CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Journal homepage:**http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EFFECTS OF SUPERHEATED STEAM DRYING ON THE ANTIOXIDANT AND ANTI-TYROSINASE PROPERTIES OF SELECTED LABIATAE HERBS

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Article history:
Article history:
Received:
15 January 2018
Accepted:
1 October 2018

Keywords:
Labiatae herbs;
Superheated steam drying;
Phenolic contents;
Antioxidant;
Anti-tyrosinase.

ABSTRACT
In this study, the antioxidant and anti-tyrosinase properties of fresh, commercial dried (CD) and superheated steam-dried (SS-D) Labiatae herbs were analysed and evaluated. Superheated steam drying (SSD) was performed at 150°C and 200°C for 5, 10 and 20 min. Fresh and CD rosemary had the highest phenolic contents and the strongest primary antioxidant activities of free radical scavenging and ferric reducing power. Fresh spearmint, CD peppermint and CD oregano displayed the strongest secondary antioxidant activity of ferrous ion chelating ability. Based on total phenolic content and free radical scavenging, three broad categories of SS-D herbs were recognized i.e. herbs that showed declines for all the drying regimes (thyme and peppermint); those that showed declines or remained unchanged (marjoram and oregano); and those that showed all three traits of increment, declines or unchanged (rosemary, sage and spearmint). Tyrosinase inhibition was strongest in fresh sage, fresh rosemary, CD thyme and CD rosemary. Reported for the first time, SS-D rosemary, SS-D thyme and SS-D marjoram showed enhanced anti-tyrosinase properties for all the drying regimes. SS-D marjoram was the most exciting as tyrosinase inhibition was not detected in fresh samples. This study on the antioxidant and anti-tyrosinase properties of selected Labiatae herbs has provided some useful insights on the effects of SSD. The drying technique can be used for the production of tyrosinase inhibitors, which are increasingly used in medicines for treating pigmentation disorders, in cosmetics for skin whitening, and in food products for inhibiting browning.

1. Introduction

Drying and cooking methods and conditions can impart changes to the biochemical properties of culinary herbs and spices. For many herbs and spices, such studies are still lacking (Yi and Wetzstein, 2011). In our previous studies, the antioxidant, antibacterial and anti-quorum sensing (anti-QS) properties of Labiatae herbs, and the effects of microwave, blanching and boiling were analysed (Chan et al., 2012a, 2012b). In another study, we reported the antioxidant, anti-tyrosinase, antibacterial and anti-QS properties of selected spices, and the effects of microwave, blanching and boiling (Chan et al., 2015). In an earlier study, we reported the antibacterial and anti-QS activities of fresh, commercial dried and superheated steam-dried Labiatae herbs (Chan et al., 2017). In this study, the antioxidant and anti-tyrosinase properties of fresh and commercial dried Labiatae herbs were analysed and evaluated. The effects of superheated steam drying on their antioxidant

and anti-tyrosinase properties were reported for the first time.

Labiatae (also known as Lamiaceae) is a large family of plants with more than 200 genera and almost 4,000 species centred mainly in the Mediterranean region (Naghibi et al., 2010). The family comprises annual or perennial herbs that are aromatic and densely glandular (Kokkini et al., 2003). Leaves are simple and opposite, and stems are quadrangular in cross-section. Flowers are hermaphrodite and form whorls arranged in spikes, heads, racemes, or cymes. With chemical constituents of terpenoids, iridoids, flavonoids and phenolic acids, the different plant parts of Labiatae species are widely used for food flavouring and preservative, and as herbal teas or traditional medicines (Kokkini et al., 2003; Naghibi et al., 2010). Some species of Labiatae are planted as ornamentals and as sources of aromatic oils. Antioxidant, antimicrobial, analgesic, anti-inflammatory, hypotensive and cardiotonic activities are among their wide array of biological and pharmacological properties.

