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Effects of a preparation based on a functional collagen polymer on the skin in the periorbital area

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Abstract: Aging is an inevitable process that manifests itself in the body through a number of physiological changes. They also include the formation of wrinkles and a decrease in collagen in the skin. Poultry protein-rich by-products can be used as a source of collagen. For the cosmetic industry, collagen hydrolyzate is used for its potential. The study tested the effect of a 1% hydrolyzate prepared from chicken stomachs mixed in a gel formulation on the condition of the skin (elasticity, change in skin relief and reduction in the amount of wrinkles). The aim was to determine, using non-invasive *in vivo* testing, whether collagen-based preparations incorporated into a carbopol gel applied topically for 8 weeks could have a positive effect on the biophysical properties of the skin in the periorbital area. For skin elasticity, the resonance times decreased on average from 257 ± 48 A.U. at 218 ± 58 A.U. to the right and from 258 ± 46 A.U. at 219 ± 57 A.U. on the left area. According to the values found, all roughness parameters R1 to R5 also decreased. For parameter R1 there was an average decrease of $38 \pm 3\%$, for parameter R2 there was a decrease of $43 \pm 4\%$, roughness parameter R3 decreased by $39 \pm 3\%$, roughness parameter R4 decreased by $34 \pm 5\%$ and finally roughness parameter R5 decreased by $39 \pm 5\%$. Visualization of the area showed a decrease in the amount of wrinkles by $16 \pm 2\%$ on the right and by $15 \pm 3\%$ on the left. The results of regular application of the formulation with the addition of 1% collagen hydrolyzate confirmed the assumption of increased elasticity, reduced skin roughness and reduced amount of wrinkles in the periorbital area. Chicken collagen could thus find application in the production of antiaging agents.

Key Words: cosmetic formulation, chicken collagen hydrolyzate, skin elasticity, skin relief, wrinkle reduction

INTRODUCTION

Collagen is a natural resource that can be used in the cosmetic field. This scleroprotein is able to reduce the amount of wrinkles and thus slow down the negative effects of aging due to both external and internal influences during human life. Substances based on natural polymers are sought-after components for the development of effective cosmetic products. A significant effect on the treated skin occurs with long-term application of such preparations. Biopolymers are an attractive raw material due to their biocompatibility to the skin and biodegradability in relation to the environment (Peng et al. 2004, Sahana and Rekha 2018).

The skin is the largest organ of the human body, occupies 16% of the body's weight and its surface covers about 1.8 m². The skin acts as a protective barrier against mechanical shocks, ultraviolet radiation, toxic substances and has a thermoregulatory, sensory, metabolic and regulatory function (Bensouilah and Buck 2006). The skin consists of three layers; these are the *epidermis*, *dermis* and *subcutis*. The *epidermis* or epithelial tissue represents the uppermost layer of the skin and protects the skin primarily against the penetration of foreign substances. The *epidermis* layer can be divided into four sublayers; namely *stratum basale*, *stratum spinosum*, *stratum granulosum* and *stratum corneum*. In the lowest basal layer of the skin or *stratum basale*, cell division and the formation of new skin cells occur. This is a very important property of the skin. The basal layer produces keratinocytes

and desmosomal proteins that form the skin cytoskeleton (Feather et al. 2020). Keratinocytes are the cornering cells that have the largest presence in the skin and are capable of so-called keratinization, as well as differentiation and migration of cells towards the surface, where they form the outer corner layer of the skin or *stratum corneum* (Caterina 2014). The *dermis* or fibrous joint is the middle part of the skin and is made up mainly of collagen and elastin, which give the skin strength and elasticity. The *subcutis* or subcutaneous ligament consists mainly of the fat part and contains a different number of fat cells in different places (Mallat and Marieb 2005). Aging can be defined as the progressive inability of an organism to maintain its internal environment in a constant, non-declining quality. Aging affects the whole organism, including the skin, where the rate of aging is individual for each individual and is influenced by many factors. The main trigger of the skin's aging process is the lack of nutrients and moisture that enter the skin through the bloodstream, from which the skin takes important nutrients and oxygen. This negative phenomenon reduces skin thickness, slows the rate of keratinocyte renewal, and decreases blood flow through the skin (Kittnar et al. 2020).

