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An Anti-Aging Facial Moisturiser Made from the Membrane of a Chicken Egg- a Collagen Face Spray

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Abstract: *Since skin is frequently exposed to a variety of stimuli, premature aging has become a public concern. Meeting the skin's collagen requirements is one way to preserve skin beauty and stop early aging. The membrane of chicken egg shells contains a protein called collagen. Egg shells from chickens are currently viewed as waste, harm the environment, and are not effectively used. As a result, the development of face spray cosmetics seems to represent a new advance in waste management and utilization. It has anti-aging properties, soothes facial skin, reduces redness on sensitive skin, nourishes and hydrates the skin, and smoothes the appearance of cosmetics.*

Keywords: Skin

I. INTRODUCTION

Skin is something that is clearly visible from a person, especially when interacting with other people, so skin aging is an unavoidable problem. The skin aging process is a dynamic process. The skin aging process causes histological changes in the skin layers. Skin aging is a biological process that occurs due to internal factors, namely genetics and DNA damage, external factors such as lack of nutrition, environmental conditions, exposure to free radicals, and so on that can affect appearance. The skin aging process is reduced to a decrease in the physiological function of the skin which appears as a clinical manifestation of the skin which will continue with age.[1]

One of the main targets of aging is the skin, which serves as our body's outermost barrier and interacts freely with an oxidative environment (Pillai et al., 2005). In addition to environmental variables including pollution, stress, and UV light, hereditary factors also play a role in skin aging (Kang, 1997). The structure and functionality of the skin are gradually altered by these causes. Due to oxidative stress, solar UV exposure in particular plays a significant role in skin aging (Rittié and Fisher, 2002). Oxidative stress is mediated by superoxide anions and hydroxyl radicals, also known as reactive oxygen species.[2]

Skin aging is a component of the "aging mosaic" that occurs naturally in humans and takes distinct paths in various organs, tissues, and cells over time. The skin offers the earliest visible indications of time passing, while the surrounding "eyes" conceal the aging symptoms of interior organs. Combinations of endogenous or intrinsic (genetics, cellular metabolism, hormones, and metabolic processes) and exogenous or extrinsic (chronic light exposure, pollution, ionizing radiation, chemicals, and toxins) elements contribute to the complicated biological process of skin aging.[3]

Fig no - 1

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In addition to being a valuable and nutrient-dense food source, the separated, processed, and/or purified egg yolk and egg white have a sizable market (Ahmed et al., 2019a). Over the past three decades, the world's egg production has increased by more than 150%, which has led to an increase in eggshell (ES) waste and egg by-products. Since calcium carbonate and the protein- based eggshell membranes (ESM) that are linked with it make up the majority of the ES (Hincke et al., 2012), these two substances are commonly disposed of together as eggshell waste. About 97 billion eggs are produced annually in the US and 10 billion in Canada, respectively (Statistics Canada, 2020; United Egg Producers, 2020). [4]

Due to increased understanding of nutrition and the consumption of eggs as a source of protein, the egg industry has expanded quickly. However, this leads to an accumulation of eggshell trash, which pollutes the environment [8]. According to the Environmental Protection Agency, one of the worst pollution issues facing the food industry is eggshell waste, which comes in at number

15. This is due to the fact that improper eggshell waste disposal can lead to the formation of fungi on the eggshell, which may pose health risks. Given these issues and the fact that eggshell waste is readily available and has not been extensively utilized in cosmetics, we are interested in turning it into a practical and affordable solution.[5]

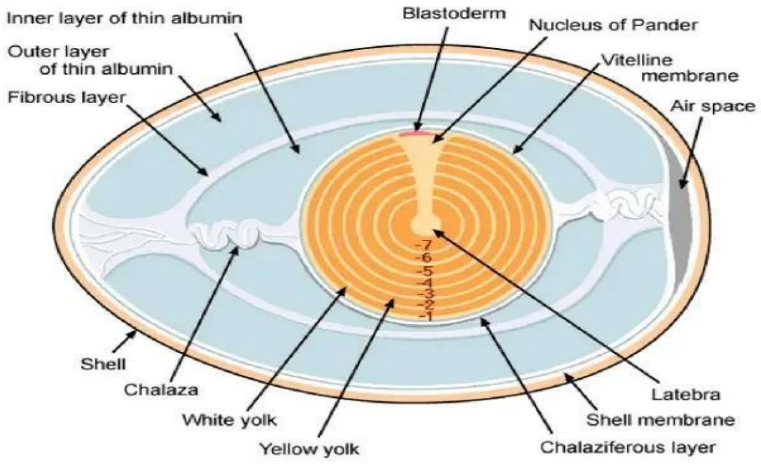

Fig no - 2

Collagen Face Spray which is made from eggshell membrane contains collagen which is suitable for teenagers to adults who have skin aging problems so that it can be used as an anti-aging agent. In addition, this product is also able to moisturize and nourish the skin, especially dry skin types, soothe facial skin, relieve redness of sensitive skin and help give a smooth impression on makeup. This Collagen Face Spray product is mainly made from eggshell membrane extract so it is safer to use at a more affordable price compared to other face spray products.[6]

