



BIO FOREXTRA
Natural Ingredient Science

ACTIVE
INGREDIENTS

Boréaline[®] Expert

THE BOREAL

REJUVENATING POWER

Red maple bark extract, obtained through
circular economy process

—
Rich in polyphenol content

—
Powerful antioxidant

—
Reduces the appearance of wrinkle



IFF | LUCASMEYER
COSMETICS

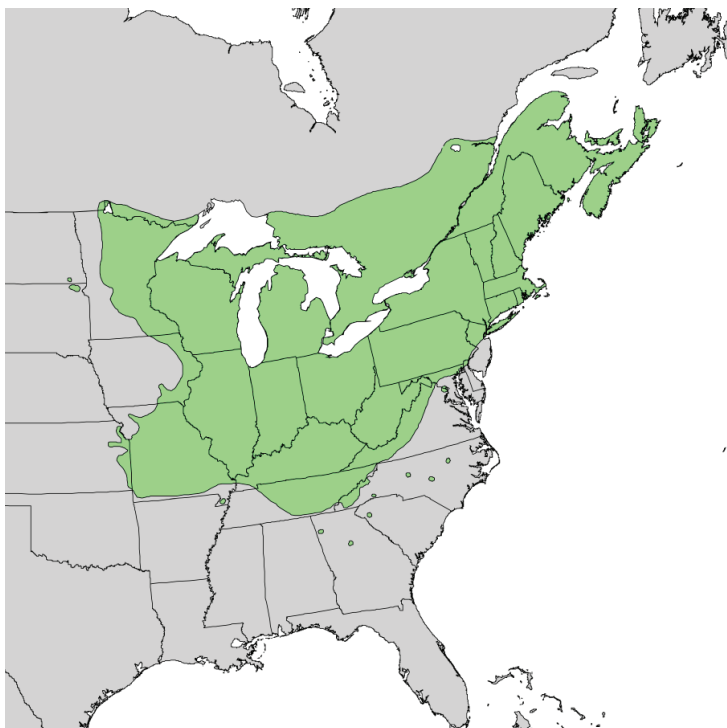
SUMMARY

DESCRIPTION	<ul style="list-style-type: none"> • 100 % natural, red maple bark extract, obtained through circular economy process • Very high polyphenol content
SKIN BENEFITS	<ul style="list-style-type: none"> • Reduces the appearance of wrinkles • Increases firmness and elasticity • Overall reduces the signs of aging
TESTED EFFICACY	<p><i>in vitro</i> tests :</p> <ul style="list-style-type: none"> • Radical species scavenging • Global antioxidant action • Anti-inflammatory • Collagen and elastin booster • Cell differentiation stimulation (involucrin) <p><i>in vivo</i> clinical test :</p> <ul style="list-style-type: none"> • Action on the skin firmness and elasticity • Reduction of the appearance of wrinkles
APPLICATIONS	<ul style="list-style-type: none"> • « Complete anti-aging » care • Plumping , repairing and anti-wrinkle products • Mature skin products • Men's care • Cosmeceuticals • Regenerating care • Day care • Night care • Sun care & After-sun care
INCI	Glycerin (and) Acer Rubrum Extract
RECOMMENDED DOSAGE	0.1 – 0.25 %

THE TREE STORY

Origin

Acer rubrum means « strong » as the robustness of its wood and bark and « red » for its beautiful flowers and leaves in bright red colors that announce the spring and fall season in Quebec. Indeed this tree native of Canada, is one of the main hardwood species of Quebec's forests. Also known as the Canadian maple, it grows in Canada mostly in the Acadian region in the Great Lakes and the St. Laurent, and in the boreal forest of Newfoundland.



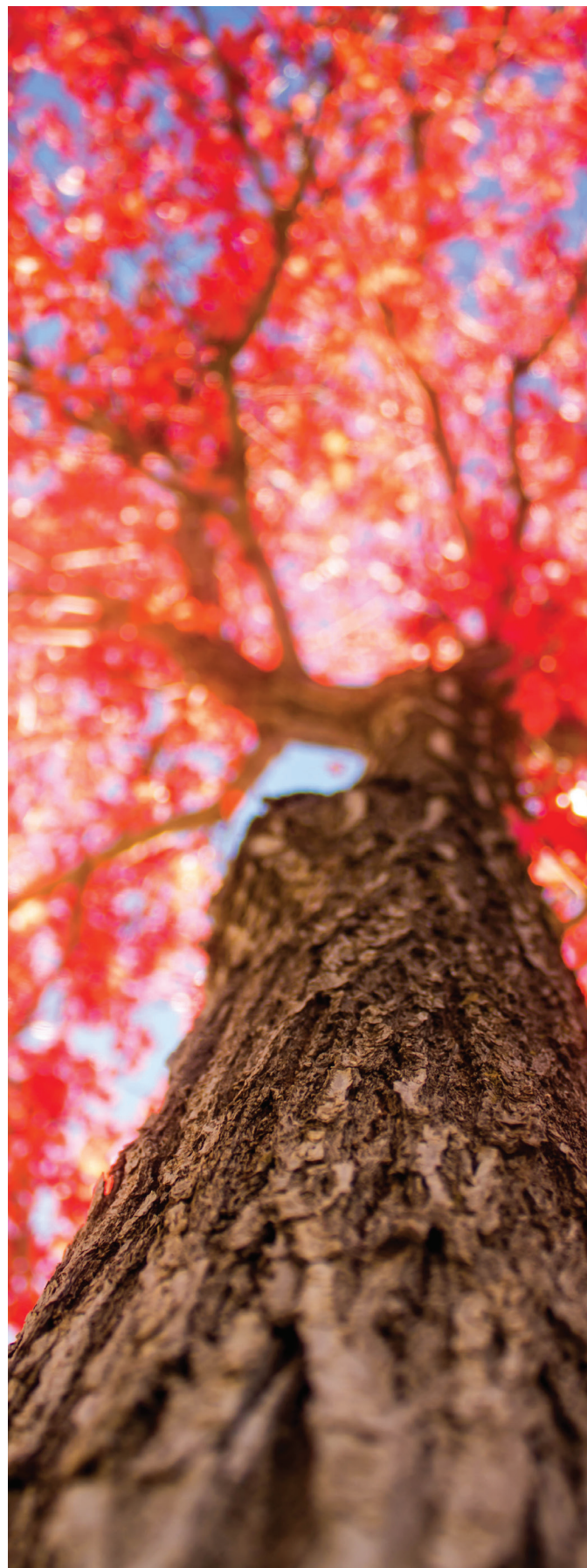
● *Acer Rubrum*

Forest industry uses

Red maple is a medium sized tree that can reach heights of 15 to 30 meters, a diameter ranging from 0.5 to nearly 2 meters. In the forest industry, it is used in the manufacture of furniture, wooden pallets and also in paper production.

Traditional uses

There is a large traditional use of the bark of this tree. Iroquois were crushing the dried bark to incorporate the powder in the flour for bread preservation. Aside from this nutritional use, red maple was recently ranked in the list of native medicinal vascular plants in Quebec. References mention that the red maple bark is also used in Indian traditional medicine (Ojibwa) as an anthelmintic, tonic and treatment for sore eyes. A bark infusion was recommended to treat cramps and dysentery.

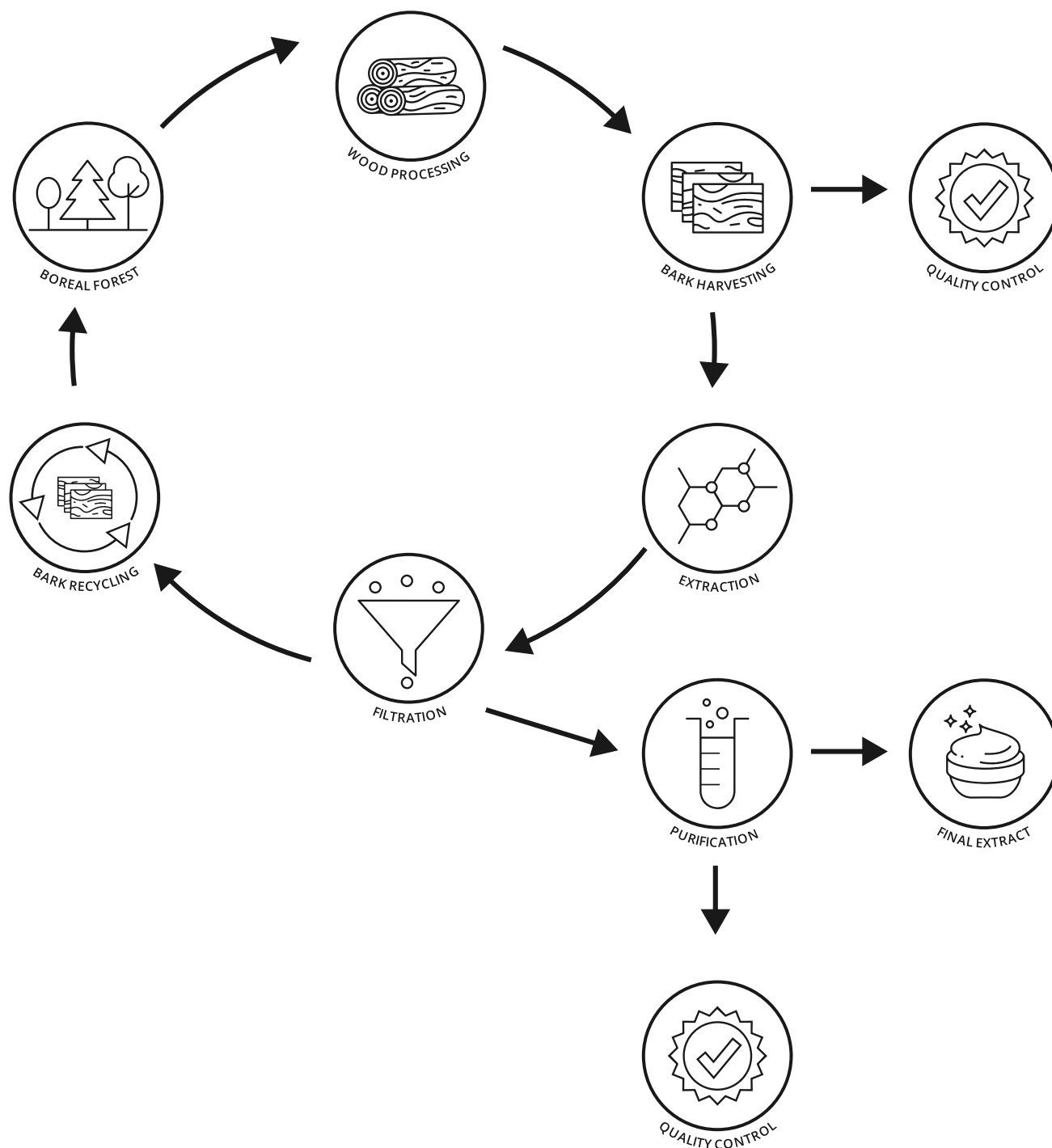


A CIRCULAR ECONOMY CONCEPT

The Canadian forest industry generates millions of tons of biomass by-products during its wood transformation process, most of which are barks. Less than 5 years ago, residual barks were buried underground, due to the lack of knowledge on how to recycle them, leading to severe environmental issues. Nowadays, these residual barks are burned in order to create renewable energy. It is a far better solution on an environmental stand point. However, all natural active compounds are destroyed when the barks are burned, losing all those precious extractives.

