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Antioxidant Activity of Peel-off Mask from Pangasius Catfish Gelatin (*Pangasius hypophthalmus*) with Astaxanthin

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ABSTRACT

Peel-off face masks are a type of mask that contains elastic materials such as gelatin, making them easy to apply and remove. These peel-off masks are applied to the face by spreading them to form a thin, transparent film layer. Pangasius catfish gelatin serves as the gelling agent in the formulation of the peel-off mask, and astaxanthin is added as an antioxidant. Astaxanthin is a carotenoid pigment with a molecular structure similar to β-carotene, exhibiting stronger antioxidant activity compared to β-carotene in neutralizing free radicals. The purpose of this research is to determine the antioxidant activity of peel-off gel masks made from pangasius catfish (*Pangasius hypophthalmus*) gelatin supplemented with astaxanthin using the DPPH method. Astaxanthin extract was used as the active ingredient in the production of the peel-off gel mask, with an astaxanthin extract concentration of 0.5%. The results of the antioxidant activity test for the peel-off gel mask made from pangasius catfish gelatin supplemented with astaxanthin yielded an IC_{50} value of 7572.84 µg/mL, while the IC_{50} value for the comparative mask (Brightening lemon blackhead brand) was 5045.74 µg/mL. These results indicate that the mask produced has lower antioxidant activity compared to the comparative masks available in the market.

INTRODUCTION

Gelatin is a water-soluble polymer capable of forming colloids and thickening solutions by creating a gel from them, making it suitable for use as a gelling and stabilizing agent. Currently, the sources of raw materials for producing gelatin from fish, including pangasius catfish, have become an alternative source of gelatin aside from mammals. Gelatin obtained from catfish possesses the necessary characteristics. The applications of gelatin in both the food and non-food industries are extensive. In food processing, it is used to impart a chewy texture to products, while in non-food applications, it is employed in cosmetics, such as masks. Gelatin is favored in mask formulations due to its transparency, lack of taste, and solid form (Lv & Huang, 2019; Derkach et al., 2020; Al-Nimry et al., 2021).

Facial masks are beauty products designed to maintain skin health and moisture. There are various types of facial masks available on the market, including powdered masks, cream masks, gel masks (peel-off masks), sheet masks, and homemade masks. Typically, the application of masks can be complex and not always suitable for people with busy lifestyles. Therefore, there is a need for innovative, user-friendly mask formulations. One such facial mask that aligns with modern trends is the peel-off face mask (Morganti et al., 2019).

Peel-off face masks are a specific type of mask containing elastic materials like gelatin, making them easy to apply and remove. These masks can be peeled off after 15-30 minutes of use. Because the active compounds in topical formulations interact with the skin for a longer period of time, they are recommended for their antioxidant and anti-acne benefits on facial skin care (Jaya et al., 2022). Polyvinyl alcohol, which produces occlusion and a tightening effect, is the basis for many peel-off masks. Masks can be made using a variety of components, such as herbal soap, moisturizer, plasticizer, scent, and preservatives. Masks come in a variety of compositions, but drying

agents such as alcohol usually regulate them. Alcohol is frequently used as a drying agent to regulate drying time since it has a lower vapor pressure than free water (Ngoenkratok et al., 2015).

The benefits of peel-off masks include therapeutic advantages such as reduced dosing schedules, comfort, and patient-friendliness. They are useful for removing blackheads, dead skin, and improving skin tone. Active ingredients in masks can enhance their value by increasing the role of peel-off masks in absorbing dust particles and unclogging pores (Kulkarni et al., 2019).

Astaxanthin is one natural component used in masks. Known for its anti-oxidant qualities, astaxanthin is a naturally occurring carotenoid pigment present in a variety of microorganisms and marine creatures (Aisoi, 2016). In the human body, this red, fat-soluble pigment has no pro-Vitamin A properties. However, some research indicates that astaxanthin has stronger biological activity in comparison to other carotenoids (Fakhri et al., 2018; Lima et al., 2021).

