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Dermatological expertise on an
eight-week lasting clinical-dermatological
application test
with hydration determination
using a corneometer

“Marine Collagen Peptide Powder (3 kDa)”

Test subjects:	20 female test persons, all with healthy skin
Test period:	8 weeks
Test area:	face and forearm
Application frequency:	once daily oral ingestion (10 g)

1. INTRODUCTION

Collagen is a fibrous protein and the main component of the skin, connective tissue and bones, contributing to their unique physiological function. Bovine was the main source of collagen until the discovery of Creutzfeld-Jacob disease (CJD) and Bovine Spongiform Encephalopathy (BSE), whose occurrence was associated with prions carried by bovine collagen. The BSE epidemic increased the interest in new sources of collagen, resulting in increased attention towards collagen peptides processed from marine by-products [1].

Type 1 is the most common types of collagen and is the main type in humans. Collagen obtained from marine sources are of type 1, and is produced and organised in cells in skin and connective tissues as well as playing an important role in bone tissue. It is in relation to not only the elasticity and structure of the bone, but also to key processes in bone mineralisation and metabolism [2]. Marine collagen is absorbed up to 1.5 times more efficiently in the body, and the bioavailability is superior to collagen of both bovine or porcine. The smaller particle size in marine collagen compared to other sources allows an easier and faster uptake and transportation of the collagen peptides to the skin, bones and joints for the synthesis of new collagen.

The collagen content of the skin is directly related to its density, strength and volume. With advancing age, the ability of the skin to replenish its collagen stores decreases. The stabilising collagen fibres of the skin also lose strength with increasing age and are even partially degraded [3, 4].

A lack of collagen can only be compensated very limited by applying appropriate superficial and short-acting cosmetics such as creams, lotions or serums. Studies have shown that by an oral intake of liquid marine collagen peptides the age-related, degenerative processes in the cell metabolism of the skin can be slowed down or even reversed. The size of these protein components is crucial for bioavailability and thus for efficacy. Only small peptides with a molecular weight of approximately 0,3 to 6 kDa can be effectively absorbed by the body and stimulate the collagen synthesis in the extracellular matrix sufficiently [5, 6].

When taken orally, the collagen peptides reach the small intestine. During digestion, di- and tripeptides or free amino acids are formed therefrom. These peptides can be easily absorbed by the intestinal mucosa, which leads to the bloodstream. In this way, the collagen peptides are distributed in the body and reach the dermis of the skin. Here, collagen peptides and free amino acids support the production of collagen fibres and elastin fibres; at the same time, collagen peptides stimulate an increase in fibroblasts, which stimulates the synthesis of collagen as well as the formation of hyaluronic acid. Some of these peptides have bioactive properties, which means they can exert positive effects in the body beyond being a nutrient [7]. The focus on biologically active peptides from different protein sources has increased among researchers. Through cell cultures and animal studies, researchers have already identified several sequences that look promising [8, 9].

Literature:

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- [8] Iwai K., Hasegawa T., Taguchi Y. et al. (2005) Identification of food-derived collagen peptides in human blood after oral ingestion of gelatine hydrolysates. J Agric Food Chem. Aug 10;53(16):6531-6.
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2. PRINCIPLE AND METHOD

The purpose of this study was to examine the tolerability of **Marine Collagen Peptide Powder (3 kDa)** according to clinical-dermatological test criteria. The participants were able to consult the physicians taking care of the test participants any day in case of any objective or subjective skin changes were noted.

An open, uncontrolled, monocentric clinical test was performed to evaluate the efficacy of the present dietary supplement with respect to the skin humidity. Before the beginning of the application test, all subjects were examined dermatologically and signed an informed consent. Only participants were included in the test group, which did not find any pathological changes in the test area or have known allergies against fish and/or shellfishes.

The above mentioned preparation was taken orally once a day. Either one package (10 g) in the morning dissolved in 300 ml liquid (water or juice) or 5 g in the morning and 5 g in the evening each dissolved in 150 ml liquid. The ingestion time was 8 weeks. The measurements of skin moisture were performed on the face and forearm before and after 8 weeks application.

