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Advances in phenolic compounds analysis of aromatic plants and their potential applications

D. Carvalho Costa [a, b], H.S. Costa [a, c], T. Gonçalves Albuquerque [a, c], F. Ramos [d, e], M.C. Castilho [d, e], A. Sanches-Silva [a, f, *]

[a] Department of Food and Nutrition, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal
[b] ICETA, Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto, Porto, Portugal
[c] REQUIMTE/LAQV, Faculdade de Farmácia da Universidade do Porto, Porto, Portugal
[d] Pharmacy Faculty, University of Coimbra, Health Sciences Campus, Coimbra, Portugal
[e] CNC — Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
[f] Centro de Estudos de Ciência Animal (CECA), Universidade do Porto, Porto, Portugal

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A B S T R A C T

Background: Aromatic plants may contribute for human health promotion due to their antioxidant properties and also by replacing added salt in foods. Phenolic compounds are one of the major groups contributing for aromatic plants properties, including the prevention of cancer, cardiovascular and neurodegenerative diseases.
Scope and approach: This review focuses on the main uses of aromatic plants generally recognized as safe (GRAS) and overviews the extraction, purification and analytical methods used to determine phenolics in these food matrices. The current state of the art is evaluated and future trends in the analysis of these compounds and food industry applications are discussed.
Key findings and conclusions: In general, aromatic plants are complex matrices regarding their content on phenolic compounds. Their composition can be affected by the chemical structure of the studied analytes, the selected methods, the composition/nature of the aromatic plant and storage conditions. The most usual separation technique to determine these compounds is High Performance Liquid Chromatography (HPLC) coupled with ultraviolet (UV) or diode array detectors (DAD), although the use of mass spectrometry (MS) detectors is increasing. The future trends include the use of more sophisticated and automated techniques in order to reduce both analysis time and the amount of solvents used.
Food industry widely uses aromatic plants, but other industries like food packaging, cosmetics, perfumery and pharmaceutical can also benefit from their properties.

1. Aromatic plants

The use of aromatic plants and spices dates back to BC in the Middle East due to their flavour, preservative, and medicinal properties (Roby, Sarhan, Selim, & Khalel, 2013). For instance, marjoram was known by ancient Egyptians, Greeks and Romans. In fact, Greeks considered marjoram a symbol of happiness (Roby et al., 2013). Both industrialized and non-industrialized countries are increasing the demand of aromatic plants as source of natural antioxidants.

The United States Food and Drug Administration has recognized more than 150 plants that present essential oils, oleoresins (solvent-free), and natural extractives (including distillates) that are safe for human consumption without limitations on intake (FDA, 2014a; FDA, 2014b). Oregano, rosemary, sage and thyme are included in this list. Examples of aromatic plants present in the referred list are *Ocimum basilicum* L. (basil), *Laurus nobilis* L. (bay), *Petroselinum crispum* (Mill.) Mansf. (parsley), *Mentha piperita* L. (peppermint), *Salvia officinalis* L. (sage), *Rosmarinus officinalis* L. (rosemary), *Artemisia dracunculus* L. (tarragon) and *Thymus vulgaris* L. (thyme) (21 CFR Ch. I, 182.10; 21 CFR Ch. I, 182.20) (FDA, 2014a; FDA, 2014b). Some of these aromatic plants belong to the Lamiaceae family, which includes around 200–250 genera and between 3200 and 6500 species. The plants belonging to this family have

* Corresponding author. Department of Food and Nutrition, National Institute of Health Dr Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, Portugal.
E-mail address: ana.silva@insa.min-saude.pt (A. Sanches-Silva).

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quadrangular stems and verticillate inflorescences (Dorman, Bachmayer, Kosar, & Hiltunen, 2004).

Table 1 compiles the culinary uses and medicinal properties of some of the aromatic plants that have the "generally recognized as safe" (GRAS) status.

Nowadays, aromatic plants seem to be "fashionable" because their use is highly recommended by health professionals due to positively affecting health by reducing the amount of salt in the diet, or by presenting health benefits related with their antioxidant properties. Indeed, the Portuguese Government in the frame of the *National Programme for the Promotion of Healthy Eating* has elaborated a document where the use of aromatic plants is encouraged in order to reduce the abusive addition of salt, which is related with hypertension (DGS, 2014). The consumption of salt by the Portuguese population (10.7 g/day) is well above the maximum recommended daily value according to the World Health Organisation (5 g/day) (DGS, 2014; Polonia, Martins, Pinto, & Nazaré, 2014; WHO, 2007).

Aromatic plants provide protein, fibre, volatile components (essential oil), vitamins (A, C and B complex), minerals (calcium, phosphorus, sodium, potassium and iron) and phytochemicals (bioactive substances present in small quantities that act, for instance, as antioxidants, bactericides or antivirals). Within phytochemicals, phenolic compounds are one of the major groups contributing for aromatic plants properties, including the prevention of cardiovascular disease and cancer. Although aromatic plants are consumed in low amounts, the biological impact of their intake cannot be ignored.