It is anticipated that this collagen face spray product from eggshell membrane will help Indonesians, particularly those with dry skin and skin aging issues, to overcome these issues. Since skin health is a major problem in the current world, this collagen face spray invention made from eggshell membrane may lead to very promising commercial potential in the economic sector.[7]

Fig No - 3

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The ESM is a polymeric fibrous meshwork, which provides a biomineralization platform for ES calcification during egg formation, while preventing mineralization towards the inner egg white (Hincke et al., 2000, 2012). In addition, ESM also functions as a physical/chemical barrier against invading pathogens and plays an important role in chick embryo development. The relative location of ESM is shown in an artistic rendition of the cross-sectional view of eggshell.[8]

It is anticipated that this collagen face spray product from Eggshell Membrane will help Indonesians, particularly those with dry skin and aging issues, to overcome these issues. Since skin health is a major problem in the current world, this collagen face spray invention made from eggshell membrane may lead to very promising commercial potential in the economic sector.[9]

The organic material known as eggshell membrane (ESM) has been demonstrated to boost collagen synthesis and cellular activity. ESM also lessens inflammation and UV-induced damage to the skin, preventing aging (Candilish et al., 1969). Keratin, an insoluble protein found in feathers, hair, horns, scales, and nails, is the building block of ESM. According to Vignardet et al. (1999), keratin has high resistance to chemical, biological, and physical responses. ESM has been proposed for usage in anti-wrinkle, skin growth, wound healing, and moisturizing formulations (Kang, 1997; Park et al., 2012).[10]

Because of its special qualities, adaptability, and sustainability, the natural biomaterial known as chicken eggshell membrane (ESM) has drawn more and more interest in the biomedical industry (figure 1). According to Torres et al. (2010), Chai et al. (2013), Park et al. (2016), Baláž (2014), Chen, Kang, and Sukigara (2014), Mensah et al. (2021), Shi et al. (2021), Zurita-Méndez et al. (2022), and Torres-Mansilla et al. (2023), it is a thin film-like structure that lines the inside surface of the eggshell and keeps the albumen apart. The ESM has exceptional biodegradability and biocompatibility because to its complex matrix of proteins, glycosaminoglycans, and minerals (Park et al., 2016; Ahmed et al., 2019a; Torres-Mansilla et al., 2023).[11]

Chondroitin sulfate, hyaluronic acid, and sulfur-rich proteins are among the bioactive substances found in the chicken eggshell membrane (ESM), a biopolymeric fibrous structure . Collagen, one of the primary components sustaining the structure of skin tissue and the control of macromolecules, is also present in the ESM . According to earlier reports, ESM in its natural form is an effective biomaterial for wound healing [48]. Although human dermal fibroblasts (hDF) facilitate the repair of injured skin tissue and encourage the release of cytokines, they are challenging to cultivate on the natural extracellular matrix.[12]

Extraction of ESM Collagen –

Method No 1

Residual ESM samples (after HA extraction) were suspended in 0.1 N NaOH at a sample to alkaline solution ratio of 1:20 (w/v) and was left in the refrigerator for 12 h to remove other proteins and to prevent the effect of endogenous proteases on collagen. The suspension was then centrifuged for 7000 rpm for 10 min, the supernatant was discarded while the precipitates were washed with distilled water twice and then with 0.025M acetic acid solution.[19]

Method No 2:

After being separated, the eggshell membrane is weighed and placed into the homogenizer tube. Within 24 hours, the extraction procedure was completed in the homogenizer tube using the macerization technique using a solvent in the form of 50% ethanol at a 1:5 ratio. At a temperature of 50°C, the filtrate from the extraction process is placed inside the evaporator machine. After evaporation, the filtrate goes into the filtration apparatus. Additionally, until a thick filtrate is created and the eggshell membrane extraction is prepared for use as the primary ingredient in collagen face spray products, the filtrate passes through a rotary evaporator machine.[20]

Identification of eggshell membrane :

Collagen Anatomical pathology test using 400x magnification microscope with Sirius Red staining. Because this test is judged to be able to show collagen fibers, this test is carried out by grading the density of collagen fibers in the histological preparations of the tested tissue from each preparation.[21]

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Identification of the Eligibility of Collagen Face Spray:

