



## Eco-enzyme from Orange Peel as a Key Ingredient in Anti Acne Peel-off Gel Mask

(Eko-enzim dari Kulit Jeruk Sebagai Bahan Dasar Masker *Gel Peel-off* Anti Jerawat)

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### Abstrak

Penelitian ini bertujuan untuk mengembangkan masker gel peel-off yang diformulasikan dari larutan ekoenzim kulit jeruk serta karakteristik fisik dan aktivitas antibakterinya terhadap *Staphylococcus epidermidis*. Masker gel yang dihasilkan memiliki karakteristik organoleptik berwarna kecoklatan transparan, konsistensi gel kental, dan aroma asam-manis segar. Uji karakteristik fisik menunjukkan bahwa masker gel sesuai persyaratan farmasi yaitu: nilai pH 4,5-6,5, daya sebar 5-7 cm, dan waktu pengeringan 15-30 menit. Masker menunjukkan homogenitas yang baik tanpa agregat, meskipun sedikit sulit dikelupas, tetapi tidak mudah robek, dan memiliki stabilitas termodinamika yang stabil. Uji antibakteri mengungkapkan bahwa masker gel peel-off yang terbuat dari eco-enzyme kulit jeruk efektif dalam menghambat pertumbuhan *Staphylococcus epidermidis*. Diameter zona hambat masker P1, P2, dan P3 masing-masing adalah  $2,5 \pm 0,5$  mm,  $2,1 \pm 0,35$  mm, dan  $1,5 \pm 0,5$  mm. Hasil ini menunjukkan potensi eco-enzim dari kulit jeruk sebagai bahan aktif untuk formulasi masker gel peel-off untuk perawatan kulit, terutama dalam menghambat bakteri penyebab jerawat.

Kata kunci: eko-enzim, kulit jeruk, masker gel peel-off, anti jerawat, *Staphylococcus epidermidis*

### Abstract

*This study aims to develop a peel-off gel mask formulated from orange peel eco-enzyme solution and its physical characteristics and antibacterial activity against *Staphylococcus epidermidis*. The resulting gel mask has organoleptic characteristics of transparent brownish color, thick gel consistency, and fresh sour-sweet aroma. Physical characteristic tests showed that the gel mask meets pharmaceutical requirements, namely: pH value 4.5-6.5, spreadability 5-7 cm, and drying time 15-30 minutes. The mask showed good homogeneity without aggregates, although it was a little difficult to peel off, but it was not easily torn, and had stable thermodynamic stability. Antibacterial tests revealed that the peel-off gel mask made from orange peel eco-enzyme was effective in inhibiting the growth of *Staphylococcus epidermidis*. The diameters of the inhibition zones of the P1, P2, and P3 masks were  $2.5 \pm 0.5$  mm,  $2.1 \pm 0.35$  mm, and  $1.5 \pm 0.5$  mm, respectively. These results indicate the potential of eco-enzymes from orange peel as active ingredients for peel-off gel mask formulation for skin care, especially in inhibiting acne-causing bacteria.*

*Keywords: eco-enzyme, orange peel, peel off gel mask, Anti acne, *Staphylococcus epidermidis**

## INTRODUCTION

The most common problem in skin health is acne. Acne is one of the skin disorders experienced by more than 90% of teenagers. Acne is not a life-threatening skin disease, but the appearance of acne can have a psychological impact that will reduce a person's self-confidence and affect their quality of life [1]. Research shows that bacteria associated with acne include *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Micrococcus* spp. One of the bacteria often found in acne is *Staphylococcus epidermidis* [2].

Acne treatment encompasses various methods, including diet, medication, and surgery. A common approach involves using antibiotics, either topically or orally [3]. While face masks are a popular treatment for facial acne, they are often deemed impractical due to modern preferences for convenience. A more practical alternative is the peel-off gel mask, a cosmetic gel applied to the face that forms an elastic membrane, making it easy to apply and remove. These masks enhance skin hydration and deliver active compounds effectively.