Superheated steam drying (SSD) is an emerging drying technique where saturated steam is heated beyond the boiling point at a given pressure (Law et al., 2013). Moisture from the food is removed by the temperature difference between the food and the superheated steam in a closed system. SSD offers several advantages (Karimi 2010; Law et al., 2013; Mujumdar 2014). It has a higher drying rate than air-drying. High steam temperature and free diffusion of water vapour are important factors for the high drying rate. The system is energy efficient as the exhausted steam can be recycled back to the system and only ~30 kJ of heat is needed to convert the saturated steam to superheated steam. No oxidation is involved and the system is free from fire or explosion hazards. SSD inactivates microbes and enzymes. Of interest to food processing is the non-oxidative reactions, ability to maintain colour and nutrients, and yielding products of higher porosity. Overall, SSD offers advantages such as, an oxygen-free environment, which lowers the percentage of oxidation and nutrient loss, improves thermal degradation due to the increase in heat transfer, improves energy efficiency, and accelerates drying rate (Cenkowski et al., 2007).

Studies have shown that some superheated steam-dried food products such as shrimp (Prachayawarakorn et al., 2002), oat groat (Head et al., 2009), rice (Rumruaytum et al., 2013), cocoa bean (Zzaman et al., 2014) and chicken sausage (Asmaa and Tajul, 2017) maintain good quality.

2. Materials and methods

2.1. Herbs studied

Seven fresh Labiatae herbs grown in Genting Highlands, Pahang, Malaysia were purchased in local supermarkets in Kuala Lumpur. They were rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), oregano (*Origanum vulgare* L.), marjoram (*Origanum majorana* L.), thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperita* L.) and spearmint (*Mentha spicata* L.). Their antioxidant and anti-tyrosinase properties were determined before and after SSD. Five commercial dried (CD) herbs of rosemary, sage, oregano, thyme and peppermint were also analysed for comparison.

2.2. Extraction of herbs

For the analysis of phenolic contents and antioxidant properties, fresh herbs (1 g) or dried herbs (0.3 g) were powdered with liquid nitrogen using a pestle and mortar. After powdering, the samples were poured into a conical flask and extracted using 50 mL of 70% methanol with continuous swirling for 1 h at 100 rpm using an orbital shaker at room temperature. The extracts were then filtered and stored at −20ºC in a freezer for further use.

For the analysis of anti-tyrosinase properties, fresh herbs (10 g) or dried herbs (3 g) were powdered with liquid nitrogen using a pestle and mortar. The powdered samples were then poured into conical flask and extracted using 100 mL of pure methanol with continuous swirling at 120 rpm using an orbital shaker. Extraction was repeated three times for 1 h each time. The filtered extracts were freeze-dried for 30 min to remove any liquid present before storage in a freezer at −20ºC for further analysis.

2.3. Superheated steam drying

Superheated steam drying (SSD) of fresh herbs was carried out at 150ºC and 200ºC for 5, 10 and 20 min. The superheated steam oven (Healsio, AX-1600, SHARP) was pre-heated at the required temperatures for 2 min before the herbs (20 g) were dried at the scheduled durations. On cooling, the dried herbs were weighed, sealed in an airtight bag and stored in a freezer at −20ºC for further analysis.

2.4. Phenolic contents

Herbs were analysed for phenolic contents of total phenolic content (TPC), total flavonoid content (TFC) and caffeoylquinic acid content (CQAC) using the Folin-Ciocalteu, aluminium chloride and molybdate assays, respectively (Chan et al., 2014, 2015).

In the Folin-Ciocalteu (FC) assay, extracts (300 µL) were introduced into test tubes wrapped with aluminium foil, followed by the addition of 1.5 mL of FC reagent (diluted 10 times) and 1.2 mL of sodium carbonate (7.5%, w/v). After incubating for 30 min in the dark, absorbance was read at 765 nm against a blank using a UV-vis spectrophotometer (Anthelie Advanced 5 Secoman). TPC was expressed as gallic acid equivalent (GAE) in mg/100 g of sample.

