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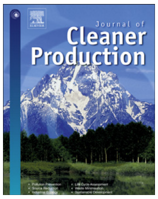

Biorecovery of antioxidants from apple pomace by supercritical fluid extraction

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ABSTRACT

This work explored the potential of supercritical fluid extraction (SFE) to recover phenolic compounds and antioxidants from apple pomace. SFE was carried out at 20 and 30 MPa and temperature of 45 and 55 °C in absence and presence of ethanol (5%) as co-solvent. The results were then compared to those obtained by Soxhlet extraction with ethanol and boiling water maceration. All the extraction techniques were performed on fresh, oven and freeze dried samples. The extracts were characterized for their antioxidants capacity with different assays, such as the Folin-Ciocalteu, the 2,2-diphenyl-1-picryhydrazyl radical (DPPH) and a flow injection coulometry technique. The results showed that the extracts obtained from SFE, carried out on freeze dried apple pomace at 30 MPa and 45 °C for 2 h with ethanol (5%) as co-solvent, led to a higher antioxidant activity (5.63 ± 0.10 mg TEA/g of extract) than conventional extraction technologies such as Soxhlet with ethanol (2.05 ± 0.21 mg TEA/g of extract) and boiling water maceration (1.14 ± 0.01 mg TEA/g of extract). The HPLC-DAD-MS analysis also confirmed the abundance of some phenolic compounds in SFE extract. Overall, the study presented here is one of the first investigations to assess the impact of supercritical carbon dioxide for the extraction of antioxidants from apple pomace.

1. Introduction

Reduction of food losses and by-products valorization is a tighten issue in food processing as the large amount of non-edible residues produced by the industries cause pollution, difficulties in the management and economic loss. Taking into consideration the huge amount of materials produced during the transformation of fruits, such as peels, seeds and bagasse, the waste disposal represents a problem (Parfitt et al., 2010). Research over the past 20 years has revealed that many food wastes could serve as a source of potentially valuable bioactive compounds, such as antioxidants, vitamins and fibers with increasing scientific interest thanks to their beneficial effects on human health (Ferrentino et al., 2017; Galanakis, 2012). In particular, industrial by-products derived from fresh fruits and vegetables are rich in antioxidants such as ascorbic acid, tocopherols, carotenoids, and polyphenols (Shi et al., 2005).

Among fruit by-products, apple pomace is a potential source of phenolic compounds. It has been estimated that apple juice production results in a juice poor in phenolic compounds and with only 3–10% of the antioxidant activity of the fruit from which it is produced. Most of the phenolic compounds remain in the apple pomace, a heterogeneous mixture of peel, core, seed, calyx, stem and soft tissue (Adil et al., 2007; Kim et al., 2005; Carson et al., 1994). In the past, this by-product was just treated as waste by incineration, anaerobic fermentation, composting, landfill, or reutilized for agricultural applications. Nowadays, many opportunities exist for converting it to marketable products such as the production of animal feed, ethanol, natural gas, citric acid, charcoal, pectin, and fiber (Dugmore et al., 2017). Some studies also show the potential applications of some recovered fractions in bakery, cosmetic and oil-based products (Galanakis et al., 2018a, 2018b, 2018c). As an example, natural antioxidants recovered from olive mill wastewater were able to induce antimicrobial properties in bakery products and subsequently prolong their shelf life (Galanakis et al., 2018a) or to prevent lipid oxidation when added to extra virgin olive oils (Galanakis et al., 2018b).

Among these opportunities, the recovery of phenolic

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compounds, using conventional or innovative technologies, represents an alternative for the valorization of apple pomace. Many studies demonstrate that conventional solvent extraction technologies (Wijngaard and Brunton, 2010; Cam and Aaby, 2010) show good recovery of polyphenols. However, solvent extraction has several drawbacks like the use of high amount of solvents, long extraction time and possible degradation of target compounds.

In the last years, innovative green technologies have been proposed for the recovery of valuable compounds from fruit by-products, such as pressurized liquid extraction (Wijngaard and Brunton, 2009), supercritical fluid extraction, microwave assisted extraction and ultrasonic extraction (Adil et al., 2007). They are able to overcome some of the disadvantages of conventional techniques shortening the processing times, accelerating heat and mass transfer rates, controlling Maillard reactions, improving the product quality, enhancing the functionality of the recovered compounds with their protection from environmental stresses and consequently extending their preservation (Galanakis, 2013, 2015; Deng et al., 2015).

Among the innovative green technologies, supercritical fluid extraction (SFE) is one of the most interesting because of the favorable properties of carbon dioxide (CO_2) that, as a dense solvent, has a low critical temperature ($T_c = 31.1\ ^\circ C$). In addition, CO_2 is colorless, odorless, non-toxic, non-flammable, safe, highly pure and easily removed from the extract following decompression.

