EASY-AGEING

A new solution from the marine world!

This active is born from a surprising discovery. A marine micro-organism lives in symbiosis with a red algae. It takes action in the digestion of a polysaccharide of the algae by secreting an enzyme called carrageenase. When the algae is attacked, the micro-organism synthesizes an important quantity of enzymes in order to release oligosaccharides, able to activate the repairing and regeneration of the algae.

An enzyme from marine origin is produced and purified via the cultivation of the marine micro-organism. The polysaccharides from the red algae harvested in their natural environment are then digested by the marine enzyme to obtain an oligo-carrageenane with high biological activity.
The enzyme enables to break the osidic bonds without damaging the monomers. The enzyme is obtained by extraction from the marine bacteria Pseudoalteromonas carrageenovora.

In the case of the red algae as Gigartina Stellata, the infection by a pathogenic agent as the P.carrageenovora takes the form of a degradation or a hydrolysis of the parietal lambda-carrageenane by the bacterial enzyme lambda carrageenase brought by the bacteria. The hydrolysis of the parietal carrageenane of the red algae generates oligosaccharidic fractions presenting different levels of polymerisation, some of which take action as mediators of transduction signals during a bacterial infection.

The « Easy-Ageing » concept: The beauty of being old

While most of the anti-ageing products promise to fight the ageing signs and to reverse time, the customers express their wish to be in adequacy with their age. As they do not feel old, they do not look for solutions to stay young, but want to keep the best possible look possible, which means to be in better health. They wish to see evolving the speech of brands towards a global and positive vision of the ageing process.
The concept of « Easy-Ageing » upsets the dictates established since decades and modifies the ideal of beauty. The ideal skincare product has to offer the possibility to obtain a perfect skin, full of vitality without artifices.
The « healthy-looking skin » is closely linked to the main function of our skin, the physical barrier function between our body and the outside world. The epidermis is responsible for

maintaining this function. The loss of efficacy of the cell renewal and the decrease of the immune competence are essential factors explaining that an ageing skin appears to be less healthy. They are the essential targets to furnish to the modern consumers the best anti-ageing products.

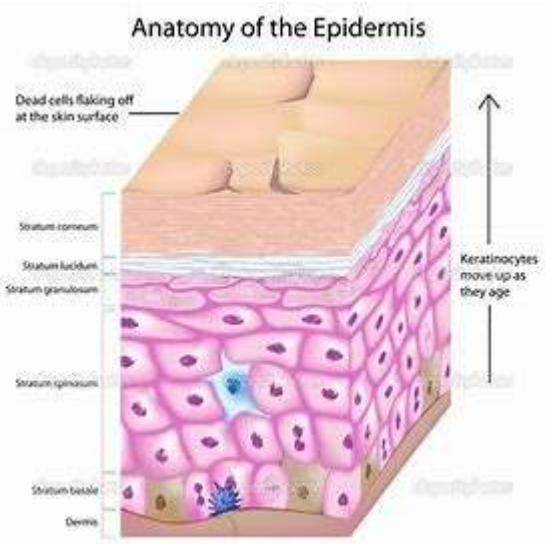
Epidermis physiology:

The epidermis, superficial layer of the skin in direct contact with the external environment, is composed of keratinocytes layers and enables to maintain the physical barrier and an effective permeability.

The efficacy of differentiation's phenomena is reduced by the drop of the synthesis of epidermal elements involved in the differentiation of the keratinocytes. The rate of epidermis cell renewal reduces with time and finishes by reaching a value near 50% on a subject of 80 years old. During the chronological ageing process and/or photo-induced, the epidermis suffers of numerous damages, reflects of functional and structural alterations and degradations.

At the cellular level, numerous metabolic functions are affected. The epidermis becomes thinner with the ageing process and the keratinocytes lose their capacity of differentiation.

