Assessment of the Properties of Emulsions Containing Different Amounts of Collagen Derived from Calf Skins

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This research aimed to assess properties of emulsions containing various concentrations of collagen hydrolysate produced from calf skins. The most optimum variant of collagen separation from calf skins was used, developed by these authors. The resulting aqueous collagen hydrolysate was employed to produce oil/water cosmetic emulsions containing from 1 to 8 wt % of hydrolysate. Hydration and sensory properties of the proposed substances were evaluated. Appearance and rubbability of the emulsions and washability of make-up were determined. Emulsions containing collagen hydrolysates were assessed by respondents of two age brackets: 18-35 and 36-55. Stability of the emulsions was appraised using centrifuge and temperature testing. On application of these preparations, the skin was highly moistened, particularly in the older respondent group. Consistency of the emulsions was homogeneous and free from clotting. The work confirmed that part of hides constituting the waste can be a potential source of collagen used as an anti-ageing ingredient of cosmetic emulsions.

Keywords: collagen hydrolysate, leather, emulsions, skin hydration, sensory assessment

Collagen is a biopolymer whose properties are frequently used by medicine and pharmacy due to its special characteristics, such as biodegradability and weak antigenicity [1]. Thus collagen, as a new type of biomaterial, has been used in drug delivery systems and tissue engineering [1-3]. Besides, collagen constitutes a third of all body proteins. It is the basic component of connective tissue, where it fulfils structural functions.

Due to its high molecular mass, collagen is unable to penetrate the epidermis. It forms a hydrophilic film on the skin surface which reduces trans epidermal water loss [4]. Its protective function also consists in preventing some of the effect of anion surfactants [5].

Skin ageing is a natural and inevitable process whereby regeneration is slowed, biological activity of the organism or its response to the lifestyle or medical conditions are reduced. Human attitudes to direct skin exposure to the sun have a role to play as well, though the expanding knowledge of hazards associated with this exposure should translate into appropriate behaviour [6,7]. The ageing process begins at around 25, when collagen synthesis is reduced [8]. Lower activity of enzymes involved in post-translation modifications can be observed at the time. Solubility of collagen is lower and its fibres become thinner. Apart from declining skin elasticity due to lower quantities of collagen and elastin fibres, quantities of subcutaneous fatty tissue diminish and the tissue is redistributed [9]. Changes of the chemical and physical properties of collagen fibres that occur as part of the ageing process are very noticeable[10]. The face's oval shape alters, facial muscles are weakened and gravitational wrinkles appear [9].

Alterations of epidermis, dermis and subcutaneous tissue are all part of the endogenous ageing process[11].

Counteracting the process of skin ageing is a considerable challenge to contemporary medicine. In many scientific centres around the world, researchers are searching for new compounds that could affect the remodelling of skin and also enhance its ability to stop evaporation of water from its surface [9,12]. Introducing collagen to everyday creams is a trend in present-day cosmetology. Application of such preparations provides an opportunity for maintaining the skin in good condition for longer [13].

A range of collagen preparations are used in cosmetics, namely, aqueous skin protein dispersions in buffering systems which contain from 300×10^{-6} to 1200×10^{-6} g/cm^3 hydroxyproline. Where 5-10% is added to cosmetics like creams, milks and lotions, they display excellent moistening and regeneration effects on the skin [14].

Collagen can only be introduced to cosmetic products containing water, e.g. emulsions, when in its soluble presentation. This form of collagen can only be produced by way of hydrolysis. The process can be chemical, in the presence of hydrogen ions in an alkaline environment, or as protein hydrolysis using enzymes [15].

All animals, including sea creatures like sponges, fish or jelly fish, can be sources of collagen proteins. Loss of structural integrity in the isolation process, heterogeneity and immunogenicity of collagen proteins occurring in nature have contributed to development of their synthetic and recombined forms [13].

