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Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol–water mixtures

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Abstract

Effects of particle size, temperature, contact time, solvent-to-sage ratio and the ethanol–water ratio on the extraction of the active compounds rosmarinic acid, carnosic compounds and essential oil from dried sage (*Salvia officinalis*) were studied. Optimal extraction conditions giving highest yield of all three active compounds were particle diameter 1 mm, extraction temperature 40 °C, solvent-to-sage ratio of 6:1 and 55–75 wt% ethanol for up to 3 h. This gave an extract equivalent to 14.9% of dry sage, containing 6.9% rosmarinic acid (55% recovery), 10.6% carnosic compounds (75% recovery) and 7.3% essential oil (42% recovery). Scale up of the process by a factor of 100 demonstrated that the optimised laboratory scale process can be carried out without any loss of efficiency at an industrial scale.

Keywords: Sage; *Salvia officinalis*; Phenolics; Rosmarinic acid; Solvent extraction; Essential oil

1. Introduction

Sage (*Salvia officinalis*) is a popular kitchen herb and is a member of the mint (*Labiatae*) family. It has been used in a variety of food preparations since ancient times. From its Latin name, "*Salvia*" meaning to cure and "*Officinalis*" meaning medicinal, it is clear that sage has a historical reputation for promotion of health and treatment of ailments (Keller, 1978; Kintzios, 2000). Modern day research has shown that sage essential oil can improve the memory and has shown promise in the treatment of Alzheimer's disease (Perry, Pickering, Wang, Houghton, & Perry, 2005; Perry, Bollen, Perry, & Ballard, 2003).

Sage is an aromatic herb and thus was previously considered mainly for its essential oil content (Heath, 1978; Keller, 1978; Perry et al., 1999; Perry, Baxter, Brennan, & van Klink, 1996; Santos-Gomes & Fernandes-Ferreira, 2003; Tucker & Maciarello, 1990). In the past few decades however, sage has been the subject of an intensive study for its phenolic antioxidant components. Several studies have shown sage to contain a range of potent antioxidants (Bisio, Romussi, Ciarallo, & De Tommasi, 1997; Chipault, Hawkins, & Lundberg, 1952; Madsen & Bertelsen, 1995; Wang et al., 1998). Due to the essential oil and antioxidant components of sage it is an economically interesting plant to study. The essential oil and flavourants of sage are used as basic material for various food, cosmetic and pharmaceutical preparations (Heath, 1978; Tucker & Maciarello, 1990). Sage antioxidants can be used as an alternative to the well-known rosemary antioxidants for the protection and preservation of certain food and nutraceutical products to extend their shelf life (Chipault et al., 1952; Cuvelier, Berset, & Richard, 1994a; Kimura & Kanamori, 1982; Shahidi, 2000; Wellwood & Cole, 2004). The possible toxicity of synthetic antioxidants and an increase in consumer preference for

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"all natural" ingredients has motivated recent studies on antioxidants from sage. Sage extracts reported in the previous literature contain a wide range of phenolic compounds (Lu & Foo, 2002). The antioxidant properties have been related to the major marker compounds carnosic acid, carnosol and rosmarinic acid (Chang, Ostric-Matijasevic, Hsieh, & Huang, 1977; Cuvelier et al., 1994a). Commercially, the quality of a sage extract is highly dependent on the content of these phenolic compounds (Nakatani, 1997). Sage extracts also contain flavonoids and other phenolics which may contribute to the total antioxidant activity (Dapkevicius, Venskutonis, van Beek, & Linssen, 1998; Djarmati, Jankov, Schwirtlich, Djulinac, & Djordjevic, 1991; Lu & Foo, 2000).

