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The effect of a 12-week dietary intake of food supplements containing collagen and MSM on dermis density and other skin parameters: A double-blind, placebo-controlled, randomised four-way study comparing the efficacy of three test products

Tina Pogačnik [a], Janko Žmitek [a], Hristo Hristov [b], Petra Keršmanc [a], Mirjam Rogl Butina [a,c], Katja Žmitek [a,b,*]

[a] VIST—Faculty of Applied Sciences, Institute of Cosmetics, Gerbičeva ulica 53, 1000 Ljubljana, Slovenia
[b] Nutrition Institute, Koprska ulica 98, 1000 Ljubljana, Slovenia
[c] Dermatologija Rogl Fabjan (Rogl Fabjan Dermatology), Derčeva ulica 35, 1000 Ljubljana, Slovenia

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ABSTRACT

Dietary supplementation with collagen has been gaining popularity as an anti-ageing remedy. The aim of this randomised, double-blind placebo-controlled four-way study was to investigate and compare the effects of 12-week daily dietary supplementation with products containing 10 g hydrolysed fish collagen (HC) or a combination of 5 g or 10 g HC with 1.5 g methylsulphonylmethane (MSM) on dermis density in healthy human subjects. The secondary objectives were to investigate the effects on a variety of other skin parameters. The study results showed improved dermis density, skin texture and reduced wrinkles for all the active products. However, products containing MSM were superior in the improvement of skin thickness and roughness, and a higher dose of HC combined with MSM was crucial for improvement in hydration. On the other hand, no significant effects of the supplementation on viscoelasticity and transepidermal water loss were determined with any of the products.

1. Introduction

The skin is a complex and dynamic organ that acts as a protective barrier between the body and the external environment. It displays the earliest indications of ageing and is therefore useful for observation of the overall ageing process. Visible signs of skin ageing can impact people's emotional, mental, and psychosocial well-being (Gilchrest & Krutmann, 2006; Gupta, 2010). While longer life expectancy has contributed to new challenges in public health worldwide (WHO, 2015), it has also sparked a growing interest in achieving a more youthful appearance, and the issue of skin ageing has become a common concern, not only in older individuals but also in younger people (Gilchrest & Krutmann, 2006).

As the skin is in direct contact with the environment and constantly exposed to external stressors, the skin ageing process is intricate and influenced by both genetic factors (intrinsic ageing) and various environmental factors (extrinsic ageing), such as exposure to ultraviolet (UV) radiation, pollution, sleep deprivation, smoking, and dietary factors (Farage, Miller, & Maibach, 2017; Krutmann, Bouloc, Sore, Bernard, & Passeron, 2017).

Collagen is the main structural protein of the dermis, and it plays a decisive role in determining skin physiology. It is primarily synthesized by fibroblasts, and in combination with elastin and other extracellular matrix (EM) components imparts mechanical strength, resiliency, and elasticity to the skin. As skin ages, increased expression of matrix metalloproteinases (MMPs) contributes to the excessive degradation of collagen and EM components, while due to the lower activity of dermal fibroblasts the synthesis of these components lags behind, leading to an imbalance favouring degradation (Calleja-Agius, Brincat, & Borg, 2013; Sardy, 2009; Varani et al., 2006). Thus, the density of collagen and other dermal extracellular matrix components diminishes, resulting in a loss of dermal volume, strength, and elasticity (Calleja-Agius et al., 2013; Naylor, Watson, & Sherratt, 2011). These changes in the dermis manifest externally as increased laxity, sagging, wrinkling, and a rough-textured

* Corresponding author at: VIST—Faculty of Applied Sciences, Institute of Cosmetics, Gerbičeva ulica 53, 1000 Ljubljana, Slovenia.
E-mail addresses: tina.pogacnik@vist.si (T. Pogačnik), janko.zmitek@vist.si (J. Žmitek), hristo.hristov@nutris.org (H. Hristov), petra.kersmanc@vist.si (P. Keršmanc), mirjam.rb@dermatologija.eu (M.R. Butina), katja.zmitek@vist.si (K. Žmitek).

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appearance of the skin. It has been shown that collagen content in skin starts to decrease gradually in mid 30s (Reilly & Lozano, 2021) and decrease becomes significant with age beyond the 40s (Castelo-Branco, Duran, & González-Merlo, 1992).

The importance of nutrition, including the intake of macronutrients, micronutrients, and other bioactive compounds, in maintaining skin health and promoting a more youthful skin appearance is widely recognized (Park, 2015; Schagen, Zampeli, Makrantonaki, & Zouboulis, 2012; Shapiro & Saliou, 2001). Dietary interventions and food supplements are gaining popularity as promotors of a youthful appearance; however, the evidence supporting their efficacy is often limited and inconsistent (Muzumdar & Ferenczi, 2021).

In the past decade, oral collagen supplementation has gained extraordinary popularity as an anti-ageing remedy (Jhawar, Wang, & Saedi, 2020). It has been shown that collagen peptides not only provide the building blocks for the body's own collagen production, but also appear to stimulate dermal cellular metabolism and enhance the synthesis of extracellular matrix proteins while inhibiting their degradation by MMPs (Asserin, Lati, Shioya, & Prawitt, 2015; Ohara et al., 2010; Vollmer, West, & Lephart, 2018). Several studies have investigated the effects of collagen peptides on skin health, of which most have utilised combinations of collagen peptides with other active ingredients such as antioxidants, vitamins, minerals, and botanicals; fewer studies have focused solely on collagen peptides (de Miranda, Weimer, & Rossi, 2021; Jhawar et al., 2020).

Methylsulphonylmethane (MSM) has also been investigated as a potentially beneficial bioactive constituent for skin health. Oral supplementation with MSM has been shown to influence skin on a genetic level, by regulating a selected number of genes responsible for inflammation, the skin barrier, and moisturisation, as well as those genes involved in the structural integrity of the skin which are associated with the ageing process (Muizzuddin & Benjamin, 2020). However, clinical data on the *in vivo* effects of MSM on skin are very limited. The combination of collagen peptides and MSM is of interest due to its potential synergistic effects on skin health. However, the specific mechanisms underlying such effects are not yet fully understood and would require further investigation.

The aim of this randomised, double-blind, placebo-controlled four-way study was to investigate and compare the effects of 12-week daily dietary supplementation containing a combination of hydrolysed fish collagen (HC; 5 or 10 g) with 1.5 g MSM or 10 g of HC without added MSM on dermis density in healthy human subjects, in comparison to a placebo product. The secondary objectives were to investigate the effects on dermis thickness, skin elasticity, hydration, transepidermal water loss (TEWL), skin roughness and wrinkles, and to investigate any differences in the effects between the test products.

2. Materials and methods

2.1. Study design

The study employed a single-centre, double-blind, randomised, placebo-controlled parallel four-way design, comparing the efficacy of continuous daily administration of three test products and a placebo for 12 weeks on dermis density and other skin parameters, in order to assess the multiple-dose effect.

2.2. Study population

A total of 109 healthy female subjects were recruited in Slovenia and included in the study. Their compliance with the inclusion and exclusion criteria was checked, and all the subjects signed a written informed consent form (ICF) before their inclusion in the study. The subjects were allocated randomly to one of four study groups, with 27–28 subjects in each group. Randomisation was achieved using a simple randomisation procedure (computerised random numbers).

Inclusion criteria: Caucasian female volunteers aged 40–65 years at the time of the signature of the ICF; a signed ICF; Fitzpatrick skin phototypes I-IV; signs of skin ageing; good general health; body mass index (BMI) <35 (severe obesity); willingness to avoid the consumption of any food supplements containing MSM, antioxidants or collagen and other protein-based food supplements during the study; willingness to follow all the study procedures and keep a diary during the study (to record compliance and palatability); willingness to maintain their living habits and not to begin or change any oestrogen or progesterone therapies during the study; willingness to avoid shaving/depilation of their forearms during the study; willingness not to change cosmetic treatment routines during the study; and willingness to avoid rejuvenation treatments during the study.

Exclusion criteria: pregnancy or breastfeeding; known or suspected allergy to any ingredient of the test products; changes in dietary habits and dietary supplementation in the last three months prior to inclusion; regular use of food supplements containing MSM, antioxidants or collagen, or other protein-based food supplements in the last three months prior to inclusion; veganism; changes in cosmetic facial and body care routines in the last month prior to inclusion; diagnosed and uncontrolled/untreated/unregulated disease; any clinically significant history of serious metabolic disease, digestive tract disease, liver disease, kidney disease, haematological disease or intake of drugs with any impact on skin reactions (e.g. glucocorticoids, antihistamines, and immunomodulators); any clinically significant acute or chronic skin diseases; skin pigmentation disorders on measurement sites; anticipated sunbathing or solarium visits before or during the study; invasive rejuvenation treatments (e.g. needle rollers, needle mesotherapy, deep/medium-deep chemical peels, abrasive laser therapy etc.) in the last 4 months prior to study entry; non-invasive rejuvenation treatments (e.g. radiofrequency, electrotherapy, ultrasound, IPL therapy) in the last month prior to study entry; shaving/depilation of the arms in the last 14 days before inclusion; and mental incapacity that precludes adequate understanding or cooperation.

