

Face and neck firming care with teff natural peptides

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In the Beauty and Personal Care Industry there is an increasing shift towards effective anti-ageing and firming products with proven benefits driven by evolving consumer needs. Besides chronological ageing, the extrinsic factors such as changing lifestyle, UV exposure, environmental pollution, lack of sleep, stress and genetics are main factors which cause skin to age and lose its firmness, elasticity, structural support, and integrity.¹ New consumer insights and scientific studies on how ageing is perceived have now concluded that the neck skin appearance and nasolabial fold areas contribute much higher weightage to overall perception of ageing than the crow's feet wrinkles and fine lines. These sites have thin and fragile skin and are often neglected from daily routines.³

NaturePep® Teff (INCI name: Hydrolyzed Eragrostis Tef Seed Extract) was specifically designed to address fragile skin areas such as neck and nasolabial folds. This natural peptide technology is obtained using vegetable superfood teff seed protein and a proprietary manufacturing process which allows for the creation of low average molecular weight peptides for enhanced skin bioavailability and multi-targeted biological activity. This technology is clinically proven to increase the production of collagens and reinforce the dermal-epidermal junction (DEJ) to enhance skin firmness, tonicity and elasticity on neck and visibly reduce nasolabial folds, volume and wrinkle depth in only 28 days.

Skin ageing and Dermal-Epidermal Junction

Skin ageing is characterised by a gradual

thinning of the epidermis and dermis, degradation of the DEJ, which lead to decrease of structural support, integrity and cell cohesion.^{1,4,5,6} The dermal-epidermal junction (DEJ) consists of a network of proteins which provides structural integrity to the skin and ensures cohesion between the epidermis and the dermis. These specific DEJ proteins provide different roles in the dermis. Collagen IV anchors the epidermis to the dermis to enhance structural support. Collagen VII anchors the DEJ to the dermis to provide mechanical stability of the DEJ.^{9,10} Fibronectin anchors cells to the extracellular matrix collagens to enhance cell cohesion and binds to collagen VII to maintain firmness and elasticity.^{9,10} Fibrillin provides structural support to elastic network, while verzican is a glycoprotein which connects epidermis to dermis for structural integrity.⁶⁻⁸ As the skin ages, DEJ becomes flatter and thinner, while collagen IV and VII decrease. Additionally, other changes occur in dermal extracellular matrix components, such as collagen I, collagen III, fibronectin, fibrillin and verzican.⁴⁻¹⁰ Decline in the collagens and DEJ proteins during ageing leads to a weakened attachment between epidermis and dermis, reduced supply of nutrients to the epidermis, decreased skin firmness, elasticity, and the appearance of aged, fatigued, and wrinkled skin.⁴⁻¹⁰

Gene expression assay

The effect of the NaturePep® Teff (now referred to as 'Hydrolyzed Eragrostis Tef Seed

ABSTRACT

It is well known that skin ageing causes a decline in collagen production and flattening of dermal-epidermal junction (DEJ) which leads to a decrease in structural support, strength, elasticity, and the appearance of fatigued, saggy, and wrinkled skin. Neck and nasolabial fold skin sites have fragile skin which requires specific care. NaturePep® Teff (INCI name: Hydrolyzed Eragrostis Tef Seed Extract) was specifically designed to target delicate skin sites such as neck and nasolabial folds, which require special care. This natural peptide technology provides clinically proven skin anti-ageing benefits via a multi-targeted biological mechanism of action. It targets the dermal-epidermal junction area, which is significantly affected during the ageing process. NaturePep® Teff stimulates the production of collagens and reinforces DEJ to strengthen skin structural support, integrity, firmness, elasticity and visibly rejuvenates the appearance of aged, fatigued skin. Clinical efficacy studies demonstrate that after only 28 days of treatment, neck skin looks visibly firmer, toned, and more revitalised, while nasolabial folds are noticeably diminished and show a smoother skin texture.

