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Improving the Skin Penetration of Cosmetics Containing Omega 3 Fatty Acids

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Abstract

Purpose: The purpose of this study is to form a new cosmetic market through the development of a composition with high skin permeability after adding omega 3 to Aloe Vera soothing gel products. **Research design, data and methodology:** In this study, omega-3 fatty acids were added to cosmetic products in the form of soothing gels. By applying nanoparticle technology to rapidly increase the penetration of raw materials into the skin, characteristics related to skin moisture and regeneration were determined. Omega-3 was used as a raw cosmetic material. Then 5% and 15% nanoparticle aqueous products containing omega-3 were prepared. The developed water hydrate was subjected to skin permeability test using artificial skin. **Results:** 53 hours of artificial transdermal penetration of the developed composition, the ethanol-based omega-3 containing nanoparticle solubilized raw material was about three times higher penetration than the ethanol-based omega-3 containing nanoparticle solubilized raw material. **Conclusions:** The raw material product (SR-1901) containing 5% of omega-3 nanoparticle water hydrate has skin regeneration ability and pain reduction effect. It can be expected that the skin cosmetics market will be reorganized into a new distribution structure and opportunity through omega-3 supplemented soothing gel cosmetics with improved efficacy than existing cosmetics.

Keywords : *Aloe Vera* Soothing Gel, Omega-3, Nanoparticle Soluble, Skin Penetration, Skin Regeneration

JEL Classification Codes : M10, M11, Q02, I10, I12

1. Introduction

Omega-3 fatty acids are polyunsaturated fatty acids that are essential to the body. There are three main omega-3 fatty acids: eicosapentaenoic acid (EPA), docosa hexaenoic acid (DHA), and alpha linolenic acid (ALA). The Inuit who lived in Greenland in the early 1970s ate mostly high-protein, high-fat, and high-cholesterol foods. Despite the fact that they rarely eat vegetables, they had low incidence of heart disease, cancer, and diabetes (Dyerberg et al., 1978; Dyerberg, & Bang, 1979; Kuhnlein et al., 2001). In the 1980s, intensive experimental studies were conducted on effects of polyunsaturated fatty acids (PUFAs), particularly omega-3 fatty acids.

Recently, it has been discovered that unsaturated fatty acids are essential nutrients for the normal growth and development of the human body. Omega-3 fatty acids play a very important role in the prevention and treatment of coronary artery disease, hypertension, arthritis, autoimmune disorders, and cancer (Dewailly et al., 2001; Goede et al., 2010; Larsson et al., 2011). Omega-3 is known to have functions in cell membrane construction, cell membrane maintenance, and intercellular signaling (Connor, 2000; Mozaffarian, & Wu, 2001). Omega-3 might also have skin regeneration effects. In the past, these fatty acids were limited to foods, health functional foods, and pharmaceuticals for product development. Recently, it has been reported that omega-3 fatty acids can be added to cosmetics for skin with various effects such as anti-aging, skin protection, moisture retention, and collagen regenerating effects (Meguro et al., 2000).

The global functional cosmetics (cosmeceutical) market is showing a continuous growth trend due to the increase of personalized needs worldwide and increasing demands of the times due to the entry into an aging society. The market for functional cosmetics production in Korea is worth more than 4 trillion won a year. Functional cosmetics production accounts for 34% of total cosmetics production. This proportion is increasing at an average annual growth rate of 17%. Soothing gel is one representative example of functional cosmetics.

Soothing gel is the most basic product used to moisturize and regenerate the skin. Many companies have already released various soothing gel products. Their roles in the summer cosmetics market are growing. Soothing gel is a representative product among products that can improve skin function by adding omega-3 to cosmetic compositions.

Recently, many attempts have been made to use omega-3 as a cosmetic ingredient. Korean Patent No. 1212899 discloses a product containing omega-3 as a cosmetic composition for preventing and improving skin wrinkles by enhancing the density of the dermal layer of the skin (Serhan, 2014). However, experimental results on whether the composition actually passes through the skin and becomes absorbed are not clearly presented.

Omega-3 by oral administration has been proven to be effective. However, it is known that omega-3 cannot be absorbed through the skin. To commercialize omega-3 in a cosmetic form with effects through the skin, a new formulation with improved skin absorption is urgently needed.

The objective of this study was to develop a functional cosmetic composition related to skin moisturizing and regeneration by adding omega-3 to cosmetics in the form of a soothing gel applying a special technology to increase skin permeability of raw materials to enhance skin penetration. Results of this study are expected to greatly contribute to the development of skin cosmetics with new technologies and compositions for the market of skin cosmetics.

2. Theoretical Backgrounds

2.1. Cosmeceuticals

The term functional cosmetic refers to a hybrid between a cosmetic and a pharmaceutical. This concept was first described by Raymond E. Reed in the 1960s. In general, functional cosmetics are substances that meet the following requirements: (1) they must be externally applicable, (2) they should produce useful and desired results, (3) they have targeted and predicted properties, and (4) they meet chemical, physical, and medical standards (Newburger, 2009; Dorni et al., 2017). Therefore, functional cosmetics combine aesthetic properties of cosmetics with skin and drug efficacy.

