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Development of Encapsulated Sage Extract Powder: Inter-comparison with commercially available powder for physical properties and metabolites composition

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Abstract

Encapsulated *Salvia officinalis* L. powder (SESP) was developed from extract obtained by supercritical fluid extraction (SFE), and it was compared with commercial sage powder of hydro-distillation extract (HESP). The optimised conditions of 30 MPa at 50 °C for laboratory scale SFE extraction were found to generate the highest yield at 40.96 ± 0.84 %. The conditions were later applied in the upscaled extraction, which yielded about 30.10 ± 1.33 % extract. Large particle size distribution at 118.46 ± 6.63 μm for SESP was notified as compared to 66.80 ± 1.50 μm for HESP. Outcomes of crystallographic analysis demonstrated that abundant metabolites from various groups existed in both powders. About 25 and 43 metabolites were identified by Gas Chromatography-Mass Spectrometry (GC–MS) in HESP and SESP, respectively. In HESP, organic acids were found to be the major group identified, while terpenoids were the largest group found in SESP.

Keywords: *Salvia officinalis* L., Encapsulated sage extract powder, Supercritical fluid extraction, Hydro-distillation, Encapsulation, Metabolite

1. Introduction

Sage, scientifically known as *Salvia officinalis* L., is a quintessential herbal ingredient in various cuisines around the globe. It is an evergreen shrub that belongs to the mint family (*Labiatae*), comprising other herbs such as thyme, oregano, basil, and rosemary [1]. Also, sage is endemic to the Mediterranean region, specifically in Greece, Albania, and former Yugoslavia, though it has been widely cultivated all over the world [1, 2]. In culinary, sage is distinctively known for its fragrant aroma and earthy taste, essential in imparting flavour to food [3].

Sage contains a wide array of bioactive compounds that comprise phenolic compounds, terpenoids, organic acids, and fatty acids [4-7]. In literature, various pharmacological studies

have reported the potential of sage to render antioxidant activities, antibacterial effects, anti-inflammatory properties, and anticancer effects [8-12]. In addition, principal metabolites in sage, namely rosmarinic acid, ursolic acid, carnosol, and rosmanol, are commonly associated with the aforementioned properties. For instance, anticancer effects attributed to rosmarinic acid and ursolic acid have been widely studied as they can inhibit the growth of various cancerous human cells [10, 13]. On the other hand, Carnosol was proven to be one of the potent antioxidants in sage, and its radical scavenging performance was deemed equal to that of α-tocopherol [14, 15].

The extraction of plant materials with conventional techniques such as maceration, Soxhlet, and hydro-distillation is cumbersome. The shortcomings such as long extraction time, low efficiency, and reliance on hazardous solvents have restrained them for potential use in commercial applications [16]. In recent years, supercritical fluid extraction (SFE) has garnered major interest among researchers and industry players as it can perform the extraction process efficiently at a shorter time [17]. Supercritical fluid extraction has been known as an efficient and environmentally friendly option to traditional extraction techniques [18]. Conceptually, SFE uses supercritical carbon dioxide as a solvent due to its gas-like transport properties and liquid-like solubility [19]. These properties are highly desirable as they could effectively diffuse into the plant matrices and dissolve all the valuable phytochemicals [20]. The utilisation of this technique is highly anticipated. Additionally, the employment of carbon dioxide in SFE was claimed to be a safe substance (GRAS) that is generally recognised by Food and Drug Administration (FDA) [21]. Hence, it affirmed that the SFE method is regarded as an environmental-friendly and promising technology compared to other extraction techniques.

In industrial practice, synthetic antioxidants have been widely used compared to natural ones due to their higher stability, low costs, and wide availability [22, 23]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate

(PG) and tert-butyl hydroquinone (TBHQ) are commonly used in the food industry [22, 24]. However, the prevalent use of synthetic antioxidants in food has caused growing concern for its safeness as a food additive. In literature, various published studies have been underlining the adverse health effects of prolonged consumption of synthetic antioxidants such as skin allergies, gastrointestinal tract problems, and potentially elevated cancer risk [24-27]. The advent of microencapsulation technology has aided in preserving natural antioxidants and serving as an alternative to synthetic antioxidants. This technique can maintain the encapsulated compounds' physical structure, prevent degradation of the compounds against environmental factors such as sunlight, oxygen, moisture, and heat, and avoid exposure of the volatile compounds to the ambient [28]. Microencapsulation technology achieved the aforementioned benefits by entrapping bioactive compounds in a bead-like structure consisting of the natural or synthetic biodegradable polymer [29]. Spray drying is one of the commonly used encapsulation techniques in the pharmaceutical and food industries as it can effortlessly encapsulate bioactive compounds while maintaining its activity [30]. Additionally, spray drying is also much better than lyophilisation in the retentions of bioactive compounds and preserves its bioactivity once exposed to harsh processing conditions [31].

Current comparative studies on sage extract mainly focus on the bioactive compounds' retention in output derived from different extraction techniques and quality changes between encapsulated and non-encapsulated extracts respective to its storage period [32, 33]. To the best of the authors' knowledge, the development of encapsulated *Salvia officinalis* L. powder derived from SFE extract (SESP) is scarce in the literature. Furthermore, it is interesting to compare the SESP's physical characteristics and metabolites composition with the encapsulated powder of hydro-distillation extract (HESP). In this sense, HESP was bought from the commercial market, and it was tested alongside SESP for comparisons. Meanwhile, the sage extract obtained from the SFE process was encapsulated using a spray drying

technique. Both encapsulated sage extract powders were studied for their structure and crystallography. Subsequently, the metabolites composition assessment in both samples was carried out using a Gas Chromatography-Mass Spectrometry (GC–MS).

2. Materials and method

2.1. Sage samples and chemicals

Dried common sage (*Salvia officinalis* L.) leaves were procured from BioComposites Centre, Bangor University, United Kingdom, mainly for supercritical fluid extraction. Additionally, encapsulated powder of the same plant species derived from the hydro-distillation extract was purchased from Xi'an Rongsheng Biotechnology Co., Ltd. (Xi'an, China). Its image (labelled as HESP) is shown in Fig. 1a. Refined grapeseed oil (Olitalia S.r.l., Forlì-Cesena, Italy) was bought from a local hypermarket in Seri Kembangan, Malaysia. All organic solvents and chemicals used in the experiments were of analytical grade. Pyridine (99%) and methoxyamine hydrochloride (98%) were procured from Sigma-Aldrich (St. Louis, MO, USA). Meanwhile, the N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) at 98% purity was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Preparation

2.2.1. Pre-processing of sage

The dried sage leaves were initially ground into powder using an RT-CR30S 3HP cutting mill equipped with a cyclone powder collector (Rong Tsong Precision Technology Co., Dawei Rd., Taichung, Taiwan). The powder was sieved using a 200 x 50 mm sieve with an aperture size of 0.220 mm to remove any large debris. In order to ensure the moisture level is below 10%, the moisture content of the powder was determined by an electronic moisture analyser (Mettler Toledo, Ohio, United States).

2.2.2. Laboratory scale supercritical fluid extraction of sage

The supercritical fluid extraction (SFE) process was optimised using a laboratory-scale high-pressure extraction plant (Deven Supercritical Pvt. Ltd., Phatak Baug, Navi Peth, Pune, India). The method used by Glisic et al. [34] was followed for this extraction process, and modifications were made to its extraction conditions for optimum efficiency. The optimisation, which involved the evaluation of various extraction pressures and extraction times were performed. In brief, about 100 g of dried sage leaves powder was precisely weighed and mixed with 20 g of GSO (20% w/w), which served as a co-extractant. The SFE extractions were performed at varied pressures of 10 MPa, 20 MPa, and 30 MPa, while the extraction temperature was fixed at 50 °C. Liquid carbon dioxide (99.0% purity, Alpha Gas Solution, Selangor, Malaysia) from a cylinder tank was pumped at a 1.6 kg/h flow rate into the extraction vessel. The extraction was performed for 6 h. The calculated solvent mass to feed mass ratio (S/F) was 80 ± 0.01. The weight of the collected extract was recorded at every consecutive 1 h, and it was stored in an amber bottle at −18 °C. The experiments were conducted in triplicate. Meanwhile, the percentage of SFE yield was calculated by applying the following formula:

$$\text{SFE yield (\%)} = \text{Mass of extract} / (\text{Mass of sample} + \text{GSO}) \times 100\ \% \quad (1)$$

2.2.3. Upscale supercritical fluid extraction of sage

The upscaling of SFE process was conducted in a pilot-scale high-pressure extraction plant equipped with four 50.0 L extraction vessels and two 27.0 L separators (Feyecon Development & Implementation B.V., Weesp, North Holland, Netherlands). Only one extraction vessel was used to load the sample in this study. The optimum laboratory-scale SFE extraction conditions were followed, except for the solvent flow rate, which was appropriately adjusted so that S/F ~ 80 was maintained. Approximately 8.0 kg of dried sage leaves powder was weighed and

mixed with 1.6 kg of GSO (20% w/w) in a rotary drum mixer until well-mixed. Subsequently, the well-mixed sample was placed into the high-pressure extraction vessel of the SFE unit for extraction by using supercritical carbon dioxide (SCO_2). The extraction vessel was 75 % filled with the sample to allow a sufficient headspace for an optimum flow of SCO_2. Liquid CO_2 from a cylinder tank was pumped into the tubing at a flow rate of 128 kg/h using a high-pressure pump and passed through a heater before entering the extraction vessel. The automated control valve regulated the heater to achieve the desired pressure (30 MPa) and temperature (50 °C) of the extraction vessel for phase conversion of CO_2 from liquid to supercritical state. The SCO_2 contacted the sample in the extraction vessel by which it deeply penetrated the microporous matrix of the sample to induce the extraction of the desired compounds.

