Moisturizing and anti-wrinkle effect of Korean Natural citrion Junos Oil and bioconverted ethylhexyl Korean citron Junos oil

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Abstract

Background: The prepared naturally-derived vegetable oil and bioconversion oil showed different properties and properties from each other. As the number of consumers seeking eco-friendly materials increases, preference for natural vegetable oils is increasing, but there are limitations in application to cosmetic formulations due to the heavy feeling and stickiness of vegetable oils.

Methods: The moisturizing and anti-wrinkle effects of natural vegetable oil and ethylhexyl bioconversion oil were confirmed. Hyaluronic Acid (HA) and Filaggrin (FLG) production were used as indicators for moisturizing efficacy evaluation using keratinocytes (HaCaT cells), and pro-collagen synthesis amount using human fibroblasts (NHDF cells) for wrinkle improvement efficacy evaluation.

Results: In the case of Korean citron oil and ethylhexyl citron oil, HA increased by sample treatment, but FLG silver increased insignificantly. Both increases in pro-collagen were weak. However, in clinical trials, both citron oil and ethylhexyl citron oil showed statistically significantly higher moisture improvement and moisture retention effects than the control group after 4 hours and 8 hours without adverse skin reactions.

Conclusion: In this study, the feeling of use was improved when applied to the skin through biocatalytic conversion technology, and it is expected that it will be continuously expanded as a cosmetic material by confirming the moisturizing and anti-wrinkle effects.

Key words: Natural vegetable oil, Bioconversion, Up-cycling, Clean beauty, Emollient

Introduction

Generally, natural vegetable oil contains about 90 to 98% or more of the component as triglycerides, and consists of less than 10% mono-glyceride and di-glyceride. Mono-glyceride is a form in which glycerol and one fatty acid are combined with ester, and di-glyceride is a form in which glycerol and two fatty acids are ester-linked. In the food industry, these were used not only to mix heterogeneous foods as a food additive, but also as a gelatinization of starch. On the other hand, in the cosmetic industry, it is mainly used as an emulsifier of cream, emulsion, and ointment because it has high emulsification stability. Di-glyceride has the properties of oil and wax and it has an intermediate property between oil and fat and mono-glyceride. It has excellent compatibility with oil, so it can be used as a DDS (Drug Delivery System) though controlling content.On the other hand, as a cosmetic ingredient, triglyceride has a disadvantage in that it is easy to remain on the skin oily because it has a larger molecular weight than mono-glyceride and di-glyceride. In addition, natural vegetable oils are more expensive than synthetic oils such as dimethicone and there is a risk of acidification because of the large number of unsaturated bonds.

Citron (YUJA, Citrus Junos) was used for this study. It originated and grows wild in central China and Tibet. It is mainly distributed and produced in Korea, China, and Japan, but Korean is known to have the strongest flavor and thickest fruit peels. In particular, citron from Goheung region, which is known as the main area of citron, was used for this study. In Goheung, about 10,000 tons of citron is processed annually, and as a by-product, citron seeds generate about 20% of raw materials. Some farms use it as fertilizer, but most of them are disposed and this causes local environmental problems.

Therefore, in this study, it is to develop natural vegetable oils by utilizing the waste citron seed resources according to the trend of clean beauty and up-cycling cosmetics trend. It is not simply a concept of recycling, but it aims to reduce environmental pollution and form a value chain structure of resources. In addition, it is to develop a light emollient derived from nature by decreasing the content of triglyceride and increasing the content of mono-glyceride and di-glyceride though bio-conversion technology using enzymes

Materials and Methods

Preparation of natural oil and bio-conversion oil

The material of natural oil used in this experiment is the seeds of Yuja. Crude Yuja seed oil containing solids was obtained using the screw type oil pressed machine. To make natural oil, Yuja from Goheung, Jeollanam-do was used. To purify the crude oil, DALSORB2500M (The Dallas Group of America Inc.) and activated carbon Nuchar SA-20 (Inngevity, USA) were used. The enzymes lipase and triacetin were used for biological conversion of natural oil. Bio-conversion yuja oil was manufactured by the principle of trans-esterification of 2-ethylhexnol though an enzymatic reaction. The molar ratio of natural yuja oil and 2-ethlyhexanol was 1:1. After the reaction, free fatty acid was removed through a distillation process to reduce odor.

Moisturizing effect

HaCaT cells were grown to high density in 24-well plates. Immediately before experiments, cells were washed two times with serum-free medium to completely remove HA accumulated during cell growth. Subsequently, HaCat cells were cultured with or without NYO for 24 hr. at the indicated time, supernatants were analyzed for HA or FLG using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology Co., LTD or Cusabio Biotech Co., LTD)

Procollagen contents effect

HDF cell culture conditions for 24 hrs. After incubation, the medium was replaced with 50 μg/ml medium containing the various concentration of PCP mixed with serum-free kit and the cells were cultured for 24 hrs. The procollagen contents in culture supernatant were measured using Procollagen type I-c-peptodie (PIP) ELASA kit according to manufacturer's manual. Results were normalized on total protein content of the supernatant.