2.3. Study products and intervention

The study was carried out at VIST - Faculty of Applied Sciences, Institute of Cosmetics (Slovenia) from October 2021 to February 2022, which was from autumn to winter in Slovenia.

All the subjects consumed 25 mL of a syrup daily for 12 weeks with a meal. One study group (the Col-HD group) received the test product Col-HD (daily dose 25 mL: hydrolysed fish collagen 10 g, vitamin C: 80 mg); one study group (the ColMSM-LD group) received the test product ColMSM-LD (daily dose 25 mL: hydrolysed fish collagen: 5 g, MSM: 1.5 g, vitamin C: 80 mg); one study group (the ColMSM-HD group) received the test product ColMSM-HD (daily dose 25 mL: hydrolysed fish collagen: 10 g, MSM: 1.5 g, vitamin C: 80 mg); and one study group received a placebo product without any active ingredients (25 mL: collagen: 0 mg, MSM: 0 mg, vitamin C: 0 mg). The other ingredients in all four study products were: water; sweetener: xylitol; acids: malic acid, citric acid; flavouring; colorant: caramel E150a; preservatives: potassium sorbate, sodium benzoate; and sweeteners: sucralose, maltodextrin. The products were packaged in 500 mL white plastic bottles in such a way that neither the study subjects nor the investigators knew which product was being used by each individual subject. To ensure daily dosing of 25 mL measuring cup was provided for participants along with the products.

All the study products were produced by TOSLA d.o.o. (Slovenia) in the form of a syrup, under established controlled conditions and produced in line with food regulations. The hydrolysed fish collagen, with molecular weight 1.5–5 kDa, was sourced from upcycled fish skin from EU territory, where it is rigorously controlled for heavy metal content.

2.4. Assessments

Regular checks of the subjects were carried out three times during the study: at baseline (T0), after 6 weeks (T6), and after 12 weeks of supplementation (T12). The results were obtained during October 2021 and February 2022. To follow their compliance with the protocol, the subjects kept a diary of test product intake for the whole 12-week intervention period, which was checked after 6 and 12 weeks of intervention (T6 and T12) in a concomitant interview with the subjects. Subjects were also asked to record any failure to follow the instructions. At the end of the study, they were required to return any leftover test products. Dermis density, thickness, skin hydration, TEWL and viscoelasticity measurements were carried out at baseline and after 6 and 12 weeks of intervention (T0, T6, T12, respectively). Assessments of roughness and wrinkles were performed at baseline and after 12 weeks of intervention. All the measurements were carried out on subjects lying (or sitting for roughness and wrinkles measurements) in a room at a temperature of 20–25 °C and a relative humidity of 40–60 %. Measuring equipment was regularly calibrated according to manufacturer's instructions.

The assessments began after a 30-min acclimatization period in the same atmospheric conditions. The subjects were instructed to wash their faces at least 2 h before the time of the measurement, and not to apply any cosmetic products on their face or forearms 2 h or less before the measurement.

2.4.1. Dermal density and thickness

The density and thickness of the dermis were measured using ultrasonography with a DermaLab Series, SkinLab Combo, 20 MHz ultrasound probe (Cortex Technology ApS, Denmark). Density was measured as a 0–100 intensity score, and dermis thickness was measured in μm. A constant gain curve was applied for each volunteer. The measurements were carried out in an outlined area (approx. 4 cm^2) in the right zygomatic area – on the right cheek, in the centre between the alar-facial groove and the earlobe under the zygomatic bone). The measurements were repeated twice, and the mean calculated.

2.4.2. Skin viscoelasticity

Skin viscoelasticity (VE) was measured using a DermaLab Series, SkinLab Combo, with an elasticity probe (Cortex Technology ApS, Denmark) in MPa. The parameter was measured in an outlined area (approx. 4 cm^2) in the right zygomatic area – on the right cheek. The measurements were repeated three times and the mean calculated.

2.4.3. Skin hydration

Skin hydration was measured using a DermaLab Series, SkinLab Combo, hydration flat probe (Cortex Technology ApS, Denmark), which measures the electrical conductivity (μS) of the stratum corneum. The measurements were carried out in an outlined area (approx. 4 cm^2) on the forearm; they were repeated eight times and the mean calculated.

2.4.4. Transepidermal water loss (TEWL)

TEWL was measured in $g/m^2/h$ using a DermaLab Series, SkinLab Combo, with a TEWL probe (Cortex Technology ApS, Denmark). The TEWL depends on the diffusion of water through the stratum corneum. The measurements were made in an outlined area (approx. 4 cm^2) on the forearm.

2.4.5. Texture – Skin roughness

Facial skin texture assessments were performed by topography measurements using an Antera 3D CS multispectral analyzer (Miravex Ltd, Ireland). The texture of the skin on the left cheek was measured as the arithmetical mean roughness – Ra (μm) (Messaraa et al., 2018). The texture was measured in the left zygomatic area in an outlined measurements area.

2.4.6. Wrinkles – Volume, maximum depth, indentation index

Measurements of the lateral periorbital wrinkles' expression were carried out by topography measurements using an Antera 3D CS multispectral analyser (Miravex Ltd, Ireland), and the following parameters used to measure the degree of wrinkle severity were evaluated: volume – volume of depressions (mm^3), wrinkle maximum depth (mm) and the wrinkle indentation index (a.u.) (Messaraa et al., 2018), at baseline and after 12 weeks of intervention. We used a medium filter, which is appropriate for analysing fine to moderate wrinkles from 0.5 mm to 2 mm in width. Profilometry of the lateral periorbital area was taken on one side of the face in the outlined measurements area; the side with more expressed wrinkles at baseline and with fewer other irregularities such as hyper-pigmentations, which could interfere with the results, was chosen.

2.5. Sample size calculations

The sample size for this study was determined based on the results of a previous study (Žmitek, Žmitek, Rogl Butina, & Pogačnik, 2020), which tested the effects of a 12-week dietary intake of a supplement containing 4 g of collagen and 50 mg of coenzyme Q10 on the primary endpoint (density of the dermis) in healthy women (age 40–65 years; BMI < 35 with no special diets) with Fitzpatrick phototype I-IV and signs of skin ageing. Considering the effect size of 0.33 in dermis density from pre-treatment status in the 12-week dietary intervention as significant for participants, and assuming a 20 % drop out rate, we determined a sample size of a minimum of 25 patients per group to be enough for detecting a difference between treatment and placebo using ANCOVA at power 80 % and for a significance level of <0.05.

2.6. Data and statistical analysis

The data were tested for all assumptions under the statistical methods used. We assumed two-tailed significance at $p < 0.05$ for all analyses. Means are reported with either a standard deviation (SD) or a confidence interval (CI). Repeated measures analysis of covariance (ANCOVA) was conducted to test the treatment effect and differences against the placebo, and also to investigate the difference between test products for parameters where a difference vs. the placebo was present. To test baseline-dependent effects, the density of the dermis and other observed parameters were centred on the mean and considered as a covariate.

After testing for significance using ANCOVA and an estimation of model marginal means, the results were converted to relative and absolute intervention effect vs. placebo (Intervention effect) according to methodology suggested by Vickers (Vickers, 2001). Using the model estimated mean, the product effect (%) was calculated according to the following equation for each time-point:

$$\left(\left(post-treatment\ value_{product}\right)-\left(post-treatment\ value_{placebo}\right)\right)/\ baseline\ value\times 100\%.$$

Data management and all statistical analyses were carried out using IBM SPSS Statistics for Windows, Version 27 (IBM Corp., Armonk, N.Y., United States). The graphs were plotted using GraphPad Prism (GraphPad Software, Version 9.5.1., San Diego, CA, USA).

3. Results

A total of 153 subjects were assessed for eligibility; 20 of these did not meet the inclusion criteria, and 24 subjects declined participation or withdrew for other reasons before the study commenced. Of the 109 enrolled subjects, 107 completed the entire 12-week trial (placebo group: 27 subjects; Col-HD group: 26 subjects; ColMSM-LD: 27 subjects; ColMSM-HD: 27 subjects). There were 2 dropouts, one in the ColMSM-HD group and one in the Col-HD group, both for personal reasons. No side effects or adverse events of any kind were reported. The trial design and the flow of subjects through the trial are presented in the Consolidated Standards of Reporting Trials (CONSORT) flow diagram in Fig. 1.

