

Research

EFFECT OF ORAL TRIPEPTIDE-COLLAGEN ON MICROCIRCULATION (NAIL FOLDS) AND SKIN WRINKLES

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Received: 25-06-2024 / Accepted: 15-11-2024

ABSTRACT

Background: Accelerated aging is associated with increased exposure to reactive oxygen species (ROS), leading to lower collagen and elastin levels in the body. This reduction reduces microvascular activity, causing microcirculatory disorders and skin wrinkles. Administering collagen tripeptides can help improve the microcirculation system.

Objective: This study aims to investigate the effects of collagen tripeptide on improving microcirculation, reducing skin wrinkles, and lowering excessive ROS toxicity.

Method: The research method used is experimental with a pre-post study approach without using a control group and descriptive quantitative data analysis. We measured microcirculation using a microscope, quantified ROS levels with the QYM-DI microscope, and assessed nasolabial fold depth with an acupuncture needle.

Results: The study results indicate that collagen tripeptide significantly improves microcirculation, reduces skin wrinkles, and decreases excessive ROS toxicity. The non-parametric Wilcoxon test results are as follows: (1) there is a significant difference before and after collagen tripeptide consumption in terms of microcirculation improvement ($p = 0.000$), reduction of skin wrinkles on the nasolabial folds ($p = 0.008$), and reduction of ROS ($p = 0.000$). This improves microcirculation, which in turn reduces skin wrinkles on the nasolabial folds.

Conclusion: Consuming collagen tripeptide for three months can improve microcirculation, reduce skin wrinkles on the nasolabial folds, and reduces ROS.

Keywords: collagen, microcirculation, nasolabial fold, reactive oxygen species

INTRODUCTION

The elderly commonly have 52% of chronic diseases and 15% of acute diseases lead to death. Aging, along with decreased microvascular activity, has been reported in many previous studies.^[1,2] Therefore, improving the microcirculation system in the elderly is important for extending lifespan.^[3] The decreased skin elasticity in the elderly may be related to age-associated structural changes in the skin.^[1] The reduction in oxygen reaching tissues and the imbalance in constructive and destructive metabolic processes can occur with aging. For example, collagen and elastic fibers deteriorate with age.^[4] Exposure to UV rays can also lead to an increase in ROS, damaging cell structure and function, and mediating an inflammatory response.^[5]

Damage to organ tissue due to microcirculation disorders can be reduced by administering peptides. Microcirculation disorders can occur due to a decrease in collagen and elastin in the blood vessel walls, leading to vessel stiffness, which can be mitigated by administering collagen tripeptides. Peptides are defined as compounds

containing two or more amino acids linked by amide bonds and can be synthesized chemically.^[6] In the study by Ichihara et al., the addition of peptides as an epicardial layer on the heart instantly reduced heart damage.^[7] This means they can enter fibroblast cells and forming collagen in the dermis, which can then regenerate cells up to the epidermal layer. Collagen tripeptides thus function as signals between cells and target molecules that trigger aging.^[8]

The process of accelerating anti-aging from within the body, particularly for the skin, can utilize oligopeptides, specifically collagen tripeptides.^[8] This roughly translates to "capable of entering fibroblast cells and forming collagen in the dermis, which can then regenerate cells up to the epidermal layer". This means that collagen tripeptides transfer signals between cells and target molecules that trigger aging.^[8] Kim et al. clarified once again that the tripeptide (Gly-Pro-Hyp) is easily absorbed intact across the intestines into the bloodstream in the human body. The Pro-Hyp amino acid component in the tripeptide can stimulate fibroblast migration and growth.

Consequently, fibroblasts can synthesize collagen and elastic fibers in the skin to aid in skin rejuvenation.^[9] Based on that explanation, oligopeptides in tripeptide form are able to repair damaged cells and prevent aging in the elderly.