In the aluminium chloride assay, extracts (1 mL) were introduced into test tubes containing 4 mL of water. Then, 0.3 mL of 5% sodium nitrite was added, followed by 0.3 mL of 10% aluminium chloride, 2 mL of sodium hydroxide solution and 2.4 mL of water to make up to 10 mL. After mixing well and incubated at room temperature for 10 min, absorbance was read at 415 nm against a sample blank of 1 mL of the respective extracts with 9 mL of water. TFC was expressed as quercetin equivalent (QE) in mg/100 g of sample.

In the molybdate assay, the reagent was prepared by dissolving 16.5 g sodium molybdate, 8 g dipotassium hydrogen phosphate and 7.9 g potassium dihydrogen phosphate in 1 L of water. The reagent (2.7 mL) was added to the extract (0.3 mL), mixed and incubated at room temperature for 10 min. Absorbance was read at 370 nm against a sample blank of the extracts with 2.7 mL of water. CQAC was expressed as chlorogenic acid equivalent (CGAE) in mg/100 g of sample.

2.5. Antioxidant activities

Antioxidant activities of free radical scavenging (FRS) activity, ferric reducing power (FRP) and ferrous ion chelating (FIC) ability were measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, potassium ferricyanide and ferrozine assays, respectively (Chan et al., 2014; 2015). In the DPPH assay, different dilutions of extracts (1 mL) were added to 2 mL of DPPH (5.9 mg in 100 mL methanol). Absorbance was read at 517 nm after 30 min. The IC_{50} was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid (AA)/100 g of sample, which was calculated as $IC_{50\ ascorbic\ acid}$ / $IC_{50\ sample}$ x 10^5 where the IC_{50} of ascorbic acid was 0.0039 mg/mL.

In the potassium ferricyanide assay, different dilutions of extracts (1 mL) were added to 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide

(1%, w/v). The mixture was incubated at 50°C for 20 min. After adding trichloroacetic acid solution (2.5 mL, 10%, w/v), the mixture was separated into aliquots of 2.5 mL, and diluted with 2.5 mL of water. Ferric chloride solution (500 mL, 0.1%, w/v) was added to each diluted aliquot and absorbance was read at 700 nm against a blank using a UV-vis spectrophotometer (Anthelie Advanced 5 Secoman) after 30 min. FRP was expressed as mg GAE/100 g of sample. The calibration equation for gallic acid (GA) was y = 16.767x (R^2 = 0.9974), where y is the absorbance and x is the GA concentration in mg/mL.

In the ferrozine assay, different dilutions of extracts (1 mL) were mixed with $FeSO_4$ (0.1 mM, 1 mL) and ferrozine (0.25 mM, 1 mL). Absorbance was read at 562 nm after 10 min. FIC ability was calculated as ($1 - A_{sample} / A_{control}$) x 100% and expressed as chelating efficiency concentration (CEC_{50}) in mg/mL or the effective concentration of extract needed to chelate ferrous ions by 50%.

2.6. Anti-tyrosinase activity

Tyrosinase inhibition of herbs was determined using the dopachrome assay with L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate (Tan and Chan, 2014; Chan et al., 2015). Samples were prepared by dissolving 10 mg of dried extracts in 1 mL of methanol and 1.5 mL of 50% dimethyl sulphoxide (DMSO). The assay was conducted in a 96-well microtiter plate using a plate reader (BIOTEK PowerWave XS Microplate) to measure absorbance at 450 nm with 630 nm as reference. Each well was filled with 40 µL of sample with 80 µL of phosphate buffer (0.1 M, pH 6.8), 40 µL of tyrosinase (31 units/mL) and 40 µL of L-DOPA (2.5 mM). Each sample was accompanied by a blank with all the components except L-DOPA. Results were compared with a control consisting of DMSO in place of sample. Tyrosinase inhibition in percent was calculated as ($A_{control} - A_{sample}$) / $A_{control}$ x 100%. The concentration of extracts used for determining tyrosinase inhibition was 0.25 mg/mL.