Marine Collagen Peptide Powder is exclusively produced in Norway, from high quality deep-sea cod skin, sustainably harvested from the crystal clear waters of the North Atlantic Ocean. Producer's R&D department carefully selects the raw materials, the enzyme mixture and the extend of hydrolysis to produce high quality collagen peptides. Marine Collagen Peptide Powder has a very high purity degree, is highly bioavailable, bioactive, neutral in taste and smell, is non-GMO, and free from carbohydrates, fat, preservatives and additives.

2. SKIN MOISTURE MEASUREMENTS

With the corneometer principle, the skin moisture of the outer layer of the skin (Stratum corneum) is determined by means of a capacity measurement. This principle is based on the fact that the dielectric constants of water and other materials are different. A relevantly shaped measuring capacitor reacts with differing capacity changes to the measurement volume that the probe has been placed on, that the equipment automatically recognises and evaluates. The active probe that is coated in special glass is pressed onto the skin position to be measured and after 1 second, the value is displayed on the corneometer as well as the degree of moisture on the upper skin. The special construction guarantees that the active forepart of the probe is always pressed down with constant pressure, even against any inaccessible skin positions.

The corneometer consists of a desk housing and the associated probe. This is connected to the desk housing that has a special socket for the helix cable. In the desk housing the measured values are displayed to a maximum of three decimal places. In addition to this, the display also fulfils other information functions.

The measurement sensor is of rectangular shape. The special glass coating on the active forepart can be moved axially and has a stroke of at least 3 mm. The measuring principle requires the plane of the forepart to lay with constant pressure. In order to guarantee the best reproduction, the forepart of the measurement probe has

- : Corneometry
- : Marine Collagen Peptide Powder (3 kDa)

to be very small (7 x 7 mm). The internal moving part - the active forepart - is pressed against the skin by a spring which is always at 3.5N.

The corneometer works completely automatically. In order to undertake a measurement, the measuring head must be pressed against the skin position to be measured. After one second the measured value will be displayed.

The value displayed on the corneometer gives the degree of moisture on the surface of the skin, e.g. before and after the skin was treated with cosmetics or pharmaceutical products, i.e. the unit displays the status or the change in the moisture on the surface of the skin.

The measurements are performed in a defined period after the application.

The device utilized in the present study is the Corneometer® CM 825 from Courage and Khazaka (Cologne, Germany) connected to an MPA system. The output are arbitrary units from 0 (no water at all) – 120 (on water). The units are well established as “Corneometer units”. Definition for the use on human skin: <30 = very dry skin; 30 – 40 dry skin; 40 – 45 normal skin; >45 sufficiently moisturized skin.

2.1 WORKING PROCEDURES FOR THE SKIN MOISTURE MEASUREMENTS

1. The test persons were acclimatised for 45 minutes at a temperature of 22 degrees centigrade and 60% relative humidity.
2. Skin measurement values were measured at three different places within the respective testing areas. The recorded values were averaged.
3. Measurements were taken before the application and after eight weeks of application. All measurements were performed at least 10-12 hours after the last application of the formerly used product resp. the test product.

2.2 EVALUATION AND STATISTICS

The skin moistness value per test area and the time were also standardised. The respective values can be found in the attached tables.

With regards to the subjects, they were specified by incrementing numbers, age and sex, standardised and the normal deviations were determined.

(Literature: L. Sachs, "Statistical Methods", 6th edition, Springer Verlag, Berlin & Heidelberg 1988).

Delta = Differences between the values of skin moistness
delta (%) = average percentage moisture change caused by the application, with reference to the starting value.

The corresponding table contains the measured values.

- : Corneometry
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3. PANELLISTS

The test panel does include 20 adult, female panellists.

3.1 INCLUSION CRITERIA

- Subjects with dry skin aged between 40 and 55 years.

