

Oral Supplementation of Hydrolyzed Fish Collagen, Acerola and Sodium Hyaluronate on Facial Skin: A Double-Blind, Randomized, Placebo-Controlled Trial

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Abstract

A person's appearance influences others' first impression of them, and as such, people of all ages and sexes, especially women, value their facial appearance. In addition to external maintenance and anti-aging products, oral health products have always been popular. The objective of this study was to investigate whether the combination of hydrolyzed fish collagen, acerola and sodium hyaluronate improve skin condition. Sixty subjects were randomly allocated to the placebo or a collagen beverage. After consuming collagen beverage (YOUNIQUE DAILY•YOU liquid collagen shot) for 8 weeks, the total antioxidant capacity (TAC) was significantly increased by 90% and the Glutathione S-transferase (GST) activity was elevated by 17% compared to baseline (week 0). After consuming collagen beverage for 8 weeks, the average value of skin collagen, texture, elasticity, hydration, lightness, melanin was improved by 9.3%, 12.9%, 4.3%, 4.4%, 3.0%, 3.4% compared to baseline (week 0). Finally, through a questionnaire survey, the subjects felt that their skin was improved. This clinical study demonstrates the combination of hydrolyzed fish collagen, acerola and sodium hyaluronate for substantial improvements in facial skin's appearance.

Keywords: Acerola, Hydrolyzed Fish Collagen, Skin Aging, Sodium Hyaluronate

Introduction

Population aging is a global trend, and the most obvious change after aging is the change in skin appearance. Quantitative and structural changes in collagen fibers are the major modifications found in aged skin [1,2]. In contrast to those in young skin, which has abundant, tightly packed, and well-organized intact collagen fibrils, collagen fibrils in aged skin are fragmented and coarsely distributed [3]. Studies have shown that both increased collagen degradation and decreased collagen biosynthesis are associated with collagen homeostasis. This process leads to clinical changes, such as skin wrinkling and loss of elasticity, which are observed in both naturally and photoaged skin [3]. Therefore, active ingredients that delay skin aging have attracted attention in recent years [4]. In recent years, supplements have become a way to prevent aging and maintain. Based on the functional demands of consumers for health care products, they hope to obtain multiple functions such as hydrating, whitening, anti-aging and protecting against exposing to sunlight when using the same product [4]. Therefore, the ingredients of health care products are gradually becoming more complex and diversified, and the use of natural raw materials is more become a trend in the development of health care products. Oral use of anti-aging skin care products was the most convenient and cheapest way to achieve skin anti-aging effects [5]. Thus, as functional foods and oral supplements, nutraceuticals are increasingly investigated to improve skin health.

Common sources of collagen on the market are animal collagen, such as pig feet and chicken feet, and fish collagen is one of the sources of collagen, which can be obtained from fish scales and skin [6]. Most of them come from freshwater fish. Contamination and less infectious diseases, the absorption rate of human body is higher than other animal collagen [7]. Some studies have shown that fish collagen supplements can improve skin wrinkles, elasticity, and moisture and have been proven to reduce wrinkles and pores [8]. The molecular weight of fish scale collagen is the key to affecting the degree of absorption, and the molecular weight below 1500 is the most suitable for human absorption [9]. Hydrolyzed collagen is a small molecule, it can be quickly decomposed and absorbed after entering the human body [10]. It is currently the most effective way to supplement collagen. Hydroxyproline is the most important amino acid in collagen, accounting for about 10%. It is the main component of collagen tissue and the main amino acid that can really be absorbed [11]. With the increase of age, the synthesis ability of collagen in the body declines. It is necessary to take in enough hy-

droxyproline to effectively supplement the collagen in the body [12]. Studies in vivo in woman between 40 and 60 years of age with hydrolyzed collagen oral supplementation during 12 weeks, showed a significant improvement in skin hydration, wrinkling, and elasticity [13]. Hydrolyzed collagen as an oral nutrient supplement in women between 35 and 65 years of age proved after three months enhancement in dermal thickness, skin firmness, and elasticity after treatment [14]. A study of sixty healthy female subjects, aged between 40 and 50 years, after 28 days of oral supplementation showed that this acted on skin elasticity and presented a more pronounced effect on dermis echogenicity, reducing skin pores, improving hydration, texture, elasticity, and firmness of the skin [15]. In addition to hydrolyzed fish collagen, natural plant extracts were recently added to skin care products.

Acerola (*Malpighia emarginata*) is a fruit that is found throughout Central America and within the northern part of South America. Because acerola has high concentrations of ascorbic acid in the edible part, it is considered to be one of the best sources of ascorbic acid [16]. Additionally, acerola contains numerous functional phytochemicals, such as carotenoids and polyphenols [17]. In the skin, the study reported that the intake of acerola polyphenol extracts prevents UVB-induced skin pigmentation in brownish guinea pigs, which possess moderate numbers of melanocytes and melanosomes in the epidermis [18]. Acerola extract has been shown to protect fibroblasts from oxidative damage, has antioxidant capacity [19]. 72 healthy women aged 35 years taking 2.5g collagen peptides, 0.6g acerola fruit extract improves skin hydration, elasticity, roughness [20].

