

## Supercritical CO<sub>2</sub> extraction of feed stocks to generate high added value bio-products

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The huge amounts of waste generated every year by the industry, in addition to the great loss of valuable materials, pose serious organisational problems, both from an economic and ecological point of view. Many of the residues, however, have the potential to be reused and utilized completely via integrated bio-refining technologies. Extraction techniques which play a very important part in biorefineries should provide swift processing and yield quantitative recovery without degradation, complemented by easy separation of extracts from the solvent.

In this work, focussed on two generic biomass examples - grape seeds and spent coffee grounds, we outline the perspectives of introducing mild, efficient and with low environmental impact techniques that apply compressed fluids in biorefineries for obtaining high value-added products with a wide spectrum of applications.

**Key words:** scCO<sub>2</sub> extraction, biomass, valorisation bio-products, bioactive compounds

### INTRODUCTION

Diverse important natural sources of bioactive compounds (e.g. plants, fats, algae and other agricultural by-products and wastes) have been reported in the literature. The focus on plants as potential natural sources is ever present and new species are being continuously explored [1]–[7]. An increasing attention is paid to the use of waste biomass as a renewable resource of high added value compounds with applications in food, cosmetics, pharmaceutical industries, biodiesel production, etc. Recycling of waste and its valorisation to non-energy and energy related products is an effective and efficient way to resolve the serious problem with the huge amounts of waste generated daily, due to the increase, in both production and consumption [8], [9]. In view of this, the circular economy concept for biomass valorisation realized via integrated biorefineries [10]–[15] is becoming very important. A sustainable smart biorefinery would allow material processing and operation virtually waste free, based on alternative and/or mixed feed stocks, and would thus contribute environmental, social and financial benefits.

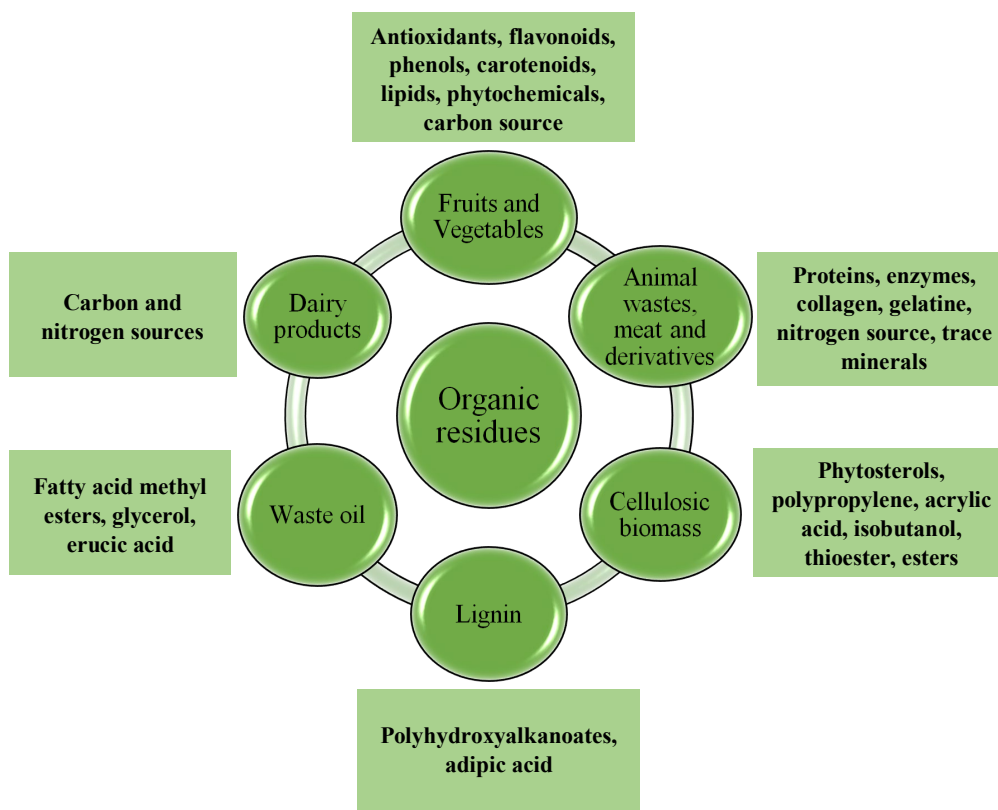
Figure 1 shows some of the options for production of food additives, antioxidants, flavonoids, enzymes and proteins, fatty acid esters, glycerol, etc. from diverse sources of raw material [8], [11].

