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# Detailed composition

One 15 ml ampoule contains :

Vitamin C 80mg Contributes to the normal formation of collagen for skin function. 100% des VNR\*

Zinc 10mg Maintains normal skin. 100% des VNR\*

Selenium 50µg Supports the structure of the skin. 91% des VNR\*

Marine Collagen 2.7 g Preserves skin firmness, elasticity and reduces signs of skin ageing.

Vitamin B3 16mg Supports the structure and function of the skin. 100% des VNR\* Maqui berries 100mg Rich in powerful antioxidants

Vitamin B5 6mg Contributes to good skin health. 100% des VNR\*

Raspberry & Red Grape Juice Known for its antioxidant and digestive virtues.

Vitamin E 12mg Protects against cell ageing caused by free radicals thanks to its antioxidant action.

100% des VNR\*

Wheat Ceramides 30mg Improves the overall appearance of the skin, retains moisture and increases firmness and elasticity.

Honey



MADE IN FRANCE

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### Abstract:

The consumption of dietary supplements for beauty purposes is becoming increasingly popular and some ingredients are already the subject of clinical studies in oral supplementation alone. Modulating skin functions, combating against skin ageing, reinforcing collagen synthesis and skin firmness, reducing wrinkles are all parameters examined with this new route of administration.

However, only a limited number of clinical trials has been conducted on finished products, and therefore on a complete formulation, entailing an interaction between the ingredients. In this comparative before-and-after study, the efficacy of LONGEVITE by EVOLEUM Paris is measured on the formation of crow's feet, skin density (stimulation of cutaneous collagen), skin firmness and elasticity and hydration. Twenty-two (22) women aged between 45 and 65 were enrolled and treated with 21 LONGEVITE ampoules (15 ml). They used one ampoule of product per day in the evening before dinner. Skin parameters were measured in all subjects before starting the treatment (Day 0), after 21 days of treatment (Day 21) and a further 21 days after the end of the treatment (Day 42) to measure the persistence of the results.

#### **Results:**

On Day 21, the following was observed: An **18.8%** decrease in the depth of crow's feet; a **7.8%** increase in skin firmness; a **28%** increase in dermal density and a **2.7%** increase in dermal thickness.

On Day 42, the anti-ageing effect was still observed with: A **27.1%** decrease in the depth of crow's feet; a **5.2%** increase in skin firmness: an **18.4%** increase in dermal density; a **7.8%** increase in the skin's surface hydration level.

### Introduction:

In 2010, the global nutricosmetics market was worth US\$2.4 billion [1]. In 2019, it is US\$5 billion and it is estimated to reach US\$9 billion by 2026 with an estimated annual growth rate of 5.5%. This is a rapidly expanding market with the emergence of numerous players and products whose promises are based on claims validated by the European Commission or on studies conducted on ingredients [2-3]. Unlike cosmetic products, nutricosmetic products rarely are subject of a study on the finished product taking into account the interactions between the ingredients and the synergies correlated to a given formulation.

In this context, the purpose of this clinical study was to illustrate the efficacy of a nutricosmetic, LONGEVITE by Evoleum, using objective instrumental techniques. The evaluation focused on the antiageing effect on the face of 22 healthy volunteers.

The tested dietary supplement was used as a treatment for 21 days. Facial hydration, dermal density, skin firmness, elasticity and depth of crow's feet were analysed on Day 0 during the initial visit, on Day 21 during the post-treatment evaluation visit and on Day 42 during the visit for persistence assessment. The visit on Day 42 allows verification, 3 weeks after the end of treatment, of the persistence of the beneficial anti-ageing effect on the skin parameters.

Photographs of the face make it possible to view the modulation of the clinical signs after treatment.

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### Material and method:

### **Tested product**

The product under review, LONGEVITE, is a dietary supplement for beauty purposes developed by Evoleum and notified to the French General Directorate for Competition Policy, Consumer Affairs and Fraud Control in October 2019. Based on marine collagen, maqui berries, wheat ceramides and a complex of vitamins and minerals, this product is manufactured in France in a certified GMP laboratory (Good Manufacturing Practices) and in accordance with pharmaceutical standards.