There are four major extraction processes used for sage: steam distillation (Dapkevicius et al., 1998; Perry et al., 1996; Tucker et al., 1990); enzyme assisted ensiling (Weinberg, Akiri, Potoyevski, & Kanner, 1999); conventional organic solvent extraction (Dapkevicius et al., 1998; Koşar, Dorman, & Hiltunen, 2005; Wang et al., 1998); and near-critical extraction (liquid or supercritical) using CO_2 (Catchpole, Grey, & Smallfield, 1996; Dapkevicius et al., 1998; Dauksas, Venskutonis, Povilaityte, & Sivik, 2001; Matsingou, Petrakis, Kapsokefalou, & Salifoglou, 2003; Reverchon, Taddeo, & Della Porta, 1995; Wang et al., 1998). Conventional solvent extraction has been reported in a laboratory scale using acetone, hexane, methanol or ethanol (Areias, Valentao, Andrade, Ferreres, & Seabra, 2000; Bauman, Knez, Murko, & Rostohar, 2000; Kimura & Kanamori, 1982; Koşar et al., 2005; Pizzale, Bortolomeazzi, Vichi, Überegger, & Conte, 2002). The yield of lipophilic and hydrophilic antioxidants and flavour compounds is highly dependant on solvent polarity. The least polar solvent, CO_2, extracts only lipophilic flavour compounds unless very high pressures are used (Bauman, Hadolin, Rizner-Hraš, & Knez, 1999; Catchpole et al., 1996; Reverchon et al., 1995; Reverchon, 1996). Mixtures of acetone and water are good solvents for polar antioxidants (Lu & Foo, 1999; Lu, Foo, & Wong, 1999; Lu & Foo, 2000) but may lead to an unacceptable level of acetone residue in the extracts. Hydroalcoholic mixtures of ethanol and water are possibly the most suitable solvent systems for the extraction of sage due to the different polarities of the active constituents, and the acceptability of this solvent system for human consumption. The extraction of sage using ethanol and water mixtures has been reported by Areias et al. (2000), Kimura and Kanamori (1982) and Nguyen, Frakman, and Evans (1994). Areias et al. (2000) used ethanol–water mixtures of 80 and 30 wt%, and Kimura and Kanamori (1982) used ethanol–water mixtures of not less than a 65 wt%, but neither reported the extraction yield. Nguyen et al. (1994) studied the extraction of carnosic acid from herbal materials and stated that a hydroalcoholic mixture containing 40–60 wt% ethanol is preferred, which gave not less than a 75% recovery of the available carnosic acid. The extraction of carnosic acid was not efficient at less than 40 wt% ethanol and at above 60 wt% ethanol the extracts obtained were green and had flavour and aroma compounds. However, there is no published data on the solvent compositions for the extraction of all the lipophilic and hydrophilic constituents simultaneously.

In view of the desirability of these compounds, the goal of this study was to simultaneously obtain the highest yield of the active constituents rosmarinic acid, carnosic type compounds (carnosol, carnosic acid and methyl carnosate) and sage essential oil from dried sage using a stirred tank extraction method. We examined the effects of the extraction parameters; temperature, extraction time, sage particle diameter, solvent-to-sage ratio and hydroalcoholic solvent ratio on the extraction yield.

2. Materials and methods

2.1. Materials

Non-flowering sage of the type "Lincoln Grey" (Perry et al., 1996) was harvested and air dried in February 2004. Analyses of the dry sage used in this study gave: total phenols (TP) equivalent to 5.5% gallic acid (all percentages are w/w); rosmarinic acid (RA) 1.8%; carnosic type (CT) (which includes carnosic acid, carnosol and methyl carnosate) 2.5%; and essential oil (EO) 2.0%.

2.2. Methods

2.2.1. Experimental procedure

Dried sage was ground in a knife mill (Wiley) to a particle size of 1–3 mm, and was separated into size fractions using sieves. A hydroalcoholic extract was prepared using food grade ethanol, 99.8% (Barwell Pacific) and deionised water. The mixture was placed in a sealed glass vial in a heated water bath shaker (Julabo SW-21C) for extraction. After extraction the glass vial was removed and left to cool for 10 min. The mixture was vacuum filtered and the solid residue and hydroalcoholic extract collected. The solid residue was dried (40 °C, overnight). Evaporation of the hydroalcoholic solvent under reduced pressure gave the sage extract, which was weighed to give the yield of total soluble solids (TSS).