Fractionation of the dissolved extract was conducted by passing through the SCO_2-containing extract into a series of separation vessels. In principle, compounds with a different molecular weight will sink into the bottom of the vessel and precipitate due to the differences of pressure-temperature combinations set in both separation vessels. Hence, it allows the fractionation process to occur in each vessel. The SCO_2-containing extract was allowed to flow into the first separation vessel and then into the second vessel before returning into its gaseous state. Pressure in the first separation vessel was set at 6 MPa while the temperature was regulated at 50 °C. Meanwhile, the second separation vessel's temperature and pressure were ramped down to 4 MPa at 30 °C, respectively. High pressure in the first separation vessel was desired to induce precipitation of the extract in the bottom of the vessel. Subsequently, the pressure and temperature drop in the second separation vessel will cause the conversion of supercritical CO_2 into gas. During this phase conversion, solubility of extract in the lower density CO_2 was reduced, and the extract was precipitated for collection. The CO_2 left the second separation vessel, which was later cooled, re-compressed, and recycled. The whole extraction process was allowed to run for 6 h, and the extract was collected at the end of extraction by dispensing it

through a manual valve. Due to the constraint of sample amount, the experiment was conducted in duplicate. The percentage of SFE yield was calculated by applying equation (1) as stated in subsection 2.2.2.

2.2.4. Spray drying encapsulation of sage extract

The encapsulation of sage extract by spray drying was performed according to the method used by Navarro-Flores et al. [35]. Before encapsulation, sage extract obtained from the SFE process was mixed with water, maltodextrin, and emulsifiers to form an emulsion. The composition of the formed emulsion at 300 g was consist of 10% sage extract, 80% water, 7% maltodextrin, 1.5% E471 emulsifier (glyceryl monostearate), and 1.5% E481 emulsifier (sodium stearoyl lactylate). The mixture was then mixed and homogenised using a Silverson L5M-A high shear industrial laboratory mixer (Silverson Machines Ltd., Chesham, Buckinghamshire, United Kingdom) at a speed of 6000 rpm for 15 min. Following that, the mixture was further homogenised using a Niro Soavi Pandaplus 2000 high-pressure laboratory homogeniser (Gea Niro Soavi SpA, Parma, Italy) at a speed of 5800 rpm for 30 min. Due to the heat generated by the high-speed rotation of the rotor blades, the process was stopped every consecutive 5-min intervals for the rotor blades to cool down. The emulsion was dried in a Büchi B-290 mini spray dryer (Büchi Labortechnik AG, Flawil, Switzerland) equipped with an atomiser nozzle of 0.5 mm diameter at 15 MPa. The operational conditions of the spray dryer were set as follows: inlet air temperature was at 130 °C; the outlet air temperature was maintained at 50 ± 1 °C, feed suspension rate at 180 mL/h and spray air flow rate was at 150 L/h. After drying, the powder was placed in an amber bottle, and the cap was tightly screwed. The bottle was kept in a refrigerator for storage at ± 4 °C. In order to generate a high yield of encapsulated sage extract powder, the experiment was performed ten times. The image of encapsulated sage extract powder obtained from the SFE process (SESP) is shown in Fig. 1b.

[Fig. 1 about here]

2.3. Characterisation

2.3.1. Particle size analysis

Particle size distributions of the encapsulated sage extract powders were analysed using a Mastersizer 2000 laser diffraction instrument equipped with a Scirocco 2000 automated dry powder dispersion unit (Malvern Instruments Ltd., Malvern, UK). Before the analysis, the particles' refractive indexes were measured using a PAL-RI refractometer (ATAGO Co., Ltd., Tokyo, Japan). The refractive indexes of both HESP and SESP were found to be at 1.37 and 1.48, respectively. About 2.0 g of samples were placed into the vibratory hopper of the dry dispersion unit, and the pressure was set at 4 bar throughout the analysis. All samples were measured in triplicate.

2.3.2. Crystallographic analysis

The crystallography of the encapsulated sage extract powders was examined using a Panalytical Empyrean X-ray diffractometer (XRD), operating at 45 kV and 40 mA. The powders were placed on nickel coated steel holder before analysis with 2 °C/min scanning rate under Cu Kα radiation.

2.3.3. GC-MS analysis

The derivatisation of the samples for the GC-MS analysis was conducted as described by Robinson et al. [36]. Approximately 25.0 mg of the encapsulated sage extract powder was added to a 50.0 μL of pyridine in a 2.0 mL centrifuge tube, and the mixture was sonicated for 10 min at 30 °C. After that, about 100 μL of methoxyamine HCl (20 mg/mL in pyridine) was added to the solution and vortexed for 1 min. The solution was first incubated for 2 h at 60 °C

and then another incubation for 30 min at 60 °C after adding 300 µL of MSTFA (N-Methyl-N-(trimethylsilyl)trifluoroacetamide). Lastly, the solution was filtered by passing through a 0.22 µm nylon syringe filter, and the filtrate was placed in an amber vial to stand overnight at room temperature.

Metabolites' identification was performed according to the GC-MS method used by Sadowska et al. [37] with some modifications. The TSQ Quantum XLS GC-MS system (Thermo Scientific, USA) was used to analyse the derivatised fraction of each encapsulated sage extract powder. Spitless aliquot injections of 1 µL were made onto a DB-5MS (J&W GC column) with the dimension of 30 m length, 0.25 mm inner diameter and 0.25 µm film thickness (Agilent Technologies, Santa Clara, California, USA). Helium was used as the carrier gas at a steady flow rate of 1.0 mL/min. The GC-MS oven temperature was initially held at 80 °C for 5 min and subsequently raised at 8 °C/min to 200 °C. Following that, the oven temperature was finally raised to 280 °C at the rate of 4 °C/min and held for 15 min. The ion source and interface temperature were set at 280 °C and 250 °C, respectively. Full scan was performed to obtain the desired mass spectra with a mass scan range of 30 to 600 m/z after a solvent delay of 5 min. The resulting spectra of each chromatographic peak were compared with mass spectral and retention time index libraries for GC-MS established by the National Institute of Standards and Technology (NIST), data version NIST17 [38].

2.4. Statistical analysis

Statistical analysis of the experimental data was performed by using the IBM SPSS Statistics 23 software (SPSS Inc. Chicago, Illinois, USA). The statistically significant difference in mean percentage of SFE yields at three different extraction pressure were assessed by a One-way Analysis of Variance (ANOVA). Following that, the possible pairs of means were compared based on Tukey HSD (honestly significant difference) test. Meanwhile, the independent t-test

was performed to compare the mean values of particle sizes for both encapsulated sage extract powders [39].

3. Results and Discussion

3.1. Supercritical fluid extraction and encapsulated powder yields

A laboratory-scale SFE unit was used in the small-scale study to perform the sage extraction at three different extraction pressure. Based on the results shown in Table 1, the percentage of SFE yield increased significantly with the rising in pressure at constant extraction temperature. The SFE operating conditions of 30 MPa at 50 °C yielded 40.96 ± 0.84 % extract, which was the highest and significantly different from the yields obtained by using other conditions. Glisic et al. [34] also demonstrated a similar trend when the same operating conditions were applied (30 MPa at 50 °C) with the highest yield at 4.82%. It is presumed that the high extraction pressure at 30 MPa might increase the density of SCO_2 in the extraction vessel [40]. Machado et al. [41] mentioned that high-density SCO_2 possess greater solvation power that induces higher solubility of the bio-active compounds, thus generating more yield. Meanwhile, as illustrated in Fig. 2, the extraction yield showed an increasing trend with the increased extraction time, and it is speculated that the yields after 6 h might reach a plateau.

