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NORDIHYDROGUAIARETIC ACID RECOVERY FROM *LARREA TRIDENTATA* BY MICROWAVE-ASSISTED EXTRACTION

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KEYWORDS

Nordihydroguaiaretic acid; *Larrea tridentata*; microwave-assisted extraction; heat-reflux extraction

ABSTRACT

Nordihydroguaiaretic acid (NDGA) is a powerful antioxidant with biological activities of great interest in several health areas, including antiviral, cancer chemopreventive, and antitumorgenic. Little information is available on extraction methods of NDGA from *Larrea tridentata*. Hence, the aim of this study was to develop a rapid and effective microwave-assisted extraction (MAE) method for NDGA recovery from *Larrea tridentata* leaves, and to compare the results obtained with those found using conventional heat-reflux extraction (HRE).

INTRODUCTION

Nordihydroguaiaretic acid (NDGA) is a lignan found in several plants, including *Larrea tridentata* (*Zygophyllaceae*), also known as creosote bush, which grows in semidesert areas of south-western USA and Northern Mexico. NDGA is mainly concentrated in the leaves and green stems (Hyder et al. 2002). This compound is known to be a powerful antioxidant (Figueiredo et al. 2008); however, recent studies have shown other very important biological activities for this compound, such as antiviral, cancer chemopreventive, and antitumorgenic activities (Hwu et al. 2008; Toyoda et al. 2007). Extraction of bioactive compounds from plants is conventionally performed by heat-reflux systems, which usually are time consuming and require large amounts of solvent. The increased need for an ideal extraction method that allows maximum bioactive compound recovery from a plant in the shortest processing time with low costs represents an important challenge. Microwave-assisted extraction (MAE) has significantly decreased extraction time and increased extraction yields in several plants (Rostagno et al. 2007; Pan et al. 2003). To our knowledge, no studies of the MAE method for NDGA recovery from *Larrea tridentata* leaves have been reported. Thus, the aim of this work was to develop a MAE technique for efficient NDGA extraction from *Larrea tridentata* leaves and to compare the results obtained with those found using conventional heat-reflux extraction.

METHODS

Air-dried leaves of *Larrea tridentata* were ground to fine powder and stored in dark bottles at room temperature for further analysis. Conventional heat-reflux extraction was performed mixing 1 g of dried powdered plant with methanol (solid/liquid ratio 1/10 g mL−1), in 250 mL Erlenmeyer flasks, which were covered with foil paper to prevent light exposure and subsequent oxidation. Reactions were performed in a water-bath at 70 ± 2 ºC, using different methanol concentrations as solvent (25 to 100% v/v) for 1 or 3 h. Microwave-assisted extraction was carried out in a microwave apparatus using a multimode closed vessel system with pressure. For reactions, 1 g of dried powdered plant was mixed with the desired amount of methanol and placed into 100 mL polytetrafluoroethylene extraction vessels. The suspensions were irradiated with microwaves at 70 ± 2 ºC and 800 W, according to the method of Zhang et al. (2008) with some modifications. After each irradiation of 1 min the sample was allowed to cool to room temperature. Different methanol concentrations as solvent (25 to 100% v/v) and solid/liquid ratios (1/5 to 1/30 g mL−1) were tested. All the extracts were filtered through a 0.2 μm membrane filter and NDGA was quantified by HPLC.

RESULTS & CONCLUSIONS

Figure 1 shows the kinetic behavior of NDGA extraction from *Larrea tridentata* leaves by MAE and HRE carried out for 4 and 60 min, respectively.

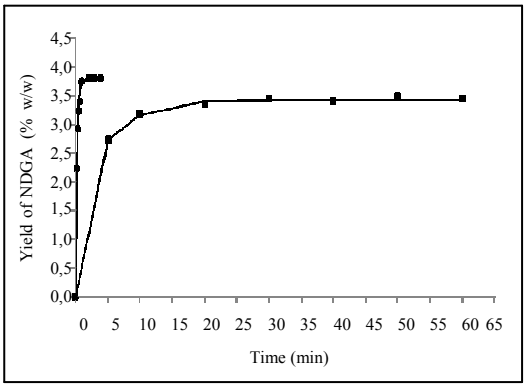

Figure 1. Kinetic behavior of NDGA extraction from *Larrea tridentata* leaves by MAE (●) and HRE (з) using 1 g plant material/ 10 mL methanol 50% (v/v), at 70 ºC and 800 W.

Kinetic parameters and extraction time for both MAE and HRE methods are presented in Table 1.

Table 1. Kinetic parameters and extraction times obtained for NDGA extracted from *Larrea tridentata* leaves by HRE and MAE.

Extraction method	$NDGA_\infty$ (%, w/w)	K (min^{-1})	Time (min)
HRE	3.42 ± 0.19	0.26 ± 0.02	18
MAE	3.79 ± 0.65	4.61 ± 0.45	1

Note that MAE was more advantageous than HRE since it reduced the extraction time from 18 to 1 min only, presenting, as a consequence, a higher extraction rate constant (4.61 ± 0.45 min−1). Moreover, slightly higher NDGA yields were found when using MAE as an extracting technique. A possible explanation for the better results for MAE compared with HRE, could be an efficient dissipation and absorption of microwave energy through the solvent and plant material, which increases the temperature inside the plant cells. This might result in cell walls breaking, allowing the release of bioactive compounds into the surrounding solvent.

In brief, microwave-assisted extraction was a faster and more efficient method for NDGA extraction from *Larrea tridentata* leaves when compared to the conventional heat-reflux extraction, mainly because it significantly reduced the extraction time. Under the optimal MAE conditions (50% methanol in water (v/v) as extraction solvent, solid/liquid ratio of 1/10 (g mL^{-1}), 70 ºC, during 1 min), maximum NDGA yield of 3.79 ± 0.65% was achieved.

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SÍLVIA MARTINS was born in Porto and received her degree in Biological Engineering (5 years BEng degree) in 2005 from the University of Minho. She was awarded a Fellowship from Food Institutional Research Measure through the Department of Agriculture and Food under the National Development Plan (2000-2006) to study at University College Cork, Cork, Ireland for her MSc in Food Science and Technology. Since 2008, she is doing her PhD project at the University of Minho.

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