Leather is an industrial commodity manufactured from skin. It is established that leather derives its mechanical strength from the structural stability of the main constituent of skin, eg. collagen[16]. As regards collagen wastes leather industry dominates in generating voluminous amounting up to 600 kg per ton of skins/hides processed [17]. Collagen being a protein tends to destabilize on exposure to heating or specific enzymes such as collagenase. Hence, collagen needs to be stabilized for its widespread applications in fields such as leather making, tissue engineering and cosmetics[16]. A novel method of obtaining collagen hydrolysates has been utilised in presented research, based on mixed acidic, basic and enzymatic hydrolysis of collagen according to our procedure [18]. Similar research was carried out by authors

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TYPE OF EMULSION										
Ingredients [%]		E I	E II	E III	E IV	E V	E VI	E VII	E VIII	E IX
Oil phase	Parafin oil	12								
	Eumulgade SE	6								
	Lanette O	1								
	Vitamin A + E	1								
Water phase	Collagen hydrolisate	0	1	2	3	4	5	6	7	8
	Glycerol	3								
	Vitamin C	1								
	Sodium benzoate	0,2								
	Water	Up to 100 %								

Table 1
RECIPE OF EMULSIONS

who showed that collagen extracted from calf limed splits at a low temperature by the pepsin-digestion method had properties similar to those of the commercial collagen [19-20]. Collagen polypeptide had also been prepared by other authors [21] from the limed hide offal in a two-step protease (EA and EA 537) treatment process at 45-60°C.

The main purpose of this study was to assess the degree of hydration of skin administered with emulsions containing different amounts of manufactured collagen hydrolysate. Another aim was to determine sensory properties of the manufactured emulsions.

Experimental part

Materials and methods

Skins of a two-month-old Lowland White calf from Sokolow S. A. abattoir in Tarnow (Poland) was the starting raw material for collagen hydrolysates.

Collagen hydrolysate was a colourless liquid of a homogeneous consistency, fully soluble in water, with a specific, yet weak odour. Acidic *p*H of the hydrolysate (3.71±0.02) slowed microorganism growth in collagen solutions. The testing was confirmed by means of microbiology analysis [18].

Eumulgade® SE – producer BASF, INCI name: Glycearyl Stearate (and) Ceteareth-20 (and) Ceteareth-12 (and) Cetearyl Alcohol (and) Cetyl Palmitate**.** According to the manufacturer's information, Emulgade® SE is a self-emulsifying base with consistency giving properties, and is suitable for preparation of cosmetic and pharmaceutical oil-in-water creams and lotions.

Lanette O – producer BASF, INCI name: Cetearyl alcohol. According to the manufacturer's information, Lanette® O is a cetyl stearyl alcohol that is used for viscosity regulation in cosmetic oil-in-water emulsions.

Paraffin oil – producer Aflofarm Farmacja Polska Sp. z o.o., INCI name: Paraffinum liquidum

Vitamin A+E – producer Hasco Lek, INCI name: Retinol and Tocopherol

Glicerolum 86% – producer Wytwornia Euceryny Laboratorium Farmaceutyczne COEL S.J. E.Z.M. Konstanty, Krakow, INCI name: Glycerin

Vitamin C – producer - Warszawskie Zaklady Farmaceutyczne Polfa S.A., INCI name: Ascorbic acid

Sodium benzoate – producer PPH Galfarm Sp. z o.o. Krakow, INCI name: Sodium benzoate

Production of collagen hydrolysate

Collagen hydrolysates was produced according to the authors' unique formulation beginning with basic hydrolysis using 10% NaOH. Enzymatic hydrolysis was then undertaken employing pancreatic protease, finally the skins were treated with a 20% citric acid solution. Application of the citric acid supplemented the earlier basic and enzymatic skin hydrolysis, though the acid also acted as a hydrolysate preservative. The detailed method of collagen hydrolysate production was published in [18]. The results presented there implied the highest concentration of hydroxyproline (the parameter identifying quantity of collagen) were obtained in a sample containing 1 part by mass of weighed lime pelt treated with 6 parts by mass of 20% citric acid. This method of deriving collagen from calf hides produced collagen solutions of high hydroxyproline content, i.e. 2202±3.2 mg/dm^3, that is, approximately 1000 mg/dm^3 greater than in hydrolysates available in the market.

Varied quantities (1-8%w/w) of the hydrolysate were added to cosmetic preparations.