However, since CO_2 is non-polar, it is not an optimal solvent for polar polyphenols. The addition of organic co-solvents like ethanol, methanol, acetone, increases the solvating power of CO_2 and the yield of extraction of polar polyphenols. The use of a co-solvent, however, needs to be limited as when it is added to CO_2, the critical temperature of the resulting mixture is elevated (Gurdial et al., 1993; Adil et al., 2007). Recently, some studies have been published dealing with the application of SFE with or without co-solvent to obtain extracts with antioxidant activity and phenolics from blueberry, cranberry and raspberry wastes (Laroze et al., 2010), wine industry by-products (Louli et al., 2004), pistachio hull (Goli et al., 2005), olive leaves (Le Floch et al., 1998), orange pomace (Benelli et al., 2010), peach kernels (Natalia et al., 2010), tomato skin and pomace (Chun et al., 2009; Lamin et al., 2008; Vagi et al., 2007).

To the best of our knowledge, just one research work has dealt with the application of CO_2 as solvent in subcritical conditions for the recovery of phenolic compounds from apple pomace (Adil et al., 2007). In that study, freeze dried apple pomace was treated with subcritical carbon dioxide using ethanol (14–20%) as co-solvent at pressures from 20 to 60 MPa and temperatures from 40 to 60 °C. Although the work showed the promising results of the technology, it did not investigate its efficiency in supercritical conditions, thus limiting the amount of ethanol used as co-solvent. Moreover, no attempts were done to assess the impact of pre-treatments (freeze drying or oven drying) on the extracts and to identify the differences between polyphenols extracted by SFE and conventional techniques.

Accordingly, this work aimed to recover phenolic compounds with antioxidant activity from fresh, oven and freeze dried apple pomace applying CO_2 in supercritical conditions without and with ethanol (5%) as co-solvent. The efficiency of the process was estimated in terms of yield. The results were compared to those obtained either by Soxhlet extraction or boiling water maceration. The antioxidants capacity of the extracts was measured applying different assays (Huang et al., 2005), such as the Folin-Ciocalteu (Singleton and Rossi, 1965) and the 2,2-diphenyl-1-picryhydrazyl radical (DPPH) (Brand Williams et al., 1995), together with a flow injection coulometry technique. Finally, besides the antioxidant activity, the chemical characterization of the extracts obtained by SFE and conventional techniques was also addressed by HPLC-DAD-MS. The study presented here is one of the first investigations that compares the impact of carbon dioxide in supercritical conditions for the extraction of antioxidants from apple pomace with that of conventional extraction technologies.

2. Materials and methods

2.1. Materials

Apple pomace, composed of peels, seeds, stems and pulp, was kindly donated by Fructus Meran S.p.A. (Vilpiano, Italy). Folin-Ciocalteu's phenol reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, ammonium formate, formic acid and gallic acid were purchased from Sigma Aldrich (Milano, Italy). 6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (Trolox, purity 97%) was purchased from Acros Organics (Geel, Belgium). Ethanol (96% purity) and acetonitrile were purchased from VWR Chemicals (Fontenay-sous-Bois, France). Methanol (HPLC grade) and lithium perchlorate were purchased from Honeywell (Steinheim, Germany).

2.2. Sample preparation

The apple pomace, separated from seeds and stems, was drained from the excess of water, packed in plastic pouches and hermetically sealed with a pulse sealer. They were then blanched at 80 °C for 20 min for an enzymatic stabilization (Valderrama and Clemente, 2004) and then cooled in an ice bath. A part of the sample was submitted to freeze drying using a laboratory scale lyophilizer (Epsilon 2-6D LSC plus freeze-drier, Martin Christ, Osterode am Harz, Germany). The process was performed freezing the pomace at −18 °C for 4 h and subsequently setting a primary drying for 20 h at a temperature of 35 °C and a vacuum of 1.01 mbar, followed by a secondary drying phase for 6 h at a temperature of 40 °C and a vacuum of 0.05 mbar. Another part of the apple pomace was dried in a moisture-controlled oven (HPP 110, Memmert GmbH & Co. KG, Schwabach, Germany) at 50 °C for 4 days. The dried samples were ground to a fine powder using a laboratory hammer mill (Perten Instruments, Hägersten Sweden) reaching a final particle size of 0.8 mm. A third part of the enzymatically stabilized apple pomace was kept fresh without any drying. All the samples were packed and stored at - 80 °C until further use.

2.3. Characterization of apple waste

The oven, freeze dried and fresh apple pomace were characterized in terms of moisture content (Sartorious MA160, Torino, Italy) and water activity (AquaLab, Steroglass, Perugia, Italy). The moisture of the fresh residue was $88.6 \pm 2.4\%$ and water activity was 0.93 ± 0.01. The drying of apple waste achieved a water reduction of around 70%, thus the freeze dried and the oven dried samples reached a moisture content of $19.2 \pm 1.1\%$ and $20.5 \pm 1.7\%$, respectively. For both dried samples, water activity was 0.15 ± 0.02.

2.4. Supercritical fluid extraction

Supercritical fluid extraction was carried out using a high pressure pilot plant (Superfluidi s.r.l., Padova, Italy) equipped with 1 L volume extractor vessel and two gravimetric separators. The flowsheet of the plant is given in Fig. 1.

The high pressure vessel contained an extraction basket of 800 mL, closed with porous stainless steel mesh filters on both ends to enable carbon dioxide to pass the cylinder without transport of solids to the exterior. The temperatures of the extractor and the two separators were automatically regulated through the recirculation