The statement above indicates that cell repair can influence microcirculation rates, which can improve tissue function. Success in cell repair among the elderly can be easily detected through nailfold capillaroscopy.^[10] Aging skin exhibits impaired barrier function, resulting in a dry appearance and increased susceptibility to skin disorders^[5], such as wrinkles in the nasolabial folds.^[11] According to Shoshani et al., the Modified Fitzpatrick Wrinkle Scale (MFWS) is an assessment of nasolabial folds in the form of a scoring system based on the severity of wrinkles.^[12] Aging is also caused by an increase in ROS within cells, which can be detected through blood tests.^[13] This study aims to investigate the effects of collagen tripeptide on improving microcirculation, reducing skin wrinkles, and lowering excessive ROS toxicity.

METHODS

The research design employed in this study is experimental, utilizing a pre- and post-study approach without incorporating a control group. Furthermore, this study received prior approval from the Research Ethics Committee of the Faculty of Medicine at Maranatha University, as evidenced by Ethics Decree number 035/KEP/IV/2024, before initiating data collection involving research subjects. The subjects involved were a group of elderly individuals aged between 60 and 80 years. They were administered collagen tripeptide supplements (specifically, the Colatrix brand containing additional Oryza Ceramide and L-glutathione) in 2x5 grams, alongside tablets that support their intake of vitamin C and cuprum. Patients are also advised to consume mushrooms and/or legumes as supportive nutrition for collagen formation. The research took place at Willy Vincent Clinic, located at 30 Gajah Lumantung Street, Taman Sari, Bandung Wetan. The research duration will span three months.

The observation was made before and after the consumption of collagen tripeptides following a three-month intake period. This technique will include all subjects who met the inclusion criteria in the study until the number of subjects has been met. The inclusion criteria for this study were as follows: both males and females, absence of gastric acid-related disorders, no intake of vitamins and/or antioxidant supplements, absence of skin diseases/dermatitis (including allergic dermatitis, atopic dermatitis, contact dermatitis, and autoimmune dermatitis such as psoriasis). Respondents who met the criteria were selected using Consecutive Sampling. The sample size was determined based on a comparative numeric formula for paired repeated measurements, with the population size per group set at 20 according to the pilot study. Therefore, the minimum required sample size for this research was 28 individuals.

The equipment utilized for this study included a microcirculation microscope, computer, QYM-DI microscope, blood lancet, object glass, pipette dropper, writing utensils, camera, and ruler. The materials employed in this research comprised immersion oil and tissue samples. Data collection was performed by capturing images of capillaroscopy (nailfold capillary fold) using a microcirculation microscope. This involved using a specialized microscope to observe and record the microcirculation of blood vessels in the nailfold capillary bed, allowing for detailed analysis and assessment of blood flow patterns and vessel health. The outcomes will be categorized into four classes on the microcirculation scale: normal (scores 1-3), mild (scores 4-6), moderate (scores 7-9), and severe (scores 10-12).

The skin wrinkles (in the nasolabial area) were then assessed using the Modified Fitzpatrick Wrinkle Scale (MFWS), which categorizes wrinkles into four main classes: no wrinkles (score 0), fine wrinkles (score 1), moderate wrinkles (score 2), and deep wrinkles (score 3).^[12] The research facility modified the measurement by inserting an acupuncture needle into the nasolabial area near the nostril, then comparing the measurement with the scoring reference. Furthermore, there was an the examination of ROS using the dry blood spot test method with the QY-MDI microscope. Data collection on ROS was done using 5 levels based on the severity of red blood cell (RBC) damage due to ROS: no ROS (Level 0), 10% of RBC damaged by ROS (Level 1), 10%-20% of RBC damaged by ROS (Level 2), 20%-30% of RBC damaged by ROS (Level 3), and >30% of RBC damaged by ROS (Level 4).^[7]

The initial data for each parameter were gathered to be compared with the final data after the consumption of collagen tripeptide. The data analysis used in this study, whether for microcirculation scores, skin wrinkles, or ROS, employed SPSS 25 with a Shapiro-Wilk normality test at a 5% confidence level. If the data followed a normal distribution, it was analysed using a dependent T-test. However, if the data did not follow a normal distribution, it was analysed using a Wilcoxon test. The first researcher confidently collected and processed observational data under the supervision of other researchers.

