Harnessing marine organic osmolytes for better ageing

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ABSTRACT

Marine life extends beyond the ocean waters to the rocky coastline. A living marine micro-ecosystem develops on this hard substrate: the epilithic holobiont. This supra-organism composed of phytoplankton and its microbiota is a source of natural organic moisturisers. By applying Phycosphere Biodynamic® Technology we biomimic the extreme environment where this holobiont lives. The organic osmolyte-rich extract is extracted to create Osmocean Phycoskin®, an original moisturising and dermoprotective bioactive ingredient reduces the impact of photoageing and restores the skin's natural well-ageing.

Marine life extends beyond the ocean waters, on the rocky coastline. A living marine micro-ecosystem develops on this hard substrate (epilithic), capable of adapting and molding its beauty to the rhythm of the changing tides.

Composed by species of microalgae and associated bacteria (microbiota), this microecosystem, which we call the epilithic holobiont, is of vital importance because it sustains the life of other organisms living on the rocky shore, providing them with food, refuge and protection.

For almost 12 hours a day at low tide, this micro-ecosystem is subjected to high solar radiation, extreme dryness and hypersalinity. With less than 10% water this fascinating microecosystem is able to survive, thanks to the mutual cooperation between these microorganisms, maintaining the cellular osmotic balance with its natural environment.

Microalgal and bacterial cells are required, in principle, to maintain an intracellular osmotic pressure higher than that of the external medium in order to generate cell turgor, generally considered to be the driving force for cell extension, growth and division.[1]

The production of low molecular weight molecules, called organic osmolytes or compatible solutes, by these microorganisms is one of the adaptive manifestations to achieve osmoregulation. Thus, there is a flow of osmolytic substances between phytoplankton and bacteria outside the cells in the phycosphere, an intimate site of metabolite exchange (Figure 1).[2]

The compatible solute answer to the problem of elevated osmolarity involves a dramatic increase of the production of these osmoprotective compounds given their compatibility with cellular functions at high internal concentrations. For this reason, they are often referred to as compatible solutes.[1]

Organic osmolytes are molecules which are compatible with cell metabolism even at molar concentrations and play protective roles in adaptation to extreme environments. These molecules have shown significant effects on biomolecules as stabilisers of native macromolecules (proteins or nucleic acid structures) and antioxidant protection. Organic osmolytes such as free amino acids, DMSP, polyols, i.e. glycerol, and saccharides have been identified in epilithic holobiont.[3]

Regulators mechanism on skin water homeostasis

Like epilithic holobionts, human skin has mechanisms to maintain cellular water homeostasis. It is a fundamental process that is vital for key functions such as cell growth and metabolism on human skin. Therefore, the skin must constantly balance water loss and hydration maintenance, as water levels are vital for maintaining structural integrity, i.e. cell volume, barrier function and immune defense, which impacts the healthy appearance of the skin.[4]

The skin has several molecules and mechanisms to maintain cellular water

homeostasis including aquaporins (AQPs), glycerol, natural moisturising factors (NMFs), lipids and tight junctions, as well as osmolytes.

- AQPs are water-permeable protein channels that allow movement of water across cell membranes having an important role in keratinocyte migration and cell volume regulation.[5]
- NMFs are free amino acids and their derivatives that act as humectants reducing the drive for water loss by absorbing water molecules in order to maintain optimal cellular hydration. Along with molecules such as hyaluronic acid and glycerol aid skin barrier function and provide skin elasticity.[6] External factors such as harsh cleansing routines and UVR exposure and internal factor like aged skin reduce NMFs content on skin via enzymatic breakdown of proteins.[7]
- Lipid composition and organization in the stratum corneum is essential for an effective water permeable skin barrier regulating diffusion levels and prevents excessive TEWL (trans epidermal water loss).[8]
- Tight junctions are cell-cell junctions that have been shown to form a paracellular barrier for solutes and water between epidermal cells by maintaining the electrochemical gradient and allowing only selective passage of ions and solutes.[9]
- When osmotic disruption occurs due to a physiological stressor these mechanisms aim to restore the cell to a homeostatic state via either accumulation or release of naturally occurring compounds called osmolytes.[10]

The cytoplasm of all organisms from bacteria to humans can contain high concentrations (10 to 100 mM) of organic osmolytes. In animal cells, these can be grouped into three classes: (1) polyols (glycerol, sorbitol and myo-inositol); (2) amino acid derivatives (taurine, alanine, glutamate and proline); and (3) methylamines (betaine, trimethylamine N-oxide (TMAO) and glycerophosphorylcholine. In humans the role of organic osmolytes has not been widely examined in many tissues apart from renal cells.