Currently, functional cosmetics must present the following two advantages: 1) immediate response such as cosmetics, and 2) long-term effects such as pharmaceuticals (Draelos, 2009). Functional cosmetics or cosmetic formulations should contain active ingredients that are safe and beneficial to the body (Draelos, 2009; Lintner et al., 2009).

2.2. Soothing-Gel

Soothing gel is a gel-type moisturizer for the purpose of soothing the skin. This product is mainly used in summer to soothe hot skin. Soothing gel can be used not only by children, but also by the whole family. If it is refrigerated, one can experience a quick cooling effect. The soothing gel ingredient is usually Aloe Vera and so on. In the manufacture of existing soothing gel products, purified water, Aloe Vera juice (skin regeneration), glycerine, propylene glycol, betaine (cell replication function) carbomer, poly-oxyethylene hydrogen, ytide Castor oil, allantoin (cell

regeneration, anti-inflammatory action) witch hazel extract, collagen, sodium hyaluronate, hexane Diol, tocopheryl acetate, fragrance, and so on are used as ingredients.

2.3. *Aloe Vera*

Aloe Vera is a plant species in the genus *Aloe*. The plant 'Aloe' is classified in the plant family of Angiosperm, monocotyledonous family liliaceae. It is native to the Mediterranean and Arabian Peninsula, India, China, and East Africa. A wild form of aloe is commonly found in Cyprus, Malta, Sicily, the Canary Islands, and India (Radha & Laxmipriya, 2015). So far, more than 350 types of aloe have been identified and 30 of them have been tested for their therapeutic properties in the human body. Aloe barbadensis, also known as *Aloe Vera*, *Aloe Ferox*, and *Aloe Aborescens*, is the most common aloe species widely used industrially. Some species of aloe can be used therapeutically, while others are toxic without the same effects on humans (Goede et al., 2010).

Aloe Vera (*Aloe barbadensis* Mill. /*Aloe Vera* Linn.) is the most common aloe cultivar. It is a perennial plant with a short medium term. Aloe plants have thick, sword-shaped leaves. Edges of their leaves have triangular thorns. Aloe contains flavonoids, terpenoids, lectins, fatty acids, anthraquinones, mono- and poly-saccharides (pectin, hemicellulose, glucomannan), tannins, sterols (camp cholesterol, β-sitosterol), enzymes, salicylic acid, minerals (calcium, chromium, copper), iron, magnesium, manganese, potassium, phosphorus, sodium and zinc), and vitamins (A, C, E, β-carotene, B1, B2, B3, B6, choline, B12, folic acid) (Boudreau & Beland, 2006; Surjushe et al., 2008; Gupta, & Rawat, 2017).

There are various types of polysaccharides in aloe. Their contents can change depending on the age of the plant. Aloe contains a soluble fiber fraction (i.e., glucomannan and hemicellulose) that can bind to fibrous receptors in plant cell walls to enhance cell proliferation. Accordingly, it has properties of accelerating wound healing. Aloe contains lignin, which aids in the absorption of ingredients through the skin. Consequently, higher amounts of collagen are produced when aloe is administered topically or externally (Shi et al., 2018). *Aloe Vera* has the ability to induce skin regeneration locally.

2.4. Omega-3

It has been reported that high dietary intake of DHA and EPA in Eskimos is associated with a very low incidence of inflammatory disease-related diseases and ischemic heart disease (Fodor et al., 2014). Thus, interest in omega-3 fatty acids has increased. PUFAs such as DHA and EPA are known to be beneficial in the treatment of rheumatoid arthritis, psoriasis, ulcerative colitis, asthma, Parkinson's disease, osteoporosis, diabetes mellitus, cardiovascular disease, cancer, and depression (Villani et al., 2013). In addition, studies have reported that PUFAs can aid the development of the nervous system, immune system, vision, and skin in infants (Wu et al., 2014).

Recent applications of fatty acids for skin-related diseases include therapies for photoaging, cancer, dermatitis, wound healing, and melanogenesis. The use of PUFAs can improves symptoms of skin diseases. Some fatty acids have been approved for clinical use or are in clinical trials for prophylactic or therapeutic use.

Omega-3 is known to be effective in regenerating skin through inflammation control and collagen synthesis (Hankenson et al., 2000; Serhan et al., 2008; Calder, 2013). Omega-3 is a good way to grow and encroach on the existing market by upgrading functions of existing products through nanoparticle-solubilization technology in inflammation control of atopic dermatitis cosmetics or acne-related cosmetics. In the case of atopic cosmetics, main ingredients in the existing market are natural oil, deep sea water, vegetable oil, and allantoin.