Product Several Collagen face spray product formulas that have been made are then re-identified through several tests to ensure the best formula to be used and to test the feasibility of using the product. Several tests were carried out, namely pH test, organoleptic test, and humidity test.[22]

Advantage and Disadvantage of eggshell membrane:

Advantages:

- Eggshell membranes are a good source of collagen, which is important for skin, bone, and joint health.
- Skin health benefits:
- Eggshell membrane collagen may improve skin barrier function, appearance, and moisture, says Ancient Nutrition.
- Bone health benefits:
- Eggshell membrane may enhance calcium absorption and deposition, potentially aiding in bone regeneration.
- Rich in collagen [23]

Disadvantages:

- Need for processing:
- Eggshell membranes often need to be processed to extract the collagen and make them suitable for various applications.
- Mesh patterns:
- The membrane's fiber arrangement is not always consistent, potentially creating mesh patterns that allow cells to pass through.
- Potential for alterations in bodily fluids:
- The membrane can change upon contact with bodily fluids, potentially altering its structure. [24]

Benefits:

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Method Of Preparation:

a) Materials :

The tools used in this study include digital scales, filtration apparatus, 100 ml glass beaker, 3000 ml glass beaker, Erlenmeyer, test tube, vacuum rotary evaporator, extraction apparatus, homogenizer tube, electric microscope. The materials used include egg shells obtained from the community around Universitas Negeri Semarang, ethanol, glycerin, PVP, aquadest.[25]

b) Separation of the egg membrane from the shell :

The sample is in the form of egg shell waste as many as 300 pieces taken from the community around Semarang State University, the collected samples are then washed with water until clean and not sticky and then dried by drying. After drying, the chicken egg shell membrane can be directly peeled off and removed from the shell.[26]

c) Production collagen face spray :

The first stage is the extract of the chicken eggshell membrane with a predetermined formula

↓

Put into a beaker glass then added glycerin and PVP which has been dissolved in warm water, then stirred until

↓

Homogeneous and then transferred into a spray bottle that has been prepared

↓

The final stage is adding Aquadest until the solution is 100 mL.[27]

Properties of Collagen of chicken eggshell membrane :

Anti-aging activitiy :

We measured the amount of peptides fragmented by collagenase using the ninhydrin reaction. Collagen Type I (Sigma Chemical Co., USA) was added to TES (N-[Tris (hydroxyl methyl) methyl]-2- aminoethanesulfonic acid sodium salt, Sigma Chemical Co., USA), buffer, diluted samples, collagenase, and Clostridiums histolyticum collagenase Type IV (Sigma Chemical Co., USA) solution, and the sample was placed in a shaking incubator for 5 h at 37℃ and centrifuged for 5 min at 2000 rpm. The supernatant was collected and mixed with ninhydrin (2,2-Dihydroxy- 1,3-indanedione, Sigma Chemical Co., USA) reaction buffer, which was heated with a circulator (Vision scientific co., Ltd., Korea) for 10 min at 80℃ and then frozen. Solutions were then mixed with isopropanol at a 1:1 ratio and centrifuged for 10 min at 12,000 rpm at 4℃. The resulting supernatant was placed in the wells of a 96-well plate, and light absorption at 600 nm was measured using an ELISA plate reader. Epigallocatechin gallate (EGCG, Sigma Chemical Co., USA) was used as a control. Inhibition of collagenase by EGCG (positive control) and egg shell membrane hydrolysates (ESMH) fractionated by molecular weight. The data presented the mean values±standard deviation of three independent experiments.[28]

Anti-inflammatory activity :

Lipopolysaccharide (LPS) was obtained from Sigma Chemical Co., USA and IFN-γ from LG Life Sciences, Korea. All laboratory supplies, including the cell culture dishes, were from Falcon. RAW 264.7 cells (mouse macrophage cell line) were obtained from the Korean Cell Line Bank (KCLB). RAW 264.7 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, WelGENE Inc., USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin (BioWhittaker Inc., USA). When cells reached 90% confluence, they were centrifuged at 2,000 rpm for 3 min and then diluted at a 1:5 ratio.[29]