3.2 EXCLUSION CRITERIA

- severe or chronic skin inflammation
- serious inner or chronic diseases
- intake of drugs that possibly can interfere with skin reactions (Glucocorticoids, antiallergics, topical immuno modulator, etc.)
- application of pharmaceutical products and skin care products with active ingredients until 7-10 days before testing
- severe allergies or occurred severe side effects after usage of cosmetic products
- sunbath or usage of tanning bed during the study period
- known cancer
- pregnancy or lactation period
- known allergy against fish and/or shellfishes

3.3 TEST SUBJECTS

Nr.	Name	Gender	Age	Skin type
1.	AhPe	f	54	dry/sensitive
2.	BaDa	f	49	dry
3.	BeMo	f	50	dry/sensitive
4.	BöTi	f	43	dry/sensitive
5.	BöCl	f	45	dry/sensitive
6.	BuSi	f	51	dry/sensitive
7.	DöSi	f	54	dry/sensitive
8.	FiMo	f	45	dry/sensitive
9.	FüAn	f	48	dry/sensitive
10.	HiNa	f	41	dry
11.	KoPe	f	55	dry/sensitive
12.	KoSu	f	53	dry/sensitive
13.	LoCo	f	55	dry
14.	LüDö	f	48	dry/sensitive
15.	MeSt	f	52	dry/sensitive
16.	ReSi	f	53	dry
17.	ScAn	f	49	dry/sensitive
18.	ScBr	f	49	dry/sensitive
19.	TeLu	f	40	dry/sensitive
20.	ZsMa	f	51	dry/sensitive

4. DERMATOLOGICAL EXAMINATIONS

1. Before the start of the application test

All participants were determined to have healthy skin in the test area. No pathological skin disorder was detected.

2. During the study

No complaint of any pathological skin disorder was reported during the course of this eight-week application test. Interruptions of the application test and/or medical intervention were not necessary.

3. After the end of the application test

During the final dermatological examination after the end of the study, none of the 20 participants showed development of any pathological skin disorder in the test area. The mentioned product was well-tolerated and did not lead to any unwanted skin reaction.

5. RESULTS

5.1 Skin moisture measurements, Test area: **FACE**, PREPARATION: **Marine Collagen Peptide Powder (3 kDa)**. Table shows the average values of 2 independent measurements.

Subjects	Before	After 8 weeks	Difference	Delta/Percent
1.	56,90	59,40	2,50	4,39
2.	56,85	58,60	1,75	3,08
3.	46,40	70,80	24,40	52,59
4.	50,30	62,80	12,50	24,85
5.	52,75	56,70	3,95	7,49
6.	37,65	67,80	30,15	80,08
7.	49,45	57,70	8,25	16,68
8.	39,15	47,50	8,35	21,33
9.	33,45	56,60	23,15	69,21
10.	36,55	54,95	18,40	50,34
11.	49,45	60,30	10,85	21,94
12.	50,00	59,25	9,25	18,50
13.	34,95	55,10	20,15	57,65
14.	52,55	57,30	4,75	9,04
15.	42,25	74,90	32,65	77,28
16.	28,00	38,95	10,95	39,11
17.	37,05	41,05	4,00	10,80
18.	36,35	54,85	18,50	50,89
19.	40,40	62,40	22,00	54,46
20.	33,45	42,05	8,60	25,71
Average	43,19	56,95	13,76	34,77
Minimum	28,00	38,95	1,75	3,08
Maximum	56,90	74,90	32,65	80,08
Stand.dev.	8,62	9,24	9,31	24,94
Change of average value: 34,77 %				

- : Corneometry
- : Marine Collagen Peptide Powder (3 kDa)

