

EXOPOLYSACCHARIDE C2 (P.0231) FOR HAIR COSMETIC FORMULATIONS: ANTIOXIDANT ACTIVITY AND KERATIN SYNTHESIS INCREASE

ABSTRACT

Dryness, frizz and breakage are all normal age-related hair changes that can be prevented and fixed by incorporating **functional ingredients** in our **hair care formulations**. More and more hair products are incorporating these ingredients into their formulas and our studies demonstrated that **Exopolysaccharide EPS C2 (P0231) increases endogenous keratin synthesis** and has a **powerful antioxidant activity**, scavenging and detoxifying the cell from free radicals through the metabolic pathways of glutathione and 8-hydroxy-2'deoxyguanosine.

INTRODUCTION

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids and DNA.

Evolution has provided the human organism with protection mechanisms against the harmful effects of free radicals, based on a complex defense system formed by antioxidant agents. These can be enzymatic mechanisms termed endogenous antioxidant agents (including enzymes SOD, catalase, glutathione peroxidase, coenzyme Q) or **exogenous antioxidant agents**, which we are responsible for making them available to our cells. Thus, it is important that the industry provides appropriate antioxidant delivery products cosmetic or

alimentary that can fulfill our need for exogenous antioxidant agents.

On the other hand, it has been shown that **keratin-1** (*KRT1* gene), among others, is expressed in the stratum corneal and keratinocytes. Several mutations in *KRT1* are associated with hair alterations, functionally linking KRT1 with hair structure. **Hydrolysed keratin** has become a common cosmetic ingredient in hair care formulations, since studies have shown that topical application of gives significant increases in skin elasticity and hydration. Due to its moisturizing properties, hydrolysed keratin has also been incorporated into shampoo and conditioner. However, there is a limitation in this cosmetic used of keratin. In fact, larger keratin structures cannot penetrate the skin so cannot be used as moisturizers. For this reason, compounds **inducing endogenous keratin synthesis** would better serve this purpose.

EXTRACELLULAR POLYSACCHARIDES (EPS)

Extracellular polymeric substances (EPS) are high-molecular weight compounds secreted by microorganisms into their environment. EPS are mostly composed of polysaccharides (exopolysaccharides) and proteins, but include other macro-molecules such as DNA, lipids and humic substances. Exopolysaccharides generally consist of monosaccharides and some non-carbohydrate substituents (such as phosphate, acetate, succinate and pyruvate) and they have found diverse applications in cosmetic, food and pharmaceutical industries.

EFFICACY STUDIES

Inkemia IUCT and Bionos Biotech S.L. have assessed the efficacy of Exopolysaccharide EPS C2 (P0231) from Inkemia IUCT.

The effect upon the global oxidative cell state of **human keratinocytes** has been evaluated through a validated metabolomics analytic technique, according to the Food and Drug Administration (FDA) guidelines. 10 cellular metabolites (reduced and oxidized forms) involved in the cell antioxidant protection system, have been quantified through **HPLC/MS-MS**; allowing the integrative analysis of the antioxidant global capability with improved accuracy than widely used in vitro antioxidant chemical reactions (TAC, ORAC...). As shown in Figure 1, **EPS C2 (P0231)** at the concentration of 1 µg/ml significantly reduced the oxidized form of glutathione, **GSSG** (Glutathione disulphide), in a **94.36 ± 15.84 %**, without decreasing the reduced form, GSH (Glutathione). Thus, the ratio GSH/GSSG, biomarker of oxidative stress, is increased in a **13.90 ± 1.36 fold**. C2 (P0231) also showed activity upon metabolite **8OHdG** (8-hydroxy-2'-deoxyguanosine), a critical biomarker of oxidative stress and carcinogenesis. P0231 significantly reduced this metabolite in **83.62 ± 17.31 %** and **80.00 ± 17.36 %**, when added to keratinocytes in culture at concentrations of 1 µg/ml and 10 µg/ml respectively.

In parallel, it has been assessed the **capacity to induce endogenous keratin-1 synthesis**, after treating human keratinocytes with the product during 24 hours and quantifying keratin content by **ELISA**. Results, shown in Figure 4, indicated that Exopolysaccharide EPS C2 (P0231) significantly **induced keratin-1 synthesis** by **39.8 ± 10.5 %**, after treating cells with 1 µg/ml.



SAFETY PROFILE

- **Patch Test and HRIPT:** Non-irritating and very good cutaneous compatibility.
- **HetCam:** No ocular irritating effects.
- **Ames Test:** Non-mutagenic nor promutagenic.
- **Phototoxicity:** No phototoxic effects.

DISCUSSION AND CONCLUSION

Antioxidant compounds such as Vit-C, Vit-E and retinol among others, have been proposed in cosmetic industry over the last decade, as functional ingredients for antiaging formulations and to prevent and modulate oxidative skin damages. However, the skin is not the only part of our bodies that should be treated with anti-aging ingredients. It has been suggested that flooding the hair with antioxidants prevents hair loss, since certain ones, like green tea, blueberries, grape seed extract and vitamins, are thought to promote hair growth and prevent the follicle from shrinking. At the same time, inducing keratin synthesis is an adequate mechanism to improve hair appearance (health and nourishment), to increase strength and resistance and to avoid hair alterations.

