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Improving the extraction of carotenoids from tomato waste by application of ultrasound under pressure

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ABSTRACT

The influence of the application of moderate pressure on the ultrasound assisted extraction of carotenoids from dry tomato pomace using a mixing of hexane/ethanol as solvent was investigated. Carotenoid extraction yield (CEY) increased by application of ultrasound and it was higher when static pressure was increased. In previous experiments it was establish that the optimum ultrasound amplitude, static pressure and sonication time for carotenoid extraction were 94 μm of amplitude, 50 kPa, 6 min. These treatment conditions increased the extraction yield 143% in comparison with the control. Response surface methodology was used for optimization extraction temperature (25–45 °C) and the proportion of hexane in the solvent (25%, 50%, 75%) for manosonication assisted extraction of carotenoids. The increment of CEY, the possibility of decreasing the percentage of hexane and the fact that the treatment did not cause degradation of carotenoid extracts are key benefits of manosonication assisted extraction of carotenoids from tomato.

1. Introduction

During the processing of fruits and vegetables a large amount of by-products are generated representing a major disposal problem for the industry in terms of costs and potential negative impact on the environment [1]. However, in some cases, these by-products represent a source of valuable compounds because of their technological and nutritional properties [2,3]. Extraction is one of the crucial steps for using these compounds in the food, pharmaceutical or cosmetic industries. Conventional extraction of these compounds is generally performed by maceration of dried by-products using water or organic solvents depending on if the component of interest is water-soluble or lypophilic water-insoluble. Generally, this technique is very time consuming and requires the use of large volume of solvents [4]. The ability of several methods such as enzyme aided extraction, supercritical CO_2, pressurized liquid extraction, microwaves, pulsed electric fields or ultrasound have been evaluated to optimize extraction of bioactive compounds from by-products by improving the extraction yield, diminishing the extraction time and/or reducing the use of organic solvent [5].

Ultrasound is a non-thermal technology which has shown to be particularly effective for improving extraction of heat labile compounds [6,7]. The effects of high power ultrasound on improving extraction are attributed to acoustic cavitation that consists on the formation, growth and collapse of microbubles inside a liquid submitted to high frequency sound waves ($\geqslant$20 kHz) [8]. This collapse is accompanied by localized extreme pressures (up to 50 MPa) and temperatures (5500 °C), strong acoustic streaming, high shear stress near the bubble wall, microjets near the solid surfaces due to asymmetric collapse of bubbles and turbulence [9]. These mechanical effects of ultrasound may facilitate the release of desired compounds from their matrices by disrupting cellular tissues and by providing a greater penetration of solvent into the cellular materials. Several studies have investigated the effect of ultrasound on the extraction of bioactive compounds such as polyphenols or carotenoids from different plant by-products. The application of ultrasound during the entire maceration step enhanced extraction yield of polyphenols of orange peels around a 40% and of carotenoids from freeze-dried tomato up to 100% [10–13].

It is known that application of moderate external pressure, from 0 to 300 kPa, during sonication (manosonication) increases the intensity of the collapse of the bubbles [14,15]. It has been demonstrated than manosonication drastically increase the inactivation effect of ultrasound on microorganisms and enzymes [16,17]. However, the effect of manosonication on the extraction of bioactive components from plant by-products has not been investigated.

Industrial processing of tomato generates a considerable amount of waste (10–40% of total processed tomato) consisting of peel, seeds and part of the pulp that is known as tomato pomace

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[18]. This tomato by-product is a rich source of carotenoids mainly in the form of lycopene and ß-carotene that are authorized as natural colorants for enhancing food color of processed foods [19]. Moreover, carotenoids have been claimed to provide health benefits such as modulation of the immune system, reduction of the risk of cancer and cardiovascular diseases or provitamin A activity [20].

Extraction of carotenoids from tomato pomace is generally performed by maceration of dried pomace using organic solvents such as hexane and ethanol because carotenoids are lypophilic, water insoluble compounds [21,22]. The process generally requires large amounts of solvents per mass of final products. These solvents define a major part of the environmental performance of the extraction process and also they have impact cost and safety issues [23]. Studies performed by Capello [23] have shown that hexane has a higher environmental impact than ethanol. Therefore, reducing the amount of this solvent without affecting the efficiency in carotenoid extraction is desired.

The aim of this study was to investigate the influence of the application of moderate pressure on the ultrasound assisted extraction of carotenoids from dry tomato pomace. The last objective was to optimize the extraction conditions under moderate temperatures to obtain the highest extraction of carotenoids yield at reduced hexane concentration.