RESULTS

This study involved 28 subjects, with their demographic data and complaints presented in Table 1. The data in Table 1 show that most respondents are female, with 17 individuals (68.93%). The most common occupation among the respondents is entrepreneurship, with 14 individuals (50%), and the highest level of education is a bachelor's degree, with 13 individuals (46.43%). There are 15 non-smoking respondents (53.57%). Half of the respondents have a habit of staying up late, frequently consume coffee, and often exercise. The study results on the effects of collagen tripeptide in improving microcirculation, reducing skin wrinkles, and decreasing excessive ROS toxicity are evaluated using scores and presented in Table 2 as follows. The research findings are presented in a histogram, as shown in Figure 1.

Table 1. Demographic Data and Respondent Complaints.

Variables	Results (n=28)	Variables	Results (n=28)
Age (Mean + SD)	(68,93 ± 7,47)	Lifestyle Habits	
Years old	(60 – 80)	Smoking (n/%)	
Gender (n/%)		1 pack/ day	2/7,14
Male	11/39,29	More than once a week	11/39,29
Female	17/68,93	Non-smoker	15/53,57
Occupation (n/%)		Staying up late	14/50
Self-employed	14/50	Coffee consumption (n/%)	
Civil Servant	1/3,57	Once a day	14/50
Housewife	12/42,86	More than once a week	14/50
Unemployed	1/3,57	Physical exercise (n/%)	
Education (n/%)		Once a week	14/50
University Graduate	13/46,43	More than once a week	14/50
High School	6/21,43	Medical history (n/%)	
Middle School	5/17,86	Memory decline	18/64,29
Elementary School	4/14,29	Diabetes Miletus	11/39,29
		Mild Stroke	15/53,57
		Gastritis	9/31,14
		Back pain	4/14,29

Table 2. Research Result on the Effects of Collagen-Tripeptide on Microcirculation, Skin Wrinkles, and ROS Toxicity (presented as scores).

Research Subjects	Microcirculation		Nasolabial		ROS	
	Pre	Pos	Pre	Pos	Pre	Pos
1	9.6	5.8	2.5	2	3	3
2	11.4	7.0	2	2	4	3
3	13.7	7.5	2.5	2.5	4	3
4	11.3	5.6	1	1	4	3
5	10.1	6.4	1.5	1.5	4	3
6	12.7	7.9	1	0.5	3	3
7	10.5	6.9	1.5	1.5	4	3
8	9.7	6.8	3	2.5	3	3
9	9.9	7.9	3	3	4	3
10	12.8	7.0	3	3	3	3
11	11.6	4.8	3	3	3	4
12	15.4	5.9	3	3	3	3
13	11.4	6.3	3	2.5	4	2
14	15.8	6.4	3	2.5	3	3
15	15.5	7.4	1	0.5	3	2
16	12.6	5.4	2.5	2	3	3
17	13.0	7.9	2	2.5	3	3
18	16.3	5.2	2.5	2	4	3
19	15.8	7.1	1.5	1	4	3
20	15.1	4.4	3	3	4	3
21	11.0	4.7	1	0.5	4	3
22	10.4	7.3	2.5	2.5	4	2
23	10.3	6.9	3	3	3	2
24	12.1	8.9	1.5	1	4	3
25	14.4	6.9	3	3	4	3
26	10.7	6.2	0.5	1	3	4
27	9.8	6.3	2	1.5	3	3
28	10.1	7.5	3	3	4	3
Average	12.25 ± 2.190	6.58 ± 1.082	2.39 ± 0.850	2.25 ± 0.856	3.54 ± 0.508	2.93 ± 0.466

The microcirculation score data, nasolabial score data, and ROS data showed non-normally distributed results, so a non-parametric Wilcoxon test was conducted on the data. The results of the non-parametric Wilcoxon test are as follows: (1) there was a significant difference before and

after the consumption of collagen tripeptides in terms of microcirculation improvement ($p = 0.000$), reduction of skin wrinkles on the nasolabial fold ($p = 0.008$), and reduction of ROS ($p = 0.000$) (Figure 1).