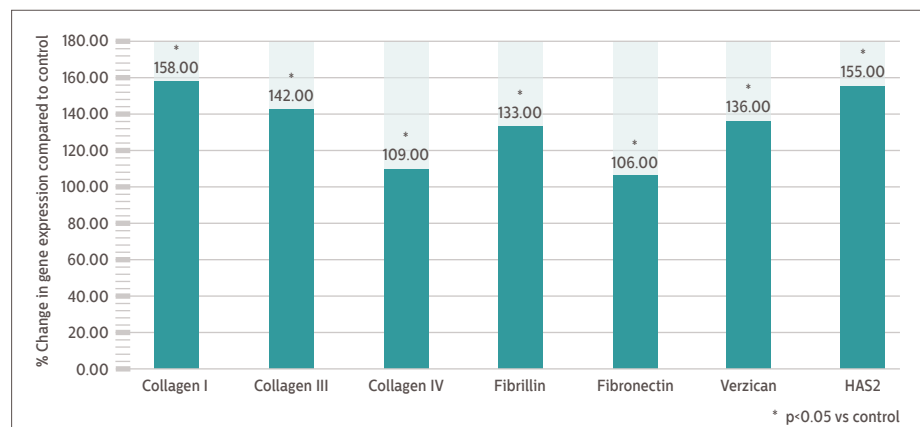


Figure 1: The percent change (%) in gene expression after NaturePep® Teff treatment compared to the control vehicle (*p<0.05).

Extract') on gene expression that involves skin ageing has been evaluated on full thickness EpiDermFTTM3D skin equivalent model (EFT-400 MatTek). Gene expression assay has been performed using a qPCR array following 24-hour exposure to the cultured media treated with this technology (0.4% active) compared to a control vehicle. Gene expression assay demonstrated statistically significant upregulation in the expression of nine critical genes involved in skin ageing. Figure 1 shows statistically significant increases in the upregulation of several critical genes involved in ageing processes compared to the control vehicle. The percentage change in each gene expression compared to the control vehicle is provided in Figure 1.

Study results show that Teff natural peptides technology upregulates the

expression of dermal collagens such as collagen I and collagen III to enhance skin strength. It modulates critical glycoproteins in the extracellular matrix such as fibrillin to enhance structural support in elastic connective tissue. It modulates fibronectin to strengthen cell adhesion, and versican to maintain firmness and elasticity. This technology has also been shown to upregulate hyaluronic acid synthase 2 (HAS2) known to stimulate the production of native hyaluronic acid to further reduce wrinkles.

Ex vivo immunofluorescence study

We measured the production of collagen I, IV and VII on ex vivo skin explants after Hydrolyzed Eragrostis Tef Seed Extract treatment using histological and immunofluorescent image analysis technique. The study was conducted on ex vivo 3D skin explants obtained from a Caucasian female volunteer of 54 years old (Fitzpatrick type III). Skin tissues were cultivated in a cultured medium for 48 hours and treated once with Hydrolyzed Eragrostis Tef Seed Extract 1% solution. Skin tissues were frozen and cryosections of 7 micrometers were stained with specific immunofluorescence staining. Skin images were acquired with a NanoZoomer S60 and quantified using image analysis software. For each image, cutaneous basement membrane corresponding to DEJ was selected and the average fluorescence intensities were calculated for collagen I, IV and VII biomarkers. These biomarkers are reported to be decreased in aged skin, causing flattening and degradation of the DEJ, which leads to loss of skin structural support, firmness, and elasticity.

Figure 2 illustrates the percent increase in the fluorescence intensities of collagen VII, I and IV compared to untreated skin. Study results demonstrate that Hydrolyzed Eragrostis Tef Seed Extract at 1% significantly increased the

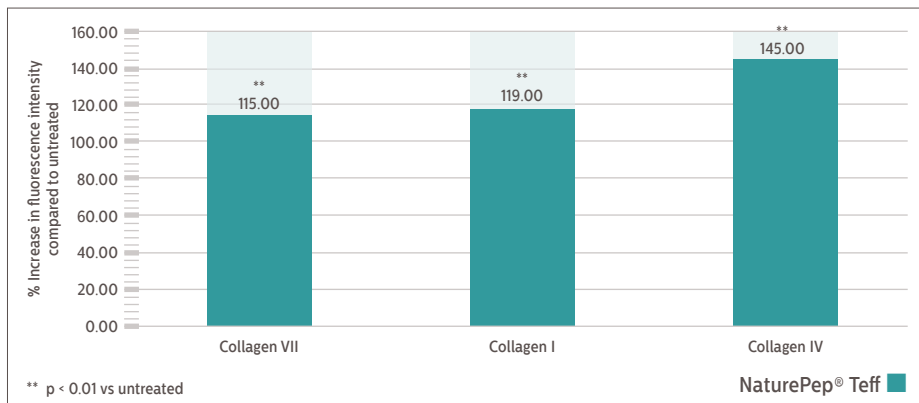


Figure 2: Percent increase in collagen VII, I and IV fluorescence intensity versus untreated skin (**p<0.01).