More and more hair products are incorporating these ingredients into their formulas and our studies demonstrated that **Exopolysaccharide EPS C2 (P0231) increases endogenous keratin synthesis** and has a **powerful antioxidant activity**, scavenging and detoxifying the cell from free radicals through the metabolic pathways of glutathione and 8-hydroxy-2'-deoxyguanosine. At the same time, security studies confirmed EPS C2 has a **very good cutaneous compatibility** and shows **no toxicity or security problem for cosmetics**. For this reason, we proposed its use as ingredient in hair cosmetic industry, showing several advantages over other compounds.

ADVANTAGES & APPLICATIONS

- **Powerful antioxidant activity** demonstrated according to FDA guidelines.
- **Oxidative stress pathways** through which it is exerting a **functional benefit** are **identified**.
- **Non-toxic** and biodegradable polymers.

- **Hair appearance** (health, nourishment and resistance) improved by inducing endogenous **keratin synthesis**.
- Dermatologically tested: **Non-irritating** and **very good cutaneous compatibility**.
- **Easy to produce** in single strain systems and **low cost** cultivation conditions (short fermentation processes).

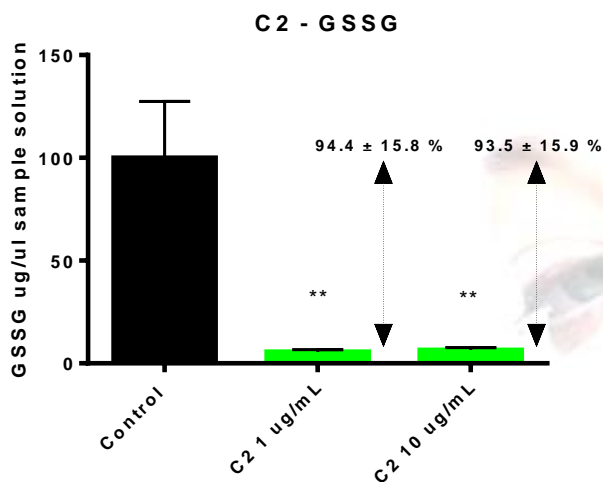


Figure 1: Graphical representation of the concentration of glutathione disulphide (GSSG), after treating cells with EPS C2 P.0231 at 1 µg/ml 10 µg/ml.

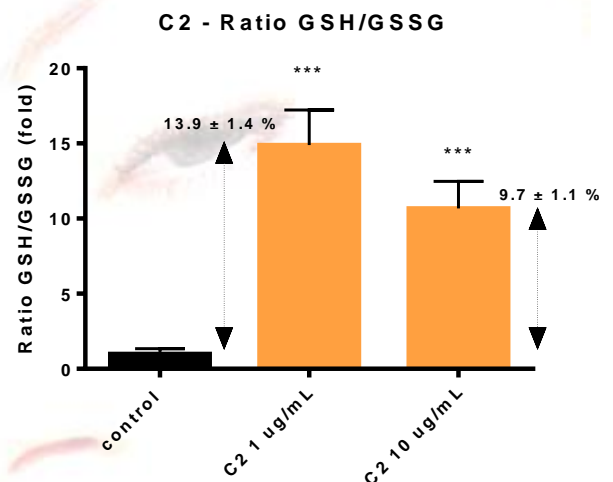


Figure 2: Graphical representation of the ratio GSH/GSSG, after treating cells with EPS C2 P.0231 at 1 µg/ml 10 µg/ml.

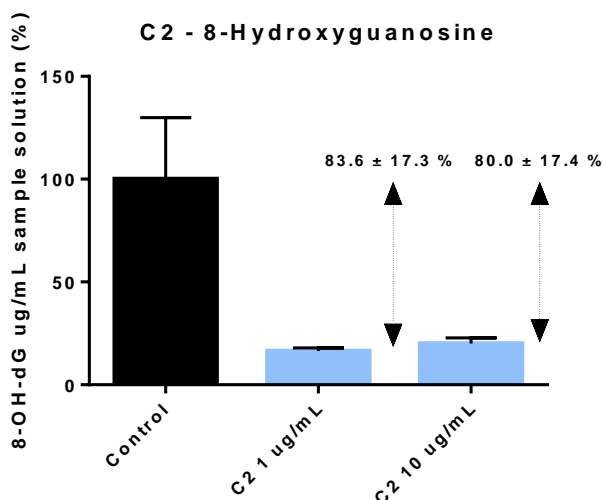


Figure 3: Graphical representation of the concentration of 8OHdG, after treating cells with EPS C2 P.0231 at 1 µg/ml 10 µg/ml.

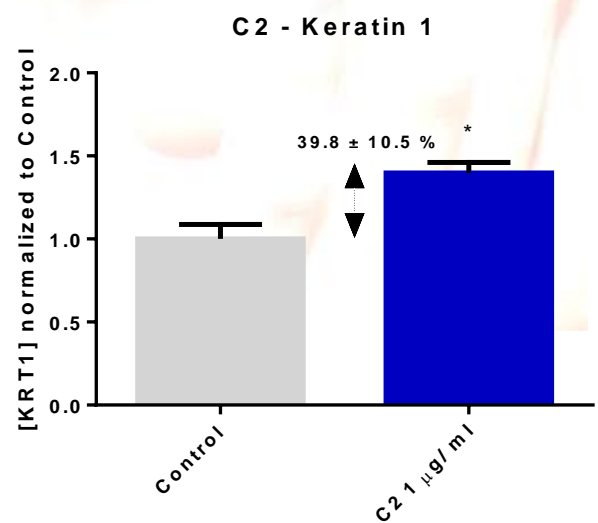


Figure 4: Graphical representation of the concentration of KRT1, after treating cells with EPS C2 P.0231 at 1 µg/ml 10 µg/ml.