Moisturizing human efficacy evaluation

Measure skin moisture using Corneometer (CM825, Courage+ Khazaka electronic GmbH, Germany). The test was conducted the number of test persons was 20, and the test site was the forearm inside the arm. 2μL/cm² of the sample was applied to the selected test site (1.5cm X 1.5cm) of the forearm, and measurements were taken immediately after application, after 4 hours, and after 8 hours.

Evaluation of human efficacy for improving eye wrinkles

The degree of wrinkles around the eyes was measured using ANTERA 3D (Miravexn Limeted, Ireland). The test was conducted the number of test persons was 44, and the test site was the face (around the eyes). The test subjects took a drop or two of the sample on the palm of the hand to make it similar to body temperature, applied it to the face, and then tapped it to absorb it. Apply a sufficient amount twice a day in the morning and evening.

Results

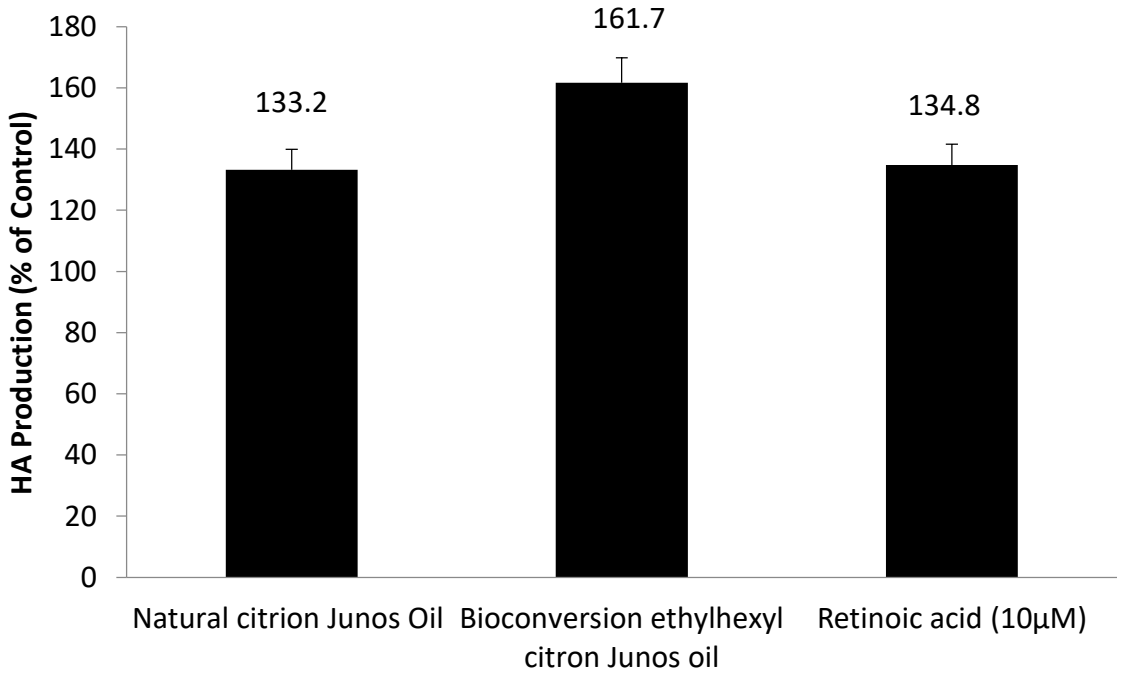
As a result of measuring the production of hyaluronic acid (HA) at a concentration without cytotoxicity, HA was increased in both natural oil and ethichexyl citron oil. In all, the production amount was insignificant. As a result of checking the procollagen production of citron seed oil and ethylhexyl citron oil, citron natural oil had a slight effect on the production, but in the case of camellia oil, it was confirmed that the procollagen production amount was increased in all cases. As a result of moisture measurement of

citron oil and ethylhexyl citron oil using a corneometer, no adverse skin reactions were observed. The moisturizing effect was shown for 8 hours.

