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Effect of particle size upon the extent of extraction of antioxidant power from the plants *Agrimonia eupatoria*, *Salvia* sp. and *Satureja montana*

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ABSTRACT

The dependence of the extent of aqueous extraction of antioxidant compounds on particle size and contact time was studied for three important medicinal plants, that are commonly used in infusions: agrimony, sage and savoury. The effect of extraction time was dependent on the plant considered; however, ca. 5 min can be taken as the minimum period required to assure an acceptable degree of extraction of those compounds. As expected, a smaller particle size led to a higher extraction extents; a typical value of 0.2 mm is accordingly recommended. Chlorogenic acid was the dominant phenolic compound extracted from agrimony, whereas caffeic acid dominated in the case of sage or savoury. A mathematical model based on Fick's law was developed from first principles, and its two parameters were suitably fitted to the experimental data generated – in attempts to predict the evolution of antioxidant capacity extracted during contact time, for each plant and each particle size.

1. Introduction

Agrimony, sage and savoury (common English names for *Agrimonia eupatoria*, *Salvia* sp. and *Satureja montana*, respectively) are plants often used in traditional medicine in Portugal, and which grow in the poor soils of the Mediterranean basin. The former has been claimed to control uric acid, favour the respiratory system, function as an analgesic or a diuretic aid, treat wounds and provide a rich source of antioxidants (Venskutonis, Škėmaitė, & Ragažinskienė, 2007). Besides application as condiment, sage has been used as an anti-diarrhoea vector, and to help in digestion, contribute to heal wounds, play an anti-inflammatory role, fight insomnia and decrease blood pressure; some of these biological activities have been associated with its contents of rosmarinic acid (Petersen & Simmonds, 2003) and L-salvianolic acid (Lu & Foo, 2001), which are two antioxidant compounds. Finally, savoury is also used as condiment, and has been prescribed to combat diarrhoea, help digestion and heal wounds, as well as a disinfectant (Gião et al., 2007). Agrimony exhibits the highest antioxidant capacity and total phenolic content within the aforementioned three plants (Gião et al., 2007, 2008) – probably because of its rich contents of coumarins, flavonoids, tannins and terpenoids (Copland et al., 2003); savoury and sage come second (in this order) in those features.

Upstream of food (or beverage) production or formulation effective, harvesting antioxidants as nutraceuticals from the aforementioned plants depends on maximisation of their extraction. However, it is widely known that the efficiency of solid/liquid extraction processes is affected by critical processing parameters, such as temperature, nature of solvent, structure of solid matrix (mainly particle size) and extraction time (Franco, Pinelo, Sineiro, & Núñez, 2007). This means that each plant matrix/extraction solvent pair behaves in a unique way, so it should be studied as such. On the other hand, both the particle size of the plant matrix and the temperature of the extraction process are easily manipulated physical conditions. In general, a smaller size and a higher temperature facilitate mass transfer (Cacace & Mazza, 2003; Waterman & Sutton, 2003), but quantification of such heuristic rules for each plant source is required before optimisation efforts can be rationally developed.

The major goal of this research study was to model the influences of particle size and time of exposure on the efficiency of aqueous extraction of antioxidant power from solid dried material from given plants. A second goal was to characterise the chemical profiles of extracts in terms of phenolic compounds (which have been often associated with comparatively high antioxidant power).

2. Materials and methods

2.1. Sample preparation

Three plants were considered: agrimony, sage and savoury, all of which were a kind gift from ERVITAL (Castro Daire, Portugal). These plants had been cultivated as organic products, and were supplied in their commercial form of dried leaves: ca. 4 g was then

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Nomenclature

a	outer specific area	k	mass transfer coefficient based on the liquid side
A_S	area of solid phase	R	ratio of volumes of solid and liquid phases
C_{AL}	antioxidant power of liquid phase	V_L	volume of liquid phase
$C_{AL,0}$	initial antioxidant power of liquid phase	V_S	volume of solid phase
C^*_{AS}	antioxidant power of solid phase, expressed as equilibrium value in the liquid phase		

crushed (using a coffee mill) for 1 min, so as to obtain the corresponding powder. This powder was consecutively passed through bolters of 0.2, 0.25 and 0.3 mm mesh sizes (Haver and Boecker, Oeld, Germany), and the four different fractions thus collected were duly weighed.

Each fraction (ca. 1 g) was finally contacted, under uniform stirring, with 110 ml of boiling distilled water – so as to mimick ready-to-drink infusion preparations; samples were collected every minute up to 10 min, and an extra two samples by 12 and 15 min. All those samples were kept in ice, so as to quench the extraction process prior to analysis. Samples were assayed for their total antioxidant capacity, as described below. All experiments were run in duplicate.