The baseline characteristics of the subjects who completed the whole 12-week trial and were included in the analysis are shown in Table 1. Only women of Caucasian ethnic origin (Fitzpatrick skin phototype I-IV) were included. All the subjects followed an omnivorous diet. The mean age of the subjects was 52.2 ± 6.7 years and their mean body mass index (BMI) was 25.4 ± 3.9 kg/m^2, with no significant difference between the groups. There was also no significant difference in the baseline values of all the measured skin parameters between the groups.

Descriptive statistics (mean and standard deviation (SD)) for all the measured parameters at baseline, at 6 weeks where relevant, and at 12-week follow-up for the placebo and all three test groups are included in Supplementary Table 1.

The findings from the outcome measures analysis are reported in Table 2 as model-estimated marginal means with 95 % confidence intervals (95 % CI) of the repeated measures ANCOVA of the intervention

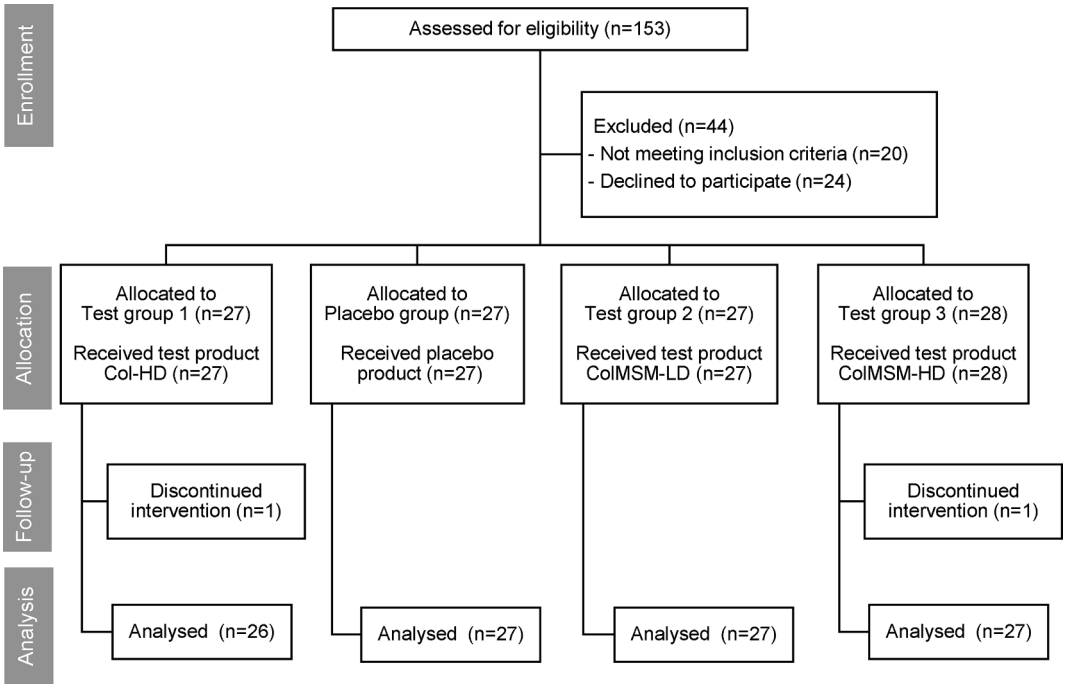

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram showing trial design and subjects' assignment and progression through the trial.

Table 1
Demographic and baseline characteristics for all four groups at baseline and the interaction between them.

	Group								p- value
	Placebo (n = 27)		Col-HD (n = 26)		ColMSM-LD (n = 27)		ColMSM-HD (n = 27)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age (years)	50.6	6.4	52.5	6.5	53.6	6.7	52.1	7.38	0.43
BMI (kg/m^2)	24. 9	4.1	25.1	3.4	25.3	3.7	26.2	4.3	0.60
Density	34.4	2.4	34.4	2.4	34.1	3.1	33.9	2.4	0.87
Thickness (μm)	1355	204	1397	204	1370	166	1296	118	0.20
Viscoelasticity (Mpa)	4.5	1.6	4.5	1.3	4.2	1.2	4.0	1.2	0.37
Hydration (μS)	35.3	13.2	33.5	12.3	37.6	13.5	34.4	13.6	0.70
TEWL (g/m*s)	8.4	2.3	8.6	1.6	8.1	1.1	8.0	1.4	0.57
Roughness – Ra (μm)	10.23	0.32	10.27	0.30	10.17	0.23	10.29	0.34	0.36
Wrinkles									
- Volume (mm^3)	7.42	0.75	7.44	0.65	7.43	0.69	7.40	0.49	1.00
- Maximum depth (mm)	0.134	0.032	0.128	0.032	0.138	0.031	0.143	0.039	0.48
- Indentation index (a.u.)	43.3	4.0	43.4	5.1	43.6	4.8	43.6	5.4	0.99

Notes: BMI: Body mass index; TEWL: transepidermal water loss; SD: standard deviation. Density is given as a 0-100 score.

Table 2
Findings from outcome measures: model estimated marginal means with 95% confidence intervals of the repeated measure ANCOVA of the intervention effect using three different test products and comparison of their effects with the placebo.

Variables	Baseline Values	Follow-up	Group			
			Placebo Group	Col-HD Group	ColMSM-LD Group	ColMSM-HD Group
Density	34.2	6 weeks	34.9 (32.6–37.2)	37.1 (34.8–39.4)*	37.3 (35.1–39.6)	38.6 (36.3–40.9)*
		12 weeks	35.3 (32.7–38.0)	40.7 (38.1–43.4)***	41.0 (38.5–43.6)**	40.5 (38.0–43.2)**
Thickness (μm)	1353	6 weeks	1341 (1317–1365)	1381 (1357–1405)	1369 (1346–1393)	1389 (1366–1413)**
		12 weeks	1359 (1331–1386)	1389 (1361–1416)	1401 (1374–1428)*	1412 (1385–1440)*
Viscoelasticity (MPa)	4.28	6 weeks	4.39 (3.82–4.97)	4.38 (3.81–4.94)	4.59 (4.04–5.14)	4.36 (3.82–4.91)
		12 weeks	4.16 (3.55–4.76)	4.74 (4.15–5.33)	4.90 (4.32–5.48)	4.58(4.01–5.15)
Hydration (μS)	35.3	6 weeks	34.4 (29.0–39.7)	32.2 (27.1–37.5)	38.31 (32.9–43.8)	33.89 (28.6–39.2)
		12 weeks	37.4 (28.1–46.8)	48.8 (39.7–58.0)	46.7 (37.1–56.2)	49.4 (40.1–58.8)*
TEWL ($g/m^2/h$)	8.26	6 weeks	9.47 (8.89–10.05)	10.063 (9.48–10.64)	8.88 (8.31–9.45)	9.21 (8.64–9.78)
		12 weeks	8.57 (8.01–9.13)	8.91 (8.36–9.48)	8.54 (7.99–9.09)	8.34 (7.8–8.89)
Roughness – Ra (μm)	10.24	12 weeks	10.44 (10.11–10.77)	9.64 (9.31–9.98)^{*,a}	9.03 (8.70–9.36)^{***,b}	8.97 (8.64–9.30)^{***,b}
Wrinkles						
- Volume (mm^3)	7.42	12 weeks	7.82 (7.27–8.37)	6.50 (5.95–7.07)*	6.55 (6.01–7.11)**	6.27 (5.72–6.82)***
- Maximum depth (mm)	0.136	12 weeks	0.144 (0.134–0.155)	0.119 (0.108–0.130)**	0.124 (0.113–0.134)*	0.110 (0.099–0.121)***
- Indentation index (a.u.)	43.5	12 weeks	44.9 (43.0–47.0)	41.0 (39.1–43.1)*	41.1 (39.2–43.2)**	40.0 (38.1–42)***

Notes: Unless otherwise indicated, the results are presented as estimated marginal means (95 % confidence interval); *p < 0.05, **p < 0.01, ***p < 0.001 indicate significant differences from baseline at follow-ups between the test products and the placebo using ANCOVA; ^{a,b} different subscript letters indicate statistical differences from baseline at follow-ups between the active products at p < 0.05. Density is given as a 0-100 score.

effect using three different test products and a comparison of their effects with the placebo. Detailed results of the contrast analysis testing for differences between each test group and the placebo are presented in Supplementary Table 2. The results of the follow-up pairwise analysis between test products showing the significant differences in their effectiveness are also indicated in Table 2, and presented in detail in Supplementary Tables 3 and 4.

The figures present the relative intervention effects for the comparison of test products to the placebo for the selected variables. The significance levels, which were determined using the repeated measures ANCOVA, relate to the differences between the products and the placebo based on the pairwise analysis. Detailed results of the relative and absolute intervention effects for all the measured parameters are presented in Supplementary Table 5.