Skin aging is also associated with loss of skin moisture. The key molecule involved in skin moisture is sodium hyaluronate or called hyaluronic acid (HA) [21]. HA is a linear glycosaminoglycan composed of 2000–25 000 repeats of the disaccharides glucuronic acid (GlcA) and N-acetylglucosamine (GlcNAc) joined alternately by β -1-3 and β -1-4 glycosidic acids [22]. HA has many important functions in the body, such as providing cushioning in human joints, stimulating the immune system and keeping skin smooth and elastic [23]. Therefore, HA has been widely used in health, beauty and clinical fields. Sodium hyaluronate is a derivative of hyaluronic acid, and is a humectant, which means that it attracts moisture [23]. Microbial HA production requires simpler downstream processing, is devoid of seasonal fluctuations, shows less batch to batch variation and reduces the risk of viral contamination [24]. In this regard, the most frequently used bacteria for industrial HA production is *Streptococcus zooepidemicus* [24]. 20 female subjects with healthy

skin in the age group of 45 to 60 years taking collagen with HA improves skin hydration, wrinkles reduction, elasticity [25].

This study used YOUNIQUE DAILY•YOU liquid collagen shot, which contained hydrolyzed fish collagen, acerola and sodium hyaluronate derived from *S.zooepidemicus*, to explore whether the skin can be improved after consuming the collagen beverage. This study evaluated the clinical benefits of an 8-week supplementation of a collagen beverage on skin appearance and blood biochemistry.

Materials and Methods

Clinical trial design

The study was registered in clinicaltrials.gov (No. NCT05376657), was performed under a protocol approved by the Antai Medical Care Cooperation Antai- Tian-Sheng Me-

morial Hospital Institutional Review Board (Approval Number: 22-010-B), and was conducted according to the code of ethics on human experimentation established by the Declaration of Helsinki (1964) and its amendments. Written informed consent was obtained from all participants after a full explanation of the study. A double-blinded, placebo controlled, randomized study was conducted (Figure 1). The subjects were randomly assigned to two groups with 30 subjects in each group. The subjects were informed to consume one sachet (25 ml) of the drink every day for 8 weeks. Before measurements, subjects were instructed to wash and wipe their face, and acclimatize for at least 30 min to the standardized laboratory conditions (room temperature 25°C, RH 55 ± 5%). Skin conditions and self-assessment questionnaires of the subjects were collected at week 0, week 4 and week 8 of the study. The fasting blood samples were collected at week 0 and week 8 of the study, and examined BUN, Creatinine, AST, ALT, TAC, GST. Inclusion criteria: Healthy adults aged above 20 years old. The exclusion criteria included:

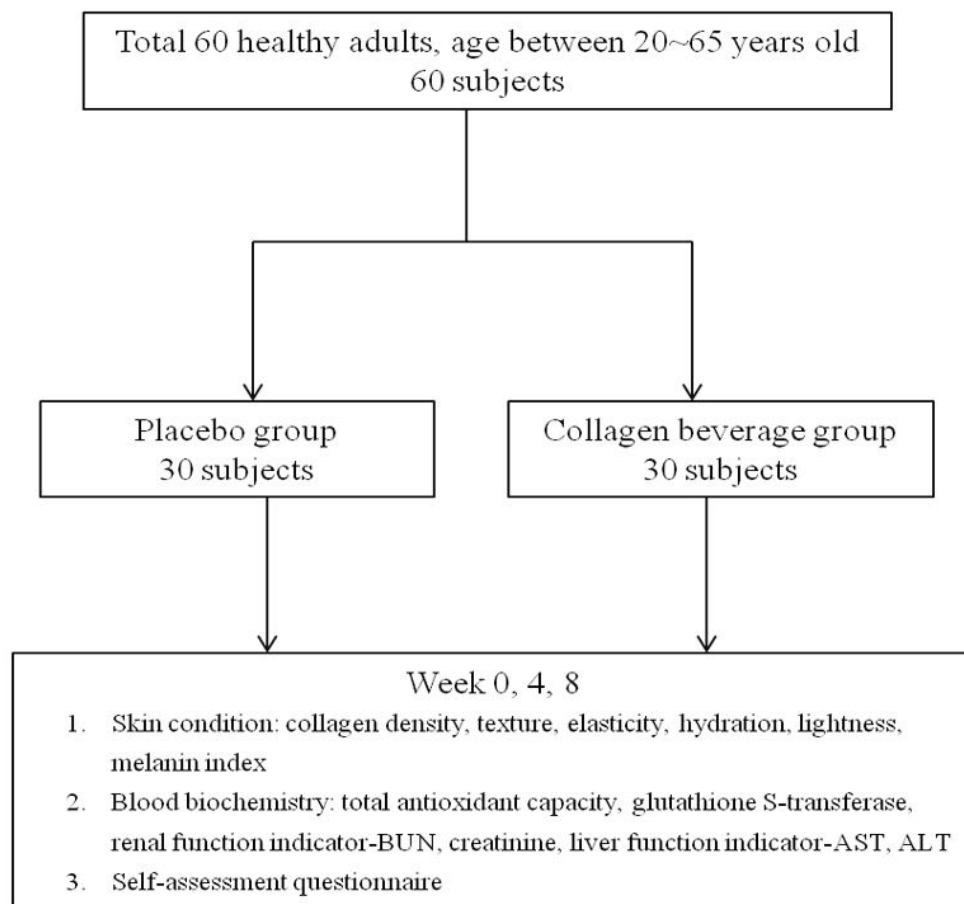


Figure 1: Clinical trial flowchart

- i) skin disease, liver cirrhosis, or chronic renal failure;
- ii) allergy to cosmetics, drugs, or foods;
- iii) pregnant and breastfeeding;
- iv) taking chronic drugs;
- v) people who had any cosmetic procedures (intense pulse light, medical peelings, or laser therapy) before 4 weeks of the study.