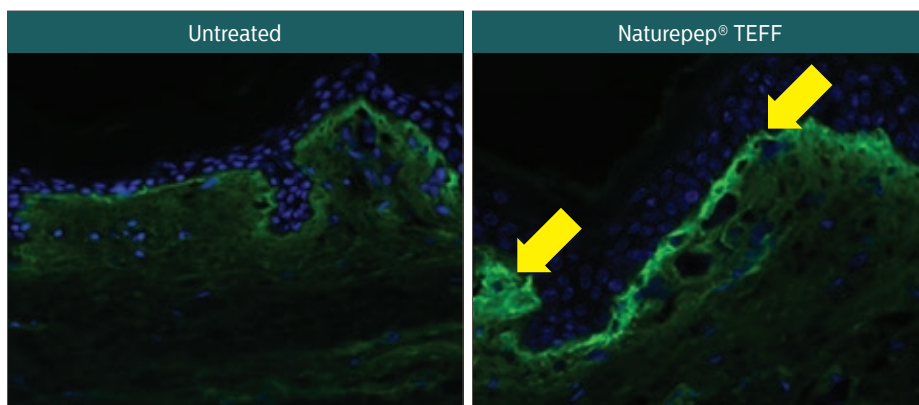


Figure 3: Untreated 3D skin explants (left image) and collagen I increase after NaturePep® Teff treatment (right image).

production of collagen VII, I, IV by 115%, 119% and respectively 145% versus untreated skin. Figure 3 represents two different cross sections of the ex vivo skin explants obtained from a Caucasian female volunteer. Left image shows untreated skin with normal presence of collagen I (seen in dark green). Right image shows a cross section of skin after Hydrolyzed Eragrostis Tef

Seed Extract treatment with visible increase in the production of collagen I seen as more intense fluorescence green colour (yellow arrows).

In vivo clinical study

The efficacy of Hydrolyzed Eragrostis Tef Seed Extract efficacy was evaluated in a parallel randomised in vivo clinical study conducted on 30 Caucasian female subjects with mature skin (ages 51-68 years old; Phototype I-III). At baseline, subjects had saggy, fatigued, and wrinkled skin on the neck and pronounced nasolabial folds. The first group of subjects received the placebo cream treatment, while the second group received Hydrolyzed Eragrostis Tef Seed Extract (at 2%) cream treatment. Products were applied two times per day (morning and night) on face, nasolabial folds, and neck. Measurements were taken at baseline (Day 0) and after 28 days of treatment (Day 28). For the neck skin sites we measured the skin biomechanical parameters such as skin fatigue, tonicity, plasticity, and elasticity with a Cutometer® (MPA 580) instrument via suction and relaxation of the skin for 3 cycles.^{11,12}

Skin fatigue (R9) measures skin resistance to stretch after 10 successive suctions. A decrease in this parameter indicates that skin is more resistant to continuous stretch and is less fatigued. Skin plasticity (Uv) measures the viscous deformation of the skin after stretch. A decrease in this parameter indicates that skin is less wrinkled, saggy, and aged. Skin tonicity (Ur) measures the immediate recovery of the skin after suction and its ability to regain its tonicity. An increase in this parameter