Numerous investigations have shown astaxanthin to possess antioxidant properties. When compared to vitamin E and β-carotene, astaxanthin has demonstrated several times the antioxidant activity against free radicals in certain instances. The antioxidant qualities of astaxanthin are thought to be important for a number of other things, including defense against UV photooxidation, inflammation, cancer, stomach issues, aging, age-related illnesses, enhanced immunological response, liver function, and general health, which includes prostate, eye, and joint health (Singh et al., 2020; Dutta et al., 2023).

The skin can be shielded against UV photooxidation by astaxanthin. In vitro, astaxanthin has higher protective effects against UV-induced photo-oxidation than β-carotene and lutein. It has been observed that supplementing rats exposed to extreme UV radiation protects the retinal photoreceptors in their eyes. These findings suggest astaxanthin has a great deal of promise as a sun protection supplement taken orally. While dietary supplements containing astaxanthin or β-carotene have demonstrated benefits in other forms of cancer, there has been little evidence from animal or clinical research on these compounds' effects on skin cancer (Eren et al., 2019).

One of the methods used to test antioxidant activity is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. This method is chosen for its simplicity, ease, speed, sensitivity, and the small amount of sample required to evaluate the antioxidant activity of natural compound materials (Ambari et al., 2021).

The purpose of this research is for creating the peel-off gel mask, evaluating its physical quality, and conducting the antioxidant activity for the pangasius catfish gelatin peel-off gel mask enriched with astaxanthin.

METHODS

The equipment used in this research are a microplate reader (Rayto), analytical balance (Fujitsu), micropipette (Socorex), water bath (Memmert), digital pH meter, mortar and pestle, glass slides, measuring pipette, beaker, measuring glass, watch glass, spatula, and stirring rod.

Materials: Pangasius catfish skin, astaxanthin (AstaLuxe™ from PT. Evergen Resources), Brightening lemon blackhead peel-off mask, 96% ethanol (Merck), methanol (Merck), DPPH (Sigma Aldrich), polyvinyl alcohol, glycerin, propylene glycol, methylparaben, and distilled water.

Extraction Gelatin from Pangasius Catfish Skin

Gelatin was extracted from the skin of pangasius catfish (*Pangasius hypophthalmus*) using an alkaline method, and the characterization was performed as described by Nasution & Harahap (2018).

Procedure for Making Peel-Off Gel Mask from Pangasius Catfish Gelatin Enriched with Astaxanthin

Polyvinyl alcohol (PVA) was mixed with aquades and heated in a glass beaker while stirring until it becomes homogeneous and clear. Pangasius catfish gelatin was dissolved in aquades at 45^0C, then glycerin was added and stirred until homogeneous. Combine both masses in a mortar, grind them, and homogenize. Propylene glycol was added to the mixture and stirred until homogeneous. Nipagin was dissolved in 96% ethanol, and then added to the gel base and aquadest was added while stirring until homogeneous. This formula is referred to as F0. In the same way as described above, a preparation was made, and in the final stage, 0.5% astaxanthin was added to the gel base, ground until homogeneous and aquadest was added while stirring until homogeneous. This formula is referred to as F1.

Evaluation of the Catfish Gelatin Peel-Off Gel Mask Enriched with Astaxanthin

Organoleptic

This involves observing the color and odor of the gel preparation.

Homogeneity

Gel preparations were applied onto glass slides, then two glass slides were pressed together and checked for homogeneity.

Spreadability Test

After weighing one gram of gel formulations, they were spread out on a glass surface, covered with a cover slip, and weighted. The diameter of the gel's spread was measured one minute later.

pH Test

One gram of gel preparations was dissolved in ten milliliters of aquadest, or distilled water. After completely submerging the pH electrode tip, the pH value was recorded.

Drying Speed Test

Gel preparations were applied to the back of the hand, and the drying speed was measured until it forms a peel-off layer using a stopwatch.

