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The enhancement of antioxidant compounds extracted from *Thymus vulgaris* using enzymes and the effect of extracting solvent

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ABSTRACT

We evaluate the total phenolic compounds (TPC) content and the antioxidant activity (AA) of extracts obtained from ground fresh thyme (FT) and depleted thyme (DT), a by-product of the process of essential oil extraction. In addition, enzymatic treatments were evaluated to improve the extraction yields of polyphenolic compounds from thyme. Extractions were performed using several solvents as methanol, ethanol, and water. Enzymes were applied prior to extraction or during the extraction process. The best results were obtained using a mixture of methanol and water, resulting in 2790 and 220 mg Gallic acid equivalent (GAE)/L of TPC for FT and DT, respectively. A similar result was observed for AA. With regard to enzymatic treatment, application of Grindamyl CA 150 enzyme as a pre-treatment resulted in the production of an extract from DT with 614 mg TE (trolox equivalent)/L of AA, 70% more than the control, and an AA of 621 mg TE/L (74% more than the control sample) was obtained using Grindamyl CA 150 during the extraction process. These results suggest that enzymatic treatment is an interesting alternative for producing antioxidant extracts from DT.

1. Introduction

The antioxidant activity of extracts from vegetable matrices depends upon the recovery techniques used, including the solvent type and polarity, particle size, condition of the raw material, and extraction parameters (Bracco, Löliger, & Viret, 1981). Because the vegetable cell wall is a highly resistant complex incorporating hemicelluloses, cellulose, pectin, lignin, and protein molecules, within which phenolic compounds are entrapped, conditioning of the raw material can promote the extraction of bioactive compounds. Traditionally, this stage may include size reduction and heat treatment by either boiling or the application of hot water or steam. It is also possible to accomplish various other tasks during this stage, including adjusting the humidity of the conditions, inactivating natural enzymes and "sterilizing" raw materials.

An alternative conditioning procedure is the enzymatic hydrolysis of structural polymers. Enzymes are highly efficient, specific, generally do not produce large amounts of unwanted products and operate under mild environmental conditions; thus, enzymatic treatment does not require complex operating systems that function only under highly restrictive conditions.

Enzymes are applied in several industrial processes. In the case of vegetable matrix treatment, it is reported that enzymatic pectin hydrolysis promotes a decrease in viscosity and neutralises the electrostatic charges between uronic acids, proteins, and tannins, reducing bonding forces and therefore the stability of the matrix. As a result, the extraction yields of either carbohydrates or phenolic compounds are improved, as is the antioxidant activity of the extract (Bagger-jørgensen & Meyer, 2004).

Antioxidant extraction using enzymes has been evaluated in a number of published studies. Several works report an increase in phenolic compounds when enzyme application was used either to improve the nutritional quality of wine (Bautista-Ortín, Martínez-Cutillas, Ros-García, López-Roca, & Gómez-Plaza, 2005), juice preparations (Bagger-jørgensen & Meyer, 2004; Koponen et al., 2008), extracted olive oil (García et al., 2001) or to increase the yield of products extracted from the vegetable matrix, such as borage and grape oil (Soto, Concha & Zúñiga, 2008; Tobar, Moure, Soto, Chamy, & Zúñiga, 2005). In addition, enzymes have been applied to agro-industrial solid wastes, such as apple, citrus and grape skins (Kim et al., 2005; Li, Smith, & Hossain, 2006; Muñoz, Sepúlveda, & Schwartz, 2004), and to berry and grape pomace

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(Kapasakalidis, Rastall, & Gordon, 2009; Laroze, Soto, & Zúñiga, 2010). In most of the studied cases, enzyme incorporation increases the presence of phenolic compounds and antioxidant activity; however, these results depend upon several variables. In particular, prior research indicates the importance of parameters such as the type of enzyme and solvent used in the extraction process.

In the case of spice antioxidant recovery using enzymes, there is one report in which rosemary and sage were treated in an enzymatic ensilage to promote greater digestibility, resulting in an observed increase in phenolic compounds (Weinberg, Akin, Potoyevski, & Kanne, 1999).

Spice incorporation in foods dates back hundreds of years, when spices were added to improve the taste and storage time of food. These plants are used as whole leaves, ground or as extracts. Among spice plants, the *Labiatae* (also named *Laminaceae*) family, which is composed primarily of basil, oregano, thyme, rosemary and sage, is considered to be an important source of potent natural antioxidants, with rosemary and sage being particularly enriched. Accordingly, the recovery and use of antioxidant compounds from spices is an interesting issue of industrial relevance (Bauman, Hadolin, Rizner, & Knez, 1999).

There are more than five hundred spices from the *Laminaceae* family that have been studied as natural sources of antioxidants because of their high content of polyphenol compounds, of which *Thymus vulgaris*, *Thymus mastichina*, *Thymus citriodotus*, *Thymus corydothymus*, *Thymus loscossi*, *Thymus pipirella* and *Thymus rumidicus* are the best known (Fecka & Turek, 2008).