The extraction variables; temperature, extraction time, sage particle size, solvent-to-sage ratio and solvent composition were systematically varied as shown in Table 1. The recovery of the active components from the raw material, Extract_i, was calculated using:

$$\text{Extract}_i\ (\%) = \frac{m_{i,\text{extract}}}{m_{i,\text{sage}}} \times 100 \quad (1)$$

where i represents the active components: total phenolic content (TP), rosmarinic acid (RA), carnosic type compounds (CT) and sage essential oil (EO). $m_{i,\text{extract}}$ is the mass of the individual component in the extract and $m_{i,\text{sage}}$ is the mass of the individual component in the starting material.

Table 1
Extraction variables used for each experiment

Experiment type	Particle size (mm)	Temperature (°C)	Time (h)	Solvent: sage ratio	Composition (wt % ethanol)
Particle size	1–3	40	3	6:1	81
Temperature	2	22–63	3	6:1	81
Time	2	40	1–6	6:1	81
Solvent: sage	2	40	1	6:1–18:1	69
Composition	2	40	3	6:1	27–100

2.2.2. Analyses

2.2.2.1. Total phenolic content. This was determined by a colorimetric method using the Folin–Ciocalteau reagent (BDH), measured as gallic acid equivalents, with various concentrations of gallic acid (BDH) dissolved in acidified aqueous methanol (2 volumes of redistilled methanol (Commodity Resources) and 4 volumes of 0.3% HCl (Scharlau) in water). Solutions of the samples or extracts were prepared at a concentration of about 5 mg/ml in the acidified aqueous methanol. Aliquots (100 μl) of the test solutions were separately added to test tubes containing 2.0 ml of 2% sodium carbonate solution. After 2 min of standing, 100 μl of the Folin–Ciocalteau reagent (diluted 1:1 with water) were added and shaken. After 30 min standing, the absorbance of the resulting mixture was measured at 750 nm using a Varian Cary 50 Bio Spectrometer (Palo Alto, CA, USA). Sage does not contain gallic acid so this method gives a relative value for the total phenolic (TP) content (Singleton, Orthofer, & Lamuela-Raventós, 1999).

2.2.2.2. HPLC. Sage (100 mg) was extracted overnight in the dark, at room temperature, with 5 ml of 1:1 acetonitrile (Unichrom):water, after initial ultra-sonication. The resultant solution was filtered through a polypropylene syringe filter (0.45 μm, 30 mm, Bonnet) and 250 μl transferred into a 2 ml vial. For HPLC analysis this was diluted to 1000 μl using 1:1 (v/v) acetonitrile:water. Analyses were carried out at 30 °C on a RP-18 column (Superspher 100 112-4 mm ID, 4 mm, endcapped, Merck, Darmstadt, Germany) with Lichrocart 100 RP-18 4-4 (5 mm) guard column. The solvent solution, **A**, was water adjusted to pH 2.5 with orthophosphoric acid (Scharlau). The solvent solution **B** was acetonitrile. **A** and **B** were mixed using a linear gradient starting with 95% **A**, decreasing to 55% over 40 min and to 0% over the next 10 min. After holding the solvent at this composition for 5 min, **A** was increased to 95% over the next minute and was held at 95% until the end of the 65 min analysis. The flow rate was 1.0 ml/min, with an injection volume of 10 μl. Peaks were detected at 280 nm. The HPLC system was a Waters (Milford, MA, USA) 600E solvent controller, a Waters 996 photodiode array detector, a Jasco (Tokyo, Japan) 851-AS intelligent sampler and Waters Millennium^{32} 3.05.01 software.

The components, rosmarinic acid and carnosic acid were identified within the samples. The other phenolic bioactive compounds were tentatively identified. The rosmarinic acid (RA) content was calculated based on the observed peak area of rosmarinic acid for each sample and a comparison with the value obtained from an authentic rosmarinic acid standard (95%, Roth). Carnosic acid equivalent content was calculated by combining the areas of carnosol, carnosic acid and methyl carnosate peaks and comparing this with the area from a carnosic acid standard (91%, Sigma). The presence of carnosic acid was determined by co-chromatography with the standard while carnosol and methyl carnosate were identified by comparing UV absorbance maxima and relative retention times with the published data (Cuvelier, Berset, & Richard, 1994b; Cuvelier, Richard, & Berset, 1996).