Supplement formulation

The YOUNIQUE DAILY•YOU liquid collagen shot contains 0.2% hydrolyzed fish collagen, 0.01% acerola and 0.004% sodium hyaluronate derived from *S.zooepidemicus*, citric acid, and water. The placebo beverage contains primarily citric acid and water. Subjects were required to consume 25 mL of the collagen beverage or a placebo beverage daily before meal for 8 weeks. The placebo and experimental product were packaged in the same appearance, shape, and size. Neither the subject nor the operator will know the content group to be taken.

Clinical skin efficacy assessment

DermaLab[®] Series SkinLab Combo was utilized to scan and analyze skin collagen density. The color scale indicates collagen density; white reflects the highest collagen density, and black reflect the lowest. Cutometer[®] dual MPA580 was utilized to measure skin elasticity, and the higher the relative value, the more significant the improvement. Corneometer[®] CM825 was utilized to measure skin moisture content, and the higher the relative value, the more significant the improvement. Chroma Meter MM500 was utilized to measure skin lightness, and the higher the relative value, the whiter the skin tone. Mexameter[®] MX18 was utilized to measure skin melanin index, and the

lower the relative value, the more significant the improvement. VISIA Complexion Analysis System was utilized to measure skin texture. Texture measures skin smoothness by identifying gradations in color from the surrounding skin tone. The lower the relative value, the greater the improvement.

Statistical analysis

The comparison of measurement results for skin parameters among groups and between groups was analyzed by student's t-test through GraphPad Prism, as $p < 0.05$ was considered statistical significance.

Results

Combined with hydrolyzed fish collagen, acerola and sodium hyaluronate had anti-oxidation effect

During the trial in safety assessment, there was no skin irritation or any other discomforts reported by the subjects. The results of blood biochemistry analysis were listed in Table 1. After consuming collagen beverage for 8 weeks, the values of liver related marker (AST, ALT), and kidney function related marker (BUN, creatinine) were not significantly changed. TAC and GST activity were utilized to evaluate the antioxidant capacity. After consuming collagen beverage for 8 weeks, the TAC was significantly increased by 90% (Figure 2A) and the GST activity (Figure 2B) was elevated by 17% compared to baseline (week 0), and the TAC was significantly increased by 86% compared to placebo group. The results showed that collagen beverage do not have adverse reactions and can have antioxidant effects.

Table 1: Demographic and results of biochemical analyses.

	Placebo		Collagen beverage	
Subject number	30		30	
Female	25		26	
Male	5		4	
Age (years)	38.8		37.7	
	Week 0	Week 8	Week 0	Week 8
AST (IU/L)	18.1 ± 1.3	20.1 ± 2.0	16.2 ± 0.8	16.2 ± 1.0
ALT (IU/L)	16.7 ± 1.9	17.6 ± 2.0	14.7 ± 1.8	15.5 ± 1.5
BUN (mg/dL)	13.9 ± 0.6	12.3 ± 0.5	11.7 ± 0.5	10.9 ± 0.4
Creatinine (mg/dL)	0.76 ± 0.03	0.77 ± 0.02	0.67 ± 0.02	0.68 ± 0.03

Data were expressed as the mean ± S.E.M; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen. Significant difference versus baseline: **, $p < 0.01$

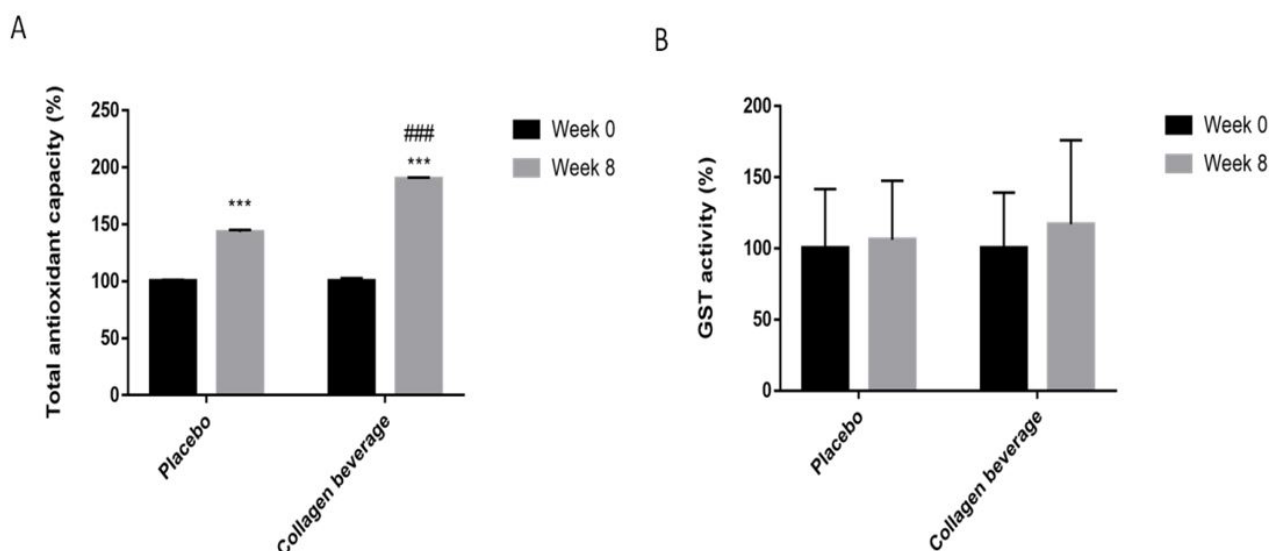


Figure 2: The collagen beverage had anti-oxidation effect. The (A) total antioxidant capacity, (B) Glutathione S-transferase, (n = 30; mean value \pm SD.) *, compared with baseline (week 0). #, compared with placebo) (***, $p < 0.001$, ###, $p < 0.001$)

