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Evaluation of the effect of high pressure on total phenolic content, antioxidant and antimicrobial activity of citrus peels

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ABSTRACT

This study aimed to assess the effect of high pressure at 300 and 500 MPa for 3 and 10 min on the phenolic compounds, antioxidant capacity and antimicrobial activity of citrus peel extracts. Total phenolic contents (TPC) and antioxidant properties of extracts were determined as free radical-scavenging ability of DPPH and using the ABTS radical cation decolorization assay. Additionally, extracts were tested for antimicrobial activity against twenty different strains of bacteria representing both Gram-positive and Gram-negative types. Citrus peel extracts demonstrated antimicrobial activity against a wide range of bacteria. The maximum level of TPC as well as antioxidant capacity were observed at 300 MPa for 3 min. Citrus peels extracts demonstrated antimicrobial activity against a wide range of microorganisms. The antimicrobial activity of orange peel extract was the highest among the four citrus peels studied. Generally, bacteria *Acinetobacter* and the strain *Listeria innocua* were more sensitive to the peel extracts.

1. Introduction

Citrus is one of the most abundant crops in the world. Its worldwide production is over 88 million tons and one-third of the crop is processed. Oranges, lemons, grapefruits, limes and mandarins represent approximately 98% of the entire industrialized crop, oranges being the most relevant with approximately 82% of the total (Izquierdo & Sendra, 2003).

Produced in tonnes per day, citrus by-products represent a problem for management, pollution, and environmental issues, due to microbial spoilage (Laufenberg, Kunz, & Nystroem, 2003; Montgomery, 2004). Citrus by-product wastes have been traditionally utilized as molasses for animal feed (Mirzaei-Aghsaghali & Maheri-Sis, 2008), fiber (pectin) (Chou & Huang, 2003) and fuel production (Llorach, Espin, Tomas-Barberan, & Ferreres, 2003). However, researchers are looking for new uses of these by-products such as production of food additives or supplements with high nutritional value (Delgado-Adámez, Gamero, Valdés, & González-Gómez, 2012b; Pokorny & Parkanyiova, 2004).

Citrus peels contain a high concentration of phenolic compounds and represent a rich source of natural flavonoids (Hayat et al., 2010; Kamran, Youcef, & Ebrahimzadeh, 2009); these are abundant in the plant. In addition, there are several compounds such as flavanones, flavanone glycosides and polymethoxylated flavones unique to citrus, which are relatively rare in other plants (Manthey & Grohmann, 2001; Montgomery, 2004). It has been reported that these compounds have high antioxidant activity (El-Seedi et al., 2012; Hayat et al., 2010) and exert antimicrobial effects against foodborne pathogens (Delgado-Adámez, Fernández-León, Velardo–Micharet, & González-Gómez, 2012b, 2012a; DelgadoAdámez et al., 2012b; Espina et al., 2011) due to their high contents of terpenoids, tannins, quinones, phenolic acids and polyphenols (Calvo et al., 2006; Lee & Lee, 2010). However, these compounds are usually present in a covalently bound form (Xu, Ye, Chen, & Liu, 2007). Therefore, reliable and practical methods for liberation of natural antioxidants and antimicrobial compounds from plant materials are of considerable interest.

For the analysis and exploitation of bioactive constituents their extraction from the cellular matrix is needed. The conditions for the extraction process depend on factors that have a direct influence on the raw material and they must be fixed to obtain the highest antioxidant and antibacterial activity (Pinelo, Rubilar, Sineiro, & Nunez, 2004).

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Several methods are used to extract and activate low-molecular weight natural antioxidants and antimicrobials using nonconventional technologies such as microwave and ultrasound-assisted extraction, supercritical fluid extraction, high temperature/high pressure extraction and pressurized liquid extraction (Ben Hamissa et al., 2012; Vilkhu, Mawson, Simons, & Bates, 2008). Several of these methods require high temperatures and many organic compounds are heat-sensitive. Therefore they will be degraded, lose biologic activity, or change into another compound (Zhang, Junjie, & Changzhen, 2004). High pressure extraction (HPE) has been initially attempted by Shouqin, Jun, and Changzheng (2005) to obtain natural plant materials. The inner membrane compounds are extracted due to cell membrane rupture under pressure. High pressure ranging from 100 to 800 MPa or even more, up to 1000 MPa, is considered as an alternative extraction method, which is proven to be fast, effective and heat degradation is avoided (Corrales, Garcia, Butz, & Tauscher, 2008; Zhang, Xi, & Wang, 2005; Zhang et al., 2004). Furthermore, this technology has been used successfully for the extraction of flavonoids from propolis (bee glue) and polyphenols from green tea (Zhang et al., 2004), anthocyanins from grape skin (Corrales, Garcia, Butz, & Tauscher, 2008) and ginsenosides from the roots of Panax ginseng (Zhang, Ruizhan, & Changzheng, 2007). However, limited information is available on the application of high pressure to extract phenolic compounds in citrus by-products.

The present study established a procedure for the extraction of phenolic compounds from citrus peels and evaluated their antioxidant and antimicrobial activity after different high pressure processes.