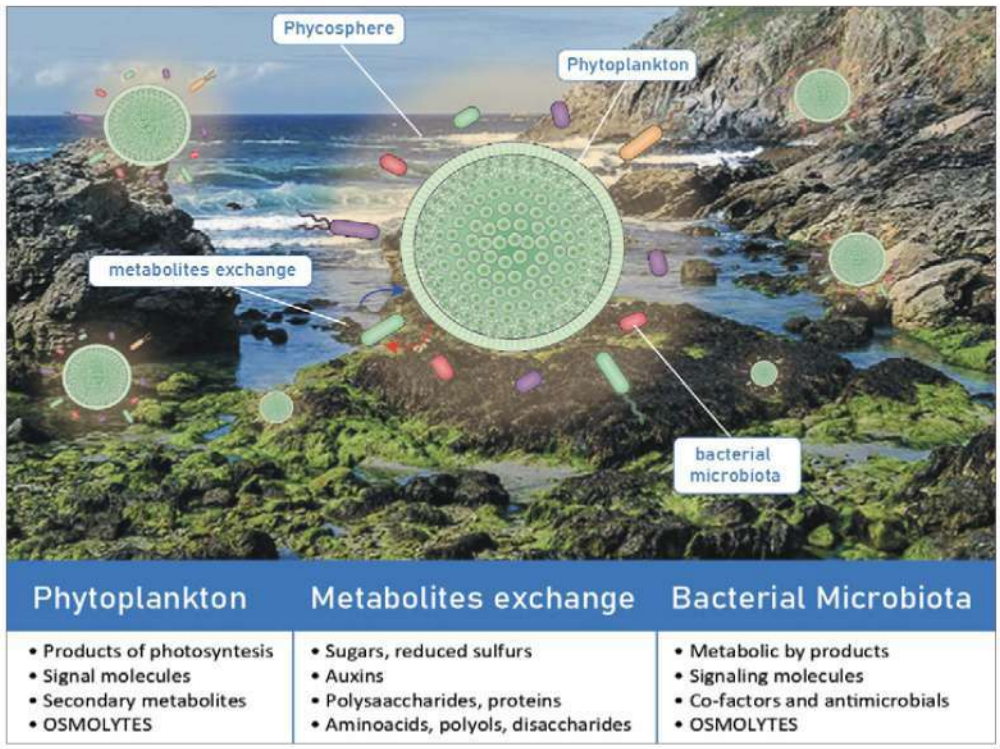

Figure 1: Organic osmolytes of epilithic holobiont interchanged by microalgae and its microbiota

Organic osmolytes strategy on skin repair

The main role of organic osmolytes is osmoprotection for cells by counteracting the effects of altered osmolarity in the surrounding microenvironment and to ensure vital cell function is not compromised.[4] Organic osmolytes respond to physiological stressors by adapting the synthesis, accumulation, and release into and out of cells. The main functions of osmolytes (Figure 2) are listed below.

