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Effect of Spray Drying Conditions on the Antioxidant and Physicochemical Properties of Clinacanthus Nutans Leaves Extracts

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Clinacanthus nutans is a medicinal plant with valuable health benefits enriched with polyphenols and flavonoids. The aim of this work was to produce spray-dried powder from C. nutans leaves using combination of κ-carrageenan (κC) and sodium carboxymethyl cellulose (NaCMC) as coating agents and to evaluate the effects of feed flow rate, inlet air temperature and concentrations of coating agent. The effects of spray drying conditions on the encapsulation yield, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, total phenolic content, total flavonoid content and four C-glycoside flavones (orientin, isoorientin, vitexin, isovitexin) of C. nutans powder were assessed for different feed flow rate (500 – 900 mL/h), inlet air temperature (110 – 150 °C) and different volume ratio of extract to coating agent (1 : 1 – 1 : 10). The result showed that vitexin was the major C-glycoside flavones compound detected in the extract. The highest encapsulation yield and antioxidant activity of the spray-dried powder was obtained from the inlet temperature of 130 °C with feed flow rate of 700 mL/h and 1 : 5 ratio (volume ratio of C. nutans extract to carrier solution). The spray-dried C. nutans had a regular spherical shape with particle size of 2.71 – 5.88 μm. In comparison to uncoated extract, the encapsulated powder showed significant improvement in particle size and antioxidant capacity. These results demonstrated that the spray-dried of C. nutans with high preservation of antioxidants is a promising source of natural products with diverse opportunities for nutraceutical and functional food applications.

1. Introduction

Clinacanthus nutans (Burm.f.) Lindau (C. nutans), a plant of Acanthaceae, is native to Southeast Asia regions of Malaysia, Indonesia, Thailand and China. The leaves have been traditionally used as folk remedies for many diseases, including the treatment of insect bites, herpes infection, allergic responses, diabetes and cancer. It is well-established that C. nutans is a good source of antioxidants, flavonoids, phytosterols, triterpenoids and other bioactive compounds (Sakdarat et al., 2009). Teshima et al. (1998) has reported the presence of C-glycosyl flavones such as vitexin, isovitexin, shaftoside, isomollupentinin in C. nutans. Since C-glycosyl flavones is one of the flavtonoids group, they act as natural antioxidants. Antioxidants play a very important role in human life which can reduce the risk of human diseases such as cancer, cardiovascular, diabetes, aging, and other chronic diseases.

Spray-drying is an economical, flexible, well-established and widely used technique produces particles with good quality for transforming liquid foods or suspensions into a powder in a one-step process (Fang and Bhandari, 2010). The microencapsulation technique of spray-drying is an effective way to protect bioactive components, preserves their stability during processing and storage, controls the release of the desired compounds and prevents undesirable interactions with food matrix against deterioration and volatile losses (Isailovic et al., 2012). Various polysaccharides and protein are used as coating agents, including

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maltodextrin, modified starch, cyclodextrins, arabic gum, kappa-carrageenan, alginates, pectin, and caseinate salt generally added to extractive solutions before spray drying in order to improve process performance and product quality (Fernandes et al., 2014). In the previous study conducted by Hazaveh and Muhamad (2012), kappa-carrageenan nanocomposite has been developed as a good carrier for controlled-release purposes. Krishnaiah et al. (2012) has reported the use of carrageenan as wall material for spray drying encapsulation of M. citrifolia L. fruit extract. A study on spray drying of C.nutans extracts in maltodextrin (10-12 DE) had reported on physico-chemical properties such as moisture content, water activity, protein, bulk density, oil content, ash, crude fibre and colour (L*, a* and b*) (Suhaimi et al., 2013). However, they did not discuss on the antioxidant activity of the spray-dried powder of C. nutans. The aim of this work was to elucidate the suitability of kappa-carrageenan and sodium carboxymethyl cellulose as a coating agent for the spray drying of C. nutans leaves extract and find the best processing parameters of spray drying to produce a powdered C. nutans extract with most optimum physicochemical properties and antioxidant content.

2. Materials and methods

2.1 Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), kappa-Carrageenan and four standard compound of C-glycoside flavones: isoorientin, orientin, isovitexin, and vitexin were purchased from Sigma-Aldrich (Malaysia). Methanol and acetonitrile (High Performance Liquid Chromatography (HPLC) grade) were obtained from RCI Labscan (Thailand), Folin–Ciocalteau's phenol reagent and gallic acid from Merck (Malaysia), Trolox (97 %) and sodium carboxymethyl cellulose from ACROS Organics (Malaysia), sodium carbonate anhydrous from Fluka (Malaysia), quercetin hydrate (95 %) and aluminum chloride hexahydrate, $AlCl_3.6H_2O$ from QReC® (Thailand). All other chemicals were of reagent grade and were used without further purification.

