Green technologies for the valorization of medicinal plants residuals

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The development of sustainable procedures for the **valorization of industrial biomass wastes** represents a major challenge for the scientific community[1]. Among biomasses, medicinal plants residuals could represent an **attractive source of bioactive compounds**. In this context, two residues from the industry of medicinal plant extracts, have been selected in our work, namely: ***Cucurbita pepo L.*** seeds and ***Serenoa repens L.*** fruits, whose oils are commercialized for the **treatment of genito-urinary tract pathologies**.

***Cucurbita pepo L.* seeds (commercial oil compositions)[2,3]**

Triglycerides: ~ 90%
Free fatty acids ~ 8%
Sterols, Carotenoids ~ 1%

Most abundant fatty acids:
Oleic and Linoleic acid
(~ 80% of total Fatty A.)

***Serenoa repens L. fruits* (commercial oil compositions)[4]**

Most abundant fatty acids:
Oleic and Lauric Acid
(~ 75% of total Fatty A.)

Free fatty acids ~ 85%
Triglycerides: ~ 8%
Alcohol. Sterols ~ 1%

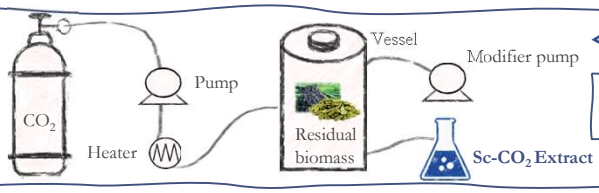
***Cucurbita pepo L.* residuals valorization through:**

Commercial **oil**

***Serenoa repens L.* residuals valorization through:**

Supercritical CO_2 technology (sc-CO_2), employed as highly selective **green** methodology that allows recovery of targeted compounds, without chemical degradation and limited operational costs, enabling **further extractions** by tuning the physico-chemical parameters of the process affecting the **supercritical fluid polarity and hence the extractable compounds**

Tuning the CO_2 density (by changing pressure and temperature) leads to ***selectivity***



The presence of low percentages of a co-solvent such as EtOH enables the extraction of ***more polar species***[5]

Extractables collection and characterization from exhausted biomasses

Three main fractions with different composition have been collected from each residual biomass. Firstly, in mild conditions, **an exhaustion of fatty acids** content was achieved. Then, increasing the CO_2 density by tuning the adopted temperature and pressure, **neutral fractions rich in alcohol components have been collected.**

Fatty acids and sterols have been characterized after their derivatization through Gas Chromatography-Mass Spectrometry (GC-MS) and the relative abundances of revealed species were calculated as relative area % detected under each peak. **Polyprenols** have been identified and quantified through Ultra-High Performance Liquid Chromatography (UHPLC) analysis.

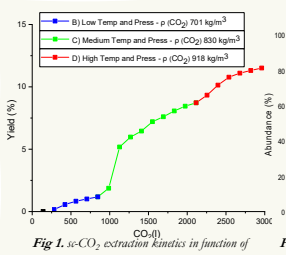


Fig 1. *sc-CO_2 extraction kinetics in function of used CO_2 of Cucurbita pepo extracts.*

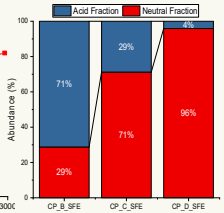


Fig 2. *Obtained fractions comparison between acid and neutral components abundances*

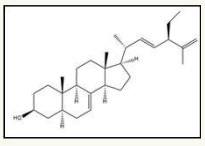


Fig 3. *Chemical structure of Δ7,22,25-Stigmastatrienol, the most abundant sterol observed*

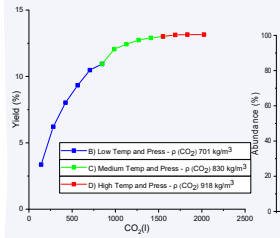


Fig 4. *sc-CO_2 extraction kinetics in function of used CO_2 of Serona repens extracts.*

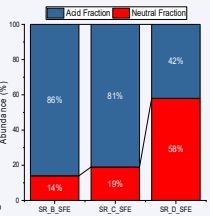


Fig 5. *Obtained fractions comparison between acid and neutral components abundances*

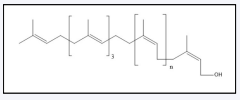


Fig 6. *General structure of polyprenols present in Serenoa repens extracts (n=1, n=2, n=3)* [6]

Extracted fractions from *Cucurbita pepo* exhausted seeds

CP_B: Fraction enriched in free fatty acids; low content of sterols (<1%).