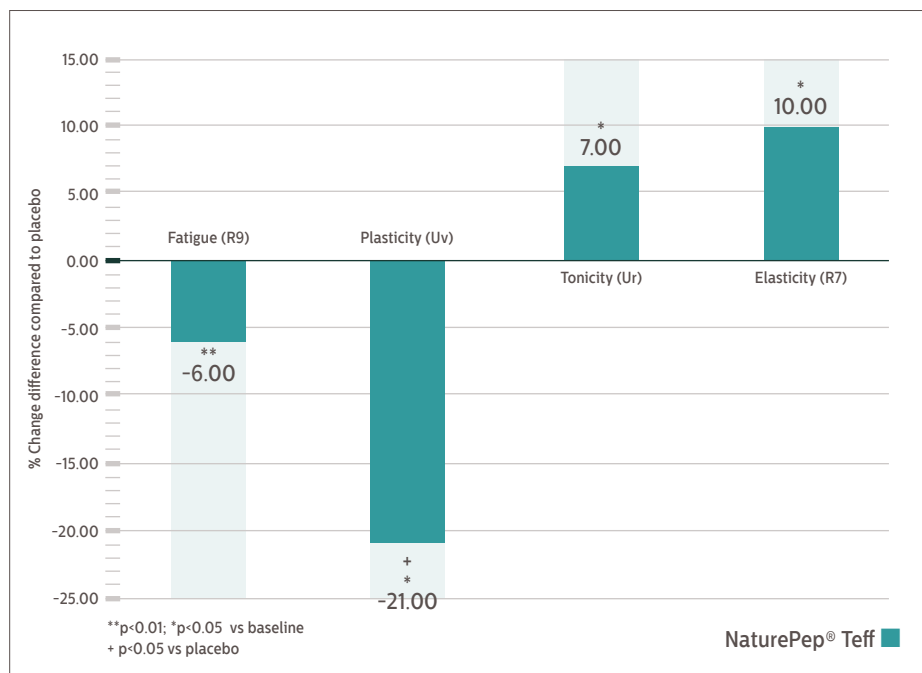


Figure 4: Percent change in neck skin biomechanical parameters after 28 days of treatment with NaturePep® Teff (difference from placebo) (** p<0.01; *p<0.05 vs baseline; + p<0.05 vs placebo).

indicates more toned skin. Skin elasticity (R7) measures skin stretch and total recovery to its original shape. An increase in this parameter indicates more elastic skin with a youthful appearance.¹²⁻¹⁴

For the nasolabial folds, we measured the average roughness, wrinkle depth and folds volume with PRIMOS® 3D Lite image analysis technique.¹⁵⁻¹⁷ Average roughness (Ra) measures the average of peaks and valleys of all heights of the folds. A decrease in this parameter means smoother skin. Average wrinkle depth (Rz) measures the difference between the highest peak and the deepest shallow inside of the nasolabial fold. A decrease in this parameter indicates decrease in fold depth and an anti-wrinkle effect. Volume measures the fold volume in mm³. A decrease indicates decrease in fold volume and less visible folds.¹⁵⁻¹⁷

Statistical analysis was performed to assess the changes from the baseline for each product using paired - Student t-test. For comparison between the products, an unpaired t-test (or Mann Whitney test) was used via SAS 9.4 statistical software. The significance level was set at p <0.05.

In vivo efficacy clinical study results: neck

The changes in neck skin biomechanical properties after 28 days of treatment with Hydrolyzed Eragrostis Tef Seed Extract compared to baseline and placebo are shown in Figure 4. Study results illustrate that Hydrolyzed



Figure 5: Digital pictures of a subject (age 58) showing saggy and wrinkled skin at Day 0 (left image). After 28 days of treatment with NaturePep® Teff, skin on the neck is visibly firmer, lifted and toned with a smoother texture and revitalized skin appearance (right image).

Eragrostis Tef Seed Extract treatment significantly decreased skin fatigue compared to baseline which indicates that skin is more resistant to continuous stretch. Hydrolyzed Eragrostis Tef Seed Extract significantly

decreased skin plasticity by 21% compared to placebo while neck lines and wrinkles were visibly reduced. Neck skin tonicity and elasticity were significantly increased compared to baseline after 28 days of treatment. Clinical



Natural ingredients are beautiful

They offer creativity, innovation and solutions that nourish and protect our body

Transform your ideas into reality and let nature be your art

Natura-Tec Marine BlueRevelation 50+™



All-in-one powerful cocktail of microalgae
 Providing an integrative approach to enhance mature skin beauty thanks to its remarkable properties, it moisturizes, nourishes and smoothes the skin while acting on cutaneous density and elasticity
 Outstandingly, the wrinkle visibility is reduced by 26,5% in 2 months.



Art of Nature

study results demonstrate that this natural technology firms, tones and rejuvenates aged skin on the neck.