2.2.2.3. GC analysis of the essential oil. The dry sage extract (1 g) was extracted with hexane (10 ml) by shaking in a water bath (40 °C, 2 h). The hexane solution was filtered, evaporated to dryness using a rotary evaporator (Bucchi), and then analysed by GC. The analyses were performed on a Hewlett–Packard series 5890 equipped with an FID detector, using a fused silica DB-5 column, 30 m × 0.25 mm i.d., film thickness 0.25 μm, with the oven temperature, 80 °C programmed to increase to 300 °C at a rate of 7.5 °C/min. The injector was set to 250 °C and the detector to 300 °C. The percentage composition of essential oil in the extracted hexane soluble compounds was computed from GC peak areas with the aid of camphor and *n*-octadecane as external and internal standards, respectively. The identification of compounds was based on comparison of the retention times of injected pure compounds and by the use of GC–MS data of sage essential oil samples obtained from supercritical CO_2 extraction and hydrodistillation.

3. Results and discussion

A systematic investigation of the parameters particle diameter, extraction temperature, extraction time, solvent-to-sage ratio and solvent composition was carried out in a laboratory scale to determine conditions which enable the production of a tincture containing optimal levels of lipophilic antioxidants, hydrophilic antioxidants and flavour/fragrance compounds. Only one process parameter was varied at a time. The extraction conditions of the experiments carried out are given in Table 1. The effects of each process parameter on the extract yield of total soluble solids (TSS), and the recovery of total phenols (TP), rosmarinic acid (RA), carnosic type compounds (CT), and essential oil (EO) are discussed below.

3.1. Sage particle size effect

The extract yield (TSS) and the bioactives recoveries (TP, RA, CT and EO) as a function of the particle size

(Table 1) are shown in Fig. 1. As the particle size was increased from 1 to 3 mm, the extraction yield and the recoveries of each bioactive component decreased. This is indicating that diffusion of the hydroalcoholic solvent into the particle, and solvent–solute diffusion out of the particle may be limiting the extraction process. At the particle sizes employed in this work the particle geometry is likely to resemble a flat plate, as observed using optical microscopy by Reverchon (1996), and as the particle size increases, the extent of undisrupted flat plate (surface of the leaf) increases.

It is possible that the waxes on the undisrupted leaf surface, which are very poorly soluble in ethanol and ethanol–water mixtures, are protecting the bioactive compounds from extraction. Under the conditions studied in this work a particle diameter of 1 mm was chosen as optimal in terms of extraction yield. A particle size of 1 mm has also been shown to give full recoveries of essential oil by supercritical extraction (Catchpole et al., 1996; Reverchon et al., 1995; Reverchon, 1996). However, small particles result in processing difficulties such as dust and heat generation during grinding; and blocked filters during extraction at a large scale, and so 2 mm particles were used in the remainder of the optimisation work.

3.2. Extraction temperature effect

The extract yield and bioactives recoveries as a function of the extraction temperature (Table 1) are shown in Fig. 2. Higher temperatures gave higher yields and higher recoveries of TP, RA and CA, due to increased solubilities and diffusion coefficients. For EO an increase in the recovery from 13.9% to 19.0% was observed when increasing the temperature from 22 to 40 °C, but a further temperature increase to 63 °C resulted in a decrease in the recovery back to 13.6%. This may be due to vaporization of volatile essential oil components during extraction at the higher temperature.

Although an increase in temperature resulted in increased extract yields, the recoveries for all the desired bioactives (except CT) were similar at both 40 and 63 °C, thus extractions at temperatures higher than 40 °C were extracting more non active compounds from the sage. Also high temperatures result in increased solvent losses. An extraction temperature of 40 °C was chosen as optimal for overall extraction and bioactives recovery.

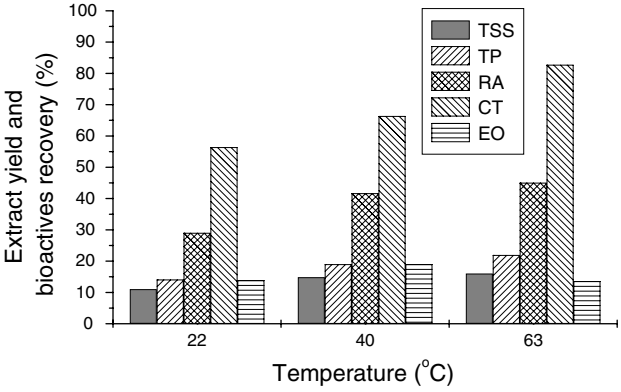

Fig. 2. Sage extract yield (TSS) and bioactives recovery (TP, RA, CT, and EO) as a function of extraction temperature.