- Cell volume regulation to maintain water homeostasis. These regulatory mechanisms exist to restore altered cell volume and these work through the accumulation or release of organic osmolytes. Upon cell shrinkage due to hyperosmolarity, the cells mechanism is employed to ensure an influx of water via the uptake of ions and an accumulation of organic osmolytes occurs through the respective transporters in order to restore cell volume.[11]
- The protein stabilisation via shifting the hydrophobic properties of the protein backbone and provide one mechanism of protection. Therefore, as skin is the outermost barrier to the external environment, age-related disruption of osmolyte strategy could impact, not only the appearance of the skin, but also susceptibility of the human system to external challenge or disease.[12]
- Organic osmolytes, have been shown to play a role in antioxidant mechanisms. When cells are exposed to physiological stressors, e.g. UVR, this can lead to accumulation of oxidative stress which is an imbalance of reactive oxygen species (ROS) in biological systems. Accumulation of ROS can lead to protein perturbation and lipid degradation, therefore, the antioxidant properties of organic osmolytes can counteract the effects of oxidative stress.[13]

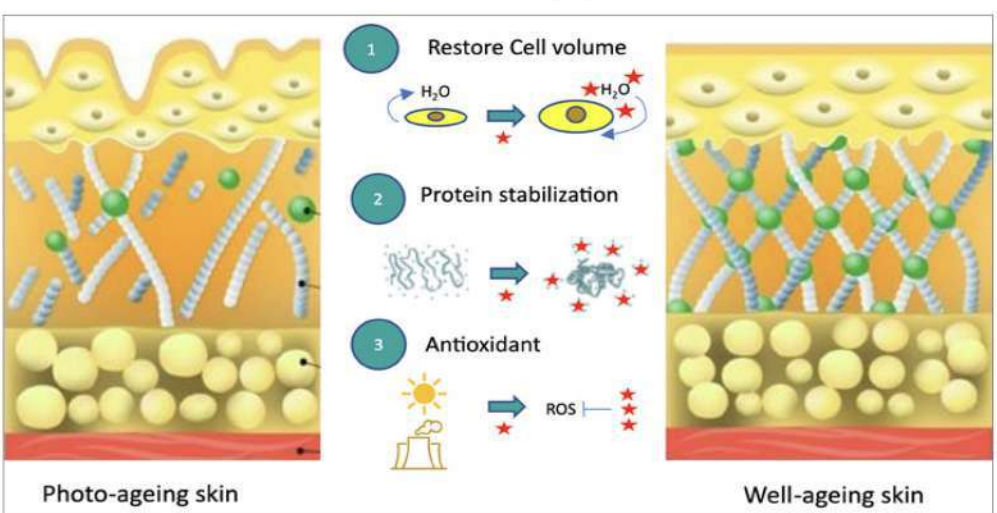

Figure 2: Model of organic osmolytes skin function and protection. Osmolytes (red stars) skin protection: 1. Restore cell volume; 2. Protein stabilisation; 3. Antioxidant protection

Osmoregulation affected by skin ageing process

Recent ageing research has directed the focus not only on age-related illnesses, but also healthy ageing. Skin ageing is associated with deterioration of tissue structure and function that leads to physical changes to the appearance of the skin such as wrinkles, loss of elasticity, dryness and hyperpigmentation.

Osmoregulation of human epidermal keratinocytes is disrupted with natural skin aged and photoageing. Therefore, this impacts skin hydration and impairs the ability of the skin to repair in response to physiological stress. Skin ageing specifically affects osmolyte transport mechanisms. Then it can cause the skin to become dehydrated and more

susceptible to damage, e.g. disrupted barrier function.[4]

Cellular hydration is fundamental in physiology cell function as it can play a role in adequate protein folding (native conformation), metabolic function and stress response. Alteration of cell volume lead an unstable form of the protein (non-native conformation) with consequent dysfunction and toxicity through processes such as aggregation, leading to various physiological disorders and diseases.

Moisturising as a first skin care well-ageing

Moisturising of the skin is recognized as the first anti-ageing skin care. Skin moisturisation is essential for its appearance, protection, complexion, softness, and the reinforcement of its barrier properties against deleterious and exogenous environmental factors. The intrinsic water binding capacity of skin is not only due to the complex natural moisturising factor present in corneocytes, but also to hyaluronic acid and a regulated water transport within the skin. With sebum, water in the stratum corneum (SC) plays an important role in keeping the barrier, the skin's surface, soft and smooth.[14]