For chromatographic analyses, samples were taken of aqueous infusions after 5 min, and were filtered through a 0.45 μm filter before injection.

2.2. Antioxidant capacity assessment

Determination of the antioxidant capacity was as detailed previously by Gião et al. (2007). The $ABTS^{\bullet+}$ stock solution was prepared via addition, at 1:1 (v/v), of 7 mM ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic) acid) diammonium salt (Sigma–Aldrich, St. Louis, MO, USA) to a solution of 2.45 mM potassium persulphate (Merck, Damstadt, Germany); the developing reaction took place in the dark, for 16 h. In order to obtain an absorbance of 0.700 ± 0.020 at 734 nm, measured with an UV 1203 spectrophotometer (Shimadzu, Tokyo, Japan), the aforementioned stock solution was diluted in as much ultra-pure water as necessary. A 10 μl-aliquot of the sample was assayed for inhibition percentage (between 20% and 80%, so as to guarantee a linear response of the analytical method), after 6 min of reaction with 1 ml of diluted $ABTS^{\bullet+}$ solution; triplicates of each sample were averaged to generate each datum point (which implies a total of six replicates per plant). The final result was expressed as equivalent concentration of ascorbic acid (in $g\,l^{-1}$), using a calibration curve previously prepared with such a reference compound.

2.3. Phenolic compound profiling

The chromatographic system consisted of a Prostar 210 LC pump (Varian, Walnut Creek, CA, USA), coupled with a 1200 triple quadrupole mass spectrometer (Varian) with electrospray ionisation (ESI) in both positive and negative modes. A 5 μm C_{18} column (4.6 mm × 100 mm, Merck) was used for separation, at a flow rate of eluant of 0.4 ml min^{-1}. The LC–MS/MS method was based on that followed by Politi, Rodrigues, Gião, Pintado, and Castro (2008). Chromatographic separation was performed within 33 min, using the following gradient pattern of eluant A (water with 0.1% (v/v) formic acid) and eluant B (methanol with 0.1% (v/v) formic acid): from 0 min with 90% A, to 12.05 min with 78% A, to 22.05 min with 50% A, to 27.05 min with 95% A, and to 30 min with 95% A. ESI–MS/MS detection used a capillary voltage of 55 V; for MS/MS fragmentation, Ar was used (under 1.20 mtorr, with a collision energy of 15 V). An in-house LC–MS/MS library was created in advance, by injecting 33 chromatographic standards of phenolic compounds, under similar analytical conditions. Identification of the phenolic compounds in the samples was by direct injection and comparison with the spectra of the in-house library; for simplicity, the results of quantification were presented as percent abundance.

2.4. Data treatment

Starting from mechanistic modelling based on Fick's law (Çengel, 2007), the balance of the antioxidant concentration throughout contact time is expected to be given by

$$V_L \frac{dC_{AL}}{dt} = kA_S(C^*_{AS} - C_{AL}) \qquad (1)$$
$$t = 0, \quad C_{AL} = C_{AL,0}$$

where V_L is the volume of the liquid phase, C_{AL} is the concentration of antioxidants in the liquid phase, t is the contact time, k is the mass transfer coefficient based on the liquid phase, A_S is the area of the solid phase, C^*_{AS} is the solubility of antioxidants in the liquid phase, and $C_{AL,0}$ is the initial value of C_{AL}. Defining a as the specific area of the powder, calculated as

$$a = \frac{A_S}{V_S} = \frac{6}{d} \qquad (2)$$

where d is the average particle diameter, and defining R as the volume ratio of the solid to the liquid phases, namely

$$R = \frac{V_S}{V_L} \qquad (3)$$

then Eq. (1) can be rewritten as

$$\frac{dC_{AL}}{dt} = kaR(C^*_{AS} - C_{AL}) \qquad (4)$$

Assuming that there is an excess of solute in the solid phase, then C^*_{AS} can be taken as essentially constant; consequently, integration of Eq. (4) from the initial condition set forth in Eq. (1) leads finally to

$$C_{AL}(t) = C^*_{AS}(1 - e^{-kaRt}) \qquad (5)$$

where (kaR) and C^*_{AS} accordingly become the only two adjustable parameters.

2.5. Statistical analyses

Non-parametric tests were applied to each set of experimental data, owing to their intrinsic heteroschedasticity. Friedman and Wilcoxon tests were thus chosen to check whether time influenced the observed results. Kruskal–Wallis tests were applied to check whether, at each time, plant source and particle size influenced the results produced. Mann–Whitney tests were, in turn, applied to unfold possible differences between plant source and particle size pairs. Principal component analysis for categorical data (PCA) was used to ascertain differences between fractional mass distributions. Finally, Tukey's tests were considered to quantify dif-