Thyme (*T. vulgaris*) is used mainly as a food seasoning, but also as a source of essential oils that are used in perfumery and as a worming and bactericidal agent in medicine (Dawidowicz, Rado, Wianowska, Mardarowicz, & Gawdzik, 2008). Additionally, thyme is known to contain a high concentration of phenolic compounds, such as thymol (40–80%) and carvacrol (55–100%), which are found in its essential oils. Fecka and Turek (2008) found a 30 mg/GMP concentration of phenolic compounds in wild thyme, with caffeic acid and rosmarinic acid derivatives being the most important of these.

The aim of this study was to evaluate the antioxidant activity of samples of ground thyme and depleted thyme (a by-product of the oil extraction process) when an enzymatic treatment is performed to optimise the extraction yield.

2. Materials and methods

2.1. Raw materials

Ground fresh thyme leaves (FT) and depleted thyme leaves (DT) were used. The DT was obtained as a residual by-product of the process of essential oil extraction from fresh thyme leaves (FT) in a steam-based process (subcritical water). Thyme leaves (FT) were harvest in the early stages of flowering bush (spring in Chile). DT samples having only residual water as a result of the extraction of essential oil.

2.2. Enzyme formulations

Two commercial enzymes were applied to the samples, namely, Macer 8 FJ (Biocatalysts Limited, UK) and Grindamyl CA 150 (Danisco A/S, provided by Pal-Harmony Chile). The enzymes have 15.46 ± 0.1 and 14.03 ± 0.33 $mg_{protein}/mL_{enzyme\ formulation}$, respectively. The biocatalysts mentioned above have cellulase (on carboxymethylcellulose) and polygalacturonase (on galacturonic acid) activities as follows: Grindamyl CA with 10.28 and 253.68 $IU/mg_{protein}$, and Macer 8 FJ with 13.37 and 200.67 $IU/mg_{protein}$, respectively.

2.3. Antioxidant compounds extraction

Samples of FT and DT were subjected to extraction at 50 °C for 15 h, using a methanol, ethanol, water or methanol/water (50/50) mixture as a solvent in a solid/solvent ratio of 1/20 (w./v.). Subsequently, the antioxidant extracts were recovered by filtration.

For the enzymatic treatment, biocatalysts were incorporated prior to antioxidant extraction either as a pre-treatment process or during the solvent extraction of phenolic compounds. In the first case, the enzymatic treatment was conducted at 50 °C for 6 h, after which the extraction solvent was added. In the second case, the enzymes were added along with the extraction solvent. In both instances, the enzyme concentration was 2.5 g/100 g substrate d.w., and antioxidant extraction was performed according to the method mentioned above.

2.4. Phenolic compound and antioxidant activity determinations

The total phenolic content (TPC) was determined by the Folin–Ciocalteu method, in which 3.75 mL of water, 0.25 mL of twofold diluted Folin–Ciocalteu reagent, 0.5 mL of phenolic extract and 0.5 mL of 10% sodium carbonate were mixed. After 1 h at room temperature, the sample absorbance was measured at 765 nm. The total phenolic content was expressed as $mg_{Gallic\ acid\ equivalents}/L_{extract}$ (mg GAE/L) (Laroze et al., 2010; Soto, Conde, Moure, Zúñiga, & Dominguez, 2008). The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method; briefly, 2 mL of a 3.6×10^{-5} M DPPH methanolic solution was added to 50 μL of phenolic extracts (diluted in methanol). The absorbance at 515 nm was recorded after 16 min. The results were expressed as $mg_{Trolox\ equivalent}/L_{extract}$ (mg TE/L) (Laroze et al., 2010; Soto et al., 2008).

2.5. Statistical analysis

Extraction and analytical assays were done by triplicate. Data were evaluated using Graph Pad Instat 3 software and the results of the test performed are given with the man value and standard deviation (SD).

3. Results

Fig. 1 shows the effects of different solvent applications in the extraction process on TPC and antioxidant activity from the FT and DT samples. It was observed that, with both FT and DT samples, an ethanol solvent produced extracts with less TPC in

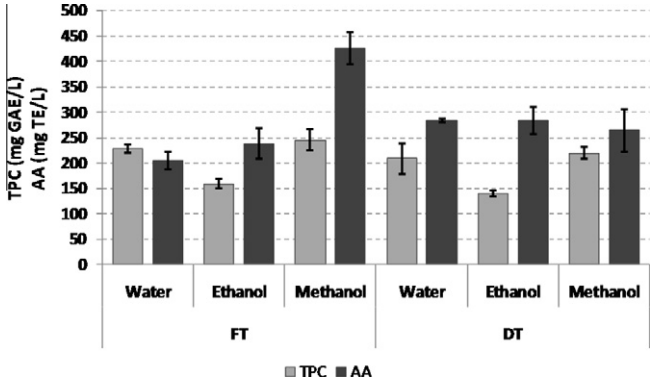

Fig. 1. The effect of the extraction solvent type on the amount of solids, total phenolic compounds and antioxidant activity (determined by the DPPH method) recovered from fresh and depleted thyme leaves. The extraction conditions were as follows: 50 °C, solid/solvent ratio 1/20 w./v., 15 h.