Dry skin represents a sign of dysfunction of the outermost layer of the epidermis, i.e. the stratum corneum functioning as the skin barrier. This condition may start at any age due to UVR and other factors but usually becomes apparent with advanced ageing.[15]

Well-ageing is an ideal natural state of health, since it allows us to enjoy the passing of the years while maintaining a rejuvenated physical appearance and external beauty. To achieve this state of well-ageing, natural moisturising products would be used to help in normal conditions of balance to the skin (cellular redox homeostasis): a correct cellular hydration and balance between the levels of ROS and detoxification systems.[16]

Well-ageing bioactive ingredient base-on marine organic osmolytes

Inspired by the epilithic holobiont, we biomimic the environmental conditions in which this supra-organism lives. By applying a patented Phycosphere Biodynamic® Technology that includes bioengineering procedures, we manage to induce an increase in the production of organic osmolytes.

Amino acids, saccharides and polyols (Figures 3 and 4) were identified as a main organic osmolytes after distilled water

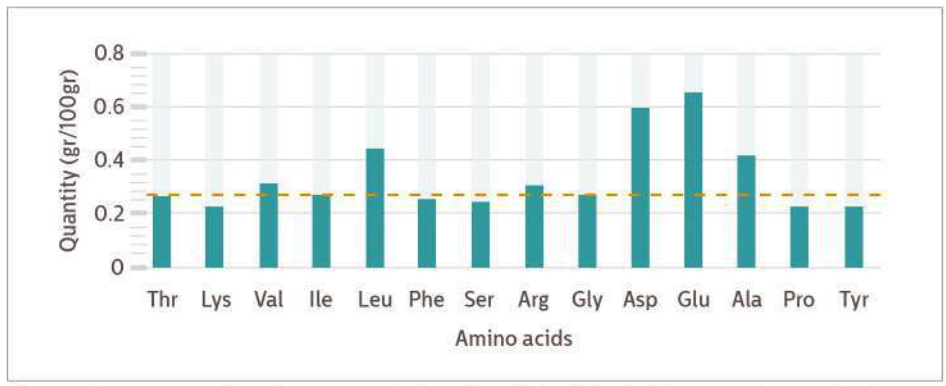

Figure 3: Organic osmolytes like aminoacids from Epylithic holobiont. Valine (Val), Leucine (Leu), Arginine (Arg) Aspartic acid (Asp), Glutamine (Glu), Alanine (Ala) most abundant (above line)

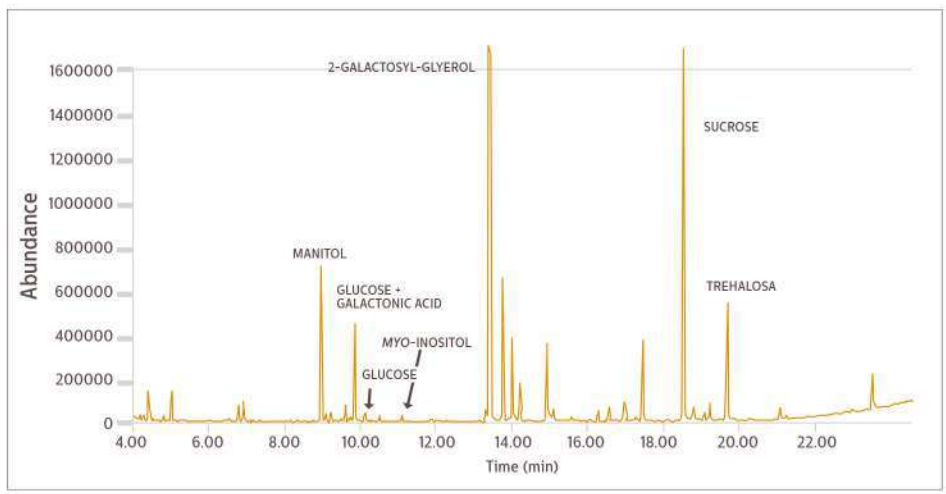

Figure 4: Organic osmolytes saccharides and polyols from Epylithic holobiont

extraction process of biomass of epilithic holobiont. This extract was used to perform Osmocean Phycoskin® (hereafter Osmocean PSK), a novel, natural well-ageing active ingredient base-on organic osmolytes.