3.3. Extraction time effect

The extract yield and bioactives recoveries as a function of the extraction time (Table 1) are shown in Fig. 3. As the extraction time was increased the extract yield and the total phenolics recovery stayed constant within experimental error, but the recoveries of the bioactives RA, CT and EO all increased. Since rosmarinic acid (RA) and carnosic type (CT) compounds are the major phenolics in sage, it seems that other oligomeric compounds (Lu et al., 1999) measured in the total phenolics (TP) must be unstable, degrading at longer extraction times. Furthermore, the increased extraction time and sustained exposure to high temperature limits throughput at a large scale and potentially increases the loss of solvent by vaporization. It is therefore suggested that an extraction time of no longer than 3 h is employed.

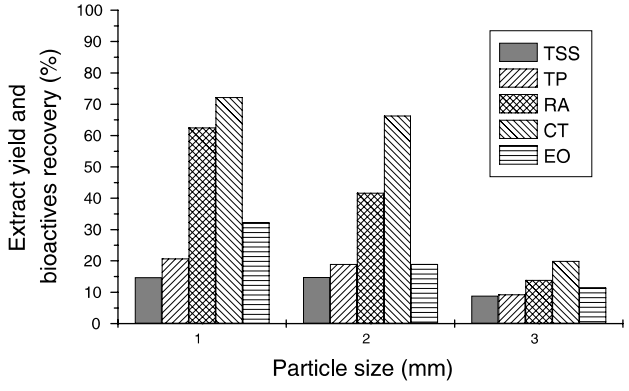

Fig. 1. Sage extract yield (TSS) and bioactives recovery (TP, RA, CT, and EO) as a function of particle size.

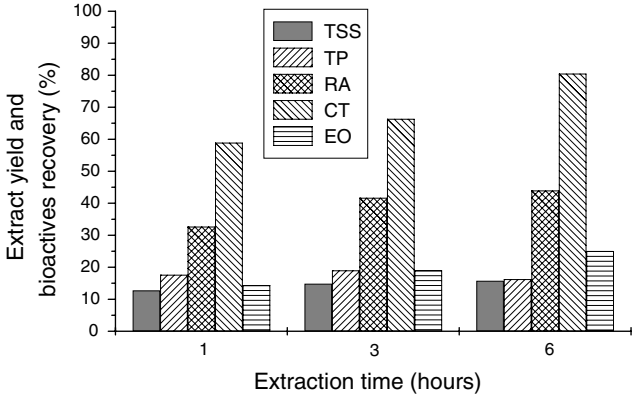

Fig. 3. Sage extract yield (TSS) and bioactives recovery (TP, RA, CT, and EO) as a function of extraction time.

3.4. Effect of solvent-to-sage ratio

The extract yield and bioactives recoveries as a function of the solvent-to-sage ratio (Table 1) are shown in Fig. 4 for ratios 6:1–18:1. The extract yield and bioactives recovery increased almost linearly with solvent ratio. An extraction at a lower solvent-to-sage ratio (3:1) was also carried out but the majority of the solvent was absorbed by the dry sage material, which resulted in minimal recovery of solvent and extract upon filtration. Analysis of the marker compounds from the 3:1 solvent ratio was not possible.

When operating with a stirred tank, the concentration of each solute within the sage leaf eventually reaches an equilibrium with the concentration of the solute dissolved in the solvent, unless the extraction is solubility limited. To assess whether the extraction of the bioactive compounds are consistent with equilibrium being reached between the solvent and solid (leaf) phases or are solubility limited (solutes are extracted in proportion to the amount of solvent used), the solubility of each bioactive compound was calculated at each solvent to sage ratio. Table 2 shows the mass of each bioactive compound extracted at each solvent-to-sage ratio and the resulting solubility. It is apparent that the extraction of the phenolics and all the bioactive compounds except EO is limited by their respective solubility in the solvent, as the solubility is constant within experimental error at each solvent concentration.