Furthermore, the protection capability of the skin is affected by a drop of the immune competence of the epidermis. Because of these deficiencies, it becomes difficult to keep an optimal cutaneous barrier function. The skin is rapidly desiccated and appears less healthy.



To keep a healthy and functional skin, it is essential to maintain the structure of the epidermis. This structure prevents the too important loss of water and plays a role of protection, this means a barrier function. The alteration of the epidermal barrier has for consequence a premature ageing and an increased sensitivity to external elements.

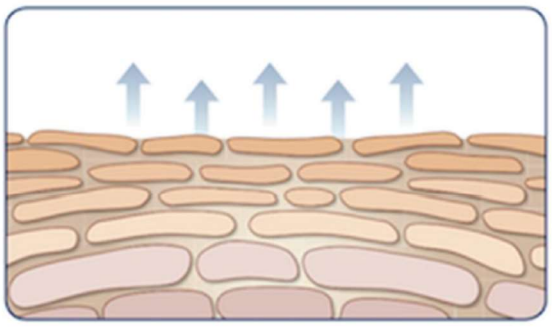
All these changes of the epidermis weaken the skin as a whole which becomes less firm, rougher, and fragile: the skin has difficulties to maintain a good level of hydration (perturbation of the barrier function).
The skin can also lose its radiance, can present alterations of its microrelief, wrinkles and fine lines can appear, giving a sense of visible fatigue and modifying the perception of the apparent age.

The cutaneous microrelief corresponds to irregularities on the skin surface. This microrelief reflects the quality of the skin texture. The microrelief is notable by a certain number of increasing depressions. Those depressions are formed by the association of furrows and folds, as well as protrusions of corneocytes.
Specific to each corporal zone, the microrelief changes over the years. An aggravation of fine lines and wrinkles is most of the time visible, while the skin can also present a rough aspect at the palpation.

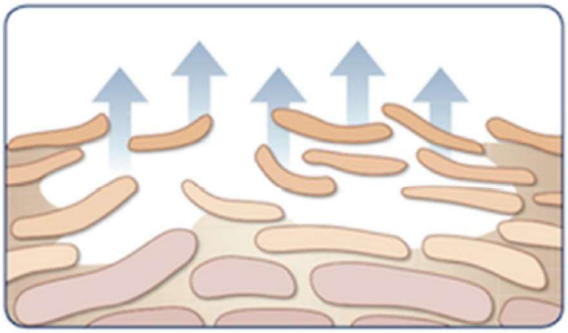
1- EASY-AGEING enhances the cutaneous barrier function

1.1- Maintaining the barrier function is the base for a healthy skin.

The skin is the primary defence. One of the essential elements to keep a healthy and functional skin, is to insure an epidermis structure in good conditions.
The skin prevents the too important loss of water and plays a role of protection. The setting and the maintaining of barrier function are the base of a healthy skin. A sensitive skin, aged or damaged, presents an impaired epidermal barrier.



Healthy skin, showing normal moisture loss



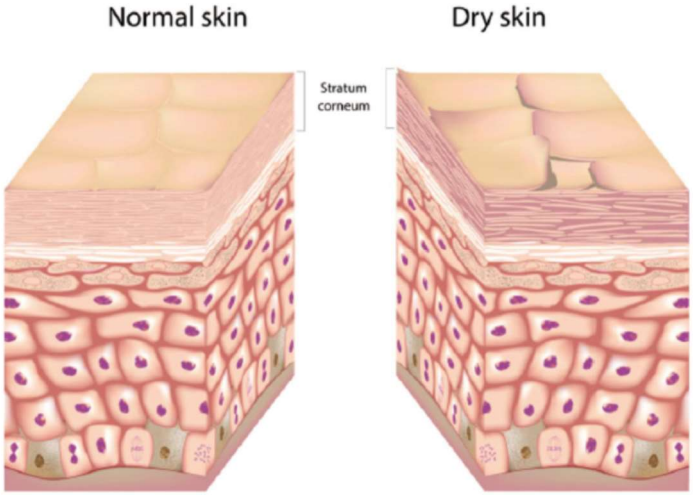
Dry skin, showing increased moisture loss