Combined with hydrolyzed fish collagen, acerola and sodium hyaluronate increased collagen, elasticity, hydration, lightness and improved texture, melanin

After consuming collagen beverage for 8 weeks, the average value of skin collagen density was respectively increased by 5.0% and 9.3% (Figure 3A), and the skin texture was respectively improved by 12.0% and 12.9% compared to baseline (week 0) on week 4 and week 8 (Figure 3B). In addition, after consuming collagen beverage for 8 weeks, the skin elasticity was respectively improved by 3.4% and 4.3% (Figure 3C), and the skin hydration was respectively improved by 4.3% and 4.4% compared to baseline (week 0) on week 4 and week 8 (Figure 3D). Finally, after consuming collagen beverage for 8 weeks, the skin lightness was respectively improved by 2.1% and 3.0% (Figure

4A), and the skin melanin index was respectively improved by 1.7% and 3.4% compared to baseline (week 0) on week 4 and week 8 (Figure 4B-C). The results showed that collagen beverage increased collagen, elasticity, hydration, lightness and decreased texture, melanin.

Combined with hydrolyzed fish collagen, acerola and sodium hyaluronate improved skin condition

The questionnaires were conducted for overall skin sagging, roughness condition, and severity of sensitive skin, dry itchy skin and dry scaly skin, and severity of uneven skin tone, dull skin and yellowish skin tone. The results showed that after consuming collagen beverage for 8 weeks, subjects felt the overall skin conditions were improved (Figure 5).

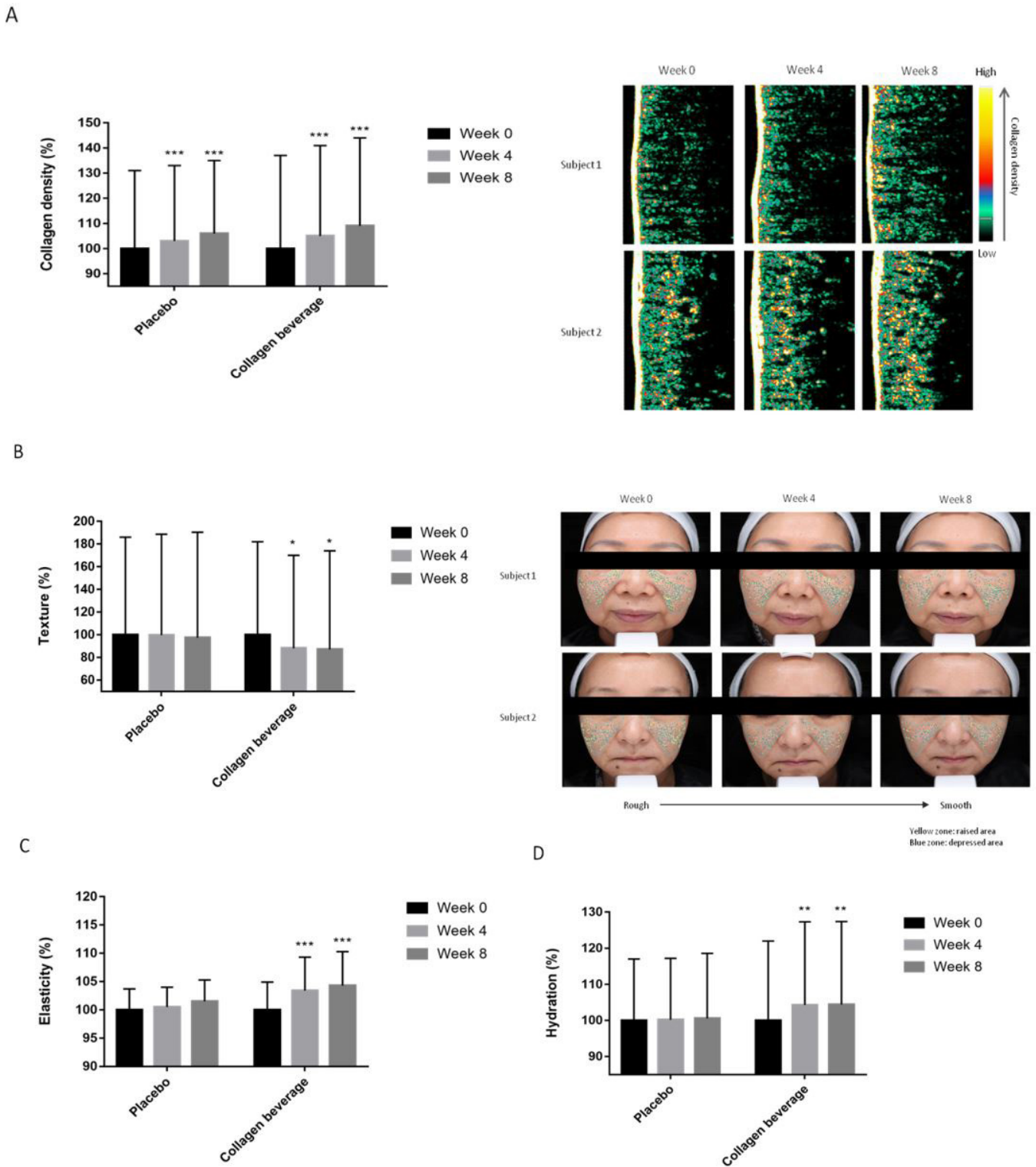


Figure 3: The collagen beverage improved skin collagen, texture, elasticity, hydration. The (A) collagen, (B) texture, (C) elasticity, (D) hydration (n = 30; mean value \pm SD.) *, compared with baseline (week 0) (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$)