2.7. Statistical analysis

Experiments were conducted in triplicate (n = 3) and results were expressed as means ± standard deviations. Analysis of variance was analysed using the Tukey Honestly Significant Difference (HSD) test based on significant difference of $p < 0.05$.

3. Results and discussion

3.1. Antioxidant properties of fresh herbs

Of the seven fresh herbs analysed (Table 1), rosemary displayed the highest phenolic contents of TPC (1280 mg GAE/100 g), TFC (215 mg QE/100 g) and CQAC (467 mg CGAE/100 g), and the strongest antioxidant activities of AEAC (1890 mg AA/100 g) and FRP (1210 mg GAE/100 g). Spearmint and peppermint had the lowest phenolic contents and weakest antioxidant activities.

The potent antioxidant properties of rosemary may be attributed to its phenolic constituents. Carnosol, carnosic acid, rosmanol and rosmarinic acid are the most important constituents that account for most of the antioxidant activity of rosemary (Ho et al., 2000; Etter, 2004). From rosemary, rosmanol and carnosol exhibited antioxidant activity more than four and two times higher than butylated hydroxytoluene (BHT), respectively (Nakatani, 2000).

In contrast, the chelating efficiency concentration (CEC_{50}) of spearmint (2.6 mg/mL) was the strongest and the weakest in rosemary (6.3 mg/mL) (Table 1). In contrast to the ranking based on phenolic contents of TPC, TFC and CQAC which showed similarity with antioxidant activities of AEAC and FRP, the ranking based on antioxidant activity of CEC_{50} was almost the reverse.

Rosemary had the highest phenolic contents of TPC, TFC and CQAC, and the

strongest antioxidant activities of AEAC and FRP but the weakest CEC_{50}. On the other hand, spearmint had the lowest phenolic contents and the second lowest antioxidant activities of AEAC and FRP but was the strongest in CEC_{50}. AEAC and FRP are measures of the hydrogen- and electron-donating abilities of primary or chain-breaking antioxidants (Antolovich et al., 2002). They prevent oxidative damage by directly scavenging free radicals (Lim et al., 2007). FIC measures the ability of secondary or preventative antioxidants to chelate metal ions. They act indirectly by preventing the generation of hydroxyl radicals *via* the Fenton's reaction. Findings of this study support our earlier report that herbal teas with strong FIC ability usually display moderate or low primary antioxidant activities (Chan et al., 2012a). This indicates that plants with potent primary antioxidant activities may not have strong secondary antioxidant activities.

The antioxidant properties of fresh non-Labiatae herbs have reported earlier by Chan et al. (2014) using the same protocols. This presented an opportunity to broadly compare between the antioxidant properties of Labiatae herbs in this study (Table 1) and those of non-Labiatae herbs. In general, non-Labiatae herbs with the strongest antioxidant properties (e.g. *Anacardium occidentale*, *Persicaria hydropiper* and *Cosmos caudatus*) surpass those of rosemary. At the same time, there is a host of non-Labiatae species with antioxidant properties weaker than or comparable to those of Labiatae herbs.

3.2. Antioxidant properties of CD herbs

Out of the five commercial dried (CD) herbs analysed, rosemary displayed highest phenolic contents (TPC, TFC and CQAC) while lowest values were observed in oregano (Table 2). Rosemary also had the strongest primary antioxidant activities with AEAC of 5710 mg AA/100 g and FRP of 3510 mg GAE/100 g. Peppermint had the weakest activities with AEAC of 3720 mg AA/100 g and FRP of 1930 mg GAE/100 g. In terms of CEC_{50}, the strongest chelating ability was observed in peppermint (1.0 mg/mL) followed by oregano (1.3 mg/mL). Both rosemary (3.0 mg/mL) and thyme (2.9 mg/mL) had the weakest activities. The ranking of secondary antioxidant activity of FIC based on CEC_{50} was in the reverse order of the ranking in phenolic contents of TPC, TFC and CQAC, and primary antioxidant activities of AEAC and FRP.

