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Multiresponse optimization of an extraction procedure of carnosol and rosmarinic and carnosic acids from rosemary

Gerlon de A.R. Oliveira [a], Anselmo E. de Oliveira [a,*], Edemílson C. da Conceição [b], Maria I.G. Leles [a]

[a] Instituto de Química, UFG, 74690-900 Goiânia, GO, Brazil
[b] Faculdade de Farmácia, UFG, 74605-220 Goiânia, GO, Brazil

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ABSTRACT

A green solvent-based optimization for rosmarinic acid (RA), carnosol (COH), and carnosic acid (CA) extraction, the three main antioxidants from rosemary, was performed. The conventional solid-liquid extraction was optimized using a central composite design (CCD) followed by the desirability approach. In the CCD analysis the quantitative effects of extraction time (4.8–55.2 min), liquid-to-solid ratio (4.6–21.4 mL g^{-1}), and ethanol content (44.8–95.2% v/v) were determined for the extracted amount of antioxidants, their concentrations in the extract, and the extraction yield. Samples were analyzed by HPLC and the antioxidants were identified by comparison with pure standard retention times and UV spectra. The desirability function that simultaneously maximizes the antioxidants extraction and their concentrations in the final product was validated. The extraction using a hydroalcoholic solution 70% v/v, at low liquid-to-solid ratio (5 mL g^{-1}), and after 55-min yielded an antioxidant recovery rate of 89.8%, and a final product 4.75 times richer in the main antioxidants than the raw material.

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1. Introduction

Rosemary is a plant known worldwide as a culinary spice and a natural preservative due to its high antioxidant and antimicrobial activities. These activities are related to the presence of phenolic compounds, mainly rosmarinic acid (RA) and diterpenes such as carnosic acid (CA) and carnosol (COH) (Collins & Charles, 1987; Moreno, Scheyer, Romano, & Vojnov, 2006).

Rosemary extract is an effective natural food preservative as on the stabilisation of sunflower oil (Urbančič, Kolar, Dimitrijević, Demšar, & Vidrih, 2014), and pork batter formulation (Hernández-Hernández, Ponce-Alquicira, Jaramillo-Flores, & Legarreta, 2009), for instance. Besides, refrigerated raw meat from animals like gilt-head seabream (Hernández, García García, Jordán, & Hernández, 2014) and sheep (Nieto, Estrada, Jordán, Garrido, & Bañón, 2011) fed with rosemary extract had extended shelf life.

The operational conditions such as extraction time, liquid-to-solid ratio, and the kind of extracting solvent for the extraction method play an important role in the conventional extraction process. Conventional solid-liquid extraction is widely used for antioxidants, plus is safe, cheap, and easy to scale up (Hernández-Hernández et al., 2009; Mulinacci et al., 2011). Extraction techniques like ultrasound and microwave assisted (Rodríguez-Rojo, Visentin, Maestri, & Cocero, 2012), or the ones using supercritical fluid (Carvalho, Moura, Rosa, & Meireles, 2005; Herrero, Plaza, Cifuentes, & Ibáñez, 2010) are mainly employed to improve the extractive process efficiency and final product quality.

The correct choice of the operational conditions leads to a higher recovery rate of the compounds of interest and a more concentrated final product (extract) using less energy, time, raw material, and solvent. All of these factors need to be optimized for antioxidant extraction of different plants or even for the same plant if it has undergone different pretreatments. Differences in matrix structure and plant composition may also require changes to the extraction process (Dorta, Lobo, & González, 2013).

Response surface methodology (RSM), proposed by Box and Wilson in 1951 (Box & Wilson, 1992), has been widely used as a statistical approach to optimize liquid-solid extractions (refer, for instance, to Pap et al., 2013; Xi & Wang, 2013). Central composite design (CCD) is one of the most popular experimental designs because it is efficient, very flexible, and can be run sequentially. Simultaneous optimization of multiple responses, e.g. the recovered amounts of many antioxidants after an extraction process, has been performed using desirability functions (Ghafoor, Choi, Jeon, & Jo, 2009; Hossain et al., 2012).

RA, COH, and CA antioxidant extraction from rosemary and other spices have been reported in the literature. Kim et al. (2010) have proposed an optimized process for RA extraction from

* Corresponding author.
E-mail address: elcana@ufg.br (A.E. de Oliveira).

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Melissa officinalis using methanol. Hossain et al. (2012) have optimized the ultrasound assisted extraction of marjoram antioxidants including RA, COH, and CA, using methanol. Several methods have been optimized for antioxidant extraction from rosemary, such as ultrasound assisted extraction (Paniwnyk, Cai, Albu, Mason, & Cole, 2009), accelerated solvent extraction (Hossain, Barry-Ryan, Martin-Diana, & Brunton, 2011), pressurized green solvent extraction (Herrero et al., 2010), and CO_2 supercritical fluid extraction (SFE) (Herrero et al., 2010; Visentín, Cismondi, & Maestri, 2011). Other studies have reported rosemary extraction without response surface modeling and optimization (Babovic et al., 2010; Couto et al., 2012).

