c]	68.886 ± 0.013[c]
10%	F1	40.295 ± 0.861[d]	63.005 ± 0.022[d]
	F2	33.699 ± 0.506[e]	52.961 ± 0.013[e]
	F3	44.731 ± 0.025[bc]	69.760 ± 0.001[bc]
	F4	47.783 ± 0.083[a]	74.407 ± 0.002[a]

(F1; control, F2; fermented whey+*L. plantarum*, F3; fermented whey+*L. bulgaricus*, F4; fermented whey+*L. plantarum*+*L. bulgaricus*)
[a] means with different superscript are significantly different (P<0.05)

Based on statistical tests, the use of fermented whey, honey, and red fruit extract in mask preparations resulted in no significant differences. The variation in antioxidant activity in the mask is due to the differing concentrations of honey added. The addition of 5% honey to the control resulted in the lowest percentage inhibition and antioxidant capacity values. Low antioxidant concentrations indicated high antioxidant activity (Purba *et al.*, 2018). Honey contains flavonoids, amino acids, and vitamins that work synergistically to protect normal cells and neutralize free radicals (Cahyaningrum 2019).

The addition of 5% or 10%,of honey to whey fermented by lactic acid bacteria, This can occur because during the fermentation process, bacterial activity produces a higher acid value than the total amount of phenols and flavonoids (Purba *et al.*, 2018). Additionally, the inclusion of red fruit extract can also affect the rate of oxidation because it contains unsaturated fatty acids such as oleic and linoleic (Ayomi 2015), which can reduce the mask's ability to ward off free radicals.

4. Conclusion

The optimal formulation for a fermented whey mask involves the use of *Lactobacillus bulgaricus* bacteria, which has a superior ability to grow in whey as it utilizes whey as a nutrient source for its growth, and the addition of 10% honey. This conclusion is based on tests of the pH value, which aligns with the skin's natural pH, a water activity value below 0.9, high spreadability, an effective drying time, and an antioxidant inhibition percentage ranging from 33-47%.

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