Producing emulsions containing different contents of aqueous collagen hydrolysates

Each emulsion was prepared in the same way, only with varied quantities of collagen hydrolysate additions to a sample. The oil phase ingredients weighed as per the formulation (table 1) were melted in a glass container at 80÷85°C and mixed with a mechanical stirrer until the consistency became homogeneous. The water phase ingredients were mixed in another vessel till their consistency was homogeneous and were heated to 80÷85°C. Both the phases were then combined and mixed intensively with a programmable homogeniser Omni Macro ES for 4 min.s at the rate of 17500 revolutions/minute [rpm]. The emulsion was cooled down to 35°C, collagen hydrolysate and sodium benzoate were added (table 1). The resultant emulsions were stored at +/- 2-8°C.

Determining appearance of the emulsions

Approximately 0.01g of the cosmetic was collected and a visual assessment was carried out under identical lighting to determine homogeneity, thickness and cohesion of the preparation. The emulsions were inspected promptly on their production and then after 1, 7 and 14 days.

Determining rubbability of the preparations

Approximately 0.01g of a given cosmetic was spread on the hand back skin and the time was measured after which the emulsions were absorbed.

Testing washability of make-up

Effectiveness of make-up removal using the emulsions with varying collagen contents was evaluated. Lines of colour cosmetics (lipstick, foundation, eye liner and eyelid shadow) were applied to hand back skin. The make-up was removed after 5 min with a cotton cosmetic wad saturated with a given preparation. The number of wad movements was the basis for scoring (1-5 very good, 6-10 good, 11-15 average, 16-20 poor washability of make-up).

Sensory testing of emulsions

Consumer testing of the collagen emulsion was conducted by a group of respondents (110 females) on days 1, 7 and 14. The respondents were trained and specifically instructed on the testing. A 5-point scoring scale was introduced, with 5 the maximum and 1 the minimum score. Details of the sensory assessments are discussed in [19]. The testing was carried out at room temperature of 20°C (±2°C) and constant air humidity of 45% (±5%). Correctness of the testing was supervised by a researcher with the University of Technology and Humanities in Radom.

Determination of emulsion stability using centrifugal test

The determination was carried out in a centrifugal machine (Nahita Centrifuges Model 2652; Auxilab, S.L., Beriain, Navarra, Spain) at 1008 RCF and for 30 min. Test tubes were filled with 15-20 mL of the emulsion and then centrifuged. They were observed every 10 min. If the emulsion remained homogeneous after 30 min, it was considered to have proper stability.

Determination of emulsion stability using the temperature test

To determine the stability, a temperature test was also used. Emulsions were kept for 24 h at different temp. (alternately 5°C±1°C) and 40°C±1°C), initially at 45°C. Emulsions were tested for 7 days.

Assays of emulsion *p*H

*p*H measurements were taken at 22ºC (±1ºC) using a pH-metre including a measurement electrode (Microcomputer pH-meter CP-315M-Elmetron).

Determining emulsions type with the dilution method

The testing was conducted in two beakers. 10 cm^3 paraffin oil was added to one and 10 cm^3 distilled water to the other. 1g of the test preparation was then added to each and contents of the beakers were carefully mixed using a 100 rpm magnetic stirrer. Solubility of emulsion drops in water and oil was assessed after a specified period of time.

Testing of hydration levels

110 females in two age brackets: 18-35 (55 women) and 36-55 (55 women) were tested. All the respondents were students at the University of Technology and Humanities in Radom at the faculties of Cosmetology, Physiotherapy and Nursing. Prior to the testing, the respondents consented in writing to measurements of their skin's functional parameters. Skin hydration levels of all the volunteers were measured by a trained individual. The testing continued for 8 weeks (20 October 2014 to 14 December 2014).

A corneometre CM 825 by Courage+Khazaka Electronic was utilised at room temperature of 20+/-2°C and constant air humidity of approximately 40%. 9 areas of 20 x 20 mm were marked on forearm skin and designated with appropriate symbols (EI – EIX). Control testing was conducted on clean, de-greased skin at all times. This, and a zero time of emulsion impact on the skin, that is, without applying a preparation, were assumed as points of reference. About 0.01 g of a test emulsion was then placed in an appropriate area for 5 min. The preparation was removed with cosmetic wads after that time. A probe was applied with a constant pressure perpendicular to the skin surface. Five measurements were carried out for a given skin area at a specified time following application of a cosmetic. The probe and a test skin fragment were wiped with clean cosmetic wads prior to and after a test. Hydration measurements were initially undertaken at 15 min intervals for 90 min. Another test commenced after 30 min and final 3 measurements were conducted every hour. The testing continued for a total of 5 h. The detailed procedure for computing changes of hydration levels which produced results for this parameter are set out in [21].

