Fucoidan hydrolysation technology analysed

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The marine environment is a rich source of biological and chemical diversity. Interestingly, algae developed adaptive responses to environmental stress (UV, temperature, bacteria, pressure, turbulence...) and thus they are able to produce saccharides or other active molecules. Brown seaweeds are the most frequent seaweed surviving in the extreme conditions of the Iroise sea, mainly due to their ability to produce and secrete some fucoidan compounds in their cellular walls. Lessonia, a French cosmetic company, developed two specialty active ingredients from fucoidans. Fucoreverse is a hydrolysed fucoidan extracted from these luminaria. In vitro and in vivo tests show anti-ageing properties. Fucowhite is a purified fucoidan-phloroglucinol complex, extracted from Ascophyllum nodosum. Fucowhite is a clinically validated skin whitening ingredient which significantly decreases skin pigmentation.

The hydrolysation technology

What are fucoidans?
Fucoidans are polymers composed of sulfated fucose. They are insoluble high molecular weight molecules: 50 to 1000 kDalton. They are known to demonstrate a wide range of biological activities including stimulation of the cellular metabolism and immunomodulation.

Fucoidans are a class of non-gelling, sulphated polysaccharides found only in brown macro algae. Their sugar backbone composition, structure and sulphation patterns differ according to their origin and the extraction techniques used. The common feature is a high content of the backbone sugar - fucose. Fucoidans have diverse bioactivity including heparin-like anticoagulant activity, inhibition of selectins and scavenger receptors, inhibition of viral entry to cells and immunomodulation.

Fucoidans also inhibit UVB-induced MMP expression in vitro, and are a good inhibitors of the dermal remodelling enzymes called matrix metalloproteinases. Interestingly, topical fucoidan was as effective as the gold standard topical steroid cream when applied to anatopic dermatitis model in mice.

The unique composition

The fucoidans can be hydrolysed until an extremely low molecular weight is obtained. Fucoreverse (now referred to as 'the anti-ageing active') is a combination of fucose mono-, di- and trimers. The second interest of this technology is to saturate the fucose molecules in sulfated groups. Figure 2 shows the lowest possible molecular weight and the highest degree of sulfation.

Figure 1: Chemical structure of fucoidan polymer.

Abstract

French company, Lessonia, has developed a hydrolysation technology applied to fucoidans. This technology involves hydrolysing these fucoidan polymers to obtain precise fractions of biologically active oligosaccharides. The fucoidan’s low molecular weight is globally unique. The mass of molecular weight < 2 kDa can be absorbed directly through the human skin. The hydrolysation process increases the skin care and anti-ageing effect of the fucoidan compared to the standard macromolecular structure. Lessonia developed two active ingredients from fucoidans, Fucoreverse™ is an anti-ageing ingredient and Fucowhite™ a whitening ingredient.

Consequences on biological activity

The biological activity of fucoidan derivatives is in inverse proportion to the molecular weight of these molecules. The shorter the saccharide chain, the greater the biological activity of the fucoidan derivative. The biological activity is also in direct correlation to the fucoidan derivative’s degree of sulfation. In effect it is these sulfated derivatives which, by attaching themselves to biological receptors, lead to cosmetic activity.

Skin absorption

By using hydrolysis technology it is possible to obtain highly ionised water-soluble oligofucoidans with an extremely low molecular weight. These 3 criteria define the skin absorption capacity of a cosmetic agent. This is why the anti-ageing active is so easily and so rapidly absorbed across the skin’s barrier.

Protecting and repairing the Extracellular matrix (ECM)

The passing of the years has negative effects on the skin and its properties, worsening them. There are biochemical, histological and physiological alterations that include ECM functional impairment, collagen and elastin degradation, and slower metabolism rate. The degenerative changes that come with age can cause facial skin to lose its vibrancy. A major factor in this process is the degradation of the extracellular matrix of the dermal connective tissue.

The extracellular matrix in the dermis is composed of a molecular network.
between the fibroblasts which ensures the skin’s structure and tone. When the deterioration and the repair of this network are imbalanced, wrinkles appear and the skin loses its firmness. Fucoidans protect this network by blocking the enzymes responsible for the deterioration of the extracellular matrix as well as the pro-inflammatory cytokines which damage the collagen. The anti-ageing active also acts as a repairing agent by stimulating the metabolism of the fibroblasts and collagen synthesis. With these 2 synergistic actions, the anti-ageing active helps to reverse the signs of ageing by bringing back the skin’s structure and firmness.

**In vitro tests**

The effect of the anti-ageing active on the fibroblasts’ proliferation and synthesis of collagen has been evaluated on human dermal fibroblasts. The anti-ageing active was tested at 2 concentrations: 0.15% and 1.5%. Untreated cells were used as negative control (CTR-).