2.2 Preparation of C. nutans Leaves Extracts

C. nutans leaves were collected in December, 2014 from TKC Herbal Nursery, Negeri Sembilan, Malaysia. The leaves and stems were separated, leaves were washed thoroughly with tap water, oven dried, homogenised and sieved (d < 350 µm) to fine powder. Decoction was prepared with 15 g of C. nutans leaves boiled in 150 mL of distilled water (10 % w/v) with constant stirring. The extract decoction was filtered, concentrated using rotary evaporator (IKA® Rotary evaporator RV 10 digital, Germany), lyophilised (Christ Alpha 1-2 LD Freeze Dryer, UK) and stored at −20 °C.

2.3 Spray-Drying Conditions

Dispersions were prepared with κ-carrageenan (κC) and sodium carboxymethyl cellulose (NaCMC) as coating agent with an optimised blend of ratio 80 : 20 following Hazaveh and Muhamad (2012). Different concentration of κC/NaCMC (0.1 - 1 % w/v) was prepared in hot water (80 °C) mixed with 0.5 g of lyophilised decoction (0.1 % w/v) and adjusted to final volume to 500 mL with distilled water and stirred until completely homogenised. The resulting mixture was then spray-dried using a lab plant spray-dryer (Triowin, China) equipped with a fluid atomiser (diameter of 10 mm) and an air compressor as well as a feed system for drying the gas consisting of a blower and an air filter. Experimental samples were fed at different feed flow rates (500 - 900 mL/h) and inlet temperature (110 °C, 130 °C, 150 °C) according to the experimental design. The outlet temperature was set at 90 °C and atomising air remained at a pressure of 0.3 psi. The spray-dried powder was collected and kept at air-tight plastics at 4 °C until further analysis.

2.4 Encapsulation Yield (EY)

The encapsulation yield was calculated as the ratio of the mass of the powder obtained at the end of the process to the mass of the initial substances added including the adjuvant and C. nutans extract (Krishnaiah et al., 2012).

$$EY\ (\%) = \frac{\text{Weight of powder after spray drying}}{\text{total weight of adjuvants and C. nutans extract added initially}} \times 100\ \% \quad (1)$$

2.5 Determination of Total Phenol Content and Total Flavonoids Contents

Folin–Ciocalteau method was used to determine total phenolic content (TPC) in sample extract (Waterhouse, 2002). Total flavonoid content (TFC) was determined using colorimetry of aluminium chloride method as described by Zhishen et al. (1999) with some modifications. The TPC and TFC was measured using UV/Vis spectrophotometer (Jenway 7305, USA). TPC was expressed as mg gallic acid equivalent per g dry mass of sample (mg GAE/g DM), while TFC was expressed as mg of quercetin equivalents (mg QE/g DM).

2.6 Determination of Free Radical-Scavenging Ability

The free radical scavenging activity of spray dried C. nutans extracts (non-encapsulated and encapsulated) was measured by using DPPH method according method of Abu Bakar et al. (2009) with slight modifications.

The antioxidant activity was expressed as mg of Trolox equivalents antioxidant capacity (TEAC) per g dry mass of sample (mg TEAC/g DM) as it showed accurate and descriptive expression than assays that express antioxidant activity as the percentage decrease in absorbance. The results provide direct comparison of the antioxidant activity with Trolox.

2.7 Identification and quantification of C-glycoside flavones by HPLC –UV/DAD

HPLC-UV/DAD analysis was performed on a Waters Alliance®e2695 (Millford, USA) system connected to 2998 PDA detector. The chromatographic separation was performed using a Waters XBridge™ C18 column (250 × 4.6 mm, 5 μm). The temperature of the column was set at 40 °C. Elution of standards and samples (10 μL) was performed with gradient solvent program, at a flow rate of 0.7 mL/min. The mobile phase consisted of 0.8 vol% glacial acetic acid in water (solvent A) and acetonitrile (solvent B) with following gradient: 5 – 19 % B (30 min), 19 – 95 % B (3 min), 95 % B (5 min), 95 – 5 % B (1 min), and 5 % B (3 min). The injection volume was 10 μL. Signal was monitored at 330 nm. Standards and samples for HPLC analysis were filtered through a 0.45 μm membrane filter (Millipore). For preparation of the calibration curve, standard stock solutions (1 mg/mL) of isoorientin, orientin, isovitexin, and vitexin was prepared in methanol and appropriately diluted (20 - 100 μg/mL) to obtain the desired concentrations in the quantification range. Identification of compounds was performed on the basis of the retention time, co-injections, and diode array spectral matching with standards.

2.8 Field Emission Scanning Electron Microscopy

The morphology of the spray-dried powder (non-encapsulated and encapsulated) were visualised using field emission scanning electron microscopy (SEM) (JEOL JSM-7600F, Japan). Samples were mounted on self-adhesive carbon sticky tape and gold coated before imaging (JFC-1600 auto fine coater operated at 20 mA for 120 s, Japan).

3. Results and Discussion

3.1 Effect of Spray Drying Conditions on Yield and Antioxidant Properties of C. nutans Powder

The encapsulation yield (EY), total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant activity (TAA) determined in C. nutans spray-dried powder obtained at different feed flow rate, inlet temperature and ratios of extract to coating agent are depicted in Figure 1. The higher EY was observed (Figure 1(a) and Figure 1€) at lower feed flow rate and at higher inlet temperature when using higher ratio of encapsulant. This could be related to the reduction of stickiness and agglomeration problems in the powders when κC/NaCMC was used as coating agent. The findings are supported by Gallegos-Infante et al. (2013) who used κC as sole coating agent to encapsulate the oak infusion extracts. This is contradictory to Tonon et al. (2008) who reported an increase in feed flow rate will result in lower process yield. They claimed that it is related to the slower heat and mass transfer occurring when the process was carried out at higher feed flow rates. These findings showed that different coating agents and their ratio to extract showed different pattern of feed flow rate. With increase in inlet air temperature, the higher yield was obtained (Figure 1(e)) at low feed flow rate which allowed the water from spray-dried sample to be vaporised completely in the enough time allotted, and the spray-dried powder can be dried sufficiently (Krishnaiah et al., 2012). As shown in Figure 1(b) - (d), at 700 mL/h feed flow rate, the highest TPC (53.4 ± 1.5 mg GAE/g DM), TFC (13.5 ± 1.2 mg QE/g DM) and antioxidant activity (AA) (4.2 ± 0.6 mg TEAC/g DM) were obtained. For inlet air temperature, increase in air temperature did not significantly affect the TPC, TFC and AA of spray-dried C. nutans (Figure 1(f) - (h)). Independent of the ratio extract to coating agent studied, the spray-drying of non-encapsulated and encapsulated C. nutans displayed small differences in term of TPC and TFC. At all ratios (1 : 1, 1 : 3, 1 : 5, 1 : 7 and 1 : 10) of Mcore/Mcoating, the percentage of DPPH scavenging activity decreased (Figure 1(h)) which could be caused by the release of active components from encapsulated C. nutans is hindered. However, at 1 : 5 ratio and inlet air temperature of 130 °C, the yield was higher and the TPC, TFC and DPPH was found to be 41.9 mg GAE/g DM, 11.6 mg QE/g DM and 1.25 mg TEAC/g DM respectively. It had been found that the increase in inlet temperature did not affect the TPC, TFC and AA values and significant differences only observed when different ratios of Mcore/Mcoating were applied. A high correlation was established between the TPC and TFC with TAA in all spray-dried C. nutans leaves extracts obtained by different feed flow rate and inlet temperature. It was observed that antioxidant activity (AA) increased proportionally to TPC and TFC. The AA increased proportionally to TPC and TFC with a correlation coefficient of feed flow rate (R^2_{TPC} = 0.6535; R^2_{TFC} = 0.8455), inlet temperature: (R^2_{TPC} = 0.9163; R^2_{TFC} = 0.9156). Other researchers also reported good linear correlation between these values (Tamuly et al., 2013) indicating that the radical scavenging activity of plant extracts depends on the amount of phenolic and flavonoid compounds in the extracts.