1.2- IN VITRO TESTS – EASY-AGEING reinforces the stratum corneum (study of loricrin and corneodesmosin markers)

The primary function of the epidermis is to produce the stratum corneum, which forms a protective and semi-permeable layer. The stratum corneum is the most external layer of the skin, and so the most visible. Its function in the aestheticism of the person is primordial. A defect of cohesion between the cells, consequence of an excessive water loss, will conduct to a roughness of the skin, a loss of elasticity, of flexibility, of pulling sensation, a whitish skin, flaked, cracked and so unsightly.

The stratum corneum is considered as brick walls inside of which the differentiated keratinocytes, mainly made of proteins are caught in a cement of specialized lipids and loricrin, a protein which replaces the keratin in the last step of maturation of the epidermis to form a functional barrier. It is a highly insoluble protein, whose the role is mainly to maintain the integrity of the skin by isolating it from the external environment.

The cohesion of the stratum corneum is insured by the specialised structure binding the corneocytes between them. Related to the desmosomes, they are the corneodesmosomes. Yet, the corneodesmosine is a protein synthetized by the keratinocytes which is the only protein identified today as specific to the corneodesmosomes. The excretion of the corneodesmosine by the keratinocytes is so, the best marker of the strength of the corneodesmosomes and so, of the link of reinforcement of the stratum corneum.



Protocol:
In this study, the effects of Easy-Ageing have been researched in a model of normal human epidermal keratinocytes (NHEK), by evaluating by RT-qPCR, their activity on the expression (RNAm) of markers selected for their importance in the reinforcement of the stratum corneum.

The keratinocytes have been sown in plaques of 24 shafts and cultivated in cultured-base tests during 24 hours. The culture medium has after been replaced by an assay medium containing or not Easy-Ageing or the reference (CaCl2) and the cells have been incubated during 24h.
At the end of the test, the RNA cellular matching to each condition has been extracted and purified within sight a reaction of RT-PCR on support multishafts to enable an analysis of involved genes into the reinforcement of the stratum corneum.

Results:
The results have shown that the treatment of cells by Easy-Ageing induces a stimulation of the gene expression of certain proteins involved into the reinforcement of the stratum corneum, notably the loricrin +519% and corneodesmosin + 214%.

Easy-Ageing increases the synthesis of the proteins involved into the reinforcement of the stratum corneum.

1.3- IN VITRO TESTS – EASY-AGEING decreases the inflammation and reinforces the innate immune (study on IL8 and defensin beta 4A markers).

The epidermis actively handles the composition of the cutaneous microflora. To do this, it prevents the colonisation of pathogenic bacteria. The differentiated keratinocytes produce antimicrobial peptides which contribute to the antimicrobial defences of the skin.
The production of these peptides is considered as a component of the healthy epidermic differentiation, as the regulation of the production of components of the stratum corneum. It has clearly shown a link between a decrease of the antimicrobial peptides and a disruption of the cutaneous barrier.

The ageing skins have more difficulties to maintain a synthesis of antimicrobial peptides on the skin surface. And so, have more difficulties to maintain a healthy skin and a healthy microbiota on the skin surface. It has been shown that ageing skins produce less peptides. At the opposite, a phenomenon of cutaneous repair is link with the rise of the production of these peptides.
The defensin beta 4A is produced and released by the differentiated keratinocytes and the corneocytes and have strong antimicrobial effects against negative gram bacterias (E. coli, Pseudomonas aeruginosa), as well as on yeasts (e.g. Candida albicans). Aside from its antimicrobials functions, this peptide is an essential component of the differentiation of the keratinocytes. It plays for example, a core role into the formation of junctions in the stratum granulosum or in the production of keratin 10.