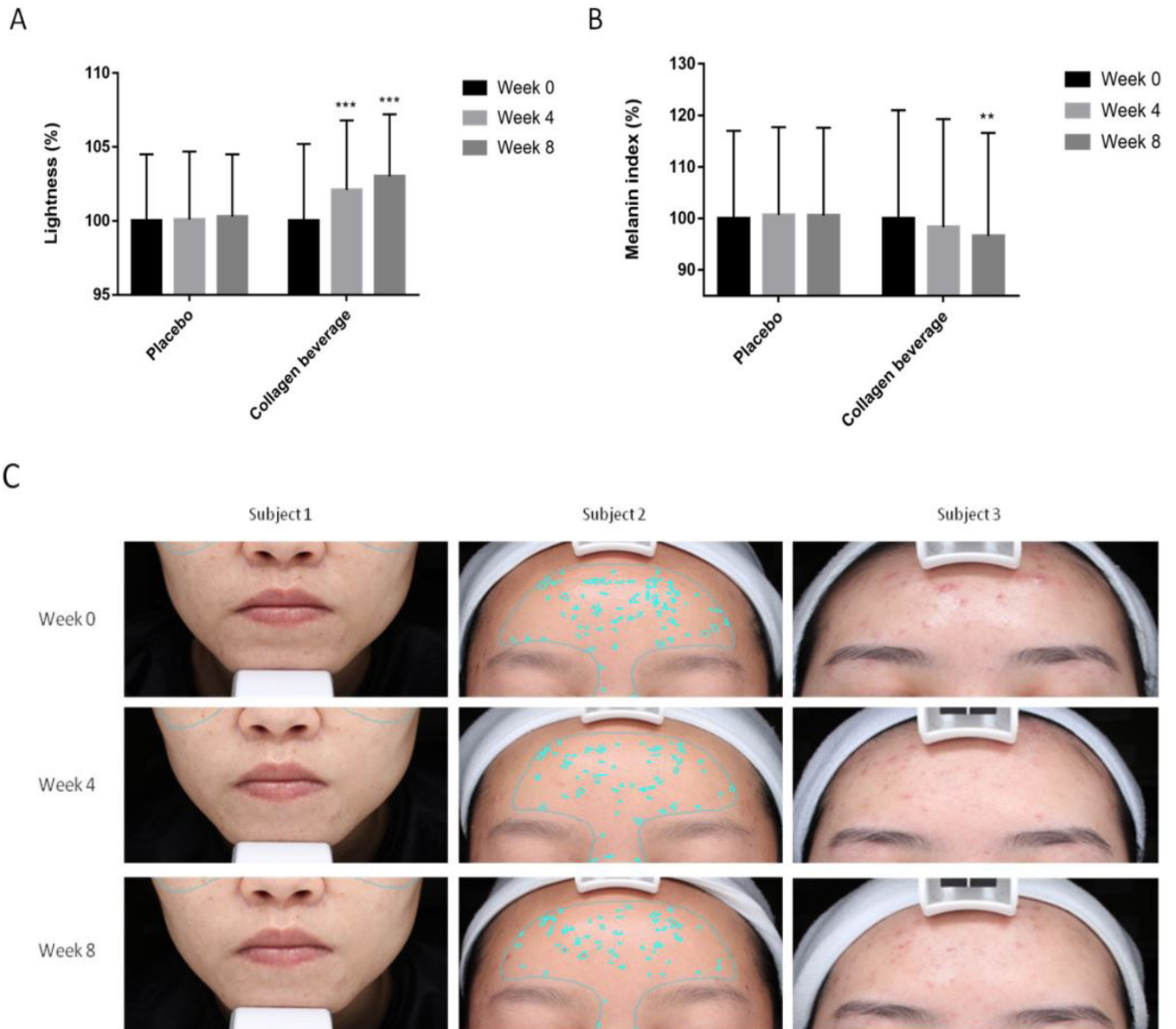


Figure 4: The collagen beverage improved skin lightness, melanin. The (A) lightness, (B) melanin, (C) image of skin pigmentation and skin tone ($n = 30$; mean value \pm SD.) *, compared with baseline (week 0) (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$)