Upon comparing pre-test and post-test averages, it is evident that collagen tripeptide (Figure 1) has a direct impact on microcirculation and ROS. This is supported by the Wilcoxon test results in Appendix 1, indicating that collagen tripeptides significantly improve microcirculation

(Figure 2) and reduce ROS in the body (Figure 4). The change in the average difference in collagen tripeptide consumption concerning nasolabial changes demonstrates the smallest change (Figure 3).

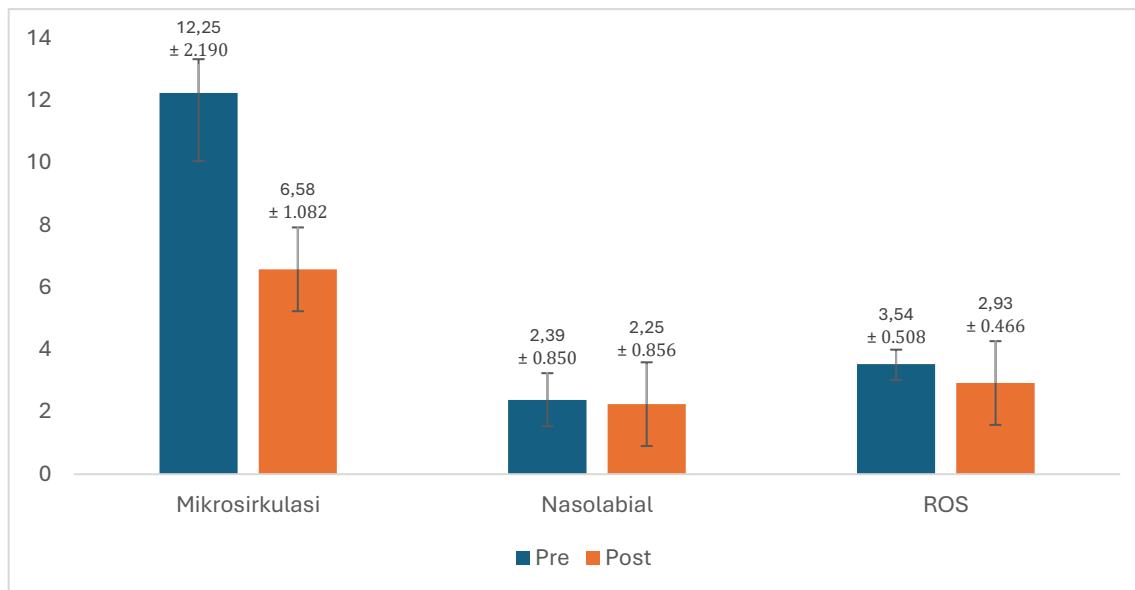


Figure 1. Histogram of Before and After Consumption of Collagen-Tripeptide.

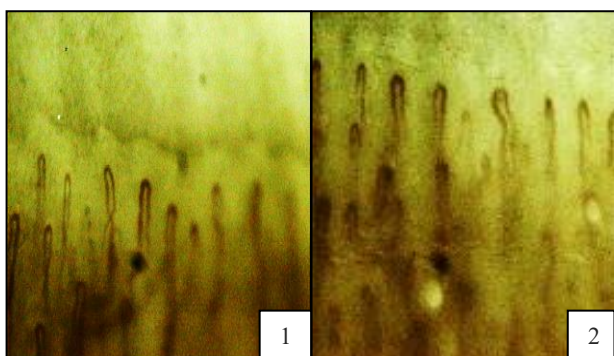


Figure 2. The difference between Microvascular (1) Pre-test and (2) Post-test.