Peel-off gel masks combine active compounds in a plastic film-forming formulation, providing an effective treatment option. One potential addition is antimicrobial enzymes, naturally abundant agents crucial in defending organisms against bacterial infections. These enzymes disrupt bacterial biofilm formation, a key factor in acne development [4]. Liquid enzyme formulations can be integrated into polymeric materials, either alone or combined with antimicrobials or anti-biofilm agents, to inhibit bacterial colony growth.

Eco-enzyme solutions, rich in various enzymes, are produced through the

fermentation of fruit and vegetable waste [5]. Orange peel is commonly used due to its pleasant aroma, high vitamin C content, and abundance of secondary metabolites. After fermentation, the eco-enzyme filtrate contains secondary metabolites such as flavonoids, alkaloids, quinones, and saponins, along with enzymes like amylase, protease, and lipase [6]. This study aims to develop an anti-acne peel-off gel mask using eco-enzyme derived from orange peel as a key ingredient. The anti-acne efficacy will be evaluated by measuring its inhibition zone against *Staphylococcus epidermidis*.

## METHODS

### Eco-enzyme production

Orange peels are collected and washed under running tap water and cut into small pieces. The orange peel pieces are rinsed again using distilled water. A total of 500 g of brown sugar is added to 5 liters of water and stirred until the sugar is completely dissolved. The sugar solution that has been made is added with 1.5 kg of prepared orange peels. Then the mixture is fermented for 3 months in an airtight jar. During the first month, the fermented mixture is stirred every day using glass or a wooden stick to release gas. In the second month, stirring occurred every two weeks and in the third month, stirring was done once in the middle of the month [6].

### Formulation of peel-off gel mask

A total of 8 grams of PVA were dissolved in 30 mL distilled water on a hotplate until the solutions formed a gel. The gel then was put in a container and labeled with A. Furthermore, 4 grams of HPMC were dissolved in 20 mL distilled water on a hotplate until fully dissolved and expanded

then stored in container B. The HPMC solution was then mixed into container A containing PVA gel until evenly blended. Next, 15 grams of propylene glycol were added to container A until evenly blended. In a separate container, 0.2 grams of methyl paraben and 0.1 grams of propyl paraben were dissolved in 15 mL of 96% ethanol until homogeneous, then added to container A. The same mixture as container A was made 3 times. Each mixture was placed in 3 different containers. Mixture A in each container was added with eco-enzyme concentrations of 75%, 50%, and 25% until each container reached a volume of 100 mL. The solution of the peel off gel mask made from 75% eco-enzyme was labeled as P1, 50% eco-enzyme was labeled as P2, and 25% eco-enzyme was labeled as P3. Furthermore, the physicochemical properties of the mask were compared with the positive control, namely the peel off gel mask containing 2% salicylic acid.

#### **Testing the physicochemical characteristics of the peel-off gel mask [7]**

**Organoleptic;** involves assessing color, consistency, and odor. Color and consistency are evaluated visually, while odor is assessed manually through smelling.

**pH;** the pH value is determined using a pH meter.

**Spreadability;** a 0.5 grams portion of the gel formulation is applied to a 20×20cm glass plate, and another glass plate is placed on top. 125 grams is placed on the plate for 15 minutes, allowing the gel to form a thin layer between the two plates. The diameter of the resulting gel layer is then recorded.

**Drying time;** one gram of gel mask is applied to the skin and left for a few minutes. The time required to dry completely is recorded.

**Homogeneity;** the homogeneity test is carried out by attaching the peel-off gel mask between two glass plates, then observing the presence of aggregates. The absence of aggregates and sandy particles indicates that the formulation is homogeneous.

**Peeling ability;** the peel-off gel mask is applied evenly to the skin surface. Then left to dry. After 15 minutes, the mask is peeled off from the skin. The ability to be easily removed or easily torn is recorded.

**Thermodynamic stability;** this test evaluated the tension and stability of the peel-off gel mask at varying temperatures. The mask was stored at 4°C in a refrigerator and at 40°C in an incubator for 24 hours. Stability was assessed by observing any changes in the gel formulation.