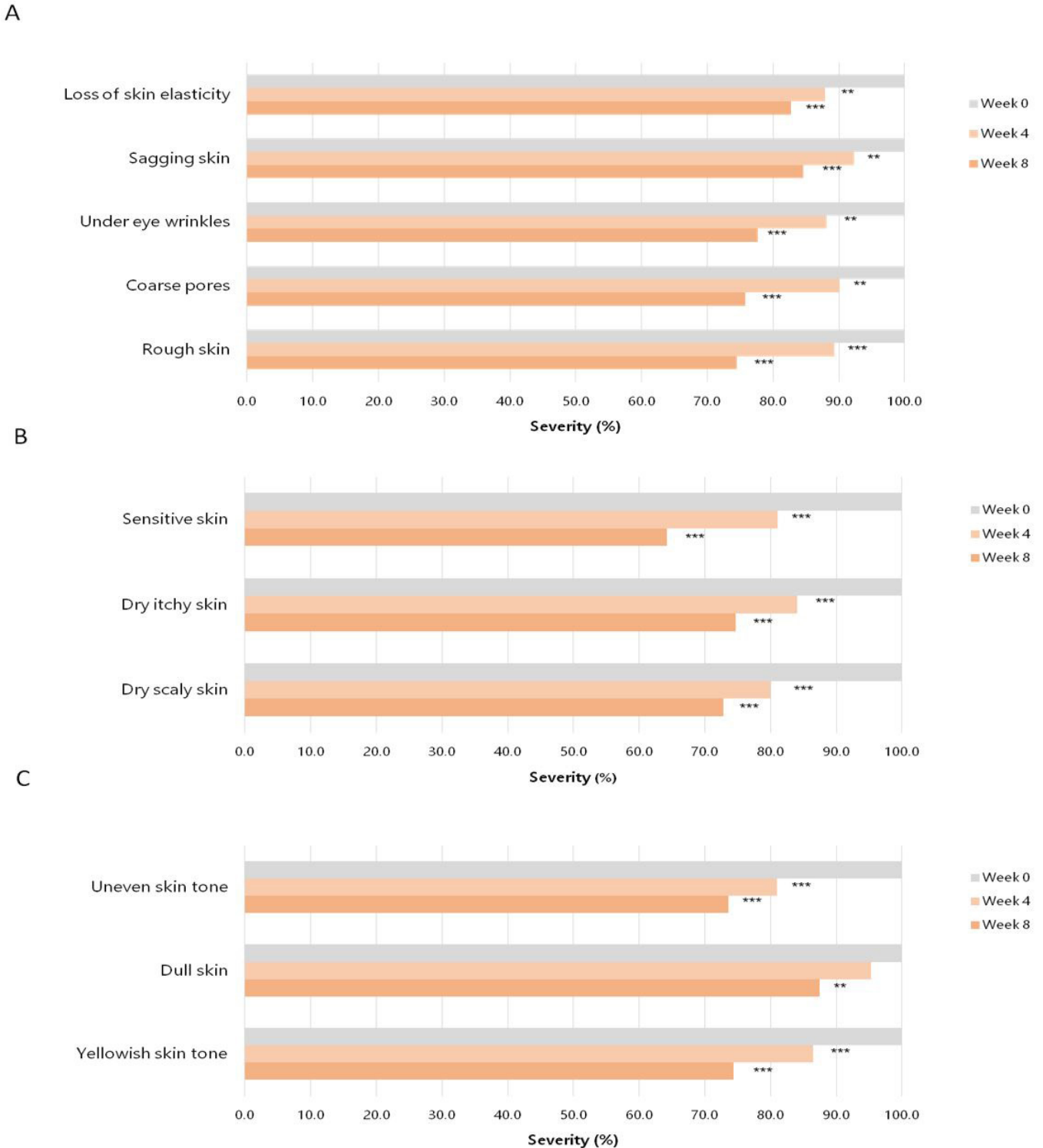


Figure 5: The collagen beverage improved skin condition. 60 subjects were recruited and divided into 2 groups, one was the placebo group (n=30) and the other was the collagen beverage group (n=30). Take one bottle of beverage daily for 8 weeks, and then examined (A) skin sagging, roughness condition, (B) severity of sensitive skin, dry itchy skin and dry scaly skin, (C) severity of uneven skin tone, dull skin and yellowish skin tone. *, compared with baseline (week 0) (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$)

Discussion

This study found that combination with hydrolyzed fish collagen, acerola and sodium hyaluronate increased antioxidant ability, collagen, elasticity, hydration, lightness and decreased texture, melanin, and improved skin condition through questionnaires, suggesting collagen beverage improves overall skin condition.

Skin appearance depends on the collagen skeleton, for example, wrinkle formation has been associated to decreased collagen synthesis and increased collagenase activity [5]. Hydrolyzed fish collagen is made up of mostly Type I collagen [26]. The function of type I collagen is to reverse the aging process and reduces the appearance of wrinkles and cellulite, to enhance skin hydration and firmness [27]. Hydrolyzed fish collagen is rich in two special amino acids: glycine and proline. Glycine is the basis for the production of DNA and RNA strands, while proline is the basis for the body's ability to naturally produce its own collagen [28]. Glycine blocks endotoxins and transports nutrients to body cells [28]. Proline acts as an antioxidant for the body, prevents free radical damage to cells, and promotes collagen synthesis [11]. Hydrolyzed fish collagen has been shown to have anti-aging properties [29]. Supplementing with hydrolyzed fish collagen can reduce the number of skin wrinkles and help improve skin hydration, firmness, smoothness, elasticity [20]. Hydrolyzed fish collagen peptides have been shown to significantly reduce crow's feet, and improve skin elasticity and moisture while reducing evaporation [30]. Hydrolyzed collagen as an oral nutrient supplement in women between 35 and 65 years of age proved after three months enhancement in dermal thickness, skin firmness, and elasticity after treatment [14]. Oral supplementation of hydrolyzed collagen in women between 40 and 59 years of age revealed a significant increment in skin hydration and collagen density at dermis level. However, fragmentation of the dermal collagen network significantly decreased after four weeks of supplementation and these effects persisted after 12 weeks [31]. Hydrolyzed collagen has been used in processed foods such as sausages to replace pork fat at 50% level of replacement. The final product results had greater water holding capacity, better stability after cooking, and improved texture such as hardness and chewiness [32]. Hydrolyzed collagen from fish can be added in beverages such as orange juice (2.5%), and the product showed an improvement in nutritional and functional properties with a higher protein content, bioavailability, and low viscosity as well as high solubility in water [33].