Bark is the skin of the tree protecting it against various environmental aggressions such as extreme nordic temperatures or insects invasion.

Bio ForeXtra creates a new eco-valorization of the Canadian forest biomass by the systematic extraction of bioactive molecules of tree barks before recycling them into green energy or composting



THE TREE SKIN FOR YOUR SKIN

The bark is a true skin for the tree. The tree concentrates in its skin a panel of bioactive molecules that allows it to resist against exterior aggressions. These natural compounds synthesized in high concentrations in barks are easily extractable. Because we know that Nature is well done and that is in its complexity that we can find its secret, our idea is to simply transfer these natural protecting and repairing agents from the trees to the benefit of the human skin. This can be done by valorizing the fabulous potential of our majestic forest trees. The results obtained through our research and development programs permit us to propose standardized extracts of high quality, safe and clinically proven.

We work with respect for nature and we use only ecological extraction methods.

Bio ForeXtra became interested in the tree species of the Canadian Boreal forest and more specifically in red maple for its resistant and robust bark which protects the tree during all seasons. This resistant and strong red maple is also the emblem on the Canadian flag. This tree can live hundreds years and it is known for its capacity to adapt itself to very hard conditions.

Boréaline® Expert is a 100% natural red maple bark extract obtained through a selective extraction process targeting the components with the highest efficiency. Greatly concentrated in polyphenols, Boréaline® Expert contains mostly phenols glycosyls, as well as flavonoids such as catechin, phenolic acids like gallic acid, pyrogallol and proanthocyanidins.



SKIN AGING

Many factors, both extrinsic and intrinsic, cause the skin to age. Those causes can easily be divided into three main categories: biological aging, environmental aging and mechanical aging.

Intrinsic aging or biological aging is the aging process that takes place over the years regardless of external influences. Biological aging is the result of changes—often genetically determined—that occur naturally within the body. Everyone has a biological clock or chronological age determined by genetic makeup. This applies to the skin as well. As our biological clock ticks, our skin gradually loses its ability to function as it once did.

After the age of 20 :

1% less of collagen is produced in the dermis each year.

- The collagen and elastin fibers become thicker, more clumped, and looser, resulting in inelastic and brittle skin and eventually in wrinkling and sagging.

After the age of 30 :

Skin's exfoliation process decreases causing dead skin cells to accumulate.

Transfer of moisture from the dermis to the epidermis is slowed and fat cells start to shrink.

- The skin looks more dull and thin.

After the age of 40 :

Collagen production is reduced. The collagen and elastin fibers break, thicken, stiffen, clump together, and lose their elasticity.

- This results in wrinkles and aging lines.

After the age of 50 :

The skin becomes dry and is easily bruised, damaged, or broken because the sebaceous (oil) glands have decreased in size. In women, menopause causes a decrease in estrogen levels, leaving the skin drier, thinner, more sensitive, and less toned.



FORTUNATELY, SOME OF THE FACTORS CONTRIBUTING TO BIOLOGICAL AGING CAN BE CONTROLLED

Scientists are beginning to understand how free radicals (unstable molecules) damage proteins, lipids, and the DNA within cells, and, in turn, accelerate the biological aging process. Antioxidants are molecules with the ability to neutralize free radicals in the skin.

Extrinsic aging or environmental aging occurs as a result of daily exposure to trillions of free radicals from a variety of sources: the sun's ultraviolet rays, pollution, smoke, harsh weather, and external stress. These free radicals damage lipids, proteins, and DNA, all of which limit the ability of cells to function and cripple the integrity of overall cell composition. Years of accumulated environmental stress on cellular structures results in the premature aging of the skin. Unlike the previous cause of skin aging, it can be controlled because it is a result of environmental damage. Extrinsic aging appears as a thickening of the cornified layer (outermost layer of epidermis), precancerous changes (an example is actinic keratosis), skin cancer, formation of freckles and sunspots, and huge losses of collagen, elastin, and glycosaminoglycans (GAGs). As a result of these processes, the skin becomes rough, uneven in tone, and wrinkled.

Environmental influences, such as pollution, smoking, and ultraviolet radiation, generate free radicals. Antioxidant enzymes and molecules can protect us from free radicals, but their damage occurs anyway. Also, because the skin is the barrier between body and environment, UV damage from the sun's rays accounts for 90 percent of premature skin aging. Photoaging damages collagen, elastin, melanocytes, and the moisture barrier, resulting in wrinkles, sagging, uneven skin tone, dark spots, and a rough, dry skin texture.

Mechanical aging is the result of continually repeated wrinkle causing behaviors, muscle movements repeated day after day and year after year (smiling and frowning). Some behaviors could be avoided to help prevent premature signs of mechanical aging (Unbalanced diet and lack of sleep).

In conclusion, aging is under the control of genetic repair mechanics and can be affected by lifestyle influences. Measures to protect our skin will keep it from aging more than it needs to.



BORÉALINE® EXPERT, A GREEN SOLUTION FOR SKIN REJUVENATION

Boréaline® Expert is an anti-aging and powerful anti-wrinkle active which functions on different biological mechanisms of action involved in the three causes of skin aging.

- **Dermal regeneration action**
 - Procollagen action
 - Proelastin action
- **Inhibitor of oxidative stress and inflammation**
 - Antioxidant and radical scavenging action
 - Targeted anti-inflammatory action
- **Booster of skin mechanical resistance**
 - Stimulation of the involucrin (action on cell differentiation)

In order to confirm its modes of actions demonstrated in vitro, the efficacy of Boréaline® Expert has also been evaluated by clinical studies:

- **Wrinkles reduction;**
- **Improvement of firmness and elasticity ;**
- **Skin rejuvenation.**



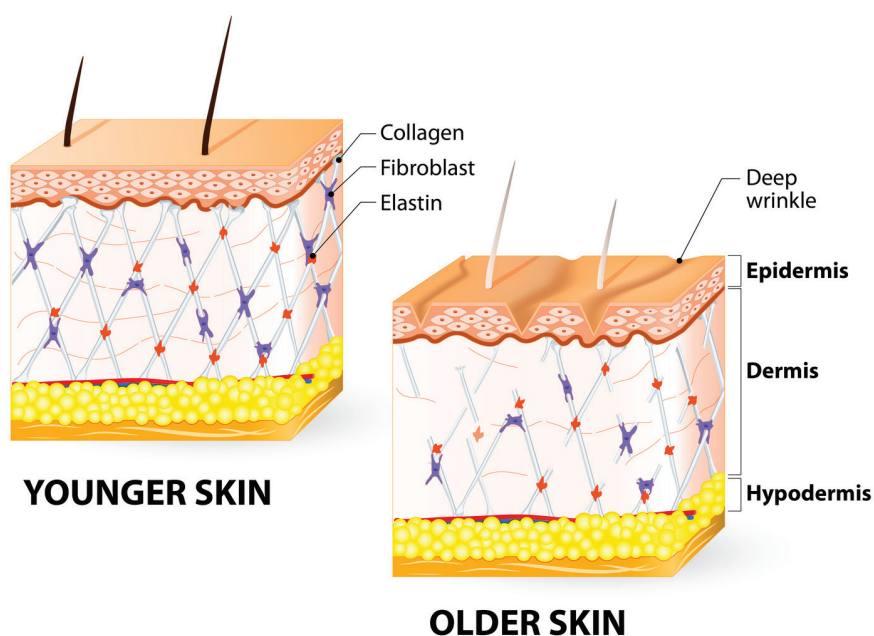
1. ACTION ON DERMAL REGENERATION

Introduction

With age a metabolic imbalance occurs between the synthesis and the degradation of the components of the dermal extracellular matrix. Making up 80 % of the dermis (the layer under the outermost layer of skin), collagen is a crucial protein that holds your skin together. Elastin is just as important, and allows your skin tissues to resume their shape after being stretched.

While collagen supplies the firmness and tautness in young skin, elastin gives the skin elasticity.

Collagen and Elastin are proteins essential to skin structure. To understand the connection of collagen and elastin with the process of skin aging, you need to look at the two primary ways that the skin, the largest organ in the body, declines over time. The healthy skin of a younger person presents an outer layer that is smooth and guarded against environmental threats. Actually, the MMP activity increases at the expense of synthesis of the collagen fibers and of the elastin, essential to the maintenance, firmness and resistance of the skin.



1.1 PROCOLLAGEN ACTION

The anti-aging efficacy of Boréaline® Expert has been evaluated on two biological mechanisms influencing skin collagen production :

Test 1 : Inhibition of the collagenase, key enzyme responsible for collagen degradation.

Test 2 : Stimulation of the collagen production.