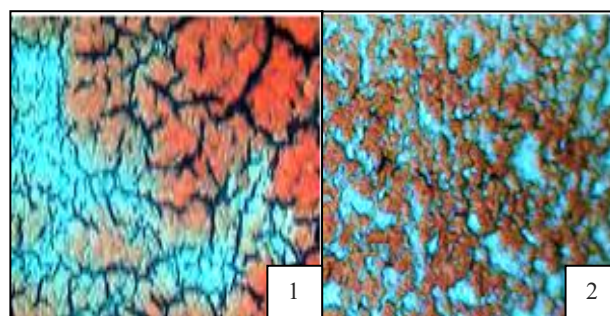


Figure 4. The difference between ROS (1) Pre-test and (2) Post-test.

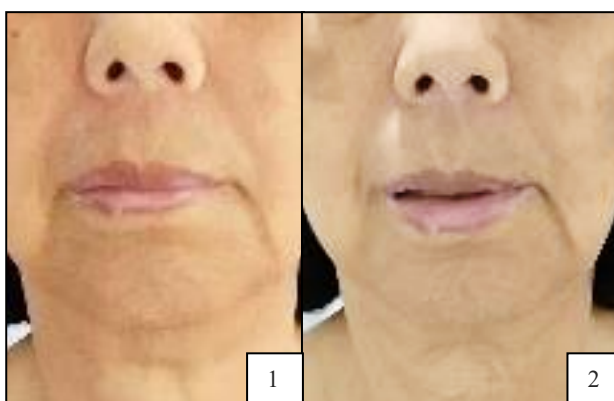


Figure 3. The difference between Nasolabial (1) Pre-test and (2) Post-test.

The ROS test observation was conducted using the QY-MDI microscope with dried blood smears. On average, the results of the changes show that most of the ROS data shifted from a value of 4 to 3, as depicted in Figure 4.

DISCUSSION

The research results indicate that consuming collagen tripeptides for 3 months has an effect on (1) improving microcirculation, as evidenced by a decrease in the score from 12.3 to 6.3 after treatment; (2) reducing skin wrinkles on the nasolabial fold, demonstrated by a decrease in the score from 2.39 to 2.25 after treatment; and (3) reducing ROS, shown by a decrease in the score from 3.5 to 2.9 after treatment, as depicted in Figure 1. Certainly, some data may show poorer results (nasolabial and ROS score) due to human error during data assessment or external patient factors beyond our control.

Arciero et al. propose that microcirculation is responsible for the functions of the body's organs; disturbances in microcirculation can lead to the deterioration of organ function, causing cell damage and the onset of diseases.^[14] Gutterman et al. add that maximizing microcirculation is necessary to distribute nutrients and oxygen throughout the body, which can optimize organ function.^[3] The oral consumption of collagen tripeptides in this study showed that administering these peptides led to improvements in

microcirculation space (microvascular), thus optimizing the repair of organ tissues in the body.

Collagen tripeptides are tripeptide molecules belonging to the oligopeptide category, consisting of 2-10 amino acids with a weight of less than 1500 Da.^[15] These tripeptide molecules are easily absorbed by the body and can reach the microcirculation areas. When consumed orally, collagen tripeptides reach the small intestine and are absorbed into the bloodstream, either in the form of di/tripeptides or free amino acids. Collagen peptides and free amino acids are then distributed throughout the human body via the bloodstream.^[16]

The consumption of collagen tripeptide influences skin wrinkles in the nasolabial fold. This is consistent with Lu et al.^[17] research, which suggests that oral collagen peptide supplementation can reduce skin wrinkles and improve skin texture. Their study indicates that after 8 weeks of consumption, there was an 18.09% reduction in skin wrinkles. This reduction is highly significant compared to this study's findings of only a 6% decrease, as age-related factors may influence the rate of collagen aesthetic formation in the skin. This study demonstrates that there were only minor changes in skin wrinkles, especially in the nasolabial fold, after three months of oral collagen tripeptide supplementation (Figure 3).