The formulation of this product aims to stimulate skin collagen and reduce the formation of wrinkles. Vitamin C, vitamin E, selenium and zinc are activators and co-factors of collagen synthesis [4-5]. Marine collagen provides the amino acids necessary for the skin to reproduce and stimulate skin collagen [6]. The maqui berry has been studied for its protective effect and its action on the inhibition of enzymes responsible for the degradation of collagen and elastin in the skin [3]. LONGEVITE contains concentrated active ingredients designed to stimulate collagen production in the skin.

The product comes in the form of 15-ml ampoules in a 21-day treatment, one ampoule per day. It should be taken in the evening before dinner.

#### Study design

The study was open-designed with a baseline assessment. Each subject is her own reference. The study focused on the improvement of skin parameters such as elasticity, firmness, hydration, density, depth of crow's feet and overall appearance. The beauty and dietary habits of the subjects were not modified. The study was conducted under dermatological supervision.

#### **Subjects**

The general criteria were as follows: Volunteer able to follow the trial procedures in their entirety, volunteer freely providing informed written consent after receiving oral and written information on the trial, benefiting from a social security system in accordance with the recommendations of French legislation (French Public Health Code L1124-3).

#### **Inclusion criteria**

The inclusion criteria were as follows: The subjects were Caucasian women aged between 45 and 65, urban, non-smokers, with a BMI < 30 and adequate self-esteem according to the Rosenberg questionnaire (9 questions). Each subject had crow's feet.

#### **Exclusion criteria**

The exclusion criteria were as follows: Prolonged exposure to the sun before and during the study; subject participating in any other clinical study evaluating medicinal products or medical devices; subject undergoing any treatment that may affect the hydration process during the month preceding inclusion in the study (corticosteroids, retinoids, dietary supplements with hydrating properties, etc.) or likely to take this treatment during the trial; subject with food allergies; subject with a progressive cardiovascular, digestive, neurological, psychiatric, genital, urinary, haematological, endocrine, or other condition; in general, any serious and/or progressive systemic disease; subject with a history of cancer, diabetes, hypercholesterolemia; subjects consuming, on average, more than one glass of wine per day; subject with a history of or associated autoimmune or connective tissue disease; subject with a skin disease on the face and/or body (atopic dermatitis, rosacea, psoriasis, couperose); pregnant or breastfeeding women.

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#### **Evaluation criteria**

Following inclusion in the study, skin firmness and elasticity, hydration, depth of crow's feet and skin density are measured during the initial visit. 2D and 3D photographs are taken for comparison purposes. A second evaluation takes place after 21 days of treatment during the post-treatment visit and 21 days later to measure the persistence of the results. Post-treatment evaluation is completed by a satisfaction survey and an assessment of tolerance to the product.

Measurement of skin firmness and elasticity using the Monaderm Cutometer (MPA 580 probe)

Firmness and elasticity are measured during the initial visit, post-treatment and after 21 days without treatment to evaluate the persistence of results via cutometric assessment. The Cutometer® is designed to measure the biomechanical properties of the upper layers of the epidermis by applying a negative pressure (suction) that deforms the skin. The measurement principle is based on the suction method. A negative pressure is created in the device and the skin is sucked into the measurement cup of the probe. After a defined period, the skin is released inside the probe. The penetration depth is determined by a non-contact optical measuring system. This optical measuring system consists of a light source and a light receptor. The intensity of the light varies according to the penetration depth of the skin.

The skin's resistance to negative pressure (firmness) is displayed as a curve (penetration depth in mm/time unit) in real time during the measurement. The R0 value corresponds to the firmness of the skin. If firmness increases, a decrease in the R0 value is observed. Elasticity is evaluated with the R5 parameter. If elasticity increases, an increase in the R5 parameter is observed.

This measurement is performed on the left cheekbone.

#### Measurement of the skin surface hydration level with the corneometer (Courage Khazaka®)

Skin hydration is measured during the initial visit, post-treatment and after 21 days of discontinuation to assess the persistence of results via corneometry. The measurement of the hydration level with the aid of a corneometer corresponds to an impedance measurement, correlated to the electrical conductivity of the water present in the stratum corneum. The hydration level is measured at 3 different points in a 4 cm<sup>2</sup> area on the left cheek (cheekbone). The final result corresponds to the 3 measurements averaged and is expressed as hydration %.

#### Ultrasound analysis of dermal density and thickness (Monaderm)

Dermal density and thickness are measured during the initial visit, post-treatment and after 21 days without treatment via a high-frequency ultrasound.

