



ORIGINAL CONTRIBUTION

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Topical application and oral supplementation of peptides in the improvement of skin viscoelasticity and density

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Summary

Background: The combination of oral supplementation and topical formulations to the improvement of skin conditions has been proposed as an innovative approach to obtain effective treatments. However, studies comparing the effectiveness of each type of treatments are still in lack. This way, the objective of this work was to evaluate the clinical efficacy of a dermocosmetic formulation with di- and tripeptides, as well the effects of an oral supplementation based on hydrolyzed collagen through biophysical and skin imaging techniques.

Methods: Sixty healthy female subjects, aged between 40 and 50 years, were enrolled, being separated in 3 groups: topical formulation, oral supplementation, and oral placebo. The stratum corneum water content, skin viscoelasticity, dermis echogenicity, and skin pores parameters were evaluated.

Results: The group with the topical formulation showed a significant increase in the stratum corneum water content and skin elasticity after 28-day period and also acted in the dermis echogenicity after 90 days with the formulation with peptides. The oral supplementation acted on skin elasticity and presented a more pronounced effect on dermis echogenicity, reducing skin pores after 90-day period.

Conclusion: The obtained results with oral supplementation and topical application of hydrolyzed proteins were considered complementary in the improvement of general skin conditions, acting in different mechanisms.

KEYWORDS

biophysical and skin imaging techniques, dermocosmetic formulation, hydrolyzed collagen, oral supplementation, rice peptides

1 | INTRODUCTION

A current innovation in the cosmetic market is the combined use of oral supplementations with classical topical formulations, creating a new approach to the treatment of skin conditions, being proposed to improve the appearance of the skin internally and externally, reaching the deepest skin layers and improving the microrelief and its surface appearance.¹

The use of topical formulations containing different active ingredients has been traditionally used, being the main form of skin care products. Although both types of products are a highlight in the

cosmetic and dermatologic areas, there is currently a lack of studies comparing the effectiveness of each treatment, their differences, and how they could contribute to improve skin health.

Skin aging is a characteristic that causes many concerns in the present population, with different types of treatments being developed.^{2,3} In general, it can be characterized by a progressive process of drying, changes on the skin microrelief, loss of firmness, and elasticity that results on wrinkles and sagging. Therefore, aged skin also shows reduced cell turnover, hyperkeratinization, abnormal elastic tissue production and pronounced loss, and degradation of collagen fibers.^{4,5}

In this context, the use of collagen peptides (or hydrolyzed collagen), that are produced by breaking collagen obtained from different sources (mainly animals, like fish and bovines) through enzymatic hydrolysis into small chains of amino acids, is highly indicated as oral supplementations to improve aged skin conditions, being easy to digest due to their size, and absorbed by the human body, which could lead to alterations on different parameters.¹

Also, di- and tripeptides extracted from rice (*Oryza sativa*) have a great potential to be added in cosmetic formulations, once they are well accepted by the skin and act in the metabolic functions of the skin cells, stimulating the synthesis of fibroblasts, collagen (type III and VII), fibronectin, and integrin, which are responsible for cell adhesion, preventing, and improving skin aging.⁶⁻⁸

Considering that dermocosmetic treatments in general act on the improvement of skin hydration, control of skin oiliness, and pores reduction, all on the epidermal layer,⁹⁻¹¹ the clinical evaluation of new treatments to also act on the dermis is very important, improving not only epidermis but also the skin density—firmness and elasticity. In addition, although the photoaging process damages the epidermis, the main target is the dermis, significantly reducing the dermis echogenicity and consequently, the skin density.¹²

Thus, the aim of this study was to evaluate the clinical efficacy of a dermocosmetic formulation added with di- and tripeptides extracted from rice as well the effects of an oral supplementation based on hydrolyzed collagen and vitamins using biophysical and skin imaging techniques.

