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Optimization of microwave-assisted extraction of hydrophilic and lipophilic antioxidants from a surplus tomato crop by response surface methodology

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ABSTRACT

Tomato is the second most important vegetable crop worldwide and a rich source of industrially interesting antioxidants. Hence, the microwave-assisted extraction of hydrophilic (*H*) and lipophilic (*L*) antioxidants from a surplus tomato crop was optimized using response surface methodology. The relevant independent variables were temperature (*T*), extraction time (*t*), ethanol concentration (*Et*) and solid/liquid ratio (*S/L*). The concentration-time response methods of crocin and β-carotene bleaching were applied, since they are suitable *in vitro* assays to evaluate the antioxidant activity of *H* and *L* matrices, respectively. The optimum operating conditions that maximized the extraction were as follows: *t*, 2.25 min; *T*, 149.2 °C; *Et*, 99.1%; and *S/L*, 45.0 g/L for *H* antioxidants; and *t*, 15.4 min; *T*, 60.0 °C; *Et*, 33.0%; and *S/L*, 15.0 g/L for *L* antioxidants. This industrial approach indicated that surplus tomatoes possess a high content of antioxidants, offering an alternative source for obtaining natural value-added compounds. Additionally, by testing the relationship between the polarity of the extraction solvent and the antioxidant activity of the extracts in *H* and *L* media (polarity–activity relationship), useful information for the study of complex natural extracts containing components with variable degrees of polarity was obtained.

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1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is the second most important vegetable crop worldwide after potato and is consumed either fresh or in the form of processed products. In 2013, about 164 million of tones were produced in the world, having been registered an increase of 2.6 million of tones over 2012 (FAOSTAT, 2015). Apart from the large amounts of solid wastes produced by the processing industry, sometimes there is also a surplus production that leads to glut in the market, distress sale and low profit to the growers (Oliveira, 2006; Sashimatsung et al., 2011). One solution for the problem of this glut may be its sustainable use for the recovery of value-added antioxidant compounds with applications in food, pharmaceutical and cosmeceutical industries. In fact, tomato is a rich source of hydrophilic and lipophilic antioxidants (Barros et al., 2012; Pinela et al., 2012). The hydrophilic fraction is constituted mainly by ascorbic acid and soluble phenolic compounds, while the lipophilic fraction contains carotenoids (mostly lycopene), tocopherols, sterols and lipophilic phenolics. Each of these compounds has their own function in the human organism, acting at different locations, but also working conjunctly, having the ability to offer protection against oxidative stress and various degenerative diseases (Carocho and Ferreira, 2013a,b; Friedman, 2013). Besides, according to some reports, antioxidants belonging to the hydrophilic fraction have a far more significant impact on total antioxidant activity than does antioxidants of the lipophilic fraction (García-Valverde et al., 2013; Kotíková et al., 2011).

The antioxidant activity can be monitored using a large variety of assays, each one based on a specific mechanism of action, including hydrogen atom transfer, single electron transfer, reducing power, and metal chelation, among others (Carocho and Ferreira, 2013a; Shahidi and Zhong, 2015). For this reason, it is important to understand the mechanisms behind the selected assay for a suitable evaluation of the antioxidant potential. Crocin and β-carotene bleaching reactions are two *in vitro* assays appropriate for the antioxidant activity evaluation of hydrophilic (*H*) and lipophilic (*L*) matrices, respectively, and can provide useful information in the study of complex natural extracts containing components with variable degrees of polarity (Prieto et al., 2013; Prieto and Vázquez, 2014). Both assays are reproducible, especially accurate, and yield a low experimental error (Prieto et al., 2014).

To recover antioxidants from plant-based products is necessary to follow suitable extraction methods that ensure and preserve its integrity and bioactivity. That's why the industry is looking for more efficient processes based on enhanced innovation capacity. Among them, microwave-assisted extraction (MAE) has gained significance due to its shortened extraction time, higher extraction rate, reduced solvent consumption and superior product's quality at lower cost (Dahmoune et al., 2015; Gallo et al., 2010), being one of the dominant trends of the "green chemistry" movement (Michel et al., 2011). However, the extraction process efficiency depends on some variables and operating conditions (Bhuyan et al., 2015; Dahmoune et al., 2015), which may not be generalized for all plant materials due to the diverse nature of existing bioactive phytochemicals. Therefore, selection and optimization of variables and operating conditions for the MAE of antioxidants from tomato is necessary.

One-factor-at-a-time approaches are commonly used to optimize extraction processes; but it is well-known that optimal operating conditions or interactions between variables cannot be predicted with this methodology. Both problems may be overcome by employing the response surface methodology (RSM), a powerful statistical tool used to predict the optimum experimental conditions to maximize or minimize various independent variables. Indeed, RSM describes the relationship between independent variables and one or more responses, enabling process optimization such as the extraction of bioactive molecules from natural sources with a reduced number of experimental trials.

This study aimed at determining the optimal extraction conditions for *H* and *L* antioxidants from a tomato surplus. Four independent variables (temperature, extraction time, ethanol concentration and solid/liquid ratio) were studied and the extraction process was optimized by RSM. The concentration-time response methods of β-carotene and crocin bleaching were applied, which are appropriate for the evaluation of antioxidant properties of *L* and *H* fractions, respectively.

2. Material and methods

2.1. Equipment and reagents

Equipments: Biotage Initiator Microwave (Biotage® Initiator+, Uppsala, Sweden) using closed high precision glass vials. Multiskan Spectrum Microplate Photometer using 96-well polypropylene microplates.

Reagents: Linoleic acid (CID 5280450); β-Carotene (CID 5280489); Crocin (CID 5281233); 2,2′-Azobis(2-amidinopropane) (AAPH or ABAP, CID 1969). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (Millipore, model A10, Billerica, MA, USA).

2.2. Plant material

A common tomato farmers' variety known as "tomate redondo or batateiro" (round tomato), and widely cultivated in rural communities from Miranda do Douro, North-eastern Portugal, was chosen for this study. Surplus tomatoes at the ripe stage were hand-harvested randomly from the middle of six plants, in selected homegardens of two villages in the studied area. The ripening stage was established according to local consumers' criteria based in morphological descriptors such as size, texture, and pericarp colour. Six tomatoes (pericarps without jointed pedicels and seeds) were frozen and lyophilized (Free Zone 4.5, Labconco, Kansas City, MO, USA), reduced to a fine dried powder (20 mesh) using a grinding machine and kept at −20 °C until analysis.

2.3. Microwave-assisted extraction of H and L antioxidants

The MAE process was performed using a Biotage Initiator Microwave apparatus in closed vials. The dried powdered samples were extracted at different time (*t*), temperature (*T*), ethanol concentration (*Et*) and solid/liquid ratio (*S/L*) ranging as defined by the RSM design (Fig. 1). The solvent volume was fixed at 20 mL. During extraction, samples were stirred at 600 rpm using a magnetic stirring bar and irradiated at 200 W. After that, the reaction mixture in the closed vial was quickly cooled in the processing chamber and then centrifuged at 6000 rpm for 10 min. The pellet was discarded and the