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Antioxidant recovery from hydrodistillation residues of selected Lamiaceae species by alkaline extraction

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ABSTRACT

Aqueous solution of potassium hydroxide (KOH) was used in order to obtain rich in antioxidant activity extracts from *Origanum dictamnus*, *Origanum hirtum, Origanum onites*, *Rosmarinus officinalis and Satureja thymbra*, after removal of the essential oil via hydrodistillation. The increase of extraction time induced lower values of total phenolic content and DPPH radical scavenging ability of the extracts, while lower alkaline concentrations, i.e. 1% and 3%, proved to be more effective than 5% (v/w) KOH. The antioxidant activity of the extracts in sunflower oil was also measured by the oxidative stability index (OSI) method. OSI values decreased as extraction time increased, while the effect of KOH concentration at short extraction time was not significant. *S. thymbra*, *O. hirtum* and *R. officinalis* extracts showed high total phenolic content and good antiradical and antioxidant activity in just 30 min of extraction with KOH 1% (w/v). LC–MS analysis showed that all extracts were rich in phenolic acids, such as caffeic acid and rosmarinic acid, while *R. officinalis* was also rich in carnosic acid.

1. Introduction

Lipid oxidation can lead to the formation of undesirable off-flavours and off-odours, and is the major cause of lipid quality deterioration and rejection in food products. In addition to product quality loss, there is also a nutritive loss due to the degradation of essential fatty acids and vitamins. The problem of ensuring a high quality of lipids and lipid-containing products and prolonging their shelf life is directly associated with stabilization by with the addition of suitable antioxidants. There is an increasing demand for natural antioxidants, following the rising consumer preference for natural products, clean labels and less use of synthetic additives in food products. The global natural antioxidants market is expected to reach USD 4.14 billion by 2022, according to a new study by Grand View Research Inc., published in June, 2015 (Globenewswire, 2015). Many plant species, particularly herbs and spices, are rich sources of natural antioxidants and represent promising alternatives to synthetic forms, although their use is limited due to characteristic aromas. Among tens of thousands of phytochemicals found in fruits, vegetables, spices and traditional medicinal aromatic plants, polyphenols (phenolic acids and flavonoids) stand out as one of the most important group of natural antioxidants (Embuscado, 2015; Yanishlieva et al., 2006). Kim et al. (2011), supported the hypothesis that phenolic compounds contribute significantly to the antioxidant activity of spices.

Phenolic compounds are found in both free and bound forms in plant cells. The free phenolics are easily extracted. In contrast, phenolic compounds covalently-bound to the plant matrix cannot be extracted by water or organic solvents. Alkaline, acidic or enzymatic hydrolysis can be used to release bound phenolic compounds (Su et al., 2014). Acidic conditions have been reported in literature to be less efficient than alkaline conditions for polyphenol extraction, probably because most of the polyphenols are linked by ester bonds (Boussetta et al., 2014; Li et al., 2008). Krygier et al. (1982) reported that losses under acidic conditions vary, depending on the nature of phenolic acid, ranging from 15 to 95% for *o*-coumaric acid and sinapic acid, respectively. Alkaline treatments were used to extract bound phenolic acids and other related compounds from cell wall material of cereal grains (Acosta-Estrada et al., 2014). Treatment with different concentrations (1–4 M) of sodium hydroxide for varying lengths of time proved to be sufficient to release bound phenolics (procyanidins) from dried cranberry pomace (White et al., 2010). Oufnac et al. (2007) used short extraction time at high alkali concentration and temperature to obtain a quantitative release of bound phenolics, while Rommel and Wrolstad (1993) reported that samples were hydrolyzed in 2N NaOH for 2 h at room temperature, in the dark and under nitrogen atmosphere.

Alkaline solutions might be a promising alternative to organic

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solvents for the extraction of both free and bound phenolic compounds from members of the Lamiaceae family. Herbs of the Lamiaceae family are often used as spices and their flavour is highly favourable to consumers all over the world. They show as strong antioxidant properties as pure commercial chemical and food additives, such as hydroquinone and BHA, (Caillet et al., 2007) and they are also valued for their antimicrobial properties. According to Møller et al. (1999), the aqueous extract of dittany had a significantly higher amount of phenolic compounds compared to the dittany extracts obtained using methanol, ethanol or acetone as solvents. Water extracts from sweet marjoram, sage and dittany have a remarkable capacity to retard lipid oxidation, related to the content and the nature of phenolic compounds (Triantaphyllou et al., 2001). Most of the Lamiaceae herbs are commercially exploited for the recovery of the essential oil through hydrodistillation.

The hydrodistillation process may generate large volumes of residual aqueous liquors. Besides the waste disposal problem, this liquor represents a potential value because of its water-soluble phenolics. Rocha-Guzmán et al. (2007), reported that the ethyl acetate extract of the residual aqueous liquors obtained from the hydrodistillation of oregano could be superior to ascorbic acid and butyl hydroxyl toluene, when used at high concentrations. Moreover, the solid residue of the hydrodistillation process is usually disposed of, although it is a rich source of antioxidant compounds. Alkaline treatment of the wet residual material (herb and residual liquor), which remains after the hydrodistillation process, could lead to extracts rich in phenolic acids, without facing the strong aroma of the essential oil and taking full advantage of the process wastes. Therefore, the objective of this study was the development of an effective procedure for the extraction of antioxidant-rich fractions, from the residual material of the hydrodistillation process, by using alkaline conditions. Five common species of the Lamiaceae family were selected, namely, *Origanum dictamnus*, *Origanum hirtum, Origanum onites*, *Rosmarinus officinalis* and *Satureja thymbra*. The effect of the alkali concentration and extraction time on the yield of phenolic compounds and antioxidant activity was evaluated.