To find out if herbs or spices have stronger antioxidant properties, data of CD herbs (Table 2) were compared to those of dried spices reported by our research group earlier using the same protocols (Chan et al., 2015). Interestingly, some distinct contrasts emerged. There was evidence that spices have a much greater range of values compared to herbs. In terms of phenolic contents of TPC, TFC and CQAC, the highs of spices (e.g. clove and cinnamon) were much higher than those of rosemary, and the lows of spices (e.g. poppy and cardamom) were much lower than those of oregano. The same trend was evident for primary antioxidant properties of AEAC and FRP. Based on secondary antioxidant activity of CEC_{50}, herbs generally had stronger chelating ability than spices.

3.3. Superheated steam drying of herbs

Average moisture loss of the herbs following superheated steam drying (SSD) at 150^{o}C for 5, 10 and 20 min was 61%, 77% and 84%, and the loss following SSD at 200^{o}C for the same durations was 71%, 83% and 86%, respectively. Acknowledging that variations exist between herbs, changes in colour are briefly described. Following SSD at 150^{o}C, the herbs turned from dark green (5 min) to dark green with slight browning (10 min), and to browning and shrivelling of leaves (20 min). After SSD at 200^{o}C, the herbs turned from dark green with slight

browning (5 min) to browning and shrivelling of leaves (10 min), and to browning, shrivelling and slight scorching of leaves (20 min).

3.4. Antioxidant properties of SS-D herbs

When subjected to drying at 150ºC and 200ºC for 5, 10 and 20 min, superheated steam-dried (SS-D) herbs showed variations in antioxidant properties when compared with those of fresh herbs. Based on TPC and AEAC, three broad categories of effects can be recognized (Table 3). They are herbs that showed declines in TPC and AEAC for all the drying regimes (thyme and peppermint); those that showed declines or remained unchanged (marjoram and oregano); and those that showed all three traits of increments, declines or remained unchanged (rosemary, sage and spearmint). In the first category, declines of 26−60% were observed in thyme and 28−73% in peppermint. In the second category, declines were 20−64% in marjoram and 21−48% in oregano. In the third category, gains of 23−52% and declines of 26−44% were displayed by rosemary, 24−35% and 25−50% by sage, and 40−60% and 21−58% by spearmint. Most of the declines in TPC and AEAC were observed following SSD for 20 min. Overall, findings of significant declines in TPC and AEAC following SSD were consistent with our earlier study on the effects of oven drying on Labiatae herbs (Chan et al., 2012a, 2012b). Earlier studies using other drying methods also showed variable effects. Compared to fresh herbs, the phenolic content of air-dried oregano and peppermint increased significantly but their radical scavenging activity declined significantly and remained unchanged, respectively (Capecka et al., 2005). The phenolic content and antioxidant activity of sun-dried rosemary and peppermint were enhanced while those oven-dried at 40ºC and 70ºC remained unchanged (Yi and Wetzstein, 2011).

Studies on other plant products also showed declines in antioxidant properties following SSD. Although the phenolic content of SS-D mate leaves was 1.9 times higher than leaves dried with conventional hot air, they were about 3 times lower than fresh leaves (Zanoelo et al., 2006). The higher phenolic content of SS-D mate leaves was credited to lower phenol oxidase activity induced by an oxygen-free atmosphere. SS-D cocoa beans at 150ºC, 200ºC and 250ºC for 10−50 min resulted in significant declines of phenolic contents of TPC and TFC, and antioxidant activities of DPPH radical scavenging and FRP with increasing time and temperature (Zzaman et al., 2014). Similarly, SSD resulted in the loss of antioxidant properties of mangosteen rinds (Suvarnakuta et al., 2011). Although SS-D avocado pulp had significantly higher TPC and TFC, and stronger DPPH radical scavenging activity than freeze-dried samples, no comparisons were made with unprocessed avocado pulp (Husen et al., 2014).