Maeda explains that there is an oral collagen-peptide test on elderly people over 50 years old with a consumption of 5 grams for 4-8 weeks that can increase skin moisture and elasticity.^[18] This is in line with Lu et al. research that consuming collagen tripeptides can significantly improve skin moisture.^[17] When consumed orally, hydrolysed collagen in the small intestine is then absorbed into the bloodstream, both in the form of small collagen peptides (di- and tripeptides) and free amino acids. These collagen peptides and free amino acids are then distributed throughout the human body, especially to the dermis.^[16]

This study also indicates that collagen tripeptides affect ROS. ROS test observations were carried out using the QY-MDI microscope with dried blood smears, and the average change results showed that most of the ROS data shifted from a value of 4 to 3, as shown in Figure 4.

Pittayapruek argues that Reactive Oxygen Species (ROS) in the body are formed due to exposure to sunlight resulting in mitochondrial dysfunction, toxic substances like cigarettes, and inflammation.^[19] According to Zhu et al., bioactive peptides can enhance cell proliferation to repair mitochondrial function after external influences like UV radiation exposure.^[20] Consistent with Chen et al., low molecular weight bioactive peptides can easily pass through the intestinal wall and have biological effects.^[21] Previous research showed positive results from administering peptides to improve ROS in the body, particularly in mitochondrial repair, thus reducing ROS production. Peptide molecules can stimulate the concentration of NAD⁺/NADH along with the expression of the PGC-1 α protein. This protein functions in mitochondrial repair due to UV radiation exposure^[20], thereby reducing internal ROS addition.

Oligopeptide consumption, especially tripeptides, needs to be stimulated by cofactors such as Vitamin C. This is because in forming collagen I and III for skin biomatrix, Vitamin C is required for collagen synthesis and acts as an antioxidant to protect proteins from oxidation.^[22] Collagen in the dermis will undergo hydrolysis through two mechanisms: 1) free amino acids providing building blocks for collagen and elastin fiber formation; 2) collagen oligopeptides acting as ligands, binding to receptors on fibroblast membranes and stimulating the production of new collagen, elastin, and hyaluronic acid.^[16]

CONCLUSION

This research can be summarized as follows: (1) The consumption of collagen tripeptides for 3 months improves microcirculation; (2) The consumption of collagen tripeptides for 3 months reduces skin wrinkles on the nasolabial fold; (3) The consumption of collagen tripeptides for 3 months reduces ROS (reactive oxygen species).

Further research is needed, such as (1) pharmacokinetic studies of collagen tripeptides to optimize their usage; (2) research on other oligopeptides that are also expected to have anti-aging effects.

ACKNOWLEDGEMENT

This research was conducted at the Anti-Aging Clinic under the permission of PT X and received academic support from Maranatha Christian University Bandung, specifically from the Medical Faculty, Master's Program in Skin Aging and Aesthetics. The research team would also like to express gratitude to all patients who participated in our study.

CONFLICT OF INTEREST AND FUNDING RESOURCES

All authors have no conflict of interest. The authors covered all the necessary costs for the study.

REFERENCES

1. Khalil A, Humeau-Heurtier A, Gascoin L, Abraham P, Mahé G. Aging effect on microcirculation: A multiscale entropy approach on laser speckle contrast images. *Med Phys*. 2016;43(7):4008–16.
2. Wlaschek M, Maity P, Makrantonaki E, Scharffetter-Kochanek K. Connective tissue and fibroblast senescence in Skin Aging. *J Invest Dermatol*. 2021;141(4):985–92.
3. Gutterman DD, Chabowski DS, Kadlec AO, Durand MJ, Freed JK, Ait-Aissa K, et al. The Human Microcirculation: Regulation of Flow and beyond. *Circ Res*. 2016;118(1):157–72.
4. Gu Y, Han J, Jiang C, Zhang Y. Biomarkers, oxidative stress and autophagy in skin aging. *Ageing Res Rev*. 2020;59:101036.
5. Kammeyer A, Luiten RM. Oxidation events and skin aging. *Ageing Res Rev*. 2015;21:16–29.
6. de la Torre BG, Albericio F. Peptide therapeutics 2.0. *Molecules*. 2020;25(10):2019–21.
7. Ichihara Y, Kaneko M, Yamahara K, Koulouroudias M, Sato N, Uppal R, et al. Self-assembling peptide hydrogel enables instant epicardial coating of the heart with mesenchymal stromal cells for the treatment of heart failure. *Biomaterials*. 2018;154:12–23.

