

# Algal Active Substances

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For the past few years, Blue Cosmetics, or ingredients from the sea, have been moving from myth to scientific reality. This marine vegetal, otherwise known as seaweed, was mainly used in marketing efforts up until the 90s. Since then, its activity has evolved toward true effectiveness. Seaweed has since then left its micromarket and has taken part, thanks to the performance of its few molecules, in the raw materials used by the international cosmetics industry. On the whole, algal substances have converged into becoming elements in true personal care cosmetics.

## Aosa Biopeptides

The aosa seaweed, or *Ulva lactuca*, lives in the rough waters of the bays facing the North-east side of the Bréhat Archipelago in France. The Aosa frond is made up with only two layers of cells where the plastid forms the photoenergetic centre of the seaweed. The elastic resistance of the cellular walls to the hydrostatic pressure is generated by the progression of water toward the shore or by the assault of waves which break on the rocks and drag along the Aosa. This resistance is due to the protein network of elastic fibres. The weight of water which hits the rocks can sometimes create pressures superior to 25 T/m<sup>2</sup>.

Aosa biopeptides<sup>2</sup> are the main protein component of the elastic fibres of the seaweed, particularly rich in proline, glycine and lysine like the elastin. Because of this likeness, Aosa biopeptides can play an important psychological part by maintaining the elasticity of the skin and by acting as an anti-wrinkle agent.

In the skin, the fibroblast is a differentiated mesenchymal cell, which is largely responsible for synthesis and remodeling of extracellular matrix laid down in the dermis. Elastin, the principal protein constituent of elastic fibres, is produced by human skin fibroblasts. Although elastin comprises only about 2% of the total protein in dermis, it is physiologically important in providing resiliency in the skin. During cutaneous aging, the disappearing of the elastic

network is partly due to the intensification of the elastolyse thanks to the action of the elastase. This elastolyse is also increased by the use of some detergents, solar exposure, just like the cutaneous flora secretes particularly active bacterial elastases.

The predominant extracellular component of the dermis is the collagen. Collagen types I and III are the major interstitial, fibre-forming collagens in normal human dermis. Collagen I represents 80% of total dermis collagen of an adult's skin, while collagen III accounts for 15%. (The remaining 5% mainly correspond to type IV and type V collagens.)

Thick fibres of type I collagen are blended with a fine felting of type III collagen, which orientates big fibres during their growth. More tensile and less fibrous, the latter is predominant in fetal and postnatal skins and during the wound healing process. Thus, type III collagen has been called a "restructuring" collagen, particular to very young skin and in the process of wound healing. Collagen I is formed later and becomes predominant. Among the numerous modifications of the extra cellular matrix (E.C.M.) during aging, collagen synthesis shows a great decrease during the aging process. Moreover, the ratio of collagen types changes throughout life. Some studies demonstrated that the content of collagen III is higher in fetal skin and in newborn infant skin than in adult skin. They found a decrease of the ratio collagen

### Key words

Sea ingredients, Aosa Seaweed, *Ulva lactuca* extract, *Codium Tomentosum* Extract, *Gelidium* Extract, *Enteromorpha* Extract

### Abstract

In recent years, ingredients from the sea have shown to be effective in cosmetic applications. In this article, the author explains how some of these ingredients can be used successfully in personal care formulations.

<sup>2</sup>Aosaine is a registered trademark of SECMA Corporation, Pontrieux, France

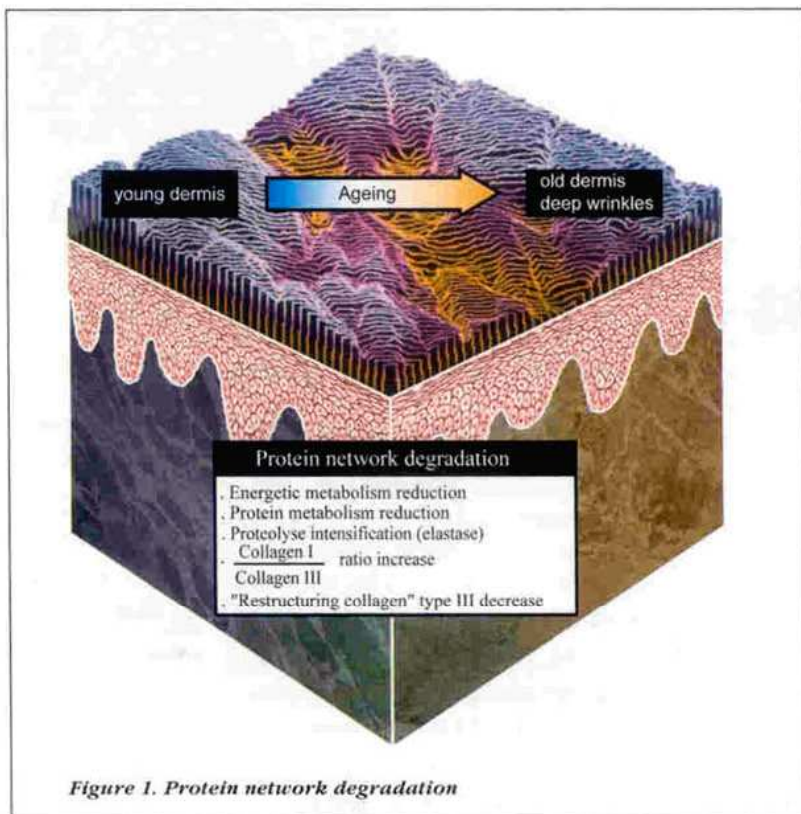


Figure 1. Protein network degradation

III/collagen I during aging, underlining the loss of capacity of old cells for producing collagen III.