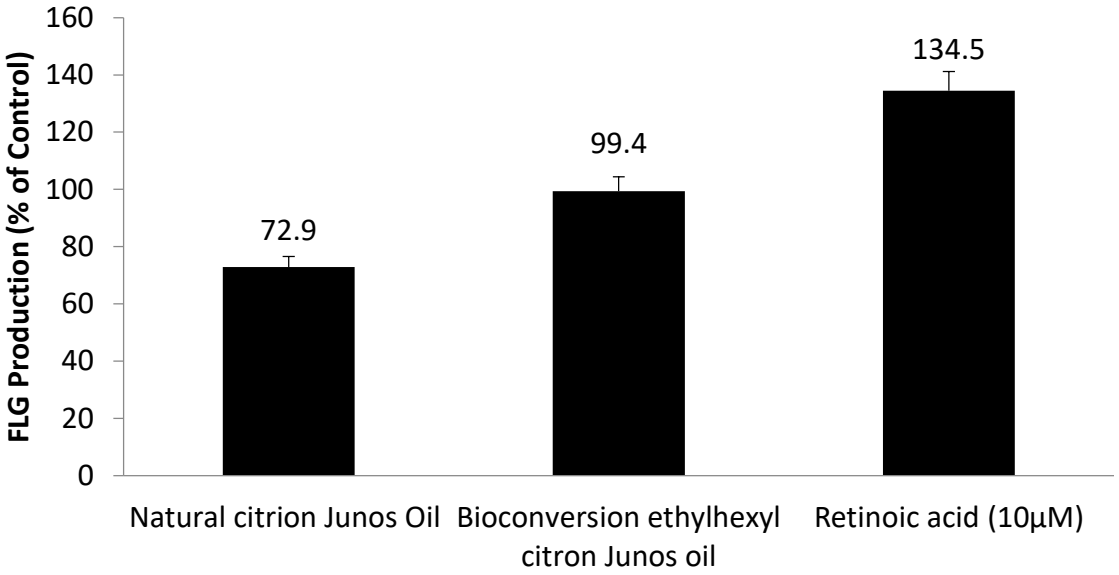
Overall size and depth of application of citron seed oil and ethylhexyl citron oil showed statistically significant improvement in wrinkles around the eyes after 4 weeks of application of the sample. In addition, no special skin adverse reactions were observed during the test period and it was safe.

Moisturizing effect of Natural citrion Junos Oil and Bioconversion-ethylhexyl citron oil

As a result of measuring the production of hyaluronic acid (HA) at a concentration without cytotoxicity, HA was increased in all compared to the control group. In particular, in the case of bioconversion(BC) ethylhexyl citron oil, it was confirmed that the amount of hyaluronic phase production was high at 161.7%(Fig. 1). As an evaluation index for moisturizing efficacy, filaggrin (FLG) production was not increased compared to the retinol acid positive control group (Fig. 2).



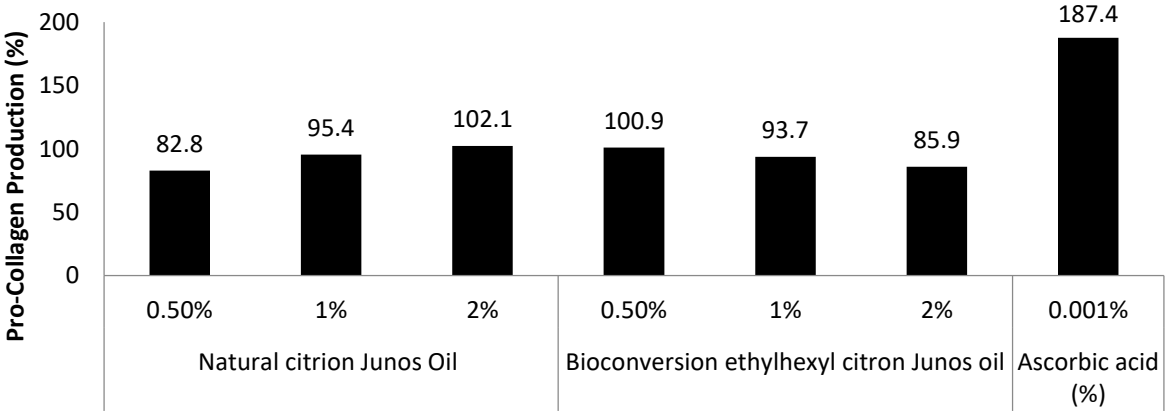
Figures 1. Effect of moisturiaing on production of hyaluronic acid (HA). HaCaT Cells were treated with 0.1% concentrations of oils for 24hr. The data represent the mean ± SD of triplicate experiments.



Figures 2. Measurement of filaggrin (FLG) production as an evaluation index for moisturizing efficacy using keratinocytes (HaCaT cells). HaCaT Cells were treated with 0.1% concentrations of oils for 24hr. The data represent the mean ± SD of triplicate experiments.

Procollagen contents effect of Natural citrion Junos Oil and Bioconversion-ethylhexyl citron oil

As a result of measuring the amount of procollagen, it was confirmed that neither citron oil nor ethylhexyl citron oil had a significant effect compared to the positive control. In particular, in the case of ethylhexyl citron oil, it decreased in a dose-dependent manner, and in the case of natural citron oil, the concentration was similar to that of the control group at 2% concentration (Fig. 3).



Figures 3. Effects of procollagen contents. HDF cell were treated with dosage(0.5, 1, 2%) concentrations of oils for 24 hrs. The data represent the mean ± SD of triplicate experiments.