Processing methods are known to have variable effects on the phenolic content and antioxidant activity of plant samples. Effects include little or no change, significant losses or enhancement (Nicoli et al., 1999). Food processing can induce the formation of new compounds so that the overall antioxidant properties increase or remain unchanged (Tomaino et al., 2005). Declines in antioxidant properties of plant samples have been attributed to thermal degradation of phytochemicals and to loss of antioxidant enzyme activities during heat treatments (Larrauri et al., 1997; Lim and Murtijaya, 2007; Chan et al., 2013).

3.5. Anti-tyrosinase properties of fresh herbs

Of the fresh herbs analysed for tyrosinase inhibition, rosemary (51%), sage (52%) and peppermint (47%) were significantly the strongest (Table 4). The

weakest tyrosinase inhibition was observed in spearmint and oregano (both 17%) while that of marjoram was not detected. Ranking of fresh herbs based on tyrosinase inhibition was rosemary ~ sage ~ peppermint > thyme > spearmint ~ oregano > marjoram. In support of findings of this study, Lin et al. (2011) studied the anti-tyrosinase activity of 48 herbs and found that oregano, marjoram, rosemary, sage and peppermint were observed to have tyrosinase inhibition, with exception of marjoram. Contrary to findings of this study, *O. majorana* was observed to exhibit the strongest tyrosinase inhibitory activity among 28 species of Lamiaceae plants, with an IC_{50} of 0.11 mg/mL (Lee et al., 2011).

Table 1. Phenolic contents and antioxidant activities of fresh herbs

Fresh herb	Phenolic content			Antioxidant activity		
	TPC	TFC	CQAC	AEAC	FRP	CEC_{50}
Rosemary	1280±164^{a}	215±40^{a}	467±74^{ab}	1890±333^{a}	1210±133^{a}	6.3±0.7^{c}
Thyme	875±109^{b}	127±10^{c}	384±45^{b}	871±119^{c}	594±50^{c}	4.7±0.1^{b}
Marjoram	735±48^{b}	170±15^{b}	503±49^{a}	1620±166^{a}	816±62^{b}	3.3±1.2^{a}
Sage	732±108^{b}	241±19^{a}	348±37^{bc}	886±120^{c}	870±54^{b}	5.5±0.4^{bc}
Oregano	524±49^{c}	86±18^{d}	281±42^{c}	1100±19^{b}	471±41^{d}	4.4±0.7^{b}
Peppermint	367±55^{d}	83±10^{d}	160±29^{d}	345±47^{e}	203±40^{f}	3.9±0.5^{ab}
Spearmint	294±14^{d}	65±2^{e}	148±22^{d}	457±18^{d}	275±7^{e}	2.6±0.3^{a}

Abbreviations: TPC = total phenolic content (mg GAE/100 g), TFC = total flavonoid content (mg QE/100 g), CQAC = caffeoylquinic acid content (mg CGAE/100 g), AEAC = ascorbic acid equivalent antioxidant capacity (mg AA/100 g), FRP = ferric reducing power (mg GAE/100 g), CEC_{50} = median chelating efficiency concentration (mg/mL), GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent and AA = ascorbic acid. Data on antioxidant properties based on phenolic contents and antioxidant activities in fresh weight are means ± standard deviations. Lower CEC_{50} values indicate stronger ferrous ion chelating (FIC) ability. Within each column, values with different superscript letters (a−e) are significant at $p < 0.05$ using the Tukey HSD test.

