Vol.5 No.1 Hal. 110-126
Juni 2022
BEST JOURNAL (Biology Education,Science & Technology) Fakultas Keguruan dan Ilmu Pendidikan
ISSN (Print) : 2614 – 8064
ISSN (Online): 2654 – 4652

Extraction Of Functionally Active Collagen From Salmon Fish As Formulation Of Clay Mask

Christine Ulina Tarigan (1), Wenny Pinta Litna Tarigan (2)

(1) State University Of Yogyakarta
(2) State University Of Medan

christineulinatarigan@uny.ac.id (1) wenny.tarigan@gmail.com (2)

ABSTRAK

Kulit salmon (Oncorhynchus nerka), produk sampingan dari jalur proses fillet, dapat berfungsi sebagai sumber kolagen akuatik yang sangat baik. Dimungkinkan untuk mengaplikasikan kolagen aktif dari ikan salmon serta formulasi bahan masker tanah liat. Kolagen membantu dalam regenerasi dan penggantian sel-sel mati dan tua untuk meningkatkan tekstur dan integritas kulit untuk menjaga kulit muda dan sehat. Tujuan dari penelitian ini adalah untuk mengetahui formulasi mana yang menghasilkan sifat fisik unggul masker clay kolagen salmonid. Ekstraksi kemurnian kulit ikan salmon dilakukan dengan proses ekstraksi konvensional. Kulit ikan salmon diolah terlebih dahulu dengan asam asetat 0,5 M. Subunit kolagen yang diekstraksi kemudian dianalisis pada gel natrium dodesil sulfat-poliakrilamida (SDSPAGE) untuk menentukan rantai peptida. Hasil kolagen dari metode konvensional adalah 35,6%. Kolagen terlarut diekstraksi sebagai bahan baku untuk bahan kosmetik seperti masker tanah liat. Formulasi masker tanah liat mengandung kolagen sebagai bahan utama karena manfaatnya yang signifikan sebagai pelembab alami. Formulasi terbaik dari clay mask kolagen salmonid adalah sediaan F3 dengan konsentrasi kaolin 25% dan konsentrasi bentonit 1%. Sediaan F3 lebih disukai responden dengan parameter bau, warna, dan tekstur yang dihasilkan baik.

Kata Kunci : Tanah Liat, Kolagen, Kosmetik, Ekstrak, Salmon, Masker

ABSTRACT

The skin of salmon (*Oncorhynchus nerka*), a by-product of the fillet process line, could serve as an excellent source of aquatic collagen. It was possible to apply active collagen from salmon fish as well as the formulation of clay mask material. Collagen helped in the regeneration and replacement of dead and old cells for improving skin texture and integrity to maintain young and healthy skin. The purpose of this study was to find out which formulations produced the superior physical properties of salmonid collagen clay masks. Extraction of salmon skin purity was conducted by conventional extraction process. Salmon rind was pretreated with 0.5 M acetic acid. The collagen subunits extracted were then analyzed on sodium dodecyl sulfate-polyacrylamide gel (SDSPAGE) to determine the peptide chains. The collagen yield of the conventional method was 35.6%. The soluble collagen was extracted as a raw material for cosmetic material such as clay masks. Clay mask formulations contained collagen as a key ingredient due to their significant advantages as natural moisturizers. The best formulation of salmonid collagen clay mask was an F3 preparation with a kaolin concentration of 25% and a bentonite concentration of 1%. F3 preparation was more preferred by respondents with good resulting odor, color, and texture parameters.

