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The Efficacy of AP Collagen Peptides Intake on Skin Wrinkle, Elasticity, and Hydration Improvement

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Abstract

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Collagen is a component of the human body, known to have a beneficial effect on human health when consumed as a food or dietary ingredient. This double-blinded, randomized, placebo-controlled study aimed to evaluate the efficacy and safety of AP collagen peptides (APCP) containing 3% glycine-proline-hydroxyproline, on improving skin wrinkles, elasticity and hydration. Totally, 105 subjects with crow's-feet wrinkles were randomly assigned to receive either placebo, APCP 1,000 mg or APCP 1,500 mg, once daily for 12 weeks. Efficacy endpoints of skin wrinkles, elasticity and hydration were measured at baseline, 6 and 12 weeks, and at the 2-week follow-up visit. Compared to the placebo group, the visual evaluations and R3 (average roughness) values of crow's-feet wrinkles were significantly decreased in both APCP groups at 12 weeks, and these effects were maintained until 2 weeks

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after completion of ingestion. During the intake period at 6 and 12 weeks, three parameters for skin elasticity (R2, R5, R7) showed significant improvement in both APCP groups, as compared to the placebo group. The APCP groups also showed a statistically significant difference in skin hydration compared to the placebo group, at 12 weeks and at 2 weeks after the end of intake. No adverse effects were encountered during the study period. These results indicate that APCP 1,000 mg and 1,500 mg can be used as a functional food ingredient to improve human skin conditions related to skin aging and hydration. R7) showed significant improvement in both APCP groups, as compared to the placebo group. The APCP groups also showed a statistically significant difference in skin hydration compared to the placebo group, at 12 weeks and at 2 weeks after the end of intake. No adverse effects were encountered during the study period. These results indicate that APCP 1,000 mg and 1,500 mg can be used as a functional food ingredient to improve human skin conditions related to skin aging and hydration. R7) showed significant improvement in both APCP groups, as compared to the placebo group. The APCP groups also showed a statistically significant difference in skin hydration compared to the placebo group, at 12 weeks and at 2 weeks after the end of intake. No adverse effects were encountered during the study period. These results indicate that APCP 1,000 mg and 1,500 mg can be used as a functional food ingredient to improve human skin conditions related to skin aging and hydration.

Keywords : collagen peptide, skin wrinkles, skin elasticity, skin hydration

Introduction

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Recently, as interest in health increases, the intake of foods having a physiologically active function is increasing. In particular, as the concept of inner beauty, which promotes health and beauty through the consumption of food or food materials, expands, the development of related functional food materials is becoming active (Jung and Lee, 2014 ; Shim and Lee, 2019). There are functional ingredients for skin health such as hyaluronic acid, chlorella, spirulina, and collagen peptides as these inner beauty food materials (MFDS, 2016). Among them, as beneficial evidence of skin health through collagen intake has been revealed, related health functional foods development is increasing (Barati et al., 2020).

Collagen is one of the major structural proteins constituting the extracellular matrix and plays an essential role in protecting the human body, such as imparting tensile strength and maintaining cellular structure (Subhan et al., 2015 ; Silva et al., 2014) . In particular, the qualitative and quantitative change in the skin of collagen, which occupies 90% of the dermal layer of the skin, induces a decrease in various metabolic activities and causes skin aging (Varani et al., 2000 ; Shin et al . , 2019). Skin aging is divided into intrinsic aging due to aging and extrinsic aging such as photoaging caused by UV exposure, all of which induce structural changes in the extracellular matrix such as a decrease in the amount of collagen and collagen fiber length, thereby reducing the elasticity of the dermal layer and causing deep wrinkles. It causes skin dryness and pigmentation (Scharffetter-Kochanek et al., 2000 ; El-Domyati et al., 2002). In order to delay skin aging, various materials that help maintain the extracellular matrix in vivo are being developed as cosmetics or health foods, and collagen is also becoming a major material (McCabe et al . , 2020).

