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Recovery of phenolics from apple peels using CO_2 + ethanol extraction: Kinetics and antioxidant activity of extracts

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ABSTRACT

Subcritical extraction (SFE) of dry and ground *Golden delicious* peels (30 g) was investigated at 25 MPa and 50 °C using CO_2 and ethanol (96%) in 75:25 mol ratio. As for conventional ethanol or methanol/acetone/water extraction, nine phenolics were identified in SFE-extracts including the sugar-based phloridzin and quercetin derivatives. Extraction kinetics of the nine phenolics and of the global yield were monitored via collection of fractions that were also characterized for their antioxidant activity (ABTS antiradical activity). Kinetics showed a constant extraction rate up to 1.1 kg of fluid and a decreasing rate afterwards, but the matrix was not exhausted after 3 h of extraction. Besides the classical continuous flow protocol, SFE was performed by introducing static periods between the dynamic collect of fractions. Static periods did not yield significant improvement in the overall yield and in the individual yield of most phenolics. Increasing the matrix loading did not improve the recovery either. Conversely, extractions from 15 g provided the highest phenolics yield of 800 mg/100 $g_{dry\ peels}$. For extracts tested for antioxidant capacity (30 g loading), values up to 5–6 mg Equivalent Ascorbic Acid/$g_{extract}$ were obtained. Activities were positively correlated with phenolics concentration in fractions only for static conditions.

1. Introduction

A diet rich in fresh vegetables and fruits is generally recommended for a healthy lifestyle because they constitute important sources of nutrients [1] like antioxidants [2], phytosterols [3] and fibre [4]. Transformation of a raw material to foodstuff creates a 'waste' that comprises as well the edible food mass that is lost, discarded, or degraded in the different stages of the food supply chain. Other remnants are peels, stems, cores, skins, seeds, husks, bran or straw from cereals, fish skin, head and bones, mill wastewaters, etc. Food 'wastes' have long been considered as undesirable materials that were disposed of, in costly manners, via animal feed, landfill or incineration, but nowadays, they are considered as promising sources of valuable nutraceuticals [1,5].

Apples are one of the most consumed fruits worldwide and are among the major sources of phytochemicals and antioxidants in the human diet. Approximately 70 million tonnes of apples are produced worldwide (http://www.wapa-association.org). Apple fruits have a varied and well-balanced composition with a large diversity of vitamins, a high content of fibres compared to other fruits and are moderately energetic in terms of caloric intake [6,7]. Prevention of various chronic diseases has been associated with apple consumption [8], in particular cardiovascular disease, in relation with the main bioactive compounds of apples, namely fibre and polyphenols [9]. Apples contain over 60 different phenolic compounds [8]. The four major phenolic groups are hydroxycinnamic acids (with chlorogenic acid as the most abundant representative), dihydrochalcone derivatives (specially phloridzin), flavan-3-ols (catechin as monomers or procyanidins as oligomers) and flavonols (quercetin and quercetin glycosides) [10,11]. The distribution and concentration of polyphenols vary greatly among apple cultivars (range of 68–165 mg/100 g edible portion [6]) and within the apple fruit. Apple peels have higher levels of total polyphenolic compounds than flesh or core and concentrate specially quercetin glycosides, chlorogenic acid and phloridzin [11–15]. As example, for eight apple cultivars, the total polyphenolics ranged from 1.0 to 2.3 mg/g of fresh weight in the peel, to be compared with the 0.33–0.93 mg/g of fresh weight in flesh [13]. Additionally, apple peels polyphenols were shown to have beneficial actions on oxidative stress and inflammation [16].

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In France, an average of 20 kg of apples per capita are consumed [17] corresponding to a 20.4% market share, far more important than that of the second most consumed fruits, bananas (14%) and oranges (12%). Apple is the largest fruit produced in France with 1.5 million tonnes in 2013. Around 30% of the production is transformed in juices, compotes, concentrates, generating large volumes of wastes including peels.

In this work, the use of compressed CO_2 to recover phenolic components from apple peel was investigated. Sub- and supercritical extraction of functional ingredients from natural sources was reviewed by Herrero in 2006 [18], whereas Diaz-Reinoso [19] focused on compounds with antioxidant activities and Marostica [20] on phenolics from plant materials. Since 2006, more than 70 papers about 'polyphenols' and 'supercritical extraction' have been published (scopus source). With special mention to food wastes, grape residues produced by the wine or distilling industry are among the most largely studied by-products. Residues comprised grape skin or seeds [21,22], bagasse [23], marc [24,25] or pomace [26,27]. As for other by-products, colouring anthocyanins were extracted from eggplant peels [28], phenolic antioxidants from several berries pressing wastes [29] or oil containing phenolics from cherry kernels [30] or grape seeds [31]. Fruits pulp or leaves were investigated as well, in particular sweet cherries [32], jamun fruits [33], pitanga leaves [34] and arrabidae chica leaves [35], for the most recent studies. To the best of our knowledge, supercritical extraction from apples was only reported once [36]. The by-product was the apple pomace, i.e. skin and pulp residues remaining after pressing the fruits for juice production. One gram of pomace was extracted by CO_2 + ethanol (14–20%) for 10–40 min varying pressure (20–60 MPa) and temperature (40–60 °C). Extracts were characterized for the total phenolic content and antioxidant activity, but no identification and quantification of the extracted phenolic species were reported.