Keywords : *Clay, Collagen, Cosmetics, Extract, Salmon, Mask*

I. PENDAHULUAN

1. Introduction

Collagen is the most notable and abundant protein in the human body. It makes up about 30-35% of systemic protein and 70-80% is a skin component. Due to the widespread use and application of collagen and its increasing commercial scale, research and development focuses on alternative sources of collagen and more environmentally friendly treatment strategies. Due to its safety, bioavailability, bioactivity, and biocompatibility, collagen is widely used in the pharmaceutical, cosmetics, and food and beverage industries. Key properties of collagen are water absorption and retention, low viscosity, moisturizing effect, emulsification and gel strength, foaming, stabilization, adhesion and aggregation Collagen from fish has less cross-linking, which improves solubility and maintains macromolecular structure during the extraction process. Collagen can come from bovines, humans, rodents, pigs, birds, or marine animals and can also be produced from recombinants (Figure 1). Collagen is commercially produced from cattle and pigs because the products of slaughtered animals such as skin, cartilage, bones and tendons are rich in collagen . The first type of raw material used in the early 1930s was pork skin, and to this day, pork is considered the main source of large-scale production of collagen. The sources of aquatic collagen are sponges, jellyfish, squid, and fish. Skin, bones, blades, heads and scales, which make up about 75% of the total weight of fish and are underutilized part of the fish fillet industry, should provide a stable and inexpensive supply of collagen resourcesFish collagen is recommended as a component for production of food, makeup, biomedical and drug applications for sketch characteristics. Collagen has an excellent ability to retain water, making it suitable for skin applications as a moisturizer. Collagen helps regenerate and replace dead and old cells, improving skin texture and integrity and maintaining young and healthy skin. Collagen extract from salmon fish is appropriate for regular to dry pores and skin. As withinside the evaluation of fish collagen content, there are protein levels, fats levels, water levels, nutrition C in brown rice that's excellent for moisturizing the facial pores and skin . The fast technological development spurred the cosmetics enterprise groups to compete to create formulations withinside the manufacture of face masks. Formulation of creating an herbal face mask wishes to be executed as an opportunity option. There had been many research at the mixture of kaolin and bentonite bases in clay face masks arrangements, such as the studies of Santoso (2017), displaying the outcomes that the interplay of kaolin and bentonite has an impact on rising viscosity, lowering spreadability, and accelerating dry time for clay masks arrangements. Then, Fauziah's studies (2018) states that the evaluation of every formula may be concluded that from a kaolin base of 35% and a bentonite awareness of 0.5% is the high-quality components. The concentration variation of kaolin and bentonite bases in the clay mask dosage formulation shows an effect on the physical properties of the preparation, in terms of color, pH, spreadability test, and the results of the preference assessment of the dosage form. Therefore, these studies become performed through formulating salmon collagen extract (*Oncorhynchus nerka* L.) withinside the shape of a clay masks using mixture of kaolin and bentonite bases and evaluate theirphysical characteristics.

2. Problem Formulation

More than 50% of waste will be generated by processing fish treatment from the viewpoint of all production. Some of this waste is used as an environmentally friendly alternative and as a source of low-cost collagen for protein extraction from fish, but most of this waste is completely unused and most of it. It is reclaimed, enhances the environment, and the environment is related to the stability of the Earth's climate. Many uses have been identified for waste from marine processing, such as animal feeds, enzyme isolation,

chitosan, make-up (collagen), soil fertilizers, and water regulators for food (hydrolysates). Facial cosmetics come in a variety of dosage forms, one of which is in the form of a mask. These face mask formulations fall into several types, with the currently popular types being washable (washed out with water) clay-based types, often referred to as clay face mask or mud mask preparation.

3. Research Purpose

Therefore, collagen is the most well-known type of collagen used in all cosmetics. High molecular weight proteins such as collagen are not absorbed by the stratum corneum of the skin. They instead remain on the surface and act as water absorbers through hydration (maintaining skin hydration) and protection from microbial invasion into damaged tissue. Local exposure to collagen in the reconstructed dermis of humans and analysis of molecular markers of irritation and inflammation did not reveal potential irritation. Therefore, the separation of collagen from fish skin for use in skin cosmetic applications may provide a sustainable and cost-effective platform for bioengineering use of fish by-products. In this study, we use salmon skin, which is rich in collagen, to archive the moisturizing quality of marine collagen contained as an ingredient in cosmetic formulations.

4. Research Benefit

We conducted an extensive physicochemical property assessment of isolated collagen and studied both its moisture capacity and its irritating potential, considering the potential use of this marine collagen in cosmetics. Cosmetics are preparation of material which are prepared to be applied in the outer parts of the body (epidermis, hair, nails and outside genitalia), enhance attraction to change the appearance, protect to keep it in good condition, to make body odor better but it does not intend to treat or therapy an illness. The technique of skin aging is characterized through the arrival of wrinkles, scales, dry and chapped pores and skin. Masks are beauty arrangements for facial skin care which have blessings to offer moisture, supply pores and skin texture, rejuvenate the pores and skin, tighten pores and skin pores, brighten pores and skin tone, enjoyable facial muscles, and therapy pimples and its scars.