Collagen, which is present in the skin/scales and bones of pigs, cattle, and fish, has biocompatibility and bioactivity in the skin and weak immunogenicity compared to other proteins, so it is a food that can be safely consumed (Avila Rodríguez et al . , 2018). When collagen is ingested, dipeptides such as glycine-proline (Gly-Pro) or proline-hydroxyproline (Pro-Hyp) or glycine-proline-hydroxyproline (Gly-Pro-Hyp)) are absorbed into the body in the form of tripeptides and distributed to tissues through the bloodstream (Daniel, 2004 ; Iwai et al., 2005 ; Ohara et al . , 2010). To consume the dipeptide or tripeptide low molecular weight collagen hydrolyzate content is high for biodistribution than intake of collagen in the polymer forms directly increase the body-absorbing efficiency aid in rapid distribution of the skin (Yamamoto, etc., 2016 ; Hong, etc. , 2019). Recently, meaningful results have been derived from skin aging studies using low molecular weight collagen hydrolyzate (Barati et al., 2020).), also in this study, using AP collagen enzyme-decomposing peptide (APCP), a low-molecular collagen hydrolyzate containing 15% or more tripeptide, to improve skin wrinkles, elasticity and moisture in adult women with skin wrinkles was to check.

Materials and Methods

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Human application test design

This human trial was designed as a double-blind, placebo-controlled, randomized trial. Subjects of this human application test participated in the study after hearing sufficient explanation from the investigator and voluntarily consenting in writing before participating, and checked whether the selection/exclusion criteria were met and received the AP collagen enzyme-decomposing peptide 1,000 mg group (APCP 1,000 mg) , A total of 105 people were randomly assigned to the AP collagenase peptide 1,500 mg intake group (APCP 1,500 mg) and the control food intake group (Placebo), 35 in each group. The registered subjects consumed the test food or control food for a total of 12 weeks , and according to the test visit schedule (Fig. 1), they visited the 0, 6, and 12 week visits and 2 weeks after the intake was finished. Efficacy evaluation was conducted on skin wrinkles (visual and device evaluation), skin elasticity and skin moisture improvement, and safety evaluation was conducted on adverse reactions after ingestion, clinical laboratory tests (hematologic/hematochemical and urinalysis), vital signs and body examination was conducted. This study was conducted in accordance with the ICH Clinical Trial Management Standards and the standard operating guidelines of ELID Co., Ltd. after approval by the ELID Clinical Trial Review Committee (IRB deliberation number: EL-90221JXZ01).

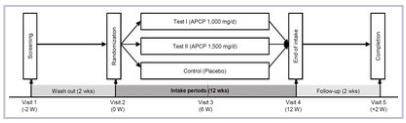

Fig 1. Clinical study overview.

Foods and intake methods for human application testing

In this test, an AP collagen enzyme-decomposing peptide (indicator component: Gly-Pro-Hyp 3.0±0.6%) made from gelatin extracted from the scales of Nemipterus virgatus was used (Jellice Co., Sendai, Japan). . A control food having the same properties and taste as the test food containing APCP 1,000 mg and 1,500 mg was used. For food for human application test, 50 mL of liquid ampoule was taken once a day at the same time for 12 weeks.

study subjects

A woman aged between 40 and 60 years who has wrinkles around the eyes and has a global photodamage score (Chung et al., 2001) of 2 points (mild) to 6 points (severe, severe) by a dermatologist . was targeted. A person with a skin disease that may affect the test, a person with irritation or allergy to food containing ingredients related to the test product, a person who has undergone dermatological procedures or management, a person with a medical condition, or a person who may affect the test at the discretion of the principal Those who could have been excluded. Subjects were prohibited from consuming health functional foods other than food for human application testing during the human application test period, and only used products (skin, lotion, sunscreen and face wash) provided for the same skin condition. For accurate measurement, after washing the face, the subject lightly drained and waited in a constant temperature and humidity room (temperature 22±2°C, humidity 40-60% RH) for 30 minutes before proceeding with the test.