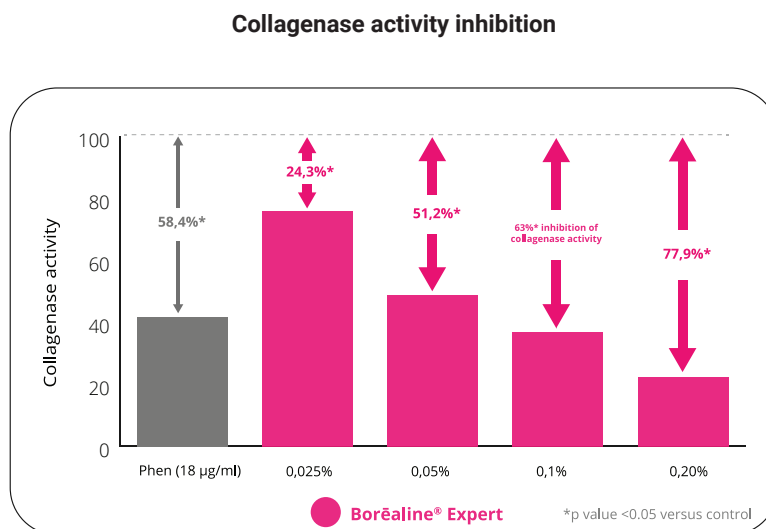
TEST 1: INHIBITION OF THE COLLAGENASE

Collagen is an helical protein found in connective tissue through out the body. At least 27 different types of collagen have been identified, with types I-IV being the most prominent in the human body. Type I collagen is the major structural protein in skin. Collagen destruction, along with damage to the other structural components of the skin (i.e., elastic and reticular fibers) occurring over decades is thought to underlie the characteristic alterations in the appearance of aged skin and the additional changes that result from chronic sun exposure

PROTOCOL

The evaluation of the effect of the extract on the inhibition of the collagenase was conducted according to the specifications in the EnzChekGelatinase/Collagenase assay. The Clostridium collagenase is used in this analysis in order to cleave the type 1 collagen combined to the fluorescein. Higher concentrations of the extract are added in the middle and the fluorescence is measured. When the collagen is degraded, a proportional fluorescence is observed in the middle. The results are revealed as a percentage of inhibition of the extract collagenase activity compared to the middle without the extract. The 1-10 phenantrolin, a well-known MMP inhibitor, was used as a positive control.

RESULTS



Boréaline® Expert inhibits 63 % and 78 % collagenase activity at 0.1 % and 0.2 % respectively. The efficacy of Boréaline® Expert is similar to the positive control, 1-10 phenantrolin, a well-known metalloprotease inhibitor.

Boréaline® Expert allows to protect the collagen for its degradation during aging process.

TEST 2: TYPE I COLLAGEN SYNTHESIS STIMULATION

Type I collagen is the most abundant collagen of the human body which forms large, eosinophilic fibers known as collagen fibers. It is well established that collagen is responsible for skin strength and elasticity, and its degradation leads to wrinkles that accompany aging.

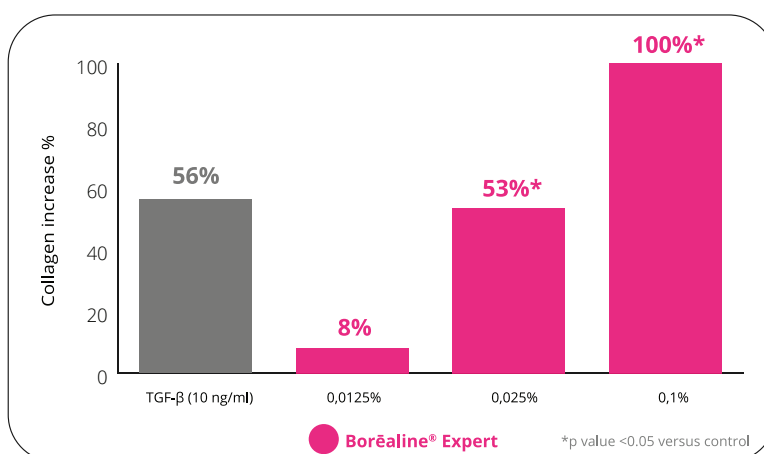
PROTOCOL

Dosage of type 1 collagen and immunolabeling

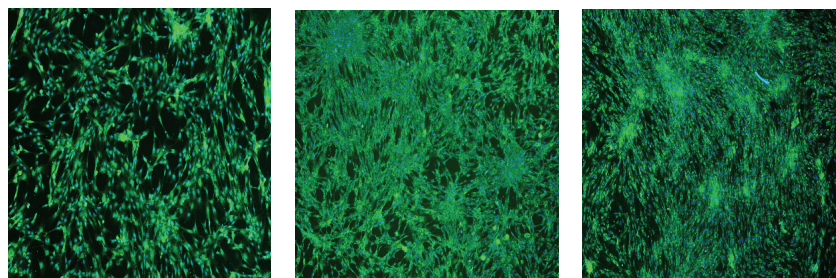
Skin fibroblasts were incubated in presence or absence of the increasing extract concentration. The cells were then fixed and incubated with an antibody against type 1 collagen. Once they were fixed to the collagen, the primary antibody is detected by a secondary antibody to which a fluorophore is grafted. The fluorescence emitted by the secondary antibodies is then measured and pictures are taken. The results are expressed as a percentage of the collagen increase by Boréaline® Expert comparatively to results obtained with cells that were not treated with the extract. The transforming growth factor, collagen synthesis stimulator, TGF- β was used as a positive control.

RESULTS

Effect of Boréaline® Expert on collagen-1 synthesis after 24H



Effect of Boréaline® Expert on collagen stimulation



Untreated

Presence of 10 ng/mL of
TGF- β (control)

Boréaline® Expert 0.1 %

Boréaline® Expert significantly stimulates collagen synthesis with a maximum increase of 100 % at 0.1 % concentration after 24H treatment. These results are confirmed by the pictures of fibroblasts treated with the same concentration and labelled with an anti-collagen antibody.

Boréaline® Expert is a great booster of type 1 collagen synthesis for skin firmness recovery.

1.2 ACTION ON ELASTIN PRODUCTION

Elastin is the most important factor in tight and elastic skin and cannot be regenerated; loss of elastin in the skin system is irreparable. Elastin is also a protein found in connective tissues—but a different type of protein than collagen. It has the actual property of being elastic. It's responsible for allowing tissues in the body to "snap back" to their original shape after being stretched or contracted. For this reason, it's often compared to a rubber band. The amount of elastin in the skin usually peaks in adolescence or early adulthood and declines thereafter. Fibroblasts in older skin have a much reduced capacity to produce new elastin. This deficiency does not appear to be a result of the loss of fibroblasts or mutations in elastin-encoding genes. More likely, age-related changes in the skin's biochemical environment shut down elastin production. Boosting elastin in the skin is a somewhat neglected topic in skin care. In part, this is a result of excessive focus of cosmetic industry and dermatologic research on collagen. However, elastin is just as important for successful skin rejuvenation.

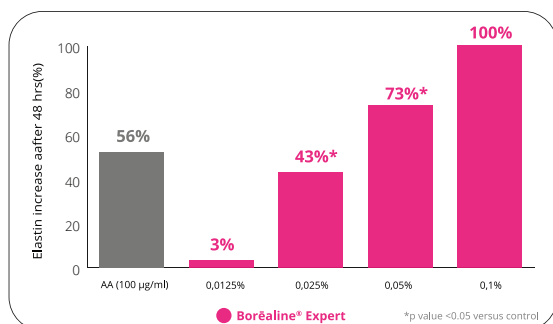
PROTOCOL

Elastin dosage and immunolabeling

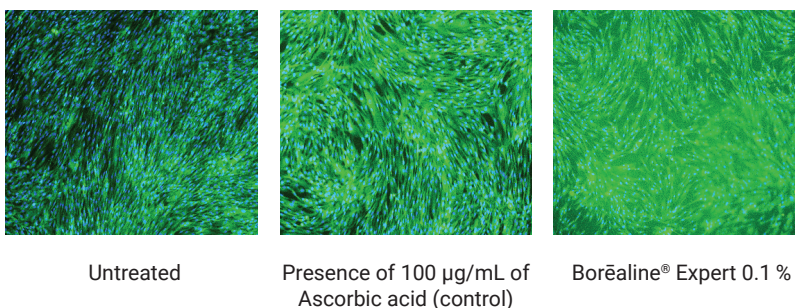
Skin fibroblasts were incubated with and without the presence of increasing concentration of the extract. The cells were then fixed and incubated with a specific antibody against elastin. Once it is fixed to the elastin, the primary antibody is detected by a secondary antibody to which a fluorophore is grafted. The fluorescence emitted by the secondary antibodies is then measured and pictures are taken. The results are expressed as a percentage of the elastin increase by Boréaline® Expert compared with the results obtained with untreated cells. Ascorbic acid (AA) at 100 µg/ml was used as a positive control.

RESULTS

Effect of Boréaline® Expert on elastin synthesis after 48H



Effect of Boréaline® Expert on elastin stimulation after 48H treatment



Boréaline® Expert is a strong booster of elastin synthesis up to 100% after 48 h treatment at 0.1% concentration. These results are confirmed by the images of the fibroblasts treated at the same concentration and labelled with an anti-elastin antibody.

Boréaline® Expert boosts elastin synthesis to maintain skin elasticity.

Boréaline® Expert is a powerful dermal regenerator with a double boosting action on collagen and elastin thus improving skin firmness and elasticity.

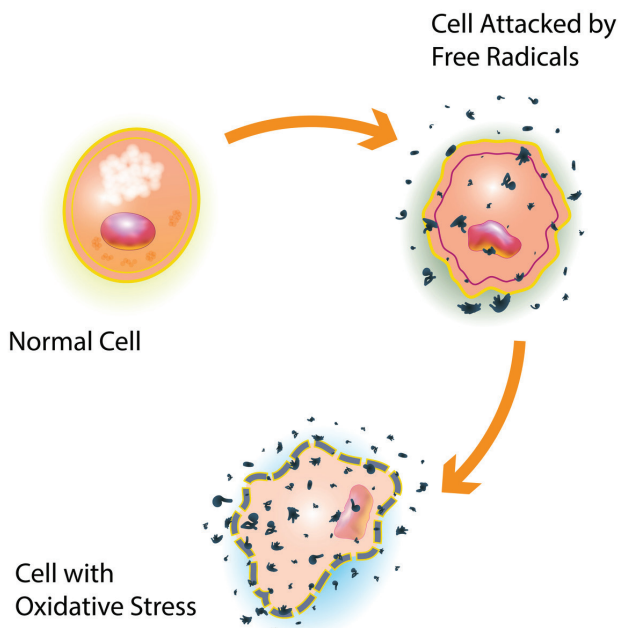
2. PROTECTION AGAINST GLOBAL OXIDATIVE STRESS

One of the theories to explain aging is the theory of the oxidative stress, according to in which very reactive molecules, linked to oxygen, damage at all times our most vital molecules such as DNA or the components of cell membranes. These highly reactive molecules are called free radicals or reactive oxygen species (ROS). The ROS are not only harmful. In fact, they have important biological functions, being involved intervening particularly in particular in the cellular signaling. So we need them. However, during oxidative stress, they are produced in excess and our natural antioxidant system is no longer sufficient to maintain the balance. These molecules cause significant damage and are the cause of several chronic diseases.