At this level, the action of these Aosa biopeptides are unique because of their numerous properties: at a cellular level, these marine biopeptides activate the energetic metabolism as well as the fibroblasts regeneration capacity. At the dermis level, one can observe a selective amplification of the collagen III biosynthesis parallel to protein fibres protection next to the proteases. This is followed by an in vivo reduction of deep wrinkles.

**Energetic metabolism:** The evaluation of the activity of Aosa biopeptides on the mitochondrial metabolism of human skin fibroblasts was performed by following the oxygen consumption (mitochondrial respiration) and measuring the cellular adenylic nucleotides (ATP, ADP and AMP) content. According to this study, the biopeptides stimulate the cellular respiration by a direct effect on the mitochondria. However, this stimulation of the basal cellular respiration is not due to a direct effect on the test product on the respiratory chain. So, the product acts on the energetic transducing systems (ATP synthesis). The increase in cellular ATP

level and the increase in ATP/ADP ratio confirm the stimulatory effect of the Aosa biopeptide on the ATP synthase.

**Cellular regeneration:** The cell regeneration in-vitro test has been made on human fibroblasts by tracing <sup>3</sup>H-thymidine in DNA and <sup>14</sup>C-uridine in RNA. Several concentrations of the Aosa biopeptide have been tested and compared to a standard containing 5 to 10% of calf fetal serum which maintains the survival of the culture. A concentration of 0.4% Aosa biopeptides produces an increase of cells -74% superior to cells of the standard in DNA, and 89% superior to cells in RNA.

**Collagen neosynthesis:** The effect of Aosa biopeptides on the collagen stimulation was performed on human fibroblasts. This study was conducted by determining the incorporation of hydroxyproline, an amino acid precursor of collagen. Hydroxyproline is necessary for collagen helix formation and, in its absence, collagen is improperly secreted from fibroblasts. The ascorbic acid, an essential cofactor in the hydroxylation of proline in hydroxyproline, was used as positive control.

A strong stimulation (+57%) of the collagen neosynthesis was observed in the presence of the biopeptide compared with vitamin C.

**Kinetics of protein synthesis:** Another study has been made on a culture of human fibroblast to follow the kinetics of protein synthesis. This kinetic was realized at 4, 6 and 24 hours by incorporation of a tracing substance. A concentration of 0.4% biopeptide gives a 35% improvement of the speed of protein synthesis after 24 hours.

**Restructuring collagen, type III:** The effect of Aosa biopeptides on the collagen type I/III ratio has been determined on human fibroblasts. The study was conducted by determining the incorporation of <sup>3</sup>H proline, an amino acid precursor of collagen, in the macromolecular fractions extracted from the cell layer. Qualitative study of collagens was evaluated after electrophoresis. The radioactivity was then determined by liquid scintillation counting. The results show that the type I/III collagen ratio is close to 3 in the control cell layer, implying a deposit of 25% type III collagen in the cell layer with reference to type I collagen. This ratio is very similar to that observed in human dermis in vivo. Aosa biopeptides, at concentrations of 1 and 100 mg/ml, induces a decrease in the type I/III collagen ratio, implying an increase of 35% and 39% type III collagen, respectively.

**Antiwrinkle activity:** The effect of Aosa biopeptides skin cream on skin relief was determined after 28 days of twice-daily application. Siliconed polymer replicas were taken of two crow's feet (wrinkles around the eyes) of volunteers before and after 28 days of twice-daily application. Skin relief was quantified with confocal microscopy and image analysis. Ten healthy female volunteers, between the ages of 40 and 50, were included in the study. No allergic