#### **Testing the anti-acne activity of the peel-off gel mask against *Staphylococcus epidermidis* bacteria**

The anti-acne activity of the peel-off gel mask was tested by measuring the inhibitory activity against the growth of *Staphylococcus epidermidis* bacteria using the disc diffusion method. A 0.1 ml of *Staphylococcus epidermidis* suspension was inoculated onto a sterile petri dish containing solid nutrient agar which was spread evenly over the surface. The disc paper that had been dripped with 0.1 mL of the peel-off gel mask solution was placed on the nutrient agar containing *Staphylococcus epidermidis* and incubated at room temperature. Anti-acne activity was assessed by measuring the clear zone using a caliper. The inhibitory activity was compared with the clear zone produced by the positive control salicylic acid peel-off gel mask formulation.

## RESULT AND DISCUSSION

### Eco-enzyme production

Eco-enzyme is produced using a ratio of brown sugar, orange peel, and water of 1:3:10. Brown sugar serves as a carbon source, orange peel provides the organic material, and water facilitates the fermentation process. The mixture is fermented for three months. The results of the fermentation process can be seen in Figure 1. Overall physicochemical observations are summarized in Table 1.



Figure 1. Eco-enzyme solution

The resulting eco-enzyme solution was cloudy brown to orange solution, emitting fresh, sweet, and sour aroma like the aroma of orange peel. The presence of white pitera fungus was observed, while no rotten aroma from fermentation was detected, nor was there any black fungus present, so the fermentation can be said to be successful. The addition of brown sugar in this mixture serves as an energy source for microorganisms such as bacteria and fungi that initiate the fermentation process [8]. These microorganisms will break down the sugar into alcohol, which is further converted into organic acids, such as acetic acid. The formation of these acids causes the pH of the solution to decrease [9]. This acid also causes the eco-enzyme solution to have a fresh sweet and sour aroma as a result of the combination aroma of sugar, organic acids, and orange peel. During fermentation, lactic acid bacteria and acetic acid bacteria convert sugar into organic acids. This acid causes the pH of the eco-enzyme solution to drop to less than 4. The decrease in pH is a key indicator that fermentation is going well, because the acidic conditions inhibit the growth of unwanted microorganisms.

Table 1. Physicochemical Observations of Eco-enzyme Solution

Physicochemical Parameters	Observation Results
Color	Brown to orange
Aroma	Fresh sweet and sour
Appearance of fungus	There is white fungus in the first week of fermentation, but after 3 months it is no longer visible
pH	< 4

The presence of white fungus, specifically resembling *pitiera*, floating on the surface of the eco-enzyme solution is a common and expected observation. This white biofilm, which is formed by the activity of microorganisms, especially fungi or yeast, during fermentation is usually harmless and can even be a sign that the fermentation process is going well. This mold appears because the surface conditions are more exposed to oxygen, allowing the growth of aerobic microorganisms. Previous studies have isolated fungi from eco-enzyme

solution, identifying 5 fungal isolates. Two isolates belong to the genera *Trichoderma* and *Paecilomyces*, while three isolates belong to the yeast group, although their genus has not yet been identified [10].

### Peel-off gel mask formulation

The physical appearance of the peel-off gel mask formulated with eco-enzyme solution exhibited a thick, transparent gel-like texture, as shown in Figure 2. The physicochemical properties of formulated masks are provided in Table 2.



Figure 2. Physical appearance of the eco-enzyme peel-off gel mask

Table 2. The physicochemical properties of formulated mask

Physicochemical Properties	Observation result			
	Positive Control	P1	P2	P3
Organoleptic				
- Color	- Transparent	- Brownish, transparent	- Brownish, transparent	- Brownish, transparent
- Consistency	- Thick like gel	- Thick like gel	- Thick like gel	- Thick like gel
- Aroma	- No sour aroma typical of orange	- Fresh sweet and sour typical of orange	- Fresh sweet and sour typical of orange	- Fresh sweet and sour typical of orange
pH	6	6	6	6
Spread power (Diameter)	5 cm $\pm$ 0,082	5,5 cm $\pm$ 0,082	5,1 cm $\pm$ 0,082	5 cm $\pm$ 0,082
Drying time	20 minutes 20 seconds	22 minutes 50 seconds	21 minutes 40 seconds	20 minutes 31 seconds

