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Novel emulsifiers from olive processing solid waste

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ABSTRACT

Emulsifiers fit for use in oil-in-water emulsions have been extracted from solid olive processing waste, using aqueous solutions under parallel and tandem extractions. SEC–MALLS/RI/UV and FTIR results showed that these products comprise of polysaccharide agglomerates, proteins and their hydrolysis products, among other constituents. The materials obtained using direct extraction at pH 7 and pH 5 of the alcohol-insoluble solid waste are effective emulsifiers for acidic and non-acidic model food emulsions, while their zeta potential values are negative at both pH. Dynamic interfacial tension measurements at the water/oil and water/air interface demonstrate surface activity. Significant differences in the dynamics of adsorption of different extracts and in their equilibrium interfacial tension are related to their emulsifying capacity and emulsion stability. SEC data of the centrifuged emulsions' serum show that most macromolecular components are interfacially adsorbed. When tested as emulsifiers, the dialysis-isolated macromolecular populations alone are moderately efficient emulsifiers, as compared to the full extracts. Smaller molecules present in the extract appear to provide a synergistic effect in the overall emulsification capacity. The above suggest that olive waste, a major pollutant in the Mediterranean and in other areas, could be used as to reclaim high added value food hydrocolloids.

1. Introduction

Olive by-products are among the primary waste produced in the Mediterranean. The olive oil industry continues to be one of the most heavily polluting among the food industries (Arvanitoyannis, Kassaveti, & Stefanatos, 2007). A number of strategies have been proposed for dealing with this important issue (Mantzavinos & Kalogerakis, 2005). The principal waste products derived from olive oil extraction are vegetation water (an aqueous colloidal effluent called “katsigaros” in Greek) and a solid residue, mainly comprising of the olive pulp, skin and stone (olive husk). Of the two, vegetation water attracted most of the attention concerning the recovery of potentially useful products (Galanakis, Tornberg, & Gekas, 2010; Papadimitriou, Maridakis, Sotiroudis, & Xenakis, 2005; Vitolo, Petarca, & Bresci, 1999), mainly focused in the reclaim of polyphenols and related antioxidant compounds (e.g. Visioli et al., 1999). Patents exist for the recovery of dietary fiber and polyphenols from olive wastewater (i.e. Tornberg & Galanakis, 2010) or of further oil, fuel and electricity (i.e. Avgoustopoulos, Vassilakos, & Seimanidis, 2008). The main post-press treatment for the solid residue is the solvent extraction of husk oil, following which the solid is compressed into briquettes and used as fuel (Vitolo et al., 1999), or is used as compost (Chandra & Sathiavelu, 2009). Of interest remains the possibility of recovering food-grade materials of high added value from the solid olive waste.

Crushing and centrifugation of olives for the removal of oil results in the rupture of the fruit cell walls. Although cellulose is the principal polysaccharide of the fruit body, other polysaccharides and proteins of putative technological importance co-exist with the insoluble polysaccharide (Gil-Serrano & Tejero-Mateo, 1988). The components of the olive paste comprise of two main fractions, one being soluble in successive elutions with aqueous buffers, and another being an insoluble cellulosic residue. Most of the non-cellulosic polysaccharides extracted under the above process can be precipitated by ethanol (i.e. Coimbra, Waldron, & Selvendran, 1994) (hence their name AIS, alcohol-insoluble solids). Aqueous extractions of olives are reported to have an AIS yield of 7–9%, while processed AIS fractions are reported to contain between 30% and 50% polysaccharides and between 20% and 25% proteins (Huisman, Schols, & Voragen, 1996). The above suggest that

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compressed oil paste is a promising source of water-soluble macromolecules.

Sequential extraction and fractionation of olive pulp has shown that the above polysaccharides compose of a number of carboxylated or non-carboxylated sugars (e.g. Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998). The different populations of polymers existing in the olive pulp, as analyzed by FTIR, are reported to be pectic polysaccharides rich in uronic acid, pectic polysaccharides rich in arabinose, arabinose-rich glycoproteins, xyloglucans, and glucuronoxylans (Coimbra, Barros, Rutledge, & Delgadillo, 1999). Olive waste has been reported to contain oligosaccharide (Lama-Muñoz, Rodríguez-Gutiérrez, Rubio-Senent, & Fernández-Bolaños, 2012). A sequential extraction process initially developed for the extraction of pectin from apple (Schols., Vierhuis, Bakx, Voragen, 1995), has been utilized for the isolation of hydrocolloids from olive (Vierhuis, Korver, Schols, & Voragen, 2003), okra (Georgiadis, Ritzoulis, Sioura, et al., 2011; Sengkhamparn, Verhoef, Schols, Sajjaanantakul, & Voragen, 2009) and salep (orchid roots) (Georgiadis, Ritzoulis, Charchari, Tsioptsias, & Vasiliadou, 2011). This process comprises of successive extractions with cold buffer, hot buffer, chelating agents and diluted alkali and, after enzyme hydrolysis, AIS is reported to yield fractions with a non-cellulosic polysaccharide content close to 50% (Vierhuis et al., 2003). This suggests, again, that compressed olives after oil extraction can be a valuable source of polymers.

The present work aims (i) in investigating the macromolecular composition of aqueous extracts obtained from solid olive processing waste; (ii) in establishing the capacity of the above extracts to serve as high added-value food additives, in the particular case as emulsifiers. Apart from extractions at pH 5 (OW5) and pH 7 (OW7), an extraction of the residues *after* OW5 removal has been also performed (CHSS), as to investigate the potential of exhaustive extractions of the solid waste.