At the skin level, oxidation is a major phenomenon responsible for cellular aging. It damages the skin cells both in the epidermis and the dermis and contributes to their degeneration. Antioxidants are therefore valuable allies to help the skin fight against the free radicals produced in high concentration during oxidative stress.

WHAT CAUSES OXIDATION ?

Constantly, the skin is exposed to different types of aggressions: they can be biological, chemical and physical. The concentration of oxygen reactive species increases dramatically after exposure to UV, pollution, tobacco or chemicals. Stress, tiredness or bad lifestyle choices can also be the source of excess free radicals in the skin.

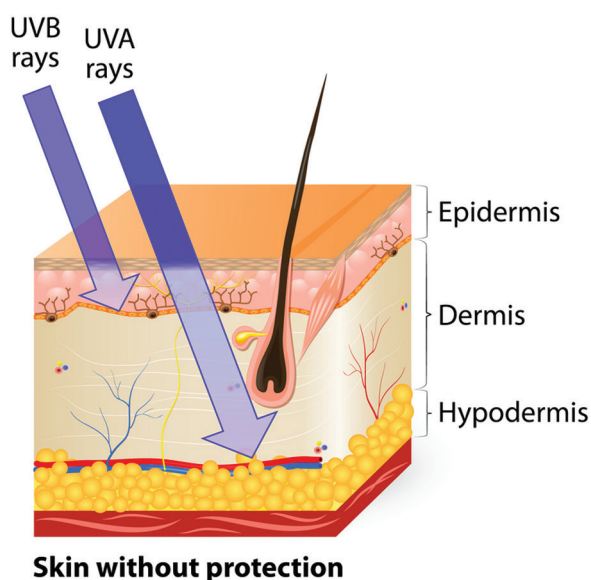


1) UV rays :

Chronic UV exposure from the sun is the predominant factor responsible of premature aging of the skin. UV-induced alterations add-up to the signs of intrinsic, genetically programmed aging; This phenomenon is called photo-aging. The so-called photo-aged skin therefore has particular characteristics and is distinguishable from photo-protected areas : it is deeply wrinkled, spotted, reddened, dehydrated, rigid and fragile. The fibers of collagen and elastin, constituting the extracellular matrix of the dermis, are disorganized and degraded, leaving the skin tissues subject to inflammation.

UVB (230-320 nm) radiation, of which only 5 % of the rays reach the surface of the Earth, penetrate up to 160-180 μm inside the skin and 10 % reach the dermis. UVB rays, although present in small quantities, are the most damaging radiation due to the large amount of energy they carry. UVA, of which 90 to 95 % of the rays reach the surface of the Earth, penetrate up to 1000 μm in the skin and 20 to 30 % reach the dermis.

UVA rays are less damaging than UVB rays, but they are constantly present in our environment and their exposure generates an oxidative stress in the skin via the excessive production of ROS in cutaneous cells and more particularly singlet oxygen, peroxide and superoxide anion. UVB are also at the root of ROS production in the skin and has also been the subject of several clinical trials¹.



1. http://www.orac-info-portal.de/download/ORAC_R2.pdf

2) Pollution

Pollution is everywhere: in the atmosphere, inside our homes, at the fingertips of smokers... It deposits daily millions of particles polluting the skin which ends up being asphyxiated. This film which forms on the surface of the epidermis, deteriorates the balance of the skin by aggressing and stifling it, all while altering the hydrolipidic film that is so crucial for the good functioning of the cells. The effects on skin beauty and health are numerous: pollution amplifies oxidative reactions, intensifies brown spots, accentuates the depth of wrinkles, increases the loss of skin radiance and promotes inflammatory reactions.

Oxides of nitrogen are also oxidative pollutants that are found in the atmosphere. The main source of anthropogenic emissions of nitrogen oxides is the combustion of fossil fuels from stationary sources (heating, power generation) and motor vehicles. Their presence is responsible for smog and acid rain. Under environmental conditions, nitric oxide is rapidly converted to nitric acid or nitrogen dioxide by atmospheric oxidants such as ozone.

A Chinese study in 2014 identified the top 5 skin problems associated with pollution. Firstly, blackheads followed by allergies and skin sensitivity, dull skin, acne and dry skin.

What is the role of antioxidants?

The endogenous antioxidant capacity of the skin is a major element of its response to oxidative stress caused by exposure to UV or other external aggressions. However, we have seen that the line of natural antioxidant defense loses much of its effectiveness during oxidative stress.

The use of antioxidants such as L-ascorbic acid (vitamin A), α -tocopherol (vitamin E) or polyphenols is therefore a way to compensate and strengthen the endogenous protection of the skin and prevent oxidative damage.

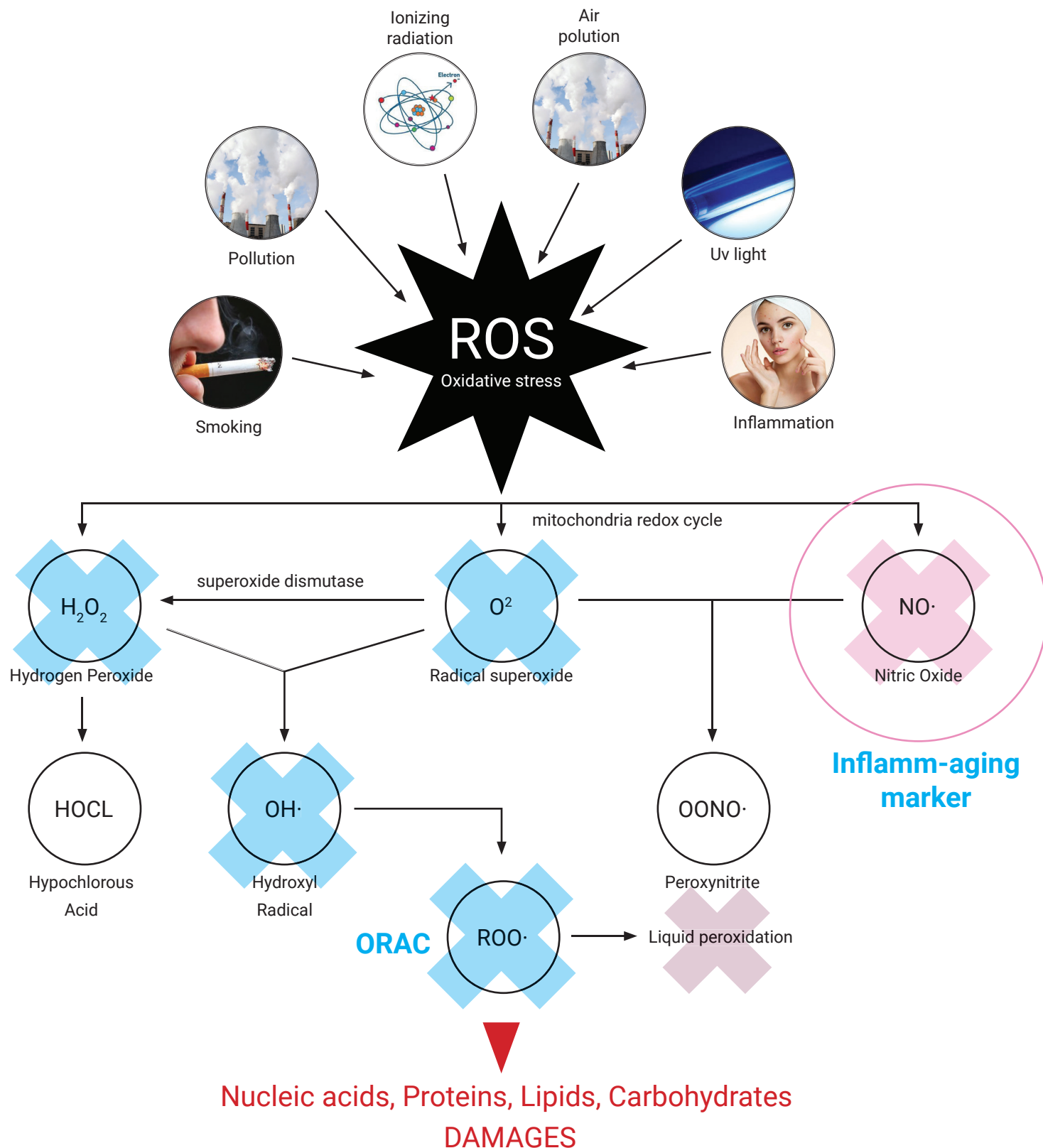
Antioxidants have also been studied in the formulation of anti-aging care because of their ability to trap free radicals and their ability to inhibit lipid peroxidation.

As the name suggests, antioxidants are oxidation inhibitors. In cosmetics, an antioxidant can be used for two main reasons :

- to protect the formula of the product from oxidation, and in particular from the rancidity of vegetable fatty substances (oils, butters, waxes ...)
- help the skin to fight free radicals, premature skin aging factors.

The antioxidant action of Boréaline® Expert has therefore been evaluated according to various methods :

- ORAC dosage to measure the global antioxidant capacity
- Anti-free radical action
- Lipid peroxidation
- Antioxidant action on cell models using cellular imaging to determine the antioxidant action in a biological medium.



DAMAGES - AGING ACCELERATION - AGE RELATED DISEASES

2.3 EVALUATION OF THE ANTIOXIDANT POWER BY ORAC MEASURE

ORAC stands for Oxygen Radical Absorbance Capacity. It's a test that permits to quantify the "total antioxidant capacity" (TAC) of an ingredient by placing a sample in a test tube, along with certain molecules that generate free radical activity and certain other molecules that are vulnerable to oxidation. In details, the assay measures the oxidative degradation of the fluorescent molecule (either beta-phycoerythrin or fluorescein) after being mixed with free radical generators such as azo-initiator compounds. Azo-initiators are considered to produce the peroxy radical (ROO·) by heating, which damages the fluorescent molecule, resulting in the loss of fluorescence.