3.1. Dermis density

Dermis density was similar in all groups at baseline (Table 1). The density adjusted baseline value was 34.2, and in the placebo group at 6- and 12-week follow-up the density values were 34.9 (95 % CI 32.6–37.2)

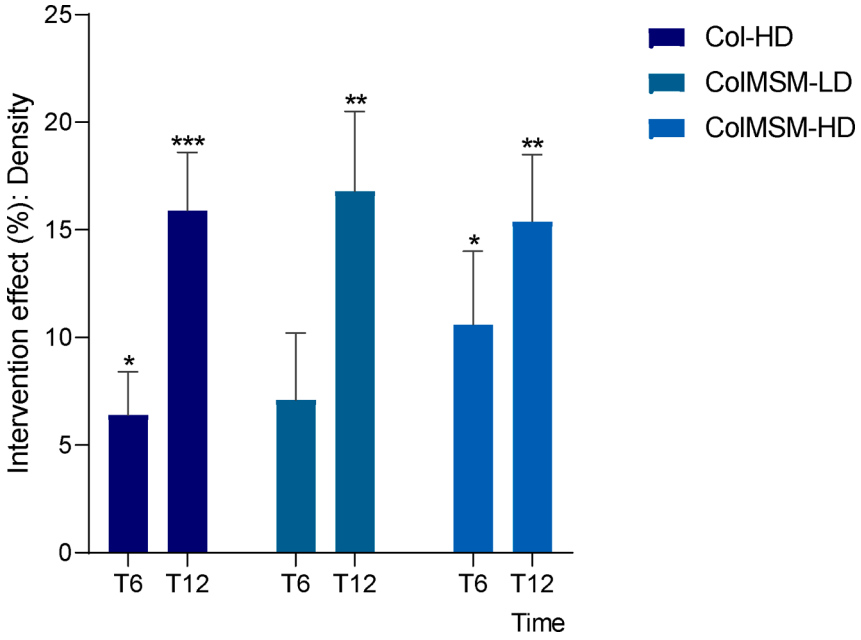

Fig. 2. Intervention effect (%) of test products on dermis density at different time points (*p < 0.05, **p < 0.01, ***p < 0.001 indicate significant differences from baseline at 6- (T6) and 12-week (T12) follow-up between the test products and the placebo using ANCOVA). Error bars indicate the standard errors of the mean.

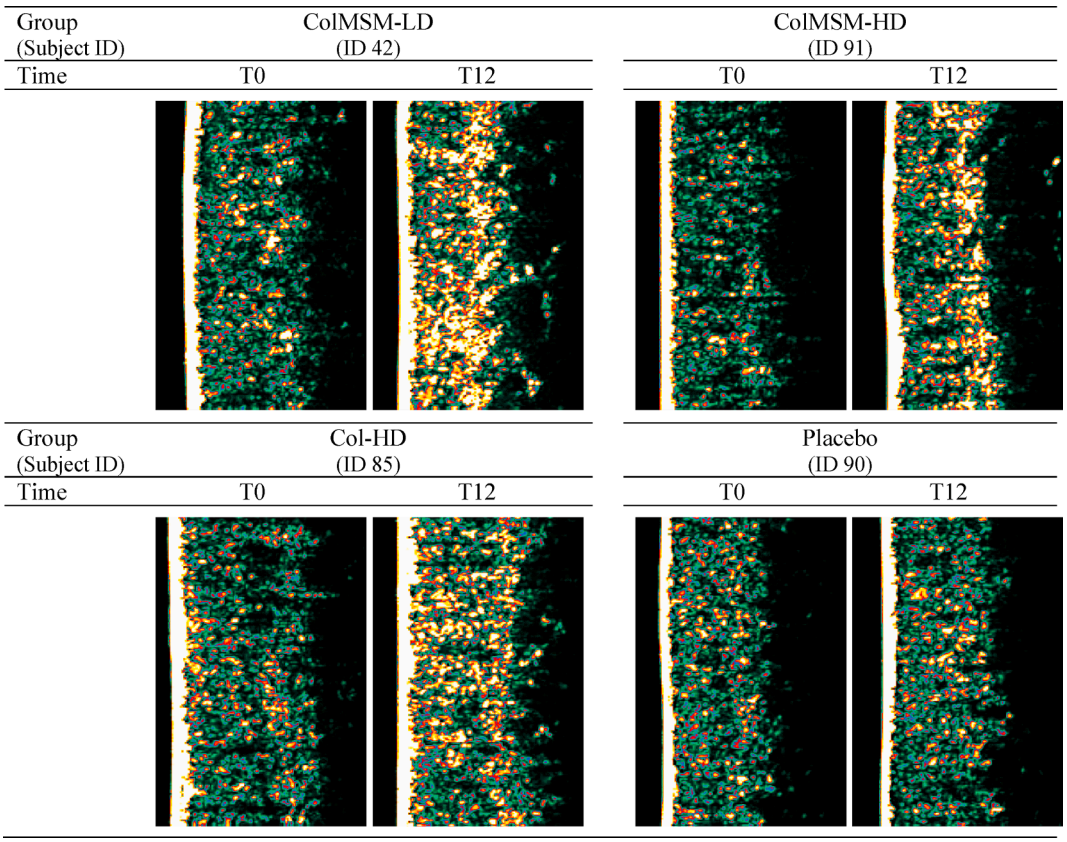

Fig. 3. Examples of the effects of intervention on the dermis on subjects from different groups: ultrasound images of the dermis at baseline (T0) and 12-week follow-up (T12). The white line to the left represents the epidermis with a water film, followed by the dermis to the right, characterized by varying intensities, and the subcutis by low-intensity areas due to a homogenous composition. Echogenicity colour scale: white > yellow > red > green > black. An increase in dermis echogenicity is visible in the images of the subjects in the ColMSM-LD, ColMSM-HD and Col-HD groups, indicating an improvement in dermal density during the study, while no important changes can be observed in the images of the subject from the placebo group.

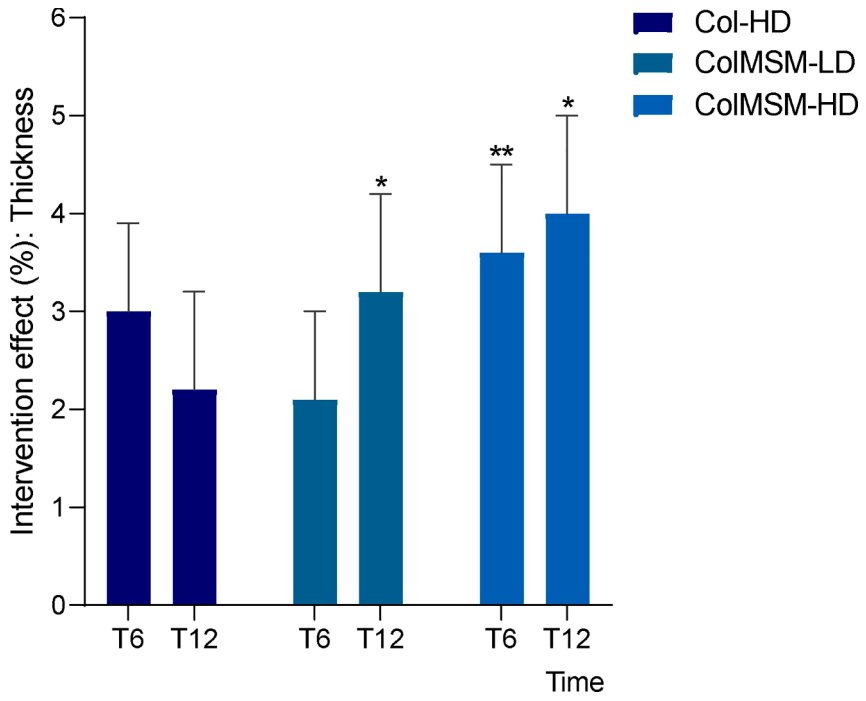

Fig. 4. Intervention effect (%) of test products on dermis thickness at different time points (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ indicate a significant difference from baseline at 6- (T6) and 12-week (T12) follow-up between the test products and the placebo using ANCOVA). Error bars indicate the standard errors of the mean.