2. Materials

A sample of olive pulp byproduct comprising of crushed fully-ripened olives (moisture content, stone content) was kindly supplied by V. Vasileiou (Thessaloniki, Greece) from his local olive mill operation in December of 2012 and was immediately stored frozen until used. Trizma base (2-amino-2-(hydroxymethyl)-1,2 propanediol), *n*-hexadecane, ETDA, ammonium oxalate, sodium azide, sodium acetate were all obtained from Sigma (St Louis, MO). Deionized water has been used for all extractions. All analytical experiments were held using ultrapure water obtained from an ELGA PurelabFlex apparatus (ELGA Process Water, Marlow, UK), unless otherwise stated. Dextran standards (molecular weights 1 kDa–1400 kDa) for HPSEC and sodium oxalate were purchased from Fluka (Steinheim, Switzerland). Glacial acetic acid and NaOH pellets were obtained form Merck (Darmstadt, Germany), petroleum ether 40–60 from Chem-lab NV (Zedelgem, Belgium). Every solution mentioned in this text used was passed through a 25 mm in diameter, 1 μm pore size glass fiber syringe filter. The ethanol used was common commercial ethanol (96°).

3. Methods

3.1. Extraction process

The initial treatment and the extraction of the material is based on protocols originally used by Huisman Schols, & Voragen (1996). The olive pulp was left to unfreeze in a fridge. The next day it was inserted in a vacuum oven at 40 mbar and left to dry until its temperature reached 70 °C, after about 12 h. It was defatted with petroleum ether (40–60) using Soxhlet and treated with 70% (v/v) ethanol at 40 °C for 1 h. This treatment aimed in removing small-molecules such as monosaccharides, oligosaccharides and phenols. The supernatant was separated and discarded using a Büchner devise with common lab filter paper and the extraction was repeated. AIS (alcohol insoluble solids) were dried by means of solvent exchange (pure acetone), then dried at room temperature, hand-destoned while ground in an mortar, keeping the particles that passed through a 1 mm sieve.

The tandem extractions of the materials OW5 and CHSS were performed as follows:

AIS (20 g) were sequentially extracted with 0.05 M sodium acetate buffer, pH 5.0 (two times, 600 ml) at 70 °C for 30 min (OW5). 0.05 M EDTA and 0.05 M ammonium oxalate in 0.05 M sodium acetate buffer, pH 5.2 (two times 600 ml) at 70 °C for 30 min (Chelating Agent Soluble Solids, CHSS). AIS (20 g) were also extracted using 5 mM phosphate buffer, pH 7.0 (two times, 600 ml) at 70 °C for 30 min (OW7). After each extraction, solubilized polymers were separated from the insoluble residue by centrifugation at 19,000 g for 25 min (25 °C), freeze dried, vacuum-packed, and stored frozen until use.

3.2. Emulsion preparation

Emulsions were prepared by means of mixing and homogenizing aqueous buffered solutions containing the emulsifier under study, and *n*-hexadecane, the latter being added as a model hydrophobic dispersed phase. The aqueous solutions for the emulsions of pH 7 were prepared comprising of 0.025 M Trizma buffer and 0.025% sodium azide, the pH being set to 7.0. The aqueous solutions for the emulsions of pH 3 were prepared comprising of 0.025 M sodium acetate and 0.025% sodium azide, the pH being set to 3.0. An amount of extract was then dissolved in each buffer and stirred as to obtain a clear 1% w/v solution. *n*-Hexadecane was then added to a volume fraction $\varphi = 0.1$, as to prepare a crude pre-mix. This pre-mix was subsequently homogenized using a laboratory ultrasonic homogenizer (Hielscher UP-100H, Germany) for 30 s. The emulsions were then transferred into sealed class tubes and were stored at 25 °C under dark and quiescent conditions. Droplet size distributions were measured 5 min after emulsification and then at regular time intervals.

3.3. Dialysis of extracts

10% solutions in SEC eluent (ultrapure water containing 0.1% sodium azide) of each extract were subjected to dialysis. The samples were dialyzed using membranes with nominal cut off threshold of 3500 Da (Orange Scientific, Braine l' Alleud, Belgium), the process lasted 24 h and the deionized water surrounding the dialysis bag has been changed 5 times at 1, 3, 6, 18 and 21 h. The content of the dialysis bag was freeze dried again and stored under vacuum.

3.4. Size exclusion chromatography (SEC)

The size exclusion chromatography setup comprised of the following in tandem: (i) a SpectraSystem SCM 1000 degasser (Thermo Separation Products, San Jose, CA); (ii) a SpectraSystem P 2000 chromatographic pump (Thermo Separation Products, San Jose, CA); (iii) a 2 μm frit (Idex, Oak Harbor, WA); (iv) a GPC/SEC PL-Aquagel-OH 50 × 7.5 mm guard column (8 μm) (Varian Inc, Palo Alto, CA); (v) two tandem GPC/SEC PL-Aquagel-OH 300 × 7.5 mm columns (Varian Inc, Palo Alto, CA), all frits and columns encased in a Model 605 column oven (Scientific Systems Incorporated, State College, PA) operating at 30 °C; (vi) a UV detector set at 280 nm (Rigas Labs, Thessaloniki, Greece); (vii) a BI-DNDC differential