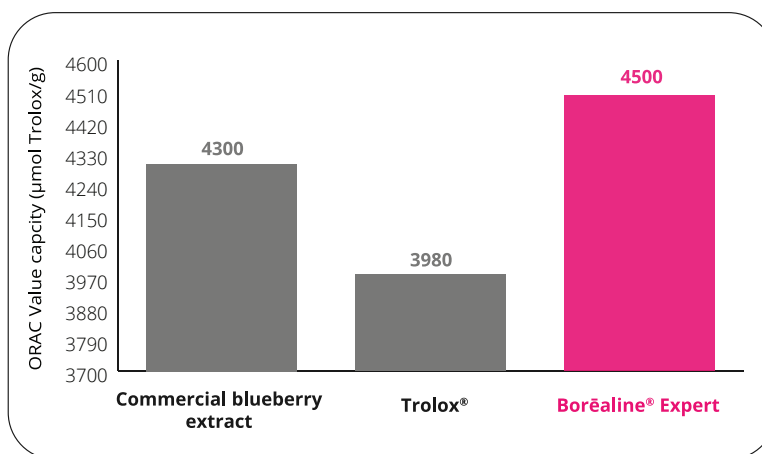
Antioxidants are considered to protect the fluorescent molecule from the oxidative degeneration. The degree of protection is quantified using a fluorometer. Fluorescein is currently used most as a fluorescent probe. The less free radical damage there is, the higher the antioxidant capacity of the test substance. There are actually a handful of different tests designed to measure total antioxidant capacity in this way, but the ORAC is probably the best known and most popular.

PROTOCOL

The measure of antioxidant power through the ORAC value (Oxygen Radical Absorbance Capacity) is an analytical robust proven industry method. The equivalent measure in Trolox® is a popular benchmark by which the antioxidant powers of a range of natural substances can be compared to.

RESULTS

Global antioxidant capacity



Boréaline® Expert has an antioxidant power (ORAC) highly superior to several natural extracts.

2.2 FREE-RADICAL SCAVENGING ACTIVITY - FIGHT AGAINST OXIDATIVE STRESS

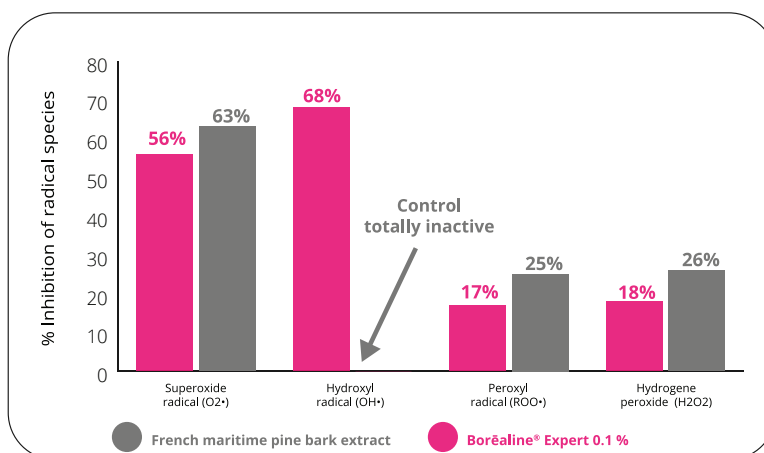
Boréaline® Expert presents a broad spectrum of anti-free radical action. Its ability to neutralize free radicals was evaluated in comparison with the standardized French maritime pine bark, largely used by the cosmetic industry for its strong antioxidant properties. This extract has been referenced in over 200 scientific publications for its biological properties and has also been the subject of several clinical trials.

PROTOCOL

The capacity of Boréaline® Expert to neutralize reactive oxygen species is measured by spectrophotometric dosage. The absorbance is then measured for solutions with or without Boréaline® Expert and a control solution with the reference. The activity of Boréaline® Expert is compared with the French maritime pine extract.

RESULTS

Anti-free radical action of Boréaline® Expert



- Boréaline® Expert demonstrates a broad spectrum of anti-free radical mechanisms acting at different stages of the oxidative stress.
- Boréaline® Expert at 0.1% inhibits the production of primary superoxide (O₂•⁻) free radicals of 56 %, thereby avoiding the production of other toxic species (NO•) and a chain reaction. This efficacy is comparable to the results with the reference.
- Boréaline® Expert inhibits drastically 68 % at 0.1% of the secondary species called radical hydroxyl (OH•) whose toxicity is one of the most significant. It acts according to three mechanisms: by ripping out either an electron or a hydrogen atom or even by adding itself on double connections. Their biological targets are most likely the organic and inorganic molecules of the cells, more specifically DNA, proteins, lipids, amino acids, sugars and metals. Our reference, the French maritime pine extract had no inhibition efficacy on this free radical specie.

Boréaline® Expert inhibits the oxidative stress at the very first stages of the oxidation phenomenon attesting its super antioxidant properties!

2.3 LIPID PEROXIDATION

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals “steal” electrons from the lipids in cell membranes, resulting in cell severe damages. In the body, the lipid peroxidation modifies the physiological functions of the cellular membrane inducing cellular aging and inflammatory reactions. In fact, unsaturated lipids sensitive to peroxidation may cause a modification of the membranes’ properties such as their fluidity, their permeability to various substances and the thickness of the double layer. This process proceeds by a free radical chain reaction mechanism. End-products of lipid peroxidation may be mutagenic and carcinogenic.

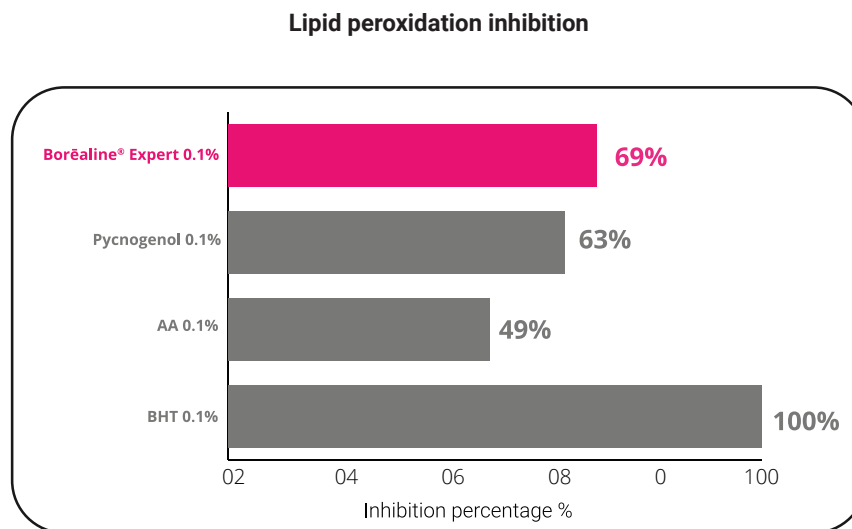
Antioxidants play a major inhibitor role on lipid peroxidation allowing the prevention of cellular aging and associated degeneration. In addition, antioxidants may also act to stabilize cosmetic formulations rich in unsaturated fatty acids prone to oxidation causing qualitative alterations, but also a toxicity due to products that are derived from lipid peroxidation.

- The antioxidant action of Boréaline® Expert was evaluated on its efficacy to inhibit lipid peroxidation.

PROTOCOL

The capacity of Boréaline® Expert to neutralize the lipid peroxidation is measured by spectrophotometric dosage. The absorbance is measured for solutions with or without Boréaline® Expert and a control solution with the reference. Boréaline® Expert activity is compared to French maritime pine extract and well known antioxidant molecules.

RESULTS



Boréaline® Expert inhibits by 50 % the lipid peroxidation.

Boréaline® Expert strongly blocks the lipid peroxidation with an activity five times superior to Vitamine C and comparable to BHT and pycnogenol.

Boréaline® Expert acts directly on skin cell's health by protecting their membranes.

2.4 EVALUATION OF ANTIOXIDANT POWER USING A CELLULAR MODEL

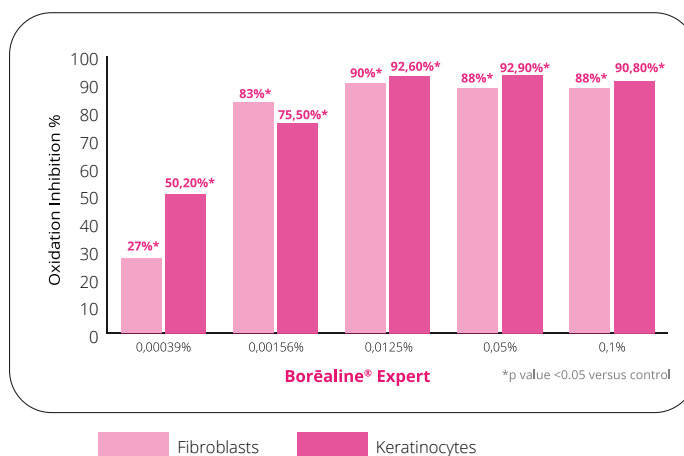
In order to confirm the antioxidant activity of Boréaline® Expert in a biological medium, two cellular models, fibroblasts and keratinocytes, were used.

PROTOCOL

The cellular antioxidant test was done as described by Girard-Lalancette et al. 2009. This test demonstrates the extract's actual biological action in the cell via the oxidation's measurement inhibition of the solution 2',7'-dichlorofluorescein-diacetate (DCFH-DA) that replaces the initial culture. The dichlorofluorescein-diacetate is not fluorescent but becomes fluorescent when oxidized by a chemical stimulation: the tert- Butyl hydroperoxide (tBH). It generates hydroxyl radicals (HO•) by Fenton reactions in the presence of intracellular iron traces. The cells are incubated with and without increased concentrations of extract and an oxidative stress is induced with the tBH. Fluorescence is measured at time zero and after three hours to calculate the percentage of inhibition of the oxidation of the DCFH.