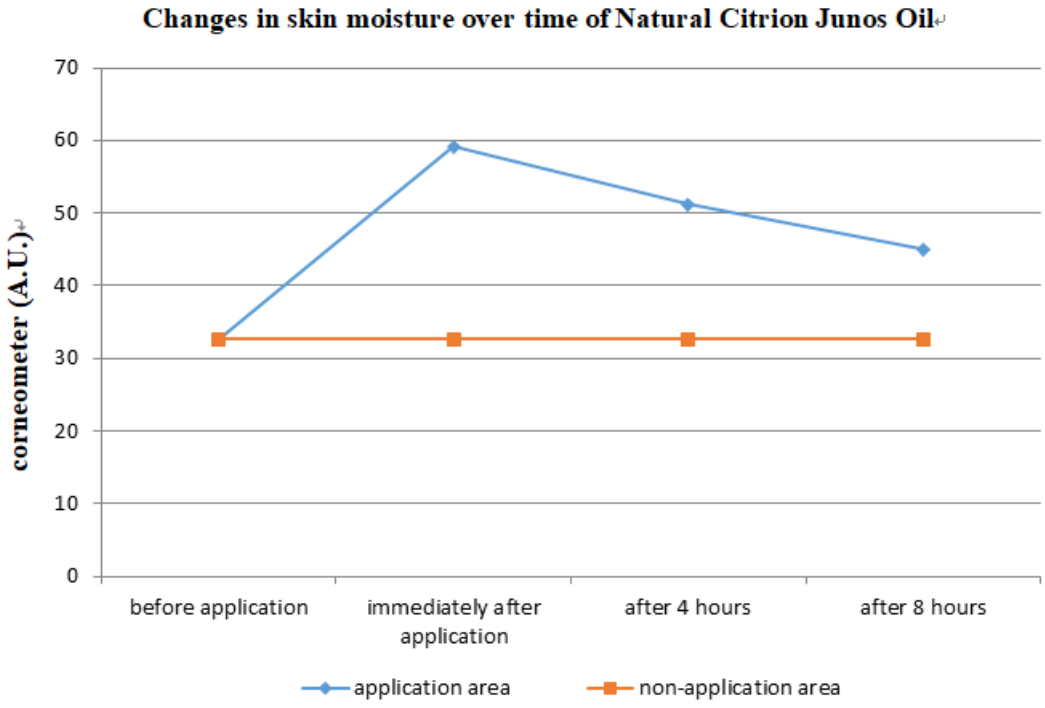
Clinical trial results for safty

As a result of the test, Natural citrion Junos Oil and Bioconversion-ethylhexyl citron oil showed no irritation at all 30 minutes, 24 hours, and 48 hours after patch removal. It was applied to the skin for 24 hours, and 30 minutes, 24 hours, and 48 hours after the patch was removed, the skin reaction at the test site was stimulated according to the criteria of the International Contact Dermatitis Research Group (ICDRG). The degree was classified, and the average skin reactivity (Mean score) was obtained according to the skin patch test result judgment table.

Moisturizing human efficacy evaluation

The result of moisture measurement of citron oil, 59.2 A.U. immediately after product application, 51.2 A.U.. after 4 hours, 45.0 A.U. after 8 hours. As compared to the non-applied site, it showed a statistically significant level of moisture improvement and 8-hour moisturizing effect immediately after product application, 4 hours, and 8 hours after application ($p < 0.05$) (Fig.4 (A)). As a result of moisture measurement of ethylhexyl citron oil, it was confirmed that there was a change in moisture to 54.2 A.U. immediately after application of the product, 47.5 A.U. after 4 hours, and 43.1 A.U. after 8 hours(Fig.4 (B)).

(A)