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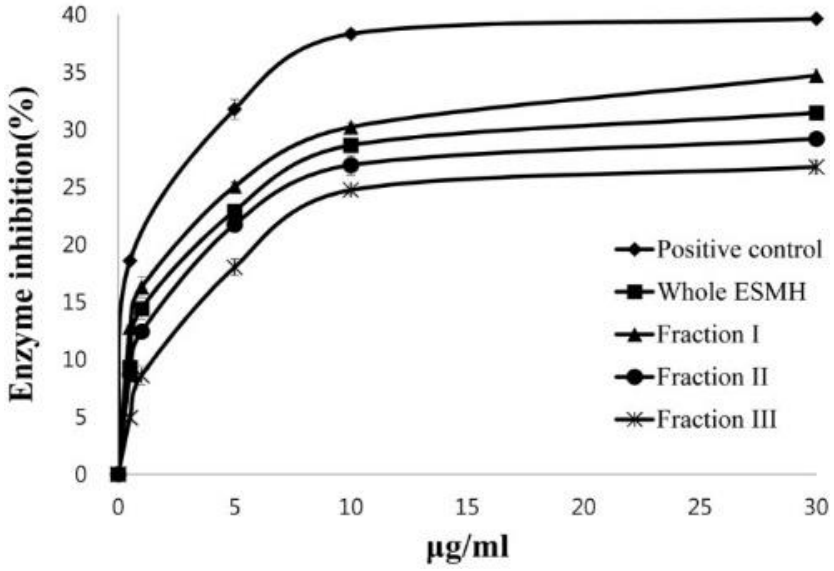

Anti-microbial activity :

All bacterial strains used in this study were supplied by the Biological Resources Center. The media used for this study, tryptic soy broth (TSB) and de Man, Rogosa, Sharpe (MRS) medium, were purchased from Difco Laboratories (Sperks, USA).To assess the antimicrobial effects of eggshell membranes against skin pathogens, we used the disc diffusion method. Pathogens were cultivated in liquid medium for 18 to 24 h, and then the solution was added to new liquid medium for 4 h before being poured as liquid agar, which set at room temperature. Sterilized filter paper discs (8 mm, Advantec, Japan) were placed in medium, and then 20 µL of eggshell sample was allowed to soak into each disk for 18 to 24 h. The clear zone around the discs was then measured. Ampicillin (Sigma Chemical Co., USA) was used as a positive control, and each test was performed three times independently.[30]

Moisture-protection:

Measurement of skin moisture and effectiveness of moisture loss reduction .National DR-1 was used to measure skin capacitance and MPA5-CK to study transepidermal water loss (TEWL) in healthy male and female subjects without any skin conditions such as allergies or any medical history of relevant diseases. Thirty minutes before the test, test subjects were placed in an indoor environment at a temperature between 20 and 22℃ and a humidity level between 40 and 60%. Skin of the subjects' lower arms was measured using the tape stripping method (Prall, 1966). Skin moisture content level (% wet basis) was measured at 0, 1, 3, and 6 h after breakage and TEWL was measured in g/h/m2.[31]

Evaluation Test :

1) pH Test Result :

Material	Formula		
	F1	F2	F3
Eggshell Membrane	30	30	30
Glycerine	20	25	30
PVP	5	5	5
Aquadest	45	40	35
PH result test	4.3	5.4	8.4

Table 1 shows the test results for collagen face spray formula. [32]

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2. Organoleptic test result of collagen face spray product

Organoleptic parameters	Organoleptic Observations		
	F1	F2	F3
Colour	Colourless	Colourless	Colourless
Smell	Odourless	odourless	Odourless
Form of Substance	Liquid	Liquid	Liquid
Homogeneity	Homogeneous	Homogeneous	Homogeneous

Table 2. Organoleptic Test Results[33]

3. Results of collagen face spray Moisture Observation in respondent

Parameters	Time Span (minutes)		
	Before Use	5	30
Water	29.5	56.0	56.7
Oil	13.2	25.2	25.5

Table 3. Humidity Test Results[34]

II. RESULT

Based on the identification of collagen levels that have been carried out at the Anatomical Pathology Laboratory using a 400x magnification microscope with Sirius red staining, the results show that there is a collagen content of 52.8% in the chicken egg shell membrane which can be seen . The collagen fibers formed based on this identification are very many and very close to each other. In each Collagen Face Spray formula there are 30 mL of chicken eggshell membrane extract in a 100 mL bottle and 18 mL of eggshell membrane extract in a 60 mL bottle.Since the face needs at least 1 milliliter of collagen per day, the face spray's collagen content can be considered to have satisfied the

III. CONCLUSION

The development of collagen face spray using eggshell membrane presents a promising and sustainable approach to skincare innovation. Eggshell membrane, a natural and underutilized byproduct, has been demonstrated to contain significant amounts of collagen and other bioactive components beneficial for skin health. This study successfully extracted collagen from eggshell membrane and incorporated it into a spray formulation suitable for topical application. Results indicate that the product has potential moisturizing, firming, and anti-aging properties, making it a viable alternative to conventional collagen sources. Furthermore, utilizing eggshell waste contributes to environmental sustainability by promoting the circular use of food industry byproducts. Future studies should focus on long-term efficacy, safety evaluations, and potential commercial scalability of this formulation.

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