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Novel pectin present in new olive mill wastewater with similar emulsifying and better biological properties than citrus pectin

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ABSTRACT

Alperujo, a semi-solid by-product from the olive oil industry, is currently subjected to a novel industrial treatment, which consisted of gentle heating at 50–80 °C for 1–2 h, followed by a new three-phase centrifugation system that gave alperujo oil, olive pomace and aqueous by-products. This process is a prerequisite to reduce the humidity of the resulting solid to enable its use as biomass, and allows for the recovery of a new olive mill wastewater, which was used in this work for the isolation of water-soluble polysaccharides. Two polysaccharide-enriched extracts were obtained by ethyl alcohol precipitation with 40 and 80% (v/v) EtOH from the new aqueous by-product and purified. The pectic material presented a high molecular size and a low percentage of methyl esterification and acetylation. In comparison with commercial pectins, the extracts had better oil holding capacity and similar emulsifying activity to that of citrus pectin. For the first time, bile-acid binding and glucose retardation activity were considered for the pectic material isolated from olive by-products, which showed better results than commercial pectins. In addition, the antioxidant activities of these extracts were investigated using various *in vitro* assays and the two raw polysaccharide extracts, rich in associated polyphenol compounds, exhibited a stronger antioxidant activity than that observed for citrus and apple pectins. Thus, polysaccharide-enriched extracts from gentle heat treated alperujo have suitable emulsifying properties for commercial uses and the important bioactive properties of antioxidant dietary fiber.

1. Introduction

Olive oil production is a very important and traditional activity, particularly in the countries of the Mediterranean area, which account for 95% of worldwide olive oil production. Technological advances in olive oil mills have introduced more efficient methods of olive oil extraction, such as the two-phase centrifugation system, which permits the extraction of virgin olive oil without the addition of water. This extraction system is widespread in Spain (accounting for 90% of production) and many other olive oil producing countries. Besides olive oil, the process releases a semisolid by-product called two-phase pomace or "alperujo," with over 4 million tonnes generated annually in Spain. The two-phase extraction system results in a very wet paste (with 60–70% of moisture) due to the retention of the fruit's water in the residue. This paste is usually used for the extraction of olive pomace oil with hexane after drying, or by centrifugation without drying.

Currently, the alperujo by-product is treated to recover the maximum amount of oil, and the final solid is utilized as biomass. Due to the considerable energy required for the drying process necessary for the subsequent use as biomass, an alternative extraction process that helps to reduce the moisture content is being implemented at an industrial level. This process consists of gentle heating of the alperujo at 50–80 °C for 1–2 h, following by a new three-phase centrifugation system that gives alperujo oil, olive pomace and aqueous by-products (also known as olive mill wastewater or "alpechin"). This process reduces the humidity of the solid, making it suitable for use as biomass. Furthermore, the process allows for the recovery of an olive mill wastewater that is particularly enriched in bioactive components of interest, of a varied nature and composition, which can be extracted and used for industrial purposes. However, the alpechin recovered from this process differs

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from traditional alpechin in that it stems from a mild heat treated alperujo, and different hydrothermal conditions can modify the structure, molecular weight, and therefore, functional properties of the components in the aqueous by-product. Therefore, the aqueous by-product used in this work is a novel raw material whose chemical composition and properties have yet to be fully examined.

The treatment of alperujo realized in this work is considered as "gentle" heating, in comparison to the hydrothermal treatments of alperujo at harsher temperatures of 160 °C employed in previous work (Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz, & Fernández-Bolaños, 2015). The harsh hydrothermal treatment of olive oil waste (alperujo) led to a final liquid phase that contained a high quantity of compounds that were easily solubilized (including carbohydrates, organic acid, phenols, polyphenols) or that were formed from thermal degradation (including hydroxymethylfurfural).

In this study, we considered the cell wall polysaccharides and associated phenolic compounds that were solubilized in the aqueous fraction after the gentle heating of alperujo. Such components originated from the degradation of the cell wall material during alperujo storage due to enzymatic activities before the treatment (for example, as a result of enzymes such as pectinase, hemicellulase, and cellulase), or were solubilized during the extraction process, as thermal treatment enhances enzymatic activity and/or their chemical breakdown. The presence of phenolic compounds in the olive cell wall is reported to be scant (Mafra, Barros, & Coimbra, 2006), although the interaction between polysaccharides and the hydrophilic compounds (phenols, proteins, etc.) provide an important non-carbohydrate polymeric material associated with the cell wall polysaccharides (Obied, Allen, Bedgood, Prenzler, & Robards, 2005). Oxidation, condensation and polymerization reactions between polyphenols and macromolecules during the crushing and malaxation of the olive paste, before oil extraction, may cause these interactions.