Improves skin wrinkles and roughness

For skin wrinkle improvement, visual evaluation and device evaluation by a dermatologist were performed for the subject's crow's feet. The eye area to be evaluated for skin wrinkles was designated before ingestion so that the same area was always evaluated, and images were taken under the same conditions using a facial fixation device for wrinkle photography and a high-resolution digital camera. Visual evaluation was performed by two dermatologists with a global photodamage score (0: none, 1: none/mild, 2: mild, 3: mild/moderate, 4: moderate, 5: moderate/severe, 6: severe, 7: very severe), but if there is a difference between each evaluator, a low value before ingestion and a high value after ingestion were selected and evaluated. For device evaluation, a silicone replica was made in the wrinkle area around the eyes, and the roughness was measured using the image analyzer software of Visiometer SV600 (Courage & Khazaka Electronics, Koln, Germany), R1 (skin roughness), R2 (maximum roughness), R3 (average). roughness), R4 (smoothness depth), and R5 (arithmetic average roughness) were analyzed. As wrinkles decreased, the degree of roughness decreased.

improve skin elasticity

Skin elasticity was measured using a Cutometer MPA580 (Courage & Khazaka Electronics) to measure the elasticity of the eyelid area of the face. The measured values were R2 (gross elasticity), R5 (net elasticity), and R7 (biological elasticity). The closer the measured value was to 1, the higher the elasticity was evaluated.

improve skin hydration

The moisture content of the stratum (*p*)rneum was measured with Corneometer CM825 (Courage & Khazaka Electronic). Corneometer CM825 is a device that measures the amount of moisture contained within the 30-40 μm depth of the epidermal layer. The same area of the subject's cheek was measured 5 times, and the average of three values excluding the maximum and minimum values was obtained.

statistical analysis

Statistical analysis of the human body test was performed using SPSS Statistics (ver. 26.0, IBM Corp., Armonk, NY, USA). $P<0.05$ level or less. Normality of data was tested by Kolmogorov-Smirnov test, and comparison between test groups was tested by Independent t-test or Mann-whitney U test.

Results and Discussion

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General characteristics of subjects

This human application test was conducted to evaluate the efficacy and safety of APCP for improving skin wrinkles, elasticity, and moisture. A total of 110 subjects who voluntarily agreed to participate in this human application study were evaluated for suitability of selection/exclusion criteria, and a total of 105 subjects were registered. A safety set was performed on a total of 104 subjects who consumed food for human application at least once after registering for the human application test and secured safety data, and the per protocol set was conducted for 12 weeks without violation of the protocol. A total of 101 subjects (34 subjects in the APCP 1,000 mg group, 33 people in the APCP 1,500 mg group, and 34 subjects in the control group) who completed the follow-up period of 2 weeks after ingestion were included. A summary of the participants' participation status and analysis group is shown in Fig. same as 2 There was no difference between groups in the analysis of the demographic information and pre-intake characteristics of subjects who participated in this human application test (Table 1), and there was no difference between the groups in the intake compliance and protein intake of food for human application testing. There were no clinically meaningful changes in clinical laboratory tests and vital signs for safety evaluation, and no adverse reactions or adverse reactions related to the test food were reported. No safety issues were reported in human application tests of similar collagen peptides using the same Gly-Pro-Hyp as APCP as an indicator (Koizumi et al., 2018 ; Kim et al., 2018b)). Based on these results, it is judged that the safety of APCP in oral intake has been confirmed.

Table 1 . Baseline characteristics of subjects

		Placebo (n=34)	APCP 1,000 mg (n=34)	APCP 1,500 (n=33)
Age (years)	Mean±SD	47.79±4.35	47.56±4.72	48.42±5.7
	Min, Max	40, 56	40, 59	41, 60
Weight (kg)	Mean±SD	59.08±7.44	58.12±8.04	57.21±6.3
	Min, Max	46.8, 78.3	45.0, 77.4	46.1, 75.(
Crow's feet visual grade	Mean±SD	4.12±0.77	4.24±0.70	4.30±0.7
	Min, Max	3, 6	3, 6	3, 6

One)*P*-value, comparisons between values in APCP 1,000 mg group and placebo group.

2)*P*-value, comparisons between values in APCP 1,500 mg group and placebo group.

Fig 2. Clinical study flow. Flow diagram showing the progress of subjects at each stage of the clinical trial.

