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Microwave-assisted extraction of phenolic acids and flavonoids and production of antioxidant ingredients from tomato: A nutraceutical-oriented optimization study

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ABSTRACT

The production of natural extracts requires suitable processing conditions to maximize the preservation of the bioactive ingredients. Herein, a microwave-assisted extraction (MAE) process was optimized, by means of response surface methodology (RSM), to maximize the recovery of phenolic acids and flavonoids and obtain antioxidant ingredients from tomato. A 5-level full factorial Box–Behnken design was successfully implemented for MAE optimization, in which the processing time (t), temperature (T), ethanol concentration (Et) and solid/liquid ratio (S/L) were relevant independent variables. The proposed model was validated based on the high values of the adjusted coefficient of determination and on the non-significant differences between experimental and predicted values. The global optimum processing conditions (t = 20 min; T = 180 °C; Et = 0%; and S/L = 45 g/L) provided tomato extracts with high potential as nutraceuticals or as active ingredients in the design of functional foods. Additionally, the round tomato variety was highlighted as a source of added-value phenolic acids and flavonoids.

1. Introduction

Phenolic compounds are a group of secondary metabolites widely spread throughout the plant kingdom. Tomato (*Lycopersicon esculentum* Mill.) fruits, apart from being a functional food rich in carotenoids, vitamins and minerals [1,2], is also an important source of phenolic compounds, including phenolic acids and flavonoids [3]. As antioxidants, these functional molecules play an important role in the prevention of human pathologies [4,5] and found many applications in nutraceutical, pharmaceutical and cosmeceutical industries [6]. Therefore, obtaining added-value functional compounds from natural sources, such as tomatoes, is highly desirable by the food industrial sector. Furthermore, the global nutraceutical market has grown in the last decade and a large percentage of the developed nutraceuticals and functional foods are driven by plant-based products [7].

Tomato is a key element of the Mediterranean diet [8] and the second most important vegetable crop worldwide, being consumed either fresh or in the form of processed products. In Trás-os-Montes, North-eastern Portugal, native population's lifestyle has highlighted the importance of local tomato varieties, which are grown using extensive farming techniques and considered as very tasty and healthy foods [9]. Among them, the common variety of tomato, locally known as "tomate Redondo" (round tomato), was reported as a source of *p*-coumaric acid and quercetin derivatives, as well as of the non-phenolic compound benzyl alcohol dihexose [3]. The *p*-coumaric acid has antioxidant, antilipidemic, antihypertrophic and cardioprotective properties [10,11]. Quercetin shows a wide range of biological and pharmacological effects, including antioxidant, anti-inflammatory, antitumor and antibacterial activities, as well as neuroprotective, hepatoprotective, cardioprotective, anti-atherosclerotic, anti-thrombotic and antihypertensive effects [12–15]. In tomato, quercetin is commonly found in the glycoside, *i.e.*, esterified with rutinose. Rutin,

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known as vitamin P, also display a remarkable array of health-promoting effects and is widely used in the industry [16]. In turn, benzyl alcohol, an aromatic alcohol, is used in cosmetic formulations, as local anesthetic, and as a flavoring substance in foods and beverages [17]. Furthermore, epidemiological studies support the protective effect of tomatoes against certain degenerative diseases associated to oxidative stress, including cardiovascular diseases and various types of cancer [18]. Meanwhile, there has been an increasing concern to develop and include phenolic-rich functional foods in the diet in order to improve the nutritional and health status.

Extraction is an important analytical step in the isolation of compounds from plant materials prior to chromatographic identification, or from a preparative point of view, to produce functional ingredients to use in new formulations [7,19]. Today, microwave-assisted extraction (MAE) is gaining many merits due to the higher extraction rate and superior products quality at lower cost. In fact, this novel green technology is considered as a potential alternative to conventional solid–liquid extraction of bioactive compounds from plant matrices [20]. However, the MAE efficiency depends on several variables which may not be generalized for all plant materials due to the diverse nature of existing bioactive phytochemicals, being necessary to select and optimize the processing conditions as a function of the used matrix and taking into account the desired responses.