Protocol:
The same tests of genes expression than the ones previously described, have enable to demonstrate that the treatment of cells by Easy-Ageing induces the stimulation of the gene expression of a protein involved in the reinforcement of the skin's defences, the defensin beta 4A (protein which destructures the exogeneous bacterias).

Results:
INNATE IMMUNITY : +2255% Defensin beta 4A

INFLAMMATION : 169% inhibition Interleukine 8
769% inhibition S100 calcium binding protein A7

1.4- Clinical evaluation : EASY-AGEING enhances the hydration and the cutaneous barrier function

A good cutaneous hydration is indispensable to confer flexibility, elasticity and a certain impermeability to the skin. The epidermis contains 70% of water at the level of the basal layer. The superficial layer of the stratum corneum contains only 10% of water. This gradient of hydration enables to the free water of the dermis to transmit from deeper layers to the most superficial ones. This phenomenon bears the name of TransEpidermal Water Loss (TEWL). The relatively low TEWL reflects the efficacy of the barrier function. The amount of water decreases lifelong, the stratum corneum only containing 7% of water in an older person.

Protocol:
The objective of the study was to evaluate the effect of Easy-Ageing on the hydration of the stratum corneum, the most superficial layer, showing the efficacy of the barrier function. The process of desquamation and of cell renewal are intimately conditioned by the good hydration of the stratum corneum.
A clinical study in double-blind against placebo has been conducted on 20 women, between 35 and 65years old, for 28 days. Each subject has applied twice a day on hemi-face a

formulation at 1% of Easy-Ageing or its placebo. The placebo base was an oil/water emulsion. The measurement of the hydration has been done at the beginning, then after 28 days by corneometry. This measurement aims to assess the hydration of the most superficial layer and so to indirectly assess the efficacy of the cutaneous barrier function; a better hydrated skin being the consequence of an effective barrier function. The measurement of the hydration of the superficial layers of the skin has been done by corneometry, method based on the existing link between the electrical properties of the tissues and their content in water. The corneometry enables to calculate the hydration index of the skin expressed in arbitrary unit.

Results
The values indicate an average increase of 16.5% in the active group when it is of 7.7% in the placebo group. The statistical analysis of the active group compared with the placebo group enables to conclude to a significant enhancement ($p<0.001\%$).

The skin is so significantly more hydrated, the cutaneous barrier function is restored.

2- EASY-AGEING acts on all the levels of the cell renewal of the epidermis

2.1- From the differentiation of the keratinocytes to the epidermis renewal

The epidermis, superficial layer of the skin in direct contact with the external environment, is for its major part composed of layers and enables to maintain the physical barrier and an effective permeability.
Differentiation, cornification and desquamation are the three steps that enable to renew and preserve the physiological mechanisms of a healthy skin. The rate of proliferation of keratinocytes in the basal membrane tail off. It is 50% weaker on a subject of 80 years old compared with a young subject. The efficacy of the differentiation process is reduced by the production drop of the cutaneous barrier's components as the keratin. Besides, the health of the skin is affected by the drop of the immune responsiveness of the epidermis. Because of these deficiencies, it becomes difficult to keep a healthy cutaneous barrier. The skin becomes rapidly dry and appears less healthy. Relaunching the renewal system of the epidermis is essential. (Proksh, 2008)

2.2- IN VITRO : EASY-AGEING boosts the main steps of the epidermis renewal (study of the keratin 10, Transglutaminase 1 and caspase 14 markers).

Marker of the keratinocytes differentiation
The keratinocytes coming from the stem cells of the basal membrane, synthetize the different keratins and migrate towards the surface in a process called keratinisation. Keratin is the predominant protein.
70 to 80 % of the epidermis mass is composed of keratin. They join to form a network of filaments which extend from the cytoplasm to the desmosomes.

