CUSTOMER:

RECIPIENT:

IN SUBSTITUTION: N/A

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T A B L E O F C O N T E N T S

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1. INTRODUCTION

Natural sea salt production in salt marshes is a process where sea water circulates from basin to basin. Due to the influence of sun action and wind, water evaporates and salt concentrates into to the crystallization basin from which it is recovered (Figure 1).

Fig. 1. Salt production on marine basins

Some microorganisms, called *Halophiles*, have developed adaptation strategies that enable them to live in environments where salt concentration can attain even five times the sea salt concentration. Such microorganisms can be found in sea salt marshes and also salt lakes.

There seems to be little in common between a human being and a microorganism that lives in salt marshes, but they have a similar challenge: fighting against dryness. To compensate the deleterious effects of the extreme conditions halophile secretes exopolysaccharides and glycoproteins building a protective shield surrounding them.

This constitutes an interesting characteristic to consider these compounds as potential cosmetic ingredients for skin care or other high-value purposes within the pharma or agrofood fields.

2. BIOCHEMIZE'S PROCESS TO OBTAIN HIGH-VALUE NATURAL BIOPRODUCTS FROM SALT MARSHES

Biochemize S.L. has developed a new green process designed to recover the compounds presents on the environment where halophilic microorganisms live. Among the different compounds recovered, it is worth mentioning a concentrated extract with a high content in glycoproteins. According to several preliminary tests conducted by Biochemize S.L., this product has shown potential cosmetic applications that could be used as a moisturizing, UV-protection, antioxidant and chelating agent.

3. PRODUCT CHARACTERISTICS

The product obtained has been slightly characterized and studied.

3.1. Composition and Appearance

Biochemize's natural bioproduct is composed of proteins, polysaccharides and is free of microorganisms (Table 1):

	%content (w/w) final extract
TOTAL PROTEIN	**30 - 40**
TOTAL GLYCOPROTEIN	**5 - 10**
TOTAL SUGAR	**20 - 40**

Table 1. Main chemical compounds of the microbial extract.

The main features of this natural product are:

- Hygroscopic
- Light pink colored
- Water soluble
- Cotton like texture (Figure 2)

Fig. 2. Microbial extract

3.2. Safety

Non allergic and toxic effects are anticipated because Biochemize's natural biproduct is directly produced within the salt production process.

Preliminary allergy tests with a panel of 6 volunteers, aged 30 to 50, have been performed. Biochemize's natural bioproduct (1% aqueous solution w/v) was applied topically on the forearm twice daily for a week. No adverse or allergic reactions have been detected at the end of the treatment (Figure 3).

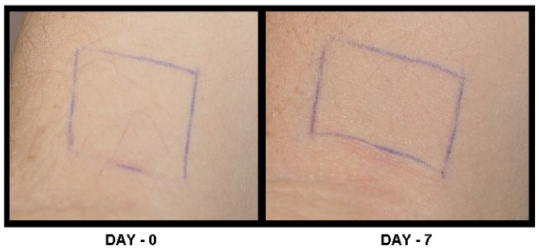

Fig. 3. Non-allergic effect on skin

Note: Biochemize's natural bioproduct **comes from a 100% natural source and no chemicals are used in the production process**

4. APPLICATIONS

Biochemize's natural bioproduct is initially intended to be used in cosmetic formulations to improve skin health, although other applications within the pharmaceutical and agrofood fields cannot be discarded.

Biochemize has conducted a number of laboratory tests to determine the product characteristics, performance and potential applications, which are described below:

4.1. Mosturizing

Moisturizing of the skin is recognized as the first anti-aging skin care. Hyaluronic acid (HA) is a natural hydrating agent considered as the gold standard for this kind of treatments. HA is involved in the structure and organization of the skin extracellular matrix (ECM).

There are many environmental factors that contribute to decrease HA content of the skin. External application with skin care products helps restoring the HA content and improve skin texture.

Biochemize's natural bioproduct can be explored as moisturizing agent according to the tests carried out by Biochemize by Dynamic Vapour Sorption (DVS) at 25ºC in comparison with HA. The experiment consisted in measuring the velocity and quantity of water absorbed by the test sample and HA when varying the vapour concentration to which the test items were subjected (Figure 4).