Finally, this study contributes to compare the clinical benefits of topical or oral treatments in the improvement of skin aging conditions, once it presents the difference between their effects in real conditions of use.

2 | MATERIALS AND METHODS

2.1 | Test Products

The topical formulation under study was based on hydroxyethylcellulose, methylphenyl polysiloxane, cyclomethicone, cyclomethicone and crosspolymer dimethicone, hydrosoluble filter UVA/UVB, propylene glycol, glycerin, and preservative (2-Phenoxyethanol (And) Methylisothiazolinone (And) Aqua/Water) and was added with 4% of the di- and tripeptides of hydrolyzed protein of rice.

To the oral supplementation product, the dose had 10 g, of which 9 g was composed of hydrolyzed collagen along with a mix of vitamins A (600 µg), C (45 mg), E (10 mg), and zinc (7,0 mg). The placebo was composed of 10 g of maltodextrin.

2.2 | Subjects

A total of 60 healthy female subjects, aged between 40 and 50 years, were enrolled for the clinical efficacy study, being separated into three separately randomized groups: topical formulation, hydrolyzed collagen, and oral placebo. For all groups, informed consent was obtained from subjects before entering in the study, which was approved by the ethics (CEP/

FCFRP n°339) of the Faculty of Pharmaceutical Sciences of Riberão Preto. In this stage of the study, the subjects applied the formulation containing 4% of the di- and tripeptides of hydrolyzed rice protein on the face twice daily or ingested 10 g of hydrolyzed collagen or its placebo daily. All oral supplementation groups were double-blinded and randomized (random assignment by blocked design in which participants are randomly assigned in equal number within a block of trials that represents the different treatment groups). The topical formulation group was not double-blinded once there was only one type of product to apply on the face region.

2.3 | Assessments

For the clinical efficacy tests, the study measurements were performed on the periorbital, frontal, and nasolabial regions of the face. On every measurement day, the subjects had to expose their clean test areas to the room's controlled climate conditions ($21.5 \pm 1^\circ\text{C}$ and $50 \pm 5\%$ relative humidity) for 20 minutes. The measures with biophysical and skin imaging techniques were made before starting the product treatment (baseline values) and after 28 (D28) and 90 (D90) days of daily product application, in terms of stratum corneum water content, skin viscoelasticity, dermis echogenicity, and skin pores. For the oral supplementation treatment, the measures were done before (baseline values) and after 90 days (D90) of daily product intake.

2.4 | Determination of the stratum corneum water content

The water content of the stratum corneum was measured with a skin capacitance meter (CorneometerTM 825, Courage and Khazaka Electronic GmbH). The device determines the water content of the superficial epidermal layers down to a depth of about 0.1 mm and expresses the values in arbitrary units. The average values of five measurements per site were used in subsequent calculations.¹³

2.5 | Determination of the viscoelastic properties of the skin

For this evaluation, the equipment Cutometer® SEM 575 (Courage and Khazaka Electronic GmbH) was utilized. It features a probe with negative pressure, and the captured light intensity is proportional to the skin penetration. The measurement of each test area was repeated 3 times. The R5 value (Ur/Ue, net elasticity - immediate recovery/elastic deformation, the closer the value is to one the more elastic the skin) was utilized as it is proven to be the most suitable in detecting age-related skin alterations. The R7 value was also utilized (Ur/Uf, biological elasticity - Portion of the elasticity compared to the complete curve, the closer the value is to one the more elastic the skin).¹⁴

2.6 | Evaluation of dermis echogenicity

To the evaluation of the dermis echogenicity, 20 MHz ultrasound equipment (Dermascan® C, Cortex Technology) was utilized. The ultrasonic wave (speed of 1.580 m/s) is partially reflected by the skin

structure, giving rise to echoes of different amplitudes. To calculate the echogenicity, the number of pixels with low echogenicity is measured by means of the image analysis software and related to the total number of pixels. This parameter is also related to retention of water between the collagen fibers and the aging and photoaging.¹⁵