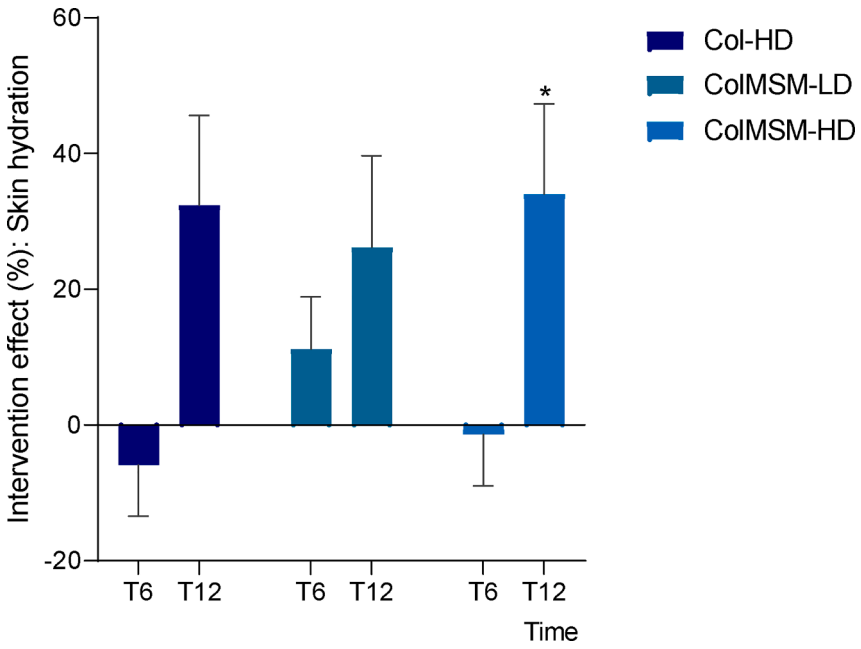

Fig. 5. Relative intervention effect (%) of test products on skin hydration at different time points (*p < 0.05, **p < 0.01, ***p < 0.001 indicate a significant difference from baseline at 6- (T6) and 12-week (T12) follow-up between the test products and the placebo using ANCOVA). Error bars indicate the standard errors of the mean.

and 35.3 (95 % CI 32.7–38.0), respectively (Table 2). At 12-week follow-up, improvement in the density was significant and statistically different in comparison to the placebo for all three test products; at 6-week follow-up an improvement trend was detected for all three test products, but it reached significance level only for the two products with a higher collagen dose (Col-HD and ColMSM-HD), as presented in Fig. 2.

In the Col-HD group, skin density increased by 15.9 % (to 40.7 (95 % CI 38.1–43.4), $p < 0.001$) after 12 weeks of intervention, and even after 6 weeks an increase of 6.4 % was detected (37.1; 95 % CI 34.8–39.4), $p < 0.05$), both with evident significant differences between the product and the placebo. In the ColMSM-LD group, skin density increased by 16.8 % (to 41.0 (95 % CI 38.5–43.7), $p < 0.01$) after 12 weeks of intervention, with evident significant differences between the product and the placebo. Insignificant improvement ($p = 0.1$) of 7.1 % was detected after 6 weeks (37.3; 95 % CI 35.1–39.6). In the ColMSM-HD group, skin density increased by 15.4 % (to 40.5 (95 % CI 38.0–43.2), $p < 0.01$) after 12 weeks of intervention; even after 6 weeks we observed an increase of 10.6 % (to 38.6 (95 % CI 36.3–40.9), $p < 0.05$), both with evident significant differences between the product and the placebo.

However, following the ANCOVA interaction analysis, we did not find a significant difference in dermis density between the test products at either the 6- or at the 12-week follow-up ($p = 0.69$, 0,97, respectively).

Examples of the effects of intervention on dermis density are presented by the images in Fig. 3. The images of the subjects in the ColMSM-LD, ColMSM-HD and Col-HD groups show a visible increase in dermis echogenicity at 12-week follow-up, indicating an improvement in dermal density, while no important changes can be observed in the images of the subject from the placebo group.

3.2. Dermis thickness

Dermis thickness was similar in all groups at baseline (Table 1). The thickness adjusted baseline value was 1353 μm (Table 2). In the placebo group at 6- and 12-week follow-up the thickness values were 1341 μm (95 % CI 1317–1365) and 1359 μm (95 % CI 1331–1386), respectively. After 12 weeks of intervention an increasing trend for thickness was observed for all three test products. However, the change from baseline in comparison to the placebo reached significance level only for the two MSM-containing products (ColMSM-LD and ColMSM-HD), as presented in Fig. 4. For the ColMSM-HD product, the thickness change had already reached a notable significance after 6 weeks.

In the Col-HD group we did not find significant difference vs. placebo at either 6-week (to 1381 μm (95 % CI 13571–1405), $p = 0.05$) or 12-week follow up (to 1389 μm (95 % CI 1361–1416), $p = 0.1$). In the ColMSM-LD group, skin thickness increased by 3.2 % (to 1401 μm (95 % CI 1374–1428), $p = 0.034$) after 12 weeks of intervention, with evident significant difference vs. the placebo, while a statistically insignificant ($p = 0.12$) increase of 2.1 % was detected after 6 weeks (1369 μm (95 % CI 1346–1393). In the ColMSM-HD group, skin thickness notably increased by 4.0 % (to 1412 μm (95 % CI 1385–1440), $p < 0.05$) after 12 weeks of intervention, and an improvement of 3.6 % (to 1389 μm (95 % CI 1366–1413), $p < 0.01$) was already detected after 6 weeks, both with evident significant differences between the product and the placebo.

According to the ANCOVA interaction analysis between the test products we did not find a significant difference in the thickness level at either 6- or at 12-week follow-up ($p = 0.53$, 0.62, respectively).

3.3. Viscoelasticity

Skin viscoelasticity (VE) was similar in all the groups at baseline (Table 1). The model adjusted baseline and follow-up values are presented in Table 2. The adjusted baseline value was 4.28 MPa. In the placebo group at 6- and 12-week follow-up, VE was 4.39 (95 % CI 3.82–4.97) and 4.16 MPa (95 % CI 3.55–4.76), respectively.

After 12 weeks of intervention, an increasing trend in skin viscoelasticity was observed for all the test products, but without significant differences vs. the placebo. For Col-HD, the increase was 13.7 % (to 4.74 MPa (95 % CI 4.15–5.33, $p = 0.13$), for ColMSM-LD 17.4 % (to 4.90 MPa, 95 % CI 4.32–5.48; $p = 0.09$) and for ColMSM-HD 10.0 % (to 4.58 MPa (95 % CI 4.01–5.15, $p = 0.17$). The changes after 6 weeks were much less pronounced and also not significant.

3.4. Skin hydration

Skin hydration (μS) was similar in all the groups at baseline

(Table 1). The adjusted baseline value for skin hydration, measured as electrical conductivity of the stratum corneum, was 35.27 μS (Table 2). In the placebo group hydration at 6- and 12-week follow-up was 34.4 (95 % CI 29.0–39.7) and 37.4 μS (95 % CI 28.1–46.8), respectively. After 12 weeks of intervention, an increasing trend in skin hydration was observed for all three test products. For Col-HD, the hydration increase was 32.4 % (to 48.8 μS (95 % CI 39.7–58.0), $p = 0.056$), for ColMSM-LD the increase was 26.2 % (to 46.7 μS (95 % CI 37.1–56.2), $p = 0.16$) and for ColMSM-HD the increase was 34.0 % (to 49.4 μS (95 % CI 40.1–58.8), $p = 0.043$), as presented in Fig. 5. However, the difference in hydration in comparison to the placebo was significant only for ColMSM-HD, while for Col-HD and ColMSM-LD it did not reach significance level. At 6-week follow-up, the differences were less pronounced and not significant for any of the test products (Fig. 5).

3.5. Transepidermal water loss (TEWL)

TEWL was similar in all the groups at baseline (Table 1). The TEWL adjusted baseline value was 8.26 g/m^2/h (Table 2). In the placebo group, at 12-week follow-up the TEWL was 8.57 g/m^2/h (95 % CI 8.01–9.13), in the Col-HD group it was 8.91 g/m^2/h (95 % CI 8.36–9.48), in the ColMSM-LD group it was 8.54 g/m^2/h (95 % 7.99–9.09), and in the ColMSM-HD group 8.34 g/m^2/h (95 % 7.8–8.89), but the changes did not reach significance level in comparison to the placebo for any of the test products. The changes after 6 weeks were also not significant.

3.6. Texture: Skin roughness

The arithmetical mean roughness (Ra) was similar in all the groups at baseline (Table 1). The roughness adjusted baseline value was 10.2 μm, and at 12-week follow-up it was 10.44 μm (95 % CI 10.11–10.77) in the placebo group (Table 2). At 12-week follow-up a notable decrease in roughness was observed in all the test groups, with evident significant differences between the product and the placebo, as seen in Fig. 6. In the Col-HD group, at 12 weeks roughness had decreased by 7.8 % (to 9.64 μm; 95 % CI 9.31–9.98; $p = 0.002$), while for both MSM-containing products the effect was almost double that of the collagen-only product, as in the ColMSM-LD group the decrease in roughness was 13.8 % (to 9.03 μm (95 % CI 8.70–9.36); $p < 0.001$), and in the ColMSM-HD group it reached 14.3 % (to 8.97 μm; 95 % CI 8.64–9.30; $p < 0.001$).