Extraction techniques are a vital part of biorefineries. Conventional extraction techniques, however, are time-consuming, require large amounts of toxic volatile organic compounds (VOCs) - solvents, and high energy spending. These shortcomings have generated interest in new advanced technologies that alleviate and/or eliminate the emission of pollutants to the environment. These green techniques are environmentally-compatible, sustainable, preserve natural resources and lower carbon footprints

### SUPERCRITICAL CO<sub>2</sub> EXTRACTION

In our days the use of organic sources to attain bioactive compounds requires the application of suitable, environmentally and ecologically friendly, innovative extraction techniques and processes. From this perspective, the methods of extraction should be technologically advanced and in line with the Green Chemistry principles related to all processes involved [16]–[18]. Supercritical fluids are gradually being used and endorsed to produce high value, natural bioactive substances from biological or organic wastes based raw materials. Supercritical fluids have the necessary transport properties that increase their ability to adjust. Moreover, supercritical fluids have low viscosity and disperse more easily within the solid matrix and have low surface tension, which allows rapid penetration of the solvent into the matrix thus increasing extraction efficiency [1], [2], [19].

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**Fig. 1.** Valorization of waste organic residues from several source of raw material.

Furthermore, because they are non-mutagenic and non-toxic are often referred to as the green solvents of the future. Supercritical CO<sub>2</sub> (scCO<sub>2</sub>) is an excellent solvent, which is cheap and abundant and is principally used to isolate nonpolar bioactive compounds (carotenoids and lipids). One option to overtake this limitation, and to enhance the extraction of more polar compounds, such as flavonoids, is the addition of modifiers such as ethanol, water, ethyl lactate, or less green modifiers like methanol, and acetone [6], [19], [20].

Moreover, a combination of scCO<sub>2</sub> with sub-critical extraction methods, mainly pressurized liquid extraction (PLE), can be used as a very effective and considerable improvement of the conventional extraction to obtain a wide range of bioactive components from different natural and organic sources. In addition, there is the possibility to join the above process to other processes within the frame of a biorefinery [7].

It is understandable that the optimal extraction conditions will be different not only with respect to the solvent and matrix, but also - to the target components, which are often with very diverse chemical structures. Hence, great care should be taken to identify and adjust the operating conditions properly.

#### GRAPE SEEDS AND SPENT COFFEE AS WASTE ORGANIC RESIDUES

Grape seeds (GS) and spent coffee grounds (SCG) wastes are two important feed stocks for development of biorefinery sustainability by circular production of high value-added products such as bioactive compounds, adsorbents and catalytic supports, polymers or nanocomposites, etc. along with products, which are of lower value but are utilized in huge quantities, such as biodiesel and other biofuels [14], [21]–[29]. Their properties complement each other, which opens up opportunities for optimization of the processes and product portfolios of sustainable biorefineries.

Grape is one of the largest fruit crops produced with an annual world production higher than 70 million tons [30] of which about 80 % is used for winemaking. Seed biomass (about 5 % of the whole grape), represents approximately (40 – 50) % of solid wastes and contains typically (8–15) % of oil.

Global coffee production creates huge volumes of waste per year. Around 650 kg of SCGs is produced from 1 ton of green coffee beans. Furthermore, during the preparation of 1 kg of soluble coffee approximately 2 kg of wet SCGs are obtained [31]. In 2014 around nine million tons of SCGs were dumped in landfills.

Extraction of the pressed GS or SCG with *n*-hexane is the current method applied to reuse the

seeds biomass and to obtain the vegetable oil. Hexane is the usual solvent used in the laboratory and in industry. However, according to the European Chemicals Agency (ECHA) it is classified as a danger that may be fatal if swallowed or inhaled, as toxic to aquatic life with long lasting effects, and as a highly combustible liquid. It is suspected also of damaging fertility. Therefore, the green alternative extraction technique, applying supercritical CO<sub>2</sub> as a solvent, is a powerful option that can lower health and safety risks, and reduce environmental footprints.