Table 2. Phenolic contents and antioxidant activities of commercial dried herbs

CD herb (brand)	Phenolic content			Antioxidant activity		
	TPC	TFC	CQAC	AEAC	FRP	CEC_{50}
Rosemary (McCormick)	6030±305^{a}	973±31^{b}	2490±41^{a}	5710±284^{a}	3510±286^{a}	3.0±0.2^{d}
Thyme (McCormick)	4670±331^{b}	1450±116^{a}	1660±194^{c}	5820 ±363^{a}	2460±118^{b}	2.9±0.5^{cd}

Sage (Heritage)	4390±269^{b}	944±88^{b}	2220±152^{b}	5100 ±308^{b}	2090±265^{c}	2.5±0.2^{c}
Peppermint (Boh)	3100±115^{c}	1510±76^{a}	1240±58^{e}	3720 ±184^{d}	1930±60^{c}	1.0±0.1^{a}
Oregano (McCormick)	2260±64^{d}	971±44^{b}	1400±55^{d}	4670 ±215^{c}	2420±129^{b}	1.3±0.1^{b}

Abbreviations: CD = commercial dried, TPC = total phenolic content (mg GAE/100 g), TFC = total flavonoid content (mg QE/100 g), CQAC = caffeoylquinic acid content (mg CGAE/100 g), AEAC = ascorbic acid equivalent antioxidant capacity (mg AA/100 g), FRP = ferric reducing power (mg GAE/100 g), and CEC_{50} = median chelating efficiency concentration (mg/mL), GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent and AA = ascorbic acid. Data on phenolic contents and antioxidant activities in dry weight are means ± standard deviations. Lower CEC_{50} values indicate stronger ferrous chelating ability. Within each column, values with different superscript letters (a−e) are significant at $p < 0.05$ using the Tukey HSD test.

Table 3. TPC and AEAC of superheated steam-dried herbs at 150°C and 200°C based on % gain or % loss compared to fresh herbs

Herb	Fresh TPC/FRS	SS-D at 150°C ($SS\text{-}D_{150}$)			SS-D at 200°C ($SS\text{-}D_{200}$)		
		5 min	10 min	20 min	5 min	10 min	20 min
Rosemary	1280±164 1890±333	+52 +37	+38 UC	UC UC	+38 +23	UC UC	−26 −44
Marjoram	735±48 1620±166	−56 −64	−29 −53	−20 −38	UC UC	UC −20	UC −44
Oregano	524±49 1100±19	UC UC	UC UC	UC −28	UC −21	−30 −28	−42 −48
Thyme	875±109 871±119	−36 −41	−49 −53	−55 −60	−41 −42	−26 −27	−32 −38
Sage	732±108 886±120	+27 UC	+35 +24	−25 −50	UC UC	UC +32	−31 UC
Spearmint	294±14 457±18	UC −41	+59 UC	−21 −58	+40 UC	+60 UC	UC −53
Peppermint	367±55 345±47	−61 −73	−46 −51	−63 −68	−41 −42	−30 −31	−30 −28

Abbreviations: TPC = total phenolic content, FRS = free radical scavenging expressed as ascorbic acid equivalent antioxidant capacity (AEAC), SS-D = superheated steam-dried and UC = unchanged. Data on TPC (mg GAE/100 g) and AEAC (mg AA/100 g) of SS-D herbs are based on fresh weight equivalent. They show significant percentage gain (+) or significant percentage loss (−) or unchanged (UC) compared to those of fresh herbs at $p < 0.05$ using the Tukey HSD test.

Table 4. Tyrosinase inhibition (%) of superheated steam-dried herbs at 150°C and 200°C compared to fresh herbs

Herb	Fresh	SS-D at 150°C ($SS\text{-}D_{150}$)			SS-D at 200°C ($SS\text{-}D_{200}$)		
		5 min	10 min	20 min	5 min	10 min	20 min
Sage	52±6	48±2	61±4↑	73±6↑	71±4↑	53±7	49±4
Rosemary	51±3	68±2↑	88±4↑	93±5↑	90±5↑	84±6↑	69±2↑
Peppermint	47±6	47±4	44±6	54±4	50±3	42±5	51±5
Thyme	31±2	59±2↑	40±2↑	39±1↑	45±2↑	51±2↑	60±4↑
Spearmint	17±6	33±5↑	39±5↑	47±6↑	42±4↑	25±5	47±5↑
Oregano	17±6	29±7	39±4↑	48±4↑	41±5↑	46±4↑	25±3
Marjoram	ND	34±6↑	16±2↑	35±5↑	21±6↑	11±6↑	22±5↑