Table 1. SFE yields (%) of sage leaves extract at different conditions using a laboratory-scale unit.

Extraction time (hr)	Extraction pressure (MPa)		
	10	20	30
1	0.41 ± 0.01	0.17 ± 0.01	1.26 ± 0.09
2	0.44 ± 0.03	1.07 ± 0.28	2.79 ± 0.28
3	0.60 ± 0.04	2.57 ± 0.22	3.42 ± 0.35
4	0.70 ± 0.04	5.65 ± 0.79	8.30 ± 0.28
5	0.90 ± 0.02	8.60 ± 0.68	12.14 ± 0.09

6	1.93 ± 0.21	10.83 ± 0.25	13.05 ± 0.25
Total SFE yield (%)	4.98 ± 0.10[c]	28.89 ± 1.71[b]	40.96 ± 0.84[a]

[a-b] Values within same row with different letters are significantly different ($p \leq 0.05$).
Values are means of triplicate determination ± SD.

[Fig. 2 about here]

GSO was used as the co-extractant as it enabled an efficient metabolites extraction from the sage leaves. In literature, the effects of co-extractant on the quality of the SFE extract were widely reported, and most researchers reported a positive outcome [42-45]. Co-extractant is believed to increase the dissolution of polar metabolites by supercritical carbon dioxide; hence it enhances the extract quality. Vegetable oil is often deemed a safe co-extractant, an appropriate choice for the extraction of natural products and food materials [44, 45]. This study used about 20% (w/w) of GSO as a co-extractant for both laboratory and pilot-scale SFE extraction. The aforementioned percentage was chosen based on a study conducted by Lee et al. [44] as the authors reported the use of 20% (w/w) vegetable oil produced the highest extraction yield, while the incorporation of vegetable oil above 20% might induce powder agglomeration. Although Lee et al. [44] used virgin coconut oil (VCO) in their study, GSO was chosen as co-extractant due to its odourless trait compared to VCO, which possesses a hint of coconut scents. Furthermore, GSO also contains abundant long-chain fatty acids compared to VCO, which contains primarily medium-chain fatty acids [46]. Medium-chain fatty acids such as lauric acid can easily oxidise compared to long-chain fatty acids [47]. This condition potentially induced the development of rancid odours, off-flavours, nutritional losses and might reduce the shelf-life of the SESP [48].

The optimum pressure-temperature combination (30 MPa at 50 °C) was used for the upscaling of the extraction process by using a pilot-scale SFE setup. The mean value of the obtained SFE yield from the upscaled extraction process was 30.10 ± 1.33 %. The spray drying encapsulation

of sage extract was conducted ten times, and the accumulated yields were at 450 g. Briefly, every 300 g emulsion used in one spray drying batch produced an average of 45 ± 1.29 g of SESP. Despite the high yield of extract obtained from the upscaled experiment, the production of SESP was limited to the small size of the spray drying unit used. Hence, the limitation constrained the yield of SESP produced and operating the small spray drying unit repeatedly might be inefficient.

3.2. Particle size distribution

Table 2 shows the particle size distributions of both encapsulated sage extract powders. The particle size of SESP was larger than that of HESP, as indicated by parameters D_{10}, D_{50}, and D_{90}. The values of all parameters for SESP, with the exception to D_{10} were found to be significantly higher than that of HESP. Based on its median diameter, D_{50}, HESP had a significantly narrower particle size distribution at around 66.80 ± 1.50 μm as compared to 118.46 ± 6.63 μm for SESP. Furthermore, as illustrated in Fig. 3, a noticeable difference in the overall shape and range of the particle size results was observed when both samples' profiles were compared. SESP demonstrated a somewhat broader particle size distribution than HESP. Given the broad particle size range of SESP, it is presumed that the presence of waxes in this sample possibly interfered with the solubility of the extract prior to the spray drying process. Hence, perfect homogeneity was unable to be attained in SESP. Additionally, the increase in agglomeration and the existence of large agglomerates were believed to cause a broad particle size range in SESP. This phenomenon was investigated by Both et al. [49] as they reported that the drying of sample at an inlet air temperature below 140 °C would increase the agglomeration tendency of the spray-dried powder. In this study, the inlet air temperature of the spray drying process was set at 130 °C. Hence, more large agglomerates could be expected in SESP. Agglomeration of SESP also could be attributed to the use of maltodextrin, as it is a

hygroscopic material. Maltodextrin is made up of relatively short-chain polysaccharides, and it is highly hygroscopic due to the presence of numerous hydrophilic groups [50]. Thus, the storage condition should be at low humidity to ensure a low water activity of SESP.

Table 2. Particle size distribution of encapsulated sage extract powders

Sample	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)	Specific Surface Area (m^2/g)	Span
HESP	18.71 ± 0.34[a]	66.80 ± 1.50[b]	153.92 ± 4.19[b]	0.15 ± 0.004[a]	2.02 ± 0.02[b]
SESP	19.19 ± 0.16[a]	118.46 ± 6.63[a]	336.82 ± 28.81[a]	0.11 ± 0.002[b]	2.68 ± 0.09[a]

[a-b] Values within same column with different letters are significantly different ($p \leq 0.05$).
Values are means of triplicate determination ± SD.
Encapsulated powder of hydro-distillation sage extract (HESP)
Encapsulated powder of supercritical fluid extraction sage extract (SESP)

[Fig. 3 about here]

3.3. Crystallographic analysis

Fig. 4 illustrates XRD diffractogram of the encapsulated sage extract powders. Both powders are amorphous material since sharp diffraction peaks did not appear on the scattered background pattern. It was likely due to inhomogeneous compounds like proteins, macromolecules and waxes, which promoted the amorphous characteristic of sage powders [51, 52]. With reduced background, sharp peaks were observed for both samples, revealing the presence of crystalline materials such as alkaloids, polysaccharides, and flavonoid components in the encapsulated sage extract powders. The distinction of these peaks for the two samples were more clearly observed on the corrected background patterns (crystalline phases) after the removal of bulky background. Interestingly, SESP showed multiple sharp peaks at 2 theta of 19.5 °C, 20.4 °C, 21.5 °C, 22.9 °C, and 23.8 °C, whereas HESP only showed singular peak at 2 theta of 19.7 °C. Meanwhile, the singular peak falling at 19.7 °C for HESP was also noted in

the crystalline phase of SESP near to 19.5 °C, signalling the content of HESP could be found in SESP sample. Additionally, the multiple peaks also implied that the variety of compounds were more available in SESP [1, 53]. Hence, it could be speculated that a higher presence of bio-active compounds in SESP might be expected compared to HESP.

[Fig. 4 about here]

3.4. Profiling of metabolites in sage extract powders by GC-MS

GC-MS chromatograms of the encapsulated sage extract powders; HESP and SESP were illustrated in Fig. 5a and Fig. 5b, respectively. Metabolites present in both encapsulated powders were identified and confirmed by scrutinising the spectral pattern. Presence of metabolites in both encapsulated powders was observed at different retention times (RTs) within a wide interval ranging from 6.0 – 55.0 min. The identification of metabolites was performed based on mass spectral and retention time index libraries for GC-MS established by the National Institute of Standards and Technology (NIST), data version NIST17 [38]. The detected metabolites were also compared with literature data to identify its potential as a bioactive compound. Altogether, about 25 and 43 metabolites were identified as potential bioactive compounds in HESP and SESP, respectively. These compounds primarily comprised organic acid, fatty acid, flavonoid, phenolic, and terpenoids, as shown in Table 3 (metabolites in HESP) and Table 4 (metabolites in SESP). The discrepancy between metabolites composition detected in HESP and SESP were quite distinctive despite the similar plant species used. HESP was found to contain more organic acids as compared to SESP. Conversely, terpenoids dominated the list of metabolites found in SESP, with 14 various kinds of terpenoids identified.

Organic acid is an important group of secondary plant metabolites, and it plays a vital role in controlling the *plant in vivo* biochemical and physiological processes [54]. In literature, organic

acid has been reported to contribute to antioxidant activities in plant materials [55-57]. The assumption was made based on output reported by Berg et al. [58]. He had proved that organic acid can scavenge reactive oxygen species such as superoxide anions. Additionally, organic acid is also widely known as a natural chelating agent, by which, in the recent decade, scientists heavily investigated its antioxidant capability [59, 60]. In brief, chelator serves as a preventive antioxidant by hindering the catalytic metals' activity in the system, thus removing the initial oxidation step [61]. Terpenoids were important metabolites in plants made up of isoprene and the isoprene polymers called terpenes [62].