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Improves skin wrinkles and roughness

Repeated UV exposure reduces collagen synthesis in the dermis through transforming growth factor (TGF) inhibition, and causes deep wrinkles while decomposing collagen in the skin by inducing matrix metalloproteinases (Brnneisen et al., 2002). APCP is a gene of type I collagen decreased after UV irradiation in human fibroblasts (*COL1A*, collagen type I alpha) and TGFβ1 gene expression (Lee et al., 2021), and induced an increase in procollagen expression to protect against structural damage to the dermal layer (Kim et al . , 2009). In addition, when APCP was orally administered to rats induced by UV exposure, epidermal and dermal thickness increased by photostimulation decreased, and skin wrinkles such as total area of wrinkles, total number of wrinkles, total length of wrinkles, and physical shape of wrinkles were improved. (Lee et al . , 2021). Based on these results, the skin wrinkle improvement effect was confirmed after ingestion of APCP in this human application test. As a result of visual evaluation of the eye wrinkles by a specialist and the Visiometer SV600 image analysis method, both the APCP intake group showed significant improvement compared to the control food intake group at 12 weeks after ingestion and 2 weeks after the end of intake in the visual evaluation (Fig. 3A , Fig. 4). The average roughness R3 of the visiometer measurement values of the eye wrinkles showed a significant improvement effect from 6 weeks on both the APCP intake group compared to the control food intake group, and the significant skin wrinkle improvement effect continued until 12 weeks and 2 weeks after the end of intake (Fig. 3B). Table 2 shows the amount of change in R1~5 for the roughness of the wrinkles around the eyes during the entire test period.and decreased in all groups. The APCP 1,000 mg group showed significant improvement compared to the control group at 6 weeks, 12 weeks, and 2 weeks after ingestion for R2, which is an indicator of wrinkle depth improvement, compared to the control food intake group, and R1 was 6 weeks after ingestion and after stopping intake. At 2 weeks, R4 and R5 showed significant improvement effect at 2 weeks after discontinuation of intake. The APCP 1,500 mg group showed significant improvement in R1~3 at least one of 6 weeks, 12 weeks, or 2 weeks after ingestion. Based on these results, it is judged that APCP has the effect of improving skin wrinkles during the intake period and up to 2 weeks after intake.

Table 2 . Changes in skin roughness parameters

Weeks		placebo	APCP 1,000 mg	APCP 1,500 mg
	6	−0.045±0.100	−0.109±0.121 *	−0.097±0.098 *
R1	12	−0.058±0.094	−0.102±0.092	−0.104±0.106
	+2	−0.060±0.084	−0.127±0.107 **	−0.093±0.113
	6	−0.011±0.063	−0.063±0.088 **	−0.061±0.059 **
R2	12	−0.035±0.054	−0.061±0.053 *	−0.064±0.081
	+2	−0.036±0.065	−0.069±0.061 *	−0.051±0.079
	6	−0.006±0.037	−0.037±0.038 ***	−0.038±0.033 ***
R3	12	−0.022±0.023	−0.041±0.029 **	−0.039±0.040 *
	+2	−0.020±0.031	−0.043±0.037 **	−0.038±0.045 *

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Week		placebo	APCP 1,000 mg	APCP 1,500 mg
R4	6	−0.023±0.061	−0.048±0.059	−0.041±0.049
	12	−0.023±0.054	−0.039±0.062	−0.042±0.052
	+2	−0.027±0.046	−0.059±0.058 *	−0.042±0.056
R5	6	−0.012±0.027	−0.019±0.030	−0.014±0.025
	12	−0.006±0.028	−0.018±0.029	−0.015±0.027
	+2	−0.009±0.025	−0.024±0.029 *	−0.016±0.027

Change in skin roughness R1 (skin roughness), R2 (maximum roughness), R3 (average roughness), R4 (smoothness depth) and R5 (arithmetic average roughness) measured by Visiometer SV 600. Results are expressed as mean±SD. A asterisk indicates comparisons between APCP 1,000 mg group and placebo group, APCP 1,500 mg group and placebo group ($^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$).

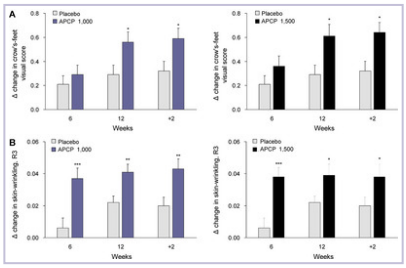

Fig 3. Changes in the skin wrinkle parameters. (A) Change (Δ) in visually assessed Crow's feet scores. (B) Change (Δ) in skin wrinkle R3 (average roughness) measured by Visiometer SV600. Results are expressed as mean±SEM. A asterisk indicates comparisons between APCP 1,000 mg group and placebo group, APCP 1,500 mg group and placebo group ($^{*}P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$).