Worldwide poultry production is growing year by year. According to the Czech Statistical Office, the consumption of poultry meat in 2020 was around 29 kg/person/year (Czech Statistical Office 2021). With the ever-increasing production of poultry meat, there is also an increasing amount of by-products, which account for up to 30% of total production. As these wastes contain significant amounts of protein, the study seeks to improve procedures for obtaining quality raw material from unused by-products that are not normally edible, thus enabling the raw material to be refined for further applications (Jayathilakan et al. 2012). When wet collagen is heated to a temperature of around 60 °C, significant structural changes occur. This is a first-order transformation, ie the conversion of crystalline biopolymer modification (ordered structure) to amorphous modification (disordered structure) (Slezák and Ryška 2006). Functional polymers, so-called collagen hydrolysates, are formed. Thanks to their properties, they can be used, for example, in the cosmetic industry in the development of formulations with an antiaging effect.

MATERIALS AND METHODS

The process of production of chicken collagen hydrolyzate, the process of preparation of cosmetic gel formulation, and also the selection of volunteers were already published in last year's paper (Polastikova et al. 2020).

Appliances, tools and chemicals

Shaker LT2 (Kavalier, Czech Republic), Schott Gerate GMBH heating plate with stirrer (Schott, Czech Republic), RZR 2020 stirrer (Ika, Germany), analytical laboratory balances (Kern & Sohn, Germany), WTB Binder E-28 dryer-TB1 (Binder, Germany), Braher P22/82 meat cutter (Braher, Spain), MPA 10 station with individual probes, Reviskometr® RV 600, Skin Visiometer® SV 700 USB, vacuum pump VP 45, Visioskop® PC 35 (Courage & Khazaka, Germany), ordinary laboratory glassware, metal sieve, mortar, plastic utensils, PA fabric, scissors, pulp, double-sided adhesive paper rings, plastic sticks and cups, transparent cover foil, paper frames, tweezers.

Protamex 6.0 T enzyme (*Bacillus* protease complex developed for protein hydrolysis in the food industry, meets strict hygiene requirements for collagen extraction; declared activity 1.5 AU/g), carbopol gel, 10% and 0.03 M NaOH solution, water, ice CH₃COOH, make-up remover Bioderma Sensibio H₂O, petroleum ether and ethanol, Jarisch's solution, 0.2 M NaCl, two-component silicone.

The objectives of this work

The study verified the effect of a cosmetic formulation based on collagen on selected biophysical parameters of the skin (skin elasticity, change of skin relief and reduction of wrinkles) using bioengineering non-invasive methods *in vivo*. Collagen was obtained by processing chicken stomachs. It was then incorporated into a gel to treat the periorbital area of the face of a group of volunteers for 8 weeks each morning and evening. The study builds on a previous study (Polastikova et al. 2020), in which the positive effects of chicken collagen on hydration and transepidermal water loss were found. Scientific hypothesis: application of a cosmetic gel formulation in the periorbital area with 1% chicken collagen hydrolyzate can be expected to increase skin elasticity and reduce eye wrinkles.

Statistical analysis

The obtained data were processed and statistically evaluated in Microsoft Excel (2010). From all the results, the arithmetic mean \bar{x} was first calculated and then the standard deviation s was determined according to the following equations (1) and (2).

$$\bar{x} = \frac{1}{N} \sum_{i=1}^N x_i \quad s = \sqrt{\frac{1}{N} \sum_{i=1}^N (x_i - \bar{x})^2} \quad (1) \text{ and } (2)$$

Where: \bar{x} – arithmetic mean, N – number of measurements, x_i – measurement value, s – standard deviation

Organization and principle of measurement

The study group consisted of 10 volunteers aged 50 ± 9 years. Wrinkle reduction and skin elasticity in the periorbital area were monitored in February, March and April 2020, always in the same laboratory, under the same conditions (air temperature 21 ± 2 °C and relative humidity $38 \pm 2\%$). The volunteers were acquainted with the organization of the work before the first measurement, and since the measurement was based on international ethical principles of biomedical research with human participants, the volunteers had to sign an individual informed consent and then complete a health questionnaire; at the same time, they could terminate the study at any time and without giving a reason. Prior to the measurement, the participants' skin was cleansed with Bioderma make-up water and Jarisch's solution and then left to stand for 20 minutes. The collagen gel was applied in a thin layer in the morning and evening to the periorbital area for eight weeks. After four weeks, the volunteers came for a control measurement and then after another four weeks for the final measurement.