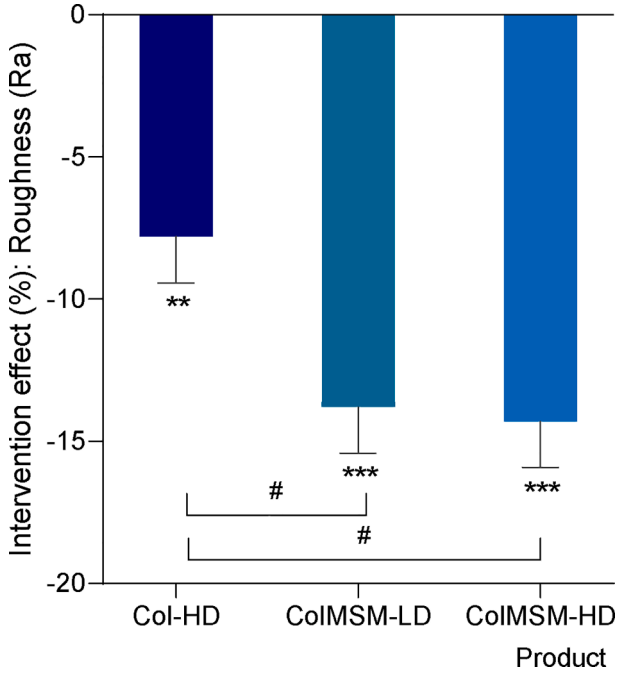

Fig. 6. Intervention effect (%) of test products on skin roughness (Ra) at different time points (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ indicate a significant difference from baseline at 12-week (T12) follow-up between the test products and the placebo; $^{\#}p < 0.05$ indicates a significant difference at T12 from baseline between active products using ANCOVA). Error bars indicate the standard errors of the mean.

As there was a significant interaction effect related to the test products ($p = 0.02$), a further follow-up pairwise comparison analysis was conducted (Supplementary Table 4). The analyses revealed significant differences between the product pairs of the Col-HD group vs. the ColMSM-LD group ($p = 0.048$) and the Col-HD group vs. the ColMSM-HD group ($p = 0.027$), while there was no significant difference between the two MSM groups (ColMSM-LD vs. ColMSM-HD; $p = 0.84$).

An example of the effects of intervention on skin roughness can be seen in Fig. 7, where visuals from a subject's cheek area using the medium filter from the Antera 3D texture mode at baseline and at 12-week follow-up are presented.

3.7. Wrinkles (topography measurements): Volume, maximum depth, indentation index

To evaluate the severity of wrinkles, three parameters were evaluated: wrinkle volume, maximum depth and the indentation index.

Wrinkle volume was similar in all the groups at baseline (Table 1). The adjusted wrinkle volume was 7.42 mm^3 at baseline. At 12-week follow-up it was 7.82 mm^3 (7.27–8.37) in the placebo group, while wrinkle volume was reduced in all the test products groups (Table 2) with significant differences vs. the placebo, as also shown in Fig. 8. In the Col-HD group it was reduced by 17.7 % (to 6.50 mm^3; 95 % CI 5.95–7.07; $p = 0.013$), in the ColMSM-LD group by 17.0 % (to 6.55 mm^3; 95 % CI 6.01–7.11; $p = 0.002$) and in the ColMSM-HD group by 20.8 % (to 6.27 mm^3; 95 % CI 5.72–6.82; $p < 0.001$). Interaction analysis did not show any significant difference in wrinkle volume between the test products at follow-up ($p = 0.69$).

Wrinkles' maximum depth was similar in all the groups at baseline (Table 1). In the placebo group, the adjusted wrinkles' maximum depth was 0.136 mm at baseline and 0.144 mm (95 % CI 0.134–0.155) at 12-week follow-up. A reduction was observed in all the test product groups with significant differences vs. the placebo, as shown in Fig. 9. In the Col-HD group, maximum depth decreased by 18.4 % (to 0.119 mm; 95 % CI 0.108–0.130; $p < 0.01$)), in the ColMSM-LD group it decreased by 14.7 % (to 0.124; 95 % CI 0.113–0.134; $p = 0.013$)) and in the ColMSM-HD group by 25.0 % (to 0.110 mm; 95 % CI 0.099–0.121; $p < 0.001$). There was no significant difference in effectiveness with regard to wrinkles' maximum depth between the test products ($p = 0.16$).

Wrinkles' indentation index was similar in all the groups at baseline (Table 1). The adjusted baseline wrinkles' indentation index was 43.5 a.u. The indentation index at 12-week follow-up in the placebo group was 44.9 a.u. (95 % CI 43–47) (Table 2), while as shown in Fig. 10, the indentation index in all the test product groups significantly improved (decrease in index) vs. the placebo group. In the Col-HD group the indentation index decreased by 9.0 % (to 41.0 a.u.; 95 % CI 39.1–43.1; $p = 0.04$), in the ColMSM-LD group by 8.7 % (to 41.1 a.u.; 95 % CI 39.2–43.2; $p = 0.005$) and in the ColMSM-HD group by 11.0 % (to 40.0 a.u.; 95 % CI 38.1–42; $p < 0.001$). There was no significant difference in effectiveness with regard to wrinkles' indentation index between the test products ($p = 0.63$).

4. Discussion

Skin ageing occurs primarily due to changes in the dermis, but topically applied skin care products often fail in the prevention of skin ageing due to their limited ability to penetrate to the dermis, which is crucial for exerting a perceivable and long-lasting influence on the skin ageing process. Oral supplementation offers an alternative approach, as

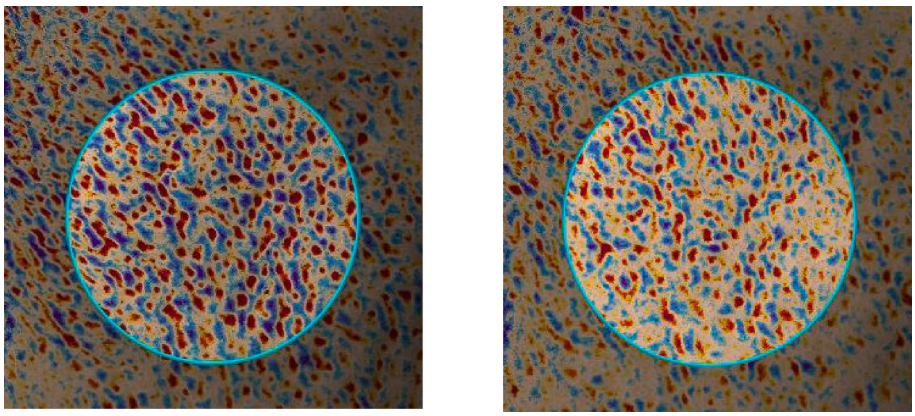

Fig. 7. Effects of intervention on skin roughness: an example of visuals from a subject's cheek area when using the Antera 3D texture mode at baseline (left) and after 12 weeks of intervention (right) with ColMSM-LD. Roughness is quantified by the vertical deviations of a real surface from its ideal form; larger deviations indicate a rougher surface. Red and yellow represent depressed areas, and blue and purple represent elevated areas.

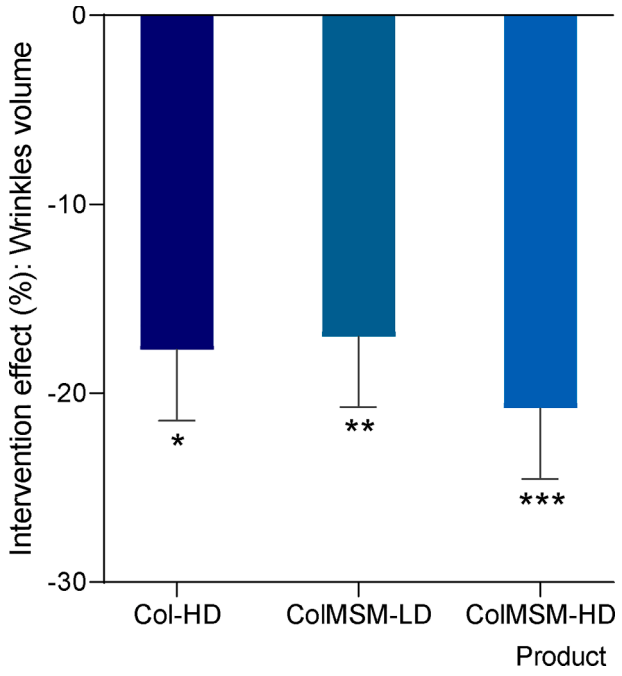

Fig. 8. Relative intervention effect (%) of test products on wrinkle volume at different time points (*p < 0.05, **p < 0.01, ***p < 0.001 indicate a significant difference from baseline at 12-week (T12) follow-up between the test products and the placebo using ANCOVA). Error bars indicate the standard errors of the mean.