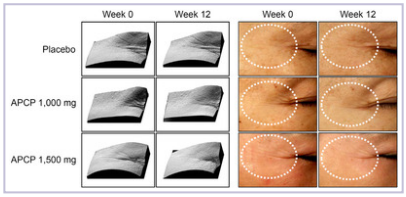

Fig 4. Image of skin wrinkle changes after 12 weeks. Representative images of the subjects in each group at baseline and week 12. Left, skin replica imaging; Right, digital photographs.

improve skin elasticity

The main cause of the decrease in skin elasticity with aging is the structural change of the extracellular matrix due to a decrease in the amount of collagen in the dermis (Kohl et al., 2011 ; Shin et al . , 2019). In particular, continuous exposure to ultraviolet light induces fibroblast hyperproliferation and inflammatory cell infiltration, and accelerates structural collapse by making the arrangement of collagen fibers and elastic fibers, which are connective tissues in the dermis, irregular (Rabe et al. , 2006). When APCP was orally administered to UV-exposed mice, inflammatory infiltration, such as histamine, an inflammatory cytokine, decreased, and collagen and elastin in the dermis increased, confirming structural improvement of the extracellular matrix, total elasticity, net elasticity, ratio of viscoelasticity, and biological elasticity. The same skin elasticity index improved (Kim et al., 2011 ; Lee et al . , 2021). Therefore, in this study, the effect of improving skin elasticity in the corners of the eyes by APCP intake was confirmed using the R2, R5 and R7 indicators. In the case of R2, which is an overall skin elasticity index, the APCP 1,000 mg group showed a significant difference compared to the control group at 6 weeks and 12 weeks, and the APCP 1,500 mg group showed a significant difference at 6 weeks, 12 weeks, and 2 weeks after the end of intake. showed a significant difference compared to the control food intake group (Fig. 5A). In R5, which is an index of skin elasticity, the APCP intake group showed a significant difference compared to the control food intake group at 6 weeks, 12 weeks, and 2 weeks after the end of intake (Fig. 5B). In R7, an indicator of immediate elastic recovery, the APCP 1,000 mg intake group showed a significant difference at 6 weeks and 12 weeks, and the APCP 1,500 mg intake group showed a significant difference compared to the control food intake group at 6 weeks, 12 weeks, and 2 weeks after the end of intake (Fig. 5C), APCP intake is judged to have an effect on skin elasticity improvement.

Rabe JH, Mamelak AJ, McElgunn PJS, Morison WL, Sauder DN. Photoaging: mechanisms and repair. J Am Acad Dermatol. 2006;55:1-19 (https://pubmed.ncbi.nlm.nih.gov/16781287) (https://doi.org/10.1016/j.jaad.2005.05.010)

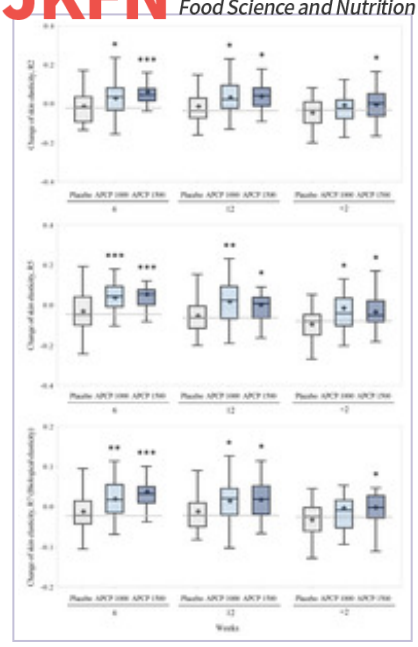

Fig 5. Changes of skin elasticity measured by Cutometer. Box plot graphs illustrating the time-dependent change in the gross elasticity, R2 (A), net elasticity, R5 (B) and biological elasticity, R7 (C). The box contains the 25th to 75th percentile, and the horizontal line within the box represents the median; the rhombus represents the mean and the error bars cover the 5th to 95th percentiles; the gray dotted line represents the median value of the placebo group. A asterisk indicates comparisons between APCP 1,000 mg group and placebo group, APCP 1,500 mg group and placebo group ($^{*}P$< 0.05, $^{**}P$<0.01, and $^{***}P$<0.001).