CP_C: Fraction enriched in neutral components such as **triglycerides** (~ 90%) and **Δ7 sterols** (~ 5%), known to be present in cucurbitaceae species.

CP_D: Fraction enriched in triglycerides (~ 95% of total fraction) and **low content of free fatty acids**

Extracted fractions from *Serenoa repens* exhausted fruits

SR_B: Fraction mostly composed by **free fatty acids**

SR_C: Fraction enriched in free fatty acids, triglycerides and in particular unsaponifiable matter such as Δ5 sterols (~5%)

SR_D: Fraction enriched in alcohols such as sterols (Δ5 sterols ~1%) and **polyprenols,** a class of unsaturated isoprenic alcohols (~4%)

Enzymatic conversion of CP_B fraction

Free fatty acids of extracted fractions provide an opportunity to obtain value-added products such as **hydroxy fatty acids**, molecules with an improved hydrophilic character, making them **appealing to cosmetics, paints and food industry**[7].

To this aim, the **Oleate hydratases A2 (OhyA2)** from *Stenotrophomonas maltophilia* was cloned and expressed in *E. coli* to evaluate its activity in the hydration reaction of commercially available fatty acids (pure oleic acid) as well as of fatty acids of the **CP_B** fraction (enriched fraction mainly containing both oleic acid and linoleic acid).

Thanks to the presence of the hexahistidine (His) tag, OhyA2 was purified from lysed cells using Ni-NTA affinity chromatography. However, the purified enzyme resulted not to be active in the hydration of the target substrates under the conditions reported in literature. Therefore, whole-cell biotransformation was considered as a possible alternative.

Optimal conditions to produce **10-hydroxystearic acid (10-HSA)** using recombinant *E. coli* cells containing Ohy are described in literature[8]. Reactions with oleic acid standard (Fig. 7) and **CP_B** fraction (Fig. 8) determined the formation of **a precipitate**. The product formation was monitored by TLC. As shown in TLC, **10-HSA** and probably other hydroxy acids were obtained (Fig. 8; lane 2). Further attempts to increase product yields are ongoing.

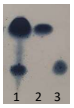


Fig 7. Lane 1: reaction; lane 2: oleic acid; lane 3: 10-HSA.

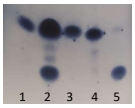


Fig 8. Lane 1: **CP_B**; lane 2: reaction; lane 3: oleic acid; lane 4: linoleic acid; lane 5: 10-HSA.

Δ7-sterols, presents at high concentration in fraction **CP_C** are known to **positive influence the prostate metabolism**[3]. Furthermore, they competitively reduced the binding of Dihydrotestosterone (DHT) which is implicated in the development of benign prostate hyperplasia (BPH) DHT to human fibroblasts[3].

Polyprenols enriched fraction SR_D , can act as **antiviral agents and positively influence the immune system**[6].

[1] Tuck C.O. et al. (2012) *Science*, 337, 695-699 [2] Rezig L. et al. (2012). *Ind Crops Prod*, 37, 82-87. [3] Procida G. et al. (2013). *J. Sci. Food Agric.*, 93, 1035-1041. [4] Marti G. et al. (2019). *Molecules*, 24, 2208-2222. [5] Viganó J. et al. (2016). *J. Supercrit. Fl.*, 110(1), 1-10. [6] Jommi G. et al. (1988) *Gazz. Chim. Ital.*, 118, 823-826
[7] Cao Y. et al. (2013). *Appl. Microbiol. Biotechnol.*, 97, 3323-3331. [8] Joo et al. (2012). *J. Biotechnol.*, 158, 17-23

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