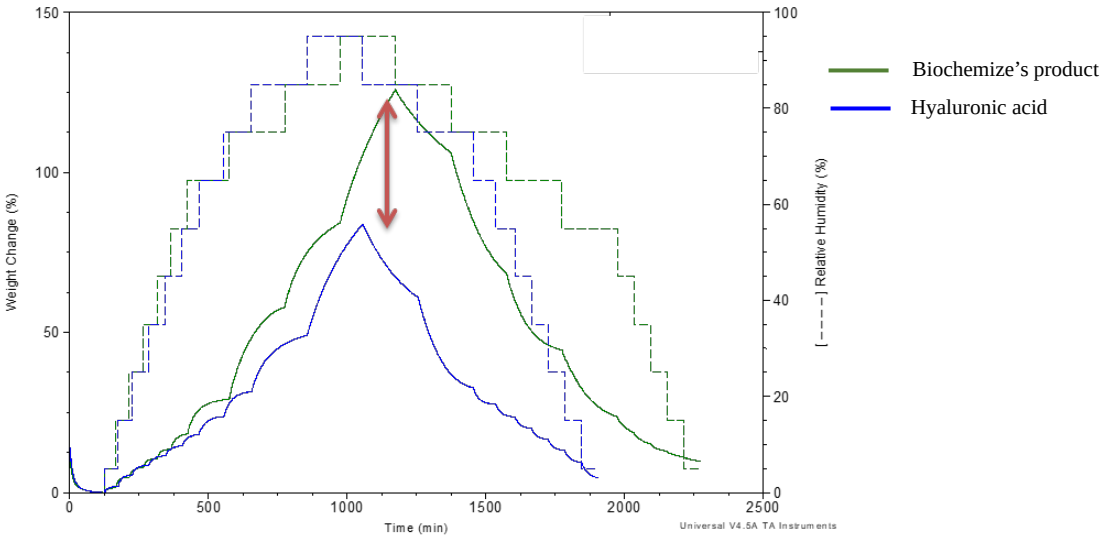

Fig. 4. Moisturizing capacity of the extract

Note: Biochemize's natural bioproduct **showed a better DVS profile than hyaluronic acid, retaining 45.7% more water at 95% RH**

4.2. UV Protection

Another skin aging factor is the solar radiation, particularly UV light. It is well known that HA acts also as a natural filter to protect skin against UV radiation, because it minimizes the effects of Reactive Oxygen Species (ROS) on the skin.

Biochemize's natural bioproduct presents a higher absorption in the UV range of 280-300 nm than hyaluronic acid which makes it ideal to improve natural skin UV-protection capacity (Figure 5).

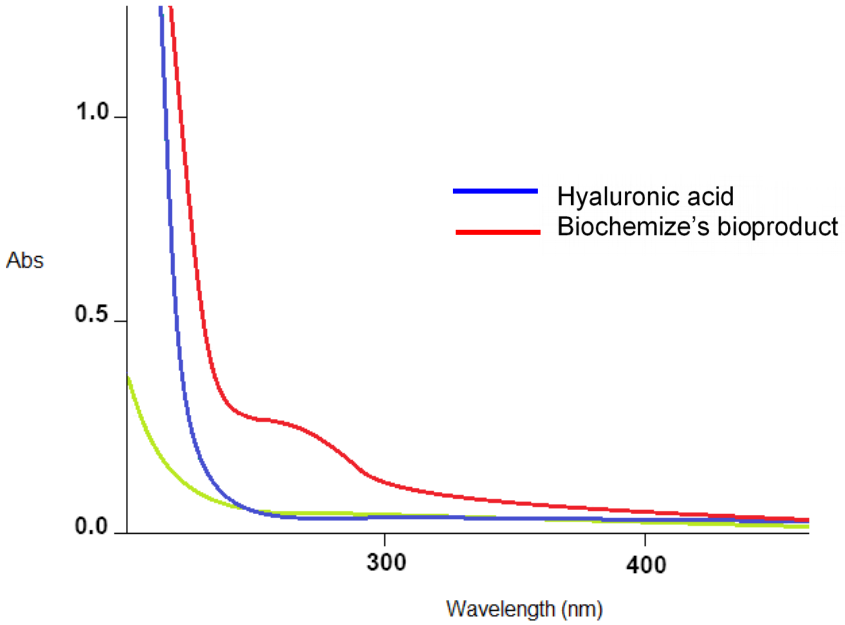

Fig. 5. UV-protection of the microbial extract

Note: Biochemize's natural bioproduct **could potentially block UV radiation better than hyaluronic acid**