Abbreviations: SS-D = superheated steam-dried and ND = not detected. Tyrosinase inhibition (%) of SS-D herbs with significant gain (↑) at $p < 0.05$ or remaining unchanged compared to fresh herbs.

3.6. Anti-tyrosinase properties of CD herbs

Of the five commercial dried (CD) herbs, tyrosinase inhibition was the strongest in thyme (61%), followed by rosemary (51%), peppermint (38%), sage (31%) and oregano (27%). Out of six dried spices, Chan et al. (2015) reported the strongest anti-tyrosinase activity in cinnamon (45%) and cumin (42%). This would mean that CD herbs have stronger anti-tyrosinase properties.

3.7. Anti-tyrosinase properties of SS-D herbs

Three of the SS-D herbs, namely, rosemary, thyme and marjoram, showed significantly enhanced anti-tyrosinase properties compared with their fresh counterparts for all the drying regimes (Table 4). Of these, rosemary had the largest gains of 17−42% for SSD at 150°C and 18−39% for SSD at 200°C. The anti-tyrosinase properties of SS-D marjoram represented the most exciting discovery as tyrosinase inhibition was not detected in fresh samples. The anti-tyrosinase properties of sage, spearmint and oregano showed significant gains or remained unchanged while those of peppermint remained unchanged for SSD at 150°C and 200°C. This is the first report on the enhancement effects of SSD on the tyrosinase inhibition of Labiatae herbs. Previous studies on the anti-tyrosinase properties have been based on fresh plant samples. Phenolic acids such as rosmarinic acid and caffeic acid, and flavonoids such as luteolin, quercetin and kaempferol found in Labiatae herbs have been reported to possess anti-tyrosinase activity (Ulubelen et al., 2005; Kim and Uyama, 2005; Fujimoto et al., 2011). Increase in bioactivity of plant materials after heat treatment has been attributed to bioactive compounds released due to the rupture of the cell matrix or produced by thermal chemical reaction, and to thermal inactivation of oxidative enzymes (Jimenez-Monreal et al., 2009; Chan et al., 2013). These reasons can also be applied for the enhanced anti-tyrosinase activity of the SSD herbs. In the literature, enhanced anti-

tyrosinase activity of plant extracts after thermal treatments has been reported. The anti-tyrosinase activity of microwave-treated cashew leaves of 49% was significantly higher than fresh leaves of 40% (Tan and Chan, 2014). Similarly, Chan et al. (2015) reported that microwave-treated cardamom (24%) also showed significant increase in tyrosinase inhibition compared to fresh cardamom (17%).

4. Conclusion

This study on the antioxidant and anti-tyrosinase properties of selected Labiatae herbs has provided some useful insights on the effects of SSD, which are reported for the first time. SS-D herbs included those that showed declines in antioxidant properties for all the drying regimes; those that showed declines or remained unchanged; and those that showed all three traits of increments, declines or unchanged. Of the SS-D herbs, rosemary, thyme and marjoram showed enhanced anti-tyrosinase properties following all drying regimes with marjoram being the most exciting as tyrosinase inhibition was not detected in fresh samples. SSD may therefore be a promising drying technique of Labiatae herbs for the commercial production of tyrosinase inhibitors, which are increasingly used in medicines for treating pigmentation disorders, in cosmetics for their skin-whitening effects, and in food products for inhibiting browning. However, further research is needed in optimising the drying conditions to maximise their anti-tyrosinase potentials for use as pharmaceutical and cosmeceutical products, and as food preservatives.

5. References

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