In vitro studies are underway to confirm the preliminary hypothesis on the effect of Osmocean PSK with mechanisms to improve natural aged and photoageing skin, due to the contribution of organic osmolytes. However, it is well known that the organic osmolytes found have been described as molecules with beneficial properties for the skin.

Glycerol is considered the quintessential of organic osmolytes. This molecule has been included for many years in topical dermatological preparations due to its diverse actions on the epidermis. It includes improvement of stratum corneum hydration, skin barrier function and skin mechanical properties, inhibition of the stratum corneum lipid phase transition, protection against irritating stimuli, enhancement of desmosomal degradation, and acceleration of wound-healing processes.[17]

Recently, it has been demonstrated that glycerol fermentation by skin bacteria upregulates the expression levels of genes associated with the skin barrier function.[18] Evidence is presented to show that the free radical scavenging properties of mannitol provide it with a two-fold mechanism of action when combined with polysaccharides like hyaluronic acid.[19]

Osmocean PSK is expected to be launched in the fourth quarter of 2022 as an original moisturising and dermoprotective bioactive ingredient that reduces the impact of photoageing and restores the skin's natural well-ageing. Its natural organic osmolytes

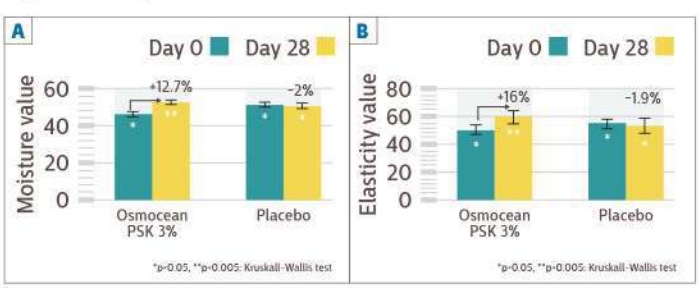

Figure 5: *In vivo* study of Osmocean PSK. Comparative values of moisture (A), elasticity (B) index of volunteers from Day 0 to 28 using active ingredient at 3%, twice a day

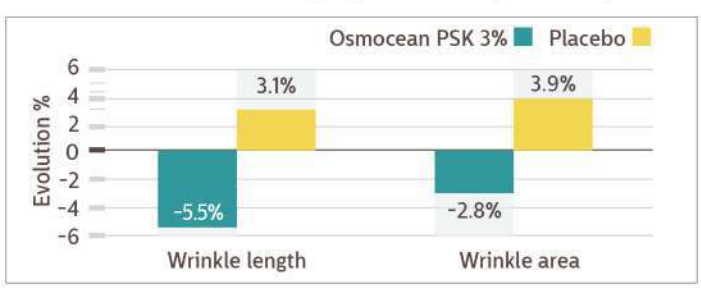

Figure 6: *In vivo* study of Osmocean PSK. Comparative values of evolution percentage of wrinkles length and wrinkle area of volunteers using active ingredient at 3%, twice a day

content helps to increase moisture and elasticity of skin.

Preliminary results of *in vivo* test of Osmocean PSK

Protocol

The clinical efficacy of Osmocean PSK bioactive was tested *in vivo* with a double-blind study. The studies were conducted with 40 volunteers aged between 35 and 75 years old, most of who had clear signs of skin ageing. A total of 20 volunteers applied the active ingredient at 3% and 20 volunteers applied placebo cream on the face, twice daily for 28 days.

Moisture and elasticity were measured on Day 0 and Day 28 by corneometer and cutometer, respectively, using a Microcaya multi-dermoscope. Additionally, eye contour wrinkles and facial expression lines were measured using ImageJ software.

Results

Osmocean PSK has a moisturising effect improving firmness and uniformity complexion of the skin and better well-ageing effect. As Figure 5 shows, moisture level was increased by 12.7% using Osmocean PSK meanwhile it was reduced by -2% in placebo treatment.