An ultrasound is performed on the right cheek coming into contact with the nasal wing by using Monaderm's 12.1 mm 2D 20 MHz narrow-focus exploration probe. The image capture and analysis of dermal density and thickness is performed via Advanced Control software. Dermal density is expressed in % and dermal thickness is expressed in mm.

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#### Profilometric evaluation of the depth of wrinkles or crow's feet (LifeViz micro®)

The depth of wrinkles is measured during the initial visit, post-treatment and after 21 days without treatment thanks to a profilometric analysis.

The analysis is performed on the deepest crow's foot (right or left). The average depth expressed in mm is equal to the total volume (= positive volume - negative volume), divided by the surface. The average depth depends on the contour used as a basis for the calculation and is useful for examining the evolution of a wrinkle between two visits.

#### **Statistical analyses**

The results are presented in the form of tables (mean  $\pm$  SD for the 22 subjects). The comparison of the different parameters is carried out with the paired Student's t test after verifying the normality of the groups on Day 0 with the Shapiro-Wilk test (with an alpha risk equal to 5%).

In the event of non-normality of the groups, a non-parametric Wilcoxon test is performed (with an alpha risk of 5%).

#### 2D and 3D photographs

Photographs of the face are taken by the LifeViz mini device and allow for the modulation of clinical signs to be viewed after treatment. The photographs taken by the LifeViz micro are used to perform profilometric analyses of the depth of crow's feet. They are taken during the initial visit, post-treatment and after 21 days without treatment.

#### Satisfaction survey

A self-evaluation questionnaire was provided by Evoleum. The volunteers answered the selfevaluation questions during the post-treatment visit only. The questions concerned the effects experienced (hydration, anti-wrinkle, firmness, softness, etc.) with respect to the use of the product and their opinion.

#### Evaluation of tolerance by the dermatologist

During the visit on Day 21, a clinical evaluation (cutaneous or systemic manifestations) made it possible to verify the tolerance of the dietary supplement.

In the event of an adverse effect, the imputability of the product is determined according to the following stages:

- very likely
- likely
- possible
- doubtful
- excluded

#### Constraints of the study

For the smooth running of the study, the volunteers committed to maintaining their usual skincare (face cream) / cleansing and make-up habits (same product and same frequency of use), to attending the appointments scheduled for the study at the GREDECO laboratory (Day 0, Day 21, Day 42); to using the dietary supplement in accordance with the Evoleum guidelines between Day 0 and Day 21; to refraining from using face cream 24 hours prior to each visit; and to refraining from using make-up on the skin on the morning of the visit.

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### **Results:**

The study was conducted on 22 women aged between 45 and 65 with an average age of  $53.3 \pm 5.4$ . The evaluation of product tolerance was excellent with no side effects observed in the volunteers. There was no loss to follow-up or discontinuation of the protocol during the study.

Measurement of skin firmness (Monaderm Cutometer, MPA580 Probe)

The significant decrease in the R0 value measured via cutometry (with 0.3020 versus 0.3274 on Day 0) testifies to a **7.8%** increase in skin firmness after 21 days of treatment with the LONGEVITE dietary supplement. On Day 42, the increase in skin firmness was equal to **5.2%**.



	± 50	± 0.0411	$\pm 0.0403$	$\pm 0.0390$			
Figure 1: Average skin firmn	ess measur	ed via cutomet	ry in 22 healt	hy volunteers	on Day 0, Da	y21 and Day	42

Measurement of skin elasticity (Monaderm cutometer, MPA580 probe)

On average, on 22 volunteers, skin elasticity was not significantly modified after 21 days of treatment with the LONGEVITE dietary supplement.



Figure 2: Average skin elasticity measured via cutometry in 22 healthy volunteers on Day 0, Day 21 and Day 42

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Measurement of the skin surface hydration level (Courage Khazaka corneometer)

On average, on 22 volunteers, the skin surface hydration level was significantly increased by **7.8%**, 3 weeks after discontinuation of the LONGEVITE dietary supplement.



Figure 3: Average skin hydration in % measured via corneometry in 22 healthy volunteers on Day 0, Day 21 and Day 42

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### Profilometric evaluation of the depth of wrinkles / crow's feet (LifeViz Micro)

The depth of crow's feet was significantly reduced by **18.8%** after 21 days of treatment with the LONGEVITE dietary supplement. Three weeks after treatment discontinuation, a significant reduction in the depth of crow's feet wrinkles (**27.1%**) was obtained with 0.063 mm as compared to 0.087 mm on Day 0.