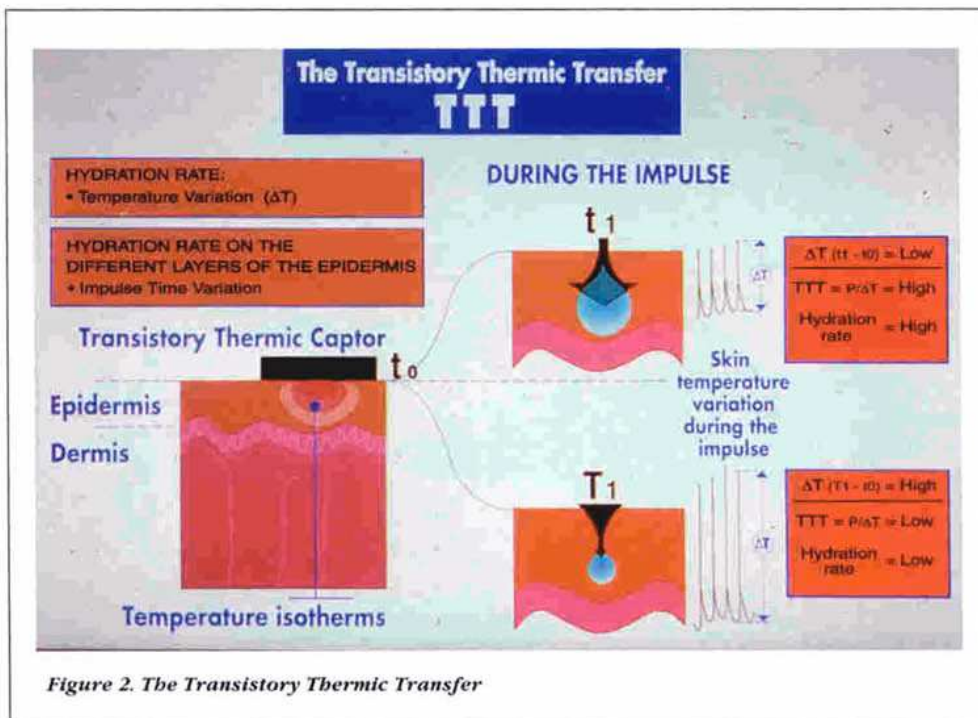


Figure 2. The Transistory Thermic Transfer

or irritation type of intolerance reaction was observed.

Skin relief was studied as 2 micron thick microclasses between 4 and 400 microns. Three relief classes corresponding to the grouping of microclasses were calculated: microrelief, medium wrinkles and deep wrinkles. The density of these three relief classes was measured before and after utilization of the product. The product reduces deep wrinkles (-23%).

### **Codium Tomentosum Extract**

*Codium tomentosum* extract<sup>b</sup> is adapted to both low and high salt concentrations by two different mechanisms. Its adaptation to salinity is based on biosynthesis of an anionic polysaccharide contained in the cell walls of the alga and different organic substances with a low molecular weight inside the cell, compatible with the protein structures and membrane systems of the cytoplasm.

A water-soluble sulfate heteropolysaccharide of sulfated arabinane type is isolated in *Codium tomentosum*.<sup>4</sup> The polymer is located outside the cell membranes forming the matrix of the cell walls. It plays a special physiological role in the alga,

<sup>b</sup>Codiavelane is a registered trademark of SECMA Corporation, Pontrieux, France

both mechanically and with regard to moisturizing and ionic regulation. These phyco colloids are strongly sulfated to preserve the metabolic and ionic equilibrium regardless of the variations of the environmental conditions.

*Codium tomentosum* extract also stores a quantity of osmotic molecules in its cells to maintain the alga at a lower pH than its environment. This is the only way the alga maintains a hydrogen activity compatible with water absorption and supply.

The cells, thus, achieve a state of equilibrium by autoregulation, whereby the osmotic effects of the marine microecosystem of the tide pools are compensated for by both the barrier and mucilage

exchange effects and the osmotic effects of the organic substances in the cytoplasm.

These water-soluble fractions are collected from the fresh algae by water-glycol extraction (50% water/50% propylene glycol). The algae are harvested by hand on the foreshore at low tide by a team of special gatherers.

*Codium* corresponds to this hydroglycolic agent with a 1.2% dry extract for a sulfur content of 1.1 g/l, i.e. the equivalent of 3.3 g/l of  $\text{SO}_4$ . This sulfate content is characteristic of sulfated polymer that represents the most water-soluble polysaccharide fraction of the extract.

### **Moisturizing activity measured by corneometer:**

An experiment was conducted using a corneometer to measure the dielectric constant of skin moisture. The skin moisture level was evaluated on a group of 10 women. The corneometer lead was placed on the forearm on an area of approximately  $4\text{cm}^2$ .

The measurements were made on treated skin (reference area). The treated areas received a dose of  $4\text{mg}/\text{cm}^2$  of five commercially available moisturizing creams. Then 5% *Codium tomentosum* extract was added to the same creams. The hydrating power of these compositions was measured over time at 0 minute, 3 minutes, 15 minutes, 30 minutes, 1 hour, 2, 4 and 6 hours.

The off-the-shelf moisturizing creams, with the exception of one, caused an immediate but short-lived increase in the moisture level. The average moisture level for the five creams was higher than 28 percent just after application but had dropped to 10% after only 3 minutes.

The addition of the extract to these creams reinforced their hydrating power. This effect lasted over time. Six hours after application, the moisture level remained high (an average of +15% compared to the control area), i.e. three times higher than moisturizing creams without the *Codium* extract.