Terpenoids were ubiquitous in sage extract and often found to be at a considerably higher portion than other metabolites [1, 63-65]. In literature, the potential of terpenoids as a naturally occurring antioxidant was highly coveted [66-68]. Many researchers have reported its potential to be used as an additives in nutraceutical products [69, 70]. The identified terpenoids in SESP consisted of diterpenoid, six compounds; and triterpenoid, eight compounds. On the contrary, terpenoid was not detected and identified in HESP.

[Fig. 5 about here]

Table 3. Metabolites composition in encapsulated powder of hydro-distillation sage extract (HESP) as identified by GC-MS.

Nr.	Metabolite	Retention time (min)	Molecular formula	Molecular weight (g/mol)	Probability (%)
	Waxes				
	Aromatic hydrocarbon				
1.	1-(3-Methylbutyl)-2,3,4,6-tetramethylbenzene	8.48	$C_{15}H_{24}$	204	8.48
	Fatty acid				
2.	Palmitic Acid, TMS derivative	23.65	$C_{19}H_{40}O_2Si$	328	95.59
3.	Stearic acid, TMS derivative	26.91	$C_{21}H_{44}O_2Si$	356	92.92
4.	1-Monopalmitin, 2TMS	33.20	$C_{25}H_{54}O_4Si_2$	474	92.91
5.	9(E),11(E)-Conjugated	26.39	$C_{21}H_{40}O_2Si$	352	64.69

	linoleic acid, trimethylsilyl ester				
	Glyceride				
6.	Glycerol monostearate, 2TMS derivative	36.52	$C_{27}H_{58}O_4Si_2$	502	74.23
7.	1,3-Dipalmitin, TMS derivative	40.77	$C_{38}H_{76}O_5Si$	640	57.05
	Non-wax metabolites				
	Carbocyclic sugar				
8.	Myo-Inositol, 6TMS derivative	24.91	$C_{24}H_{60}O_6Si_6$	613	60.77
	Flavonoid				
9.	Dihydroquercetin, 5O-TMS	39.99	$C_{30}H_{52}O_7Si_5$	664	32.43
10.	Catechin (2R-E)-, 5TMS derivative	39.99	$C_{30}H_{54}O_6Si_5$	650	18.87
11.	cis-Dihydroquercetin,	39.99	$C_{30}H_{52}O_7Si_5$	664	15.5
12.	Cardamonin, bis(tert-butyldimethylsilyl) ether	50.00	$C_{28}H_{42}O_4Si_2$	498	9.57
	Organic acid				
13.	Citric acid, 4TMS derivative	20.60	$C_{18}H_{40}O_7Si_4$	480	98.35
14.	Malic acid, 3TMS derivative	15.77	$C_{13}H_{30}O_5Si_3$	350	95.95
15.	Quininic acid (5TMS)	21.29	$C_{22}H_{52}O_6Si_5$	552	86.23
16.	Glycolic acid, 2TMS derivative	7.81	$C_8H_{20}O_3Si_2$	220	81.75
17.	Butanedioic acid, 2TMS derivative	12.70	$C_{10}H_{22}O_4Si_2$	262	75.74
18.	Lactic Acid, 2TMS derivative	7.55	$C_9H_{22}O_3Si_2$	234	67.11
19.	Butanoic acid, 2-(methoxyimino)-3-methyl-, trimethylsilyl ester	11.42	$C_9H_{19}NO_3Si$	217	47.40
20.	2-Furoic acid, TMS derivative	9.1	$C_8H_{12}O_3Si$	184	36.24
21.	3-Furoic acid, TMS derivative	9.1	$C_8H_{12}O_3Si$	184	25.58
	Oxoacid				
22.	Phosphoric acid, bis(trimethylsilyl) 2,3-bis[(trimethylsilyl)oxy]propyl ester	19.84	$C_{15}H_{41}O_6PSi_4$	460	74.83
	Phenolic acid				
23.	Protocatechuic acid,	20.47	$C_{16}H_{30}O_4Si_3$	370	85.67

	3TMS derivative				
	Sugar acid				
24.	Galactaric acid, (R,S,R,S)-, 6TMS derivative	24.21	$C_{24}H_{58}O_8Si_6$	642	88.22
25.	D-Gluconic acid, 6TMS derivative	23.51	$C_{24}H_{60}O_7Si_6$	628	85.58

Table 4. Metabolites composition in encapsulated powder of supercritical fluid extraction sage extract (SESP) as identified by GC-MS.

Nr.	Metabolite	Retention time (min)	Molecular formula	Molecular weight (g/mol)	Probability (%)
	Waxes				
	Aldehyde				
1.	2,4-Decadienal, (E,Z)-	12.68	$C_{10}H_{16}O$	152	13.52
	Fatty acid				
2.	Palmitic Acid, TMS derivative	23.67	$C_{19}H_{40}O_2Si$	328	90.81
3.	1-Monopalmitin, 2TMS derivative	33.43	$C_{25}H_{54}O_4Si_2$	474	90.28
4.	Stearic acid, TMS derivative	26.96	$C_{21}H_{44}O_2Si$	356	89.32
5.	1-Monomyristin, 2TMS derivative	29.78	$C_{23}H_{50}O_4Si_2$	446	80.52
6.	9(E),11(E)-Conjugated linoleic acid, trimethylsilyl ester	26.37	$C_{21}H_{40}O_2Si$	352	63.43
7.	Heptadecanoic acid, TMS derivative	25.19	$C_{20}H_{42}O_2Si$	342	41.46
8.	Heptadecanoic acid, glycerine-(1)-monoester, bis-O-trimethylsilyl-	34.83	$C_{26}H_{56}O_4Si_2$	488	20.47
9.	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-eth anediyl ester	29.44	$C_{35}H_{68}O_5$	568	19.23
	Glyceride				
10.	Glycerol monostearate, 2TMS derivative	36.74	$C_{27}H_{58}O_4Si_2$	502	84.61
11.	Glycerol, 3TMS derivative	12.11	$C_{12}H_{32}O_3Si_3$	308	69.01
12.	Diglycerol, 4TMS derivative	19.83	$C_{18}H_{46}O_5Si_4$	454	66.34
13.	Monocaproin, di-(trimethylsilyl)-	17.90	$C_{15}H_{34}O_4Si_2$	334	1.38
	Triterpenes				

14.	Lupeol trimethylsilyl ether	49.06	$C_{33}H_{58}OSi$	498	89.06
15.	α-Amyrin, TMS derivative	48.65	$C_{33}H_{58}OSi$	498	67.43
16.	α-Amyrin, TMS derivative	47.50	$C_{33}H_{58}OSi$	498	66.73
17.	Stigmast-5-ene, 3β-(trimethylsiloxy)-, (24S)-	47.38	$C_{32}H_{58}OSi$	486	40.72
18.	β-Sitosterol, TMS derivative	47.38	$C_{32}H_{58}OSi$	486	40.72
19.	Olean-18-en-3-ol, O-TMS, (3α)	47.75	$C_{33}H_{58}OSi$	498	19.61
20.	12-Oleanen-3-yl acetate, (3α)-	48.60	$C_{32}H_{52}O_2$	468	2.17
21.	Lup-20(29)-en-28-al, 3-(trimethylsilyl)oxy, (3α)-	49.06	$C_{33}H_{56}O_2Si$	512	2.06
	Vitamin E				
22.	α-Tocopherol, TMS derivative	42.48	$C_{32}H_{58}O_2Si$	502	74.54
	Non-wax metabolites				
	Anhydro sugar				
23.	Levoglucosan, 3TMS derivative	19.02	$C_{15}H_{34}O_5Si_3$	378	57.34
	Coumarin				
24.	Scopoletin, TBDMS derivative	7.32	$C_{16}H_{22}O_4Si$	306	16.91
25.	3-(3',4'-Dimethoxyphenyl)-7-hydroxycoumarin, trimethylsilyl ether	31.72	$C_{20}H_{22}O_5Si$	370	4.81
	Diterpenes				
26.	Dehydroabietic acid, TMS derivative	29.61	$C_{23}H_{36}O_2Si$	372	82.76
27.	3-Hydroxyferruginol, di(trimethylsilyl) ether	30.31	$C_{26}H_{46}O_2Si_2$	446	72.87
28.	2-Hydroxyferruginol, di(trimethylsilyl) ether	30.31	$C_{26}H_{46}O_2Si_2$	446	17.18
29.	Carnosol, O,O-bis-TMS	31.05	$C_{26}H_{42}O_4Si_2$	474	16.53
30.	Rosmanol, O,O-bis-TMS	31.57	$C_{26}H_{42}O_5Si_2$	490	15.16
31.	3-Hydroxytotarol, di(trimethylsilyl) ether	30.31	$C_{26}H_{46}O_2Si_2$	446	6.27
	Flavonoid				
32.	Cardamonin, bis(tert-butyldimethylsilyl) ether	49.96	$C_{28}H_{42}O_4Si_2$	512	8.76
33.	Cannabinolic acid, TMS	49.96	$C_{28}H_{42}O_4Si$	498	4.85
	Organic acid				
34.	Citric acid, 4TMS derivative	20.59	$C_{18}H_{40}O_7Si_4$	480	97.23
35.	Lactic Acid, 2TMS	7.59	$C_9H_{22}O_3Si_2$	234	74.01

	derivative				
36.	Butanoic acid, 2-(methoxyimino)-3-methyl-, trimethylsilyl ester	11.41	$C_9H_{19}NO_3Si$	217	39.86
37.	Lactic acid dimer, bis(trimethylsilyl)-	14.10	$C_{12}H_{26}O_5Si_2$	306	18.83
38.	Succinic acid, di(2,2,3,3,4,4,5,5-octafluoropentyl) ester	26.58	$C_{14}H_{10}F_{16}O_4$	546	11.15
39.	Glycolic acid, 2TMS derivative	9.36	$C_8H_{20}O_3Si_2$	220	6.21
	Phenolic acid				
40.	3,5-Dihydroxybenzoic acid, 3TMS derivative	31.72	$C_{16}H_{30}O_4Si_3$	370	5.44
	Sugar acid				
41.	D-(+)-Gluconolactone, 4TMS derivative	21.5	$C_{18}H_{42}O_6Si_4$	466	75.92
42.	Ribonic acid, 2-desoxy-tetrakis-O-(trimethylsilyl)-	18.30	$C_{17}H_{42}O_5Si_4$	438	59.59
	Sugar alcohol				
43.	Pentitol, 3-desoxy-tetrakis-O-(trimethylsilyl)-	17.90	$C_{17}H_{44}O_4Si_4$	424	88.62

Both encapsulated sage extract powders were identified with the presence of phenolic compounds. Phenolic compounds are major secondary metabolites in plants and could be categorised into phenolic acids and polyphenols [71]. The identified phenolic compounds in HESP were primarily polyphenols, specifically flavonoids, namely dihydroquercetin, cis-dihydroquercetin, cardamonin, and catechine. Additionally, protocatechuic acid, a type of phenolic acid, was also identified in HESP. Meanwhile, two polyphenols in the coumarin class, scopoletin and 3(3',4'-Dimethoxyphenyl)-7-hydroxycoumarin, were identified in SESP. In addition, one flavonoid in the class of chalcones, which was cardamonin and phenolic acid, 3,5-Dihydroxybenzoic acid were also identified in SESP. Phenolic compounds identified in both encapsulated sage extract powders were found to be in agreement with those reported by Sarrou et al. [63]. Many studies in recent literature have reported that phenolic compounds

could render an antioxidant function due to their intrinsic properties [72-76]. Phenolic compounds were reported to scavenge free radicals, chelate metal cations, and donate hydrogen atoms or electrons, which contributed to its antioxidant activity [77].

Variations of terpenoids found in HESP and SESP were also potentially attributed to the extraction technique employed. Researchers have reported that SFE can extract more terpenoids compared to hydro-distillation extraction of plant materials [78, 79]. However, according to Pavlić et al. [78], the selectivity of terpenoids was heavily relied on the SFE conditions, specifically the pressure and temperature during the extraction process. Despite the efficiency of the SFE technique employed, SESP was produced via the spray drying encapsulation process. Hence, a direct comparison of the identified metabolites found in SESP with pure extract (essential oil) could not be possible. Metabolites could be susceptible to degradation due to intense heat throughout the process chain. According to Leyva-Jiménez et al. [80], the use of high air inlet temperatures during the spray drying process could trigger the degradation of metabolites in the extract. This assumption was supported by the reported percentage of the recovered total polar compounds in the encapsulated lemon verbena (*Lippia citriodora*) extract powder which was below 50%. Similarly, Tülek et al. [81] also reported that the amount of volatile compounds in the encapsulated lemon balm (*Melissa officinalis L.*) extract powder were significantly reduced after the encapsulation process. Despite that, maltodextrin has been reported to be an efficient encapsulating agent as it can provide better heat protection to flavonoids compared to other ingredients [80]. Therefore, the use of maltodextrin to prepare SESP was ideal for metabolites retainment throughout the encapsulation process.

The presence of waxes in both encapsulated sage extract powders was identified, and the compounds are shown in Table 3 and Table 4. Plant waxes are complex mixtures of very long-chain acyclic hydrocarbons (alcohols, aldehydes, alkanes, fatty acids, ketones, and esters) and

cyclic wax components (triterpenoids) which exist in an amorphous layer on the outer surface of the leaves [82-84]. Based on the results, seven waxes composed of aromatic hydrocarbon, fatty acids, and glycerides were identified in HESP. Meanwhile, 22 different waxes of primarily fatty acids and triterpenes were found in SESP. Commonly, the presence of waxes in essential oils is undesirable as it may cause sedimentation and cloudiness, attributed to the non-soluble components in the waxes [85]. Hence, a process called "dewaxing" often being performed to remove the insoluble portion of the waxes. This procedure usually consists of sequential steps that start with separating the oil by centrifugation, chilling the oil in the refrigerator, and filtration of the soluble oil portion [85]. In this study, the presence of waxes in encapsulated powders was not assumed to affect the quality of the products. However, it is presumed that the dewaxing of the extract before the spray drying process might improve the solubility of the encapsulated powder, which would be beneficial for application in foods.

4. Conclusions

Present study revealed the development of encapsulated *Salvia officinalis* L. powder (SESP) by using the extract from supercritical fluid extraction (SFE) and its comparison with the ones obtained from hydro-distillation extract (HESP). In the production of SESP, the conditions of 30 MPa at 50 °C were proven to be optimum operating conditions as it produced the highest yield (40.96 ± 0.84 %) in the laboratory scale SFE process. The optimised conditions were later used to upscale the extraction process and generated SFE yield at 30.10 ± 1.33 %. Based on particle size analysis, SESP had a larger particle size distribution at 118.46 ± 6.63 μm as compared to 66.80 ± 1.50 μm for HESP, which was potentially caused by the presence of waxes and the increase in powder's agglomeration. From the diffraction analysis, multiple crystalline phases had been tracked for both encapsulated sage extract powders, evidencing the mixture of different crystalline bio-substances like alkaloids, polysaccharides, and flavonoids in the

samples. Subsequently, around 25 and 43 metabolites of different groups were identified as potential bioactive compounds in HESP and SESP, respectively. Metabolites belonging to the organic acid group were identified the most in HESP than other groups, while SESP was found to contain a multitude of terpenoids. Therefore, it implies that the extract obtained by SFE and followed by encapsulation technique can produce a plant-based powder with high metabolites' retention, which might serve as a promising antioxidant food additive.

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References

[1] A. Ghorbani, M. Esmaeilizadeh, Pharmacological properties of *Salvia officinalis* and its components, J. Tradit. Complement. Med. 7 (2017) 433-440. https://doi.org/10.1016/j.jtcme.2016.12.014.

[2] NParks, Flora and Fauna Web. https://www.nparks.gov.sg/florafaunaweb/flora/4/6/4664, 2020 (accessed 7 July 2020).

[3] S.M. El-Sayed, A.M. Youssef, 2019. Potential application of herbs and spices and their effects in functional dairy products. Heliyon. 5, e01989. https://doi.org/10.1016/j.heliyon.2019.e01989.

[4] M. Jakovljević, S. Jokić, M. Molnar, M. Jašić, J. Babić, H. Jukić, I. Banjari, Bioactive Profile of Various *Salvia officinalis* L. Preparations, Plants 8 (2019) 55. https://doi.org/10.3390/plants8030055.

[5] J. Živković, M. Ristić, J. Kschonsek, A. Westphal, M. Mihailović, V. Filipović, V. Böhm, 2017. Comparison of Chemical Profile and Antioxidant Capacity of Seeds and Oils from *Salvia sclarea* and *Salvia officinalis*. Chem. Biodivers. 14, e1700344. https://doi.org/10.1002/cbdv.201700344.

[6] N. Martins, L. Barros, C. Santos-Buelga, M. Henriques, S. Silva, I.C.F.R. Ferreira, Evaluation of bioactive properties and phenolic compounds in different extracts prepared from *Salvia officinalis* L, Food Chem. 170 (2015) 378-385. https://doi.org/10.1016/j.foodchem.2014.08.096.

[7] C.S. Garcia, C. Menti, A.P. Lambert, T. Barcellos, S. Moura, C. Calloni, C.S. Branco, M. Salvador, M. Roesch-Ely, J.A. Henriques, Pharmacological perspectives from Brazilian *Salvia officinalis* (Lamiaceae): antioxidant, and antitumor in mammalian cells, An. Acad. Bras. Cienc. 88 (2016) 281-292. https://doi.org/10.1590/0001-3765201520150344.

[8] A.H. Mansourabadi, H.M. Sadeghi, N. Razavi, E. Rezvani, Anti-inflammatory and Analgesic Properties of *Salvigenin, Salvia officinalis* Flavonoid Extracted, Future Nat. Prod. 2 (2016) 31-41. https://doi.org/futurenatprod.skums.ac.ir/article_14340.html.

[9] M. Beheshti-Rouy, M. Azarsina, L. Rezaie-Soufi, M.Y. Alikhani, G. Roshanaie, S. Komaki, The antibacterial effect of sage extract (*Salvia officinalis*) mouthwash against *Streptococcus mutans* in dental plaque: a randomized clinical trial, Iran. J. Microbiol. 7 (2015) 173-177. https://doi.org/PMID: 26668706; PMCID: PMC4676988.

[10] A. Jedinák, M. Mučková, D. Košťálová, T. Maliar, I. Mašterová, Antiprotease and Antimetastatic Activity of Ursolic Acid Isolated from *Salvia officinalis*, Z. Naturforsch. C 61 (2006) 777-782. https://doi.org/10.1515/znc-2006-11-1203.

[11] P. Badiee, A.R. Nasirzadeh, M. Motaffaf, Comparison of *Salvia officinalis* L. essential oil and antifungal agents against candida species, J. Pharmaceut. Tech. Drug. Res. 1 (2012). https://doi.org/10.7243/2050-120X-1-7.

[12] E. Horváthová, A. Srančíková, E. Regendová-Sedláčková, M. Melušová, V. Meluš, J. Netriová, Z. Krajčovičová, D. Slameňová, M. Pastorek, K. Kozics, Enriching the drinking water of rats with extracts of *Salvia officinalis* and *Thymus vulgaris* increases their resistance to oxidative stress, Mutagenesis 31 (2015) 51-59. https://doi.org/10.1093/mutage/gev056.

[13] C.P.R. Xavier, C.F. Lima, M. Fernandes-Ferreira, C. Pereira-Wilson, *Salvia Fruticosa*, *Salvia Officinalis*, and Rosmarinic Acid Induce Apoptosis and Inhibit Proliferation of Human Colorectal Cell Lines: The Role in MAPK/ERK Pathway, Nutr. Cancer 61 (2009) 564-571. https://doi.org/10.1080/01635580802710733.

[14] K. Miura, H. Kikuzaki, N. Nakatani, Antioxidant Activity of Chemical Components from Sage (*Salvia officinalis* L.) and Thyme (*Thymus vulgaris* L.) Measured by the Oil Stability Index Method, J. Agric. Food Chem. 50 (2002) 1845-1851. https://doi.org/10.1021/jf011314o.

[15] M.-E. Cuvelier, H. Richard, C. Berset, Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary, J. Am. Oil Chem. Soc. 73 (1996) 645-652. https://doi.org/10.1007/BF02518121.

[16] O. Wrona, K. Rafińska, C. Możeński, B. Buszewski, Supercritical Fluid Extraction of Bioactive Compounds from Plant Materials, J. AOAC Int. 100 (2017) 1624-1635. https://doi.org/10.5740/jaoacint.17-0232.

[17] R.P.F.F. da Silva, T.A.P. Rocha-Santos, A.C. Duarte, Supercritical fluid extraction of bioactive compounds, TrAC, Trends Anal. Chem. 76 (2016) 40-51. https://doi.org/10.1016/j.trac.2015.11.013.

[18] E. Ibáñez, J.A. Mendiola, M. Castro-Puyana, Supercritical Fluid Extraction, in: B. Caballero, P.M. Finglas, F. Toldrá (Eds.), Encyclopedia of Food and Health, Academic Press, Oxford, 2016, pp. 227-233.

[19] A.K. Sunol, S.G. Sunol, K. Cogswell, 20 - SUBSTITUTION OF SOLVENTS BY SAFER PRODUCTS, in: G. Wypych (Ed.), Handbook of Solvents (Third Edition), Chemtec Publishing, Ontario, 2019, pp. 1455-1634.

[20] T. Arumugham, R. K, S.W. Hasan, P.L. Show, J. Rinklebe, F. Banat, Supercritical carbon dioxide extraction of plant phytochemicals for biological and environmental applications – A review, Chemosphere 271 (2021) 129525. https://doi.org/10.1016/j.chemosphere.2020.129525.

[21] FDA, Part 184 Direct Food Substances Affirmed as Generally Recognized as Safe. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1240, 2020 (accessed 13 June 2020).

[22] L. Xiu-Qin, J. Chao, S. Yan-Yan, Y. Min-Li, C. Xiao-Gang, Analysis of synthetic antioxidants and preservatives in edible vegetable oil by HPLC/TOF-MS, Food Chem. 113 (2009) 692-700. https://doi.org/10.1016/j.foodchem.2008.07.072.

[23] B. Saad, Y.Y. Sing, M.A. Nawi, N. Hashim, A.S. Mohamed Ali, M.I. Saleh, S.F. Sulaiman, K.M. Talib, K. Ahmad, Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC, Food Chem. 105 (2007) 389-394. https://doi.org/10.1016/j.foodchem.2006.12.025.

[24] S.C. Lourenço, M. Moldão-Martins, V.D. Alves, Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications, Molecules 24 (2019) 4132. https://doi.org/10.3390/molecules24224132.

[25] A.B. Engin, N. Bukan, O. Kurukahvecioglu, L. Memis, A. Engin, Effect of butylated hydroxytoluene (E321) pretreatment versus L-arginine on liver injury after sub-lethal dose of endotoxin administration, Environ. Toxicol. Pharmacol. 32 (2011) 457-464. https://doi.org/10.1016/j.etap.2011.08.014.

[26] S.-H. Jeong, B.-Y. Kim, H.-G. Kang, H.-O. Ku, J.-H. Cho, Effects of butylated hydroxyanisole on the development and functions of reproductive system in rats, Toxicology 208 (2005) 49-62. https://doi.org/10.1016/j.tox.2004.11.014.

[27] A.A.M. Botterweck, H. Verhagen, R.A. Goldbohm, J. Kleinjans, P.A. van den Brandt, Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands Cohort Study, Food Chem. Toxicol. 38 (2000) 599-605. https://doi.org/10.1016/S0278-6915(00)00042-9.

[28] A. Mooranian, R. Negrulj, N. Chen-Tan, H.S. Al-Sallami, Z. Fang, T.K. Mukkur, M. Mikov, S. Golocorbin-Kon, M. Fakhoury, G.F. Watts, V. Matthews, F. Arfuso, H. Al-Salami, Microencapsulation as a novel delivery method for the potential antidiabetic drug, Probucol, Drug Des. Devel. Ther. 8 (2014) 1221-1230. https://doi.org/10.2147/DDDT.S67349.

[29] L. Chen, C. Gnanaraj, P. Arulselvan, H. El-Seedi, H. Teng, A review on advanced microencapsulation technology to enhance bioavailability of phenolic compounds: Based on

its activity in the treatment of Type 2 Diabetes, Trends Food Sci. Technol. 85 (2019) 149-162. https://doi.org/10.1016/j.tifs.2018.11.026.

[30] M.R.T. Zorzenon, M. Formigoni, S.B. da Silva, F. Hodas, S. Piovan, S.R. Ciotta, C.A. Jansen, A.S. Dacome, E.J. Pilau, C.E. Mareze-Costa, P.G. Milani, S.C. Costa, Spray drying encapsulation of stevia extract with maltodextrin and evaluation of the physicochemical and functional properties of produced powders, J. Food Sci. 85 (2020) 3590-3600. https://doi.org/10.1111/1750-3841.15437.

[31] E. Assadpour, S.M. Jafari, Advances in Spray-Drying Encapsulation of Food Bioactive Ingredients: From Microcapsules to Nanocapsules, Annu. Rev. Food Sci. Technol. 10 (2019) 103-131. https://doi.org/10.1146/annurev-food-032818-121641.

[32] E. Langa, G.D. Porta, A.M.F. Palavra, J.S. Urieta, A.M. Mainar, Supercritical fluid extraction of Spanish sage essential oil: Optimization of the process parameters and modelling, J. Supercrit. Fluids 49 (2009) 174-181. https://doi.org/10.1016/j.supflu.2008.12.007.