Marker of the cornification
The transglutaminases (TGM) are dependent calcium enzymes involved in the formation of covalent bonds between proteins. In the skin, the transglutaminases are found in the most superficial layer of the epidermis where their activity is necessary for the binding of cornified envelop. The cornified envelop is an essential structure in the barrier function. In the race of the final differentiation of the keratinocytes, the surexposure of the transglutaminases contributes to the assemblage of the stratum corneum, link between them the constitutive proteins as involucrine, filagrine and loricrin.
The bridges formed thanks to the TGM enable a high resistance to the proteolytic degradations

inducing a good resistance at the skin surface. The increase of the transglutaminases expression is going to promote the maturation of the stratum corneum and optimise its structure for a better cutaneous texture. (Richard L, 2005)

Marker of the desquamation

Proteolytic enzymes, notably the caspase 14, are implied in the desquamation of the skin. They degrade the structures of intercellular cohesion which are called corneodesmosomes in the stratum corneum. This mechanism enables to eliminate the old cells on the surface is a prerequisite for a continuous renewal of the epidermis. The desquamation is linked to the hydration of the enzymes which need water for their activity. A decrease of the expression of the caspase 14 is associated with an increase of the transepidermal water loss (TEWL), indicating that the caspase 14 is vital for the quality of the cutaneous barrier function.
The caspase 14 is essential to maintain a healthy epidermic differentiation, the cornification and the formation of the stratum corneum. In surface, the proteolytic enzymes degrade the filagrine in free amino acids which form the natural skin hydration. It is an essential mechanism to maintain the water balance at the stratum corneum level. An increase of the caspase 14 activity will contribute also to increase the skin hydration. (Hoste E, 2013)

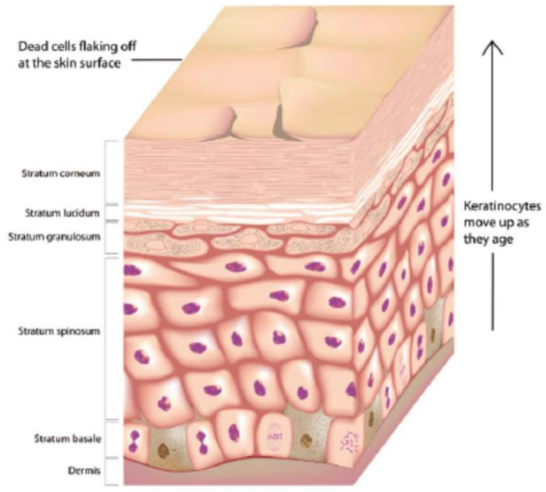
EASY-AGEING increases the Caspase 14 synthesis, enzyme of the desquamation. It enables to protect against the hyperkeratose and to bring suppleness for a better skin texture.

Protocol

The same tests of gene expression that the ones previously described have enable to demonstrate that the treatment of cells by Healthyskin induces a stimulation of the gene expression.

Results

The results have shown that the treatment of cells by Easy-Ageing induces a stimulation of the gene expression of some proteins involved in the keratinocytes differentiation, notably the transglutaminase 1 (enzyme enabling the molecular association at the level of the cornified envelop) +982%, the keratin 10 (predominante protein of the epidermis) +1801%, the caspase 14 + 3366 %.



DESQUAMATION: +3366% Caspase 14
DIFFERENCIATION: +982% Transglutaminase 1
+1801% Keratin 10