**Moisturizing activity measured by transitory thermal transfer:**

Evaluation of the moisture level by transitory thermal transfer (TTT) is based on the capability of the skin to exchange heat when in contact with another substance. The moisture level expressed as TTT is measured by a thermal device including a heating element generating a thermal wave and a temperature probe. The thermal wave generated is transitory and pulsed. The thermal pulses with a constant power and duration and is followed by a quiescent period, also with a constant duration. This pulse/quiescence cycle is the transitory characteristic of the measurement. The thermal wave propagates through the skin.

The skin temperature variation is measured during the pulse, and the device records this temperature variation as a signal. The TTT is then defined as the ratio of the power (p) of the thermal pulse applied by device to the temperature variation of the skin during the pulse:  $TTT = P/DT$ . The values are in  $mw/^\circ C$ . The higher the skin moisture level, the smaller the temperature variations due to the thermal pulse, because of heat transfer with the water. The TTT is, thus, inversely proportional to the temperature variation and directly proportional to the moisture level. The pulse duration can be adjusted to explore the superficial, mid-layer and deep moisture levels of the epidermis successively.

The effect of a product on the skin moisture level is expressed as the percentage of variation of the TTT measurement. This percentage is equivalent to the variation in moisture level observed between time after application of the product and time before application. The experiment was conducted with a microeffusivimeter with measurement of the moisture levels in the three layers of the epidermis (superficial, mid-layer, deep layer) by TTT. The products were applied twice a day for seven days.

The hydrating power was determined as follows:

- Measurement on day 0: just

before the first application and 1 hour and 3 hours after.

- Measurement on day 8: just before application (12 hours after the last application on day 7) and one hour after.

The use of 5% *Codium tomentosum* extract in a cream has various effects on the skin moisture level:

- Immediate hydrating action: The *Codium* extract doubles the skin moisture level on the first application ( $t = 1$  hour).
- Lasting hydrating action: The cream with the extract maintains the moisture level in the skin for more than three hours, whereas the skin moisture level drops by 25 percent for the placebo cream. After the 8-day application period, the moisture level in the skin remains more than 20 percent higher than the skin treated with the placebo cream.
- Moisturizing of all the layers of the epidermis: Application of the placebo cream is characterized by a systematically higher water loss in the superficial layers of the skin. By contrast, at the outcome of the treatment with the extract, regulation and control of the moisture level through the entire stratum corneum was observed. The measurement made on day 8 following application shows that *Codium* increases the action of the placebo cream by 50 percent.

### **Gelidium Extract**

In the world of the alga, there exist mechanisms for the storage and release of lipids. In order to meet the energy needs of the algal cell, lipids are transformed to energy and the excess is stored. This reserve is used by the alga in periods of stress, exposure to cold and weak sunshine (reduced photosynthesis). Algal agents that can transmit messages to instigate lipolysis are sterols or derivatives and are particularly active in the rhodophyta, or red algae. Algal sterol<sup>5</sup> is obtained from a red alga of the species *Gelidium* sp, where it plays an important role in algal metabolism. *Gelidium* extract<sup>c</sup>, which contains 1.5 % of active sterol, can be used in the same way as a lipolytic and firming agent in cosmetic products.

**Lipolytic activity:** Lipolytic activity in cells of adipose tissue is assessed in culture media by measuring the concentration of liberated glycerol. Adipocytes released glycerol after application of *Gelidium*. At concentrations between 2.5 and 5%, it exerts a clear lipolytic activity compared to traditional lipolytic agents such as caffeine and theophylline.

**Slimming effect:** A balm containing 5% *Gelidium* extract was applied to 16 female volunteers. All presented a localized adipose overload. Each subject applied the product twice a day on the thighs for four weeks in the direction of venous circulation (from the bottom to the top of the thighs) during a period of about 3 minutes. The slimming effect was appraised by means of tape measurement and echography.

<sup>c</sup>Rhodysterol is a registered trademark of SECMA Corporation, Pontrieux, France

