Journal of Applied Sciences

ISSN 1812-5654

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Applied Sciences 9 (17): 3092-3097, 2009
ISSN 1812-5654

Study on Retention of Bioactive Components of *Morinda Citrifolia L.* Using Spray-Drying

[1]D. Krishnaiah, [1]R. Sarbatly, [1]A.M.M. Hafiz, [1]A.B. Hafeza and [2]S.R.M. Rao
[1]Chemical Engineering Programme, School of Engineering and Information Technology,
University Malaysia Sabah, 88999, Kota Kinabalu, Sabah, Malaysia
[2]Department of Chemical Engineering, College of Technology, Osmania University,
Hyderabad-500007, India

Abstract: The aim of this study was to evaluate the influence of spray drying conditions on the chemical and biological properties of *Morinda citrifolia* L. powder. The process was carried out on a lab scale spray dryer using carrageenan as a coating agent. The effect of inlet temperature and M_{core}/M_{wall} on Encapsulation Yield (EY), particles morphology and antioxidant potential were investigated. The evaluation of antioxidant potential was assessed using DPPH radical, total phenolic and flavonoid content. The highest quality of powder in terms of antioxidant capacity was produced at optimum drying temperature of 130°C using M_{core}/M_{wall} of 1/4 and at constant temperature of 150°C, the highest M_{core}/M_{wall} ratio was 1/2. The encapsulation yield was higher at higher temperature (150°C) and using M_{core}/M_{wall} of 1/4. Particle size analyzer and scanning electron microscope were used to monitor the structure and size of the powders. The results indicated that all the powders obtained were smooth spheres with size range of 1-20 μm.

Key words: Microencapsulation, morinda citrifolia, spray-drying, carrageenan

INTRODUCTION

Herbal and natural plants have been used as medicine in centuries in many cultures in the world. Scientists and medicinal professionals have shown great interests in doing research in this field recently as they are acknowledged the health benefits of the plants. Moreover, plants are potential sources of natural antioxidants. They produce a very imposing group of antioxidant compounds which inclusive of carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols (Hollman, 2001). These antioxidant compounds are important and beneficial for food protection as well as a defense mechanism of living cells against oxidative damage (Vimala and Adenan, 1999).

Morinda citrifolia L., the Mengkudu, is one of the important traditional Polynesian medicinal plants that have been used for over 2000 years. Known commercially in Malaysia as Mengkudu, it is also referred as Cheese fruit, Ba Ji Tian, Nhau, Indian Mulberry throughout the world. Mengkudu is a native plant found around Southeast Asia to Australia and is cultivated in India, Polynesia, the Caribbean, Central and Northern South America. Mengkudu is noted for its wide range of usage as a medicinal plant or as an edible food source. The young leaves of this plant can be cooked as vegetables and even the dried leaves or fruits are used to make infusions and teas. Besides that, the roots and bark can be turn into dyes, while the trunks part can be use as firewood and tools (Yanine *et al*., 2006).

Mengkudu has recently been the object of many claims concerning its nutraceutical properties. It has been recognized as a plant that contains a broad range of therapeutic effects such as antioxidant, antibacterial, antiviral, antifungal, antitumor, anti-inflammatory and immune enhancing effects (Wang *et al*., 2002). Various publications have shown that mengkudu can be used to relieve different kinds of illnesses including arthritis, diabetes, high blood pressure muscle aches and pains, blood vessel problems and cancers.

Two clinical studies reported a relief of arthritis and diabetes associated with mengkudu consumption (Elkins, 1998; Solomon, 1999): the observed beneficial effects may result from certain compounds such as scopoletin, nitric oxide, alkaloids and sterols and also to the antioxidant potential of Mengkudu. Therefore, consumption of this fruit is currently high, not only in the producing countries but also in the United States, Japan and Europe.