Physicochemical Properties	Observation result			
	Positive Control	P1	P2	P3
Homogeneity	Homogeneous texture, no large aggregates found	Homogeneous texture, no large aggregates found	Homogeneous texture, no large aggregates found	Homogeneous texture, no large aggregates found
Peeling ability	Not easy to peel, but not easy to tear	Not easy to peel, but not easy to tear	Not easy to peel, but not easy to tear	Not easy to peel, but not easy to tear
Thermodynamic stability at freezer temperature (4°C)	Stable	Stable	Stable	Stable
Thermodynamic stability at freezer temperature (40°C)	Stable	Stable	Stable	Stable

Physicochemical observations showed no significant difference between the control peel-off gel mask (salicylic acid) and the three variations of peel-off gel masks P1, P2, and P3. The pH value of all samples was 6. This value meets the 1996 SNI standard that all preparations for facial skin treatment have a pH of 5.98-6.4. The spreadability values of masks P1, P2, P3 and positive control, also meet the standard for good

spreadability, ranging from 5 to 7 cm [10]. Drying time observation for P1, P2, P3, and the positive control showed that the drying ability was in accordance with pharmaceutical recommendations, the range of 15-30 minutes [11]. A summary of the evaluation of the peel-off gel mask made from eco-enzyme solution can be seen in Table 3.

Table 3. Summary of Physicochemical Evaluation of Eco-enzyme Peel-off Gel Mask

Parameter	Meet the Pharmacy Requirements	Does Not Meet Pharmacy Requirements
pH	✓	-
Spread power	✓	-
Drying time	✓	-

### Anti-acne activity testing of the peel-off gel mask against *Staphylococcus epidermidis* bacteria

The inhibitory activity test aims to assess the potential of the peel-off gel mask as an anti-acne agent by evaluating its effect on

*Staphylococcus epidermidis*. The clear zone can be seen in Table 4.

The results showed that the diameter of the clear zone produced by masks P1, P2, and P3 was on average smaller than that of the positive control mask from salicylic acid.

Salicylic acid has been known to have antibacterial activity with a mechanism of disrupting bacterial cell walls [12].

Table 4. Clear Zone Diameter of Peel-off Gel Mask

Gel peel-off mask	Clear Zone Diameter (mm)
Positive Control	3.2 ± 0.1
P1	2.5 ± 0.5
P2	2.1 ± 0.35
P3	1.5 ± 0.5

The clear zone value was also small, this is thought to be because the thick peel-off gel mask preparation makes it difficult to diffuse in to nutrient agar. Although the peel-off gel masks P1, P2, and P2 have smaller inhibition zones, they still show the growth inhibition of *Staphylococcus epidermidis* bacteria by forming a clear zone area. This suggests that the peel-off gel masks P1, P2, and P3 have potential as anti-acne agents through their inhibition of *Staphylococcus epidermidis*. The observed inhibition is due to the role of enzymes and secondary metabolite compounds present in the eco-enzyme solution used in the formulation. Previous studies have shown that after the fermentation process in the eco-enzyme filtrate, yields various secondary metabolites such as flavonoids, alkaloids, quinones, saponins. Amylase, protease and lipase enzymes were also found in the eco-enzyme filtrate [6].

### CONCLUSIONS

The peel-off gel mask shows several organoleptic characteristics including a brownish transparent color, thick gel consistency, and fresh-sweet-and-sour aroma. The mask's pH, spreadability, and drying time meet pharmaceutical standards: the pH ranges from 4.5 to 6.5, spreadability

is between 5-7 cm, and drying time is 15 to 30 minutes. It demonstrates good homogeneity, with no visible aggregate. While its peeling ability is somewhat limited, the mask is not easily torn and shows good thermodynamic stability. The inhibitory activity test of the peel-off gel mask, made using an orange peel eco-enzyme solution, showed that masks P1, P2, and P3 inhibited the growth of *Staphylococcus epidermidis*. The corresponding clear zone diameters were 2.5 ± 0.5, 2.1 ± 0.35, and 1.5 ± 0.5, respectively.

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### CONFLICT OF INTEREST

I hereby declare that there is no conflict of interest in writing this scientific paper.

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