RESULTS

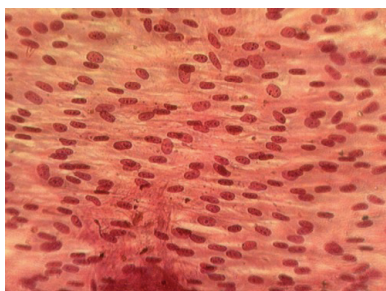
Boréaline® Expert antioxidant activity in cell models



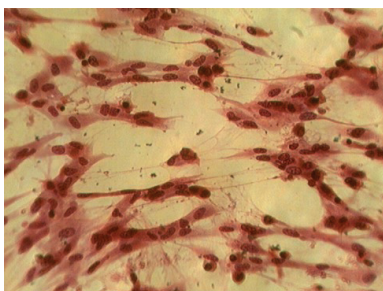
Boréaline® Expert shows a very high capacity to inhibit DCFH oxidation induced by the tBH in skin cells. Since very low concentration (0.0125%), the red maple bark extract inhibits 90% of the oxidation phenomenon in cells.

The protective effect of Boréaline® Expert following an oxidative stress was also evaluated by cell imaging. Human skin fibroblasts were both treated and untreated with 0.1% of Borealine(R) Expert®. The oxidative stress was induced by a dose of 125 µM of tBH during 4 hours. Cells were then set and coloured with Hema Stain. The pictures clearly show that the treatment with tBH is cytotoxic for skin fibroblasts in comparison with untreated control groups whose cellular layer remains compact. At a concentration of 100 µg/mL Boréaline® Expert has a nearly total protective effect on fibroblasts which keeps the viability.

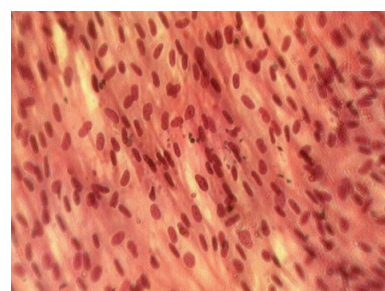
Boréaline® Expert effect on skin cells subjected to a powerful oxidative stress



Untreated



Presence of 100 µg/mL of Ascorbic acid
(control)



Boréaline® Expert 0.1 %

Boréaline® Expert assures a total protection against oxidation

Boréaline® Expert is a powerful antioxidant fighting extrinsic aging damages

3. ACTION AGAINST INFLAMMAGING

Chronic inflammation, known as silent inflammation, also appears as a major cause of premature aging and more particularly in sensitive skins. It is as well known by the term “inflamm-aging”. Multiple aggressions may generate free radicals which provoke the production of inflammatory markers that spread the inflammation and induce, over time, skin aging markers.

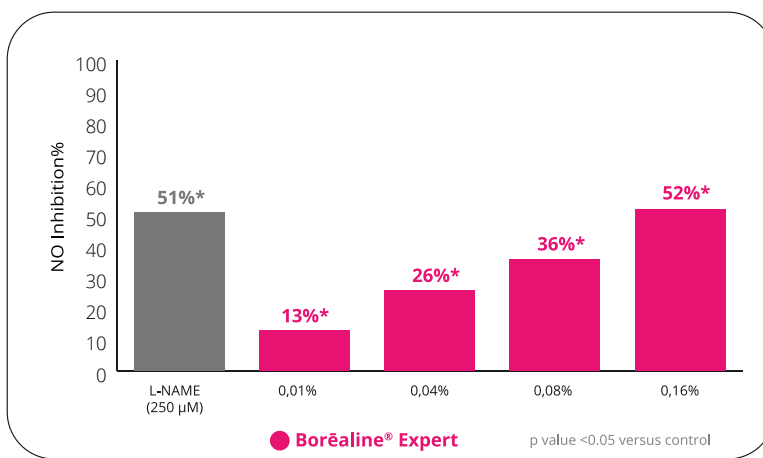
- The anti-inflammatory activity of Boréaline® Expert was evaluated on stimulated macrophages with the LPS inducing the production of nitric oxide (NO), major inflammatory marker.

PROTOCOL

Macrophages were treated with increasing concentrations of extracts. Afterwards, the LPS is added in order to stimulate the macrophages to produce NO. After a 24-hour incubation, supernatants are collected and the NO concentrations are dosed with the Griess reactive. The absorbance measures are taken and the NO production inhibition percentage of the extracts compared to the stimulated cells with LPS only is calculated. The results are expressed as a percentage of the oxidation inhibition of NO production. The anti-inflammatory efficacy is compared the L-NAME reference (Nitro-L-Arginine Methyl Ester hydrochloride), specific inhibitor of the NO production.

RESULTS

Nitric oxide inhibition by Boréaline® Expert



At 100 µg/mL Boréaline® Expert inhibits just over 40 % of the NO inflammatory marker production. This anti-inflammatory efficacy is superior to the L-NAME reference with a dose-response activity as well.

Boréaline® Expert has an anti-inflammatory action thus contributing to the overall fight of the aging process.

4. MECHANICAL RESISTENCE OF THE SKIN - ACTION ON CELL DIFFERENTIATION

The epidermal barrier is renewed as an average of every 4 weeks after desquamation of the Stratum corneum in order to maintain its integrity and cohesion. One of the major functions of the Stratum corneum is to control hydric flows and dermal absorption. Its main components are corneocytes, corneous envelope, as well as intercellular bilayer lipids. The corneous envelope is composed of strongly cross linked proteins, such as loricrin, involucrin and filaggrin that mostly provide the skin with mechanical resistance.

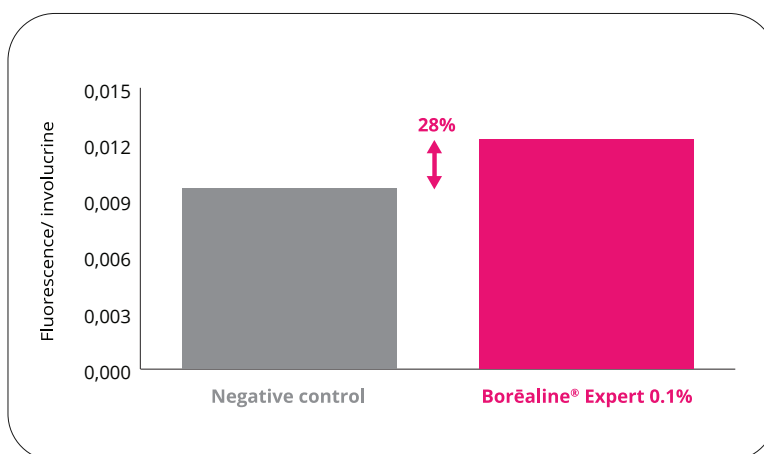
- Boréaline® Expert was tested on its ability to stimulate the involucrin production, major marker of the barrier function and the cell differentiation.

PROTOCOL

Skin keratinocytes were incubated with the presence and absence of the increasing concentrations of the extract. Cells were then set and incubated with a specific antibody against involucrin. Once fixed to the involucrin, the primary antibody is detected by a secondary antibody to which a fluorophore is grafted. The fluorescence emitted by the secondary antibody is then measured and pictures are taken. Results are expressed as percentage of the increased involucrin production by Boréaline® Expert compared to results obtained with untreated cells.

RESULTS

Involucrin activity by Boréaline® Expert

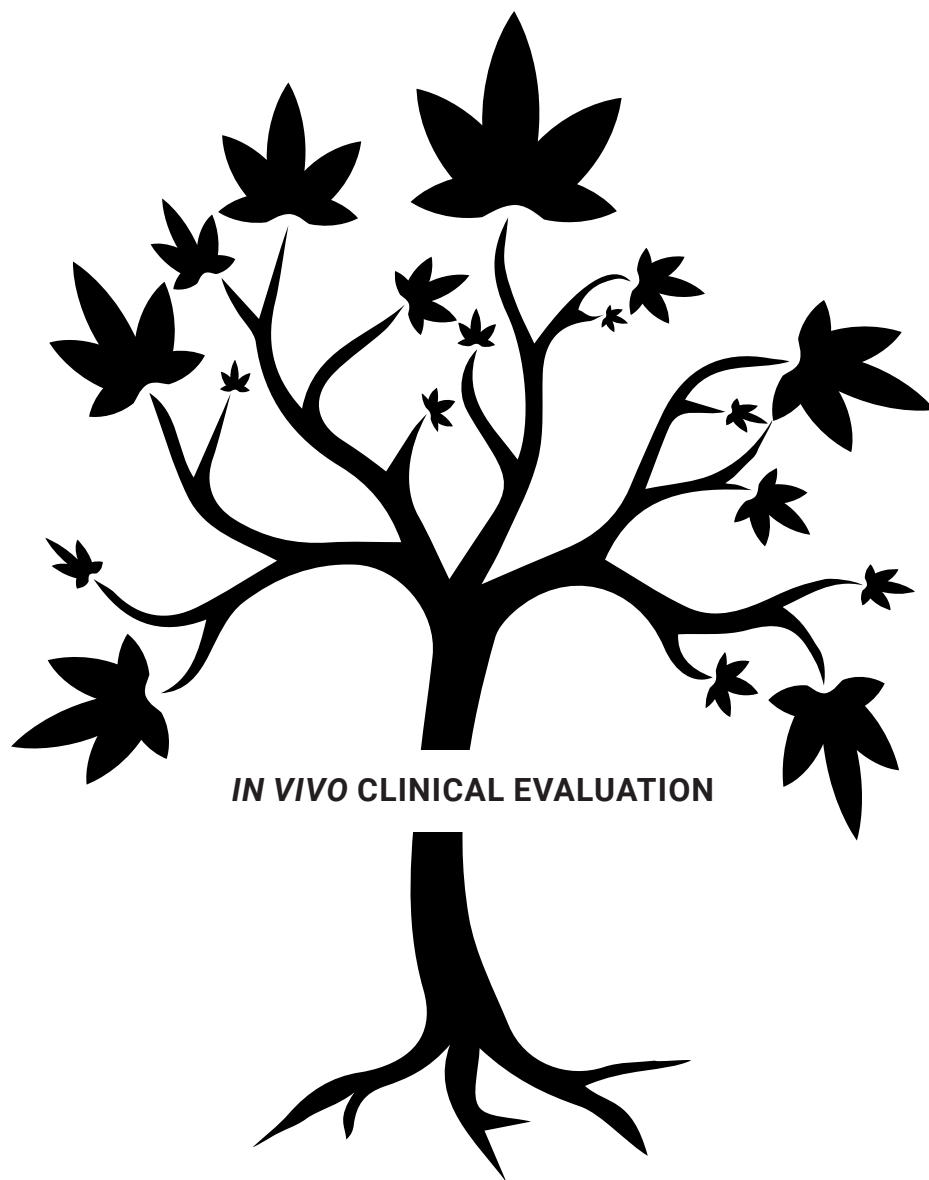


Boréaline® Expert significantly stimulates by 28% the involucrin synthesis.