This review draws from the great interest on these plants due to their abundance in diet, potent antioxidant properties, which are related with their effect on the prevention of oxidative stress associated with diseases, namely cardiovascular, neurodegenerative diseases and cancer. As a result of the great number of different phenolic compounds in aromatic plants, it is difficult to determine them individually, evaluating their antioxidant and biological potential. The aim of this review is to overview the extraction, purification and analysis methods used to determine phenolics in aromatic plants, to evaluate the current state of the art and to reflect the future trends in the analysis of these compounds in plant matrices. Moreover, present and future industrial applications are also discussed.

2. Properties and structure of the most common phenolic compounds

Phenolic compounds are ubiquitous in the plant kingdom but their distribution depends on the part of plant/tissue (Robards, 2003). Phenolic compounds derive from phenylalanine and tyrosine, they have an aromatic ring bearing one or more hydroxyl substituents and they are secondary metabolites of plants (Muchuweti et al., 2007; Naczk & Shahidi, 2004). They are water soluble and can occur as glycosides, when combined with a sugar molecule, such as glucose, galactose, rhamnose, arabinose, xylose and rutinose (Justesen, Knuthsen, & Leth, 1998; Muchuweti et al., 2007).

Around 8000 polyphenols have been described and can be divided into several sub-groups that range from simple molecules, like phenolic acids, to polymerized compounds such as tannins (Muchuweti et al., 2007). Table 2 shows the chemical structure of some phenolic compounds found in aromatic plants, as well as some physical and chemical properties. These properties can greatly influence phenolics extraction from aromatic plants and also the analytical approach used in their determination.

Flavonoids, the most common in our diet, are considered primary antioxidants, chelators and superoxide anion scavengers. They are divided in flavones, flavanols, flavanones, isoflavones and anthocyanins (Dai & Mumper, 2010). Their antioxidant activity depends on the position and degree of hydroxylation of the molecule (Robards, 2003). Glycosides are less active than aglycones (Peña-Neira, Hernández, García-Vallejo, Estrella, & Suarez, 2000).

In plants, phenolic compounds are essential for diverse biological activities such as contributing for seed dispersal and pollination due to its attractive colours, participating in signalization and structural roles, and in defence strategies under stress conditions such as wounding, infection, UV light or excessive light (Muchuweti et al., 2007; Naczk et al., 2004). They can also act as phytoalexins or inhibit normal feeding behaviour (antifeedant) (Naczk & Shahidi, 2004).

In foods they may contribute to oxidative stability, colour, odour, flavour bitterness or astringency. Polyphenols activities include antioxidant, antimutagenic, anticancer, antiallergenic, anti-inflammatory, antiviral, antiulcer, antidiarrheal, anthelmintic, molluscicidal, antihepatoxic and antiproliferative (Carocho, Barreiro, Morales, & Ferreira, 2014; Muchuweti et al., 2007). Epidemiological studies showed that the intake of natural phenolic antioxidants is correlated with a reduced incidence of diseases such as coronary heart disease, age related eye degeneration and cancer (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Hertog & Hollman, 1996; Knekt, Järvinen, Reunanen, & Maatela, 1996; McCullough et al., 2012; Steinmetz & Potter, 1991). In general, polyphenols can protect from diseases with an etiology and pathophysiology related with reactive oxygen species (Armatu, Colceru-Mihul, Bubueanu, Draghici, & Pirvu, 2010). In fact, phenolic compounds were found to inhibit human immunodeficiency viral replication (HIV), human simplex virus (HSV) and glucosyl transferases of *Streptococcus mutans* (associated with dental caries) (Proestos, Chorianopoulos, Nychas, & Komaitis, 2005).

Phenolics act as reducing agents, hydrogen donators and singlet oxygen quenchers (Proestos, Boziaris, Nychas, & Komaitis, 2006). Due to the importance of phenolic compounds they have already been the target of several reviews (Dai & Mumper, 2010; Lule & Xia, 2005; Molnár-Perl & Füzfai, 2005; Naczk & Shahidi, 2004; Prasain, Wang, & Barnes, 2004; Rijke et al., 2006; Robards, 2003).

3. Determination of phenolic compounds in aromatic plants

3.1. Extraction procedures

One of the most critical steps in the quantitative analysis of compounds in samples is the extraction, which aims to selectively separate, with good recovery, target compounds from the sample and eliminate interferences (Dobiás et al., 2010). Many factors can influence extraction of phenolic compounds from foods, such as the chemical structure of phenolic compounds, the extraction method used, the composition of the food and the storage time. Table 3 summarises some of the extraction procedures found in the literature to extract phenolic compounds from aromatic plants. Most of the extraction procedures of polyphenols correspond to a solvent extraction due to being easy to use, efficient, and having a wide applicability (Dai & Mumper, 2010). Many factors influence the solvent extractions, such as the polarity of the solvent, time, temperature, sample-to-solvent ratio and properties related with aromatic plants such as their composition in phenolics. Extraction of phenolics from aromatic plants, requires that samples are previously milled, grounded and homogenised. These samples can be fresh, frozen or dried. Drying processes include air-drying and freeze-drying. Although freeze-drying is recognized as a method that allows retaining higher levels of phenolics, it should be used with caution because it can also originate undesirable effects on the properties of the plants (Dai & Mumper, 2010).

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