The principle of measuring the elasticity of the skin with the Reviscometer[®] RV 600. The device evaluates the elasticity, or elasticity of the skin, using the speed of propagation of the acoustic wave. The measurement is focused on measuring the skin at different angles and the results depend on the anisotropy and isotropy of the skin. The Reviscometer[®] RV 600 is a device used to measure the elasticity of the skin using two sensors, one sensor emitting a shock wave and the other sensor receiving a wave; it all depends on the speed at which the wave spreads through the skin fibers. An adhesive paper ring is glued to the measuring point on both sides and then a probe is attached, which allows measurements in 30° steps, and the data are immediately displayed on a computer monitor, which is connected to the MPA station. For each volunteer, 13 skin elasticity values were measured for each angle of rotation (0° to 360°) (The Reviscometer[®] RV 600: Technical charges 2013).

The principle of measuring the change in the relief of wrinkles using Visiometer[®] SV 700. It is a device that is used to analyze the microrelief of the skin, where light passes through a very thin blue silica replica and is displayed on a CCD camera. The amount of light transmitted depends on the thickness of the silicone casting. The procedure for measuring skin relief was as follows; first it was necessary to glue a double-sided adhesive paper ring to define the measured area and then to prepare a two-component silicone, which consisted of a base and a catalyst. While mixing, the substances were applied in a ratio of 1 : 1 to a plastic cup and the excess air was sucked off by means of a vacuum pump, mixing time 20 s. After solidification, about 5 minutes, the replica was glued to a paper stencil, which was inserted into a Skin Visiometer[®] SV meter. Roughness parameters R1 to R5 were determined for each replica obtained (Courage & Khazaka: Technical charges 2016).

The principle of measuring wrinkle reduction using Visioscope[®] PC 35. It is a USB camera equipped with polarizing filters, which is able to take images with a magnification of thirty times, which can then be analyzed. The camera was applied to the periorbital area, then an image was taken, which was immediately evaluated. Using this method, we visually used the photo to determine the smoothing of the skin after eight weeks of application of the collagen formulation and also the percentage of wrinkles in the area. The change in skin relief and the reduction of eye wrinkles were recorded using the CSI-Complete Skin Investigatio program (Courage & Khazaka: Technical charges 2016).

RESULTS AND DISCUSSION

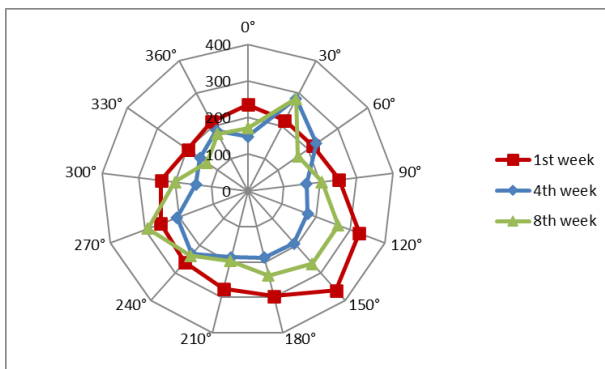
Evaluation of the effect of collagen gel on skin elasticity

The study verified the effect of a cosmetic product with collagen on the elasticity of the skin. Resonance time (RRT) values were obtained at different angles of rotation (0° to 360°) and are reported in reviscometric units [A.U.]. Due to the circular shape of the probe, ray graphs were constructed for better clarity, which show the RRT results for individual angles in the right and left areas of the face; we can see the graphs in Figure 1. It is true that the higher the RRT values, the higher the stiffness or lower elasticity of the volunteers' skin. The data obtained show that the cosmetic formulation had an effect on increasing elasticity, as RRT values decreased in more than 85% of cases during the eight-week application. On average, there was a decrease in RRT from 257 ± 48 A.U. at 218 ± 58 A.U. on the right temple and from 258 ± 46 A.U. at 219 ± 57 A.U. on the left temple. Thus, it has been shown that collagen hydrolyzate in a cosmetic formulation has a positive effect on the skin and also improves its elasticity, as it directly promotes the formation of type I collagen and fibroblasts in the *dermis* layer.