Corresponding Author: D. Krishnaiah, Chemical Engineering Programme, School of Engineering and Information Technology, University Malaysia Sabah, 88999, Kota Kinabalu, Sabah, Malaysia

Microencapsulation technique is an effective way and widely used to protect drug or food ingredients against deterioration and volatile losses. The protective mechanism is to form a membrane wall to enclose droplets or particles of the encapsulated material. There are currently a variety of means available in regards of preparing microparticles. The practice of each method is depending on the types of desired microparticles. It can include the properties of the wall structures, sizes, shape quantity and quality of final product. Another factor that often comes into consideration is cost of operation, practicality of the process and overall time frame. Of all the means, fluid bed coating, centrifugal extrusion and spray drying are the major commercial processes usually used in terms of practicality and product volume. However, in food and drug industry, spray drying is still favoring the most. It is due to its continuous production and easiness of industrialization (Shu *et al*., 2008). Nevertheless, microencapsulation of *Morinda citrifolia* L. extracts by spray drying still has not been reported.

For microencapsulation by spray drying, carrageenan is a good choice as wall material due to its pseudoplastic properties. These properties will allow it to act as plasticizer, promoting the formation of spherical and smooth-surfaced microcapsules, enhancing adhesion force between wall and core materials (Shu *et al*., 2008). Moreover, it has good properties of emulsification, edibility and biodegradation. Carrageenan is also one of polysaccharides, which can be used as excipient in drug industry.

In this study, a spray drying method is developed for preparation of *Morinda citrifolia* L. mirocapsules using carrageenan as wall material and to investigate the effect of different temperatures on the antioxidant content and activity of the powders.

MATERIALS AND METHODS

Materials: Mengkudu fruit was obtained from various places around Kota Kinabalu, sabah, Malaysia. The fruits are obtained at unripe stage, tap washed and separated or the seed and pulp. The pulp is sun dried and further oven dried until constant weight is obtained. Afterwards, the dried pulp is pulverized and kept for storage at 40°C.

Extraction of mengkudu: Pulverized antioxidant sample of Mengkudu were extracted using ethyl acetate using 10% solvent concentration. One hundred gram of blended powder was mixed with 1000 mL of ethyl acetate inside a conical flask and left on shaking incubator for 3 days at the temperature of 37°C. The solution was filtered and the filtrate portion was kept at 4°C for further study.

Preparation of emulsion and spray drying: Carrageenan solution was set to 0.5% of concentration. About 0.5 g of carrageenan powder was added into 100 mL of distilled water. During the addition of carrageenan powder, the distilled water in the beaker was stirred and heated up to 80°C in order to obtain a well-mixed solution.

The emulsion of Mengkudu extract and carrageenan was set to 400 mL with selected ratio based on Table 1. The resulting emulsions then were vigorously stirred before fed into a lab plant spray dryer SD05 with the main chamber 215 mm in diameter and height of 500 mm. The spray drier were operated at 5 different temperatures (Table 1).

The pump speed is set to -5.83 to 210 mL h^{-1}, air flowrate of -114 to 69 m^3 h^{-1} and compressor air pressure of 1.2 bar.

Extraction of antioxidant from powder: Two hundred milligram of spray dried powder was carefully weighed and mixed with 2 mL of ethanol and 0.5 mL of distilled water. The solution was then agitated using a vortex for 1 min and centrifuged at 5000 rpm for 10 min. The supernatant was collected as Mengkudu powder extract and kept at 4°C for further analysis.

DPPH Radical Scavenging Activity (RSA): The effect of the microcapsules extract as an antioxidant source was estimated using free-radical-scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical with slight modification (Alothman *et al*., 2008). An aliquot of 200 μL^{-1} powder extract was mixed with 4 mL of 25 mg L^{-1} methanolic solution of DPPH. The result solution was

Table 1: EY (%), DPPH RSA, Total phenolic content (TPC) and flavonoid content of microcapsules obtained at different spray-drying condition