Boréaline® Expert optimizes the keratinocytes differentiation to improve the mechanical resistance of the skin, increasing its integrity and hydration control.

4. SUMMARY OF THE MODES OF ACTION OF BORÉALINE® EXPERT

Targeted actions		Efficacy tests	Benefits for the skin
DERMAL REGENERATION	Collagen	Collagen synthesis in cell	<ul style="list-style-type: none"> Provides a plumping effect Restores skin resistance to tensions and tractions Fights against the appearance of fine lines and wrinkles
		Collagenase inhibition (collagen degradation enzyme)	
	Elastin	Elastin synthesis in cell	<ul style="list-style-type: none"> Ensures the maintenance of skin's elasticity Fights against the appearance of fine lines and wrinkles
OXIDATIVE STRESS	Antioxidant	ORAC	<ul style="list-style-type: none"> Antioxidant action Maintains the physiological balance Prevents signs of aging
		Lipid peroxidation	
		Cell models (DHCP-CA)	
	Free radical scavenger	Primary radicals : superoxide, hydrogen peroxide	<ul style="list-style-type: none"> Antioxidant action Fights against oxidative stress Reduces signs of aging by protecting
		Secondary radicals : hydroxyl, peroxy	
INFLAMMATION	Inflammation	Inhibition of nitric oxide	<ul style="list-style-type: none"> Soothing action
CELL DIFFERENCIATION	Involucrin	Involucrin synthesis in the cells	<ul style="list-style-type: none"> Protects the cutaneous barrier



Boréaline® Expert was subject to clinical studies allowing the evaluation in vivo of its anti-aging global efficacy.

PROTOCOL

- Tested material :

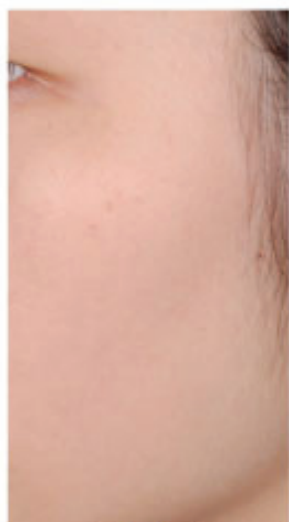
References	Cream A is a placebo, white basic cream. Cream B is the active cream containing 0.25% Borealine® Expert
Aspects	1. White emulsion. 2. White emulsion.
Storage	Ambient temperature.
Application frequency	Twice daily (in the morning and in the evening) during 56 days.
Application site	Face (focusing on the crow's feet and temples).
Application method	Apply the product on dry and clean skin of the face and massage gently until complete penetration under normal conditions of use.

- Studied population :

The study is carried on 34 subjects (with preselection on 60 subjects) in two parallel groups of 17 subjects, for the results on 30 subjects minimum.

Specific criteria
Sex : female.
Age : between 50 and 65years old.
Phototype : I to IV on the Fitzpatrick scale.
Subjects presenting all skin types.
Subject having wrinkles and fine lines on the crow's feet with grade from 4 to 6 on the Bazin® scale

Grade of wrinkles according to the Bazin® scale



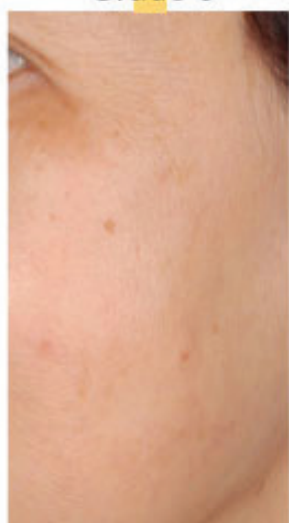
Grade 0



Grade 5



Grade 6



Grade 1



Grade 4



Grade 2



Grade 3

CLINICAL STUDY 1- GLOBAL SHORT-TERM EFFICACY (2 WEEKS TREATMENT)
SUBJECTIVE EVALUATION

PROTOCOL

Volunteers who tested the product evaluated its anti-aging efficacy after 14 days of using it twice a day.

The subjective evaluation was carried out through a questionnaire at D14.

The volunteers has to evaluate the efficacy of the product comparing signs of aging before and after. The product is applied twice a day in the morning and the evening on the face (focusing on the crow's feet and temples).

RESULTS

Evaluated criteria	Positive responses collected at D14
Flexibility	100 %
Hydration	100 %
Anti-wrinkle	88 %
Reduced signs of fatigue	94 %
Firmness	94 %
Improved skin texture	94 %
Softer skin	94 %
Reduced sings of aging	82 %
Diminished wrinkles	82 %
Pulped up skin	82 %

After of 2 weeks of use, volunteers were in majority satisfied about the ant-aging efficacy of the product. 88 % of volunteers recommend this product and are ready to continue using it.

The subjective evaluation showed that women using Boréaline® Expert cream perceived rapidly improvement of several signs of aging. For 88 % of them, the product has a real efficacy on wrinkle, firmness, elasticity and their skin was looking younger after only two-weeks of application.

Consumers using Boréaline® Expert products perceived a reduction of the skin of aging after only 14 days of use

CLINICAL STUDY 2- ANTI-WRINKLE SHORT-TERM EFFECT (1 MONTH TREATMENT)
DOUBLE-BLIND *IN VIVO* ANTI-WRINKLE EVALUATION

Skin texture (or cutaneous relief) and facial wrinkles are the earliest and the most important signs in skin aging. When we observe the cutaneous surface, we notice that it is traveled by many furrows and folds that form the skin microrelief. Furrows and plains modify themselves over the years. The deepness of furrows increases with age, the deepest ones accentuate, smaller ones disappear, and the cutaneous surface find itself distended and wrinkled.



PROTOCOL

The 3D PRIMOS® system (Phaseshift Rapid In vivo Measurement Of Skin) was used to evaluate the smoothing and anti-wrinkle effect of Boréaline® Expert on the cutaneous relief parameter R_t , representing the maximum amplitude of the relief.

This technique consists in calculating a phase image from images with interference fringe projection. This image then allows to determine the height of each point.

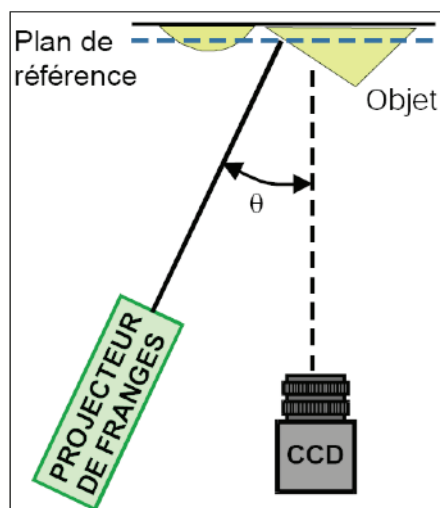
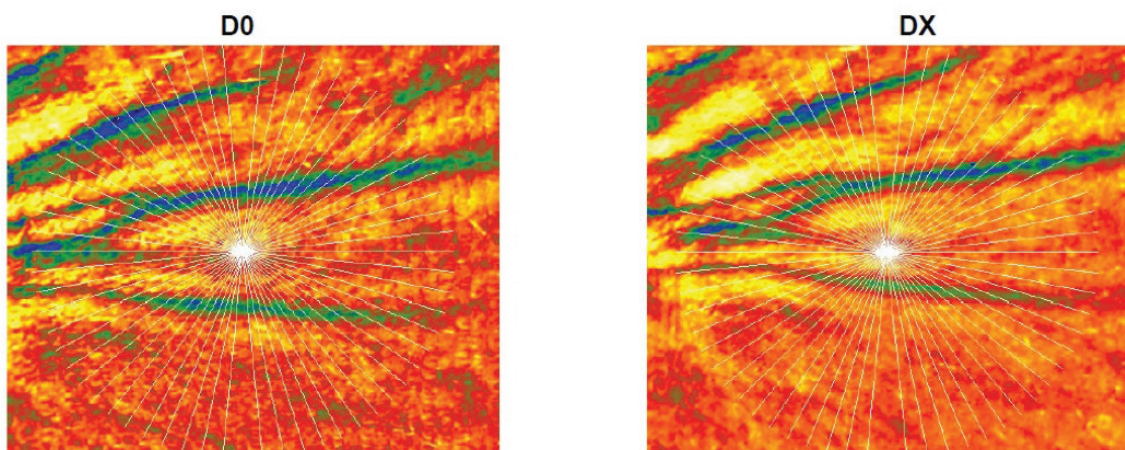


Image sensor CCD - Charge Coupled Device - arrangement of a plurality of light sensitive elements, each of which captures and allows to read an electrical signal proportional to the amount of light incident on it.

PROJECTOR FRINGES

The acquisition software allows to obtain 2D and 3D measurements and to determine parameters of the cutaneous relief on 32 radiuses distributed like a star on the zone of interest. An automatic system of repositioning allows the precise re-identification of the zone of measurement.

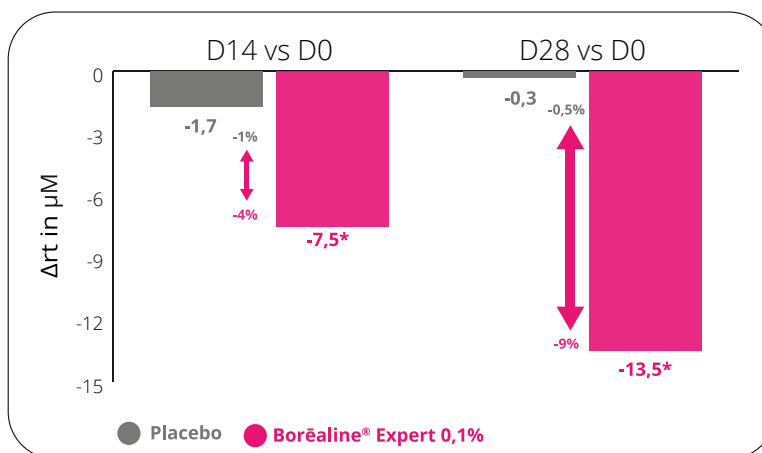


Rt is the height difference from the highest peak to the deepest valley within the total measurement section (ln).