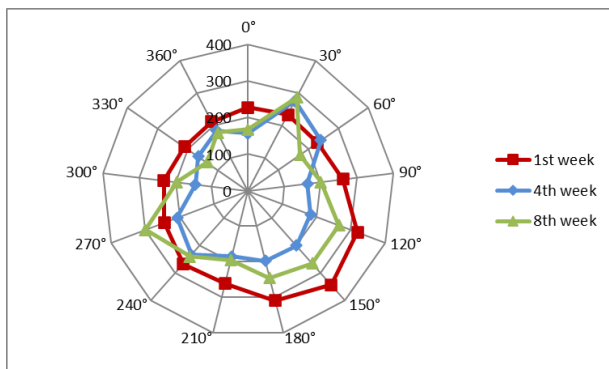
Similarly, in 25 Japanese women taking 7 g of collagen hydrolyzate orally, a significant improvement in forearm elasticity was found. In another innovative study involving 60 women, topical and oral administration of collagen hydrolyzate were combined. After only one month, the condition of the skin improved, specifically the hydration increased. After ninety days, the elasticity of the skin improved and even the wrinkles on the forehead were reduced (Aguirre-Cruz et al. 2020).

Figure 1 Comparison of average RRT values for individual angles of rotation of the probe in the periorbital region

A) Right sleeping area



B) Left sleeping area



Evaluation of the effect of collagen gel on skin roughness

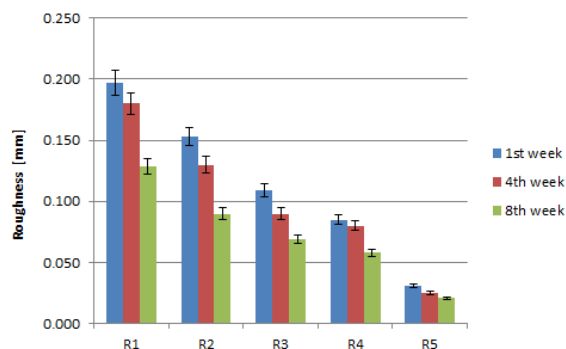
Skin roughness was evaluated using roughness parameters R1 to R5 obtained by processing replicas removed from the periorbital area using a Visiometer[®] SV 700. The definition of roughness parameters is based on DIN 4762-4768. The roughness parameter R1 (Rt) defines the distance between the highest peak and the deepest depression; the roughness parameter R2 (Rmax) represents the maximum local roughness from the roughness values of the different segments; the roughness parameter R3 (Rz) calculates the average roughness from the five highest and at the same time from the five lowest segments; the roughness parameter R4 (Rp) indicates the maximum height of the profile and its value is related to the length of the wrinkle, and finally the roughness parameter R5 (Ra) determines the average deviation of the actual profile from the average. The relationship between the individual parameters is: $R1 \geq R2 \geq R3 \geq R4 \geq R5$ (DIN 4762–4768: Determination of water content in Honig. Deutsche Industrie Norm). From the average values of the roughness parameters R1 to R5, bar graphs were constructed in individual weeks of measurement for both the left and right areas. It is evident from the graphs that wrinkles were gradually smoothed out due to the cosmetic formulation, as all roughness parameters decreased during the eight-week application. According to the calculated values, there was an average decrease of the parameter R1 by $35 \pm 3\%$ (right area) and by $41 \pm 2\%$ (left area); roughness parameter R2 decrease by $41 \pm 5\%$ (right area) and by $44 \pm 3\%$ (left area); roughness parameter R3 decrease by $37 \pm 4\%$ (right area) and by $40 \pm 2\%$ (left area); roughness parameter R4 decrease by $32 \pm 5\%$ (right area) and by $36 \pm 4\%$ (left area) and finally roughness parameter R5 decrease

by $32 \pm 6\%$ (right area) and by $45 \pm 4\%$ (left area). Thus, the results shown in Figure 2 show that collagen has a significant effect on reducing the depth of wrinkles in the periorbital area when applied for a long time.