Since omega-3 is not currently included in major product groups released in the cosmetic market, a technology to upgrade the efficacy of products by adding it as a main ingredient of functional cosmetic ingredients is needed. In addition, it is difficult for oily skin to absorb the composition. To enhance skin absorption of the composition, only liposome technology is applied. Nano-solubilization technology applied in this study was developed after the liposome technology. This technology is a next-generation technology that can promote skin absorption. It is a technology with a high penetrating power by dispersing smaller particles and putting them between water molecules so that they could penetrate the skin. Therefore, omega-3 additive composition manufacturing technology to which a nanoparticle-water-soluble technology is applied can also be applied to atopic cosmetics or anti-wrinkle products through collagen enhancement in inflammation-related cosmetics. This technology will have a large ripple effect so that a new product can be launched.

2.5. Patented Nanoparticle Technology

Oil-in-water, a patented technology of K&P Nano Co., Ltd., was used in this study. It is known that one oxygen atom and two hydrogen atoms in a water molecule are covalently bonded as shown in Figure 1.

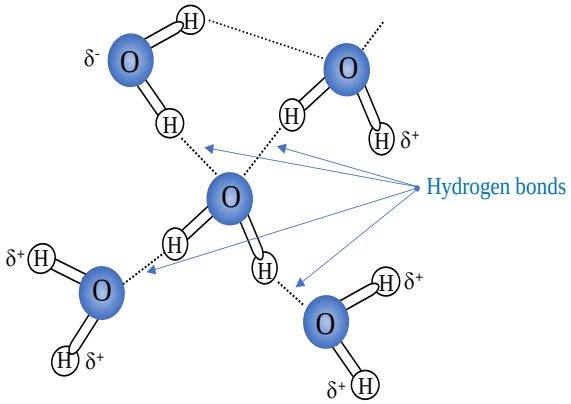

Figure 1: Molecular Structure of Water

Oxygen atoms have a weak negative charge. Hydrogen atoms have polarity. They are nanoparticle-soluble in these structures to form fat-soluble particles with cations as multi-layered complex factors. This substance can help penetrate the skin of fat-soluble omega-3 and promote the secretion of collagen from fibrous cells of the dermis, thereby showing ability to regenerate damaged skin. After oil-soluble nanoparticles through the above technology, the first coating was performed using an emulsifier or surfactant. As shown in Figure 2, fat-soluble particles were repeatedly coated several times to make multi-layer coated particles with positive ions. Then, it is a technology that makes the solubilisation of lipid by forming a structure in which the fat-soluble particles combine with the anions in the oxygen of the water molecule.

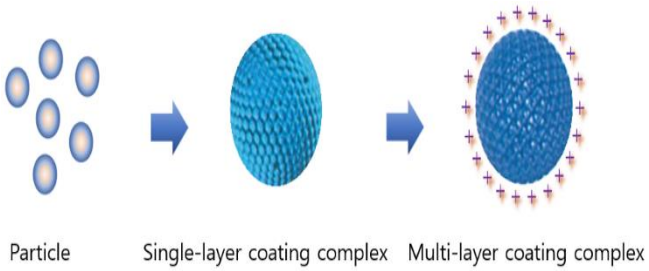

Figure 2: Nano-solution Process of Fat-soluble Particles

When a fat-soluble substance (e.g., omega-3) is solubilized, solubilized nanoparticles will become smaller in size to facilitate skin absorption. The soothing gel composition with omega-3 can enhance skin absorption as shown in Fig. 3. Properties of omega-3 and this nanoparticle water-soluble penetration technology are expected to increase the effect of soothing gel's skin regeneration function.

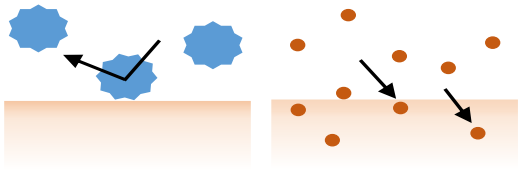

Figure 3: Principle of Skin Absorption Enhancement

3. Research Methods

3.1. Experimental Samples and Reagents

3.1.1. Experimental Samples and Materials

Perilla oil containing omega-3 produced by Green Tech Korea with a low-temperature wearing pressure method was used in this study. It was selected considering its light and soft scent as advantages.

Table 1: Test Results of Perilla Oil Containing Omega-3

Items	Criteria	Result	Evaluation
1. Appearance	Light yellow Clear liquid	Conformity	Conformity
2. Ph	3.0 ~ 9.0	5.5	Conformity
3. Pb	20ug/g below	Non-detection	Conformity
4. As	10ug/g below	Non-detection	Conformity

Omega-3 nanoparticle water-soluble raw material was commissioned to K&P Nano Co., Ltd. and manufactured. Artificial skin (Strat-M membrane, Millipore, USA) used in this study had two layers of poly-sulfone (epidermis, dermis) similar to human skin and polyolefin (porous) material. It was made of a material similar to the human body for skin penetration test as a substitute for human and animal skin tests.

3.1.2. Experimental Reagents

Reagents used in this experiment included n-hexane, C19:00 standards, and purified water. For other reagents, analytical grade reagents were used.