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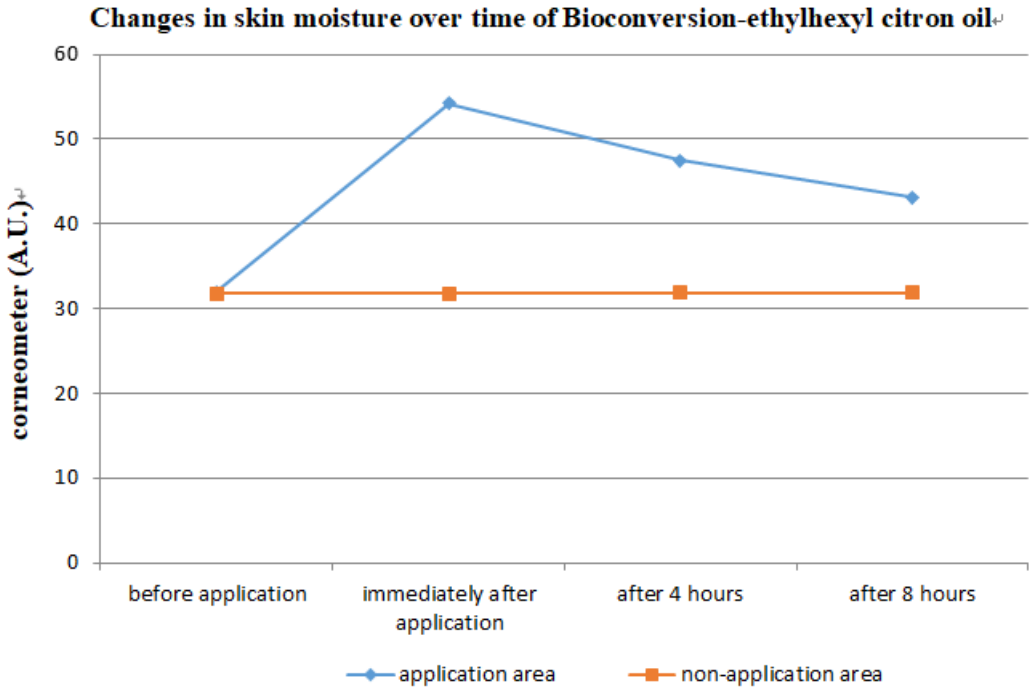


Figure 4. (A) Changes in skin moisture over time of Natural Citrion Junos Oil, (B) Changes in skin moisture over time of Bioconversion-ethylhexyl citron oil. As compared to the non-applied

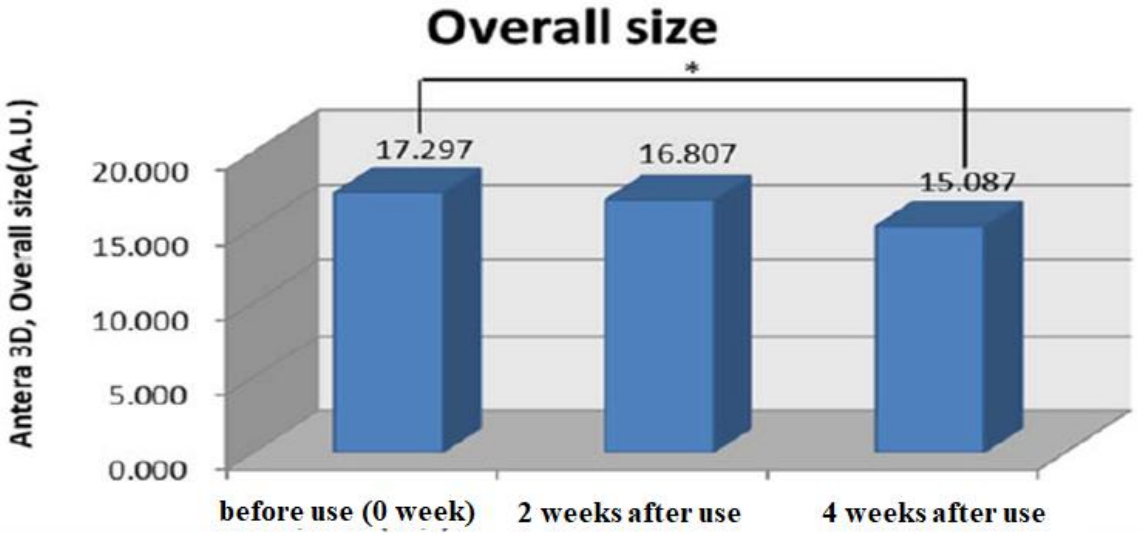
site, it showed a statistically significant level of moisture improvement and 8-hour moisturizing effect immediately after product application, 4 hours, and 8 hours after application ($p < 0.05$) .

Evaluation of human efficacy for improving eye wrinkles

The result of measuring wrinkles around the eyes of the group using , Natural Citrion Junos Oil before (0 weeks) using the product Overall size17.297 A.U., Depth 0.058 ㎜, After 2 weeks of using the product Overall size 16.807 A.U., Depth0.057 ㎜, Overall size after 4 weeks of using the product 15.087 A.U., Depth 0.050 mm (Fig. 5). Compared to before product use (week 0), overall size 3.054 %, depth 3.009 % after 2 weeks of product use, and overall size 12.782 % and depth 15.204 % after 4 weeks of product use were shown(Fig. 6).

As a result of measuring wrinkles around the eyes of the group using , Bioconversion-ethylhexyl citron oil before (0 weeks) using the product Overall size16.373 A.U., Depth 0.054 ㎜, After 2 weeks of using the product Overall size 16.176 A.U., Depth0.053 ㎜, Overall size after 4 weeks of using the product 14.309 A.U., Depth 0.046 mm (Fig. 7). Compared to before product use (week 0), overall size 1.310 %, depth 2.735 % after 2 weeks of product use, and overall size 12.837 % and depth 14.606 % after 4 weeks of product use were shown(Fig. 8).