The selectivity of the solvent was determined as a function of the solvent-to-sage ratio and the solute loading (the mass of extract per kg of solvent). The solute loading is plotted against the solvent-to-sage ratio in Fig. 5, left hand

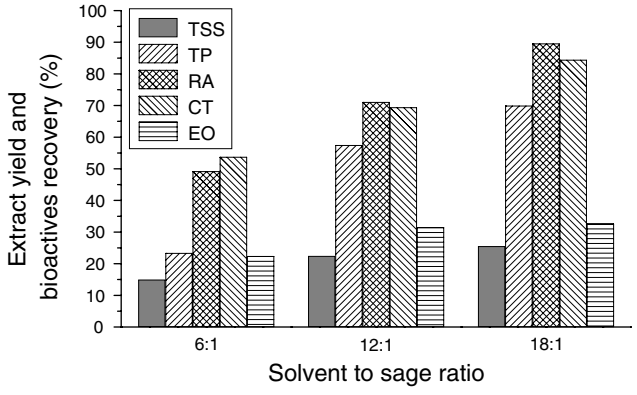

Fig. 4. Sage extract yield (TSS) and bioactives recovery (TP, RA, CT, and EO) as a function of solvent-to-sage ratio.

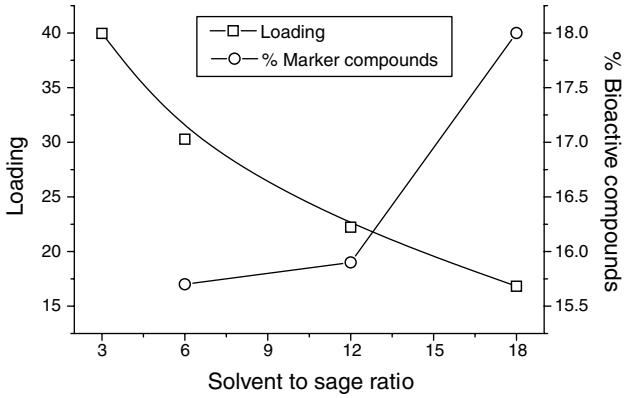

Fig. 5. Total soluble solids loading and percentage of bioactive compounds in the total soluble solids as a function of solvent-to-sage ratio.

y-axis and the percentage of bioactive compounds in the total extract (Fig. 5, right hand *y*-axis). The loading drops dramatically with increasing solvent-to-sage ratio, whilst the purity of the extract increases the solvent becomes slightly more selective for the desired bioactive components. However, the increase in selectivity is outweighed by the increase in the cost of the solvent required.

Three sequential extractions were carried out on a single sage sample to see whether a second and third extraction using a 6:1 solvent ratio resulted in a similar extraction yield to single extractions at a 12:1 and 18:1 solvent-to-sage ratio. All other extraction conditions were kept constant. The sequential extractions simulate the multi-vessel packed bed extraction of sage, whereby the fresh solvent contacts a fully to partially extracted bed of material. The cumulative extract yield and recoveries for each bioactive compound are shown in Fig. 6. As expected as the number of extractions is increased the total extract yield increases, but the yield of each marker compound obtained after two extractions has decreased by half and after three extractions it has decreased by half again. Inspection of Figs. 4 and 6 shows that there is no great difference between the extract yields and recoveries of all bioactive compounds for two extractions using a 6:1 solvent-to-sage ratio as compared with a single extraction using a 12:1 solvent-to-sage ratio. After three extractions at 6:1 there is a greater recovery of EO compared to the single 18:1 extraction but all other bioactive compound recoveries are similar.