Statistical analysis

The results of sensory evaluation were performed by using the Statistica 6.2 software (StatSoft, Tulsa, USA) using multi-factorial ANOVA test. The results of Changes of the skin hydration degree were performed using ANOVA test with repeated measurement. Significance of Differences Between the average values you Evaluated by Means of Duncan test at $p < 0.05$

Results and discussions

The cosmetics industry uses collagen preparations in the form of nutriceutics (diet supplements) and skin preparations (masks, gels or creams). Aesthetic medicine commonly utilises collagen as a tissue filler [13]. The preparations (EI - EIX) containing collagen hydrolysates were white, thick, opaque substances of a unique, weak odour. Visual assessments of the emulsions promptly on as well as 1, 7 and 14 days after their production showed all the emulsions were easy to apply, adhered well to and did not run from hand surfaces. They were also easy to rub into the skin and free from clotting. All the emulsions were absorbed by the skin surface after 20 s, leaving a light polish only in the initial 5 min. Evaluation of effective make-up removal by means of collagen emulsions, regardless of quantities of collagen in formulations, demonstrated all the tested preparations washed make-up effectively. An average of 8±1 movements were made as part of make-up removal. All the preparations had pleasant, delicate odours for the entire period of testing. The unchanging appearance of the emulsions was proof the systems were adequately protected from growth of undesirable microorganisms.

A sensory assessment by volunteers of two age groups (tables II and III, fig. 1) of the emulsions produced proved they were completely homogeneous preparations, easy to apply and not running off the skin, building a thin film once applied. The emulsions did not contain any clots or air bubbles, exhibited homogeneous structures and their consistency fully met expectations of the older respondent grouping (fig. 2). The younger group assessed emulsions E II – E V as homogeneous, cohesive and dense compared to the emulsions without hydrolysate additions. The remaining emulsions (E VI – E IX) displayed more varied consistencies and less homogeneity (fig. 3). In general, the cushion effect (the parameter determining palpability of a spread preparation between thumb and index finger) was felt more as the quantity of collagen in a test preparation rose relative to the control sample. Both groups of volunteers felt the preparation showed minimum palpability on application of emulsions (E VIII and E IX). Some respondents mentioned a minor resistance to spreading of the preparations. This parameter scored above 4.4 on the reference scale of 1 to 5. Taking assessments by all respondents into consideration, spreading of the preparations on the skin was better for emulsions containing collagen hydrolysate than for the control sample without collagen hydrolysate. Volunteers in the age bracket 36-55 pointed out the resistance to spreading was minimum in the case of emulsions E VI, E VII, E VIII and E IX. Smoothing and viscosity were the most variable parameters in the sensory evaluation by all the respondents (fig. 1).

Both the age groups noted improved skin smoothing and flexibility as quantities of collagen in the formulations increased (fig. 2 and 3). However, according to the authors [23], there is no correlation between skin hydration levels

FEATURE	TYPE OF EMULSION								
	E I	E II	E III	E IV	E V	E VI	E VII	E VIII	E IX
C	4,80 ± 0,41	4,80 ± 0,41	4,80 ± 0,41	4,90 ± 0,31	4,85 ± 0,37	4,85 ± 0,37	4,85 ± 0,37	4,90 ± 0,31	4,90 ± 0,31
H	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	4,90 ± 0,31	4,90 ± 0,31	4,85 ± 0,37	4,75 ± 0,44
CE	4,25 ± 0,64	4,40 ± 0,60	4,35 ± 0,67	4,35 ± 0,67	4,45 ± 0,69	4,40 ± 0,60	4,40 ± 0,60	4,55 ± 0,60	4,65 ± 0,59
D	4,60 ± 0,50	4,70 ± 0,47	4,70 ± 0,47	4,60 ± 0,50	4,60 ± 0,50	4,70 ± 0,47	4,60 ± 0,50	4,65 ± 0,49	4,70 ± 0,47
SM	3,50 ± 0,51	3,65 ± 0,49	3,65 ± 0,59	3,70 ± 0,57	3,75 ± 0,55	3,75 ± 0,44	3,80 ± 0,41	3,95 ± 0,22	3,90 ± 0,31
ST	3,05 ± 0,60	3,25 ± 0,55	3,55 ± 0,51	3,60 ± 0,50	3,55 ± 0,51	3,60 ± 0,50	3,65 ± 0,49	3,60 ± 0,50	3,65 ± 0,49
G	4,20 ± 0,62	4,45 ± 0,60	4,40 ± 0,60	4,45 ± 0,60	4,50 ± 0,61	4,45 ± 0,60	4,65 ± 0,49	4,70 ± 0,47	4,60 ± 0,50
A	4,25 ± 0,55	4,50 ± 0,51	4,45 ± 0,51	4,50 ± 0,51	4,60 ± 0,50	4,60 ± 0,50	4,60 ± 0,50	4,60 ± 0,50	4,55 ± 0,51