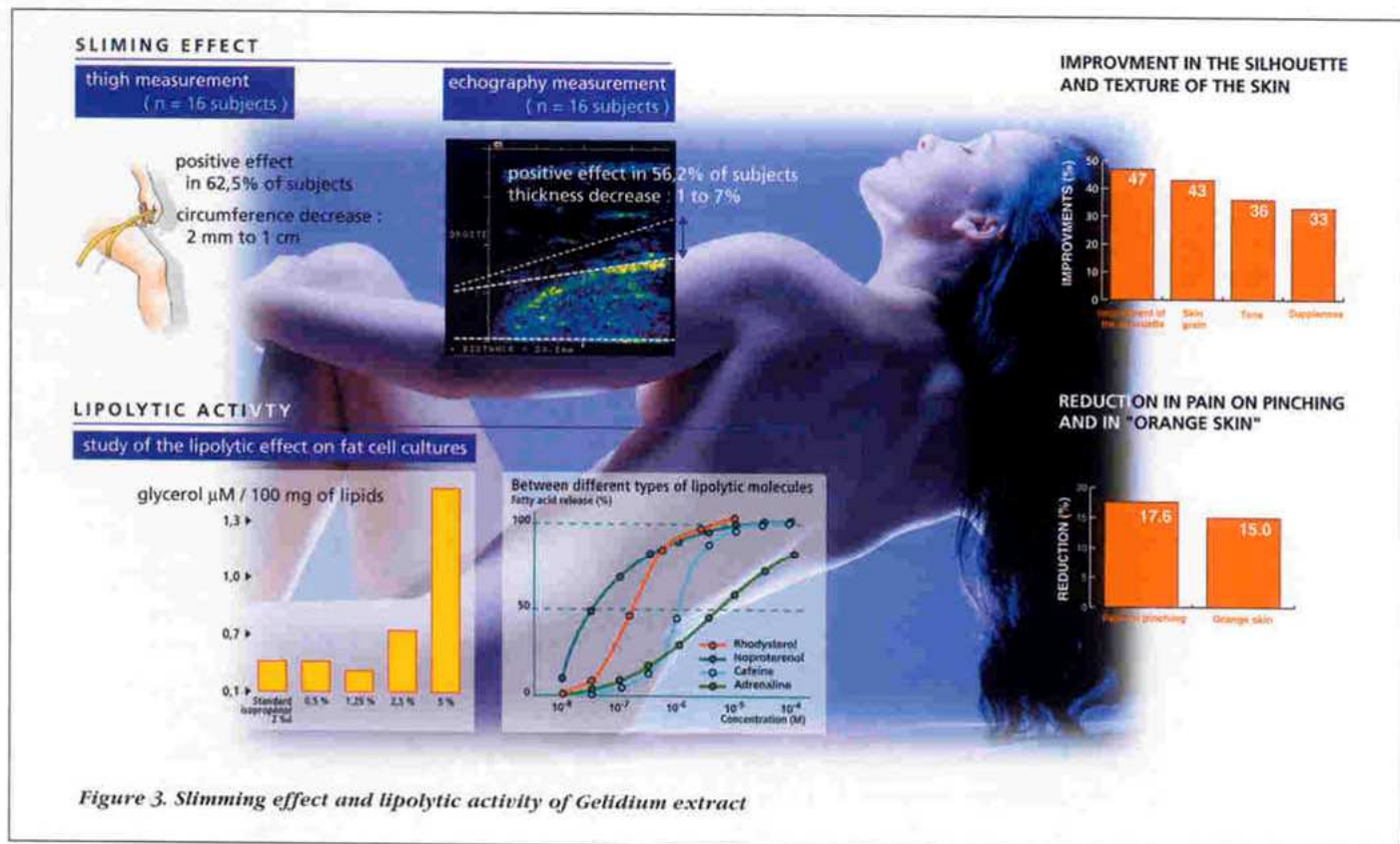


Figure 3. Slimming effect and lipolytic activity of *Gelidium* extract

**Centimetric measurement:** The circumference of the thighs was evaluated before and after local treatment on the subject, who was standing close to a wall. We measured the diameter about mid-way between the knee and the hip and determined the distance from the floor and the wall. To ensure that diameters were measured parallel to the floor and perpendicular to the leg, we placed two levels on the tape measure. Thigh circumference slimming effects were observed following the treatment with *Gelidium* extract. These measurements showed a slight (-3 mm) to clear-cut (-10 mm) decrease in 10 subjects out of 16 (62.5%). Tape measurement of the thighs showed a 2 mm to 1 cm decrease in the thickness of the adipose tissue in 10 of the 16 subjects.

**Echographies:** These were performed on the same days as the tape measurements by means of an echograph. We scanned the thigh area in the regions of interest. Digitally stored scans were analyzed for epidermal, dermal and fat layer thickness. Echography showed between 0.2 to 1.8 mm (i. e., -1% to 7% variation) decrease in the thickness of the adipose tissue in 9 of the 16 of the subjects.

**The signal of lipolysis:** Lipolysis, the breakdown of fat, begins with the degradation of triglycerides into glycerol and free fatty acids. Following triglycerides hydrolysis, the fatty acids are directed to the interior of the mitochondria where oxidation to CO<sub>2</sub> and H<sub>2</sub>O occurs or are eliminated by the lymphatic system. This lipolysis can be mediated through action on the β and α adrenergic receptors, adenylyl cyclase, the intercellular cyclic AMP (adenosine 3', 5' cyclic monophosphate) the phosphodiesterase and the lipase.

In this area, the sterol of *Gelidium* extract acts as a signal to the receptors of the adipocyte by stimulating lipolysis. Fields of applications of "signal molecules," now called

"chemoreception," are very interesting in cosmetology. This enables a substance applied to the skin to act on physiological mechanisms of the underlying layers. It seems to be capable of breaking up the excess fats stored in the cells to allow their removal and of preventing fat from entering the normally balanced cell.

It also appears to stimulate the fibroblasts during its lipolysis action. The connective tissue appears to undergo a reorganization which gives it back its tonicity and elasticity.