A decrease in Rt characterizes an anti-wrinkle effect.

RESULTS

Anti-wrinkle efficacy of Boréaline® Expert versus placebo



After one month of using Boréaline® Expert cream, the Rt representative parameter of the wrinkle depth has diminished significantly, deep wrinkles are corrected and smoothed compared with skin before treatment. This effect was observed on 73 % of women and an average 9 % reduction of the wrinkle depth has been measured. However, on some subjects, the application of Boréaline® Expert cream allows the wrinkle reduction up to 28 %.

Boréaline® Expert has significant anti-wrinkle effect after 14 days and keeps on going after 28 days compared to placebo.

**CLINICAL STUDY 2- ELASTICITY AND FIRMNESS SHORT-TERM EFFECT
(1 MONTH TREATMENT)**

DOUBLE-BLIND IN VIVO EVALUATION EFFECTS ON SKIN BIOCHEMICAL PROPERTIES

PROTOCOL

The skin has two rheological properties :

- a visco-elastic behavior with high elastic component,
- a natural tension which varies depending on the geographical inter-zones variations.

This tightness is bound to a directional networks lines of the skin (Langer network)

The rheological properties of the conjunctive tissue is linked to its structure: collagen fibers and elastin arranged in three-dimensional network play an important role.

In vivo measurements of the rheological properties of the skin by non-invasive methods are able to determine variations induced over time by active pharmaceutical, cosmetic or topical product.

The procedure used enables the evaluation of biological extensibility and elasticity variations of the epidermis layers.

Measurement is done with a MPA 580 Cutometer® (Courage & Khazaka) connected to a computer.

The technique consists in skin aspiration by a measurement probe. These measurements define different parameters characterizing the biomechanical properties of tegument.

The skin is sucked into the orifice of a probe by constant vacuum pressure for a set length of time. The depth to which the skin penetrates into the probe is measured by two optical prisms located at the opening of the probe's orifice to eliminate the effects of friction and mechanical strain.

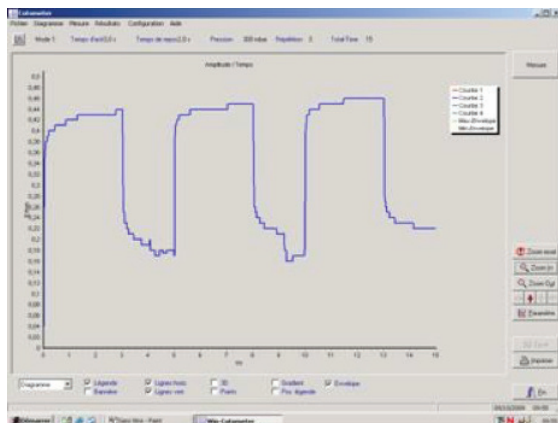
According to the principal claim searched (evaluation on skin firmness, elasticity and plasticity) and also according to the choice of the measurement zone, the adjustments of the Cutometer® probe differ :

Cutometer® probe adjustments

Measurement zone	Face (temples)
Cutometer® probe	2mm
Pression (constant vacuum pressure)	450mbar

Principal claim	Firmness, elasticity and plasticity
Type of inhalation	3 seconds
Time of relaxation	3 seconds
Consecutive cycles	3 repetitions

Consecutive cycles of three deformations of the skin measured using Cutometer®



Each measurement is an average of two acquisitions in close zones.

BASIC PARAMETERS :

The effects of Boréaline® Expert cream versus a placebo were evaluated on skin biomechanical properties with Cutometer® and according to the following rheological parameters :

- U_v : delayed elongation (viscoelastic component)
- U_f : final elongation (total deformation : elastic and viscoelastic)
- U_e : immediate elongation (purely elastic component)
- U_v : delayed elongation (viscoelastic component)
- U_r : immediate retraction (tonicity)
- U_a : recovered elongation at the end of the stress cycle

The analysed biomechanical parameters are the followings :

- Firmness :
 - R_0 (U_f) :

A decrease of this parameter features a tensing effect and a stronger skin.

- Elasticity :
 - R_2 (U_a/U_f) : total deformation. This parameter refers to the skin total elasticity. It is used along with R_7 in order to evaluate the skin elasticity and aging. As the value increases, skin is more elastic.
 - R_5 (U_r/U_e) : immediate elastic deformation. This parameter refers to the net elasticity of the skin. R_5 is a selection parameter which quantifies skin aging process. It represents the skin ability to regain its form after a deformation due to its elasticity, and that, independently of the skin thickness. This recuperation diminishes with age. Therefore, as the value increases, skin gains elasticity.
 - R_7 (U_r/U_f) : This parameter refers to the skin biological elasticity. This parameter, independent of the skin thickness, measures the skin capacity to regain its initial position after deformation. Thus, as the value increases, skin is more elastic.

An increase of either one of these three parameters features improved skin elasticity and demonstrates the ability Boréaline® Expert has to diminish skin aging signs.

We considered an average of this three parameters to represent effect on global elasticity.

- Tonicity or Elastic recovery
 - R_1 ($U_f - U_a$) : It represents the ability of the skin to return to its original state after total and long elongation.

A decrease of this parameter indicates skin elasticity improvement.

- Plasticity or viscoelasticity

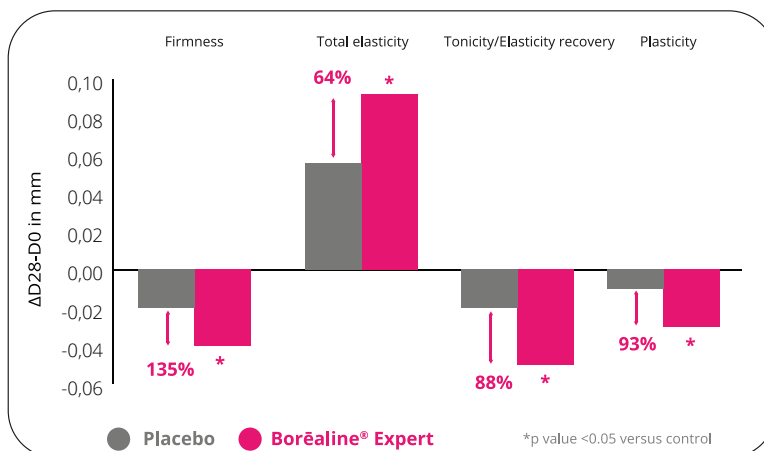
U_v ($U_f - U_e$) : It corresponds to the part of viscous deformation of the skin (due to collagen fibre of dermis) and tends to increase in aged skin.

A decrease of this parameter indicates skin plasticity improvement, therefore, skin has a younger aspect.

Boréaline® Expert cream (0.25%) was compared to the basic cream (placebo).

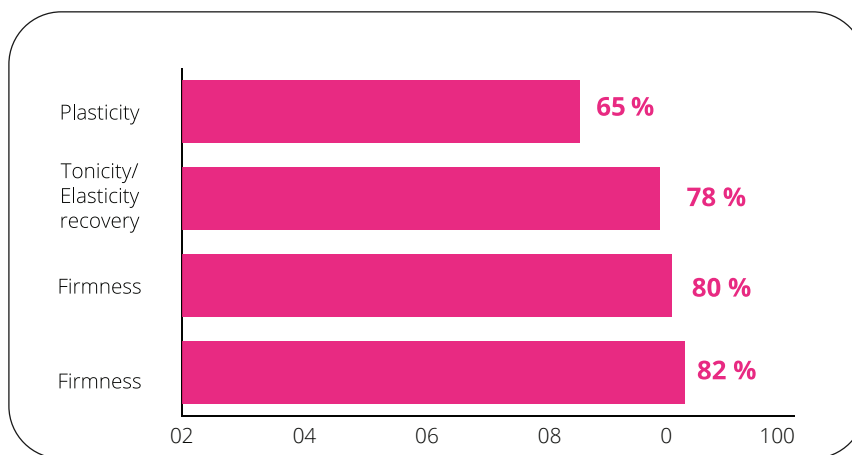
RESULTS

Effect of Boréaline® Expert on skin biomechanical parameters after 1 month treatment



Skin biomechanical properties have been significantly improved after only one month of using Boréaline® Expert cream compared to placebo. Significant improvement on all measured parameters have been measured.

% of women presenting a positive measured effect



For each parameter, more than 70 % of the subjects presented a positive measure showing firmer and more elastic skin.

Boréaline® Expert is a global anti-aging agent acting on skin elasticity and firmness and contribute to a younger looking skin.

CLINICAL STUDY 3- LONG-TERM VISIBLE EFFECT (2-MONTHS TREATMENT)
***IN VIVO* EVALUATION OF THE VISUAL EFFECT ON CROW'S FEET AREA**

PROTOCOL

The device used is the Visia® from CANFIELD® imaging systems.

The Visia® allows taking pictures in standard light and a very rapid capture of images.

The control of the repositioning takes place directly on data-processing screen using an overlay visualization of the images at each time of acquisition.

On D0 and after 14, 28 and 56 days of the product use, a photography of the full face at front is taken in normal light to illustrate the visual effect.

Anti-wrinkles effects of Boréaline® Expert



D0



D14



D28



D56

The pictures showed clear visual effect of using Boréaline® Expert cream. Wrinkles of the crow's feet area and fine lines under the eye were significantly reduced after only one month of treatment and have quasi-totally disappeared after two-months. This study permits also to demonstrate a continuous effect as well as a long-term effect of this active. Boréaline® Expert acts in depth on skin biomechanical parameters allowing a replumping effect on the skin. The skin looks smooth, firmer and younger.

Boréaline® Expert acts in depth to reduce wrinkles of high grade and fine lines. The skin is rejuvenated, replumped, firm and more elastic and its appearance is improved day after day.

	% of subject (yes)
Would you continue to use the product ?	88 %
Would you like to buy this product at the end of the study ?	88 %

Boréaline® Expert is a global anti-aging agent acting on wrinkles depth reduction and skin elasticity and firmness improvement.