Legendary:
C, Consistency; CE, Cushion effect; D, Distribution; SM, Smoothing; ST, Stickiness; G, Greasiness; A, Absorption

Table 2
SENSORY PROFILE OF EMULSIONS FOR THE RESPONDENTS AGED 18-35 YEARS

FEATURE	TYPE OF EMULSION								
	E I	E II	E III	E IV	E V	E VI	E VII	E VIII	E IX
C	4,90 ± 0,31	4,90 ± 0,31	4,90 ± 0,31	4,95 ± 0,22	4,95 ± 0,22	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	4,95 ± 0,22
H	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00	5,00 ± 0,00
CE	4,30 ± 0,57	4,45 ± 0,51	4,40 ± 0,50	4,35 ± 0,49	4,45 ± 0,51	4,35 ± 0,49	4,30 ± 0,47	4,55 ± 0,51	4,50 ± 0,51
D	4,45 ± 0,51	4,55 ± 0,51	4,60 ± 0,50	4,55 ± 0,51	4,50 ± 0,51	4,65 ± 0,49	4,65 ± 0,49	4,70 ± 0,47	4,70 ± 0,47
SM	3,50 ± 0,51	3,90 ± 0,31	4,35 ± 0,49	4,60 ± 0,50	4,65 ± 0,49	4,75 ± 0,44	4,75 ± 0,44	4,80 ± 0,41	4,75 ± 0,44
ST	3,20 ± 0,62	3,55 ± 0,51	3,85 ± 0,37	3,85 ± 0,37	4,05 ± 0,39	3,95 ± 0,22	3,95 ± 0,39	3,90 ± 0,31	4,05 ± 0,22
G	4,15 ± 0,59	4,55 ± 0,51	4,45 ± 0,51	4,55 ± 0,51	4,50 ± 0,51	4,55 ± 0,51	4,60 ± 0,50	4,55 ± 0,51	4,45 ± 0,51
A	4,10 ± 0,64	4,50 ± 0,51	4,60 ± 0,50	4,50 ± 0,51	4,40 ± 0,50	4,40 ± 0,50	4,50 ± 0,51	4,65 ± 0,49	4,70 ± 0,47

Legendary of C, H, CE, D, SM, ST, G, A is given in Table II

Table 3
SENSORY PROFILE OF EMULSIONS FOR THE RESPONDENTS AGED 36-55 YEARS

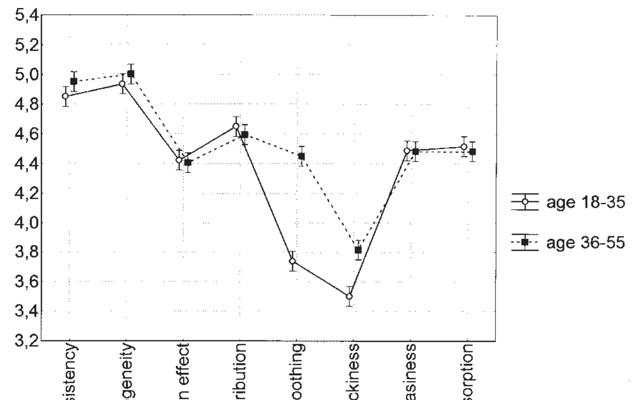

Fig.1. Average sensory profile for the respondents aged 18-35 and 36-55 years. The current effect: F(7.2736)=29.335, p<0.001. Vertical bars indicate 0.95 confidence intervals

and skin elasticity. In their opinion, hydration level of skin relates to the hydration level of epidermis, therefore, hydration of the epidermis does not increase elasticity of the dermis layer. Thus, the growing skin elasticity noted by the respondents was their individual perception, which is of course a major point of product evaluations.