(A)



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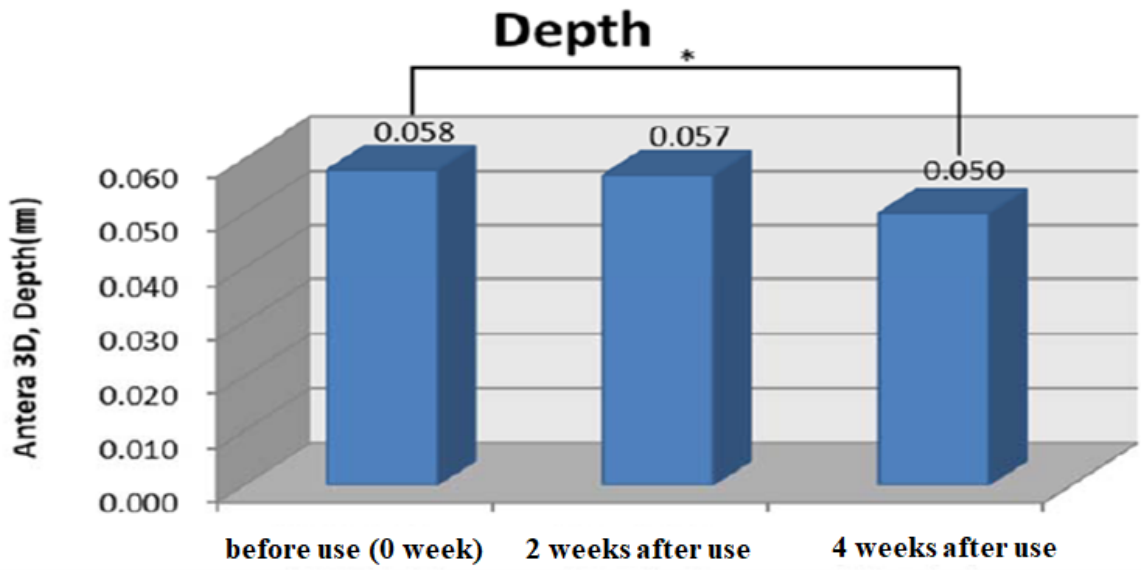
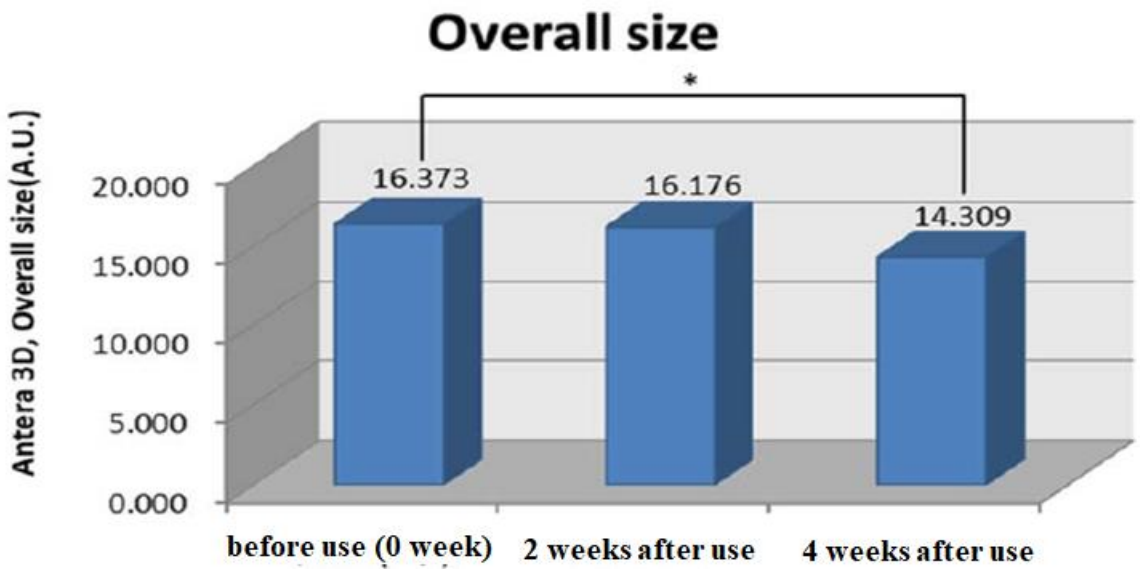


Figure 5. Changes in wrinkles around the eyes after using Natural Citrion Junos Oil. (A) Overall size (B) Depth. Measurement result of wrinkles around the eyes using ANTERA 3D. Analysis using ANTERA 3D was expressed in overall size and depth, the overall size unit was A.U., and the depth unit was mm. Overall size and Depth showed a statistically significant level of improvement in wrinkles around the eyes after 4 weeks of using the product ($p<0.05$).