II. METHOD

a. Materials

Salmon fresh skins (non salted) were provided by a local supermarket, kaolin, bentonite, glycerin, nipagin, xanthan gum, rose oil and distilled water. The salmon skin was transported to the laboratory facilities and stored at −20 °C until use. All preparations and experiments outlined below were carried out in quadruplicates at 5°C or lower, unless stated otherwise, and the means ± standard deviation(SD) was reported in the result section.

b. Extraction and Purification of Collagen

For the extraction, the skin was plunged in 0.5 M of acetic acid solution (1:10 w/v) for 72 hours under stirring, followed by centrifugation at 20,000 g for 1 hour at 5^0C. The supernatants, containing Acid Soluble Collagen (ASC), were collected and saved at a cold temperature (5^0C). To precipitate the collagen, the supernatants were salted out by adding NaCl to a final concentration of 0.7 M, followed by precipitation by adding NaCl to a final concentration of 2.6 M in 0.05 M Tris–HCl (pH 7.5), and left overnight. The resultant precipitates were separated by centrifugation at 20,000 g for 1 hour at 5^0C and resuspended in 0.5 M of acetic acid. These solutions were dialyzed with 0.1 M acetic acid for two days. Then, 0.02 M acetic acid for two days and finally, soak in distilled water to pH 7. The

solution was then lyophilized and stored at room temperature until further use. The extraction procedure was performed as described previously. The wet ASC yield of fish skin is calculated using equation (1):

$$\text{Yield of collagen (wet) (\%)} = \frac{\text{Weight of collagen (g)}}{\text{Weight of wet skin (g)}} \times 100$$

c. Humidity Regain

Collagen's ability to keep water from the atmosphere has been evaluated by evaluating the change of weight during the incubation in the atmosphere that can be controlled. Collagen froze was decorated and placed in a desiccator for over 72 hours. Later, collagen samples were transferred to a closed atmospheric system at room temperature in a relatively constant humidity environment of 33%, maintained on saturated $CaCl_2$ solution. The innovated humidity has been calculated and expressed according to the percentage of dry weight.

d. Formulation of Clay Mask

Sample of clay masks created to 100 grams per formula. Each component is weighed according to its corresponding formula. The preparation method was first dissolved with bentonite and methyl paraben with 25 ml and 20 ml of hot water, respectively. After that, let stand for 15 minutes. Bentonite and methyl paraben have been added to mortar, added with xanthan gum, crushed with homogeneity. Then add glycerin, propylene glycol and collagen extract, mix and crush until homogeneous. In addition, Kaolin has gradually been put into mortar while continuing to be overwhelmed until the preparation is homogeneous. Rosae oleum has been added for aroma. After that, the evaluation was done on the preparation formula.

e. Evaluation of Avaliability

There are 9 types of tests used in this research :

1. Organoleptic Test
2. Homogenity test
3. pH Test
4. Vicosity Test
5. Spreadability Test
6. Preparation of Test Drying Time
7. Ease of Cleaning Test
8. Hedonic Test
9. Irritation Test

f. Data Analysis

The test data for pH, viscosity, dispersibility, drying time were analyzed using the one-way anova test. Meanwhile, the test data for the physical properties of homogeneity and organoleptic, and ease of cleaning were analyzed descriptively.

III. RESULT AND DISCUSSION

Collagen Extraction

Among marine sources, fish skin was widely selected for the extraction of type I collagen. Fish skin was widely available, had no risk of disease transmission, and had no religious ties. Considering that 75% of a fish's weight was eliminated as fish skin, scales or bones, it can be used as a valuable source of collagen. Salmon skin was selected as the raw material

for isolating type I collagen. During ASC extraction, we observed that it was easier to dissolve salmon skin collagen in acidic solution. No need re-extraction to further dissolve collagen, as there was no skin left after 72 hours in acetic acid solution. Regarding the collagen extraction yield for salmon skins, we obtained a yield of 20.3% of collagen extract. The salmon skin had a high moisture content (81 %), reasonably good protein (14 %) and lipid contents (4.3 %) and a very low ash content (0.7 %). Furthermore, these values were consistent with those observed in other studies with the same or different species.