Runs	Tinlet (°C)	Toutlet (°C)	M_{core}/M_{wall}	EY (%)	DPPH RSA (%)	TPC (mg TAE/100 g powder)	Flavonoid (mg CE/100 g powder)
1	150	81.0±1.25	1/2	31.05	20.46	197.86	282.75
2	150	81.0±1.25	1/4	36.38	14.27	77.50	218.25
3	150	81.0±1.25	1/6	33.54	13.40	81.43	171.00
4	150	81.0±1.25	1/8	31.33	10.23	69.32	152.34
5	140	76.2±1.37	1/4	35.78	15.32	95.23	231.54
6	130	70.5±0.83	1/4	33.44	17.40	168.93	220.00
7	120	65.4±0.34	1/4	31.93	17.35	158.98	260.43
8	110	61.5±0.83	1/4	30.44	16.94	151.79	244.25

and left under dark condition for 60 min. Afterwards, the decreased absorbance of the DPPH solution was evenly mixed measured at 515 nm against blank methanol with Perkin Elmer UV-VIS lambda 25 spectrophotometer. Results were expressed as percentage of inhibition of the DPPH according to Eq. 1:

$$\text{DPPH RSA (\%)} = \left[\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right] \times 100\% \quad (1)$$

where, DPPH RSA is the radical scavenging activity of antioxidant, Abs control is the absorbance of DPPH solution without extracts an abs sample is the absorbance of dpph with sample taken after 60 min.

Total phenolic content: The procedure is based on Folin-Ciocalteau assay, developed by Singleton and Rossi (1965) with some modification (Singleton and Rossi, 1965). 1 mL of Folin-Ciocalteau reagent (1:9:Folin Ciocalteau:distilled water) and 200 µL of sample was mixed in a tube and left for 5 min at room temperature. After that, 800 µL of sodium carbonate solution was added; the mixture was then homogenized and left at room temperature for 2 h. The total phenolic content was further determined using Perkin-Elmer UV-VIS lambda 25 spectrophotometer at 765 nm. Tannic acid was used as the standard. The value of phenolic content were expressed in mg of tannic acid equivalents (TAE)/100 g of powder based on the equation $y = 0.007x$ ($r^2 = 0.997$).

Total flavonoid content: Total Flavonoid (TF) contents of the Mengkudu extracts were determined according to the colorimetric assay developed by Zhishen *et al*. (1999). One milliliter of powder extract was added with 0.3 mL of (5% w/v) $NaNO_2$. 0.3 mL of (10% w/v) $AlCl_3$ was added after 5 min, followed by 2 mL of NaOH (1 M) 1 at 6th min. The mixture was shaken vigorously and the absorbance of the mixture is read at 510 nm. The results were expressed as mg Catechin Equivalents (CE)/100 g of powder. A calibration curve was prepared using a standard solution of catechin (20 to 100 mg L^{-1}, $r^2 = 0.996$).

Encapsulation yield: The Encapsulation Yield (EY) was calculated using Eq. 2. It was expressed as the ratio of the mass of microcapsules obtained after spray drying and the mass of initial substances added (Shu *et al*., 2008):

$$\text{EY (\%)} = \frac{\text{weight of micropsules after spray drying}}{\text{The total weight of carrageenan + extracted sample}} \times 100\% \quad (2)$$

Particle size distribution: The size distribution of resulting powder was determined by a laser scattering particle size distribution analyzer instrument (Model LA-300, Horiba instruments Inc).

Scanning Electron Microscopy (SEM): The powder samples were analyzed in a SEM, HITACHI Model S-3700 N (Hitachi High Tech Science Systems Corporation, Japan) in order to examine the external appearance of the particles. The encapsulated samples were fixed in stubs on an adhesive carbon paper. The images of the specimen were obtained at an accelerating voltage of 5 KV and taken at different magnifications ranging 2500 to 7000 SE as indicated on the images.

RESULTS AND DISCUSSION

Antioxidant activity: In this study, the antioxidative activities of spray dried Mengkudu powder were quantified through the effect of free radical of DPPH. Table 1 shows the percentage of radical scavenging activity for DPPH at selected parameter. In this study, M_{core}/M_{wall} is defined as the volume ratio of Mengkudu extracted sample against wall material (0.5% carrageenan). Among the M_{core}/M_{wall} ratio, it shows a higher significant percentage of DPPH inhibition at ratio of 1/2. As the ratio of M_{core}/M_{wall} decrease from 1/4 to 1/6, the values also decreasing but only on slight changes. At different drying temperature, the ratio was set constant at 1/4 and can be compare using runs 2, 4 and 5 (Fig. 1a). The highest antioxidant activity was measured at drying temperature of 130°C, followed by 110 and 150°C (Fig. 1b). During the process, the drying temperature must be optimized so that the quantity of the heat is sufficient to produce microparticles and in the same time retain a high content of antioxidant. Drying at a higher temperature will lead to degradation of antioxidative compound and also possibility of losses of volatile substances (Georgettia *et al*., 2008).

However, the temperature effect on the relative antioxidant of the extracted sample may be related to differences in stability or to differences in antioxidant potencies at various temperatures (Hove and Hove, 1944).

Flavonoid and total phenolic content: Flavonoids and Total Phenolic Content (TPC) of powdered samples are given in Table 1. Runs 1, 2 and 3 shows results for powder dried at fixed drying temperature of 150°C, the highest total phenolic content (mg of TAE/100 g of powder) were observed at ratio M_{core}/M_{wall} of 1/2 with 197 mg of TAE. At

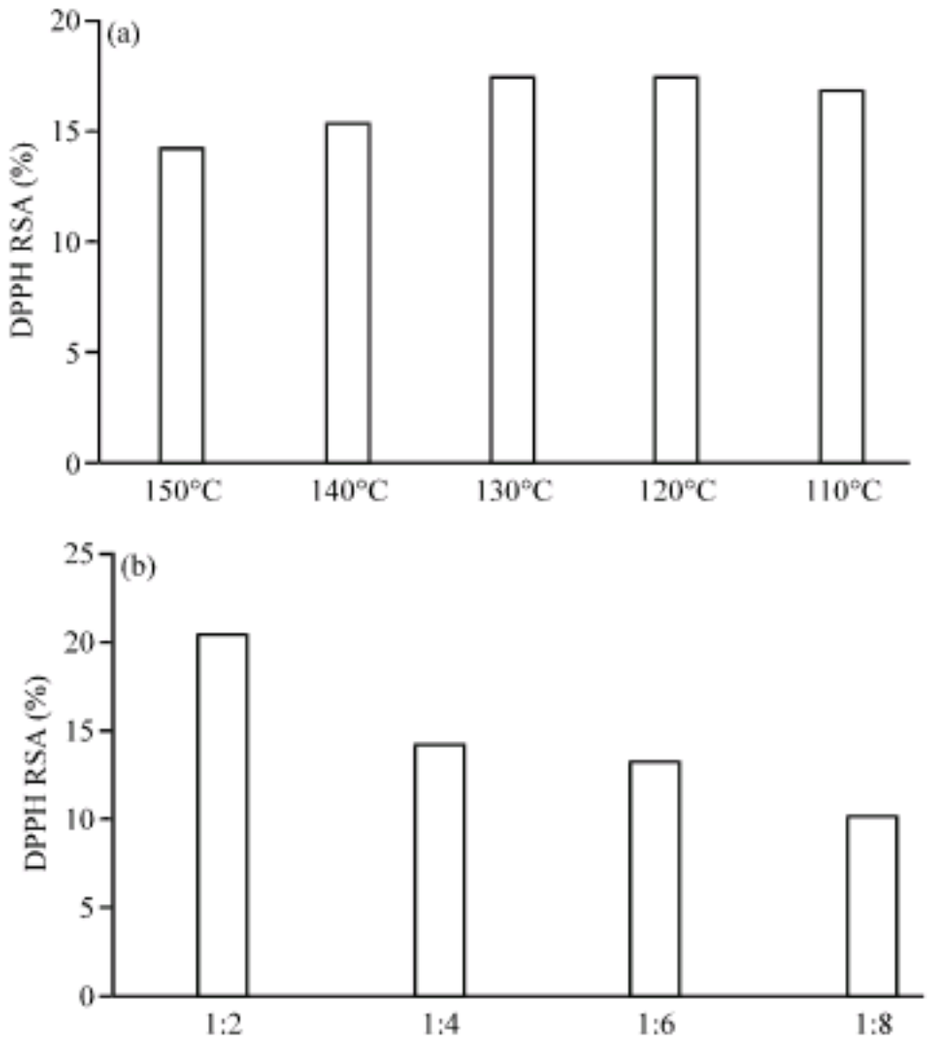

Fig 1: Free Radical Scavenging activity of the spray dried Mengkudu powder: (a) Constant Mcore/Mwall ratio of 1/4 and (b) Constant temperature at 150°C

lower ratio of 1/4 and 1/6, the TPC values only slightly varied at 77.5 and 81.43 mg of TAE, respectively. Flavonoid content (mg of CE/100 g of powder) shows the same trend obtained for TPC analysis. The content of flavonoid was only significantly high when dried at M_{core}/M_{wall} of 1/2.

At fixed drying ratio M_{core}/M_{wall} of 1/4, the highest TPC value was achieved at 130°C. TPC shows the lowest content when dried at a higher temperature. At this condition, there are possible losses or degradation of certain types of phenolic compounds (Amin *et al*., 2006). The highest flavonoid obtained at 120°C with 260 of mg CE, followed by drying at 110°C. From the results obtained by spray drying of Mengkudu, it can be concluded that DPPH RSA have an excellent correlation with TPC ($r^2 = 0.848$) and flavonoid ($r^2 = 0.855$). It had been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic group with result found by Miliauskas *et al*. (2004) during RSA analysis of plant extract.

The Encapsulation Yield (EY): In the experiment, it is obvious that temperature and ratio of M_{core}/M_{wall} have a significant effect on the encapsulation yield of resultant microcapsules. Based on Table 1, comparing at temperature drying rate ranged from 110 to 150°C, the highest yield was obtained at the highest temperature. At lower temperature, the quantity of the heat is not sufficient to dry the product and thus there will be a quantity of water exists in the product. The powder with more water content will easily stuck on the chamber wall and lead to a low EY (Shu *et al*., 2008).

Table 2: Mean diameter of powder produce at different M_{core}/M_{wall} ratio, at temperature of 150°C

M_{core}/M_{wall}	Diameter (μm)
1/2	8.98±0.25
1/4	10.94±0.26
1/6	11.35±0.58

Table 3: Mean diameter of powder produce at different temperatures, with M_{core}/M_{wall} of 1/4

Temperature (°C)	Diameter (μm)
150	10.94±0.26
130	10.00±0.55
110	7.60±0.30

The EY will increase further with temperature increment until an optimum point is achieved before decreasing, as observed by Shu *et al*. (2008). This is because at high temperature, the balance between the rate of water evaporation and film-formation may break leading to broken microcapsules that will accumulate at the chamber wall and further decreasing the EY.

Decreasing the M_{core}/M_{wall} from 1/2 to 1/8 while keeping other parameters unchanged, at first the EY increased but then decreased when the ratio reached 1/4. Such negative effect happened probably due to feed viscosity which exponentially increased with decreasing ratio of M_{core}/M_{wall}. A greater feed viscosity can cause more solids to paste in the main chamber wall due to the higher amount of solids in contact with the wall, therefore, reducing the EY (Tonon *et al*., 2008). The result is in agreement with results published by other researcher that stated M_{core}/M_{wall} = 1/4 was the optimal choice for effective microencapsulation yield (Shu *et al*., 2008). Scanning electron micrographs of spray dried powders of Mengkudu using carragaeenan as wall material are shown in Fig. 2a-e.

Particle size analysis: Table 2 and 3 show the mean diameter at different temperature and ratio, respectively. According to Table 3, the resultant particle size increased with the increasing of temperature. Based on Tonon *et al*. (2008), higher temperature is related to a higher swelling of droplets and further results in faster drying rate that produces larger particles than drying under slower rates. Higher wall material content also led to the production of larger particles (Table 2). This correlation maybe due to the feed viscosity used, which increased exponentially with wall concentration. The result obtained also in agreement with Keogh *et al*. (2003)

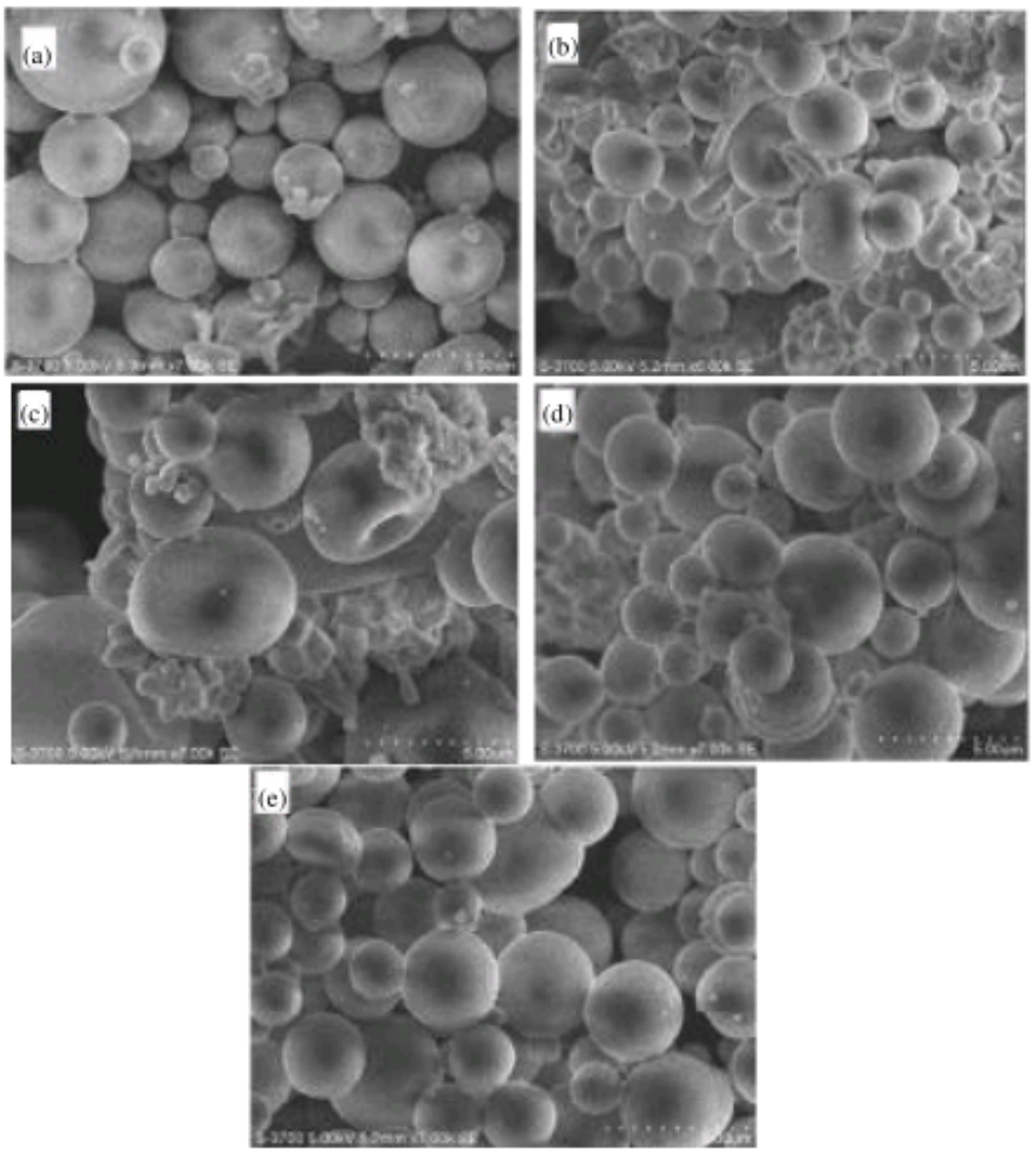

Fig. 2: Scanning electrom micrographs of spray dried powders of Mengkudu using carragaeenan as wall material: (a) 150°C, 1/2, (b) 150°C, 1/4, (c) 150°C, 1/6, (d) 110°C, 1/4 and (e) 130°C, 1/4

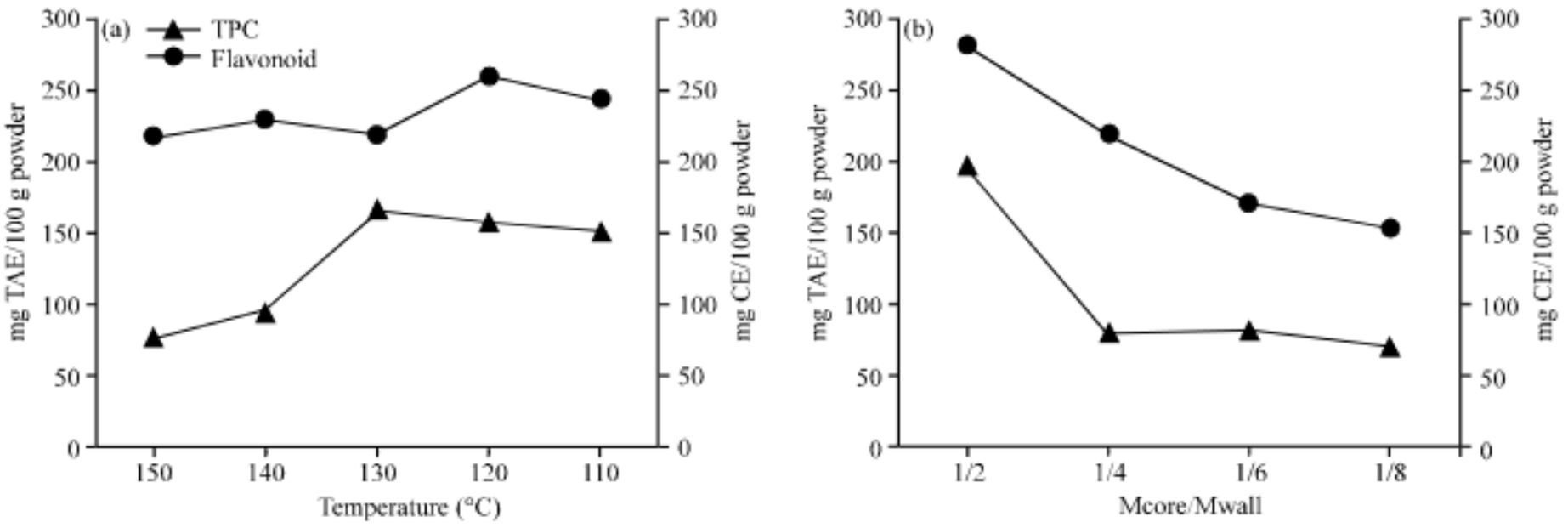

Fig. 3: Correlation of TPC and TF at: (a) constant Mcore/Mwall ratio of 1/4 and (b) constant temperature at 150°C

that observed a linear increasing of particle size with feed viscosity. Gharsallaoui *et al*. (2007) stated that at higher liquid viscosity, a larger droplet is formed during the atomization process thus, will produced larger particle size.

SEM: Powders morphology: SEM micrograph of Mengkudu spray-dried at various parameters (Fig. 2a-e) confirmed that the particles powders is in the range of micro size (1-20 μm). Most of the particles had spherical shape and less agglomerated. All of the spray dried microcapsules containing carrageenan with different antioxidant equivalent appeared to be a smooth spheres. The presence of coating agent such as carrageenan will react as plasticizer and is important on promoting the formation of spherical and smooth surfaced microparticles (Bruschi *et al*., 2003). Correlation of TPC and TF is shown in Fig. 3a and b.

CONCLUSION

Mengkudu microparticles were prepared by means of spray drying. Carrageenan at different ratio were used to prepare the fine particles and showed significant effect on all the response studied. The main factor affecting the antioxidant content is drying temperature. Drying at higher temperature had dramatically decreased the antioxidant potential of resultant powder. However, it was observed that M_{core}/M_{wall} also plays an important role on determining the highest and lowest possible range for optimized outcome antioxidant content. In respect to powder morphology, increasing the temperature resulted particles with larger sizes and higher yield. While, increasing M_{core}/M_{wall} ratio led to the production of smaller particles, which is related to the increase on feed viscosity.

ACKNOWLEDGMENTS

This research was fully supported by Ministry of Science, Technology and Innovation of Malaysia under the project SCF0049-IND-2007. Author also would like to acknowledge Awang Bono from Universiti Malaysia Sabah for his expertise and knowledge.

REFERENCES

Alothman, M., R. Bhat and A.A. Karim, 2008. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem., 115: 785-788.

Amin, I.Y., K.I. Norazaidah and E. Hainida, 2006. Antioxidant activity and phenolic content of raw and blanched Amaranthus species. Food Chem., 94: 47-52.

Bruschi, M.L., M.L.C. Cardoso, M.B. Lucchesi and M.P.D. Gremião, 2003. Gelatin microparticles containing propolis obtained by spray-drying technique: Preparation and characterization. Int. J. Pharm., 264: 45-55.

Elkins, R., 1998. Hawaiian Noni (*Morinda citrifolia*) Prize Herb of Hawaii and the South Pacific. Woodland Publishing, Utah.

Georgettia, S.R., R. Casagrandeb, C.R.F. Souzaa, W.P. Oliveiraa and M.J.V. Fonsecaa, 2008. Spray drying of the soybean extract: Effects on chemical properties and antioxidant activity. LWT., 41: 1521-1527.

Gharsallaoui, A., G. Roudaut, O. Chambin, A. Voilley and R. Saurel, 2007. Applications of spray-drying in microencapsulation of food ingredients: an overview food research international. Food Res. Int., 40: 1107-1121.

Hollman, P.C.H., 2001. Evidence for health effects of plant phenols: Local or systemic effects?. J. Sci. Food Agric., 81: 842-852.

Hove, E.L. and Z. Hove, 1944. The Effect of Temperature on the Relative Antoxidant Activity of α, β and γ-Tocopherols and of Gossypol. Laboratoty of Animal Nutrition, Alabama Polytechnic Institute, Auburn.

Keogh, M.K., C.A. Murra and B.T. O'Kennedy, 2003. Effects of ultrafiltration of whole milk on some properties of spray-dried milk powders. Int. Dairy J., 12: 995-1002.

Miliauskas, G., P.R. Venskutonis and T.A. van Beek, 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem., 85: 231-237.

Shu, Y.L., Z.Y. Fu, J.Y. Zhang, W.M. Wang, H. Wang, Y.C. Wang and Q.J. Zhang, 2008. Microencapsulation of *Radix salvia miltiorrhiza* nanoparticles by spray-drying. Powder Technol., 184: 114-121.

Singleton, V.L. and J.A.J. Rossi, 1965. Colorimetry of total phenolics with 315 phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Viticulture, 16: 144-158.

Solomon, N., 1999. The Noni Phenomenon. Direct Source Publishing, Utah, USA.

Tonon, R.V., C. Brabet and M.D. Hubinger, 2008. Influence of process conditions on the physicochemical properties of aÇai (*Euterpe oleraceae* Mart.) powder produced by spray drying. J. Food Eng., 88: 411-418.

Vimala, S. and M.I. Adenan, 1999. Malaysian tropical forest medicinal plants: A source of natural antioxidants. J. Trop. Forest Prod., 5: 32-38.

Wang, M.Y., B.J. West, C.J. Jensen, D. Nowicki, C. Su, A.K. Palu and G. Anderson, 2002. *Morinda citrifolia* (Noni): A literature review and recent advances in noni research. Acta Pharmacol. Sin., 12: 1127-1141.

Yanine, C.B., V.A. Fabrice, M. Perez, M. Reynes, Jean-Marc Brillouetc and P. Brat, 2006. Critical Review. The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. J. Food Comp. Anal., 19: 645-654.

Zhishen, J., T. Mengcheng and W. Jianming, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.