2.7 | Evaluation of skin by high-resolution photography

The Visioface® digital photography imaging system (Courage and Khazaka Electronic GmbH) was utilized for the evaluation of facial

skin, consisting of a cabin attached to a high-resolution digital camera (10 megapixels) and 200 white LED. This apparatus is connected to research software that enables evaluation of visible pores and wrinkles.¹⁶

2.8 | Statistical analysis

Two-way ANOVA and Bonferroni post-test were used in this study. Statistical differences between the treatments baseline and T90 were ascertained by paired Student's *t* tests for each parameter evaluated (GraphPad Software Inc, La Jolla, CA, USA). Differences were accepted as statistically significant at $P < 0.05$.

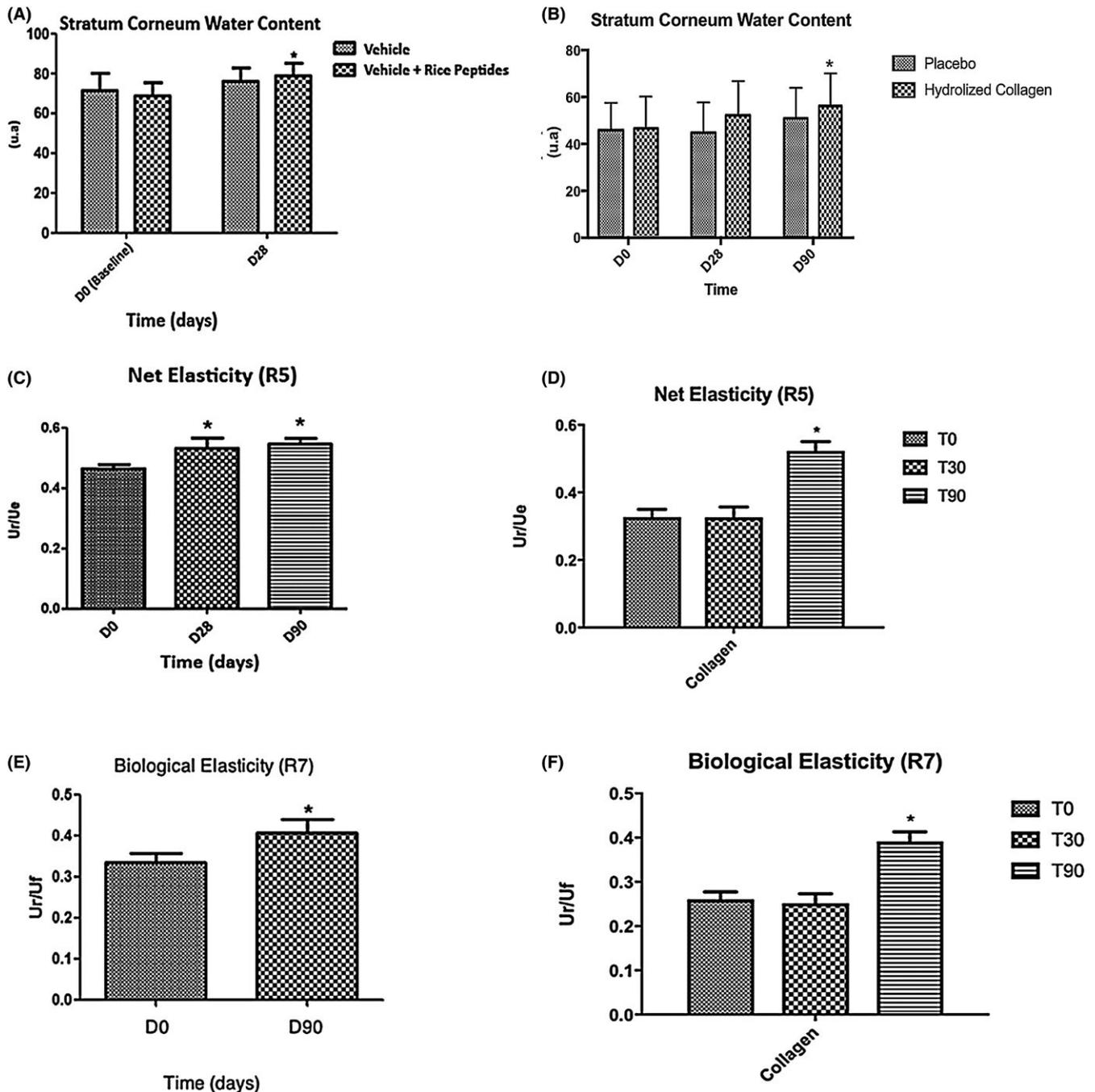


FIGURE 1 A and B, Stratum corneum water content, (C) and (D) net elasticity R5 (Ur/Ue), (E) and (F) biological elasticity R7 of rice peptides and oral collagen treatments, respectively. * Significantly different from the baseline values ($P < 0.05$)

3 | RESULTS

3.1 | Determination of the stratum corneum water content

The topical treatment with the cosmetic formulation containing rice protein peptides showed a significant increase in the stratum corneum water content when compared to the baseline values after 28 days of treatment (Figure 1). The group that applied the topical formulation on the face utilized the same product without the active ingredient (vehicle) in the forearm region, and no significant differences between the measurement times and baselines of both products were noted in all parameters (data not shown).

In contrast, when compared to the use of oral supplementation based on hydrolyzed collagen and vitamins, significant differences in skin hydration were observed on the group that received the treatment when compared to placebo only 90 days of treatment (Figure 1).

3.2 | Determination of the viscoelastic properties of the skin

The application of the cosmetic formulation containing di- and tripeptides also acted in the improvement of the mechanical properties of the skin (Figure 1). Skin elasticity and viscoelasticity parameters increased significantly (R5—net elasticity and R7 biological elasticity parameters) after 90 days of treatment with the topical formulation.