Increasing the solvent-to-sage ratio increases both the recovery of bioactive compounds, and the concentration of the bioactive compounds relative to the total soluble

Table 2
Bioactive compound solubility as a function of solvent-to-sage ratio

Solvent (ml)	Amount extracted (g)				Solubility (g/100 ml)			
	TP	RA	CT	EO	TP	RA	CT	EO
60	0.13	0.06	0.07	0.04	0.22	0.11	0.12	0.07
120	0.29	0.13	0.16	0.06	0.24	0.11	0.13	0.05
180	0.40	0.18	0.22	0.06	0.22	0.10	0.12	0.04

TP = total phenolics, RA = rosmarinic acid, CT = carnosic type compounds, EO = essential oil.

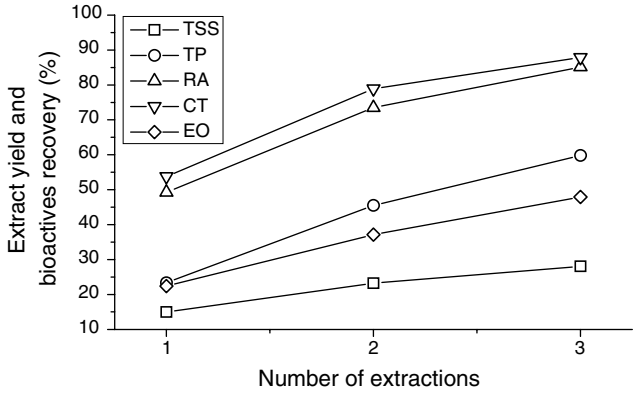

Fig. 6. Cumulative extraction yield and bioactives recovery over 3 extractions using 69% ethanol, a particle diameter of 2 mm at a temperature of 40 °C for 1 hour with a solvent-to-sage ratio of 6:1.

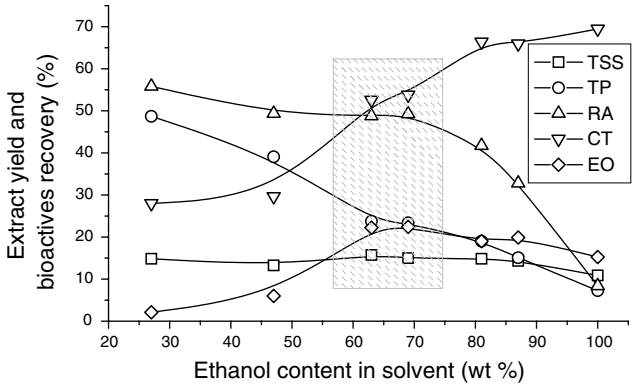

Fig. 7. Effect of hydroalcoholic solvent ratio on the extraction of sage bioactive constituents.

solids. However, exhaustive extraction is restricted by solubility limitations of the bioactive compounds. The packed bed extraction of sage is likely to be more efficient than the stirred tank method used in this work. As the extraction of bioactive compounds are limited by their solubility with the solvent composition used, a compromise must be reached between concentration in the solvent, cost of the solvent and energy costs associated with evaporation of the solvent. The optimal solvent-to-sage ratio is chosen as 6:1.

3.5. Effect of hydroalcoholic solvent ratio

The extract yield and bioactives recoveries as a function of the ethanol content in the hydroalcoholic solution (Table 1) are shown in Fig. 7. Of all the extraction variables studied in this work, the hydroalcoholic solvent ratio had the most pronounced effect on the recoveries of sage bioactive compounds. The total yield of solids extracted from the sage material remained constant within experimental error over the hydroalcoholic solvent ratios studied. However, the recovery of the individual bioactive compounds is highly dependant on the hydroalcoholic solvent ratio employed for the extraction. At high ethanol contents there was a high recovery of the CT compounds (more lipophilic) but a low recovery of RA (more hydrophilic). As the ethanol content was decreased from 100 to 70 wt% there was a rapid rise in the recovery of RA, which levels off as the ethanol content was decreased below 70 wt%. The recovery of CT compounds gradually decreased as the ethanol content in the solvent decreased. The recovery of EO was low below ethanol contents of 55 wt%. Therefore, ethanol had a higher selectivity for EO and CT, whereas water had a higher selectivity for RA and total phenolics (TP). The optimal hydroalcoholic solvent composition was between 55 and 75 wt% ethanol, as shown by the hatched area in Fig. 7.