3.2. Experimental Design

The main objective of this study was to develop a product with rapid efficacy that could promote skin penetration by adding omega-3 to cosmetics in the form of

a soothing gel and applying nanoparticle technology to this soothing gel cosmetic. Ultimately, the goal is to revitalize the skin moisturizing and regenerating cosmetics market through the developed omega-3 soothing gel.

To achieve this objective, the detailed research process consisted of four major steps with an experimental process. The first step was to select omega-3 ingredients that could be used as cosmetic ingredients. In the second step, a nanoparticle aqueous solution containing 20% or more of omega-3 content was prepared. The third step is to prepare 5% of omega 3 and 15% aqueous nanoparticles of omega 3 with improved skin permeability again among the aqueous nanoparticles containing 20% or more of omega 3. In the fourth step, an optimal soothing gel plus prototype, the final product containing omega-3 and 5% nanoparticle hydrate, was prepared.

3.3. Experimental Analysis and Methods

3.3.1. Analysis of Fatty Acid Content, an Omega Trivalent Content

First, 1mg of perilla oil, a raw material containing omega-3, was placed into a 2mL screw-cap vial. Then 1 mg of a fatty acid analysis sample (e.g., omega-3 fatty acid content) into the 2mL tube. After 0.9mL of a solvent mixture of acetyl chloride and methanol prepared at a ratio of 1:10 (v/v) was added to the tube, 0.1mL of 3 mg/mL internal standard (methyl nonadecanoate) was also added. After that, the tube was heated at 80 °C for 60 min. After 60 minutes, the reaction was terminated by cooling the 2mL tube. After reaction completion, 1 mL of hexane was added into the 2mL tube followed by vortex for 5 minutes to move the dissolved FAME to the hexane layer. It was then allowed to stand at room temperature for separation of the hexane layer. The separated hexane layer was transferred to a vial for GC analysis using a pipette and analysed. To analyse the sample with gas chromatography, a gas chromatography instrument (Perkin Elmer Clarus 600, USA) equipped with an HP-INNOWAX column (30 m in length, 0.53 mm in I.D.) was used. Initial oven temperature was 140℃. It was heated to 180℃ at 8℃/min and 230℃ at 50℃/min. It was then held at 230℃ for 20 min. Carrier gas (He) flow rate was 3 mL/min. Injector and detector temperatures were set at 250°C.

3.3.2. Analysis of Nanoparticle Soluble Containing Omega-3

Freeze-drying process was used in this study to remove moisture. The omega-3 containing nanoparticle aqueous solution was frozen at –70°C overnight. After freeze-drying, the weight of the remaining solid (called concentration yield, e.g., if the weight remaining was 10% of the weight before lyophilisation, the concentration yield was 10%) was measured. After reaction completion, 1 mL of hexane was placed into a 2 mL tube followed by vortex for 5 minutes to move the dissolved FAME the hexane layer. The tube was then allowed to stand at room temperature to separate the hexane layer. The separated hexane layer was then transferred to a vial for GC analysis using a pipette and analysed.

3.3.3. Skin Penetration Enhancement Test

For the skin penetration test of the omega-3-containing nanoparticle water-soluble raw material, the experiment was conducted in the following order. First, a transdermal absorption device (FDC-6T Semi-Automated Franz Diffusion Cell Drive Console System, Logan Inc., USA) was washed with sterile distilled water as shown in Figure 4. An artificial transdermal Strat-M membrane (SKBM02560) was placed between the sample loading chamber and the collection tube. Both the sample loading chamber and the collection tube were filled. Distilled water was then used to fill the collection tube container for receiving the permeated sample under the chamber. Then 1 mL of each sample was loaded into the sample loading chamber.

Two of each of the two experimental groups including the control group among the six loading chambers in the equipment are loaded into the chamber. When time elapsed after loading the sample, the sample permeated and the sample in the loading chamber decreased. The permeation time of all samples was 53 hours. Samples collected in the permeation sample collection tube through percutaneous penetration in the loading chamber were collected and freeze-dried. Fatty acid analysis of the omega-3-containing sample was then performed to compare the permeability (amount) of the sample.