Elasticity was increased by 16% in volunteers using Osmocean PSK and reduced by -1.9% in volunteers using placebo cream. These results evidence an improvement in a lifting effect probably due to organic osmolytes content of Osmocean PSK.

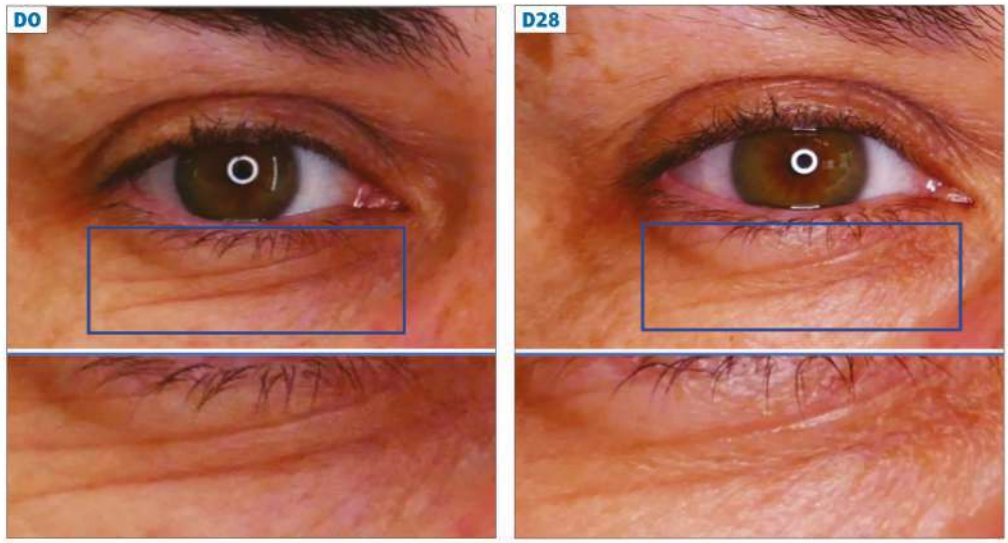

Figure 7: *In vivo* study of Osmocean PSK. Visual effect of Osmocean PSK on eye contour wrinkles D0 to D28

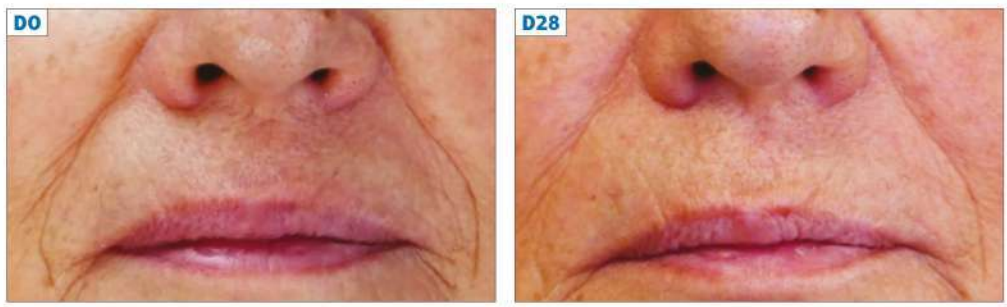

Figure 8: *In vivo* study of Osmocean PSK. Visual effect of Osmocean PSK on facial expression line D0 to D28

TABLE 1: WELL-AGEING CREAM WITH OSMOCEAN PSK

Phase	Ingredients	INCI	w/w (%)
A	Water	Aqua	q.s 100
	Propylene glycol	Propylene glycol	10.0
	Glycerin	Glycerin	3.00
	Vitamin B3	Niacinamide	2.00
	Xantan gum	Xantam gum	0.20
	Guar gum	Guar gum	0.20
B	Sunflower oil	*Helianthus annuus* seed oil	29.0
	Glyceryl stearate	Glyceryl stearate	5.10
	Vitamin E	Tocopherol	1.00
	Oramix G110	Caprylyl capryl glucoside	0.90
C	Osmocean PSK	Aqua, *Populus termuloides* bark extract, Plankton extract	3.00
D	Pentano-1,2-diol	Pentylene glycol	0.85
	3-(2-ethylhexloxy) propane-1,2-diol	Ethylhexylglycerin	0.15
E	Parfum	Parfum	0.20