8. Bojarska J. Amino acids and short peptides as anti-Aging "superfood." *Int J Nutr Sci.* 2021;5(1):1039.
9. Kim DU, Chung HC, Choi J, Sakai Y, Lee BY. Oral intake of low-molecular-weight collagen peptide improves hydration, elasticity, and wrinkling in human skin: A randomized, double-blind, placebo-controlled study. *Nutrients.* 2018;10(7):826.
10. Bertolazzi C, Cutolo M, Smith V, Gutierrez M. State of the art on nailfold capillaroscopy in dermatomyositis and polymyositis. *Semin Arthritis Rheum.* 2017;47(3):432–44.
11. Pathak Y. The Skin Aging Process and Anti-Aging Strategies. *Biomed J Sci Tech Res.* 2022;42(2).
12. El-Mesidy MS, Alakloulk WT, Azzam OA. Nasolabial fold correction through cheek volume loss restoration versus thread lifting: a comparative study. *Arch Dermatol Res.* 2020;312(7):473–80.
13. Zhang Y, Dai M, Yuan Z. Methods for the detection of reactive oxygen species. *Anal Methods.* 2018;10(38):4625–38.
14. Arciero JC, Causin P, Malgaroli F, 1 Department of Mathematical Sciences, IUPUI, 402 N. Blackford, LD 270, Indianapolis IN 46202, USA, 2 Department of Mathematics, University of Milan, via Saldini 50, 20133 Milano, Italy. Mathematical methods for modeling the microcirculation. *AIMS Biophys.* 2017;4(3):362–99.
15. Chen XL, Peng M, Li J, Tang BL, Shao X, Zhao F, et al. Preparation and functional evaluation of collagen oligopeptide-rich hydrolysate from fish skin with the serine collagenolytic protease from *Pseudoalteromonas* sp. SM9913. *Sci Rep.* 2017;(15716):1–13.
16. Sibilla S, Godfrey M, Brewer S, Budh-Raja A, Genovese L. An overview of the beneficial effects of hydrolysed collagen as a nutraceutical on skin properties: Scientific background and clinical studies. *Open Nutraceuticals J.* 2015;8(1):29–42.
17. Lu S, Zhang S, Wang Y, Ni J, Zhao T, Xiao G. Anti-Skin Aging Effects and Bioavailability of Collagen Tripeptide and Elastin Peptide Formulations in Young and Middle-Aged Women. *J Dermatol Sci Cosmet Technol.* 2024;1(2):100019.
18. Maeda K. Skin-Moisturizing Effect of Collagen Peptides Taking Orally. *J Nutr Food Sci.* 2018;08(02):1–8.
19. Pittayapruerk P, Meehansan J, Prapapan O, Komine M, Ohtsuki M. Role of Matrix Metalloproteinases in Photoaging and Photocarcinogenesis. *Int J Mol Sci.* 2016;17(6):868.
20. Zhu N, Xu MH, Li Y. Bioactive Oligopeptides from Ginseng (*Panax ginseng* Meyer) Suppress Oxidative Stress-Induced Senescence in Fibroblasts via NAD⁺/SIRT1/PGC-1 α Signaling Pathway. *Nutrients.* 2022;14(24):1–14.
21. Chen T, Hou H, Fan Y, Wang S, Chen Q, Si L, et al. Protective effect of gelatin peptides from pacific cod skin against photoaging by inhibiting the expression of MMPs via MAPK signaling pathway. *J Photochem Photobiol B.* 2016;165:34–41.
22. Laing S, Bielfeldt S, Ehrenberg C, Wilhelm KP. A Dermoneutrient Containing Special Collagen Peptides Improves Skin Structure and Function: A Randomized, Placebo-Controlled, Triple-Blind Trial Using Confocal Laser Scanning Microscopy on the Cosmetic Effects and Tolerance of a Drinkable Collagen Suppleme. *J Med Food.* 2020;23(2):147–52.