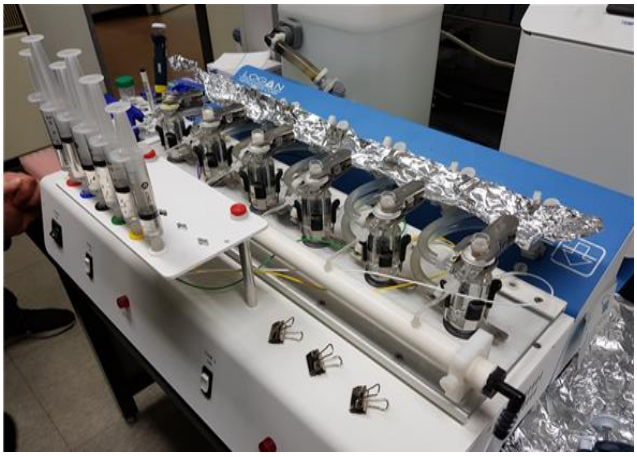

Figure 4: Transdermal Penetration Device Particles

4. Research Results

4.1. Analysis of Omega-3 Content Raw Material (Perilla Oil) Fatty Acid

The test method for omega-3 (18:3) content in perilla oil was based on 'Korean Industrial Standard KS M 2413 Fat and Oil Derivatives - Fatty Acid Methyl Ester - Ester and Linolenic Acid (C 18:3, Omega-3) Methyl Ester Content Analysis Method'. Compositions and contents of fatty acids of general perilla oil and commercially available perilla oil from Green Tech Korea containing omega-3 containing substances were compared. Results of analysis of compositions and contents of perilla oil in the market and those of Green Tech Korea perilla oil showed no difference (Figure 5). However, Green Tech Korea's perilla oil was desorbed by low temperature pressure from the fragrance. Thus, it was chosen for this study because it was more sensual to use it as a cosmetic composition.

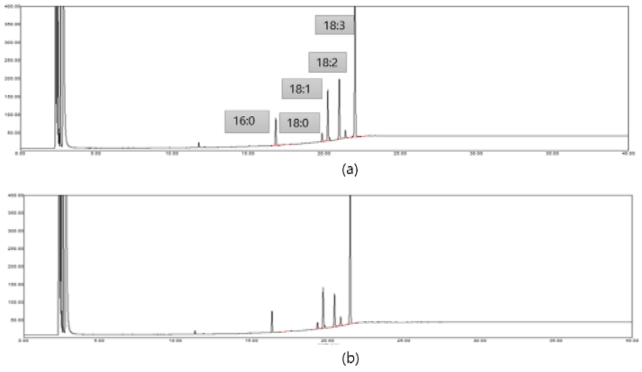

Figure 5: Fatty Acid Analysis Using Gas Chromatography of General Perilla Oil (a) & Greentech Korea's Perilla Oil (b)

4.2. Preparation and Analysis of Nanoparticle Soluble (SR-1904) Containing 20% or More of Omega-3 Content

Oil-in-water technology, a patent (registration 10-1062904) of K&P Nano Co., Ltd., was used for the manufacture of nanoparticles. This technology was jointly developed using the patented technology of K&P Nano Co., Ltd. by placing an order for manufacturing when producing an aqueous solution of omega-3 containing nanoparticles. For the preparation, first, monostearate or poly-fatty acid ester, etc., as mega-3 fatty acid components were mixed with perilla oil and heated to 40~75°C. Glycerine, an aqueous component, was also heated to 75°C. The oil phase component was then stirred and added to the aqueous phase component and the mixture was appropriately stirred at 1500 to 3,000 rpm for 5 minutes to 2 hours using a homogenizer to form an O/P (oil-in-polyol) type translucent gel. Purified water was then added to the above translucent gel and stirred again for 2 hours using a homogenizer at 1500~2,500rpm. Thereafter, oil-in-water (O/W) nanoparticles were prepared by slowly cooling and degassing. At this time, monostearate, polyfatty acid ester, glycerine ghatti gum, etc. were used to prepare the omega-containing nanoparticle aqueous product in consideration of the fragrance of the final product, skin permeability, and production cost. Among them, the glycerine-based nanoparticle aqueous solution showed a phenomenon of layer separation after about a month as shown in Figure 6.

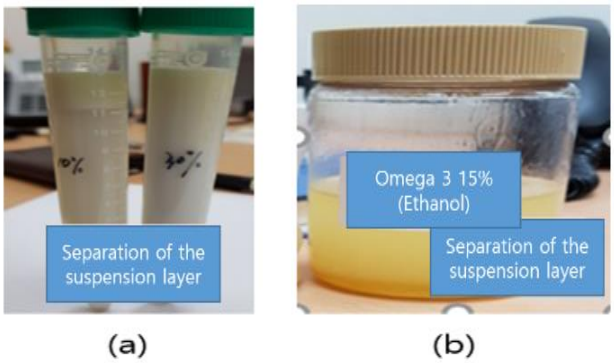

Figure 6: Omega-3-Including Nanoparticle Soluble

Monostearate and ghatti gum were excluded from the composition because they might cause layer separation and interfere with skin permeation. By improving problems of the sample according to the composition and manufacturing process found through optimization studies so far, an aqueous solution of nanoparticles containing 20% or more of the omega-3 content that did not separate layers was prepared as shown in Figure 7.

Figure 7: Nanoparticle Hydrate Containing at Least 20% Omega-3

Content analysis test was self-analyzed with a previously developed method. Results are shown in Figure 8.