The increase in skin elasticity was also observed with the Hydrolyzed Collagen oral supplementation, where the group receiving the treatment showed significant differences in the mechanical properties of the skin when compared to baseline and placebo after 90 days of treatment only in the net elasticity parameter in the periorbital region (Figure 1).

3.3 | Evaluation of dermis echogenicity

The dermis echogenicity improved significantly after the 90 days of treatment with the cosmetic formulation under study (Figure 2).

However, in the study with the ingestion of hydrolyzed collagen oral supplementation, it was possible to observe a significant

improvement of this parameter on the forehead and nasolabial regions (Figure 2).

3.4 | Evaluation of skin by high-resolution photography

The Figure 3 represents the high-resolution images from skin before and after 90 days of hydrolyzed collagen oral supplementation. It is possible to observe an improvement of skin wrinkles on frontal region of the face and a decrease in skin pores on the molar region. In addition, the topical formulation also improved skin wrinkles after 90-day period of treatment (Figure 4).

4 | DISCUSSION

During the evaluation of moisturizing effect through the stratum corneum water content analysis, it was possible to observe a statistically significance after a month of treatment only on the topical application group, showing the importance of the use of cosmetic products for the maintenance of skin hydration. Furthermore, the active ingredient under study, di- and tripeptides, is already known for enhancing the moisturizing effect of these formulations.¹⁰

Previous studies from our research group¹⁴ showed a significant improvement of skin microrelief after a single application of a cosmetic formulation containing the mixture of di- and tripeptides (immediate effects). This result suggests that the moisturizing effect observed in this study after use of a topical formulation could be also be observed in deeper layers of the epidermis. This way, the results obtained through the use of hydrolyzed collagen suggest that the use of cosmetic formulations is important to obtain a moisturizing effect on the skin in a short period of time. A similar study in the literature also showed no significant differences in skin hydration between the placebo and collagen hydrolyzed supplementation group.¹