### *Enteromorpha Compressa* Extract

The green alga *Enteromorpha compressa* that lives in the upper strand is one of the species that represents the transition between seaweed and land plants. It is indifferent and insensitive to land-based substances borne by fresh water, so its preferred niche is in zones directly influenced by rivers and runoff.

An active fraction of peptides<sup>4</sup> is extracted from the alga *Enteromorpha compressa* and constitutes an original approach to hypersensitivity by its novel

<sup>4</sup> Enteline is a registered trademark of SECMA Corporation, Pontrioux, France

action on neuropeptides. Hypersensitivity is a global phenomenon, widespread around the world. During the past several years, dermatologists have noted a significant increase in the number of people suffering from skin reactions linked to hypersensitivity, which is characterised by a skin with a lower-than-normal tolerance and involves both disorders of the stratum corneum and a disturbance of the microcirculatory system. Two clinical studies on skin with a tendency to atopy or hypersensitivity have shown the efficacy of the active ingredient toward skin sensitivity.

**Desensitisation as target:** The desensitising effect of a body lotion containing 2% *Enteromorpha compressa* on the sensation of pruritus (severe itching) was determined by thermography. Ten volunteers, otherwise healthy but hypersensitive to pruritus and with a propensity to atopy (skin allergy) were selected. Skin was sensitised by thermal aggression using a probe in contact with the internal side of the wrists. The 3 cm diameter probe combined a temperature sensor with 0.1°C accuracy and a heat control system that supplied heat to the skin, also with a 0.1°C accuracy. The physical method, more specifically sensorimetric, enabled the perception of pruritus to be studied and quantified by evaluating variations in sensations it caused. This technique is preferred over less precise visual methods.

Initial stimulation was applied in the absence of the product in order to determine "control" values. At first, the temperature corresponding to "very hot" - as perceived by the volunteers at the interior of the wrists - was determined for each subject. After this determination, the time required for the sensation of pruritus to appear on the symmetrical zone (control) of the wrist was noted. Each subject announced the onset and termination of the sensation of pruritus. These values were recorded by the operator. Since the results were given by the volunteers, the use of a placebo enabled the psychological effect of product application to be eliminated.

The measurement zone was

again stimulated two hours later. *Enteromorpha compressa* formulated at 2% in a lotion was applied at the dose of 8 µl/cm<sup>2</sup> on one of the wrists. The placebo lotion was applied at the same dose on the other wrist. The three parameters studied were the time for the sensation of heat to appear, its duration and its intensity. The latter was expressed as a numerical scale between 0 and 4, corresponding to a gradient of increasing intensity of the sensation.

The results of this experiment showed that in comparison to the control zone, the placebo lotion tended to accentuate the problem of pruritus as judged by the three evaluation parameters. When the placebo lotion was applied, the sensation of pruritus appeared very rapidly, after 28 seconds. When *Enteromorpha compressa* was incorporated in the placebo formula, sensitisation effects were greatly retarded, since the time of appearance of the sensation of pruritus increased to 112 seconds. This improvement was obtained in 10 of the 10 volunteers when compared to the placebo, and on 8 of the 10 volunteers when compared to the control.

Lotion containing *Enteromorpha compressa* also considerably reduced the duration of the sensation of pruritus: control, placebo and *Enteromorpha* extract lotion values were 89, 67 and 47 seconds. This duration decreased in 8 of 10 volunteers compared to the control, and in 6 of 10 compared to the placebo. Similarly, the *Enteromorpha compressa* containing milk also reduced the intensity of the sensation of pruritus. Thus, in the case of the control and placebo lotions, intensities were judged to be strong (2.8-2.9) in contrast to the extract lotion, where the intensity of the sensation of pruritus was genuinely attenuated, as shown by the low value (1.1). Compared to the placebo, improvement was seen in 9 of 10 volunteers.

*Enteromorpha compressa*, thus, has considerable soothing properties when applied to skin that is hypersensitive to pruritus. In this study, the reactivity phenomenon was delayed by a factor of 4 in terms of time. This reactivity was also reduced in terms of intensity (55%) and duration (30%).

**Comfort as target:** This study was conducted by 10 dermatologists on 45 subjects, included because of their very dry hypersensitive skin, and a sensation of pruritus.

The lotion, identical to that in the preceding study containing 2% *Enteromorpha* extract, was applied to the body once or twice daily (depending on the case) for 20 days. Clinical evaluations were conducted by the dermatologists and volunteers in order to quantify treatment efficacy as determined by the following criteria: desquamation (peeling), erythema sensation of skin straining and pruritus. Efficacy was scored from 0 (good) to 4 (poor). The improvement of well being was also evaluated according to characteristics such as dryness of the skin, its comfort and the quality of life.