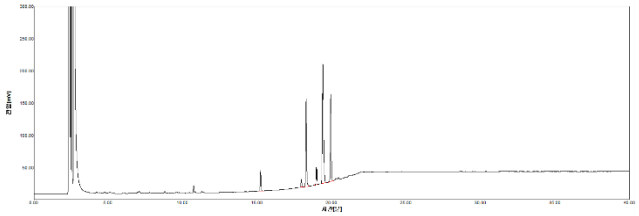

Figure 8: Product Content Analysis of Nano Water Hydrate (SR-1904) Containing 20% Omega-3

As shown in Table 2, after lyophilization of aqueous nanoparticles, total fatty acid contained in the solid sample was analyzed to be 47g/100 g (%). The sample after freeze-drying from which the moisture in nanoparticles was removed corresponded to 66% of the sample weight before freeze-drying. That is, since the concentration yield was 66%, the raw material content was calculated to be 31.4%, confirming that the sample was prepared with omega-3 content of 20% or more.

Table 2: Composition and Content of Nanoparticle Water Hydrate Containing 20% or More of Omega-3

Ingredients	Analysis of total fatty acid content in the sample before freeze-drying (g/100g)
Palmitic acid (C16:0)	4.2
Stearic acid (C18:0)	1.7
Oleic acid (C18:1)	19.0
Linoleic acid (C18:2)	4.0
Linoleic acid (C18:3, omega-3)	18.8
Total	47.5
Total fatty acid content considering the concentration yield *	**31.4**

Note: *(Concentrate content, g/100g) × (Concentrated rate, %) = 47.5g/100g × 66% = 31.4g/100g

The same product was analyzed by Incheon (Songdo) Techno park. Results are shown in Figure 9 and Table 3. After lyophilization of aqueous nanoparticles, total fatty acid contained in the sample in the solid was analyzed to be 46.5 g/100g. The sample after freeze-drying from which the moisture in nanoparticles was removed corresponded to 66% of the sample weight before freeze-drying. That is, since the concentration yield was 66%, the raw material content was calculated to be 30.7%, confirming that a suitable sample with omega-3 content of 20% or more was prepared.

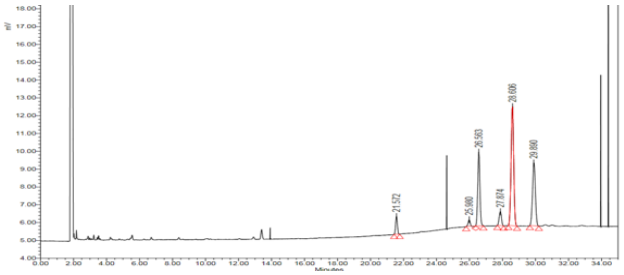

Figure 9: Content Analysis of Nano Water Hydrate (SR-1904) Containing 20% Omega-3 by Techno Park in Incheon

Table 3: Composition and Content of Nanoparticle Water Hydrate Containing 20% or More of Omega-3

Ingredient	Analysis of total fatty acid content in the sample before freeze-drying (g/100g)
Palmitic acid (C16:0)	3.5
Stearic acid (C18:0)	1.7
Oleic acid (C18:1)	17.9
Linoleic acid (C18:2)	3.6
Linolenic acid (C18:3, omega-3)	20.0
Total	46.5
Total fatty acid content considering the concentration yield *	**30.7**

Note: *(Concentrate content, g/100g) × (Concentrated rate, %) = 46.5 g/100g × 66% = 30.7 g/100g

Results of the company's content analysis and Incheon (Songdo) Techno Park were first checked. The same sample was finally confirmed as shown in Table 4 through the official test report of the Korea Food Science Research Institute. After lyophilization of aqueous nanoparticles, total fatty acid contained in the sample in the solid was analyzed to be 46.5g/100g (%). The sample after freeze-drying from which the moisture in nanoparticles was removed corresponded to 66% of the sample weight before freeze-drying. That is, since the concentration yield was 66%, the raw material content was calculated to be 30.7%, confirming that a suitable sample with omega-3 content of 20% or more was prepared.

Table 4: Composition and Content of Nanoparticle Water Hydrate Containing 20% or More of Omega-3

Ingredient	Total fatty acid content in the sample before freeze-drying (g/100g)
Palmitic acid (C16:0)	2.9
Stearic acid (C18:0)	1.0
Oleic acid (C18:1)	20.7
Linoleic acid (C18:2)	3.4
Linolenic acid (C18:3, omega-3)	18.6
Total	46.6
Total fatty acid content considering the concentration yield*	**30.8**

Note: *(Concentrate content, g/100 g) × (Concentrated rate, %) = 46.6 g/100 g × 66% = 30.8 g/100 g

4.3. Manufacture of Omega-3 Containing Nano-Aqueous Product with Skin Permeability Improved by 50%

Table 5 below shows a process chart with an attempt to prepare an omega-3 containing nanoparticle-aqueous product with skin permeability improved by 50%. In the first experiment, samples T1806 and T1808 prepared in the previous study were first tested for skin penetration in order to prepare an optimal omega-3-containing nanoparticle aqueous solution suitable for skin penetration. However, they did not penetrate. Samples prepared in advance in the first and second transdermal permeation tests failed the percutaneous permeation test.