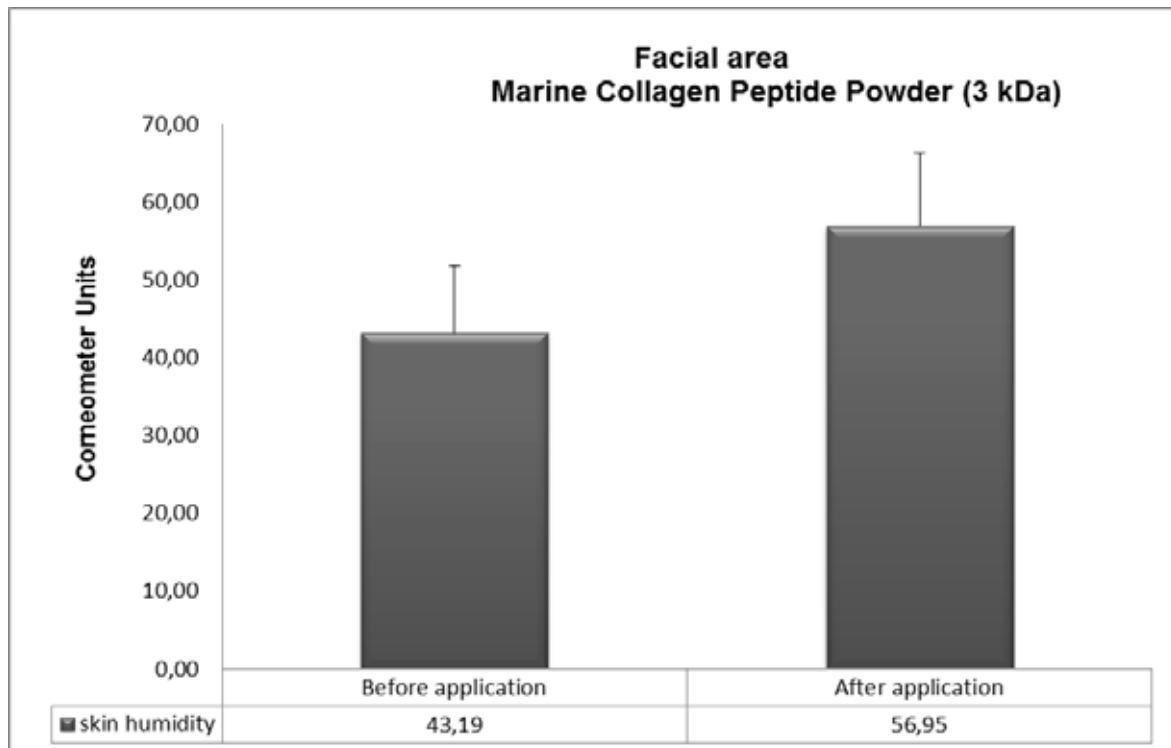


Fig. 5.1 Illustration of the average skin humidity before and after 8 weeks application of the product **Marine Collagen Peptide Powder (3 kDa)**. Definition: < 30 = very dry; 30 – 40 = dry; 40 – 45 normal; >45 sufficiently moisturized

After application of the test product **Marine Collagen Peptide Powder (3 kDa)** the average skin humidity of the 20 participating subjects increased by 13,76 corneometer units in the facial area which is equally to 34,77 % humidity improvement.

: Corneometry
 : Marine Collagen Peptide Powder (3 kDa)

5.2 Skin moisture measurements, Test area: **FOREARM**, PREPARATION:
Marine Collagen Peptide Powder (3 kDa). Table shows the average values of
 2 independent measurements.

Subjects	Before	After 8 weeks	Difference	Delta/Percent
1.	26,60	30,95	4,35	16,35
2.	34,15	36,10	1,95	5,71
3.	32,60	32,95	0,35	1,07
4.	35,30	36,00	0,70	1,98
5.	35,85	35,65	-0,20	-0,56
6.	30,10	37,05	6,95	23,09
7.	33,35	39,35	6,00	17,99
8.	32,15	33,75	1,60	4,98
9.	27,40	29,50	2,10	7,66
10.	36,75	38,98	2,23	6,07
11.	31,25	42,30	11,05	35,36
12.	35,95	35,85	-0,10	-0,28
13.	23,55	25,10	1,55	6,58
14.	28,10	29,50	1,40	4,98
15.	25,30	33,30	8,00	31,62
16.	32,05	32,75	0,70	2,18
17.	35,05	36,45	1,40	3,99
18.	26,95	32,25	5,30	19,67
19.	27,90	32,10	4,20	15,05
20.	20,80	24,55	3,75	18,03
Average	30,56	33,72	3,16	11,08
Minimum	20,80	24,55	-0,20	-0,56
Maximum	36,75	42,30	11,05	35,36
Stand.dev.	4,54	4,47	3,01	10,53
Change of average value: 11,08 %				

- : Corneometry
- : Marine Collagen Peptide Powder (3 kDa)