Figure 4: Average crow's feet size in mm measured via profilometric analysis in 22 healthy volunteers on Day 0, Day 21 and Day 42



Image 1: Visible reduction in the depth of crow's feet after 21 days of treatment. Example of a volunteer before (left) and after (right) 21 days of oral treatment with LONGEVITE

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Ultrasound analysis of dermal density (Monaderm)

Dermal density was significantly increased by **28.0%** after 21 days of treatment with the dietary supplement LONGEVITE. The increase in dermal density 3 weeks after treatment discontinuation was equal to **18.4%** (significant decrease in dermal density by 7.5% compared to Day 21).



Dermal density (%)	Day 0	Day 21	Day 42
Average	32.43	41.51	38.40
± SD	± 6.37	± 8.15	± 7.60

Figure 5: Average skin density measured by high-frequency ultrasound in 22 healthy volunteers on Day 0, Day 21 and Day 42



Image 2: Visible increase in skin density after 21 days of treatment. Example of a volunteer before (top) and after (bottom) 21 days of oral treatment with LONGEVITE

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### Ultrasound analysis of dermal thickness (Monaderm)

On average, on 22 volunteers, a significant increase by **2.7%** in dermal thickness was observed after 21 days of treatment with the LONGEVITE dietary supplement. Dermal thickness, 3 weeks after discontinuation of treatment with LONGEVITE, was similar to that measured on Day 0.



Figure 6: Average dermal thickness (mm) measured via a high-frequency ultrasound on Day 0, Day 21 and Day 42

### 2D / 3D photographs

2D/3D photographs were taken on Day 0, Day 21 and Day 42 to illustrate a more global impact on the overall appearance of the skin. These photographs revealed an improvement in forehead wrinkles, improved skin tone evenness and regulation of seborrhoea. As the results are unique for each volunteer, no significant results can be drawn from these photographs.



Image 3: Visible improvement in the overall appearance of the skin after 21 days of treatment. Example of 2 volunteers before (left) and after (right) 21 days of oral treatment with LONGEVITE

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#### Self-administered satisfaction questionnaire

Following the completion of a self-administered questionnaire on Day 21, the volunteers observed: For 95.5% of them, an improvement in skin firmness (95.5% were at least quite satisfied). For 90.9% of them, an improvement in their wrinkles (90.9% were at least quite satisfied). For 82% of them, an improvement in skin youthfulness (82% saw at least a slight improvement). 18% observed an improvement in the first week, 46% in the second week and 23% in the third week.

90.9% of them would like to extend their treatment. 95.5% would recommend the treatment to a friend. 1 in 2 volunteers received spontaneous positive comments on the beauty of their skin and face from their friends and family.

#### Discussion

The appearance of wrinkles is one of the main signs of skin ageing in Caucasian skin [7]. Their formation is partly due to a decrease in the level of collagen, a structural protein, in the skin. A decrease linked to oxidative stress, glycation and genetics is observed from the age of 30. A plethora of studies has emphasised that oral supplementation of different types of collagen has a positive impact on collagen synthesis in the skin and on wrinkle reduction [2, 8].

In this study on a complete formulation based on collagen, maqui berry, wheat ceramides and a specific vitamin and mineral complex, the average improvement in the depth of crow's feet was equal to 18.8% after 21 days of treatment and 27.1% after further 21 days without treatment. An improvement in dermal density and firmness was also observed, reflecting the increase in collagen levels. The improvement in the reduction of wrinkles after treatment discontinuation shows that the collagen synthesis mechanisms activated by the treatment continue after treatment completion.

With regard to skin elasticity and hydration, these do not display any significant improvement after 21 days of treatment. Since LONGEVITE does not act directly on elastin, the protein responsible for skin elasticity, but only indirectly with the preservation of elastin thanks to the maqui berry extract [3], this lack of results over 3 weeks can undoubtedly be improved by extending the treatment. With regard to hydration, the measurement was carried out via corneometry, a measurement of skin surface hydration. The study period was marked by significant cold spells which could have affected the hydration results.