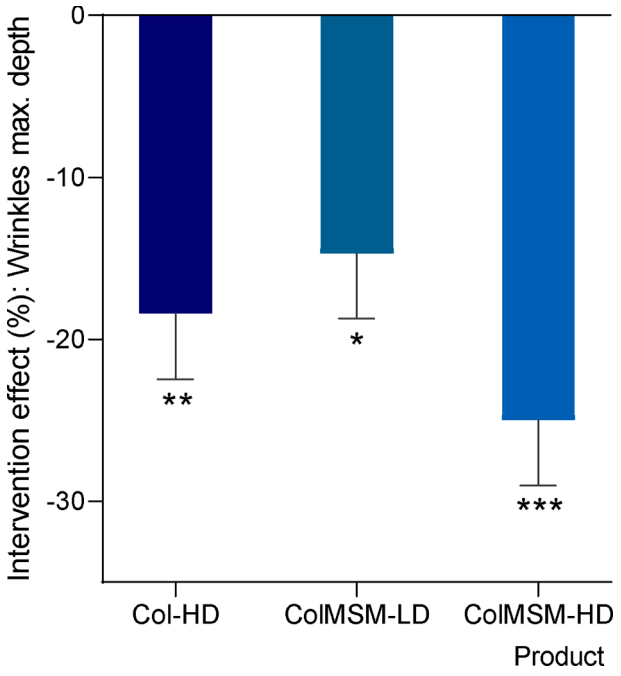

Fig. 9. Relative intervention effect (%) of test products on wrinkles' maximum depth at different time points (*p < 0.05, **p < 0.01, ***p < 0.001 indicate a significant difference from baseline at 12-week (T12) follow-up between the test products and the placebo using ANCOVA). Error bars indicate the standard errors of the mean.

in this way nutrients and other bioactive constituents can be delivered through the bloodstream to the dermis and can therefore demonstrate effects.

Hydrolysed collagen (HC) peptides have emerged as a promising anti-aging supplement, demonstrating beneficial effects on the skin in various scientific studies. Recently, the mechanistic aspects of collagen supplementation were reviewed in paper by Barati et al. (2020). The findings suggest two possible mechanisms of action for collagen. Collagen peptides that are products of collagen digestion, are absorbed into the bloodstream from the gut and can directly stimulate synthesis of extracelullar matrix components by fibroblasts. For example, it has been shown that after supplementation of collagen peptides to fibroblast cultures an increase of type I collagen expression could be observed (Proksch, Segger, et al., 2014). On the other hand, collagen and its fragments could induce regulatory T cells, which can then suppress the immune response against endogenous collagen by polarizing macrophages towards M2-like macrophages. This can help to improve skin health by reducing inflammation and damage to the skin (Barati et al., 2020).

The effects of hydrolysed collagen supplementation on skin ageing were reviewed in a paper by de Miranda et al. (2021) which included a meta-analysis of the results of randomized, double-blind, controlled trials which followed a variety of skin-related outcomes, such as wrinkles, hydration, elasticity, or firmness. The meta-analysis of 19 selected studies, with a total of 1,125 participants aged between 20 and 70 years (95 % women), showed favourable results of hydrolysed collagen supplementation compared with a placebo in terms of skin hydration, elasticity, and wrinkles. Beneficial effects on skin hydration and elasticity were confirmed also in meta-analysis by Pu et al. (2023). However, it is important to note that more about half of the included studies in

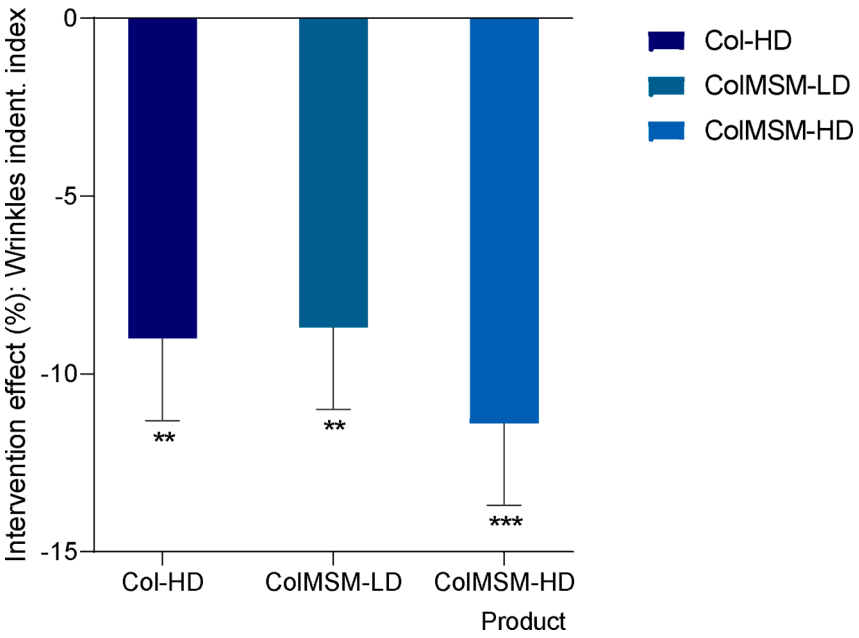

Fig. 10. Relative intervention effect (%) of test products on the wrinkles' indentation index at different time points (*p < 0.05, **p < 0.01, ***p < 0.001 indicate a significant difference from baseline at 12-week (T12) follow-up between the test products and the placebo using ANCOVA). Error bars indicate the standard errors of the mean.

both meta-analyses examined collagen supplements in combination with other active ingredients, which may have influenced the outcomes.

Proksch, Schunck et al. (2014) showed that daily ingestion of 2.5 g of collagen peptides (CP) for 8 weeks increased dermal matrix synthesis and resulted in a statistically significantly higher content of procollagen type I and elastin. A significant reduction in eye wrinkle volume (by 20 %) was observed with a long-lasting effect at 4-week follow-up. They concluded that a direct effect on the dermal matrix could explain the long-lasting improvement in skin wrinkles. In another study they showed that a daily intake of 2.5 g or 5 g HC for 8 weeks was able to improve skin elasticity in women with dry skin, while no significant effects on roughness, skin moisture or TEWL, an indicator of skin barrier function, were observed (Proksch, Segger, et al., 2014). Asserin et al. (2015) showed that ingestion of 10 g of CP (either fish or porcine) over 8 weeks was able to improve skin hydration but not TEWL in women with dry skin, while 12 weeks of daily supplementation improved collagen density in the dermis. *Ex-vivo* experiments demonstrated that collagen peptides induce collagen and glycosaminoglycan production, offering a mechanistic explanation for the observed clinical effects (Asserin et al., 2015).

Choi et al. (2014) concluded that daily supplementation with 3 g CP may improve skin hydration and elasticity, but not the TEWL, melanin or erythema index; concomitant intake of 500 mg of vitamin C did not enhance the effect of CP on skin properties. Koizumi et al. (2018) showed that a daily intake of 3 g CP (fish origin) over 12 weeks resulted in a decrease in periorbital wrinkles, and also enhanced facial skin moisture and skin elasticity. A significant improvement in elasticity, hydration and skin roughness was also observed in a study by Sugihara et al. (2015) which included 2.5 g HC daily supplementation for 8 weeks. In a recent placebo-controlled study by Miyanaga et al. (2021), supplementation with 1 g or 5 g CP daily over 12 weeks increased epidermal water content and decreased TEWL in both groups, while skin elasticity and skin thickness remained unchanged. In other studies, different active constituents have been added to collagen. In our previous placebo-controlled study (Žmitek et al., 2020), supplementation with 4 g CP and 50 mg CoQ10 over 12 weeks resulted in improved dermis density, reduced periorbital wrinkle area and the total wrinkle score, and improved skin smoothness. On the other hand, changes in skin hydration, dermis thickness, TEWL and viscoelasticity were not significant. In a study by Bolke et al. (2019), 12 weeks supplementation with 2.5 g CP with concomitant acerola fruit extract, vitamin C, zinc, biotin, and a native vitamin E complex improved skin hydration, elasticity, roughness, and density.

MSM is an organosulfur compound found in a variety of foods including milk, grains, fruits, and vegetables (Butawan, Benjamin, & Bloomer, 2017; Magnuson, Appleton, & Ames, 2007). Evidence indicates that it acts as a source of dietary sulfur and may affect the compartmentalization and metabolism of sulfur (Richmond, 1986). Sulfur plays an essential role in a variety of physiological processes, including the synthesis of collagen, hyaluronic acid, and keratohyalin. Besides that, MSM may help protect ECM proteins from damage and degradation by attenuating inflammation and reducing oxidative stress (Butawan et al., 2017; Muizzuddin & Benjamin, 2020). Excessive inflammation can lead to the degradation of structural matrices in the skin, which can contribute to the signs of aging. MSM has been shown to reduce inflammation by inhibiting the production of pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), nitric oxide (NO), prostaglandin E2 (PGE2), and nuclear factor (NF-κB) (Butawan et al., 2017). Additionally, it was shown that MSM supports the body's intrinsic antioxidant pathways through increased levels of antioxidants, such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Butawan et al., 2017). Furthermore, MSM has been shown to reduce levels of homocysteine, a molecule with damaging effects on collagen crosslinking (Toohey, 2008), which is important for maintaining the strength and elasticity of the skin. In a recent placebo-controlled study by Muizzuddin & Benjamin (2020) it was shown that daily supplementation with 3 g of MSM over 16 weeks reduces visual signs of aging such as wrinkles and roughness according to expert grading and self-evaluations. In the second part of this study, daily supplementation with 1 g or 3 g of MSM over 16 weeks was tested and measurements showed improvement of net elasticity and skin hydration for both dosages. According to expert grading also skin texture/smoothness and wrinkles were improved. However, there was no placebo control in the second part of the study.