The aim of this work was to evaluate the potentialities of supercritical technology to extract phenolic compounds from apple peels, focusing on three issues:

(1) Identification and quantification of the extracted polyphenols.
(2) Monitoring the extraction kinetics of polyphenols and of the total extracted amount.
(3) Studying the impact of process conditions onto kinetics and yields (implementation of static steps during extraction, variation of the amount of matrix loaded in the vessel).

In this work, dry apple peels were extracted by CO_2 + 25% mol cosolvent (ethanol at 96%) at 25 MPa and 50 °C during 3 h. Being an apolar fluid, CO_2 has a limited capacity for dissolving polyphenolics so ethanol is required as cosolvent to overcome this limitation [37–39]. The use of high proportion of ethanol was justified by the fact that phloridzin and quercetin glycosides, abundant in apple peels, contain a sugar moiety that is too polar to be soluble in neat CO_2. The selected temperature and pressure are in the range of those used for supercritical extraction of phenolic compounds [20] and moderate temperature and high pressure are generally associated with low thermal degradation and high solubility. Due to the 25% of cosolvent, extractions were performed in subcritical conditions since 50 °C is below the critical temperature of that CO_2 + cosolvent mixture [36]. During the extraction course, fractions were regularly collected in order to describe accurately the extraction kinetics and possibly fractionate the phenolics pool. Extracts were characterized in terms of global yield, phenolic composition and antioxidant activity. A new procedure of extraction that alternated static and dynamic periods was also investigated with the aim of giving longer time for mass transfer and diffusion to occur.

2. Materials and methods

2.1. Apple peel preparation and chemicals

Golden delicious apples were purchased from a local conventional orchard and were stored at 1 °C. Apples were peeled mechanically (Kali, France) and the obtained peels were immediately packed into polyethylene bags and frozen at −18 °C for 24 h. Samples were then freeze-dried during 48 h (Heto Lab Equipment, Heto FD 2.5, Denmark) and were further stored in the dark under vacuum at room temperature. The moisture content of the dried apple peels was measured by weight loss at 68 °C in oven under vacuum (Multilab20, Le Matériel Physico-Chimique, France) and was in the range of 5–7%. For extractions, the dried apple peels were ground using a kitchen-type grinder (Moulinex, France). The obtained ground material was not sieved so the samples consisted in grains of various sizes below 1 mm.

Carbon dioxide (CO_2, 99.5 wt.%, Air Liquide, France) and ethanol (EtOH, 96%, Xylab, France) were used for supercritical extractions. Solvents for liquid chromatography were of HPLC grade (acetonitrile, 99.98%; acetic acid, 99.5%) and were purchased from Fisher Chemical and Acros Organics, respectively. Several standards of polyphenols (HPLC-grade, purity higher than 97%) were purchased from Sigma–Aldrich and Extrasynthese (France): (+)-catechin, (−)-epicatechin, phloridzin, chlorogenic acid, quercetin-3-D-glucoside, quercitrin (quercetin-3-O-arabinoside), hyperoside (quercetin-3-*O*-galactoside).

2.2. Supercritical fluid extraction

Extractions were performed using a home-made system which consists of an extractor vessel of 490 cm^3 (length of 25 cm and inner diameter of 5 cm) heated by heating mantle (Watlow) and two Gilson pumps for fluids admission (model 305, heads of 25SC and 10SC for CO_2 and cosolvent, respectively). The CO_2 and cosolvent flow rates were checked by a gas-meter and the cosolvent volume consumption over time, respectively. Multiple stop valves (Autoclave France) enable to bypass the extractor to stabilize the extracting flux, and a metering valve is used to control the overall flow rate. The set-up comprises a pre-cooling unit (Julabo, Germany) for CO_2, a pre-heater cartridge (TOP Industrie, France), a relief valve and various pressure and temperature sensors. The extractions were performed at 50 °C and 25 MPa which is close to the pressure limit of the equipment. The extractor was filled with a weighted amount of dried apple peels, using alternated beds of matrix and glass beads of 2 mm to avoid caking. The extracting CO_2 + cosolvent mixture circulated from bottom to top. Dissolved species were recovered in a home-made cyclonic collector whose bottom was plunged in ice and that operated at atmospheric pressure. Fractionation of extracts was performed by changing regularly the collector bottom.

Extractions were carried out following two procedures. As common steps, the vessel was charged with the matrix, heated, and pressurized with CO_2 up to 25 MPa. A static period of 20–30 min was applied before starting the extraction by activating the CO_2 and cosolvent pumps. The first fraction was collected until a continuous flow of ethanol appeared in the collector, typically after 20–25 min, for an overall CO_2/EtOH flow of 10 g/min. This fraction, labelled F0, corresponds to the ethanol breakthrough and contains mostly the matter that was solubilized during the static period in neat CO_2. In the so-called dynamic procedure, the extraction continued with regular collects of extracts every 20 min, i.e. for one breakthrough time, over 200 min. The extractor was sometimes subjected to 10 min of static step when recovering F0 and F1 in order to wash the collector. After 200 min, the cosolvent pump was stopped and neat CO_2 was used to flush the matrix from ethanol