improve skin hydration

The stratum corneum of the skin consists of keratinocytes and intercellular lipids such as ceramides, cholesterol, and free fatty acids, and has a skin barrier function that absorbs moisture from the outside and maintains moisture in the skin (Blanken et al . Ceramide is known to play an important role in maintaining the moisture content of the stratum corneum of the skin (Coderch et al., 2003 ; Wertz and Downing, 1982). When collagen peptide is treated, ceramide kinase, a ceramide kinase, is activated and Increases ceramide synthesis by inducing the expression of serinepalmytoyl transferase, a biosynthetic enzyme (Kim et al., 2018a ; Kim et al . , 2011). Hyaluronic acid, known as another moisturizing factor, is a major component of the extracellular matrix, along with collagen, and has a hydrophilic structure, so it has excellent water retention ability and plays an important role in maintaining skin moisture (Nudecker et al., 2000). Collagen increases the expression of hyaluronan synthase, a hyaluronic acid synthase, and induces hyaluronic acid synthesis by inhibiting the hyaluronic acid synthase by stimulating the proliferation of fibroblasts in the dermal layer and chemotaxis. is known (Shigemura et al., 2009 ;Ohara et al., 2010 ; Kang et al . , 2018). It was confirmed that the hyaluronic acid in the dermal layer was recovered and the skin moisture content increased when collagen was orally administered to mice whose skin moisture content was decreased by UV exposure (Oba et al., 2013 ; Kim et al., 2009 ; Lee et al . , 2021). In other words, it can be explained that the collagen peptide induces the synthesis of skin moisturizing factors such as ceramide and hyaluronic acid to increase epidermal homeostasis, thereby helping the barrier function of the stratum corneum of the skin and maintaining moisture (Gu et al., 2010 ; Cha et al . , 2016). Accordingly, in this study as well, the change in the moisture content of the stratum corneum of the skin was confirmed using Corneometer CM825. As a result, both the APCP 1,000 mg and 1,500 mg intake groups increased water content from 6 weeks, and compared to the control food intake group at 12 weeks and 2 weeks after the end of intake. It showed a significant increase (Fig. 6). As a result, it is judged that APCP has an effect of improving moisture retention in the stratum corneum of the skin.

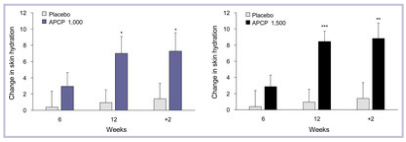

Fig 6. Changes of skin hydration measured by Corneometer. Results are expressed as mean±SEM. A asterisk indicates comparisons between APCP 1,000 mg group and placebo group, APCP 1,500 mg group and placebo group ($^{*}P$<0.05, $^{**}P$<0.01, and $^{***}P$<0.001).

summary

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In this study, a double-blind, randomized, comparative human application test of APCP for skin wrinkles, elasticity and moisture improvement was conducted on adult women aged 40 to 60 years. In the visual evaluation of the wrinkle area around the eyes and the skin wrinkle improvement evaluation using a visiometer, both the APCP 1,000 mg and 1,500 mg groups showed significant improvement compared to the control food group at 12 weeks, and skin wrinkle improvement was maintained until 2 weeks after the end of ingestion. was able to confirm In the evaluation of skin elasticity improvement, both the APCP 1,000 mg and 1,500 mg intake groups showed significant improvement compared to the control food intake group from the 6th week on the R2, R5 and R7 indicators, and showed a statistically significant difference until the 12th week. In the skin moisturizing improvement evaluation, both the APCP 1,000 mg and 1,500 mg intake groups showed significant improvement compared to the control food intake group at 12 weeks, and the skin moisture improvement status was maintained until 2 weeks after the end of intake. During the study, there were no significant changes in adverse events, laboratory tests, and vital signs tests, confirming the safety of APCP. Through the results of this human application test, it was confirmed that APCP is a health functional food that can help improve skin health such as skin wrinkles, roughness, elasticity and moisture.

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