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Profile of Bound Phenolic Compounds from Olive Pomace

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The olive oil production is characterized by significant amounts of residues and their management is an economic and environmental challenge issue for the olive mills. Nowadays, the more relevant waste of olive oil extraction is Olive Pomace (OP), due to the large implementation of two-phase system [1]. OP contains large amount of water (50-70%), fibre [2] and carbohydrates [3], but also phenolic compounds (~98% of olive's phenolic compounds remains in OP) [4]. Several studies have been performed with OP as a source of polyphenol compounds. However, an appreciable amount of polyphenols, called "Non-Extractable Polyphenols" or "Bound Polyphenols" (BP) can remain on the extraction residue [5]. The BP are associated with fibre and/or proteins and its extraction can't be performed using the common aqueous-organic solvents. In rich fibre plant foods like olives, BP and fibre are intimately associated [6], therefore the polyphenol content of OP could be underestimated. In several studies have been demonstrated that BP are representative group of total polyphenols. Besides that BP could be bioaccessible and bioavailable in the human gut and they may have an important role in gastrointestinal health and contribute to the systemic effects associated with dietary antioxidants [5].

The overall objective of present study was to study the relevance of Bound Phenolics (BP) of OP. Therefore, the present study aimed to determine the content of phenolics (BP and FP) using chromatographic method (HPLC and LC/MS) to identify the major classes of phenolic compounds present in BP and FP. The antioxidant activity of FP and BP was also studied.

The OP samples were supplied from olive mills (2-phase system) from Coimbra district and was stored in in a freezer at -80 ºC. Extraction procedures of polyphenols (FP and BP) followed the method described by Xie *et al.* (2015) with some modifications [3]. Total Phenolic Compounds (TPC) and Antioxidant Activity (AOX) of FP and BP were analysed using the methods Folin-Ciocalteau [mg gallic acid equivalents (GAE)/ g DW] and ABTS [mg Trolox equivalents/g DW], respectively. FPC and BPC analyses were performed by HPLC followed the method described by Oliveira *et al.* [8].

The TPC and AOX of FP were greater (at least 6 times) than BP. However, the amount of BP was significant (3,62 ± 0,38 and 3,20 ± 0,12 mg GAE/g DW) as source of phenolic compounds. The principal FP quantified in OP samples was 3-hydroxytyrosol (FP: 2.07 ± 0.14 mg/g DW; BP: 0.19 ± 0.05) and the principal BP was caffeic acid (greater in BP – 0.25 ± 0.02 mg/g DW - than in FP – 0,22 ± 0.02 mg/g DW). The protocatechuic acid only was found in BP in the range of 0.11 ± 0.01 to 0.08 ± 0.01 mg/g DW. Derivatives of hydroxybenzoic acid, such as the protocatechuic acid, can be found as part of complex structures such as lignin, while the derivatives of hydroxycinnamic acid such as caffeic acid, are mainly linked to structural components of cell wall (cellulose, lignin and protein) through ester linkages [9].

Although, the OP's amount of BP was lower than FP, they could significantly contribute to polyphenol intake from OP and provide a significant biological activity associated with gastrointestinal health and potential systemic effects in large intestine. Based on previous studies BP reach the colon, where they are released and metabolized in different bioavailable metabolites by the action of bacterial microbiota [5].