The soothing effect of *Enteromorpha* extract was evaluated in a clinical study of a population providing a wide range of manifestations characteristic of reactive skin. Depending on the individuals, these manifestations can be character-

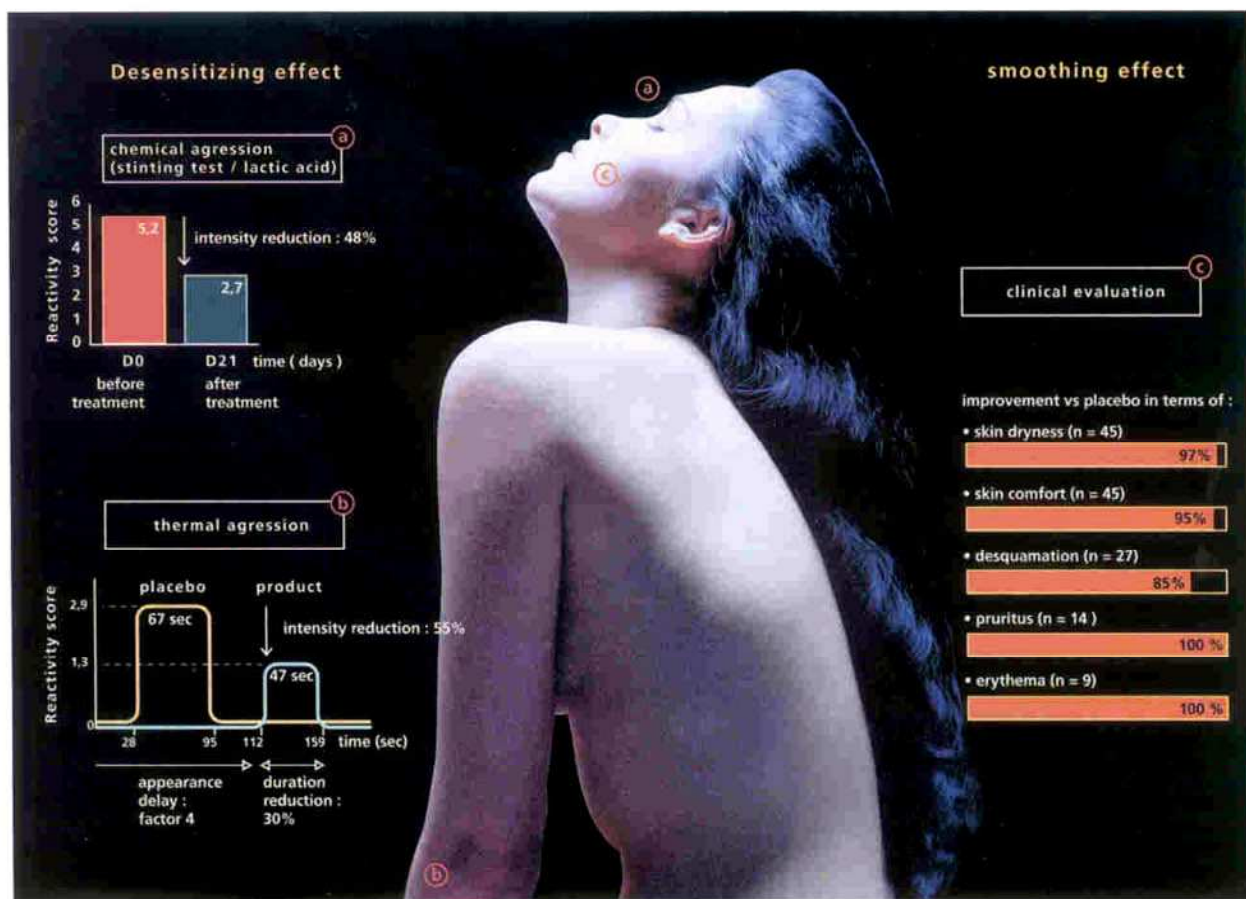


Figure 4. Effect of *Enteromorpha* extract on skin

ized by problems of desquamation, erythema, pruritus and skin straining. The lotion containing *Enteromorpha compressa* was effective against all these symptoms. In the 9 subjects with problems of erythema, this phenomenon disappeared following treatment. In addition, pruritus totally disappeared in the 14 subjects concerned.

At the end of treatment, the number of subjects still reporting problems of skin straining was reduced from 18 to 1. At the same time, the number of individuals complaining of desquamation decreased from 27 to 7. This efficacy was also encountered in terms of the criteria of well being, since among all the subjects, 97% confirmed improvement in terms of dryness, 95% for skin comfort and 92% for general well being.

***Enteromorpha* extract, the soothing and comfort response:** Faced with the extent of the phenomenon of hypersensitivity, "sensitive skin" lines of cosmetics have multiplied during the past several years,

with different paths of development being undertaken. Understanding the problem of skin sensitivity has been gained not only by testing formulations with soothing action against external aggression, but also by the quest for better acceptability of the formulation itself. Thus, the cosmetics industry has, in general, become oriented toward lighter and non-occlusive textures. The products are adapted to sensitive skin by eliminating unwanted products and remaining within the pH range of the skin.

Companies have also tackled hypersensitivity by acting either on protection against deleterious environmental effects or by reconstituting the hydrolipid film (hydrating film, natural moisturizing factor (NMF), vegetable oils, etc.), or by applying venotonic agents or anti-irritants. In this setting, *Enteromorpha compressa* represents a novel approach to hypersensitivity, by initially considering it to be a neurosensorial problem before being erythematous.