## MATERIALS AND METHODS

Grape seeds and spent ground coffee were supplied by a wine industry and a coffee shop, with a particle size of  $0.62\pm 0.04$  and  $0.273\pm 0.023$  mm, respectively. The waste organic residues were dried for a period of 48 h at 343 K, to guarantee lowering excessive humidity which is harmful to scCO<sub>2</sub>.

Supercritical extraction with CO<sub>2</sub>, at flow rates of 0.11 kg/h, pressures up to 40 MPa, and temperatures up to 333 K, was performed in an apparatus, equipped with a 50 mL internal volume vessel, manufactured from AISI 316 stainless steel tubing (32 cm long with an internal diameter of 1.41 cm), following the previously described methodologies [17], [18], [24]. The conventional extraction used for comparison in our study, was carried out with *n*-hexane in a Soxhlet system.

The quantitative analysis of fatty acids distribution of the extracted esters was performed by transesterification of the glycerides in a 2M methanol solution of KOH, as recommended in the Annex I to Commission Regulation (EEC) No 2568/91(1). A GC-FID system with a fused-silica capillary column SP-2380, 60 m length, 0.25 mm of internal diameter and 0.20  $\mu$ m film thickness, with helium as the carrier gas at a constant flow rate of 1.0 mL/min was employed for the analysis of the fatty acid methyl esters (FAMES) [24].

## RESULTS AND DISCUSSION

The effect of the scCO<sub>2</sub> operating parameters - pressure and temperature, on the extraction yields, as well as a comparison with the *n*-hexane extraction yield, can be deduced from Table 1.

The results of applying the scCO<sub>2</sub> technique, confirmed that at the same temperature the extraction time, needed to achieve the maximum yield is influenced by pressure. At higher temperatures this trend is more pronounced with the extraction time decreasing considerably with increasing pressure. This behavior is explained by

the rise in solvent density, which leads to enhancing the solvation power of CO<sub>2</sub> [31]. It should be noted that, considering the standard deviation, the maximum oil yields achieved by the scCO<sub>2</sub> extraction were, for both matrices, within the range of the *n*-hexane extraction oil yield. However, the scCO<sub>2</sub> times to obtain the maximum yields, compared to *n*-hexane extraction, were shorter - around 110 - 130 min for the higher pressure (40 MPa) and 200 min - for the case when pressure was 30 MPa (Table 1).

Figures 2 and 3 show the main fatty acid composition (% of total fatty acids) results of the GS and SCG oils, obtained by *n*-hexane and scCO<sub>2</sub> extraction. Fatty acids like myristic (C14:0), palmitoleic (C16:1), gadoleic (C20:1), behenic (C22:0), nervonic (C24:0) are also present in the oil but in very low quantities - to the total of less than 1.3 %.

According to Figures 2 and 3, the distribution of the fatty acids in the extracted oils does not depend significantly on the extraction method. The main fatty acid in both oils is linoleic (C18:2). However, the grape seed oil contains more linoleic acid esters (64 - 67 %) when compared to the SCG oil (41 - 43 %). On the other hand, the amounts of palmitic acid (C16:0) in the first and the second oil types are 8 and  $\approx$  33%, respectively. These results are confirmed also by the fact that monounsaturated MUFA and di-unsaturated DUFA are the prevailing compounds in both oils, when compared to the saturated. However, the two oil types are complementing each other, for instance as biorefinery feed stocks, since the grape seeds oil is significantly more unsaturated (with  $\approx$ 86 % in total) in comparison with the SCG oil (around 56 %).

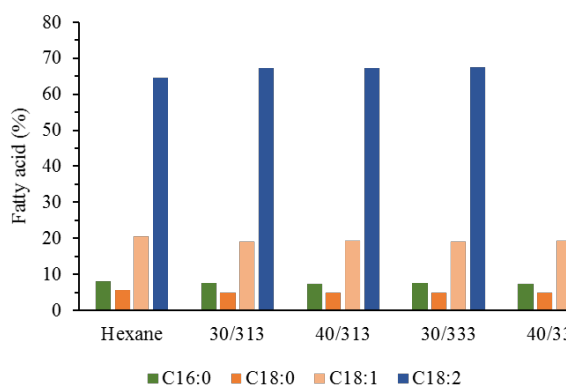
## CONCLUSION

The effects of the scCO<sub>2</sub> technique operating parameters - temperature and pressure, on the yield and the fatty acid profile of the oils extracted from grape seeds and from spent ground coffee biomass were evaluated in detail and reported.

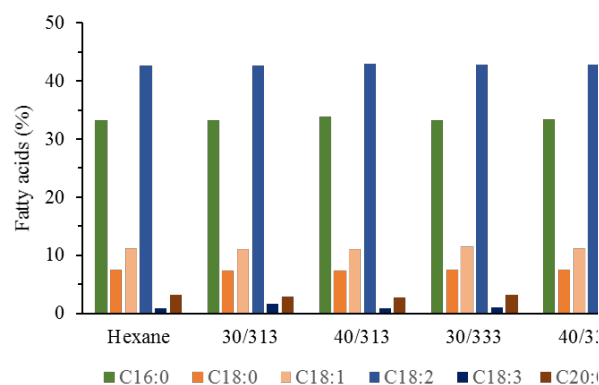
Taking into consideration the higher amounts of essential MUFA and DUFA, the grape seeds oils can be designated to human consumption. In biodiesel application, after separating high value bioactive compounds like diterpenes, caffeine, polyols, etc., mixtures of the two types of oils can provide properties like cetane number, lubricity, cold filter plugging points, etc. with complementing qualities.

**Table 1.** Oil yields and extraction times, applying scCO<sub>2</sub>, as a function of the operating conditions. Comparison to *n*-hexane extraction results.

scCO <sub>2</sub> conditions	Extraction method	Grape seeds Oil Yield (%)	Time (min)	Spent coffee Oil Yield (%)	Time (min)
$p(\text{MPa})/T(\text{K})$ ;	<i>n</i> -hexane	12.28±0.35	240	10.38±0.82	240
	30/313	11.96±0.60	191	10.60±0.54	191
	40/313	12.07±0.55	148	10.10±0.72	110
CO <sub>2</sub> , flow rate 0.11 kg/h	30/333	12.17±0.38	214	9.41±0.59	223
	40/333	12.83±0.56	134	10.11±0.65	110



**Fig. 2.** Fatty acid composition (% of total fatty acids) GC-FID analysis of GS oils obtained by hexane and scCO<sub>2</sub> extraction  $p(\text{MPa})/T(\text{K})$ .



**Fig. 3.** Fatty acid composition (% of total fatty acids) GC-FID analysis of SCG oils obtained by hexane and scCO<sub>2</sub> extraction  $p(\text{MPa})/T(\text{K})$ .

The scCO<sub>2</sub> oil extracts, as compared to those from the conventional *n*-hexane extraction, are obtained by an integrated technique with reduced time for processing. They do not require use/regeneration of harmful solvents and generation of hazardous wastes. Furthermore, extraction with scCO<sub>2</sub> has low environmental impact, and in combination with similar techniques are a prerequisite for circular waste-free production in sustainable smart biorefineries.

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#### REFERENCES

- O. Catchpole, S. Tallon, P. Dyer, F. Montanes, T. Moreno, E. Vagi, W. Eltringham, J. Billakanti, *Am. J. Biochem. Biotechnol.*, **8**(4), 263 (2012).
- J.P. Coelho, A.F. Palavra, in: High Pressure Fluid Technology for Green Food Processing, T. Fornari

