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Seeds of pomegranate, tomato and grapes: An underestimated source of natural bioactive molecules and antioxidants from agri-food by-products

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ABSTRACT

Pomegranate, tomato and grape seeds are quantitatively relevant agri-food by-products rich in molecules beneficial to human health. To valorize this resource, the composition and antioxidant activity of seeds and deriving supercritical CO_2 (SC-CO_2) extracted oleoresins were evaluated. Grape seeds showed the highest content of total phenolic compounds (33.9 mg GAE/g), flavonoids (15.6 mg CE/g) and condensed tannins (14.0 mg CE/g), while tomato seeds presented the highest content of tocochromanols (159.6 μg/g). Grape seeds showed the highest total antioxidant activity (178.2 μmol TE/g), as evaluated by TEAC assay, followed by pomegranate (19.8 μmol TE/g) and tomato (9.8 μmol TE/g). Oleoresin yields obtained by SC-CO_2 extraction from the seeds ranged between 3.1 (pomegranate) to 7.8 (tomato) g oleoresin/100 g. Total tocochromanols were abundant in pomegranate (2008 μg/g) and tomato (1769 μg/g) oleoresins; a relatively low amount was instead detected in the oleoresin extracted from grape seeds (636 μg/g). Carotenoids were not detected in all oleoresins. Pomegranate oleoresin had a higher antioxidant activity than the others. Mono- and polyunsaturated fatty acids were more abundant than saturated in all oleoresins, with the highest percentage of unsaturated fatty acids detected in pomegranate seed oleoresin (~90%), mainly due to punicic acid (~70%).

1. Introduction

Agri-food by-products produced during handling and processing of fruits and vegetables, including peels, seeds, leaves, bracts, stems, roots and bark, represent a major waste disposal problem for industry (Ezejiofor et al., 2014). Besides large amounts of storage (sucrose, starches, inulin, pectin like polysaccharides, etc.) and cell wall structural (cellulose, hemicelluloses and pectins) carbohydrates, proteins and lipids, potentially useful for animal and human food supplementation and/or bioenergy production, high-value natural compounds such as carotenoids, phenols, tocochromanols, vitamins, or phytosterols can be found in most of these by-products, many of them having health-promoting properties (Kamal-Eldin and Appelqvist, 1996; Kohno et al., 2004; Lenucci et al., 2013).

Seeds represent a quantitatively abundant by-product of fruit industrial processing. They are often discarded within the so called pomace together with skins, and vascular tissues of the fruits, but can be easily recovered by separation and sifting technologies. An estimated 13–20% by weight of the grapes processed by the wine industry ends up as pomace after pressing, with seeds accounting for approximately 8–20% of the total waste depending on the grape cultivar and processing method, equivalent to 0.5–2.0 million tons per year (Schieber et al., 2001; Dwyer et al., 2014). Similarly, seeds account for approximately 60% of the total waste of tomatoes (3–6 million tons per year) and 22% of the waste (rind plus seeds) of pomegranate juice industry (FAO, 2011; Abid et al., 2017). Seeds represent the portion of the fruit with the highest concentration of bioactive molecules so that their waste represents a double loss for agri-food industry that has to face the cost of disposal and the loss of profits for their re-use and valorization. Pastrana-Bonilla et al. (2003) reported that among the different portions of grape fruit, seeds exhibit the highest antioxidant activity followed by the skin and the flesh; similarly, the seed fraction of tomato

Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid); CE, catechin equivalents; GAE, gallic acid equivalents; HAA, hydrophilic antioxidant activity; LAA, lipophilic antioxidant activity; MUFA, monounsaturated fatty acids; ORAC, oxygen radical absorbance capacity; PUFA, polyunsaturated fatty acids; SC-CO_2, supercritical CO_2; SFA, saturated fatty acids; TAA, total antioxidant activity; TE, trolox equivalents; TEAC, trolox equivalent antioxidant capacity; UFA, unsaturated fatty acids

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was shown to contain higher phenolic content than pulp, thus representing an important reservoir of these compounds whose bioactivity has been unequivocally established (Chandra and Ramalingam, 2011). The antiproliferative and apoptotic effects of proanthocyanidins from grape seeds on colon cancer Caco2 cells has been demonstrated (Engelbrecht et al., 2007; Kaur et al., 2009). Further, tomato and pomegranate seeds have attracted interest since their oil is rich in unsaturated fatty acids (UFA), especially linoleic acid (> 50% in tomato), phytosterols and antioxidants which make it particularly suitable as edible oil (Roy et al., 1996; Eller et al., 2010). Pomegranate seed oil is a major source of punicic acid, a distinctive ω-5 trienoic fatty acid with emerging evidences for important therapeutic uses in human health including inhibition of cancer cell proliferation (Albrecht et al., 2004; Jeune et al., 2005; Aruna et al., 2016).

The increasing awareness of the potential commercial value of most agri-food by-products has stimulated the exploitation of efficient extraction techniques of their bioactive compounds with undeniable environmental sustainability benefits and a more effective use of the harvested plant material (González-Paramás et al., 2004; Vági et al., 2007).

In recent years, supercritical CO_2 (SC-CO_2) extraction technology has been widely proposed to prepare food-grade oleoresins from a variety of by-products, including seeds, as an alternative to conventional extraction techniques. Being non-toxic, non-flammable, non-corrosive and highly selective, the use of CO_2 as unique extraction solvent is, in fact, safer and more environmentally friendly compared to the use of most conventional organic solvents (Reverchon and De Marco, 2006; Liu et al., 2012; Durante et al., 2012). The use of seeds as co-matrix to increase the solubility and extractability of carotenoids (especially lycopene from tomato dehydrated matrices) in SC-CO_2 and for the enrichment of the extract in bioactive compounds has been also described (Lenucci et al., 2015; Durante et al., 2016).