Acerola is a tropical fruit, well known as a healthy fruit due to its high content of nutraceutical compounds like phenolics and vitamin C. Acerola has also been demonstrated to exhibit beneficial effects, such as anti-inflammatory effect, and suppresses excessive UVB-induced skin pigmentation [34]. Polyphenol extracts (cyanidin-3- α -O-rhamnoside, pelargonidin-3- α -O-rhamnoside and Astilbin) from acerola inhibit tyrosinase activity. Acerola fruit exerts superoxide radical scavenging activity [34]. Acerola extract is a potent source of antioxidants and hydrating polysaccharides, some of which have been shown to protect fibroblasts in skin from suffering oxidative damage [34]. In addition to high levels of ascorbic acid (vitamin C), it has been reported that the vitamin C of acerola is better absorbed by human beings than the synthetic ascorbic acid [35]. It was found that the mixture of the polyphenol, and vitamin C could act synergistically to protect lipid peroxidation and collagen degradation [36]. Vitamins E, C and flavonoids are major natural antioxidants capable of preventing damage generated by oxidative stress [37], and synergistically in quenching free radicals [38]. In addition, acerola contains a variety of phytonutrients such as carotenoids, phenolics, flavonoids, and anthocyanins, with a variety of biofunctional properties [16]. Carotenoids and flavonoids act as antioxidants for the skin, protecting it from UV light exposure, and improving tone, brightness, photo-protection, and increase the collagen I synthesis through Phosphoinositide 3-kinases (PI3K) [39].

HA has traditionally been extracted from animal tissues such as synovial fluid, rooster combs, cartilage, vitreous humour and umbilical cords [40]. However, fermentative HA production by *Streptococcus* generates yields with higher concentrations of HA at lower costs and with more efficient downstream processes [41]. Among the strains of this bacteria, *S. zooepidemicus* is one of the most commonly used [42]. The size of the HA polymers in the skin gradually decreases with age. The epidermis loses its primary molecules responsible for binding and retaining water molecules, resulting in a loss of skin moisture, leading to the marked dehydration, atrophy, and loss of elasticity of aging skin [21]. Hyaluronic acid supplements improve skin moisture and brighten. Consistent with our results, combined with hydrolyzed fish collagen, acerola and sodium hyaluronate, can improve skin conditions. This study had confirmed that collagen beverage included hydrolyzed fish collagen, acerola and sodium hyaluronate improves skin condition. This product can be used as one of the choices for future skin care products.

Conflicts of Interest

All authors declare no conflict of interest.

References

1. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S et al. (1997) Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 337: 1419-28.
2. Quan T, Fisher GJ (2015) Role of Age-Associated Alterations of the Dermal Extracellular Matrix Microenvironment in Human Skin Aging: A Mini-Review. *Gerontology* 61: 427-34.
3. Shin JW, Kwon SH, Choi JY, Na JI, Huh CH et al. (2019) Molecular Mechanisms of Dermal Aging and Antiaging Approaches. *Int J Mol Sci* 20.
4. Shanbhag S, Nayak A, Narayan R, Nayak UY (2019) Anti-aging and Sunscreens: Paradigm Shift in Cosmetics. *Adv Pharm Bull* 9: 348-59.
5. Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC (2012) Skin anti-aging strategies. *Dermatoendocrinol* 4: 308-19.
6. Leon-Lopez A, Morales-Penaloza A, Martinez-Juarez VM, Vargas-Torres A, Zeugolis DI, et al. (2019) Hydrolyzed Collagen-Sources and Applications. *Molecules* 24.
7. Jafari H, Lista A, Siekapen MM, Ghaffari-Bohlouli P, Nie L et al. (2020) Fish Collagen: Extraction, Characterization, and Applications for Biomaterials Engineering. *Polymers (Basel)* 12.
8. Al-Atif H (2022) Collagen Supplements for Aging and Wrinkles: A Paradigm Shift in the Fields of Dermatology and Cosmetics. *Dermatol Pract Concept* 12: e2022018.
9. Carvalho AM, Marques AP, Silva TH, Reis RL (2018) Evaluation of the Potential of Collagen from Codfish Skin as a Biomaterial for Biomedical Applications. *Mar Drugs* 16.
10. Wang H (2021) A Review of the Effects of Collagen Treatment in Clinical Studies. *Polymers (Basel)* 13.
11. Wu G, Bazer FW, Burghardt RC, Johnson GA, Kim SW, et al. (2011) Proline and hydroxyproline metabolism: implications for animal and human nutrition. *Amino Acids* 40: 1053-63.
12. de Paz-Lugo P, Lupianez JA, Melendez-Hevia E (2018) High glycine concentration increases collagen synthesis by articular chondrocytes in vitro: acute glycine deficiency could be an important cause of osteoarthritis. *Amino Acids* 50: 1357-65.
13. Kim DU, Chung HC, Choi J, Sakai Y, Lee BY (2018) Oral Intake of Low-Molecular-Weight Collagen Peptide Improves Hydration, Elasticity, and Wrinkling in Human Skin: A Randomized, Double-Blind, Placebo-Controlled Study. *Nutrients* 10.
14. Addor FAS, Cotta Vieira J, Abreu Melo CS (2018) Improvement of dermal parameters in aged skin after oral use of a nutrient supplement. *Clin Cosmet Investig Dermatol* 11: 195-201.
15. Maia Campos P, Melo MO, Siqueira Cesar FC (2019) Topical application and oral supplementation of peptides in the improvement of skin viscoelasticity and density. *J Cosmet Dermatol* 18: 1693-9.
16. Prakash A, Baskaran R (2018) Acerola, an untapped functional superfruit: a review on latest frontiers. *J Food Sci Technol* 55: 3373-84.
17. Nowak D, Goslinski M, Wojtowicz E, Przygonski K (2018) Antioxidant Properties and Phenolic Compounds of Vitamin C-Rich Juices. *J Food Sci* 83: 2237-46.
18. Hanamura T, Uchida E, Aoki H (2009) Skin-Lightening Effect of a Polyphenol Extract from Acerola (*Malpighia emarginata* DC.) Fruit on UV-Induced Pigmentation. *Bioscience, biotechnology, and biochemistry* 72: 3211-8.
19. Alvarez-Suarez JM, Giampieri F, Gasparrini M, Mazzoni L, Santos-Buelga C et al. (2017) The protective effect of acerola (*Malpighia emarginata*) against oxidative damage in human dermal fibroblasts through the improvement of antioxidant enzyme activity and mitochondrial functionality. *Food Funct* 8: 3250-8.
20. Bolke L, Schlippe G, Gerss J, Voss W (2019) A Collagen Supplement Improves Skin Hydration, Elasticity, Roughness, and Density: Results of a Randomized, Placebo-Controlled, Blind Study. *Nutrients* 11.