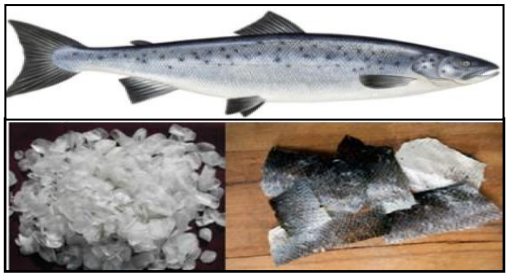

Figure 1. Fish processing waste; fish frame, scales and skin (Jafari et al., 2020)

Humidity Regain Analysis

Collagen's ability to hold water was fundamental to its success as a cosmetic ingredient, with its primary role being to control skin moisture. Its ability to be a natural humectant was assessed by measuring the relative absorbance of water from the atmosphere, using samples lyophilized under constant air humidity conditions. When the samples were exposed to 63% relative humidity, the water absorption increased significantly, approximately 6% of the sample weight. This result is in agreement with other measurements performed on collagen suitable for cosmetic applications.

Organoleptic Test

Organoleptic testing of a clay mask with fish collagen extract (*Oncorhynchus nerka* L.) was performed by direct observation of the color, odor and texture of the sample. There were no significant differences in texture and fragrance in each clay mask formulation. Each formulation has a distinctive aroma that comes from the addition of rose oleum as a flavoring or fragrance agent, and has a semi- solid, mud-like texture. The difference in sample color was not too significant, this difference in color was due to the difference in the basic concentration of bentonite in each formulation, describing the bentonite itself, which was a very fine powder with pale yellow to grayish cream in color.

Table 1. Organoleptic Observations

Formula	Shape	Color	Aroma
F1	Semi Solid	Beige	Aromatic Typical
F2	Semi Solid	Beige	Aromatic Typical
F3	Semi Solid	Beige	Aromatic Typical
F4	Semi Solid	Beige	Aromatic Typical

Homogenity Test

Homogeneity test of collagen fish extract (*Oncorhynchus nerka* L.) clay mask preparation obtained homogeneous results. The preparation could be stated to be homogeneous because each formula shown a homogeneous structure and there were no coarse grains found in the clay mask sample.

Table 2. Homogeneity Test

Formula	Homogeneity
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous
F4	Homogeneous

Table 3. pH, Viscosity, Spreadability Test

Formula	pH	Viscosity (cP)	Spreadability (cm)
	Mean ± SD (n=4)	Mean ± SD (n=4)	Mean ± SD (n=4)
F1	4.43 ± 0.31	20218.1 ± 141.1	6.63 ± 0.26
F2	5.70 ± 0.20	24132.5 ± 82.32	5.62 ± 0.17
F3	6.51 ± 0.18	29191.2 ± 106.7	4.92 ± 0.12
F4	7.42 ± 0.09	33277.6 ± 376.3	4.41 ± 0.07

IV. Conclusion

The combination of materials concentration in the collagen clay mask formulation showed an effect on the physical properties of the preparation, in terms of color, pH, viscosity, dispersibility, and rapid drying of the preparation. The best formula for collagen clay masks from salmon (*Oncorhynchus nerka* L*)* collagen extract was the F3 preparation where the concentration of kaolin was 25% and bentonite was 1%. F3 preparation was more preferred by respondents in hedonic testing with odor, color and texture parameters that are generated.

REFERENCES

Addor, F. a. S. A., Vieira, J. C. & Melo, C. S. A. (2018). 'Improvement of dermal parameters in aged skin after oral use of a nutrient supplement'. *Clinical, Cosmetic and Investigational Dermatology,* 11**,** 195.

Arvanitoyannis, I. S. & Kassaveti, A. (2008), 'Fish industry waste: treatments, environmental impacts, current and potential uses'. *International Journal of Food Science & Technology,* 43**,** 726-745.

Clapp, J. M., & Giaccotto, C. (1998). Residential hedonic models: A rational expectations approach to age effects. *Journal of Urban Economics, 44*(3), 415-437. https://doi.org/10.1006/juec.1997.2076

Fauziah, DW (2018). *Effect of Kaolin and Bentonite Base on Physical Properties of Combination Mud Mask of Olive Oil (Olive Oil) and Green Tea (Camelia sinensis).* Journal of Pharmacy Scienceand Health, 3 (2), 9–13.