Thus, topical products with the addition of peptides are indicated to promote an immediate increase in the stratum corneum water content, protecting, and moisturizing the skin.¹⁵

For the determination of the viscoelastic properties of the skin, an improvement of skin elasticity on the periorbital region was observed with the topical treatment, as the skin in this region is thinner and is

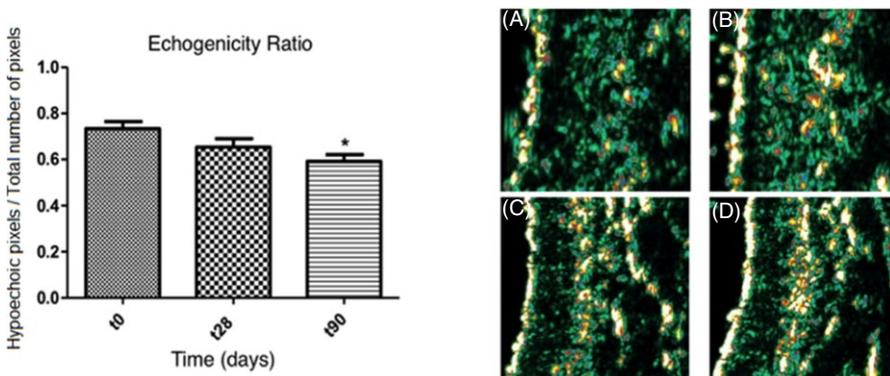


FIGURE 2 Dermis echogenicity ratio after the treatment with the topical formulation under study and ultrasound images of the collagen supplementation (A and B) and topical formulation peptides of rice protein (C and D) before (baseline) and after 90 days of treatment, respectively. Echogenicity color scale: white>yellow>red>green>blue>black

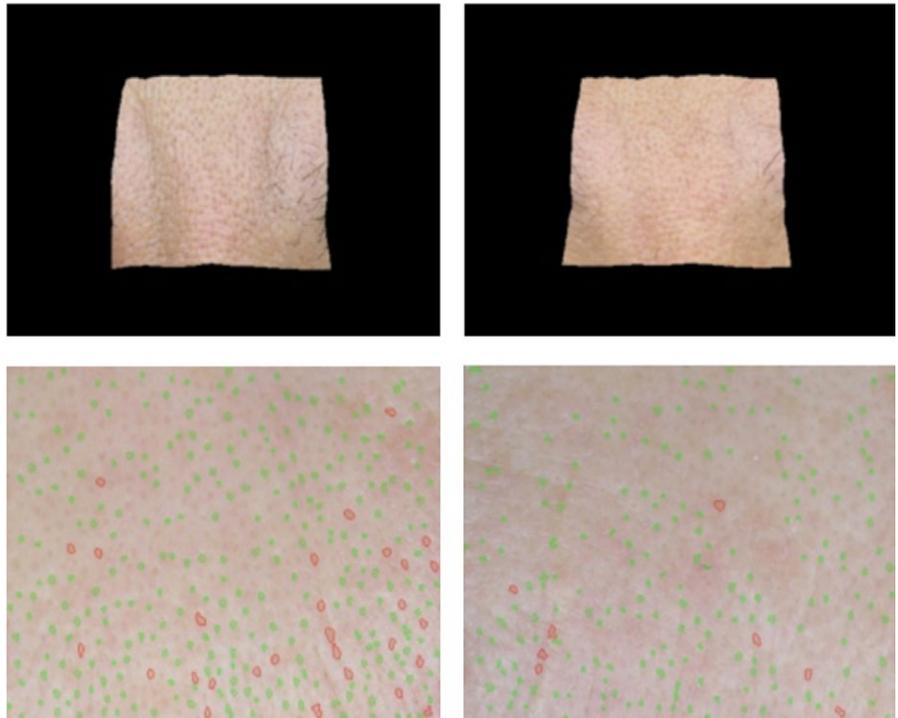


FIGURE 3 High-resolution images of pores and the glabellar wrinkle on the frontal region of the face before and after 90 days of oral supplementation by the Visioface Quick® software. The green pores are considered fine pores and the red, large pores