Skin Irritation Test

An irritation test was conducted on 10 panelists by applying the preparation to the back of the hand with surface area ± 2.5 cm^2.

Adhesion Test

The adhesion test was performed by placing 0.5 grams of gel on a glass object and covering it with another glass object. 1 kg weight was added for 3 minutes, and the time it takes for the two glass objects to detach was measured.

Antioxidant Activity Test of the Pangasius Catfish Gelatin Peel-Off Gel Mask Enriched with Astaxanthin

Preparation of DPPH Solution

Preparation of DPPH solution 80 µg/mL concentration in methanol (Ambari et al., 2021).

Preparation of Sample Solution

80 mg of the sample was dissolved in 8 mL of methanol, resulting in a concentration of 10000 µg/mL. Subsequently, dilutions were made with methanol to achieve concentrations of 5000 µg/mL, 2500 µg/mL, 1250 µg/mL, 625 µg/mL, and 312.5 µg/mL.

Preparation of Reference Solution (Brightening lemon blackhead brand)

80 mg of the reference substance was dissolved in 8 mL of methanol, resulting in a concentration of 10000 µg/mL. Dilutions were made with methanol to achieve concentrations of 5000 µg/mL, 2500 µg/mL, 1250 µg/mL, 625 µg/mL, and 312.5 µg/mL.

Antioxidant Activity Test

The antioxidant activity test was conducted using a microplate reader with the DPPH method (1,1-Diphenyl-2-picrylhydrazyl). The plate consists of rows A-H, each containing 12 wells. There were specific steps involving DPPH, samples, negative controls, and blanks to measure antioxidant activity.

RESULT AND DISCUSSION

The antioxidant testing for the formula of pangasius catfish gelatin peel-off mask, which is added with astaxanthin, is created from various raw material mixtures. These include catfish gelatin, which serves as the gel base for the mask. The second ingredient is polyvinyl alcohol, known as a film-forming agent, skin protectant, and commonly used in gel, lotion, mask, and other cosmetic and skincare applications. The next component is glycerin, which is often used in gel preparations as a humectant to improve the softness and spreadability of the product and protect it from drying out due to its high water content. Another ingredient is propylene glycol, which is typically used as an antimicrobial preservative, disinfectant, humectant, plasticizer, solvent, and stabilizing agent. Nipagin is a commonly utilized preservative in cosmetic, food, and pharmaceutical products, frequently found in cosmetic formulations. Lastly, distilled water and 96% ethanol are used as solvents in the peel-off mask (Rowe et al., 2009).

The peel-off mask's physical quality is evaluated, including organoleptic tests for color and odor. F0, which is yellowish and has a distinctive smell, is due to not containing astaxanthin extract. The yellow color and unique scent result from pangasius catfish gelatin used as the gel base. In contrast, F1 is reddish and has a distinct smell because it contains astaxanthin extract. The reddish color in F1 comes from the astaxanthin extract (Ambati et al., 2014). The

result of the physical evaluation of peel-off mask F0 and F1 describe in Table 1.

Homogeneity testing checks the distribution of active ingredients in the product. Good homogeneity is indicated by the absence of lumps on the glass object, and both formulas exhibited good homogeneity.

The spreadability test determines how well the gel mask spreads when applied to the skin. The diameter of the spreadability for F0 was 6.8 cm, while F1 was 6.4 cm. Both formulas met the requirement for good spreadability. Spreadability requirement 5 to 7 cm, so this gel mask meet the requirement (Brendler & Williamson, 2019).

Table 1. Result of the physical evaluation of peel-off mask formulation

Parameter	F0	F1
Color	Yellowish	Reddish
Odor	Distinctive smell	Distinctive smell
Homogeniety	Homogen	
Spreadability	6.8 cm	6.4 cm
pH	6.8	5.4
Drying speed	28 minutes	30 minutes
Skin Irritation	Not irritating	Not irritating
Adhesion Test	14 second	15 second

The pH test checks the acidity of the gel mask. F0 had a pH of 6.7, and F1 had a pH of 5.4. Topical products should have a pH that matches the skin (around 7.4). A safe pH range for topical products is 4-8 because an overly alkaline pH can lead to dry skin, and an overly acidic pH can cause skin irritation (Shukry & Ghada, 2013).