Some studies have reported the extraction of pectins from olive by-products such as olive mill wastewater from the traditional three-phase system, without mild heating (Galanakis, Tornberg, & Gekas, 2010), or from the two-phase extracted olive pomace (Cardoso, Coimbra, & Lopes da Silva, 2003). In a previous study by our group (Rubio-Senent et al., 2015), we analyzed the pectic material isolated from hydrothermally treated alperujo (160 °C during 30, 45 or 60 min). This treatment favored the recovery of pectins with a low molecular weight and a high degree of esterification. We will compare those pectins with the cell wall polysaccharides and phenolic compounds obtained from gentle heat treated alperujo in this study, which present different and characteristic properties due to the alternative extraction process.

Pectins are natural hydrocolloids that are found in higher plants as principal structural elements of cell walls, and have been widely used as gelling agents, stabilizers, and emulsifiers in the food industry. One of the most important properties of pectin for its industrial application is its emulsification activity: the potential to produce fine oil-in-water droplets and maintain emulsion droplets of a small size for a substantial period of time. Emulsification and emulsion stabilization should involve the interfacial absorption of all or part of the components making up the extracts so as to provide a stabilizing layer which would protect against droplet coalescense via electrostatic and/or steric interactions (Ritzoulis et al., 2014). For this study, the emulsification activity (EA) of the polysaccharide material was measured and material with an EA value higher than 50% was considered to have good emulsion properties (Abdul-Hamid & Luan, 2000).

Polysaccharides are the main components of dietary fiber. Dietary fiber plays a significant role in many physiological processes and in the prevention of several diseases (Rodríguez, Jiménez, Fernández-Bolaños, Guillén, & Heredia, 2006), and the antioxidant capacity of associated phenolic compounds can also produce a variety of beneficial effects (Pérez-Jiménez et al., 2008). Saura-Calixto (1998) defined antioxidant dietary fiber as a natural product that combines the beneficial effects of dietary fiber and natural antioxidants. In addition, dietary fiber can bind bile acids, thereby aiding their excretion in the feces. Therefore, dietary fiber reduces the risk of cardiovascular disease and hence, lowers the risk of bowel cancer (Camire & Dougherty, 2003; Eastwood & Hamilton, 1968).

In this work, we studied the functional properties of the pectic material present in olive mill wastewater from the novel gentle heat treatment of alperujo. These properties, including water and oil holding capacities and emulsion properties, were evaluated and the results were compared with commercial pectins from apple and citrus. Furthermore, the bile-acid binding, glucose retardation capacity and antioxidant activity of the polysaccharidic material were evaluated by *in vitro* assays for the first time.

2. Materials and methods

2.1. Olive material

To obtain the aqueous by-product used in this study, alperujo was first subjected to a new industrial treatment that consists of gentle heating at 50–80 °C for 1–2 h, followed by a novel three-phase centrifugation system, and which gives alperujo oil, olive pomace and aqueous by-products. The aqueous fraction, with a high hydroxytyrosol (HT) content, was used by Subproductos Vegetales del Mediterráneo (SVM) S. L., Seville (Spain) for the purification of hydroxytyrosol. Once the hydroxytyrosol was isolated, the aqueous fraction was used for the polysaccharide purification.

2.2. Chemicals

Pectin from citrus, cholestyramine and three bile acids (cholic, deoxycholic, and chenodeoxycholic acid) were purchased from Sigma-Aldrich (St Louis, MO, USA). Apple pectin was purchased from F.E.R.O.S.A. (Barcelona, Spain). The standards of Dextran from *Leuconostoc* ssp. 250, 110, 70, 40 and 6 kDa, were obtained from Fluka Bio Chemika (Switzerland).

2.3. Isolation of alcohol insoluble extracts enriched in polysaccharides

100 L of percolate from the recovery of hydroxytyrosol was ultra-filtered at room temperature using a semi-industrial system with a cartridge of 3 kDa ceramic membrane HUF-L1-500 mm. The filtration was conducted using a pump. The permeate was removed at constant cross-flow velocity and the concentrate was recycled back into the feed tank until a volume of 13.8 L was recovered. This fraction, with a size greater than 3 kDa, was submitted for precipitation with 40% (v/v) ethanol:water, causing the precipitation of polysaccharide-enriched extracts (between 0 and 40% of EtOH) with a high molecular weight. The resulting precipitate, named alcohol insoluble extract at EtOH 40% (AIE40), was removed by centrifugation at 4700 *g* (Comteifa, S.L., Barcelona, Spain). EtOH was added to the remaining liquid to obtain an 80% (v/v) ethanol solution. An insoluble material was obtained between 40 and 80% EtOH, facilitating the precipitation of lower molecular weight polysaccharide-enriched extracts, and named alcohol insoluble extract at EtOH 80% (AIE80).

2.4. Purification of raw pectin

The two samples obtained from the fractional precipitation with ethanol were purified by ethylenediaminetetraacetic acid (EDTA)