Procedure: Solubilise Vitamin B3 in a few millilitres of water. Mix xanthan gum with glycerin and add it to the rest of the water under gentle stirring and heating at 45-50°C for 25-30 minutes. Add guar gum to the previous mixture while continuing to stir and heat. Finally add pentylene glycol to complete phase A, heating to 75ºC until perfectly homogenised. Stir until a homogeneous mixture phase B (leave tocopherol to the end) at 75°C. Disperse phase B in phase A and emulsify using homogeniser. Cool to room temperature, stirring constantly. Add the tocopherol, phase C, D and E to the previous mix phase A-B and emulsify until a homogeneous final mixture for 20 minutes with 3500 rpm

TABLE 2: EYE CONTOUR CREAM WITH OSMOCEAN PSK

Phase	Ingredients	INCI	w/w (%)
A	Water	Aqua	q.s 100
	Camomila extract	*Chamomilla recutita* flower extract	10.0
	Glycerin	Glycerin	4.00
	Vitamin B3	Niacinamide	2.00
	Vitamin C	Sodium Ascorbyl Phosphate	2.00
	Hyaluronic acid	Hydrolized sodium hyaluronate	0.30
	Xantan gum	Xantan gum	0.20
B	Argan seed oil	*Argania spinosa* seed oil	5.00
	Jojoba seed oil	*Simmondsia chinensis* seed oil	3.00
	Sucrose stearate	Sucrose stearate	2.00
	Vitamina E	Tocopherol	1.00
C	Osmocean PSK	Aqua, *Populus tremuloides* bark extract, Plankton extract	3.00
D	Verstatil SL	Aqua, sodium levulinate, potassium sorbate	1.00

Procedure: Stir until a homogeneous mixture phase A. Stirring and heating until a homogeneous mixture phase B. Disperse phase B in phase A and emulsify using homogenizer until a homogeneous mixture for 20 min. Cool to room temperature, stirring constantly. Add phase C at room temperature. Add phase D to phase A, B and C and emulsify until a homogeneous mixture for 25 minutes with 3400 rpm

Osmocean PSK has biomechanical repair properties that show visible wrinkle improvement and improved skin uniformity. The length and area of wrinkles around the eyes and facial expression lines (Figure 6) were reduced by -5.5% and -2.8% respectively after 28 days of Osmocean PSK use.

Volunteers using the placebo did not show a reduction in wrinkles, but an increase of 3.1% and 3.9% respectively. Visual evidence confirm the wrinkle reduction effect on the eye contour (Figure 7) and facial expression lines (Figure 8).

Formulation examples

The extraction process uses unique green chemistry principles and do not involve the use of environmental harmful chemicals. Thus, Osmocean PSK are non-sensitising, non-dermal and ocular irritating. This ingredient is over 100% natural origin content (ISO 16128). It is expected to achieve COSMOS-certified, Vegan shield and microbiota-friendly.

The main applications should be in dermocosmetics night and daily creams and serums and eye contour creams. The well-ageing action of Osmocean PSK will help to create an extraordinarily effective, natural and original end product with some marketing claims, such as:

- Moisture protection: repair of dry skin.
- Lifting effect: reinforcing the evenness of the complexion.
- Anti-wrinkle effect: repairing the eye contour and expression lines of the face.

As the examples in Tables 1 and 2 show, the formulation well-ageing cream and eye contour repair cream.

Conclusion

Amino acids, saccharides and polyols were identified as a main organic osmolytes in aqueous extract of the biomass of epilithic holobiont. These molecules are well-know recognized to play an essential role in skin osmoregulation strategies.

The active ingredient Osmocean PSK has a moisturising effect improving firmness and uniformity complexion of the skin and better well-ageing effect. It repairs biomechanical properties (elasticity) showing a visible improvement of wrinkles and better skin's uniformity. **PC**

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