In addition to feedback on the improvement of skin parameters, the study revealed beneficial side effects on sleep quality (time required to fall asleep, depth and quality of sleep, energy on awakening) for 6 out of 22 subjects (27.3%) and on tone (physical activity, help with concentration, decision-making) for 5 out of 22 subjects (22.7%). This phenomenon could be due to the composition of vitamins B3 and B5, as studies have shown that supplementation with B vitamins can improve sleep, lucidity of dreams, and recall [9]. The toning action can be associated with vitamin C which is known to reduce fatigue.

The 2D/3D photographs also illustrate the action of LONGEVITE on the overall appearance of the skin. Specifically, an improvement in seborrhoea is noted, undoubtedly correlated to zinc supplementation [10], skin tone evenness, reduced redness and fading dark spots, potentially correlated to the antioxidant effects of the maqui berry [11] and the vitamin and mineral complex.

An in vitro or in situ study could complete this study in order to better identify the biological mechanisms activated and stimulated by treatment with LONGEVITE.

The parameters relating to skin tone evenness and reduced seborrhoea could also make up the subject of an instrumental study to quantify the improvement of these parameters with the 21-day treatment.

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#### **Conclusion:**

Based on the results obtained during the study, it is possible to confirm the anti-ageing efficacy of the LONGEVITE dietary supplement. A significant reduction in the depth of crow's feet occurs after 21 days of treatment and continues to decrease after further 21 days of treatment discontinuation, proving a persistence of the anti-wrinkle effects. This effect is correlated to an increase in the density of the connective tissue, which is composed of more than 80% collagen, thus translating into an increase in and stimulation of the collagen level in the dermis. It is important to note that these results were obtained with the oral treatment of the LONGEVITE dietary supplement by Evoleum, composed of hydrolysed marine collagen, maqui berries, wheat ceramides and a complex of specific vitamins and minerals. These results are not relevant to all other collagen-based formulations.

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Bibliography

[1] Yong-Li McFarland et coll, The Nutricosmetics Market : a Global Health Wellness Megatrend [archive] - Présentation du cabinet d'études Frost&Sullivan

[2] E. Proksch, M. Schunck, V. Zague, D. Segger, J. Degwert, S. Oesser, Oral Intake of Specific Bioactive Collagen Peptides Reduces Skin Wrinkles and Increases Dermal Matrix Synthesis, Skin Pharmacol Physiol. 2014;27(3):113-9.

[3] Sergio Davinelli, Juan Carlos Bertoglio, Armando Zarrelli, Riccardo Pina & Giovanni Scapagnini (2015) A Randomized Clinical Trial Evaluating the Efficacy of an Anthocyanin-Maqui Berry Extract (Delphinol<sup>®</sup>) on Oxidative Stress Biomarkers, Journal of the American College of Nutrition, 34:sup1, 28-33.

[4] Geesin JC, Darr D, Kaufman R, Murad S, Pinnell SR. Ascorbic acid specifically increases type I and type III procollagen messenger RNA levels in human skin fibroblast. J Invest Dermatol. 1988 Apr;90 (4):420-4

[5] McClain, Wiley & al. Influence of zinc deficiency on synthesis and cross-linking of rat skin collagen. Biochim Biophys Acta. 1973 Apr 28;304(2):457-65.

[6] Etude in vitro sur explant de peau humaine avec supplémentatation de Cartidyss et vitamin C sur la néosynthèse de glucoaminoglycane après 3 et 12 jours.

[7] Machková L, Švadlák D, Dolečková I. A comprehensive in vivo study of Caucasian facial skin parameters on 442 women. Arch Dermatol Res. 2018 Nov;310(9):691-699.

[8] Liane Bolke & al. A Collagen Supplement Improves Skin Hydration, Elasticity, Roughness, and Density: Results of a Randomized, Placebo-Controlled, Blind Study. Nutrients 2019, 11, 2494.

[9] Aspy DJ, Madden NA, Delfabbro P. Effects of Vitamin B6 (Pyridoxine) and a B Complex Preparation on Dreaming and Sleep. Percept Mot Skills. 2018 Jun;125(3):451-462

[10] Burton JL, Goolamali SK. Zinc and sebum excretion. Lancet. 1973 Jun 23;1(7817):1448.

[11] Junji Tanaka, Takashi Kadekaru, Kenjirou Ogawa, Shoketsu Hitoe, Hiroshi Shimoda, Hideaki Hara, Maqui berry (Aristotelia chilensis) and the constituent delphinidin glycoside inhibit photoreceptor cell death induced by visible light, 2013