Fauzi, Y., Widyastuti, Y. E., Satyawibawa, I., & Hartono, R. (2012). Kelapa Sawit: Budidaya, Pemanfaatan Hasil dan Limbah, Analisis Usaha dan Pemasaran. Penebar Swadaya. Jakarta.234.

Fernandes, R., Neto, R. C., Paschoal, C., Rohling, J. & Bezerra, C. (2008), 'Collagen films from swim bladders: preparation method and properties'. *Colloids and Surfaces B: Biointerfaces,* 62**,** 17-21.

Gomez-Guillen, M. C., Turnay, J., Fernandez-Dıaz, M., Ulmo, N., Lizarbe, M. A. & Montero, P. (2002), 'Structural and physical properties of gelatin extracted from different marine species: a comparative study'. *Food Hydrocolloids,* 16**,** 25-34.

Harborne, J. B. (1987). Metode Fitokimia: Penuntun cara modern menganalisis tumbuhan. *Bandung. Penerbit ITB*, *78*.

Jafari, H., Lista, A., Siekapen, M. M., Ghaffari-Bohlouli, P., Nie, L., Alimoradi, H. & Shavandi, A. (2020), 'Fish Collagen: extraction, characterization, and applications for biomaterials engineering'.*Polymers,* 12**,** 2230.

Kuncari, E. S., I. Iskandarsyah, P. Praptiwi. 2014. Evaluasi, Uji Stabilitas Fisik dan Sineresis Sediaan Gel yang Mengandung Minoksidil, Apigenin dan Perasan Herba Seledri (Apium graveolens L.). Buletin Penelitian Kesehatan. 42 (4): 213-222.

Liang, J., Pei, X.-R., Zhang, Z.-F., Wang, N., Wang, J.-B. & Li, Y. (2012), 'A chronic oral toxicity study of marine collagen peptides preparation from chum salmon (*Oncorhynchus keta*) skin usingSprague-Dawley rat'. *Marine Drugs,* 10, 20-34.

Li, P.-H., Lu, W.-C., Chan, Y.-J., Ko, W.-C., Jung, C.-C., Le Huynh, D. T. & Ji, Y.-X. (2020), 'Extraction and characterization of collagen from sea cucumber (*Holothuria cinerascens)* and its potential application in moisturizing cosmetics'. *Aquaculture,* 515, 734590.

Mo, W. Y., Man, Y. B. & Wong, M. H. (2018), 'Use of food waste, fish waste and food processing waste for China's aquaculture industry: Needs and challenge'. *Science of the Total Environment,*613, 635-643.

Nagai, T., Yamashita, E., Taniguchi, K., Kanamori, N. & Suzuki, N. (2001), 'Isolation and characterisation of collagen from the outer skin waste material of cuttlefish (*Sepia lycidas*)'. *FoodChemistry,* 72, 425-429.

Peng, Y.; Glattauer, V.; Werkmeister, J.A.; Ramshaw, J.A. Evaluation for collagen products for cosmetic application. Int. J. Cosmet. Sci. 2004. 26, 313. [CrossRef]

Petcharat, T., Benjakul, S., Karnjanapratum, S. & Nalinanon, S. (2020), 'Ultrasound-assisted extraction of collagen from clown featherback (*Chitala ornata*) skin: yield andmolecular characteristics'. *Journal of the Science of Food and Agriculture*.

Raman, M. & Gopakumar, K. (2018b), 'Fish collagen and its applications in food and pharmaceutical industry: A review'. *EC Nutr,* 13, 752-767.

Reiger, MM (2000). *Harry's Cosmetology 8th ed.* Chemical Publishing Co. Inc. New York 523- 572.

Roininen, K., Lähteenmäki, L., & Tuorila, H. (1999). Quantification of consumer attitudes to health and hedonic characteristics of foods. *Appetite*, *33*(1), 71-88. https://doi.org/10.1006/appe.1999.0232

Accepted Date	Revised Date	Decided Date	Accepted to Publish
20 Mei 2021	21 Mei 2021	23 Mei 2021	Ya