The 3rd transdermal permeation test was a primary screening of permeable cosmetic solvents. There were failures and successes. In the 4th and 5th transdermal permeation tests, secondary cosmetic solvent screening was performed after identifying characteristics of artificial skin permeation. In the 4th transdermal permeation test, the permeability of ethanol and glycerine was good. In the 2nd to 4th skin permeation test, the artificial percutaneous permeation solvent selection screening was mainly performed. In the fifth step, a nanoparticle aqueous solution was prepared using selected solvents glycerine and ethanol and the transdermal penetration test was performed. The final product, HSS-2, was also prepared and tested for transdermal penetration as shown in Table 5. These developed samples were finally remanufactured and a transdermal permeation test was commissioned to Incheon Techno park based on the pre-tested skin permeation test.

The transdermal permeated sample obtained there was requested for analysis by the Korea Food Science Research Institute and an official test report was obtained. In the 5th, 6th, and 7th tests, the samples used for the transdermal permeation test were confirmed through preliminary tests. Samples SR-1901 and HSS-2 confirmed in these results were commissioned to Incheon Techno park for skin permeation test.

Table 5: Development of an Omega-3-containing Nanoparticle Aqueous Solution

No.	Date	Sample names						Results
		1	2	3	4	5	6	
1	1/29	T1806	T1806	T1806	T1808	T1808	T1808	Fail
2	3/28	DMSO	DMSO	DMSO	Water50%	Water 50%	Water50%	Fail
3	4/15	Toluene (o)	Chloroform (×)	p-Xylene (o)	Chloro-benzene (o)	Tetralin (×)	Tetrachloro-ethane (×)	Success (o) Fail (×)
4	5/10	TW(o)	TP(o)	TP(o)	Glycerol(o)	Water (o)	Ethanol (o)	Ethanol good permeability
5	5/31	Glycerol base(o)	Glycerol base(o)	Glycerol base(o)	Ethanol base(o)	Ethanol base(o)	Ethanol base(o)	Nanoparticle undiluted solution all successful
6	7/15	HSS-1 (o)	HSS-1 (o)	HSS-1 (o)	HSS-2 (o)	HSS-2 (o)	HSS-2 (o)	Success
7	8/12	Perilla oil (Omega-3)	Perilla oil (Omega-3)	5% Omega-3 content	5% Omega-3 content	10% Omega-3 content	15% Omega-3 content	Nanoparticle undiluted solution all successful

4.4. Comparison of Skin Permeability of Soothing Gel Containing Ethanol-Based Water-soluble Raw Material

Prototype HSS-1 was prepared to contain 3% of omega-3 nanoparticles and 5% solubilized raw material in the final soothing gel product. A prototype named HSS-2 was prepared to contain 5% of omega-3 nanoparticles in 5% water-soluble raw material in the final soothing gel product. A preliminary skin permeation test was conducted at Incheon Techno Park using this prototype sample. Both HSS-1 and HSS-2 succeeded in skin penetration as shown in Table 6 and Figure 10. At this time, HSS-1 and HSS-2 contained several types of compositions added to the prototype that delayed skin permeation.

As a result, the skin permeability time was delayed rather than the permeation time of ethanol- and glycerin-based omega-3-containing nanoparticle aqueous solution. Among the above two prototypes, 5% of omega-3 nanoparticles in HSS-2 was contained in the final product soothing gel. Thus, it was determined as the composition of the final prototype.

Table 6: Skin Permeability of HSS-1 and HSS-2

Artificial skin of the sample Permeation time (h)	HSS-1 nanoparticle[1] Cumulative amount of skin penetration (UL)			HSS-2 nanoparticle[2] Cumulative amount of skin penetration (UL)		
	chamber 1 (a)	chamber 2 (b)	chamber 3 (c)	chamber 4 (d)	chamber 5 (e)	chamber 6 (f)
22	500	500	500	400	400	400
46	900	900	900	700	800	600
70	1,300	1,300	1,200	900	1,000	800
94	1,800	1,700	1,400	1,000	1,100	900

Note: 1) HSS-1: Omega 3 aqueous solution 5% soothing gel (3%) prototype, omega 3 5% nanoparticle water-soluble raw material contains 3% in the final product soothing gel
2) HSS-2: Omega 3 aqueous solution 5% soothing gel (5%) prototype, omega 3 5% nanoparticle water-soluble raw material contains 5% in the final product soothing gel

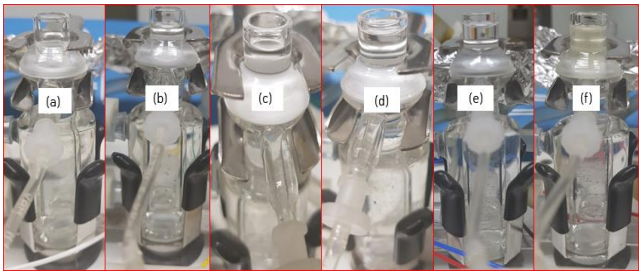

Figure 10: Skin Permeability of HSS-1 and HSS-2

4.5. Comparison of Omega-3 Undiluted Solution (Perilla Oil) and Aqueous Solution Containing Ethanol Base

An aqueous solution of omega-3 containing nanoparticles based on ethanol, a solvent selected through a solvent screening test for easy skin penetration in the previous test, was prepared as a test sample. It was confirmed that the test-prepared ethanol-based omega-3 5%-containing nanoparticle water-soluble raw material had skin permeation performance. An omega-containing 15% aqueous nanoparticle solution with increased omega content up to 15% was additionally prepared as a test sample and a comparison test was conducted with non-nanoparticle omega-3-containing (perilla oil) and skin penetration test by a certification agency. As a result, the omega-3 containing stock solution did not penetrate the artificial dermis used in the skin permeation test as shown in Table 7.

