Cascade use of olive waste towards a highly competitive olive sector: high value byproducts, advanced biobased materials and advanced biofuels integrated production

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Introduction

The olive value chain represents one of the most important bioeconomic sectors for Portugal, having this country the opportunity to emerge as the third-largest worldwide olive oil producer in the upcoming decade due to the modernization of its olive groves. Traditional rainfed production systems have been replaced by new intensive olive systems, with high-density, irrigated and mechanically harvested, which are leading to significant changes in the Portuguese olive sector. In fact, the olive oil worldwide production has been constantly rising, with a 20% increase between 2008/ 2009 (2.67E+06 tons (t)) and 2019/ 2020 campaign years (3.21E+06 t) (IOC, 2020), despite yearly fluctuations attributed to the uncertainty of climate and alternate-year bearing (Espadas-Aldana et al., 2019). Regarding the olive oil global consumption, a similar tendency was also observed, from 2.83E+06 t in 2008/ 2009 to 3.23E+06 t in 2019/ 2020, representing a 14% increase.

Olive waste is known as a rich source of functional and bioactive compounds, as well as bioenergy. The potential combination between by-products and bioenergy of this biosource is a key option for selecting new technologies and pathways for their valorisation and waste management. Traditional technologies - such us direct combustion in controlled chamber or farms directly for environment – do not promote the maximisation value of this bioresidue and generate some environmental problems, related with gaseous emissions, low energy efficiency and generation of others residues. In the present work, the annual potential biomass production in olive sector for Portugal case study is analysed and the conversion olive solid waste by the cascade use principle was evaluated in order to develop a circular bioeconomy approach for the olive sector, by using advanced technologies, under the "ValorMais", "iCeres" and "Recursos Biológicos: da Biologia Molecular aos Resíduos" and "BeirInov" Projects. These projects are mutually complementary and are aligned with a sustainable and circular bioeconomy. This work concluded that the olive sector generates high amount of solid waste, in average scenarios, 540,000 ton/year of pomace and 723,000 ton/year of tree bioresidues. Additionally, these biowastes have a high potential of conversion into by-products for the (1) food industry (multifunctional ingredients with higher health-promoting effects than dietary fibre and polyphenols and polysaccharides for edible coatings to increase the food preservation with extended food shelf life), (2) chemical sector and (3) the production of advanced biofuels in the same bioprocess designed over cascade use concept.

This work represents a complex cross funds realized in the last 5 years, demonstrating the relevance of these scenarios for Portugal, with high potential for olive oil industry, transforming problems into opportunities and minimizing the environmental impact of this sector. The ValorMais project aims to provide a platform for the recovery of waste and by-products from the agricultural, agri-food and forestry sectors, through material, organic and energy recovery. The exemplary mobilizing effect of this operation, "ValorMais: Creating value with agri-food and forestry by-products", applied in five tiers (nuts, olive oil, forest and the tomato industry), may act as a driver for other ranks in the agricultural, agri-food and forestry sectors. The projects "iCeres" and "Recursos Biológicos: da Biologia Molecular aos Resíduos" entails the transfer innovative knowledge and entrepreneurship to the business fabric, based on emerging technologies and "BeirInov" the development of advanced biobased

material from lignocellulosic biomass rich in polysaccharides and bioingredients to increase the quality and extended food shelf life with the decrease of food losses.

Material and Methods

The work was divided into two parts. One related to the life cycle inventory and moisture content analysis, and the other concerning the main characteristic necessary to evaluate the potential for conversion into by-products for food, fine chemical and advanced biofuels industries.

Part 1: Life Cycle Inventory and Moisture Content

Life Cycle Inventory divided the value chain into two parts. Process A, related to the production of tree bioresidues, originated during the cultivation and harvesting operation. Process B, related to the conversion of olives into olive oil. The collection of data was performed both in an agricultural and industrial environment, as well as in an already created database. The procedure moisture determination (protocol adapted from NREL National Renewable Energy Laboratory - Technical Report NREL / TP-510-42621, Revised March 2008) is intended to determine moisture after 105 ºC drying of a sample. For previously dried crucibles, weigh between 0,5 to 2 g of sample. Each sample must be analyzed in triplicate. Place the crucibles with the sample in the oven at 105 ± 3 ºC and do the first weighing at least after 4 hours. After the aforementioned time has elapsed, move the crucibles to a desiccator and allow them to cool for approximately 30 minutes before weighing. This procedure must be repeated (keeping the sample for at least 1 hour in the convection drying oven at the temperature mentioned above) until three constant weighings are obtained (with a variation of less than ± 0,1%).