In the present study, we investigated the effects of liquid food supplements containing different dosages of hydrolysed fish collagen (5 or

10 g) in combination with 1.5 g MSM (ColMSM-LD and ColMSM-HD, respectively), or 10 g of HC (Col-HD) without MSM in comparison to a placebo over a 12-week intervention period on skin density as a primary objective, and other skin parameters, i.e. thickness, hydration, TEWL, texture (roughness) and wrinkles.

After 12 weeks of supplementation, all three test products, Col-HD, ColMSM-LD and ColMSM-HD, efficiently improved skin density (by 15.9 %, 16.8 % and 15.4 %) with no significant differences between them. The observed improvements in skin density are consistent with the results of previous studies (Asserin et al., 2015; Bolke et al., 2019; Žmitek et al., 2020). A lower collagen dose (5 g collagen/day) was sufficient for a detected improvement in skin density, as there was no significant difference in effectiveness between the ColMSM-LD and ColMSM-HD products, and the lack of difference between Col-HD and ColMSM-HD showed that the addition of MSM to a higher dose of collagen did not have any additional significant effects on skin density. However, improvement in density with higher dose (HD) products was significant earlier – after only 6 weeks.

The study results showed that the addition of MSM to the test products had a beneficial effect on dermis thickness, as the thickness improvement after 12 weeks was only statistically different in comparison to the placebo for the products containing MSM (ColMSM-LD and ColMSM-HD). While the effectiveness with regard to skin thickness of both the ColMSM products vs. the placebo was similar and significant at 12-week follow-up (3.2 % and 4.0 % intervention effect, respectively), only the MSM product with a higher dose of collagen (ColMSM-HD) reached a significant improvement in thickness vs. the placebo (3.6 %) after only 6 weeks of intervention. Therefore, a higher collagen dose in combination with MSM was obviously superior as it gave faster results in skin thickness improvement than a lower collagen dose in combination with MSM.

The results for viscoelasticity showed an increasing trend in all test products, but the differences failed to reach significance level in comparison to the placebo for any of them. Similar observations have been reported in previous studies, using comparable collagen doses (Miyanaga et al., 2021; Žmitek et al., 2020).

The results of skin hydration showed increasing trends for all three test products, but the difference after 12 weeks in comparison to the placebo only reached significance for the product containing MSM in combination with a higher collagen dose (ColMSM-HD). The product with the same collagen dose (Col-HD) but without MSM did not result in a significant effect on hydration, suggesting the beneficial effect of the addition of MSM for hydration improvement, as it was crucial for effectiveness. On the other hand, a lower HC dose, even though it was combined with MSM, was obviously not sufficient. Failure to reach a significant increase in skin hydration for the Col-HD and ColMSM-LD products could be also due to the fact that our study was not primarily focused on skin hydration and therefore, in contrast to several other studies showing the influence of CP on hydration in subjects with dry skin, the latter was not an inclusion criteria in our study. As in several other studies (Asserin et al., 2015; Choi et al., 2014; Proksch, Segger, et al., 2014; Žmitek et al., 2020), we did not detect any important effects of the products on TEWL.

All three test products, Col-HD, ColMSM-LD and ColMSM-HD were effective in reducing skin roughness in comparison to the placebo (by 7.8 %, 13.8 % and 14.3 %, respectively) after 12 weeks of supplementation. However, both the products with MSM (LD and HD) had superior effectiveness in comparison to the collagen-only product, as the addition of MSM to collagen almost doubled the effect, regardless of the collagen dose. Superior effectiveness of products with added MSM on roughness is also in accordance with observed effects of MSM in study by Muizzuddin & Benjamin (2020). On the other hand, since the effectiveness of the two MSM products (ColMSM-LD and ColMSM-HD) was comparable, we can conclude that a lower dose of collagen (5 g/day) in combination with MSM is sufficient for an effective improvement in roughness. Interestingly, better results for improving skin roughness can be achieved by a daily intake of 5 g collagen with 1.5 g MSM than by a higher dose of collagen (10 g/day) without MSM. These results suggest a synergistic effect of collagen and MSM for improving skin texture.

With regard to the severity of wrinkles, all the test products, Col-HD, ColMSM-LD and ColMSM-HD, were able to improve wrinkle volume (by 17.7 %, 17.0 % and 20.8 %, respectively), maximum depth (18.4 %, 14.7 % and 25.0 %, respectively) and indentation index (9.0 %, 8.7 % and 11.4 %, respectively), with no significant difference between them. As the intervention effect on all the wrinkles parameters (volume, max. depth, indentation index) of the product with a lower collagen dose containing MSM (ColMSM-LD) was comparable to the two products with a higher collagen dose (ColMSM-HD and Col-HD), it can be concluded that 5 g collagen/day in combination with MSM is also sufficient for improvement in the severity of wrinkles.

Several previous studies have shown that supplementation with HC has the potential to reach the dermis in order to improve collagen synthesis (Barati et al., 2020; Ohara et al., 2010; Yazaki et al., 2017). The current study further validates these achievements, particularly in the improvement in skin density and thickness, which is reflected in reduced wrinkles and roughness, and it unequivocally demonstrates that the effects of all the tested products extend beyond the epidermis. The observed beneficial effects suggest that HC in the tested supplements was efficiently broken down to biologically active collagen peptides, exerting effects on dermis regeneration which were also clearly visible and cosmetically relevant. Due to the superior effects of the HC/MSM combination over HC with regard to skin thickness and texture, it also appears that HC and MSM can act synergistically and further boost the effectiveness of each other.

The key strength of this human intervention study was the comprehensive evaluation of the effects of supplementation on various skin parameters, alongside a comparison with a placebo. It is also important to acknowledge the study's limitations. All test products also contained vitamin C (80 mg), which might also had some effects to measured parameters (Pullar, Carr, & Vissers, 2017), but we should note that previous studies did not observe effects of much larger dosage of vitamin C (500 mg) (Choi et al., 2014). Another limitation is that all participants were women within a restricted age range. Such criteria were used to enable better homogeneity, helping to reduce variability within a study population. We should also mention relatively short intervention time. As the average epidermal skin cycle in young healthy individuals lasts 30–40 days (Farage et al., 2017), 12 weeks may not be sufficient to obtain detectable significant nutritional effects on the skin, and therefore an extended duration for testing the supplementation could be advantageous. However, longer intervention times increase the risk of dropout and seasonal effects. In relation to this it is worth noting that the study was conducted during late autumn to winter, a period characterised by a seasonal drop in skin hydration and elasticity due to the lower air humidity caused by indoor heating and colder outdoor temperatures (Nam, Baek, Koh, & Hwang, 2015; Wan et al., 2015). Thus, the comparison of the test products to the placebo was crucial to overcome such seasonal influences.

5. Conclusion

In the present randomised, double-blind placebo-controlled four-way study the 12-week administration of liquid food supplements, characterised by a combination of hydrolysed fish collagen (5 or 10 g) with 1.5 g MSM or 10 g of collagen without added MSM, was tested. The results showed several beneficial effects on the skin. All the test products were able to improve dermis density, reduce wrinkle severity and improve skin texture. However, the products with added MSM were superior in the improvement of skin thickness and twice as efficient as the collagen-only product in the reduction of skin roughness. Also, a higher dose of collagen with MSM was crucial for the improvement in skin hydration. On the other hand, no significant effects of the supplementation with either of the products on viscoelasticity or TEWL were

determined.

Ethics statement

The study was in full compliance with the principles laid out in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the VIST – Faculty of Applied Sciences (approval ID: 2021/6-ET-SK, date of approval: 12.7.2021) and included in the ClinicalTrials.gov PRS register under the record NCT04988412. The study was carried out in compliance with the requirements of local authorities. Before participation in the study, all the subjects signed a written informed consent form (ICF).

CRediT authorship contribution statement

Tina Pogačnik: Methodology, Investigation. **Janko Žmitek:** Supervision, Project administration, Funding acquisition, Conceptualization. **Hristo Hristov:** . **Petra Keršmanc:** Methodology, Investigation. **Mirjam Rogl Butina:** Writing – review & editing, Supervision, Conceptualization. **Katja Žmitek:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2023.105838.

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