Transdermal permeation test of the ethanol base omega-3 containing nanoparticle solubilized raw material and the ethanol base omega-3 containing nanoparticle solubilized raw material with 15% ethanol base of omega-3 content was performed for 53 hours from the start of permeation.

The ethanol base omega-3 containing nanoparticle solubilized raw material was about 3 times better than the ethanol base omega-3 containing nanoparticle solubilized raw material with 15% ethanol base of omega-3 content. It

seems that even if the omega-3 content was nanoparticle, the raw material containing 5% rather than 15% is advantageous for percutaneous penetration as shown in Table 7. Considering manufacturing cost and skin permeability, 5%-containing nanoparticle solubilized raw material with an ethanol-based omega-3 was selected as a sample for enhancing skin permeability by 50%.

In addition, it was decided to manufacture HSS-2 raw material using this.

Table 7: Final Preliminary Test of 15% Nanoparticle Aqueous Solution with Ethanol Base Omega-3 at 5%

Sample percutaneous penetration Time (h)	Undiluted solution containing omega-3, perilla oil (Cumulative amount of sample permeation, UL)		Ethanol base Omega-3 at 5% containing nanoparticle water-soluble raw material (Cumulative amount of sample permeation, UL)		Ethanol base omega-3 at 15% containing nanoparticle water-soluble raw material (Cumulative amount of sample permeation, UL)	
	chamber 1	chamber 2	chamber 3	chamber 4	chamber 5	chamber 6
5	0	0	300	300	200	200
29	0	0	1,100	1,100	400	400
48	0	0	1,700	1,700	600	600
53	0	0	2,500	2,500	800	800

5. Conclusions

The purpose of this study was to develop a product that could further strengthen characteristics of Aloe Vera, the main raw material of commercially available soothing gel products. The skin regeneration ability and pain reduction effect of Aloe Vera could strengthen by adding omega-3. Thus, perilla oil containing omega-3 with a soft light and fragrance produced by Green Tech Korea at low temperature wearing pressure to the soothing gel was made with Aloe Vera as a basic raw material.

Raw materials for optimizing omega-3 nanoparticle water-soluble raw materials were produced. The content of omega-3 was analyzed by gas chromatography. Transdermal permeability characteristics were revealed with a transdermal permeation device. The final product of the raw material (SR-1901) containing 5% of omega-3 and nanoparticle-water hydrate was mixed with various compositions to prepare the final cosmetic prototype HSS-2 as Soothing Gel Plus. This composition was used in a percutaneous penetration test together with SR-1901 to confirm characteristics of skin regeneration ability and pain reduction efficacy. As one of the achievements of this study, a patent application (10-2019-0124159) for this composition has been completed.

Results obtained from this study are summarized as follows:

- Comparison of omega-3 (18:3) analysis between commercial perilla oil and perilla oil to be used as raw material showed that omega-3 (18:3) contents in mart products and Greentech Korea's perilla oil were 58.7% and 53.3%, respectively, showing almost the same content.

- For the production of nanoparticles containing 20% or more of omega-3 content, compositions of omega-3, glycerol, gattigum, and purified water were investigated to prepare omega-3 solubilized nanoparticle raw materials. As a result of GC analysis of omega-3 nanoparticle-solubilized raw material 10% content test, the omega-3 content in perilla oil was 65% and the omega-3 content in the nanoparticle solubilized solution was 55%. Omega-3 raw material maintained the content of omega-3 at 84% after the production of raw material (SR-1904) in perilla oil nanoparticle aqueous solution.

- The final product (SR-1901) of raw materials containing 5% of omega-3-containing nanoparticle-water hydrates was manufactured using the technology for manufacturing aqueous nanoparticles containing 20% or more of omega-3 (SR-1904). Omega-3 nanoparticle water-

soluble raw material content was confirmed.

● Omega-3 containing nanoparticles (5% aqueous solution) SR-1901 was mixed with various compositions to prepare the final cosmetic prototype (HSS-2) as Soothing Gel Plus. This composition also penetrated the artificial skin with SR-1901. It was confirmed that it exceeded the target of skin penetration improvement by 50%.

It can be expected that the skin cosmetics market will be reorganized into a new distribution structure and opportunity through omega-3 supplemented soothing gel cosmetics with improved efficacy than existing cosmetics.

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