Skin smoothing effects were far more palpable for the older group of respondents, who considered their skin very smooth and soft on application of emulsions E VI - E IX compared to the control sample an hour. All the respondents noticed diminishing viscosity of the skin on application of emulsions containing maximum quantities of hydrolysate (fig. 2 and 3). They felt the skin exhibited minor fatty deposits, though they finally said it was appropriately moistened, pleasant and delicate to touch. Respondents of both the age groups were unanimous in claiming the preparations showed good absorbability in the range of 30 s to 1 min.

Stability analysis by means of centrifugal and temperature testing demonstrated all the emulsions were successful at the tests. The preparations exhibited no destabilisation changes, remaining homogeneous and durable until the end of both the tests.

The emulsions' reactions corresponded to the skin's *p*H and were within 5.6 ± 0.2. All the emulsions dissolved in water, producing light milky solutions. The emulsions applied showed no changes in paraffin oil, on the other hand, which suggested they are all oil in water systems. The authors [24] believe mixes of hydrophilic and lipid (hydrophobic) ingredients which form O/W emulsions on the skin are the ideal form of cosmetic that can supply water and oils to the skin. This hydrates the skin and prevents its degreasing at the same time.

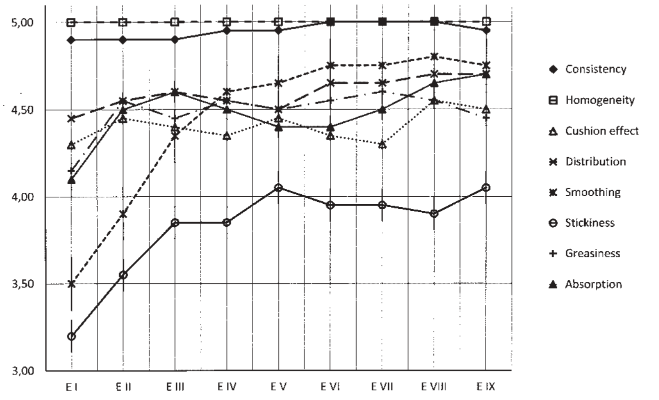

Fig.2. Sensory profile of emulsions done for the respondents aged 35-55 years.The current effect: F(56.1368)=3.1317, p<0.001

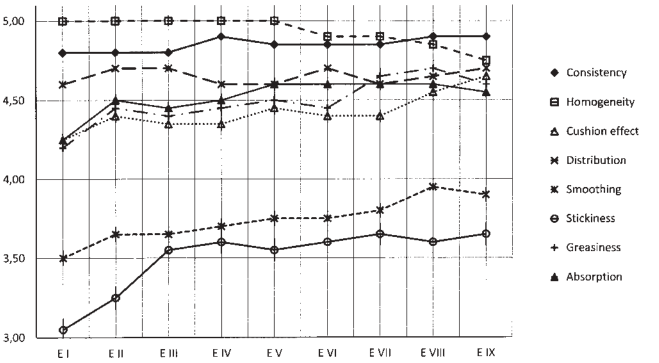

Fig. 3. Sensory profile of emulsions done for the respondents aged 18-35 years.The current effect: F(56.1368)=0.86591, p=0.74868

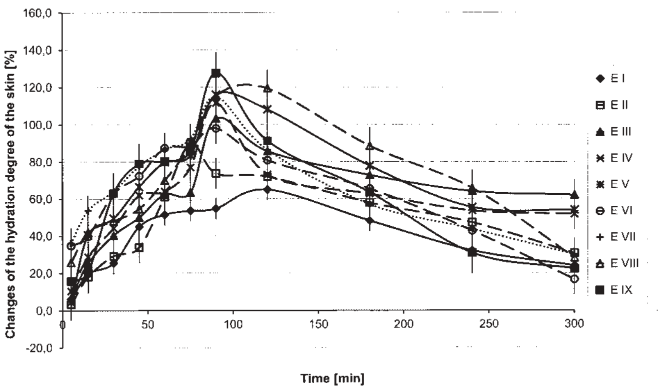

Fig. 4. Changes of the hydration degree of the skin with the passage of time for the women aged 18-35 years.
The current effect: F(80.360)=55.090, p<0.001