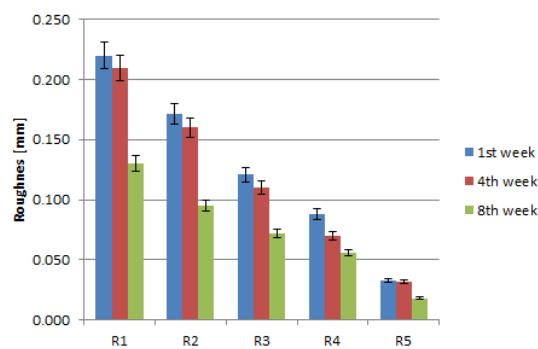
Proksch et al. tested the effect of collagen hydrolyzate administered orally on the reduction of wrinkles in the periorbital area in women aged 45–65 years. One group received placebo and the other group collagen hydrolyzate. After eight weeks, wrinkles were reduced by 20.1% compared to the placebo-only group (Proksch et al. 2014).

Figure 2 Average values of roughness parameters R1 to R5 in individual weeks of measurement in the periorbital area

A) Right sleeping area



B) Left sleeping area

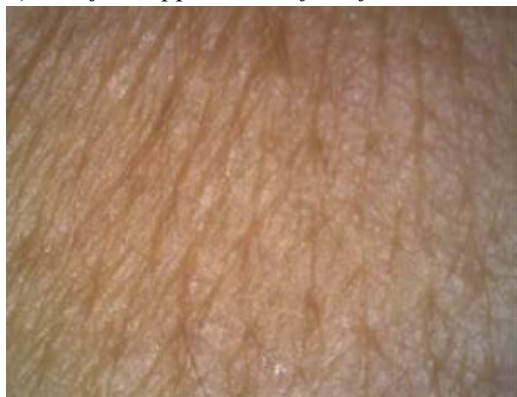


Evaluation of the effect of collagen gel on wrinkle reduction

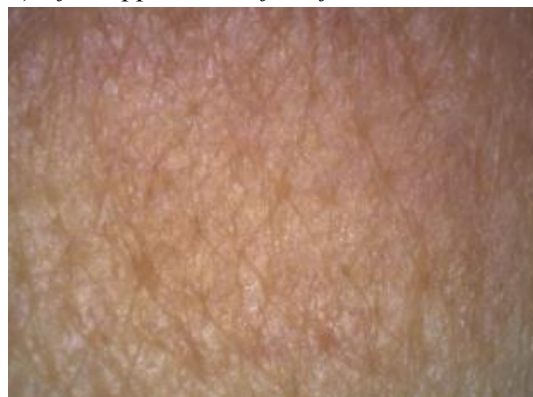
The amount of wrinkles found after the end of the study correlated with the evaluation of the previous annotated parameters. In volunteers, the mean amount of wrinkles on the right side of the study was $16 \pm 2\%$ on the right side and $15 \pm 3\%$ on the left side. During the eight-week application, it decreased to $13 \pm 1\%$ (right side) and to $12 \pm 2\%$ (left side). In Figure 3, we can observe the change in skin relief before and after the eight-week application of the gel formulation in volunteers, in which there was an average reduction in wrinkles from $12.2 \pm 0.6\%$ to $10.6 \pm 0.4\%$.

Figure 3 Change in skin relief in the periorbital area before and after application of the gel cosmetic formulation (age of the volunteer 45 years)

A) Before application of the formulation



B) After application of the formulation



CONCLUSION

The study tested the effect of a gel cosmetic formulation enriched with 1% addition of chicken collagen hydrolyzate. During eight weeks of regular two-day application in the periorbital area, skin parameters such as elasticity, skin roughness and wrinkle reduction were monitored. The change of parameters before the application of the model formulation and after the eight-week application was monitored. It was expected to increase skin elasticity, reduce skin relief, and reduce wrinkles.