2. Material and methods

2.1. Plant material

Red tomatoes (commercial variety: canario) were purchased from a local supermarket. Tomatoes were passed thought a laboratory peeler-pulper to obtain the tomato pomace composed of skin, seeds and part of the pulp. The tomato pomace was dried in an oven with air circulation at 25 °C until constant weight and dried pomace was grounded in a laboratory mill. Tomato particle size was 4 ± 1 mm. The dry sample was stored in the dark at 4 °C until needed.

2.2. Chemicals

Hexane and ethanol, analytical grade, where purchased from VWR International (Fontenay-sous-Bois, France). All solvents for HPLC analysis (acetonitrile, hexane and methanol) were of HPLC gradient grade and were obtained from Fisher Scientific (Fair Lawn, NJ). All-trans lycopene was purchased from Sigma Chemical Co. (Sigma–Aldrich Company, St. Louis, MO).

2.3. Reference extraction process

In order to identify the advantages of application of manosonication on extraction of carotenoids, conventional maceration of dried tomato pomace with solvent (hexane–ethanol) during the same time as ultrasound assisted extraction was used as control experiments. The first series of experiments were conducted with a mixture of equal volumes (50:50) of both solvents. The extraction of carotenoids was performed into a 250 ml vessel placed in a temperature-controlled (±1 °C) water bath with agitation at 25, 35 and 45 °C. Dry tomato waste (3 g) was placed in the extraction vessel containing 100 ml of solvent tempered at the extraction temperature. When the effect of extraction time was investigated 1 ml of the extract were removed at different time intervals.

2.4. Ultrasound and manosonication assisted extraction

Ultrasound and manosonication treatments were carried out in equipment previously described that permits to apply ultrasound treatments of different amplitude and at different hydrostatic pressures [17]. A treatment chamber of 100 ml pressurized with nitrogen was used for extraction experiments. The tip of a sonication horn (13 mm diameter) connected to a Digital Sonifier® ultrasonic generator (Branson Ultrasonics Corporation, Danbury, Connecticut, USA) that emits sound vibration at a frequency of 20 kHz and different amplitudes (34–145 μm) was located in the bottom of the chamber. A cooling coil located into the treatment chamber was used to dissipate the heat generated by ultrasound and to maintain a constant temperature (25, 35 and 45 ± 2 °C) by circulating cooled water–ethylen glycol mixture. The temperature was monitored by a thermocouple located into the treatment chamber. An entry to the treatment chamber closed by a rubber septum was used to sampling the extraction medium along extraction time using a syringe.

Dried tomato pomace (3 g) was placed into the treatment chamber with 100 ml of solvent (hexane/ethanol) and samples of 1 ml were taken at different time intervals.

Optimal manosonication treatment conditions were established in the first series of experiments, changing the ultrasound amplitude from 58 to 94 μm, the static pressures from 0 to 100 kPa, and sonication time up to 10 min. These experiments were conducted in a mixture of equal volumes (50:50) of both solvents at 25 °C.

The power input (W) into the treatment medium was determined through the calorimetric method previously described by Raso [24].

2.5. Carotenoid quantification

The extracts obtained at different extraction times were centrifuged at 5400 × g for 6 min to eliminate impurities. 0.1 ml of water was added to 1 ml of the extract, in order to separate supernatant into distinct polar and non-polar layers. The absorbance of the non-polar layer (hexane layer) containing carotenoids was measured at 472 nm on a spectrophotometer (Jenway 6505 UV/VIS, Jenway, Felsted, UK). Absolute hexane was used as blank. The carotenoid extraction yield (CEY) was determinate using the molar extinction coefficient of lycopene in hexane at 472 nm (E1%1 cm 3450) [25] and it was expressed as mg of carotenoids/100 g of tomato pomace dry weight (dw).

2.6. Kinetics of carotenoid extraction

Eq. (1) commonly used to describe the solid–liquid extraction of different intracellular compounds [26,27], was fitted to the experimental data corresponding to the evolution of the carotenoid extraction yield along the time.

$$CEY = CEY_{max}(1 - \exp(-kt)) \quad (1)$$

where Y_t is the carotenoid extraction yield at time t (min), Y_{max} is carotenoid extraction yield at equilibrium and k (min^{-1}) is a rate constant.

2.7. HPLC analysis of carotenoids

Before injection, the extracts were concentrated on a miVac concentrator (GeneVac Ltd., UK) for 15 min at 30 °C by vacuum evaporation of 10 ml of the hexane layer and re-dissolving in 2 ml of hexane.

HPLC/DAD analyses were performed on a Varian ProStar high performance liquid chromatograph (Varian Inc., Walnut Creek,