Part 2: Chemical and Bioactive Characterization, Energy Content and Bioenergy conversion

Chemical components of ash, protein and lipids and carbohydrates:

- Ash Determination (protocol adapted from NREL National Renewable Energy Laboratory - Technical Report NREL / TP-510-42622, January 2008) - CARBOLITE (serie number 21 – 100993; ELF 11/148): Once properly identified, place the crucibles in the muffle furnace for 4 hours at 575 ºC. After the aforementioned time has elapsed, these must be moved to a desiccator. After reaching room temperature, the crucible must be weighed and the process must be repeated until the variation in mass is less than 0,1 mg. Weigh between 0,5 to 2,0 g of the previously dried sample in the crucibles. Each sample must be analyzed in triplicate. As soon as the program ends, the muffle furnace must be allowed to cool and the crucibles must be moved to a desiccator. Weigh the crucibles along with the ashes once they reach room temperature. Repeat this procedure until the variation in mass is less than 0,3 mg.

-The free sugar and organic acid content were obtained 34 and determined by Beckman Coulter System Gold HPLC (Knauer, Berlin, Germany) coupled to RI and UV detector using Aminex 37-H column (Bio-rad, Berkeley, USA) at 55 °C and 35 mM H_2SO_4 as mobile phase (flow rate: 0.5 mL/min). The quantification was achieved using standard calibration curves (0.2 - 2.0 mg/mL). Total amino acids content of each powder was performed using pre-column derivatisation with orthophthalaldehyde (OPA) methodology 35 and quantified using a calibration curve built with amino acids pure standards. The profile of fatty acids was obtained and analysed following the methodology of Pimentel, Fontes, Gomes, & Rodríguez-Alcalá (2015) with some modifications regarding the internal standard used (glyceryl tritridecanoine, TG-C13) and the derivatisation process (methanol and sodium methoxide were added to 50 mg of sample in the amounts of 2.26 mL and 240 µL, respectively). All measurements were done in triplicate and expressed as g/ 100 g DW.

Bioactive Characterisation:

- Total phenolic compounds extraction and quantification (Free and Bound Phenolic Compounds): The extracts of free phenolic compounds were obtained according to the Alu'datt et al.. The free phenolics compounds extract (FPC) were obtained using methanol as solvent (1:10, 1h of agitation on orbital shaker 200 RPM, two successive extractions). The residue obtained after FPC extraction was hydrolysed with 20 mL of 4 M NaOH at room temperature 37. The collected fraction of phenolic compounds were designated as bound phenolic compounds (BPC). The total phenolic content (TPC) of FPC and BPC extracts was determined according to the Folin-Ciocalteu method 38. Results were expressed as mg gallic acid equivalents (GAE)/ 100 g DW.

- Antioxidant Activity: The free and bound phenolic extracts were used to evaluate the radical scavenging capacity of crude olive pomace, liquid and pulp fraction samples according to 3 methods: DPPH [Alexandre et al.], ABTS [Cano et al.] and Oxygen Radical Absorbance Capacity (ORAC) [Oliveira et al.].

- Antioxidant activity evaluation: The FPC, BPC, IDF-BPC and SDF-BPC extracts were used to evaluate the AOX of OP powders according to the methods of DPPH, ABTS and ORAC using a microplate reader (Fluostar, Optima; BMG Labtech, Ortenberg, Germany). The radical stock solutions were freshly prepared. All analysis were performed in triplicate and expressed in μM of Trolox-equivalents (TE)/ g DW.

Determination of dietary fibre composition:

TDF content was estimated using the enzyme-gravimetric method, according to AOAC method 991.43 (1990), with slight modifications according to Deng et al. (2011). The results were expressed as TDF, IDF and SDF g/100 g DW. The IDF and SDF profile were assessed in agreement with Deng et al. (2011) methodology.

The chromatographic analysis of FPC, BPC, IDF-BPC and SDF-BPC extracts were performed following the methodology described by Oliveira et al. (2015). The main phenolic compounds identified (3-hydroxytyrosol, protocatechuic acid, tyrosol, vanillin, caffeic acid, p-coumaric acid and luteolin) were quantified by HPLC using external calibration curves constructed based on their maximum UV signal. The results were expressed as mg/100 g DW. The hydroxytyrosol glucoside and tyrosol glucoside were respectively expressed as hydroxytyrosol and tyrosol equivalents in mg/100 g DW.

The phenolic compounds were released from IDF and SDF fractions using the same hydrolysis . This methodology is in agreement with the procedure applied in other food matrices as whole-grain cereals [Guo and Beta] to release BPC from IDF and SDF. The BPC extract obtained from IDF and SDF were denominated as IDF-BPC and SDF-BPC, respectively. These extracts were used to measure TPC and AOX.

Energy Content and Bioenergy conversion:

- Lower Heating Value: Once the 6200 Isoperibol Calorimeter, the pump and the heating system are switched on, turn on the water handling system and set the oxygen pressure to 30 bar. Afterwards, pour 1 g of sample (previously dried at 105 ºC) into the combustion crucible, which must then be placed on the pump head support. To allow the combustion of the sample, a wick must be inserted in the sample to allow the connection between the electrodes and the sample. The pump head with the sample is then inserted in the pump itself and oxygen must be injected, in order to ensure that the essential conditions inside the pump are met for the complete combustion of the sample. The pump must then be soaked in a bath prepared through the water handling system and the electrodes must be connected to the pump. Finally, the test can begin. (The procedure should take approximately 20 minutes).

- Bio-liquid conversion: Thermochemical pilot equipament to convert solid waste olive into bio-liquid, under the conditions of 350ºC in an inert atmosphere was used and its characterization through Gas Chromatography/Mass Spectrometry (GC-MS) technique. The GC-MS characterization was performed in a Shimadzu GCMS-QP2010 Ultra, equipped with an autosampler and an electron impact source. The compound analysis by GC/MS starts with the identification of the volatile compounds, by comparing their mass spectra and retention times with those from widely spread libraries, followed by its respective quantification, using the chromatogram peaks areas by relating them with standard solutions response.

Results and Discussion

Part 1: The Life Cycle Inventory made it possible to obtain an average annual approximation of the quantities produced of solid wasted in the olive oil production value chain. Process A, based on data collection in the field and database of other olive production systems, averaged a production of 723,000 ton/year dry basis, obtained throughout the Portuguese territory, in a total 361,483 ha of production. In terms of moisture content, there was a variation between 10.50-45.50% weight. The moisture content varies significantly due to factors such as the age of the pruning branch, the physical state of the pruning branch (dry or green), the time and season. In process B, the pomace generation inventory was performed, resulting from the inventory of 10 olive oil producers, where the percentage of pomace generation was obtained for each litre of oil produced, in a 2-phase mill system (mostly in Portugal). In this process, an average generation of 540,000 ton/year dry basis was obtained in Portugal.

Part 2: The Lower Heating Value of the different samples characterized obtained a range value between 4,200 e 4,350 Kcal/kg dry basis, and 3.2 – 4,3 % the ashes. A 64-68% balance mass conversion of olive solid waste to bio-liquid was obtained, that represents 33% of the overall efficiency of advanced biofuel production. The present work fractionated and valorised the liquid and pulp fraction of olive pomace obtaining two stable and safe powdered ingredients, namely a liquid-enriched powder (LOPP) and a pulp-enriched powder (POPP) with the ambition to design an integrated production process. These powders were characterized chemically, and their bioactivity was assessed. LOPP exhibited a significant amount of mannitol (141 g kg−1), potassium (54 g kg−1) and hydroxytyrosol derivatives (5 mg g−1). POPP exhibited a high amount of dietary fibre (620 g kg−1) associated with a significant amount of bound phenolics (7.41 mg GAE g−1 fibre DW) with substantial antioxidant activity. POPP also contained an unsaturated fatty acid composition similar to that of olive oil (76% of total fatty acids) and showed potential as a reasonable source of protein (12%). A significant antioxidant activity not only in free phenolics (ORAC: 455–503 μM trolox equivalents/g) but also in bound phenolics (ORAC: 121–130 μM trolox equivalents/g). Their functional properties (solubility, water-holding and oil-holding capacity), antioxidant capacity and antimicrobial activity were also assessed, and their biological safety was verified, with high potential for advanced biobased materials integration to apply in the food industry packing.

Conclusions

An integrated valorization bioprocess of olive solid waste is a key option for Portugal to (1) implement new technologies that allow the maximum valorization of this bioresource; and (2) integrate the cascade use principle. An integrated bioprocess concept is proposed by the present work from the conversion of olive biowaste into non energetic by-products, with high value, (multifunctional bioingredients and biobased materials with technical proprieties based on bifunctionality), in combination with the energetic byproducts production (advanced biofuels for the transport sector, Fischer Tropsch Diesel).

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