- and R. P. Stateva (eds), Springer, Cham Heidelberg New York Dordrecht London, 2015, p. 357.
- R. Ravindran, A.K. Jaiswal, *Trends Biotechnol.*, **34**(1), 58 (2016).
- F.J. Barba, Z. Zhu, M. Koubaa, A.S. Sant'Ana, V. Orlien, *Trends Food Sci. Technol.*, **49**, 96 (2016).
- J.E. Sosa-Hernández, Z. Escobedo-Avellaneda, H.M.N. Iqbal, J. Welti-Chanes, *Molecules*, **23**(11), 2953 (2018).
- M.B. Soquetta, L. de M. Terra, C.P. Bastos, *CYTA - J. Food*, **16**(1), 400 (2018).
- R. Gallego, M. Bueno, M. Herrero, *TrAC - Trends Anal. Chem.*, **116**, 198 (2019).
- D. Pleissner, Q. Qi, C. Gao, C.P. Rivero, C. Webb, C.S.K. Lin, J. Venus, *Biochem. Eng. J.*, **116**, 3 (2016).
- J. Moncada B, V. Aristizábal M, C.A. Cardona A, *Biochem. Eng. J.*, **116**, 122 (2016).
- H.R. Ghatak, *Renew. Sustain. Energy Rev.*, **15**(8), 4042 (2011).
- N. Mirabella, V. Castellani, S. Sala, *J. Clean. Prod.*, **65**, 28 (2014).
- U. De Corato, I. De Bari, E. Viola, M. Pugliese, *Renew. Sustain. Energy Rev.*, **88**, 326 (2018).
- S. Maina, V. Kachrimanidou, A. Koutinas, *Curr. Opin. Green Sustain. Chem.*, **8**, 18 (2017).
- F.B. Shinagawa, F.C. De Santana, L. Rabelo, O. Torres, J. Mancini-filho, *Food Sci. Technol.*, **35**(3), 399 (2015).
- J. Cristóbal, C. Caldeira, S. Corrado, S. Sala, *Bioresour. Technol.*, **259**, 244 (2018).
- W. Wardencki, J. Namieceniak, *Green Chemistry Current and Future Issues*, **14**(4), 389 (2005).

17. P. Anastas, N. Eghbali, *Chem. Soc. Rev.*, **39**, 301 (2010).
18. H. Machida, M. Takesue, R.L. Smith, Jr, *J. Supercrit. Fluids*, **60**, 2 (2011).
19. M. Herrero, E. Ibáñez, *J. Supercrit. Fluids*, **96**, 211 (2015).
20. E. Ibáñez, M. Herrero, J. A. Mendiola, M. Castro-Puyana, in: *Marine Bioactive Compounds: Sources, Characterization and Applications*, M. Hayes(eds), Springer, 2012, p. 55–98.
21. S. S. Georgieva, J.A.P. Coelho, F.C. Campos, M.P. Robalo, R.P. Stateva, *J. Chem. Technol. Metall.*, **53**(4), 85 (2018).
22. J.A.P. Coelho, M.P. Robalo, G.P. Naydenova, D.S. Yankov, R.P. Stateva, *Bulg. Chem. Commun.*, **50**(C), 74 (2018).
23. J.P. Coelho, R.M. Filipe, M.P. Robalo, R.P. Stateva, *J. Supercrit. Fluids*, **141**, 68 (2018).
24. A.E. Atabani, A.H. Al-Muhtaseb, G. Kumar, G.D. Saratale, M. Aslam, H.A. Khan, Z. Said, E. Mahmoud, *Fuel*, **254**, 115640 (2019).
25. S.V Caetano, F.M. Silva, T.M. Mata, N.S. Caetano, V.F.M. Silva, *Clean Technologies and Environmental Policy*, **16**(7), 1423 (2014).
26. A. Kovalcik, S. Obruca, I. Marova, *Food Bioprod. Process.*, **110**, 104 (2018).
27. S. Bail, G. Stuebiger, S. Krist, H. Unterweger, G. Buchbauer, *Food Chem.*, **108**(3), 1122 (2008).
28. J. Garavaglia, M.M. Markoski, A. Oliveira, A. Marcadenti, *Nutr. Metab. Insights*, **9**, 59 (2016).
29. V. Radovanovic, S. Dekic, B. Radovanovic, *J. Process. Energy Agric.*, **15**(4), 263 (2011).
30. M. Bordiga, F. Travaglia, M. Locatelli, *Int. J. Food Sci. Technol.*, **54**, 933 (2019).
31. S. K. Karmee, *Waste Manag.*, **72**, 240 (2018).
32. R.M. Couto, J. Fernandes, M.D.R.G. da Silva, P.C. Simões, *J. Supercrit. Fluids*, **51**, 159 (2009).