The drying speed test aims to see how fast the mask forms a film on the skin. Ideal drying time is between 15-30 minutes. The results showed that F0 dried in 28 minutes, and F1 dried in 30 minutes. Both formulas met the requirements (Rum & Suherman, 2021).

The skin irritation test is done to assess the potential irritation of the gel mask when applied to the skin to ensure its safety. In this research, both formulas did not cause skin irritation and are safe for use. There were no alterations in the form of redness, itching, or swelling of the skin (Syakri et al., 2021).

The adhesion test measures the ability of the mask to adhere to the skin during application and shows the mask's ability to perform its function while it dries. Both formulas showed excellent adhesion, with more than 1 second of adhesion time (Ningrum, 2018).

The DPPH technique was utilized to ascertain the antioxidant activity of the mask. According to the findings, the comparative product's IC_{50} value was 5045.74 µg/mL, but the mask with an addition of 0.5% astaxanthin had an IC_{50} value of 7572.84 µg/mL. The results of the antioxidant activity of the astaxanthin mask and the comparison mask were in the very weak category (Leksono et al., 2018). have very weak Tables II and III display the findings of the measurement of antioxidant activity. This suggests that the study mask has less antioxidant activity than the commercial mask, which is constructed of a combination of vitamin C-rich components. Astaxanthin's low water solubility, bioaccessibility, bioavailability, and strong flavor or odor all contribute to its low stability when exposed to high temperatures, acid, oxygen, or light (Dutta et al., 2023; Martínez-Álvarez et al., 2020; Honda et al., 2021).

Other research related to the antioxidant activity in peel-off gel mask preparations using banana peel extract resulted in an IC_{50} value of more than 200 µg/mL, and the same goes for masks containing sesewanua leaf extract, which had an IC_{50} value of 504.74 µg/mL. However, preparations using guava leaf extract had a lower IC_{50} value of 79.56 ppm (Rompis et al., 2019; Sosalia et al., 2021; Apriani et al., 2022).

Table 2. Result of antioxidant activity of pangasius catfish gelatin peel-off mask with added 0.5% astaxanthin

Concentration (µg/mL)	Absorbance	%Inhibition	IC_{50} (µg/mL)
10000	0.20	61.58	7572.84
5000	0.31	41.71	
2500	0.39	25.96	
1250	0.42	20.99	
625	0.44	17.04	
312.5	0.49	8.19	

Tabel 3. Results of antioxidant activity measurement of commercially available peel-off mask brands

Concentration (µg/mL)	Absorbance	%Inhibition	IC_{50} (µg/mL)
10000	0.15	73.26	5045.74
5000	0.34	40.59	
2500	0.39	31.27	
1250	0.45	20.82	
625	0.51	11.93	

CONCLUSIONS

Based on the obtained results, it can be concluded that the physical quality of the gel peel-off mask made from pangasius catfish gelatin with astaxanthin meets the standard quality requirements and the antioxidant activity has an IC_{50} value of 7572.84 µg/mL, while the reference product has an IC_{50} value of 5045.74 µg/mL.

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AUTHORS' CONTRIBUTIONS

Preformulation investigations were carried out and the document was written by Azlaini Yus Nasution. Novia Novita Sari assessed the peel-off mask, while Annisa Fauzana was in charge of data interpretation and analysis. After carefully examining the findings, all authors gave their approval to the manuscript's final draft.

CONFLICT OF INTERESTS

No conflict of interest in this publication.

ETHICAL CONSIDERATION

Evaluation in this research has obtained ethical clearance from the Research and Health Ethics Committee Abdurrab University, No. 062/KEP-UNIVRAB/VII/2023.

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