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ENZYMATIC AQUEOUS EXTRACTION: AN ALTERNATIVE AND EFFICIENT METHOD TO REMOVE CYANOGENIC COMPOUNDS FROM PLUM KERNEL EXTRACT

INTRODUCTION

Perfectly suited for skin care products, plum kernel oil possesses antioxidant, moisturizing, soothing and protective properties which help to prevent signs of ageing. Despite apparent benefits, opportunities for new high value extracts remain very limited because of high levels of cyanogenic compounds contained in kernels of Prunus genus that release hydrogen cyanide upon breakdown. Among the most described, amygdalin, is freely soluble in water. This hydrophilic character often presents drawbacks and therefore limits the use of water-based cosmetic ingredients obtained from Prunus.

Biolie has developed and patented[1] an innovative enzymatic process dedicated to the biorefinery of plant raw material in aqueous media. Thanks to Screenzym®, a bio-targeting platform dedicated to the formulation of specific enzymatic mixtures, the extraction process procures the greatest actives compounds from the plant. Moreover, the soft conditions of extraction help preserving the integrity and the activity of biomolecules, and the enzymatic process yields to the release of fractions enriched in exclusive biomolecules.

In the framework of developing an original cosmetic active ingredient from the seeds of French Ente plums, we have relied on this innovative technology in order to investigate the effect of the processing on cyanide concentration. Our strategy was to integrate a full proteomic analysis, particularly well adapted to a better understanding of the plum extract activity on the skin, including potential toxic effects.

Key words : Enzymatic Aqueous Extraction, Proteosome, cyanogenic compounds, Plum

Structural Analysis of plant cell walls – Bibliography
Study of the plant components (polysaccharides, phenols, proteins, oil…)

1

Design of a customized enzymatic cocktail
Screening of enzymes and formulation depends on yield, efficacy, process, costs, ….

2

Enzymatic Aqueous Extraction
Optimization of physico-chemical parameters

3

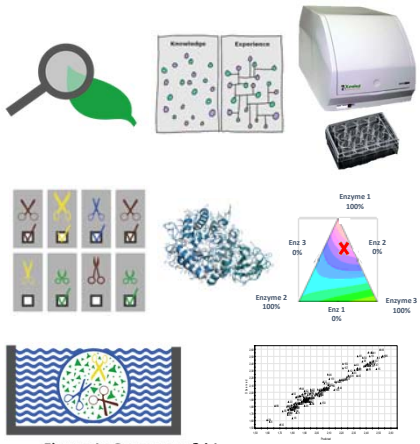

Figure 1 : Screenzym® bio-targeting tool for Enzymatic Aqueous Extraction (EAE)

METHODS

1. Enzymatic extraction

The knowledge of the chemical structure of the cell walls allowed to design targeted enzymatic mixtures. Then, plum almonds were crushed to the right milling to enhance enzymes-substrates interactions. The enzymatic hydrolysis was performed with temperature control and monitored on-line thanks to dedicated analytical method. Finally, a physical separation was done thanks to centrifugation. Calculation of oil yields and HPLC characterization of the aqueous phase allowed to define the most effective enzymatic cocktail. Total cyanide content was measured by HS-GC/MS.

2. Proteomic analysis

Skin explants were obtained from a 40 year-old female donor and were treated with the plum extract at different concentrations. Total peptides were extracted and the proteomes of pooled skin samples were characterized. High resolution LC-MS/MS allowed to identify and quantify thousands of proteins. Huge amounts of data were submitted to the bioinformatics tools CORAVALID™, dedicated to identify potential effect and define relationships between proteins, biological processes and concepts. Protein abundance ratios of the treated samples were compared with untreated samples.

RESULTS

Total polyphenols (g/L)	4.604
Reducing sugars (g/L)	85.23
Total protein (g/L)	189
Cyanide (mg/mL)	4.507

Figure 2 : Characterization of the aqueous phase
The very low levels of cyanide in the aqueous extract obtained by enzymatic process limit the possible risk associated with cosmetic use (dry matter : 28%)

	Oily phase (EAE)	Control (cold pressure)
Saponification index	182,4	178,1
Peroxide index	1,6	8,6
Acid index	1,8	1,6
Acidity (%)	1,6	1,4
Iodine value	91,2	86,8

Figure 3 : Charaterization of the oily phase vs control obtained by cold pressure
The oil obtained by enzymatic extraction has been compared with a control almond oil : the enzymatic process produces a similar oil with the exception of the peroxide index. Our oily phase is more stable over time, probably due to a greater extraction of antioxidant compounds. The fatty acid profile is quite similar to that of the control oil (Data not shown)

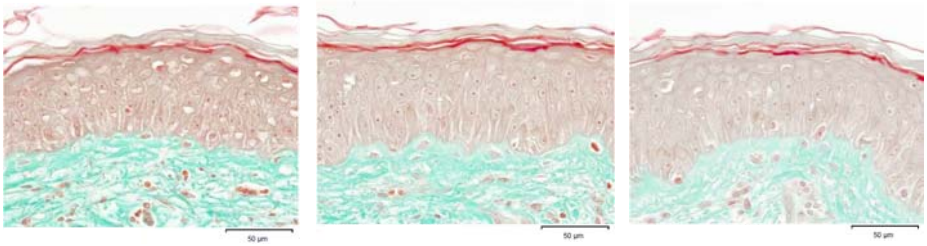

Figure 4 : Cell viability and general morphology
J8 : at 0,5% (P1) and 1% (P2), the plum aqueous extract exhibits a slight dermal activity by regulating the density of the dermal extracellular matrix

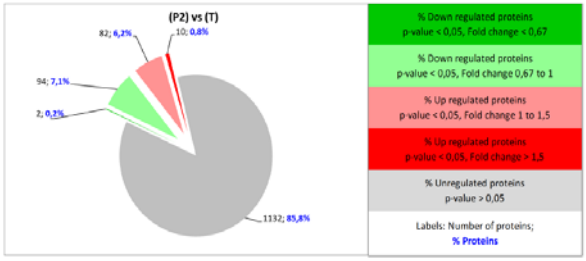

Figure 5 : Proteins distribution according to their p-value and fold change (FC)
The expression of 1% of the quantified proteins appears to be significantly different in the P2 explants compared to control group T (p-value < 0,05 ; FC > 1,5 or < 0,67).
10 proteins are overexpressed
2 proteins are under-expressed
In addition to enrichments, the elements point to regulation of proliferation, apoptosis, cytokine and Wnt signaling pathway. The first results tend to a plum extract regulatory role of skin barrier homeostasis.

DISCUSSION AND CONCLUSION

In one step, two ingredients were generated with the enzymatic process: an oily active ingredient with high concentrations of mono and polyunsaturated acids and an aqueous extract enriched in water-soluble active molecules such as polyphenols, reducing sugars and proteins. In this study, we provide evidence that enzymatic aqueous extraction is effective for cyanogenic glucoside removal because of cell compartments ruptures, thus allowing direct contact between cyanogenic compounds and the enzyme that catalyzes the hydrolytic breakdown. Proteomic analysis (still in progress) identified new candidate biomarkers and will lead to a better understanding of the underlying biological mechanism regulated by the new botanical extract. In view of the first results, it seems that through a homeostasis effect the ingredient helps to protect skin and leads to the regulation of the density of the extracellular matrix and tissue repair.

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[1] Muniglia L., 2010, Enzyme Method of Extracting Oils and Proteins from Vegetable Matter in an Aqueous Medium, WO/2011/045387