2. Material and methods

2.1. Solvents and reagents

Ethyl acetate (EtOAc) was obtained from Acrōs Chemicals (Tech-Line S.A., Athens, Greece). Potassium hydroxide, pellets (> 85%), sodium sulphate anhydrous (> 99%), 2,2-diphenyl-1-picryl hydrazyl, Folin Ciocalteu phenol reagent (2N), gallic acid, and sodium carbonate anhydrous (> 99,5%), were obtained from Sigma-Aldrich (Life Science Chemilab S.A., Athens, Greece). LC–MS standards, namely rosmarinic acid, and caffeic acid, were obtained from Sigma-Aldrich (Life Science Chemilab S.A., Athens, Greece), and carnosic acid was obtained from Dayang Chemicals Co (Hangzhou, China). Refined sunflower oil was obtained from Minerva S.A. (Inofyta, Greece).

2.2. Plant material

Dry herbs of *Satureja thymbra* and *Origanum vulgare* ssp. *hirtum* were obtained from the Institute of Plant Breeding and Genetic Resources (member of the Hellenic Agricultural Organization-DEMETER), while *Origanum onites* and *Rosmarinus officinalis* from the company Alexopoulos Alexandros & Co (Athens, Greece). The air-dried plant material *Origanum dictamnus* was purchased from the local market of Crete (Greece). The herbs were collected during the maximum blooming period from regions of the wider mainland of Greece, dried and screened by hand. The dried herbs were ground in a laboratory mill (Retch ZM 1; Haan, Germany), equipped with a 0.5 mm sieve.

2.3. Extraction procedures

The herbs were subjected to hydrodistillation in a laboratory scale water-steam distillation apparatus, for 6 h. Batches of 200 g were used in each experiment, mixed with 2.5 L of water and steam was supplied through the mixture. The collected essential oil was dried over anhydrous sodium sulphate and kept in sealed glass vial in the refrigerator until used. The distilled water (hydrosol) was not recycled, and, therefore, was collected with the essential oil and not further studied. Upon completion of the hydrodistillation process, the residual wet material (herb and water) was divided, under stirring, into three equal parts. An amount of KOH was added in each part, so that the final concentration of alkali was 1, 3 and 5% (w/v) respectively. All the experiments were conducted in 1 L three-necked round bottles with magnetic stirring, at room temperature. Samples (150 g) were taken at four different digestion times, 0.5, 3, 6 and 24 h. Subsequently, the samples were filtered to remove the herb, and the liquid was acidified with citric acid to reach a pH equal to 2.5, so that most phenolic compounds prevailed in non-ionic forms. Citric acid occurs naturally in fruits and vegetables, and can be used as acidifying agent without effectuating any bitter taste. The acidified liquor was subjected in 3 successive extractions in a separatory funnel, with 100 mL of ethyl acetate each, to recover the antioxidant compounds. Ethyl acetate was selected because it exhibits low boiling point and non-toxicity, which allows its use in the food industry and reduces the cost of the process. Furthermore, ethyl acetate proved to be a good solvent to recover antioxidants, such as caffeic acid, after the hydrolysis process (De Leonardis et al., 2005). The extracts were combined and the solvent was removed by using a rotary evaporator (Rotavapor R-3000, Büchi, Switzerland) equipped with a water jet vacuum pump (Thermostat Tom Jet-1, Genser, Germany). Then the samples were re-diluted in ethyl acetate (25 mL) and stored in amber glass bottles at 4 °C, until further processed for analysis. Experiments were run in duplicate and the presented results are mean values.

2.4. DPPH free radical scavenging assay

The antiradical activity of the samples collected from the different extraction procedures was determined by the DPPH radical assay. A UV–vis instrument (T90+, UV–vis Spectrometer, PG Instruments, Leicestershire, England) was used to monitor the reaction of DPPH radical with the sample. Samples (0.1 mL) of extract solution in methanol were added to 3.9 mL of $6*10^{-5}$ M DPPH radical solution in methanol and the absorbance at 515 nm was recorded over time. Several concentrations of the extracts were used and the quantity of dry sample needed to reduce 50% of the initial DPPH radical concentration (EC_{50}) was evaluated according to the methodology reported by Brand-Williams et al. (1995).

2.5. Determination of total phenolic content

The total phenolic content (TPC) of the extracts was determined by the Folin-Ciocalteu reagent using the method of Singleton et al. (1999). The absorbance of all samples was measured at 765 nm using a T90+ UV–vis Spectrometer, (T90+, PG Instruments, Leicestershire, England). The results are expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g d.e.), though the construction of a reference curve.

2.6. Oxidative Stability Index (OSI)

The antioxidant activity of the different extracts was tested on refined sunflower oil by subjecting it to forced dynamic oxidation and measuring the lengthening of the OSI time (AOCS Official Method Cd 12b-92 Revised 2013). A six-channel oxidative stability instrument (Omnion Inc., Rockland MA, USA) was used. Five grams (± 0.2 g) of