2. Materials and methods

2.1. Plant materials

Samples of lemon (*Citrus limon*), tahiti lime (*Citrus latifolia*), mandarin (*Citrus reticulata*) and sweet orange (*Citrus sinensis*) used in this study were obtained from a local supermarket in Porto, Portugal. Prior to extraction, fruits were peeled; peels were cut into pieces (ca. 0.25 cm^2) and stored at 4 °C until use.

2.2. Bacterial strains and media

Twenty bacterial strains, ten Gram-positive and ten Gram-negative bacteria, deposited in the culture collection of Escola Superior de Biotecnologia as frozen concentrates (−80 °C) in Tryptone Soy Broth (TSB; Lab M, UK) containing 30% (*v*/*v*) glycerol were used as target strains to assess the antimicrobial activity of the extracts (Table 1).

2.3. Sample preparation

Each sample of citrus peels (ca. 15 g) was placed in a double-low permeability polyamide–polyethylene bag and vacuum-sealed. Treatments were conducted in a high pressure system (Unipress Equipment, Model U33, Institute of High Pressure Physics, Warsaw, Poland). The equipment has a pressure vessel of "35-mm diameter" and "100-mm height" "100-mL capacity" surrounded by an external jacket, connected to a thermostatic bath (Huber Compatible Control CC1, New Jersey, USA) to control the temperature. As recommended from the manufacturer for this HPP equipment, a mixture of propylene glycol and water (40:60) (Dowcal N fluid, Dow Chemical Company) was used as the pressurizing fluid and to control the temperature in the external jacket. Pressure was built up slowly (100 MPa/min) to minimize temperature increases due to adiabatic heating, and depressurization took place in less than 60 s. After pressure release, samples were immediately cooled in an ice-water bath.

Five different batches were prepared: (i) control without pressure treatments; pressure treatment of (ii) 300 MPa, 3 min; (iii) 300 MPa, 10 min; (iv) 500 MPa, 3 min; (v) 500 MPa, 10 min. All pressure treatments were applied at 10 °C. Each condition was made in triplicate.

Table 1
Bacterial strains used as target organisms to assess the antimicrobial activity of the extracts.

Bacterial strains	Source
Gram-positive	
Bacillus subtilis	ESBCC 01
Enterococcus faecalis	ATCC 29212
Staphylococcus aureus	ESBCC 81
Listeria innocua	PHLS 2030c
Listeria monocytogenes	ESBCC 3391
Listeria monocytogenes	ESBCC 2264
Listeria monocytogenes	ESBCC 1940/1
Listeria monocytogenes	ESBCC 1853/3
Clostridium sporogenes	ESBCC 01
Clostridium difficile	ESBCC V591359
Gram-negative	
Acinetobacter lwoffii	ESBCC T7BT5
Acinetobacter junii	UGCC 889
Acinetobacter pittii	ESBCC T1BP1
Acinetobacter baumannii	ESBCC 05
Klebsiella spp.	ESBCC 01
Escherichia coli	ATCC 25922
Proteus vulgaris	ESBCC 01
Salmonella Typhimurium	ESBCC 01
Pseudomonas spp.	ESBCC 01
Cronobacter sakazakii	ATCC 51329

ESBCC: Culture Collection of Escola Superior de Biotecnologia.
ATCC: American Type Culture Collection.
PHLS: Public Health Laboratory Service, Colindale, London.
UGCC: University of Gotemburg Culture Collection.

2.4. Total phenolic content of peel

Ten grams of each citrus peel was homogenized with 60 mL of solvent (80% aqueous ethanol, containing 1% conc. HCl). Phenolic compounds were extracted as described by Casquete et al. (2014). Extraction was performed using a magnetic mixer for 1 h in the absence of light at room temperature (25 °C) and filtered. This process was repeated twice. Excess ethanol was removed by heating at 37 °C in a rotary evaporator under vacuum. The resultant aqueous extracts (crude extracts) were combined to a final known volume and total phenolic content (TPC) was measured spectrophotometrically (λ 760 nm) in a UV-2401PC spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD, USA) using the Folin–Ciocalteu reagent (Wettasinghe & Shahidi, 1999). Results were expressed as equivalent of gallic acid (mg GAE/100 g of fresh peel extracts).

2.5. Purification of the crude extract

The crude extract was purified using HyperSep C18 SPE Cartridges (Thermo Scientific, Cartridge 500 mg, USA). In an initial stage, the cartridge was preconditioned by sequentially passing 10 mL absolute methanol and 10 mL deionized water from a Milli-Q water purification system (Milipore, Bedford, MA, USA) through the cartridge. In a second stage, the crude extract was loaded into the cartridge. The cartridge was then washed by adding 10 mL of Milli-Q water. Finally, HPLC-grade methanol was added to elute the phenolic compounds, and the fraction was collected. Before antioxidant and antimicrobial activity analysis, excess methanol was removed by heating at 37 °C in a rotary evaporator under vacuum. The resultant extracts were combined to a final known volume with Milli-Q water.

2.6. Total antioxidant activity

2.6.1. Determination of the total antioxidant activity by DPPH assay

The electron donation capacity of aqueous extracts diluted to 0.1 mg/mL with Milli-Q water was measured by bleaching of the purple-coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical