

Microencapsulation of Polyphenols from *Orthosiphon stamineus* Leaves Extracts using Polysaccharide and Protein

Sook Fun Pang¹, Jolius Gimbut^{1,2}, Mashitah M. Yusoff³

¹Faculty of Chemical & Natural Resources Engineering

²CoE for Advanced Research in Fluid Flow (CARIFF)

³Faculty of Industrial Sciences & Technology

Universiti Malaysia Pahang, 26300 Gambang

Pahang, Malaysia.

Corresponding E-mail: jolius@ump.edu.my

Luqman Chuah Abdullah⁴

⁴Dept. Chemical and Environmental Engineering

Faculty of Engineering

Universiti Putra Malaysia, 34300 Serdang

Selangor, Malaysia

Abstract—This paper presents the *Orthosiphon stamineus* polyphenol extraction and microencapsulation study using whey protein isolate, gum arabic and maltodextrin. Polyphenol content was analysed using ultra-performance liquid chromatography. The highest yield of rosmarinic acid (38.70 mg RA/g DW) was obtained using 70% aqueous methanol, whereas the highest yield of sinensetin (261.21 µg Sin/g DW) and eupatorin (2.71 mg Eup/g DW) was obtained using isopropanol. Aqueous solvent such as 70% ethanol provide a broader range of polarity than the pure solvent, and hence enhances a simultaneous extraction of both methoxylated and hydroxylated compounds. It was found that microwave assisted extraction is capable to perform an efficient and very fast extraction of polyphenols within 2 minutes, compared to the ultrasonic assisted extraction which require 90 minutes. Microencapsulation of polyphenols from *O. stamineus* using WPI, maltodextrin and gum arabic has successfully reduced polyphenol degradation during spray drying. The highest polyphenol retention of rosmarinic acid (84.25%), sinensetin (83.96%) and eupatorin (85.61%) was achieved by microencapsulation using gum arabic. The gum arabic derived microcapsule has a smooth spherical surface which enhance particle flowability.

Keywords—*Orthosiphon stamineus*; misai kucing; phenolic content; flavonoid; microencapsulation; gum arabic

I. INTRODUCTION

Orthosiphon stamineus is locally known as 'misai kucing' is consumed widely as a herbal tea in Malaysia. Previous studies revealed that extract of *O. stamineus* contained many medically useful bioactive compounds such as terpenoids, polyphenols and sterols that poses a diuretic [1], antidiabetic [2], antiangiogenic and antiproliferative properties [3].

The active components from *O. stamineus* leaves such as rosmarinic acid, sinensetin and eupatorin, is recovered through the extraction process. The yield of bioactive component in the extract is affected by the type of solvent used, extraction

method and condition. Most of the previous work related to extraction of polyphenol from *O. stamineus* used a thermal intensive extraction such as maceration [4] and accelerated solvent extraction [5]. These methods are often time consuming besides affected by thermal degradation of polyphenols due to heat exposure over a prolonged period. Nowadays, better extraction methods of polyphenols have been developed such as the ultrasonic-assisted extraction (UAE) [6, 7], microwave-assisted extraction (MAE) [8, 9] and accelerated solvent extraction (ASE) [5]. However, ASE is normally performed at high temperature to increase the diffusivity of solvent and to maintain the solvent in liquid form using high pressure which may cause thermal degradation of thermolabile active component. Extraction is a mass transfer process involving solvent transport to the solid phase (inner transport), dissolution of the solutes (solubility) and release of solutes from the solid matrix to the bulk phase (external transport). The UAE technique reduces the inner and external mass transfer limitation and hence increases the yield of extraction. Zhang *et al.* [10] for instance, showed that ultrasonic wave can break the cell membranes reducing control of inner mass transport. Similarly, rapid temperature rise during MAE also breaks the plant cell wall. Thus, extractions using MAE and UAE methods were chosen for this work.

Solvent type plays an important role to ensure successful extraction of bioactive compounds from plant material. Solvent diffuses into the solid plant material and solubilize compounds with similar polarity during extraction [11]. It is understood that phenolic compound concentrations in extracts from the same plant material may vary accordingly with the solvent used. A combined effect of the different extraction methods (ME and UAE) and varying solvent polarity to the polyphenol extraction from *O. stamineus* has never been studied previously, and hence this is one of the aims of this work. In the present study, ultra-performance liquid chromatography (UPLC) was used for the determination of polyphenols from *O. stamineus* extracts. UPLC provides improved separation in a

shorter analysis time of *O. stamineus* extracts without compromising peak capacity and sensitivity [12].

A powder-based product is desirable for convenience of consumption besides having a longer shelf life and ease of handling. Spray drying is a common method of producing powder, but it requires very high temperature, which may adversely affect the quality of product. Furthermore, the polyphenol from *O. stamineus* extracts is prone to thermal degradation [12, 13]. The thermal degradation is undesirable because the degraded product is of low nutritional value and consequently, hampers the intention to produce a functional food or nutraceutical product. However, very limited study concerning drying and preservation of polyphenol in *O. stamineus* extract is available in the literature [12]. The microencapsulation by spray drying is an effective way to reduce thermal degradation. Microencapsulation is a process in which small particles are enclosed by a coating, or embedded in a homogeneous or a heterogeneous matrix of wall material or encapsulating agent. The choice of wall material is one of the main concerns for microencapsulation process. Common microencapsulation agents such as whey protein isolate (WPI), gum arabic, maltodextrin are often used for herbal or plant-related product. WPI was used for microencapsulation of phenolic compounds from the bilberry extracts [14], while Shahin-Nadeem et al. [15] used maltodextrin for microencapsulation of phenolic compounds from herbal tea (*Salvia fruticosa*). Krishnan et al. [16] found that gum arabic offered greater protection to the oleoresin than maltodextrin and modified starch. Pang et al. [12] studied the microencapsulation of *O. stamineus* using WPI and maltodextrin, however, no study on gum arabic was done. Therefore, this work aims to study the performance of gum arabic as encapsulating agents for *O. stamineus* derived polyphenols in comparison to WPI and maltodextrin.

II. MATERIALS AND METHODS

A. Chemicals and Plant Material

The HPLC grade solvents such as the acetonitrile (ACN) and analysis grade solvent such as methanol, isopropanol and ethanol were purchased from Merck (Darmstadt, Germany) and trifluoroacetic acid (TFA) was purchased from Fisher Scientific (Leics, UK). The dimethyl sulfoxide (DMSO), standard of rosmarinic acid, eupatorin and sinensetin were obtained from Sigma Aldrich (St. Louis, MO). The lactose-free whey protein isolate powder was obtained from Ultimate Nutrition (Fleetwood, UK) with 99% of undenatured proteins meanwhile Maltodextrin DE10 was obtained from San Soon Seng Food Industries (Malaysia). The leaves were collected in Gambang, Pahang, Malaysia from a white flowered *O. stamineus* similar to one that has been deposited at the Forest Research Institute, Malaysia (voucher no. ZAS1113). Freshly collected leaves were washed with deionised water and dried at 37 °C for 3 days before crushed to powder. Prior to use the powder was kept in an air-tight plastic bag in a desiccator at room temperature to prevent moisture absorption.

B. Extraction of Phenolic Compounds

The powdered plant material was weighed (1 wt. %) and mixed with solvent in a 250 ml sealed Erlenmeyer flask. UAE was carried out in an ultrasonic bath (CREST P1800D, US) at 45 kHz for 90 minutes and temperature was set at 40 °C. A similar plant material to solvent ratio (1 wt. %) was placed in a 50 ml PTFE Teflon vessel (Figure 3.2) for MAE using a domestic microwave oven (Samsung, MW71E, Korea). Effect of solvent study was performed at microwave power of 200 W for 2 minutes. The supernatant was then separated from the plant residue by vacuum filtration through 0.45 µm nylon membrane.

C. Microencapsulation by Spray Drying

The extracts were encapsulated by three types of wall material which are whey protein isolate gum Arabic and maltodextrin DE10. The encapsulant concentration of 10 wt.% was set for all samples where the optimum polyphenols retention can be achieved [13]. *O. stamineus* extract (0.7 wt.% solid content) was mixed with WPI, gum arabic or maltodextrin. The solution was mixed with magnetic stirring at 40 °C for 30 minutes to obtain a homogeneous solution. The resultant solution was spray dried using a lab scale spray dryer (Lab Plant SD06A, UK) fitted with 0.5 mm atomizer and air velocity of about 4.1 m/s was set constant throughout the experiment. The inlet air temperature was set at 180 °C and maintained at ±1°C by the proportional-integral-derivative controller. The feed was metered into the dryer by means of a peristaltic pump at 407.1 ml/hr. Similar setups were employed in all experiments to ensure a fair comparison. Dried powder samples were collected from a Schott bottle attached at the bottom of the cyclone separator.

D. Analysis of Polyphenols Content

The total solid content from *O. stamineus* extract was determined by evaporating the liquid from 5 ml solution completely in an oven. Moisture content for all dried powder samples is determined using a moisture analyser, and the water content is subtracted during preparation of solution for UPLC analysis of polyphenol after spray drying. The same dry weight of solid (bioactive compounds) is set for the initial solution (extract) and after drying the solution to ensure a fair comparison of polyphenol retention [12]. The predetermined amount of dried powder was dissolved in 60% aqueous methanol with the aid of vortex mixer to ensure dissolution of less polar compounds. The stock solution of rosmarinic acid (10 mg/ml) was prepared in methanol, whereas eupatorin (10 mg/ml) and sinensetin (5 mg/ml) were dissolved in DMSO. The three analytical standards were further diluted to 0.08 µg/ml to develop an eight points calibration curve. Qualitative and quantitative determinations of *O. stamineus* extracts major constituents (rosmarinic acid, sinensetin and eupatorin) were performed on a Waters Acquity UPLC H-Class (Milford, MA) fitted with Acquity UPLC HSS T3 column (2.1 x 75 mm, 1.8 µm) and a Acquity UPLC HSS T3 VanGuard column guard (2.1 x 5 mm, 1.8 µm). The UPLC system is equipped with photodiode array detector and connected to a computer running Waters Empower 2 software. The mobile phase consists of solvent A:water:TFA (20:0.001; v/v) and solvent B: ACN:TFA

(20:0.001; v/v) and the following gradient elution: 0–2.0 min, 26% B; 2.0–3.9 min, 26–50% B; 3.9–6.9 min, 50–95% B and finally washing the column with 95% B for 0.6 min and reconditioning the column with 26% B isocratic for 1.4 min. The temperature was maintained at room temperature (24 °C), with the injection volume of 2 µl and flow rate at 0.17 ml/min. The sample was filtered with 0.2 µm PES membrane filter before injected to the UPLC system. The peaks for rosmarinic acid (3.10–3.30 min), sinensetin (5.50–5.60 min) and eupatorin (5.65–5.75 min) were detected at 340 nm.

E. Particle Surface Morphology

The morphologies of the spray-dried particles were evaluated with field emission scanning electron microscope (JEOL JSM-7800F, Japan). The dried powder was mounted on specimen stubs with double-sided adhesive carbon tapes. The specimen was coated with platinum and was examined at 1 to 3 kV with a magnification ranging from 500x to 20000x.

F. Particle Moisture Content

The moisture content of the spray dried powder was determined using a moisture analyzer (AND MS-70, Japan). Initially, about 1g of powder sample was placed on the heating pan of the moisture analyser. The moisture content evaporates as a result of continuous heating, and the experiment stopped automatically once the mass of the sample become constant.

G. Statistical Analysis

Each experiment was repeated in triplicates. Analysis of variance (ANOVA) was performed by using the data analysis tools in Microsoft Excel 2010, and a least significant difference (LSD) test was used to compare the means with a confidence interval of 95%.

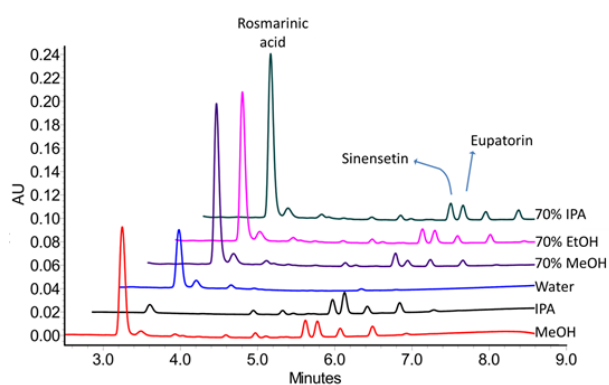


Fig. 1. UPLC chromatogram for *O. stamineus* extract by MeOH, IPA, water, 70% MeOH, 70% EtOH and 70% IPA.

III. RESULTS AND DISCUSSION

A. UPLC Quantification of Polyphenols

The phenolic compounds were identified by means of the retention time and UV spectra of the standard (rosmarinic acid, sinensetin and eupatorin). The UV spectra and retention time for all three target components were found to be good matched with each standard compound obtained from Sigma-Aldrich.

Active components were quantified by comparing peak areas with the results of a calibration series of the standard. The calibration curves of all target compounds showed good linearity ($R^2 > 0.997$) for the whole range of concentration studied (0.08 – 250 µg/l). Limit of detection was determined by setting the signal to noise ratio of 3:1. The UPLC method developed for the first time by Pang *et al.* [12] is capable of a fast and accurate qualitative and quantitative analysis of polyphenols from *O. stamineus* extract. The analysis time is less than 7 minutes as shown in Fig. 1, which is about three times faster than those of the other reported method [17]. The UPLC detection is not affected by the different solvent type and encapsulant used in this work. Thus, similar method for determination of polyphenol content was employed throughout this work.

B. Influence of Solvent Type and Extraction Method to Polyphenols Extraction

Influence of solvent type on polyphenols extraction was examined by employing solvent of various polarities (MeOH, IPA, H₂O, 50% MeOH, 50% IPA, 70% MeOH, 70% IPA and 70% EtOH) for both MAE and UAE methods. The extraction time for MAE and UAE were set at 2 and 90 minutes, respectively, following the preliminary study which indicates their optimum extraction time. Prolonging UAE extraction beyond 90 minutes decreased the yield due to thermal degradation. The solubility of the bioactive component in different solvent is affected by its structural characteristic. The highly methoxylated compound such as sinensetin and eupatorin, which are lipophilic were more stable in low polar solvent such as isopropanol. Similar findings are also reported by Akowuah *et al.* [4] who found that amount of sinensetin and eupatorin is higher in low polar solvent, i.e. chloroform extract. However, a highly hydroxylated compound such as rosmarinic acid is hydrophilic, thus more soluble in methanol than in isopropanol. For the same reason, rosmarinic acid can be found in water extract, but not sinensetin and eupatorin. The results suggest that aqueous alcoholic solvent has a higher (> 30%) extracting capacity of flavonoid and phenolic content compared to pure solvent which is consistent with findings from other researchers [18]. Wach *et al.* [18] for instance, found that aqueous methanol ranged from 40 to 80% is preferable for rutin and quercetin extraction from *H. perforatum*. It is thought that aqueous solvent provides a wider range of polarity in contrast to the pure solvent, and hence enhances simultaneous extraction of both methoxylated and hydroxylated compounds. This phenomenon can be seen clearly in the case of aqueous isopropanol, which increased extraction of rosmarinic acid (hydroxylated compound) over ten folds from 2.93 to 34.78 mg/g DW without adversely affecting the extraction of the methoxylated flavonoid (sinensetin and eupatorin).

Water is capable of extracting phenolic and flavonoid content using UAE method. Presence of sinensetin, eupatorin and rosmarinic acid cannot be observed from the UPLC chromatogram in Fig. 1 for the extract of the same dilution because their concentration is below the detection limit of the UPLC. However, as mentioned earlier in section 2.5 the water extract is freeze-dried prior to UPLC analysis, which enable

detection of the targeted phenolic compounds. UPLC analysis shows a tiny presence of sinensetin (26.75 µg Sin/g DW), eupatorin (0.07 mg Eup/g DW) and rosmarinic acid (8.41 mg RA/g DW) from water extract. This is in agreement with the previous study by Akowuah et al. [4] who found very little phenolic compounds from water extract and in some case undetectable.

The UPLC analysis of MAE extracts shows a significant amount of rosmarinic acid (13.47 mg RA/g DW), which is expected due to its solubility in aqueous solution. Tiny presence of sinensetin (76.85 µg Sin/g DW) and eupatorin (0.06 mg Eup/g DW) are also detected. Amount of RA and Sin obtained from MAE is much higher (about 2x) to that of UAE. This is due to microwave heating, which interacts selectively with the free solvent molecules present in the homogenized solution; this leads to localized heating, and the temperature increases rapidly. Thus, such systems undergo a dramatic expansion, with subsequent rupture of cell walls, allowing the polyphenol to flow outwards from the finely crushed leaves [19]. Although UAE may enhance inner mass transfer by breaking the cell membrane wall, they are lacking the rapid localized heating, which could alter the polyphenol solubility in the bulk region. Extraction of polyphenol using a pure solvent (MeOH or IPA) favours UAE with over 20% higher yield for rosmarinic acid, eupatorin and sinensetin compared to MAE. Extraction using aqueous solvent shows no significant differences between UAE and MAE methods, although it can be seen that eupatorin extraction in UAE favours aqueous isopropanol, while for MAE aqueous methanol is better. Maceration which was performed only for the 70% aqueous isopropanol showed as good polyphenol extraction as the UAE, and somewhat better than the MAE. Nevertheless, the MAE method has the advantage of being the fastest at 2 minutes compared to 90 minutes for UAE and 4 hours for maceration technique. Conventional solvent extraction without microwave assistance is a time-consuming process based on heat to increase the mass transfer rate in the extraction system. In contrast, microwave-assisted extraction is a fast extraction process where microwave energy is delivered efficiently to materials through molecular interaction with the electromagnetic field and offers a rapid transfer of energy to the extraction solvent and raw plant materials [20, 21]. Furthermore, the direct interaction of microwave with solvent also results in the rupture of the plant cells, triggering a quicker release of intracellular products into the solvent [22].

Solvent with high dielectric constants (e.g. high polarity solvent like water) can absorb more microwave energy, which makes water molecule rotates as they try to align themselves with the alternating electric field of the microwaves, and resulting in rapid heating. This is also one of the reasons why extraction efficiency of MAE differs to those of UAE. As shown in Fig. 2, water under microwave irradiation is capable of extracting rosmarinic acid (13.57 mg RA/DW) but not during ultrasonic extraction. At the same time total flavonoid content from MAE (73.28 mg QE/DW) is almost double of that obtained using UAE (40.66 mg QE/DW). It is also interesting to note that only a mere 5.16% increase in rosmarinic acid extraction yield for UAE when 50% aqueous methanol is used as opposed to pure methanol. Meanwhile, a noteworthy

54.34% increase in rosmarinic acid extraction yield for MAE using a similar solvent. Although water can extract rosmarinic acid, it cannot extract sinensetin and eupatorin from the plant matrix. Therefore solvent in which the target analyte is soluble must be considered. Hence, aqueous solvent is still preferable in microwave extraction in order to obtain higher yield of the targeted analyte. Finding from this work suggests that solvent with lower amount of water added such as 70% MeOH, 70% EtOH and 70% IPA are the preferred solvent to achieve simultaneous extraction of hydrophilic component (rosmarinic acid) and lipophilic component (sinensetin, eupatorin). *O. stamineus* extracts using MAE with 70% EtOH was used for the microencapsulation study because MAE can perform fast extraction and ethanol GRAS status (Generally Recognized as Safe).

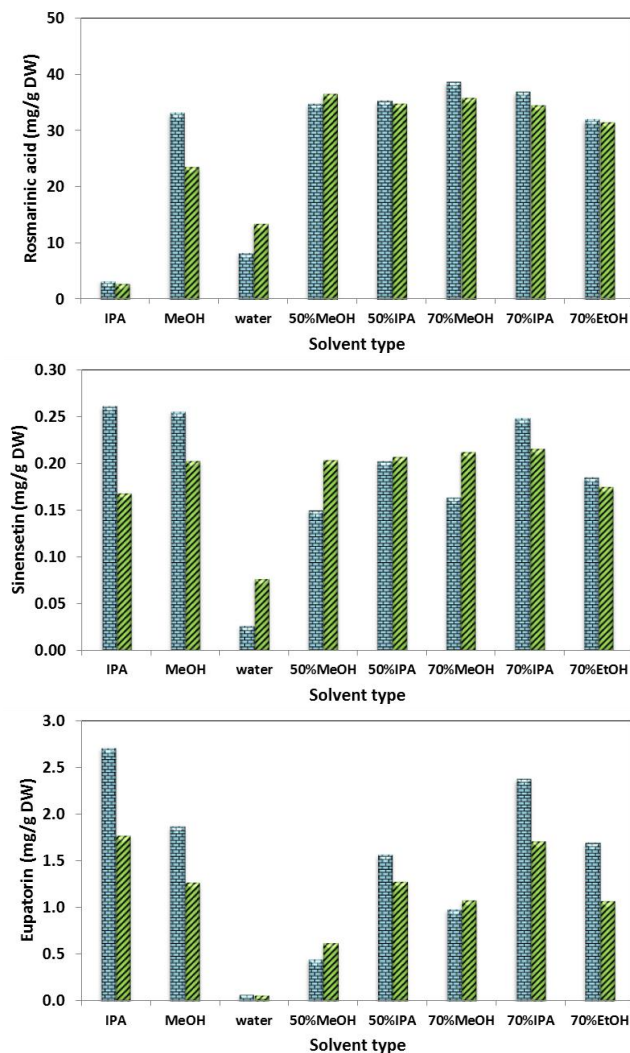


Fig. 2. Effect of solvent and extraction method on polyphenols extraction from *O. stamineus*

C. Microencapsulation of Phenolic Compounds from *O. stamineus*

The polyphenols retention of WPI, gum arabic and maltodextrin encapsulated powder were compared to the initial solution to assess the level of polyphenols preservation. The

initial solution (denoted as 'extract' in Fig. 3) and the dried powder were examined for their rosmarinic acid, sinensetin and eupatorin content using UPLC. All microencapsulant tested in this work showed a good retention (ranging from 66.67% to 85.60%) of the targeted components in *Orthosiphon stamineus* extract (Fig. 3). According to the finding from Pang et al. [12], spray dried powder without microencapsulation showed severe degradation of polyphenol content i.e., rosmarinic acid (54.24%) and eupatorin (39.81%). The retentions of individual polyphenol without microencapsulation are 45.76%, 78.31% and 60.19% for rosmarinic acid, sinensetin and eupatorin, respectively. Both the rosmarinic acid and eupatorin showed higher degradation than sinensetin due to presence of hydroxyl groups in their molecular structure. Flavonoid with more hydroxyl groups is known to be more susceptible to thermal degradation [23]. However, these degradation issues can be reduced by microencapsulation. Results from this work shows that microencapsulation using 10 wt.% of gum Arabic provides the highest polyphenol retention of rosmarinic acid (84.25%), sinensetin (83.96%) and eupatorin (85.61%). Similar finding was obtained by Krishnan et al. [16] found that gum Arabic offered greater protection to the oleoresin than maltodextrin and modified starch. The efficient entrapment of the constituents and volatiles in gum arabic is due to good film forming capability and their plasticity rather than a glassy property. Plasticity is known to prevent cracking of the protective matrix [24, 25]. However, Tonon et al. [26] found there is not a significant difference between açai juice powders produced with maltodextrins and gum Arabic respect to both anthocyanin content and antioxidant activity. From this study suggests that microencapsulation using gum Arabic provides slightly better (0.8- 5.51%) preservation of polyphenol during spray drying compared to maltodextrin. WPI encapsulated powders have lower retention compare to gum arabic and maltodextrin encapsulated powder this might be due to the protein denaturation during spray drying, entrapped polyphenols may not be as accessible as those encapsulated by undenatured soluble protein [27].

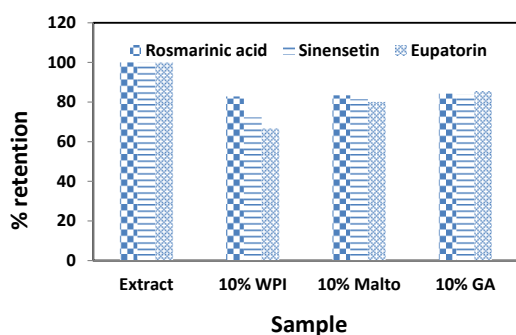


Fig. 3. Retention of rosmarinic acid, sinensetin and eupatorin using a different encapsulation agent.

D. Moisture Content

The maltodextrin encapsulated powder (5.57%) has a lower moisture content compared to WPI (6.66%) and gum arabic (7.16%) encapsulated powder. Higher values were obtained for microcapsules produced with gum arabic, due to its more hydrophilic characteristics. Similar finding was made by

Frascareli et al. [28] who reported that microcapsules of coffee oil produce by gum arabic have higher moisture content than WPI microcapsules. Nevertheless, the moisture content for all three encapsulating agents tested in this work is below 10%, which is a desired moisture for spray dried powder.

E. Particle Morphology

Spray dried powder of *O. stamineus* polyphenols encapsulated by WPI, gum arabic and maltodextrin were observed to have a granular structure using FESEM as shown in Fig. 4. The microcapsules encapsulated by gum arabic has a shallow wrinkle than the particle encapsulated by WPI. The dried powder obtained without encapsulant contains broken particles, and the surface morphology comparable to the one encapsulated by maltodextrin, which contains many rough wrinkle. The broken particle is not desirable because it exposes the entrapped polyphenol with the hot drying condition. Formation of dented surfaces of spray-dried particles is attributed to the shrinkage of the particles during the drying process [29]. The dried powder encapsulated with maltodextrin and the one without encapsulation has a much rougher surface that those encapsulated by WPI and gum arabic. The increase in surface roughness may decrease the amount of contact adhesion between two particles and between a particle and surface of the spray dryer, but at the same time may adversely affect particle flowability. WPI based powder has a deeper wrinkle than other particle, but the surface is rather smoother than that of maltodextrin. Overall, the gum arabic based powder has a smoother surface with almost spherical appearance. Theoretically, spherical particle has a better flowability than the particle with wrinkle surface.

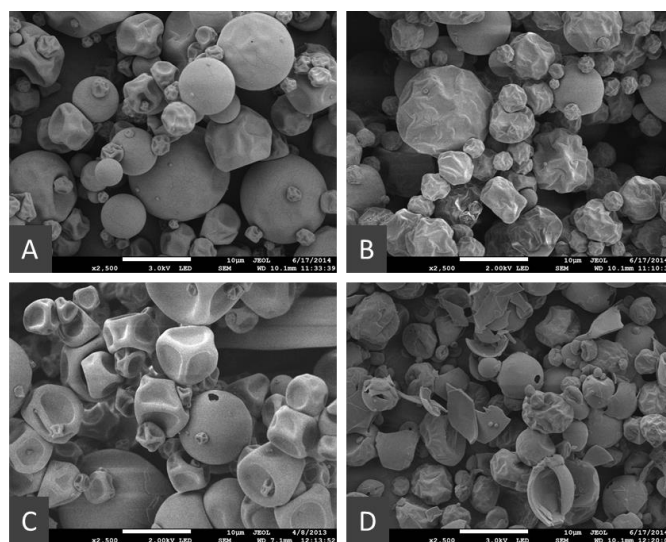


Fig. 4. The surface morphology of microcapsule obtained using a different encapsulation agent. A) Gum arabic, B) Maltodextrin, C) WPI, D) without encapsulation.

IV. CONCLUSIONS

The results suggest that the polyphenol extraction from *O. stamineus* leaves is affected by the solvent type. The highest

yield of rosmarinic acid (38.70 mg RA/g DW) was obtained using 70% aqueous methanol. The highest yield of sinensetin (261.21 µg Sin/g DW) and eupatorin (2.71 mg Eup/g DW) was obtained using isopropanol. Aqueous solvent such as 70% methanol and 70% ethanol provide a wider range of polarity than the pure solvent, and hence enhances simultaneous extraction of both methoxylated and hydroxylated compounds. It was found that MAE is capable to perform an efficient and very fast extraction of polyphenols within 2 minutes, compared to the UAE which needs 90 minutes. Microencapsulation of polyphenols from *O. stamineus* using WPI, maltodextrin and gum arabic has successfully reduced polyphenol degradation during spray drying. The highest polyphenol retention of rosmarinic acid (84.25%), sinensetin (83.96%) and eupatorin (85.61%) was achieved by microencapsulation using gum arabic. The gum arabic derived microcapsule has a smooth spherical surface which enhance particle flowability.

ACKNOWLEDGMENT

Pang Sook Fun thanks Ministry of Education Malaysia for the MyPhD scholarship through MyBrain15 programme. This project is funded by Ministry of Education Malaysia through FRGS/2/2013/TK05/UMP/02/4 and RACE RDU121308.

REFERENCES

- Arafat, O.M., Tham, S.Y., Sadikun, A., Zhari, I., Houghton, P.J. and Asmawi, M.Z. 2008. Studies on diuretic and hypouricemic effects of *Orthosiphon stamineus* methanol extracts in rats, *Journal of Ethnopharmacology*, 118: 354-360.
- Mohamed, E.A.H., Yam, M.F., Ang, L.F., Mohamed, A.J., & Asmawi, M.Z. (2013). Antidiabetic properties and mechanism of action of *Orthosiphon stamineus* Benth bioactive sub-fraction in streptozotocin-induced diabetic rats. *JAMS Journal of Acupuncture and Meridian Studies*, 6, 31-40.
- Dolečková, I., Rárová, L., Grúz, J., Vondrusová, M., Strnad, M., Kryštof, V. (2012). Antiproliferative and antiangiogenic effects of flavone eupatorin, an active constituent of chloroform extract of *Orthosiphon stamineus* leaves. *Fitoterapia*, 83, 1000-1007.
- Akowuah, G.A., Ismail, Z., Norhayati, I., & Sadikun, A. (2005). The effects of different extraction solvents of varying polarities on polyphenols of *Orthosiphon stamineus* and evaluation of the free radical-scavenging activity. *Food Chemistry*, 93, 311-317.
- Pouralinazar, F., Yunus, M.A.C., Zahedi, G. (2012). Pressurized liquid extraction of *Orthosiphon stamineus* oil: Experimental and modeling studies. *Journal of Supercritical Fluids*, 62, 88-95.
- Tabaraki R. and Nateghi A., 2011, "Optimization of ultrasonic-assisted extraction of natural antioxidants from rice bran using response surface methodology," *Ultrason. Sonochem.*, 18, 1279-1286.
- Zhang, H.F., Yang, X.H., Zhao, L.D. and Wang, Y. 2009. Ultrasonic-assisted extraction of epimedin C from fresh leaves of *Epimedium* and extraction mechanism. *Innovative Food Science and Emerging Technologies*. 10(1): 54-60.
- Elez Garofulić, I., Dragović-Uzelac, V., Režek Jambrak, A., & Jukić, M. (2013). The effect of microwave assisted extraction on the isolation of anthocyanins and phenolic acids from sour cherry Marasca (*Prunus cerasus* var. Marasca). *Journal of Food Engineering*, 117(4), 437-442.
- Spigno G. and Faveri De D.M. Microwave-assisted extraction of tea phenols: A phenomenological study. 2009. *Journal of Food Engineering*. 93: 210-217
- Zhang, H.F., Yang, X.H., Zhao, L.D. and Wang, Y. 2009. Ultrasonic-assisted extraction of epimedin C from fresh leaves of *Epimedium* and extraction mechanism. *Innovative Food Science and Emerging Technologies*. 10(1): 54-60.
- Ncube, N.S., Afolayan, A.J. and Okoh, A.I. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*. 7: 1797-1806.
- Pang S.F., Yusoff M.M., Gimbin J. 2014. Assessment of phenolic compounds stability and retention during spray drying of *Orthosiphon stamineus* extracts. *Food Hydrocolloids*. 37: 159-165.
- Gimbin, J., Pang, S.F., Lau, S.J., Yusoff, M.M., Chuah, L. (2014), Thermal degradation kinetics of flavonoid derived from *Orthosiphon stamineus*, 1st International Conference on Food Properties (iCFP2014), Kuala Lumpur, Malaysia, January 24-26, 2014.
- Betz, M., Steiner, B., Schantz, M., Oidtmann, J., Mäder, K., Richling, E., et al. (2012). Antioxidant capacity of bilberry extract microencapsulated in whey protein hydrogels. *Food Research International*, 47, 51-57.
- Sahin-Nadeem, H., Dinger, C., Torun, M., Topuz, A., & Özdemir, F. (2013). Influence of inlet air temperature and carrier material on the production of instant soluble sage (*Salvia fruticosa* Miller) by spray drying. *LWT e Food Science and Technology*, 52, 31-38.
- Krishnan, S, Kshirsagar, A. and Singhal, R. 2005a. The use of gum arabic and modified starch in the microencapsulation of a food flavoring agent. *Carbohydrate Polymers*. 62(4): 309-315.
- Mohamed, E.A.H., Yam, M.F., Ang, L.F., Mohamed, A.J., Asmawi, M.Z. (2013). Antidiabetic Properties and Mechanism of Action of *Orthosiphon stamineus* Benth Bioactive Sub-fraction in Streptozotocin-induced Diabetic Rats. *JAMS Journal of Acupuncture and Meridian Studies*, 6, 31-40.
- Wach A., Pyrzynska K. and Biesaga M. 2007. Quercetin content in some food and herbal samples. *Food Chemistry*. 100(2): 699-704.
- Eskilsson, C. S., and Bjorklund, E. 2000. Analytical-scale microwave assisted extraction. *Journal of Chromatography A*. 902: 227-250.
- Criado, M.R., Torre, S.P., Pereira, I.R., and Torrijos, R.C. 2004. Optimization of a microwave-assisted derivatization-extraction procedure for the determination of chlorophenols in ash samples. *Journal of Chromatography*. 1024: 155-163.
- Zhou, H.Y., and Liu, C.Z. 2006. Microwave-assisted extraction of solaneseol from tobacco leaves. *Journal of Chromatography*. 1129: 135-139.
- Lay-Keow, N., and Michel, H. 2003. Effects of moisture content in cigar tobacco on nicotine extraction similarity between Soxhlet and focused open-vessel microwave assisted techniques. *Journal of Chromatography*. 1011: 213-219.
- Buchner, N., Krumbein, A., Rhon, S., & Kroh, L. W. (2006). Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Communications in Mass Spectrometry*, 20, 3229-3235.
- Bertolini, A.C., Siani, A.C., & Grosso, C.R.F. (2001). Stability of monoterpenes encapsulated in gum arabic by spray drying. *Journal of Agricultural and Food Chemistry*, 49, 780-785.
- McNamee, B.F., O'Riordan, E.D., & O'Sullivan, M. (1998). Emulsification and microencapsulation properties of gum arabic. *Journal of Agricultural and Food Chemistry*, 46, 4551-4555.
- Tonon, R.V., Brabet, C., and Hubinger, M.D. 2010. Anthocyanin stability and antioxidant activity of spray-dried açai *Euterpe oleracea*. juice produced with different carrier agents. *Food Research International*. 43(3), 907-914.
- Anandharamakrishnan, C., Rielly, C.D., & Stapley, A.G.F. (2008). Loss of solubility of α -lactalbumin and β -lactoglobulin during the spray drying of whey proteins. *LWT Food Science and Technology*, 41, 270-277.
- Frascarelli E.C., Silva V.M., Tonon R.V., Hubinger M.D. Physicochemical properties of coffee oil microcapsules produced by spray drying. *Agrobio Envases*. 27-28 September 2011. Brazil.
- Rosenberg, M., Kopelman, I.J. Talmon, Y. 1985. A scanning electron microscopy study of microencapsulation, *Journal of Food Science*, 50: pp. 139-144.