**Stimulating fibroblast proliferation**

**Protocol**

Culture medium containing the tested product was added to the wells containing cells in the G0 phase of cell cycle. At the end of incubation period, the cell viability was evaluated and the increasing proliferating rate compared to untreated control cell culture.

**Results**

The data are reported as the percentage increase of cell proliferation after cells’ exposure to the test item, in respect to untreated cells. The treatment with the anti-ageing active has positively modulated cell proliferation in the considered experimental system. All recorded variations are significant vs negative control (CTR-). A significant increase in the proliferation of fibroblasts compared to the non-treated group of: +35.5% in the 0.15% dose; 51.1% in the 1.5% dose.

**Stimulating type 1 collagen synthesis compared with the negative control**

**Protocol**

The determination of collagen synthesis is carried out by quantitative dye-binding method. The chromogen agent used in the assay is Sirius Red. The collagen concentration is calculated by means of date interpolation on a standard curve obtained with known and increasing collagen concentrations.

**Results**

The data are reported as the percentage increase of collagen synthesis after cells’ exposure to the test item respect untreated cells. The treatment with the anti-ageing active has positively modulated collagen synthesis in the considered experimental system. All recorded variations are significant vs negative control (CTR-). The anti-ageing active has a significant stimulatory effect on collagen synthesis compared to the non-treated control group: +23.7 % in the 0.15% dose; +31.1 % in the 1.5% dose.

**Clinical study**

**Protocol**

The aim of the study is to evaluate the safety of use, the cosmetic acceptability and the efficacy of the anti-ageing active. It is considered whether the product works on the wrinkles depth. In order to reach this goal, a clinical-intrumental study is carried out on 42 volunteers showing wrinkles on the crow’s foot area, divided in two groups according to the randomisation lists: group A received the active product whereas group B received placebo. Product efficacy is evaluated after 28 days of product use by means of non-invasive skin bioengineering technique to evaluate skin profilometry. The active product used contain 1.5% of the anti-ageing active.
Skin surface is quantitatively assessed by Lifeviz Micro. It is a non-contact in vivo skin measurement device based on structured light projection. In conjunction with a comprehensive 3D measurement and evaluation software, the sensor enables the evaluation of wrinkle depth.

**Results**

**Efficacy on crow’s feet area after 28 days**

After using 1.5% anti-ageing active twice daily for 28 days, statistics show that the anti-ageing active significantly decreases the depth of crow’s feet wrinkles by 9.1% on average, versus placebo. \( P<0.05 \) Student test.

**Efficacy on anti-irritant properties**

The study showed a significant decrease in irritations and redness as shown in Figure 5. The subjects who used the product containing the anti-ageing active had good skin tolerance whereas those who used the placebo cosmetic base had moderate tolerance with a few cases of skin irritation. These results show the use of the anti-ageing active to calm sensitive skin.

**Whitening the skin**

Fucowhite is a clinically validated skin whitening ingredient which significantly decreases skin pigmentation. Fucowhite (now referred to as ‘the skin whitening active’) is a unique purified fucoidan-phloroglucinol complex, extracted from the brown algae *Ascophyllum nodosum* by lixiviation in water. This unique complex of fucoidan with antioxidant polyphenol, makes it an effective and versatile ingredient for whitening applications.

**Tyrosinase inhibitory activity**

The skin’s pigmentation results from the presence of melanin in the epidermis. This pigment is synthesised in vesicles known as melanosomes of specialised cells, melanocytes. The melanin pigment is produced using tyrosine, an essential amino acid that is submitted to a series of reactions, linked to tyrosinase, as the key enzyme in this biosynthesis, to form melanin.

The enzyme tyrosinase was incubated with its substrate tyrosine. The product of this reaction (melanin) was analysed by measuring the optical density at 480 nm. The skin whitening active was an effective tyrosinase inhibitor at very low concentrations.

**Lightening efficacy on Asian skin type**

Twenty-four Asian subjects were used in a placebo controlled study during 28 days to assess the ability of a 2% skin whitening active emulsion formulation to decrease the skin pigmentation. The colour of the skin was measured by a chromameter. This appliance makes it possible to define a luminance parameter: L, clarity from dark to pale; an individual typological angle ITA which defines the degree of pigmentation of an individual’s skin.

The results show a significant increase of skin clarity and decrease of the skin pigmentation. Statistical analysis highlights a significantly better effect using the ‘active cream’ on L parameter and ITA compared with placebo. It induces a lightening effect characterised by a significant increase in L and ITA parameters.

**References**