21. Papakonstantinou E, Roth M, Karakiulakis G (2012) Hyaluronic acid: A key molecule in skin aging. *Dermatoendocrinol* 4: 253-8.
22. Lu JF, Zhu Y, Sun HL, liang S, Leng FF et al. (2016) Highly efficient production of hyaluronic acid by *Streptococcus zooepidemicus* R42 derived from heterologous expression of bacterial haemoglobin and mutant selection 62: 316-22.
23. Gupta RC, Lall R, Srivastava A, Sinha A (2019) Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory. *Front Vet Sci* 6: 192.
24. Zakeri A, Rasaei MJ, Pourzardosht N (2017) Enhanced hyaluronic acid production in *Streptococcus zooepidemicus* by over expressing HasA and molecular weight control with Niscin and glucose. *Biotechnol Rep (Amst)* 16: 65-70.
25. Gollner I, Voss W, von Hehn U, Kammerer S (2017) Ingestion of an Oral Hyaluronan Solution Improves Skin Hydration, Wrinkle Reduction, Elasticity, and Skin Roughness: Results of a Clinical Study. *J Evid Based Complementary Altern Med* 22: 816-23.
26. Sanchez A, Blanco M, Correa B, Perez-Martin RI, Sotelo CG (2018) Effect of Fish Collagen Hydrolysates on Type I Collagen mRNA Levels of Human Dermal Fibroblast Culture. *Mar Drugs* 16.
27. Reilly DM, Lozano J (2021) Skin collagen through the lifestyles: importance for skin health and beauty. *Plastic and Aesthetic Research* 8: 2.
28. Holwerda AM, van Loon LJC (2022) The impact of collagen protein ingestion on musculoskeletal connective tissue remodeling: a narrative review. *Nutr Rev* 80: 1497-514.
29. Geahchan S, Baharlouei P, Rahman A (2022) Marine Collagen: A Promising Biomaterial for Wound Healing, Skin Anti-Aging, and Bone Regeneration. *Mar Drugs* 20.
30. Evans M, Lewis ED, Zakaria N, Pelipyagina T, Guthrie N (2021) A randomized, triple-blind, placebo-controlled, parallel study to evaluate the efficacy of a freshwater marine collagen on skin wrinkles and elasticity. *J Cosmet Dermatol* 20: 825-34.
31. Asserin J, Lati E, Shioya T, Prawitt J (2015) The effect of oral collagen peptide supplementation on skin moisture and the dermal collagen network: evidence from an ex vivo model and randomized, placebo-controlled clinical trials. *J Cosmet Dermatol* 14: 291-301.
32. Sousa SC, Fragoso SP, Penna CRA, Arcanjo NMO, Silva FAP et al. (2017) Quality parameters of frankfurter-type sausages with partial replacement of fat by hydrolyzed collagen. *LWT - Food Science and Technology* 76: 320-5.
33. Bilek SE, Bayram SK (2015) Fruit juice drink production containing hydrolyzed collagen. *Journal of Functional Foods* 14: 562-9.
34. Sato Y, Uchida E, Aoki H, Hanamura T, Nagamine K et al. (2017) Acerola (*Malpighia emarginata* DC.) Juice Intake Suppresses UVB-Induced Skin Pigmentation in SMP30/GNL Knockout Hairless Mice. *PLoS One* 12: e0170438.
35. Carr AC, Vissers MC (2013) Synthetic or food-derived vitamin C--are they equally bioavailable? *Nutrients* 5: 4284-304.
36. Dai F, Chen WF, Zhou B (2008) Antioxidant synergism of green tea polyphenols with α -tocopherol and l-ascorbic acid in SDS micelles. *Biochimie* 90: 1499-505.
37. Fabre G, Bayach I, Berka K, Paloncýová M, Starok M et al. (2015) Synergism of antioxidant action of vitamins E, C and quercetin is related to formation of molecular associations in biomembranes. *Chemical Communications* 51: 7713-6.
38. Rattanawitpong P, Wanitphakdeedecha R, Bumrungrert A, Maiprasert M (2020) Anti-aging and brightening effects of a topical treatment containing vitamin C, vitamin E, and raspberry leaf cell culture extract: A split-face, randomized controlled trial 19: 671-6.
39. Darvin ME, Lademann J, von Hagen J, Lohan SB, Kolmar H et al. (2022) Carotenoids in Human Skin in Vivo: Antioxidant and Photo-Protectant Role against External and Internal Stressors 11: 1451.

40. Fallacara A, Baldini E, Manfredini S, Vertuani S (2018) Hyaluronic Acid in the Third Millennium. *Polymers (Basel)* 10.
41. Vazquez JA, Montemayor MI, Fraguas J, Murado MA (2010) Hyaluronic acid production by *Streptococcus zooepidemicus* in marine by-products media from mussel processing wastewaters and tuna peptone viscera. *Microb Cell Fact* 9: 46.
42. Skive B, Rohde M, Molinari G, Braunstein TH, Bojesen AM (2017) *Streptococcus equi* subsp. *zooepidemicus* Invades and Survives in Epithelial Cells. *Front Cell Infect Microbiol* 7: 465.

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