[33] N.K. Mohammed, A.S. Meor Hussin, C.P. Tan, M.Y. Abdul Manap, A.M. Alhelli, Quality changes of microencapsulated *Nigella sativa* oil upon accelerated storage, Int. J. Food Prop. 20 (2017) S2395-S2408. https://doi.org/10.1080/10942912.2017.1371189.

[34] S. Glisic, J. Ivanovic, M. Ristic, D. Skala, Extraction of sage (*Salvia officinalis* L.) by supercritical CO_2: Kinetic data, chemical composition and selectivity of diterpenes, J. Supercrit. Fluids 52 (2010) 62-70. https://doi.org/10.1016/j.supflu.2009.11.009.

[35] M.J. Navarro-Flores, L.M.C. Ventura-Canseco, R. Meza-Gordillo, T.d.R. Ayora-Talavera, M. Abud-Archila, Spray drying encapsulation of a native plant extract rich in phenolic compounds with combinations of maltodextrin and non-conventional wall materials, J. Food Sci. Technol. 57 (2020) 4111-4122. https://doi.org/10.1007/s13197-020-04447-w.

[36] A.R. Robinson, R. Gheneim, R.A. Kozak, D.D. Ellis, S.D. Mansfield, The potential of metabolite profiling as a selection tool for genotype discrimination in *Populus*, J. Exp. Bot. 56 (2005) 2807-2819. https://doi.org/10.1093/jxb/eri273.

[37] U. Sadowska, A. Kopeć, L. Kourimska, L. Zarubova, P. Kloucek, The effect of drying methods on the concentration of compounds in sage and thyme, J. Food Process Preserv. 41 (2017) e13286. https://doi.org/10.1111/jfpp.13286.

[38] NIST, NIST/EPA/NIH Mass Spectral Library with Search Program, (Data Version: NIST v17, Software Version: 2.3), National Institute of Standards and Technology (NIST), 1 June, 2017.

[39] D. Granato, V.M. de Araújo Calado, B. Jarvis, Observations on the use of statistical methods in Food Science and Technology, Food Res. Int. 55 (2014) 137-149. https://doi.org/10.1016/j.foodres.2013.10.024.

[40] T. Tolcha, T. Gemechu, S. Al-Hamimi, N. Megersa, C. Turner, High Density Supercritical Carbon Dioxide for the Extraction of Pesticide Residues in Onion with Multivariate Response Surface Methodology, Molecules 25 (2020) 1012. https://doi.org/10.3390/molecules25041012.

[41] B.A.S. Machado, C.G. Pereira, S.B. Nunes, F.F. Padilha, M.A. Umsza-Guez, Supercritical Fluid Extraction Using CO_2: Main Applications and Future Perspectives, Sep. Sci. Technol. 48 (2013) 2741-2760. https://doi.org/10.1080/01496395.2013.811422.

[42] C. Da Porto, D. Decorti, A. Natolino, Water and ethanol as co-solvent in supercritical fluid extraction of proanthocyanidins from grape marc: A comparison and a proposal, J. Supercrit. Fluids 87 (2014) 1-8. https://doi.org/10.1016/j.supflu.2013.12.019.

[43] S.A. Radzali, M. Markom, N.M. Saleh, Co-Solvent Selection for Supercritical Fluid Extraction (SFE) of Phenolic Compounds from *Labisia pumila*, Molecules 25 (2020) 5859. https://doi.org/10.3390/molecules25245859.

[44] W.J. Lee, C.C. Ng, J.S. Ng, R.L. Smith, S.L. Kok, Y.Y. Hee, S.Y. Lee, W.K. Tan, N.H. Zainal Abidin, S.A. Halim Lim, G.H. Chong, Supercritical carbon dioxide extraction of α-mangostin from mangosteen pericarp with virgin coconut oil as co-extractant and in-vitro bio-accessibility measurement, Process Biochem. 87 (2019) 213-220. https://doi.org/10.1016/j.procbio.2019.09.009.

[45] S.L. Kok, W.J. Lee, R.L. Smith, N. Suleiman, K.N. Jom, K. Vangnai, A.H. Bin Sharaai, G.H. Chong, Role of virgin coconut oil (VCO) as co-extractant for obtaining xanthones from mangosteen (*Garcinia mangostana*) pericarp with supercritical carbon dioxide extraction, J. Supercrit. Fluids 176 (2021) 105305. https://doi.org/10.1016/j.supflu.2021.105305.

[46] J. Garavaglia, M.M. Markoski, A. Oliveira, A. Marcadenti, Grape Seed Oil Compounds: Biological and Chemical Actions for Health, Nutr. Metab. Insights 9 (2016) NMI.S32910. https://doi.org/10.4137/NMI.S32910.

[47] J.P. DeLany, M.M. Windhauser, C.M. Champagne, G.A. Bray, Differential oxidation of individual dietary fatty acids in humans, Am. J. Clin. Nutr. 72 (2000) 905-911. https://doi.org/10.1093/ajcn/72.4.905.

[48] S. Calligaris, L. Manzocco, M. Anese, M.C. Nicoli, Shelf-life Assessment of Food Undergoing Oxidation–A Review, Crit. Rev. Food Sci. Nutr. 56 (2016) 1903-1912. https://doi.org/10.1080/10408398.2013.807222.

[49] E.M. Both, R.M. Boom, M.A.I. Schutyser, Particle morphology and powder properties during spray drying of maltodextrin and whey protein mixtures, Powder Technol. 363 (2020) 519-524. https://doi.org/10.1016/j.powtec.2020.01.001.

[50] Y.Z. Cai, H. Corke, Production and Properties of Spray-dried *Amaranthus* Betacyanin Pigments, J. Food Sci. 65 (2000) 1248-1252. https://doi.org/10.1111/j.1365-2621.2000.tb10273.x.

[51] M.S. Abu-Darwish, C. Cabral, I.V. Ferreira, M.J. Gonçalves, C. Cavaleiro, M.T. Cruz, T.H. Al-bdour, L. Salgueiro, Essential Oil of Common Sage (*Salvia officinalis* L.) from Jordan: Assessment of Safety in Mammalian Cells and Its Antifungal and Anti-Inflammatory Potential, Biomed Res. Int. 2013 (2013) 538940. https://doi.org/10.1155/2013/538940.

[52] K. Li, Q. Liu, H. Cheng, M. Hu, S. Zhang, Classification and carbon structural transformation from anthracite to natural coaly graphite by XRD, Raman spectroscopy, and HRTEM, Spectrochim. Acta A Mol. Biomol. Spectrosc. 249 (2021) 119286. https://doi.org/10.1016/j.saa.2020.119286.

[53] K.M. Zia, M. Zuber, M. Barikani, A. Jabbar, M.K. Khosa, XRD pattern of chitin based polyurethane bio-nanocomposites, Carbohydr. Polym. 80 (2010) 539-543. https://doi.org/10.1016/j.carbpol.2009.12.017.

[54] M.F. Drincovich, L.M. Voll, V.G. Maurino, Editorial: On the Diversity of Roles of Organic Acids, Front. Plant Sci. 7 (2016) 1592-1592. https://doi.org/10.3389/fpls.2016.01592.

[55] Q. Liu, G.-Y. Tang, C.-N. Zhao, R.-Y. Gan, H.-B. Li, Antioxidant Activities, Phenolic Profiles, and Organic Acid Contents of Fruit Vinegars, Antioxidants 8 (2019) 78. https://doi.org/10.3390/antiox8040078.

[56] R. Lo Scalzo, Organic acids influence on DPPH scavenging by ascorbic acid, Food Chem. 107 (2008) 40-43. https://doi.org/10.1016/j.foodchem.2007.07.070.

[57] Q. Zafra-Rojas, N. Cruz-Cansino, A. Delgadillo-Ramírez, E. Alanís-García, J. Añorve-Morga, A. Quintero-Lira, A. Castañeda-Ovando, E. Ramírez-Moreno, Organic Acids, Antioxidants, and Dietary Fiber of Mexican Blackberry (*Rubus fruticosus*) Residues cv. Tupy, J. Food Qual. 2018 (2018) 5950761. https://doi.org/10.1155/2018/5950761.

[58] A.J.J.V.D. Berg, S.B.A. Halkes, H.C.Q.V. Ufford, M.J. Hoekstra, C.J. Beukelman, A novel formulation of metal ions and citric acid reduces reactive oxygen species in vitro, J. Wound Care 12 (2003) 413-418. https://doi.org/10.12968/jowc.2003.12.10.26552.