Apart from the large amounts of industrial by-products derived from tomato processing, sometimes a surplus production of this fruit occur, which can be sustainably used for functional ingredients recovery. In a previous study conducted by Li et al. [20], optimal extraction conditions were determined based on the ferric reducing antioxidant power (FRAP) and oxygen radical absorption capacity (ORAC) assays. These optimized conditions were then used in the analysis of phenolic compounds. However, non-phenolic compounds can influence antioxidant responses. Therefore, an RSM optimization based on chromatographic analysis is more accurate and desired, once the optimal conditions obtained from antioxidant responses may not match the conditions for the extraction of individual compounds. In addition, the low range of extraction time ($\leqslant$3.68 min) originated non significant results. Our study aimed at determining the optimal MAE conditions for maximizing the recovery of functional phenolic compounds and the antioxidant capacity of extracts from tomato. Different variables (processing time, temperature, ethanol concentration, microwave power, and solid/liquid ratio) were investigated and the extraction process optimized using a central composite design coupled with response surface methodology (RSM). The content of the major phenolic compounds (two phenolic acids: benzyl alcohol dihexose and a *cis p*-coumaric acid derivative; and two flavonoids: quercetin pentosylrutinoside and quercetin-3-*O*-rutinoside) and the antioxidant activity (DPPH free-radical scavenging activity and reducing power) were evaluated as responses.

2. Material and methods

2.1. Standards and reagents

HPLC-grade acetonitrile was from Fisher Scientific (Lisbon, Portugal). Formic acid was purchased from Prolabo (VWR International, France). The phenolic compound standards (*p*-coumaric acid, caffeic acid and rutin) were from Extrasynthese (Genay, France). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from Sigma (St. Louis, MO, USA). All the other chemicals 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. Preparation of tomato extracts

2.2.1. Plant material

A common farmers' variety of tomato, known as "tomate redondo or batateiro" (round tomato), widely cultivated in rural communities from Miranda do Douro, North-eastern Portugal, was chosen for this study. Fruits at the ripen stage were hand-harvested randomly from the middle of six plants, in selected homegardens of two villages in the studied area. Ripeness was established according to local consumers' criteria based in morphological descriptors such as size, texture, and color patterns of pericarp. According to local standards, the visual tonality of mature tomatoes was evaluated as corresponding to no. 42 in Red Group, using the color chart of the Royal Horticultural Society. Six tomato fruits (pericarps without jointed pedicels and seeds) were lyophilized (Free Zone 4.5, Labconco, Kansas City, MO, USA), reduced to a fine dried powder (20 mesh) and kept at −20 °C until analysis.

2.2.2. Microwave-assisted extraction

The MAE process was performed using a Biotage Initiator Microwave (Biotage® Initiator+, Uppsala, Sweden) in closed vessels of high-precision glass. Ethanol:water mixtures were used since ethanol has low toxicity and efficiency for the extraction of phenolic compounds. The presence of a polar hydroxyl group and a non-polar end was also taken into account. The solvent volume was fixed at 20 mL. The powdered samples were extracted using different time (t), temperature (T), ethanol concentration (Et) and solid/liquid ratio (S/L) conditions that ranged as defined by the RSM design (Table 1). During processing, samples were stirred at 600 rpm using a magnetic stirring bar and irradiated at 200 W (a preliminary study presented in Fig. A.1 of the supplementary material indicated that the microwave power has no effect on the extraction process). After that, the mixture in the extraction vessel was quickly cooled in the processing chamber. The mixture was centrifuged at 6000 rpm for 10 min, the pellet was discarded and the supernatant was carefully collected for further analysis. The dry weight (dw) obtained from each solution was evaluated to determine the extraction yield (g extract/g sample). A schematic representation of the sequential steps followed in this work is shown in Fig. A.2 provided in the supplementary material.

2.3. Chromatographic analysis of the main phenolic compounds

After the MAE process, the extract solutions were purified using Sep-Pak® C-18 3 cc Vac Cartridges (Phenomenex, Torrance, CA, USA), wetted and activated with methanol followed by water; sugars and other polar substances were removed with 10 mL of water, and phenolic compounds were further eluted with 5 mL of

Table 1
Coded and natural values of the optimization parameters used in the RSM analysis. The four independent variables X_1 (time, min), X_2 (temperature, °C), X_3 (ethanol concentration, %) and X_4 (solid/liquid ratio, g/L) were combined in a 5-level full factorial design of 25 combinations and 7 replicates at the center of the experimental domain.

Coded values	Natural values			
	X_1: t (min)	X_2: T (°C)	X_3: Et (%)	X_4: S/L (g/L)
−2	0	60	0	5
−1	5	90	25	15
0	10	120	50	25
+1	15	150	75	35
+2	20	180	100	45