FIGURE 4 High-resolution images of the glabellar wrinkle on the frontal region of the face before and after 90 days of oral supplementation by the Visioface Quick® software

easier treated with a cosmetic product.¹⁶ On the other hand, with the oral supplementation, an improvement in the nasolabial region was observed, which, according to the skin characteristics on this area, needs a different type of treatment, as the supplementation with hydrolyzed collagen, as an alternative to more invasive treatments that are known to act on this region, as the application of fillers.¹⁷

In the dermis echogenicity parameter, the treatment with the ingestion of hydrolyzed collagen oral supplementation promoted a significant improvement in the forehead and nasolabial regions. This result is related to the dermis morphology, presenting an increase in fibroblast density, greater formation of collagen fibrils, and repair of the present damages, helping in the chronological aging and photoaging process, improving the skin density.¹⁸ According to dermis imaging analyses, it is possible to observe that both treatments were effective in the improvement of the dermis echogenicity, but the effects with the oral treatment were more pronounced, as it acts on the skin from deeper layers, which are the mechanism to the improvement of dermis.¹⁹

In summary, the topical application of peptides acted in the clinical efficacy with significant results on the hydration, skin elasticity, and wrinkles reduction parameters, with short-term effects. Meanwhile, the oral supplementation with hydrolyzed collagen and vitamins improved skin elasticity and density (dermis), along with a reduction in pores and wrinkles, with a progressive effect, long-term.

These results suggest that the treatments, when combined, act on different regions of the skin and with different mechanisms that may enhance the benefits and thus, promote the improvement of the general conditions of the skin. In summary, the results highlight the importance of the traditional use of cosmetic formulations combined with the intake of oral supplementation, as they can be considered complementary, not equivalent.

It is important to notice the limitations of this study, as the nonuse of a vehicle treatment group for the topical formulation as control, the differences in the composition of topical and oral formulations and the differences between the clinical images before and

TABLE 1 Determination of the main composition of total amino acids in both study products

Determination of the composition of total amino acids in hydrolyzed rice extract		
	Results	%
Arginine	57 mg/100 mL	10.5
Glycine	25 mg/100 mL	4.6
Lysine	22 mg/100 mL	4.0
Serine	41 mg/100 mL	7.6
Tyrosine	49 mg/100 mL	9.1
Glutamic acid	100 mg/100 mL	18.5
Alanine	33 mg/100 mL	6.1
Histidine	16 mg/100 mL	3.0
Methionine	6 mg/100 mL	1.1
Phenylalanine	44 mg/100 mL	8.1

Determination of the composition of total amino acids in the hydrolyzed collagen	
	Results
Arginine	1026 mg
Glycine	1574 mg
Lysine	378 mg
Serine	288 mg
Tyrosine	18 mg
Glutamic acid	1026 mg
Alanine	801 mg
Histidine	72 mg
Hydroxyproline	1161 mg
Proline	1206 mg

after treatment. More studies should be developed to complement these informations.

Furthermore, the use of peptides in both forms helps to enhance the hydration, elasticity, and firmness of the skin.

Finally, the obtained results with the oral supplementation and topical application of hydrolyzed proteins were complementary in the improvement of general skin conditions.

5 | CONCLUSIONS

Under the experimental conditions of this study, it was possible to conclude that the use of topical formulations and the intake of an oral supplementation, both containing peptides, are a good alternative to maintain the skin health as a whole, as they act in different skin mechanisms. The combination of these treatments can be considered a new concept for skin care products, once it would fulfill different skin parameters, being a full treatment, improving the hydration, texture, elasticity, and firmness of the skin.

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