Thus, we present, for the first time, a comparative analysis of the main bioactive constituents from pomegranate, tomato and grape seeds, as well as of the oleoresins obtained from their extraction by SC-CO_2, including fatty acid composition and the content of tocochromanols, in order to better assess the potential of these agri-food by-products as sources of nutraceutical or functional food natural ingredients.

2. Materials and methods

2.1. Chemicals

Tocopherols (α-, β-, δ- and γ-forms), myristic, palmitic, stearic, arachidic, margaric, palmitoleic, oleic, linoleic and linolenic acids used as standards, PBS solution (pH 7.4), 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) diammonium salt, 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), catechin, Folin & Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), gallic acid, vanillin, sodium nitrite ($NaNO_2$), aluminium chloride hexahydrate ($AlCl_3{\cdot}6H_2O$), sodium chloride (NaCl), as well as all solvents were purchased from Sigma–Aldrich (Milan, Italy). Punicic acid and tocotrienols (α-, β-, δ- and γ-forms) were purchased from Cayman chemicals (Ann Arbor, MI, USA). Carotenoid standards were purchased from CaroteNature (Lupsingen, Switzerland). High purity carbon dioxide (99.995%) for supercritical fluid extraction was purchased from Mocavero Ossigeno (Lecce, Italy).

2.2. Plant material processing

The analyses were carried out on pomegranate (*Punica granatum* L., cultivar Dente di cavallo), tomato (*Lycopersicon esculentum* L., cultivar San Marzano) and grape (*Vitis vinifera* L., cultivar Nero di Troia) seeds, which were manually separated from the pomaces obtained from the mechanical pressing of the fruits to produce juices. All fresh fruits were grown in open-field in the province of Lecce in southern Italy (latitude 40°23′16″80N, longitude 17°57′41″40E; decimal degrees 40.3881; 17.9615) by local farmers. Approximately 10 kg of fully ripe healthy fruits were processed into juices. For pomegranate an electrical juice squeezer (Fimar S.p.A., Rimini, Italy) was used, while tomato and grape were processed by a Kuvings CS600 cold-press juicer (NUC Electronics Co., Daegu, Korea). Approximately 0.3 kg of seeds were collected from each pomace. Isolated seeds were vacuum-dried to constant weight at 60 °C by a Salvis Lab IC40 vacuum-drying oven (Bio Instruments S.r.l., Firenze, Italy) and then ground in a laboratory ultra-centrifugal mill (ZM200, Retsch GmbH, Haan, Germany) through 18 mesh (1 mm) sieve. These cultivars were selected since they are traditional Italian and largely used for industrial processing.

2.3. Extraction of soluble and insoluble-bound phenolic compounds

Extraction of phenols was carried out according to Adom et al. (2003). Briefly, 0.05 g of samples (three independent replicates from the same seed batch) were mixed with 1 mL of 80% (v/v) chilled ethanol for 10 min. After centrifugation at 2500*g* for 10 min, the supernatant containing the soluble phenolic compounds was recovered. The extraction was repeated twice and the supernatants were combined.

The insoluble-bound phenols were extracted from the pellets resulting from the soluble phenolic extraction. The pellets were sequentially washed twice with 100% methanol (1 mL) and chloroform/methanol/water (1/1/1 v/v/v – 1.5 mL) and once with 100% acetone (1 mL). The supernatants were discarded after each extraction and centrifugation at 9000*g* for 7 min. The residues were then digested with 1 mL of 1 M NaOH at room temperature for 1 h with shaking under nitrogen gas. The mixtures were acidified to pH 2 with 12 M HCl and extracted three times with 500 µL of ethyl acetate. The ethyl acetate fraction was evaporated to dryness under vacuum by a rotary evaporator. Phenolic compounds were dissolved in 500 µL of 80% (v/v) ethanol.

2.3.1. Determination of total phenolic content

Total phenolic content was determined on each extract (soluble and insoluble-bound phenolic extracts) according to the method of Xu et al. (2008). Briefly, 50 µL of extract were mixed with 50 µL of Folin-Ciocalteu's phenol reagent and 450 µL distilled water. The mixture was kept at room temperature for 5 min, and then 500 µL of 7% (w/v) Na_2CO_3 added, reaching the final volume of 1250 µL with distilled water. The mixture was left at room temperature in the dark for 90 min. Absorbance was measured at 750 nm using a Beckman DU650 spectrophotometer (Beckman Coulter Ltd., High Wycombe, UK). The amount of total phenols was calculated using gallic acid as calibration standard within the range of 0–12 µg GA/100 µL in 80% ethanol. The results were expressed as mg gallic acid equivalents (GAE)/g seeds. A single technical replica was performed for each independent extract.

2.4. Extraction of flavonoids and condensed tannins

To 0.1 g of sample seeds (three independent replicates from the same batch) 1.5 mL of 100% methanol were added. The mixture was then shaken at 4 °C for 16 h, centrifuged at 9000*g* for 10 min and the supernatant was recovered.

2.4.1. Determination of total flavonoid content

The total flavonoid content was determined as described by Zhishen et al. (1999). 50 µL of extract were diluted with distilled water to a final volume of 500 µL and 30 µL of 5% $NaNO_2$ (w/v) were added. After 5 min, 60 µL of 10% $AlCl_3$ were added, followed, after further 6 min, by 200 µL of 1 M NaOH and 210 µL of distilled water. The absorbance was read at 510 nm in a Beckman DU650 spectrophotometer. The linear calibration curve was from 0 to 400 µg catechin/mL in 100% methanol. The results were expressed as mg catechin equivalents (CE)/g seeds. A