This present paper reports a CCD optimization for the conventional solid-liquid extraction of the main antioxidants from rosemary using a desirability approach. A green solvent-based optimization was performed for RA, COH, and CA extraction, the three main antioxidants from rosemary. In the CCD analysis the quantitative effects of extraction time, liquid-to-solid ratio, and ethanol content were determined for the extracted amount and concentration in the extract of RA, COH, and CA, and the extraction yield. Next, the desirability function that simultaneously maximizes the antioxidants extraction and their concentrations in the final product was validated. Lastly, a RA, COH, and CA enriched rosemary extract was obtained with a high recovery.

2. Materials and methods

Two extractive methods were carried out in this work. In the first one, due to the importance of knowing the total antioxidant content available for extraction, high dilution and ultrasound were employed to quantitatively extract RA, COH, and CA from the powder. In the second method, conventional extraction using green solvents (water and ethanol) and low liquid-to-solid ratio were optimized to be suitable for future applications in the food field.

2.1. Materials

Rosmarinic acid (98%) was purchased from Sigma (Germany). Carnosic acid and carnosol, both with over 95% purity, were obtained from Chromadex (USA). Methanol was of HPLC grade (Tedia, Brazil). Acetic acid and absolute ethanol were from Vetec (Brazil). Ultrapure water (Millipore, USA) was also used. Fine rosemary powder (dried ground leaves from Morocco, having particle size distribution as follows: <0.125 mm: 28.2%; 0.125–0.180 mm: 25.7%; 0.180–0.250 mm: 26.0%; and 0.250–0.425 mm: 20.1%; average particle size of 181 μm, and 8.4% of volatile) was purchased from Santosflora (Brazil). RA, COH, and CA were quantified in the powder.

2.2. Quantification of antioxidant compounds in the rosemary powder

Approximately 200.00 mg of rosemary powder were weighed and transferred to a 50.0 mL volumetric flask. The extractor liquid was added to the flask and the extraction was carried out for 10 min using an ultrasound bath (Unique, Brazil). Water, methanol, ethanol, acetone, and their aqueous mixtures (Fig. 1) were employed in trials to quantitatively extract the three main rosemary antioxidants. Following the extraction, samples were filtered through a 0.45 μm Durapore® PVDF membrane (Millipore, Brazil),

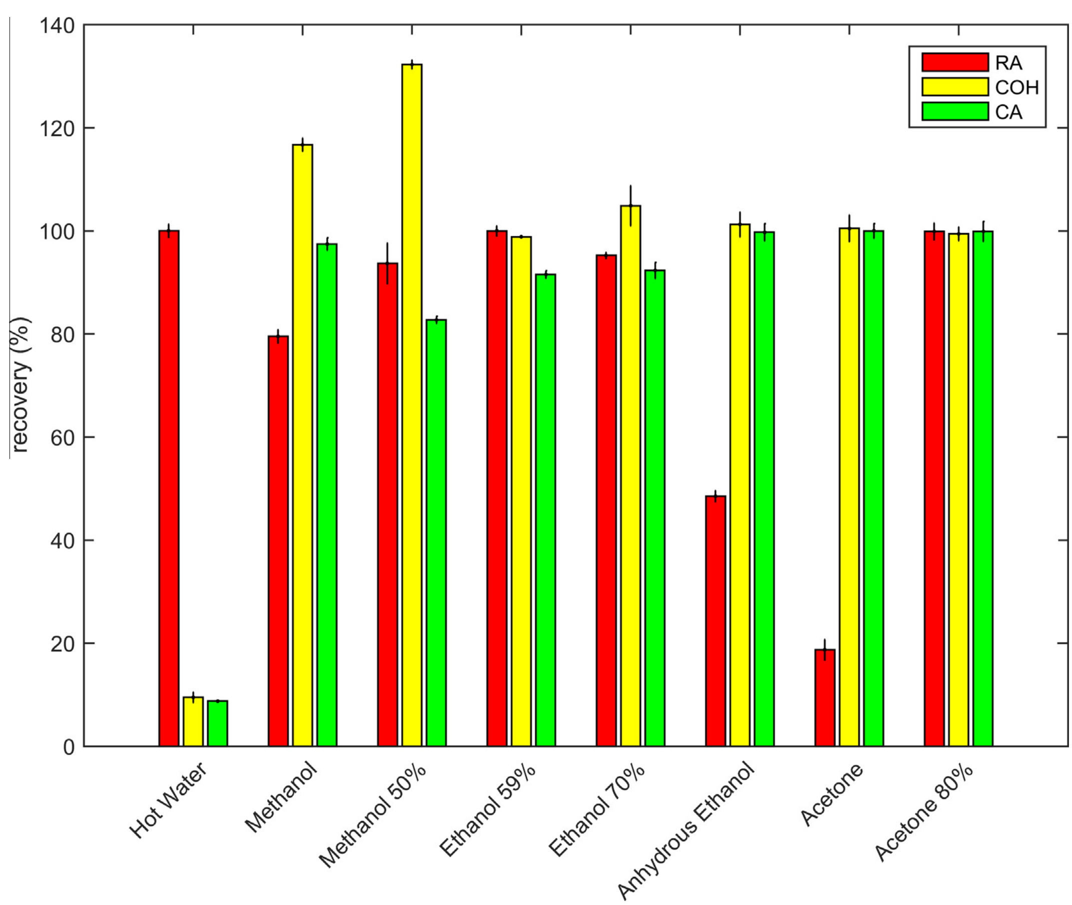

Fig. 1. RA, COH, and CA recovery from rosemary powder using different extraction solvents. Error bars represent mean ± 1 standard deviation.

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