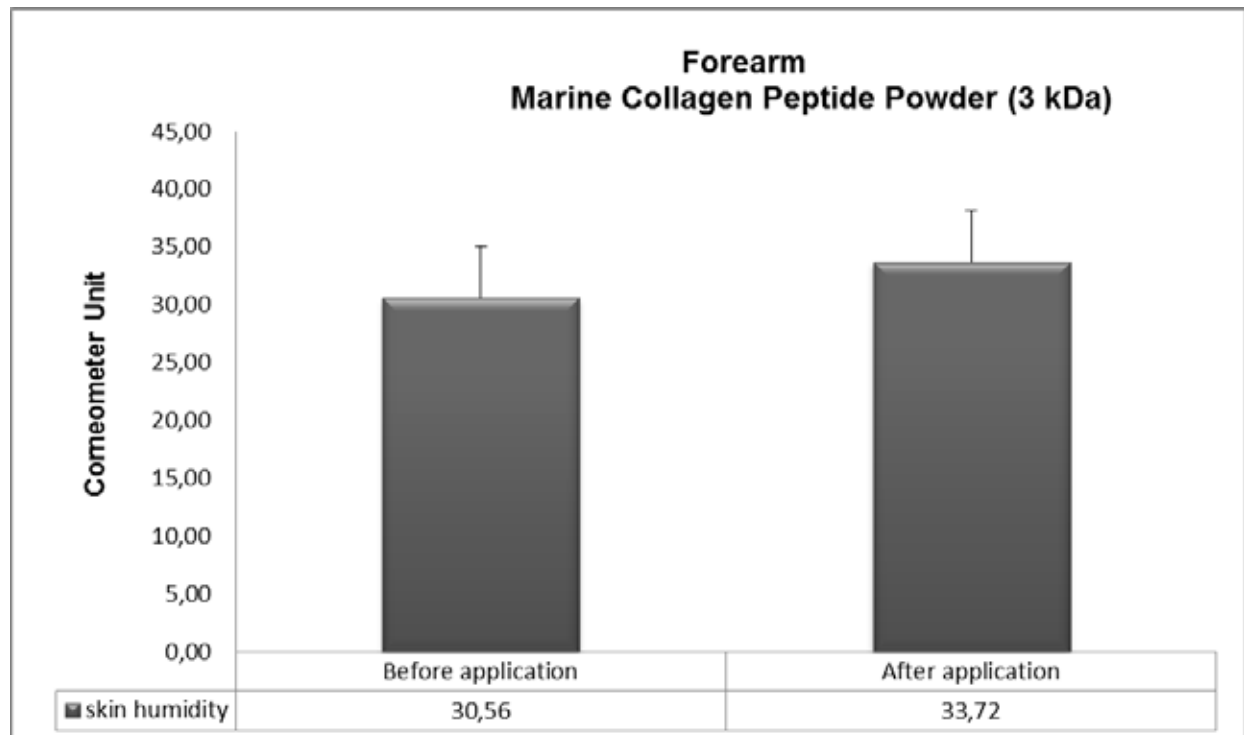


Fig. 5.2 Illustration of the average skin humidity before and after 8 weeks application of the product **Marine Collagen Peptide Powder (3 kDa)**. Definition: < 30 = very dry; 30 – 40 = dry; 40 – 45 normal; >45 sufficiently moisturized

After application of the test product **Marine Collagen Peptide Powder (3 kDa)** the average skin humidity of the 20 participating subjects increased by 3,16 corneometer units at the forearm which is equally to 11,08 % improvement.

- : Corneometry
- : Marine Collagen Peptide Powder (3 kDa)

6. ASSESSMENT OF THE TEST RESULTS

All of the 20 study participants tolerated the product **Marine Collagen Peptide Powder (3 kDa)** well during the course of the eight-week application test under dermatological and clinical criteria. There were no undesired or even pathological skin reactions in the test area.

The skin moisture has been measured on 20 subjects to determine the effect of the preparation **Marine Collagen Peptide Powder (3 kDa)**. The skin moisture values were obtained using the Corneometer CM 825 (Courage&Khazaka).

The change in the skin moisture value was determined in the application area before and after using the preparation for a period of eight weeks. In the facial area an improvement of skin moisture of about 34,77 % was measured. On the forearm an increase of 11,08 % in average was determined.

In summary, it can be concluded from a dermatological point of view that the product

Marine Collagen Peptide Powder (3 kDa)
was tolerated
very well

according to clinical dermatological criteria and led to an improvement in skin moisture.

The average improvement of skin moisture after 8 weeks amounted in the facial area to **34,77 %** and on the forearm to **11,08 %**.



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