4.3. Metal chelation

Allergic dermatitis to metal ions, for example nickel, may take place at any age and occurs once the metal has touched the skin. Once this allergy is developed, it persists for many years, often lifelong.

One approach in skin treatment for people with allergic dermatitis is to remove metal ions from the skin using creams or designed soaps containing agents with chelating properties (Figure 6).

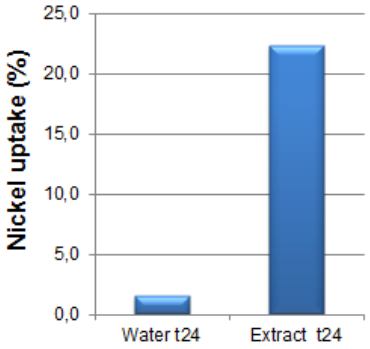

Fig. 6. Metal chelation capacity regarding nickel

Note:Biochemize's natural bioproduct has shown good Nickel chelating properties

4.4. Anti-oxidant

Skin aging is accelerated by reactive oxygen species (ROS), mainly generated by exposure to solar ultraviolet radiation (UVR), in a process known as photo-aging. Because cutaneous iron catalyzes ROS generation, it is thought to play a key role in photo-aging. Topical iron chelating agents might have a potential application in cosmetic formulations to remove harmful cutaneous iron excess.

Following-up the experiments carried out by Biochemize to test the chelating properties of its natural bioproduct, the chelation of iron was also assessed (Figure 7).

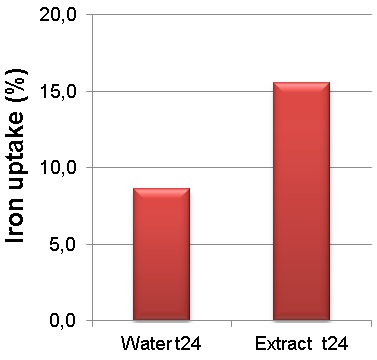

Fig. 7. Chelation of iron and anti-oxidant properties of the microbial extract.

Note: Biochemize's natural bioproduct has high affinity for iron and could potentially reduce free ROS generation in skin

5. FURTHER STUDIES AND APPLICATIONS

In vitro and in vivo studies are to be scheduled to be performed using different formulations in development in order to reinforce the results.

Biochemize's natural bioproduct can be fractionated into its different components and further studied to explore its potential use in a variety of applications.

Biochemize's natural bioproduct is an innovative source of natural compounds in the cosmetic and pharmaceutical field.

An important point to remark is that this process has been developed for the production of the microbial extract, as it has been described.

It is clear that each one of the different microbial strain species (alone and/or in consortium with other species) present in this type of water has developed its own strategies for the synthesis of different secondary metabolites, with specific metabolic functions each.

There is significant literature about it, and some of the molecules produced and its functions are reasonably well known (i. e. surfactins, UV-radiation absorbers, carotenes, etc.). From the microbial extracts obtained and/or directly through fermentative procedures where single or consorted microbial cells are grown up; a wide portfolio of products of natural origin can be obtained.

In this case and for each one of these metabolites of possible interest, an specific fermentation/ accumulation process using living microbial cells should be developed.

From this standing point, the industrialization step can be approached by the usage of a specific fermentation broth, based on the saline water medium; to support the growth and metabolites expresion of the target microbial flora, selected on the basis of the extract fraction which is pursued. On the top of all, the Biochemize's knowledge of the natural environment, where these biofunctional extracts happen, is a potent ally for the design of a controlled production process, and it's a powerful driver for speeding up the overcoming of the industrialization gap.