Its originality resides in the fact that it acts at the initiation stage of inflammation, pain or pruritus by blocking access of neuromediators to cell receptors. *Enteromorpha* extract modulates their activity and the attendant biological response. This leads to a reduction in the activation of epidermal cells. As a result, the extract has a positive effect on the tolerance threshold and breaks the vicious cycle of sensitive skin - the skin becomes more intolerant as aggression is more frequent and, as its reactions intensify, crises multiply. *Enteromorpha* extract, thus, leads to a return of the situation toward a more

normal state, with the skin reacting less violently and less rapidly to external aggressions.

When *Enteromorpha compressa* is incorporated at 1-2%, it provides an overall cosmetic response to the problem of hypersensitivity. It is intended for all care or hygiene lines as well as specific "sensitive skin" lines. It is a positive addition to face, body and hair products, examples of which are makeup removers, shower gels, foaming baths, etc. While *Enteromorpha* extract provides a solu-

tion to problems of intolerant skin, it also is recommended in more specific cases of irritable and atopic skin. In this case, it acts as a genuine active ingredient by reducing the degree of skin reactivity and minimising skin reactions.

The skin is subjected to a number of aggressive factors every day. Taken individually, they may cause only minor damage, but when the entire arsenal is present over long periods of time, it may potentiate harmful effects in the epidermis and the dermis. The skin also retains a certain degree of reactivity during a renewed contact with the initial factor. *Enteromorpha* extract can thus be recommended in sun or after-sun products to enable the skin to recover from prior traumas.

*Enteromorpha compressa* is a new response to problems of

skin that is atopic, irritable or intolerant. Starting with the initiation of the sensitisation process, it leads to the inactivation of epidermal receptors involved in inflammatory reactions or in pain. Over the course of time, these reactions may cause damage which, if repeated, can lead to functional and structural changes in the skin and, even if in an indirect manner, to the formation of free radicals by lipoperoxidation of cell membranes. Hypersensitivity can lead to skin ageing.

*Enteromorpha compressa* formulations have a several objectives: to improve tolerance to cosmetic products, improve the capacity for adaptation and the comfort of sensitive skin, and afford anti-wrinkle protection in skin ageing. *Enteromorpha compressa* pushes back the frontiers of hypersensitivity.

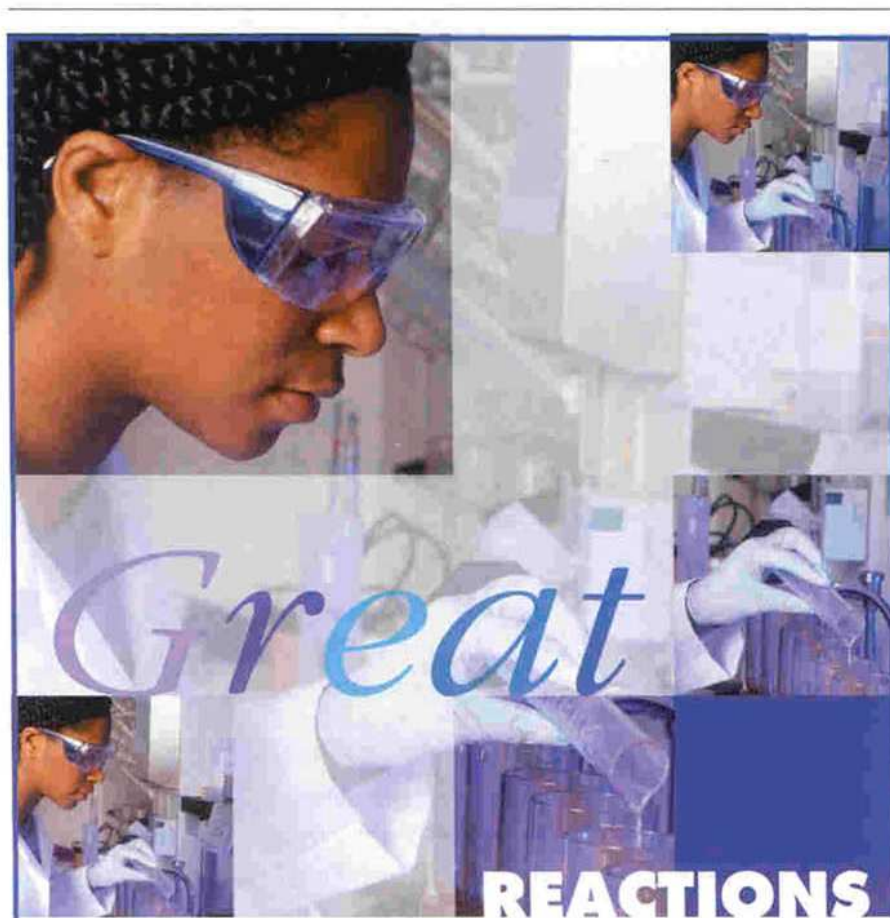
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