[59] D. Arsenov, M. Zupunski, M. Borisev, N. Nikolic, S. Orlovic, A. Pilipovic, S. Pajevic, Exogenously Applied Citric Acid Enhances Antioxidant Defense and Phytoextraction of Cadmium by Willows (*Salix* Spp.), Water Air Soil Pollut. 228 (2017) 221. https://doi.org/10.1007/s11270-017-3405-6.

[60] J.A. Mahmud, M. Hasanuzzaman, K. Nahar, A. Rahman, M.S. Hossain, M. Fujita, Maleic acid assisted improvement of metal chelation and antioxidant metabolism confers chromium tolerance in *Brassica juncea* L, Ecotoxicol. Environ. Saf. 144 (2017) 216-226. https://doi.org/10.1016/j.ecoenv.2017.06.010.

[61] K.E. Allen, 4 - Metal chelators as antioxidants for food preservation, in: F. Shahidi (Ed.), Handbook of Antioxidants for Food Preservation, Woodhead Publishing Inc., Cambridge, 2015, pp. 79-104.

[62] M. Nigam, Chapter 3 - Phytomedicine: Scope and current highlights, in: C. Egbuna, A.P. Mishra, M.R. Goyal (Eds.), Preparation of Phytopharmaceuticals for the Management of Disorders, Academic Press, Cambridge, 2021, pp. 39-54.

[63] E. Sarrou, S. Martens, P. Chatzopoulou, Metabolite profiling and antioxidative activity of Sage (*Salvia fruticosa* Mill.) under the influence of genotype and harvesting period, Ind. Crops Prod. 94 (2016) 240-250. https://doi.org/10.1016/j.indcrop.2016.08.022.

[64] M. Ali, P. Li, G. She, D. Chen, X. Wan, J. Zhao, Transcriptome and metabolite analyses reveal the complex metabolic genes involved in volatile terpenoid biosynthesis in garden sage (*Salvia officinalis*), Sci. Rep. 7 (2017) 16074. https://doi.org/10.1038/s41598-017-15478-3.

[65] A. Raina, K. Negi, M. Dutta, Variability in essential oil composition of sage (*Salvia officinalis* L.) grown under North Western Himalayan Region of India, J. Med. Plant Res. 7 (2013) 683-688. https://doi.org/10.5897/JMPR.

[66] M. Greeshma Geetha, K. Murugan, Antioxidant Activities of Terpenoids from *Thuidium tamariscellum* (C. Muell.) Bosch. and Sande-Lac. a Moss, Pharmacogn. J. 10 (2018) 645-649. https://doi.org/10.5530/pj.2018.4.106.

[67] R. Torres-Martínez, Y.M. García-Rodríguez, P. Ríos-Chávez, A. Saavedra-Molina, J.E. López-Meza, A. Ochoa-Zarzosa, R.S. Garciglia, Antioxidant Activity of the Essential Oil and its Major Terpenes of *Satureja macrostema* (Moc. and Sessé ex Benth.) Briq, Pharmacogn. Mag. 13 (2018) S875-s880. https://doi.org/10.4103/pm.pm_316_17.

[68] C.-Y. Wang, Y.-W. Chen, C.-Y. Hou, Antioxidant and antibacterial activity of seven predominant terpenoids, Int. J. Food Prop. 22 (2019) 230-238. https://doi.org/10.1080/10942912.2019.1582541.

[69] X. Lyu, J. Lee, W.N. Chen, Potential Natural Food Preservatives and Their Sustainable Production in Yeast: Terpenoids and Polyphenols, J. Agric. Food Chem. 67 (2019) 4397-4417. https://doi.org/10.1021/acs.jafc.8b07141.

[70] L. Caputi, E. Aprea, Use of terpenoids as natural flavouring compounds in food industry, Recent Pat. Food Nutr. Agric. 3 (2011) 9-16. https://doi.org/10.2174/2212798411103010009.

[71] I.O. Minatel, C. Borges, M.I. Ferreira, H. Gomez Gomez, O. Chen, G. Pace, G. Lima, Phenolic Compounds: Functional Properties, Impact of Processing and Bioavailability, in: M. Soto-Hernandez, M. Palma-Tenango, M.d.R. Garcia-Mateos (Eds.), Phenolic Compounds - Biological Activity, IntechOpen Limited, London, UK, 2017.

[72] A.A.H. Kadhum, A.A. Al-Amiery, A.Y. Musa, A.B. Mohamad, The antioxidant activity of new coumarin derivatives, Int. J Mol. Sci. 12 (2011) 5747-5761. https://doi.org/10.3390/ijms12095747.

[73] F. Topal, M. Nar, H. Gocer, P. Kalin, U.M. Kocyigit, İ. Gülçin, S.H. Alwasel, Antioxidant activity of taxifolin: an activity–structure relationship, J. Enzyme Inhib. Med. Chem. 31 (2016) 674-683. https://doi.org/10.3109/14756366.2015.1057723.

[74] D. Zhang, S. Nie, M. Xie, J. Hu, Antioxidant and antibacterial capabilities of phenolic compounds and organic acids from *Camellia oleifera* cake, Food Sci. Biotechnol. 29 (2020) 17-25. https://doi.org/10.1007/s10068-019-00637-1.

[75] C.-Y. Shaw, C.-H. Chen, C.-C. Hsu, C.-C. Chen, Y.-C. Tsai, Antioxidant properties of scopoletin isolated from *Sinomonium acutum*, Phytother. Res. 17 (2003) 823-825. https://doi.org/10.1002/ptr.1170.

[76] C. Sarawong, R. Schoenlechner, K. Sekiguchi, E. Berghofer, P.K.W. Ng, Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour, Food Chem. 143 (2014) 33-39. https://doi.org/10.1016/j.foodchem.2013.07.081.

[77] I.B. Afanas'ev, A.I. Dcrozhko, A.V. Brodskii, V.A. Kostyuk, A.I. Potapovitch, Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation, Biochem. Pharmacol. 38 (1989) 1763-1769. https://doi.org/10.1016/0006-2952(89)90410-3.

[78] B. Pavlić, O. Bera, N. Teslić, S. Vidović, G. Parpinello, Z. Zeković, Chemical profile and antioxidant activity of sage herbal dust extracts obtained by supercritical fluid extraction, Ind. Crops Prod. 120 (2018) 305-312. https://doi.org/10.1016/j.indcrop.2018.04.044.

[79] S. Maksimovic, Z. Kesic, I. Lukic, S. Milovanovic, M. Ristic, D. Skala, Supercritical fluid extraction of curry flowers, sage leaves, and their mixture, J. Supercrit. Fluids 84 (2013) 1-12. https://doi.org/10.1016/j.supflu.2013.09.003.

[80] F.J. Leyva-Jiménez, J. Lozano-Sánchez, M.d.l.L. Cádiz-Gurrea, Á. Fernández-Ochoa, D. Arráez-Román, A. Segura-Carretero, Spray-Drying Microencapsulation of Bioactive Compounds from Lemon Verbena Green Extract, Foods 9 (2020) 1547. https://doi.org/10.3390/foods9111547.

[81] Z. Tülek, H. Alaşalvar, B. Basyigit, S. Berktaş, P. Salum, Z. Erbay, I. Telci, M. Çam, Extraction Optimization and Microencapsulation of Phenolic Antioxidant Compounds from Lemon Balm (*Melissa officinalis* L.): Instant Soluble Tea Production, J. Food Process Preserv. (2020). https://doi.org/10.1111/jfpp.14995.

[82] F.D. Lavergne, C.D. Broeckling, D.M. Cockrell, S.D. Haley, F.B. Peairs, C.E. Jahn, A.L. Heuberger, GC-MS Metabolomics to Evaluate the Composition of Plant Cuticular Waxes for Four *Triticum aestivum* Cultivars, Int. J Mol. Sci. 19 (2018) 249. https://doi.org/10.3390/ijms19020249.

[83] D.S. Seigler, Plant Waxes, in, Plant Secondary Metabolism, Springer US, Boston, MA, 1998, pp. 51-55.

[84] A.-C. Schuster. 2016. Chemical and functional analyses of the plant cuticle as leaf transpiration barrier [Doctoral thesis, University of Würzburg]. Online Publication Service of Würzburg University. Würzburg, Germany. https://opus.bibliothek.uni-wuerzburg.de/frontdoor/index/index/docId/13347

[85] C.A. Correa, M.B.G. de Doz, C.M. Bonatti, H.N. Sólimo, Recovery of Lemon Essential Oil, Lemon Wax, and Diatomaceous Earth from the Filter Cake of the Lemon Essential Oil Dewaxing Process at Pilot-Plant Scale, Ind. Eng. Chem. Res 47 (2008) 9573-9580. https://doi.org/10.1021/ie7017355.