The results presented here on the effect of hydroalcoholic solvent ratio are in good agreement with those of Nguyen et al. (1994) for carnosic acid, where at high ethanol contents it is found that flavour and aroma compounds were extracted and at low ethanol contents the extraction yield of CT compounds had substantially diminished.

3.6. Reproducibility

The reproducibility of the measurements was assessed for sage extractions in triplicate at three different parameter combinations, which are summarized in Table 3. The reproducibility of these results was good, at between 2% and 10% relative error, except for CT and EO for extraction method 3. In this case, the relative error was increased to just under 15%. This is because the levels of these bioactives were near the detection limit and manual integration of the peaks was necessary, which introduced a larger degree of error into the results.

3.7. Optimal extraction conditions and scale-up

Sage with a particle diameter size of 1 mm was extracted at a temperature of 40 °C for 1 h with a solvent-to-sage ratio of 6:1 (v/w) using a hydroalcoholic solution of

Table 3
Reproducibility of sage extraction yields

Experimental	Method					Average yield and (standard deviation)				
	Particle size (mm)	Temp (°C)	Time (h)	Solvent:sage ratio	Composition (wt% ethanol)	TSS	TP	RA	CT	EO
1	2	40	3	6:1	81	14.2 (1.1)	18.4 (0.8)	39.2 (2.5)	60.3 (6.1)	20.3 (1.4)
2	2	40	3	6:1	69	14.5 (0.9)	29.6 (3.0)	52.1 (3.3)	52.0 (1.6)	24.9 (2.5)
3	2	40	1	12:1	27	23.5 (1.0)	72.4 (1.7)	90.7 (1.9)	23.9 (3.5)	2.2 (0.3)

TSS = total soluble solids, TP = total phenolics, RA = rosmarinic acid, CT = carnosic type compounds, EO = essential oil.

69 wt% ethanol to assess whether the chosen extraction conditions would result in optimal extraction yields with reduced extraction time. This gave an extract of 14.9% total soluble solids, containing 6.9% rosmarinic acid (55% recovery), 10.6% carnosic compounds (75% recovery) and 7.3% essential oil (42% recovery). Comparison with the optimisation program results was satisfactory, as all bioactive compound recoveries were similar to or higher than those obtained previously.

The stirred tank extraction process was scaled up by a factor of 100 (1 kg of sage was extracted per trial) to establish whether the optimised conditions hold at a larger scale of operation. The average extraction yield results (and standard deviation from the mean) for the five large scale trials are a TSS yield of $14.9 \pm 1.1\%$, with an extract content of $5.3 \pm 0.7\%$ rosmarinic acid ($55.6 \pm 4.2\%$ recovery), $9.7 \pm 0.8\%$ carnosic compounds ($66.1 \pm 6.4\%$ recovery) and $6.1 \pm 0.4\%$ essential oil ($39.8 \pm 2.9\%$ recovery). Agreement between the optimal extraction yields at laboratory scale and at the pilot scale was good, giving confidence that the optimised process can be carried out without any loss of efficiency at an industrial scale.

4. Conclusions

The aim of this work was to study the extraction efficiency of three classes of compounds: rosmarinic acid, carnosic acid (and related compounds) and essential oil from sage as a function of the process conditions: sage particle size, extraction temperature, extraction time, ratio of solvent-to-sage and solvent composition. The extractions were carried out by a stirred tank method. Optimised extraction yields of rosmarinic acid, carnosic acid (and related compounds) and essential oil were achieved by decreasing the particle diameter as far as practical (1 mm), operating at a moderate extraction temperature at which all actives are stable, an extraction time sufficient to overcome diffusion limitations, solvent-to-sage ratio which is efficient and feasible and a solvent composition capable of extracting the lipophilic and hydrophilic bioactive components. The optimal extraction conditions that satisfied the above constraints were found to be a particle diameter of 1 mm, at an extraction temperature of 40 °C, an extraction time of 1–3 h, solvent-to-sage ratio of 6:1 and 55–75 wt% ethanol for up to 3 h.

Semi-pilot scale extractions (scale factor 100) at optimal conditions were also carried out. Extraction yield results were found to be in good agreement with those at a laboratory scale, which gives confidence that the optimised process can be carried out without loss of efficiency at an industrial scale.

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