Figure 5 shows digital pictures of a subject (age 58) at baseline (Day 0) and after 28 days of treatment with Hydrolyzed Eragrostis Tef Seed Extract. At baseline, neck skin looks saggy, fatigued and with pronounced lines and wrinkles. As is evident from Figure 5, neck area after the treatment with this technology is noticeably firmer, lifted and toned, while skin texture is smoother, revitalized and neck lines are visibly reduced.

In vivo efficacy clinical study results: nasolabial folds

The percent change in the average roughness, wrinkle depth and fold volumes on the nasolabial folds in the skin sites treated with Hydrolyzed Eragrostis Tef Seed Extract and placebo are shown in Figure 6. After 28 days of treatment, Hydrolyzed Eragrostis Tef Seed Extract significantly decreases the average roughness by 7%, wrinkle depth by 8% and volume folds by 9% compared to baseline and performed significantly better than placebo.

Figure 7 illustrates pictures of a subject (age 66) with pronounced nasolabial folds at baseline (left picture). After 28 days of treatment with Hydrolyzed Eragrostis Tef Seed Extract, nasolabial folds are visibly reduced by 16% for this subject, wrinkles are diminished, while skin looks smoother and rejuvenated (right picture).

Conclusion

NaturePep® Teff technology provides a multi-targeted biological activity with clinically proven multiple skin benefits. The unique composition of NaturePep® Teff natural peptides increases the production of dermal collagens and key dermal-epidermal junction components to strengthen skin structural foundation, firmness, elasticity and rejuvenate the appearance of aged, fatigued, and fragile skin on the neck and nasolabial folds. In vivo clinical efficacy studies demonstrate that after only 28 days of treatment, neck skin looks visibly firmer, lifted, toned, and revitalised, while nasolabial folds are noticeably diminished and smoother. This highly effective Teff natural peptide anti-ageing technology is quite versatile and can be used across various

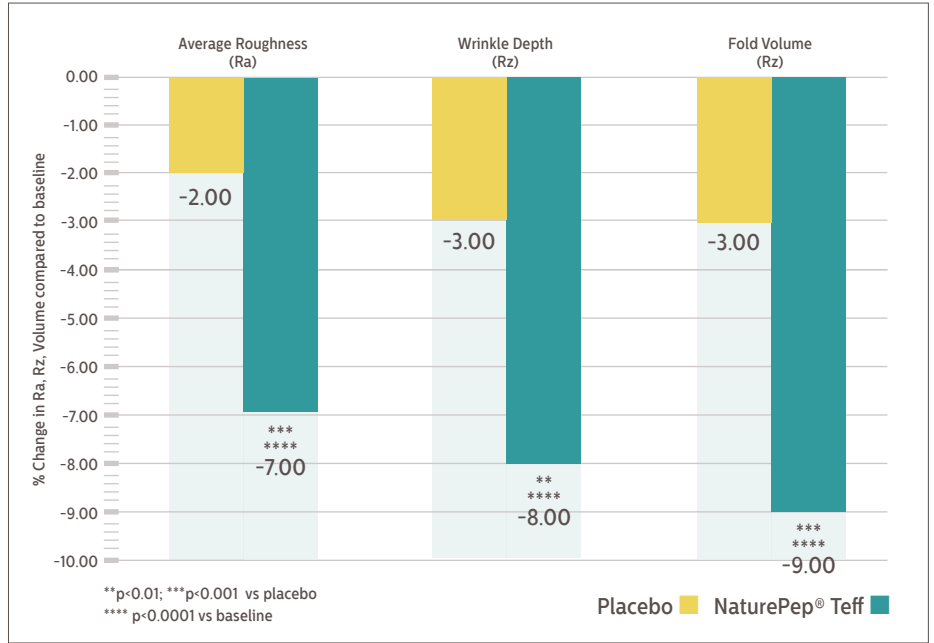


Figure 6: Percent change in average roughness, wrinkle depth and fold volume after 28 days of treatment with NaturePep® Teff and placebo compared to baseline (**p<0.01; ***p<0.01 vs placebo; ****p<0.0001 versus baseline).

formulations types and is suitable for face, neck, and décolletage cosmetic categories. **PC**

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Figure 7: Left picture illustrates pronounced nasolabial folds at baseline (Day 0). Right picture illustrates the benefits of NaturePep® Teff, including reduction of nasolabial folds and smoother skin (Day 28).