2.3- Used at 1%, EASY-AGEING enhances the cell renewal of the epidermis

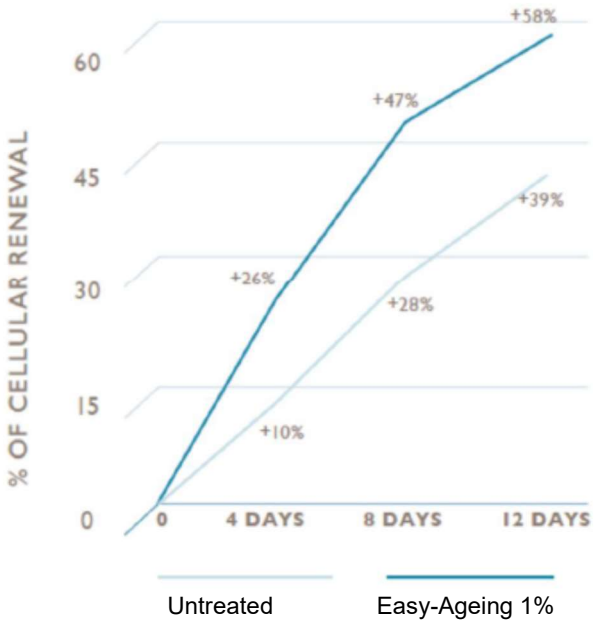
A clinical study has been conducted on 20 women from 35 to 65 years old, for 28 days. Each subject has applied twice daily on the forearms a formulation containing Easy-Ageing.

A solution at 10% of dihydroxyacetone (DHA) has been applied on the forearms during 4 hours under occlusive with the aim to tint the skin. A total of 2 patches tests have been applied on each subject (control zone, active product zone). The coloration of the skin is measured by spectrophotometry by using a spectrophotometer CM-700D (Konica Minolta). The obtained value corresponds to a ITA parameter. An increase of the ITA value corresponds to a skin clarification. The principle of the measure is that more the stratum corneum renewal is important, faster the skin coloration disappears and so faster the ITA value increases. The measurement has been done at T0, then 4, 8 and 12 days.

The results expressed in ITA index and the associated “p” index are summarized below in the 2a and 2b spreadsheets:

	T0	T4	T8	T12
Reference	24.2	26.6	31.0	33.7
Easy-Ageing	23.6	29.8	34.8	37.3

Easy-Ageing improves skin renewal

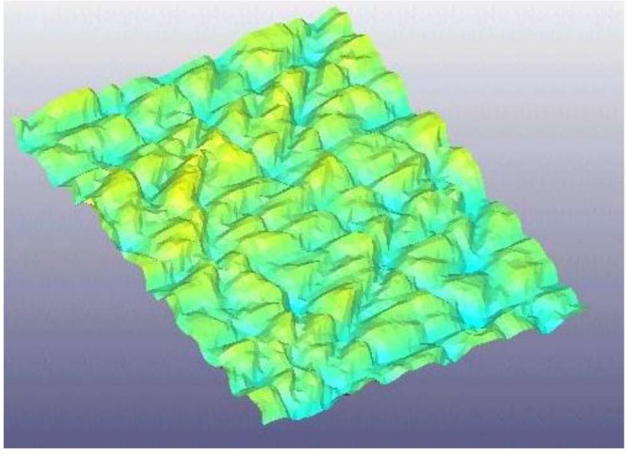


The rate of cell renewal doubles in 4 days only.

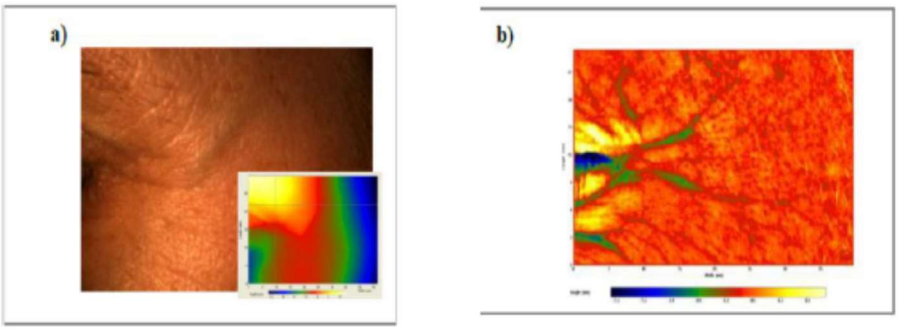
3- EASY-AGEING improves the cutaneous texture