The corneometric analysis of the respondent group aged 18-35 demonstrated a marked rise of skin hydration to reach its maximum 90 min after application of the emulsions to the skin (fig. 4). The change of skin hydration 90 min on the application was maximum for the emulsion E IX (127.6%) with the greatest concentration of collagen hydrolysate (8% mass). The parameter declined dramatically, by 36.3% over the following 30 min. For E IX after 180 minutes, the skin hydration changed by 63.5% compared to the skin without any application, nearly equal to the condition after 30 min. The skin hydration reduced over successive hours of the testing to reach 30.8% after 4 hours and become, after 5 h, barely 22.2% higher than that of the skin to which the preparation had not been applied (fig. 4). Variations of the skin hydration levels were similar for all the emulsions tested. Analysis of the curves suggests that, in all the cases, the hydration rose for some time (normally between 5 and 90 min) to reach its maximum and then decline and attain, after 3-4 h, levels comparable to those measured after 30 and 15 min (fig. 5). Testing of skin hydration on application of the preparations E II - E IX to the volunteers aged 36-55 also demonstrated the parameter to grow. The greater the hydrolysate concentration, the higher the degree of hydration (fig. 5,6). Corneometric analysis of this respondent grouping proved the skin hydration grew distinctly to reach its maximum 90 min after application of emulsions E II, E III and E IV (fig. 6). Emulsions containing between 4 and 8% mass of the collagen (E V – E IX) produced grater skin hydration, with a maximum to be reached after 120 min. Contrary to perceptions of the younger group of respondents, the parameter declined slowly on attaining its highest level (fig. 4). As far as skin hydration results for emulsions VII, VIII, IX after 90 min are concerned, its changes averaged 119.3±7.2% for the respondents aged 18-35 and were far greater (174.7±11.17) for the volunteers aged 36-55 (fig. 4 and 6). 5 h after application of hydrolysate creams, skin hydration levels of volunteers aged 18-35 were highest in respect of E III, E IV and E V, that is, 51.7 - 62%. The respondents aged 36-55, meanwhile, exhibited maximum skin hydration changes for the formulations E VI – E IX, i.e. from 100.4 to 123.8% (fig. 6). These skin hydration trends conform with findings by the authors [5]. They claimed collagen is active on the skin surface by restricting trans epidermal water losses by means of a hydrophilic film or partial prevention of activity of anion surfactants.

Collagen in the emulsions generally showed greater hydration capability in mature complexions than in the younger respondent grouping (fig. 7). Sensory assessment was also higher evaluated by the volunteers aged 36-55

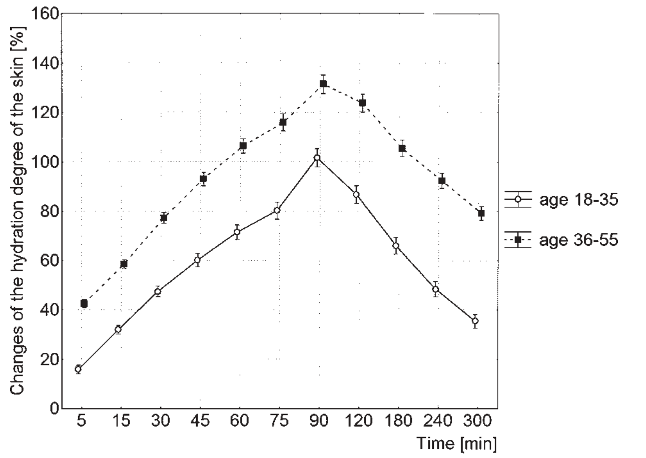

Fig.5. Average changes of the hydration degree of the skin with the passage of time for women aged 18-35 and 36-55 years.
The current effect: F(10.720)=33.764, p<0.001. Vertical bars indicate 0,95 confidence intervals