(A)



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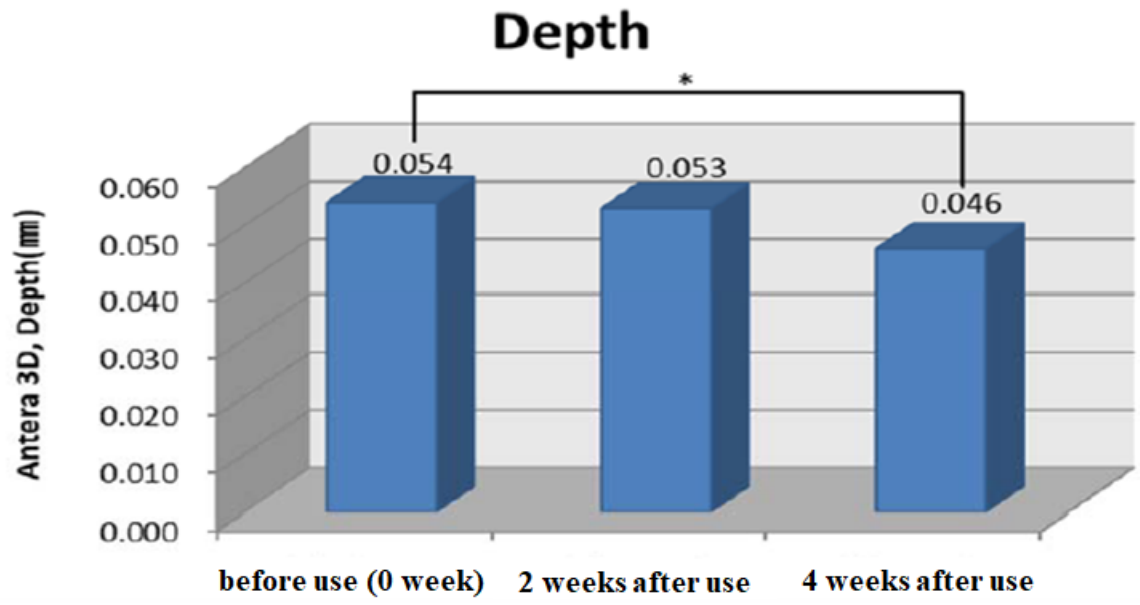

Figure 7. Changes in wrinkles around the eyes after using Bioconversion-ethylhexyl citron oil. (A) Overall size (B) Depth. Measurement result of wrinkles around the eyes using ANTERA 3D. Analysis using ANTERA 3D was expressed in overall size and depth, the overall size unit was A.U., and the depth unit was mm. Overall size and Depth showed a

statistically significant level of improvement in wrinkles around the eyes after 4 weeks of using the product ($p<0.05$).

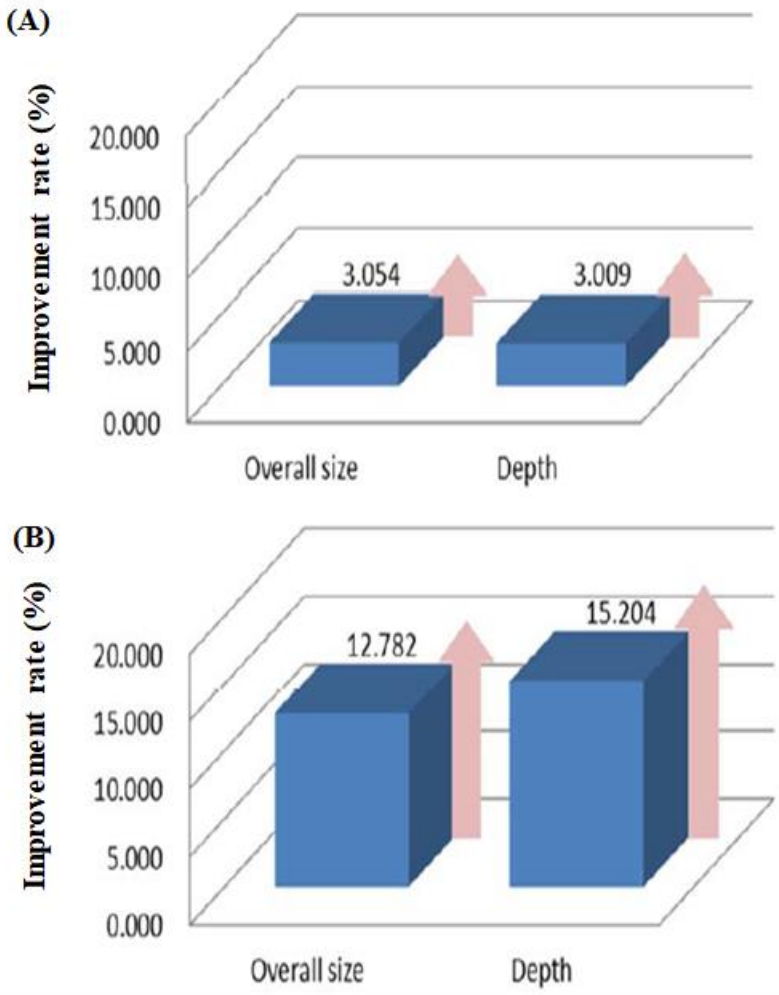

Figure 8. Improvement rate of wrinkles around the eyes after using Bioconversion-ethylhexyl citron oil. (A) Improvement rate of wrinkles around the eyes after 2 weeks of use, (B) Improvement rate of wrinkles around the eyes after 4 weeks of use