3.1- Microrelief and cutaneous structure

At its surface, the skin presents irregularities defined as the cutaneous microrelief.
The microrelief reflects the quality of the cutaneous texture. It is characterized by a certain number of depressions that intersect. These depressions are formed by the association of furrows and of folds and also by the projection of the corneocytes. Specific to each corporal zones, the microrelief changes withyears. So, the formed cutaneous lines are oriented in all the directions on a young subject (isotropic state) and aline when the subject becomes old (anisotropic state).
Tension lines can be observed on a dehydrated or aged skin while polygons are better defined on a hydrated and young skin, reflecting an improvement of the cutaneous microrelief.



3.2- Used at 1%, Easy-Ageing improves the cutaneous microrelief



A clinical study in double-blind against placebo has been conducted on 20 women between 35 and 65 years old during 28 days. Each subject has applied twice a day on a hemi-face Easy-Ageing or its placebo. The placebo base was oil/water emulsion without active.

The measure of the microrelief has been done at the beginning, then after 28 days by a profilometric assessment. The roughness of the skin on the crow's-feet wrinkles area has been measured by a microtopography 3D system (Primos GFMesstechnikTM Gmbh). A 3D image reproduces the skin's surface.
The measured parameter is named « Sa » and represents an arithmetic value of the skin's roughness. A decrease of this value is correlated to an improvement of the skin's smoothing.

The results show a significant decrease of the « Sa » parameter in the active group compared to the placebo group ($p < 0.05$). In the active group, the decrease of the « Sa » value has been around 6.3% against 1.3% in the placebo group.

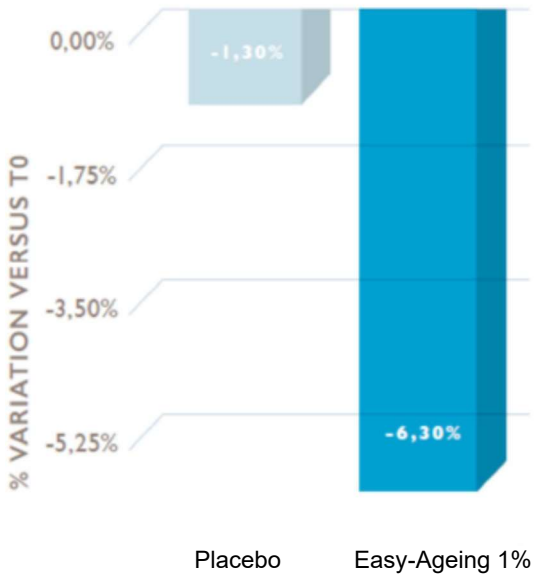
The measured and shown values are detailed as follows:

Crow's feet wrinkles		Sa, T28 (%)
Face cream incorporating Healthyskin composition	Measurement of the variation in % according to à T0 (vs To)	- 6,3
	Maximum variation	- 42,3
	Minimum variation	+ 13
	t-test vs. To	0,018
	t-test vs. placebo	0,136
Face cream - placebo	Measurement of the variation in % according to T0 (vs To)	- 1,3
	Maximum variation	- 10,4
	Minimum variation	+ 10,3
	t-test vs. To	0,401

Thanks to Easy-Ageing, the skin is softer, less rough and the microrelief is smoothed.

Easy-Ageing significantly improves the cutaneous microrelief compared to the placebo. The cutaneous microrelief is the result of the structure, the texture and the hydration.
The enhancement of this parameter is to be interpreted as a better global health of the skin. The improvement of the microrelief is associated to a visibly younger skin.

Easy-Ageing decreases skin microrelief



Applications

- Healthy ageing lines,
- Skin perfectors,
- Creams and lotions for dry skin,
- Anti-Ageing creams and lotions,
- Dermocosmetic products,
- Skin renewal serum.

Recommended usage level: 1%

INCI name : Glycerin & Aqua & Gigartina stellata extract

REFERENCES AND NOTES

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