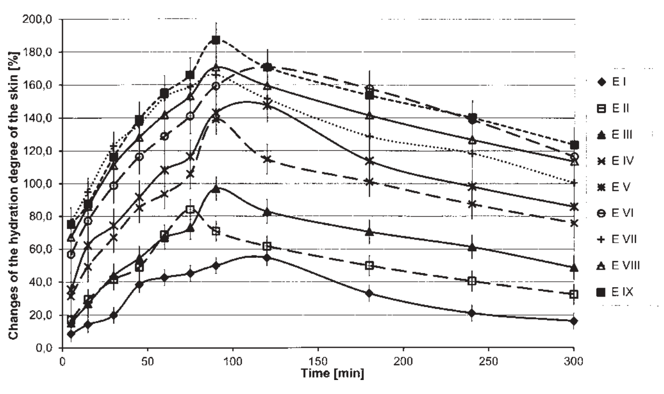

Fig. 6. Changes of the hydration degree of the skin with the passage of time for the women aged 36-55 years.
The current effect: F(80.360)=13.206, p<0.001

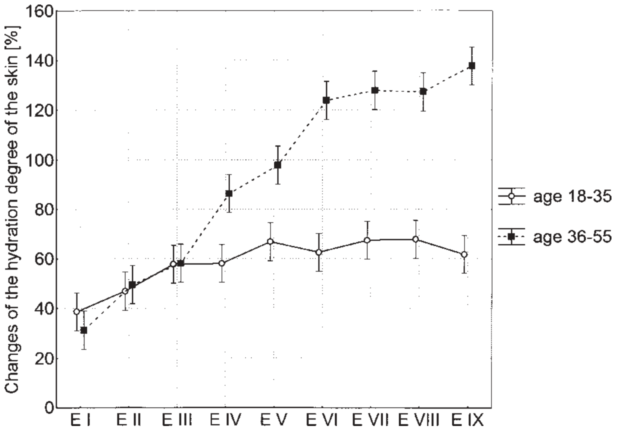

Fig. 7. Average **c**hanges of the hydration degree of the skin in dependence on the type of emulsion for women aged 18-35 and 36-55 years

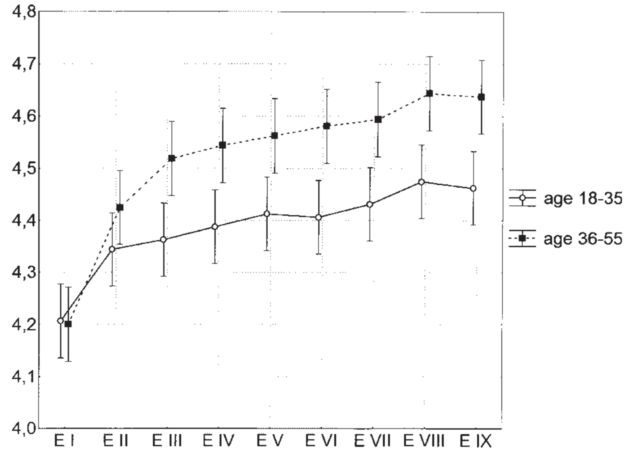

Fig. 8. Average sensory profile in dependence on the type of emulsion for the respondents aged 18-35 and 36-55 years. The current effect: F(8.2736)=1.3866, p=0.19706. Vertical bars indicate 0,95 confidence intervals

(fig.8). Skins of the older respondents, most likely due to higher water deficits, exhibited greater hydration on application of the test preparations. The authors [25] claim, however, the ultimate effects of a preparation depend on molecular responses and kinetics of skin penetration and distribution which determine concentration of ingredients in target skin areas. It is well known water retention is a factor helping skin to delay ageing processes and remain in good condition for longer. According to [9], peptide preparations are able to improve skin hydration provided the right formulation is selected. In their opinion, it is believed that regular use of such preparations can improve and increase skin hydration.

Conclusions\

Hydration and improvements of the skin condition by the emulsions produced have been confirmed. Emulsions containing greater concentrations of collagen hydrolysate displayed maximum skin hydration. Hydration properties of the collagen were most palpable in mature females. Consistency of the emulsions was homogeneous, free from clotting and acceptable to the respondents. Visual evaluation of the emulsions found no microbiological alterations. The preparations retained a pleasant and delicate odour for the entire period of storage, which affirms absence of any adverse bacterial flora.

The results corroborated the authors' hypothesis that collagen hydrolysate can be applied as an ingredient of cosmetic emulsions with hydration and anti-ageing properties and thus it confirms that waste hides manufactured during tanning process could be a potential source of collagen used for cosmetic preparations.

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