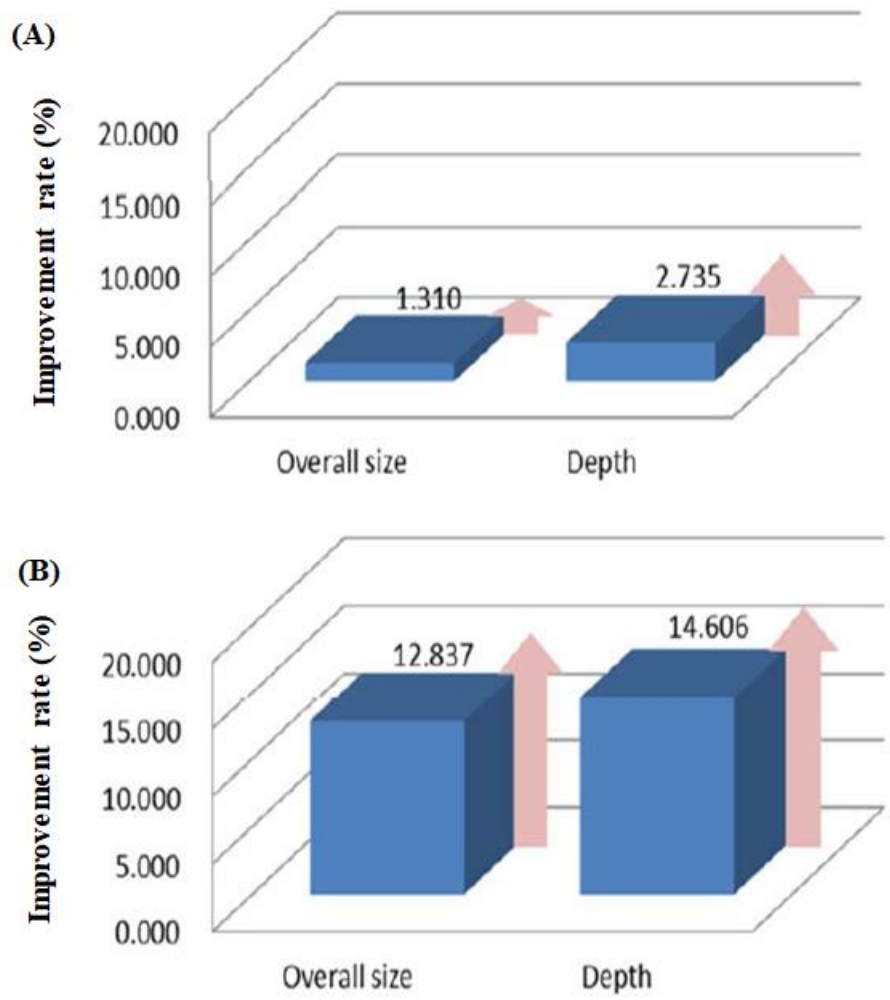

Figure 8. Improvement rate of wrinkles around the eyes after using Natural Citrion Junos Oil. (A) Improvement rate of wrinkles around the eyes after 2 weeks of use, (B) Improvement rate of wrinkles around the eyes after 4 weeks of use.

Discussion and Conclusion

In this study, a cosmetic emollient that meets upcycling and clean beauty trends was developed using the by-product of citron in Korea. In addition, despite the bioconversion technology using enzymes, the disadvantages of natural vegetable oils, which are sticky and oily, have been supplemented. Unlike natural milk oil mainly composed of triglycerides by the transesterification reaction principle, the content of triglycerides in bioconverted milk oil was significantly reduced, and the content of mono- and di-glycerides increased relatively. Both natural milk oil and bioconverted milk oil increased the production of hyaluronic acid by 30-40%, confirming the moisturizing effect and the improvement of wrinkles around the eyes.

Natural oils extracted from plants can satisfy consumers who are looking for eco-friendly materials, but there are limitations in cosmetic formulations due to stickiness and oily feeling. Bio-conversion technology improves light feeling, distribution, absorption and softness when applied to the skin. In addition, anti-inflammatory and moisturizing effects are improved, so it is expected to continue to expand into cosmetics and external skin applications.

Conflict of Interest Statement.

The authors do not have any conflict of interests regarding the publication of this paper.

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