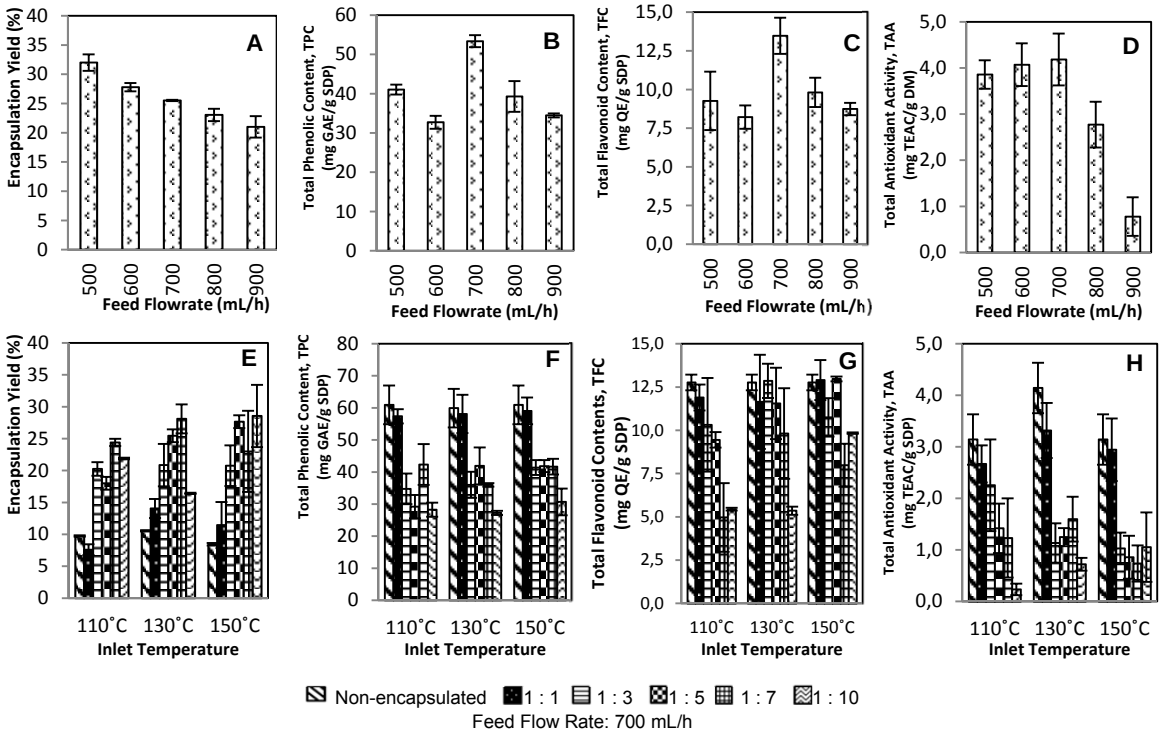

Figure 1: (a) - (d) Effect of feed flowrate and (e) - (h) effect of inlet temperature and different volume ratio of extract to carrier agent towards encapsulation parameters of spray-dried C. nutans powder (SDP)

3.2 Effect of Spray Drying Conditions on Four C-glycoside Favones of C. nutans Powder

The retention time, regression equation and correlation coefficient of each marker are summarised in Table 1. Four C-glycoside flavones (1 - 4) that might contribute to the antioxidant behaviour of the C. nutans were identified and quantified in all spray-dried C. nutans leaves extracts. Quantification was carried out by integration of the peak using an external standard method as presented in Figure 2. The feed flow rate did not exhibit significant differences to all four C-glycoside flavones compositions. The inlet temperature and ratio of Mcore/Mcoating differed significantly as increase in temperature resulted decrease in the amount of C-glycoside flavones. The distribution of C-glycoside flavones in all spray-dried C. nutans was mainly vitexin (14.4 to 23.5 µg/mL) followed by isoorientin (2.9 to 22.4 µg/mL), isovitexin (1.9 to 6.4 µg/mL) and orientin (1.5 to 5.1 µg/mL). The varied composition of four individual C-glycoside flavones in all spray-dried samples was depended on the spray drying conditions such as feed flow rate, inlet air temperature and concentration of coating agent used. The inlet temperature of spray drying seems to have no significant effect to the concentration of other compounds (isovitexin, isoorientin, orientin) than vitexin could be attributed to the higher boiling point properties of other compounds. Therefore, it can be concluded that the spray drying conditions does not significantly affects the composition of C-glycoside flavones in spray-dried powder of C. nutans.

Table 1: Retention time and regression equations for four C-glycoside flavone compounds: isoorientin, orientin, isovitexin and vitexin

Peak no.	Compound	R_t (min)	Regression equations	Correlation coefficient (R^2)
1	Isoorientin	29.12	y = 36,659x – 49,973	R^2 = 0.9990
2	Orientin	30.24	y = 31,605x – 30,533	R^2 = 0.9995
3	Isovitexin	32.92	y = 42,969x – 48,449	R^2 = 0.9972
4	Vitexin	33.29	y = 12,370x – 17,3439	R^2 = 0.9985

*y = mx + c, where x is concentration in µg/mL and y is area under curve at UV 330 nm wavelength.

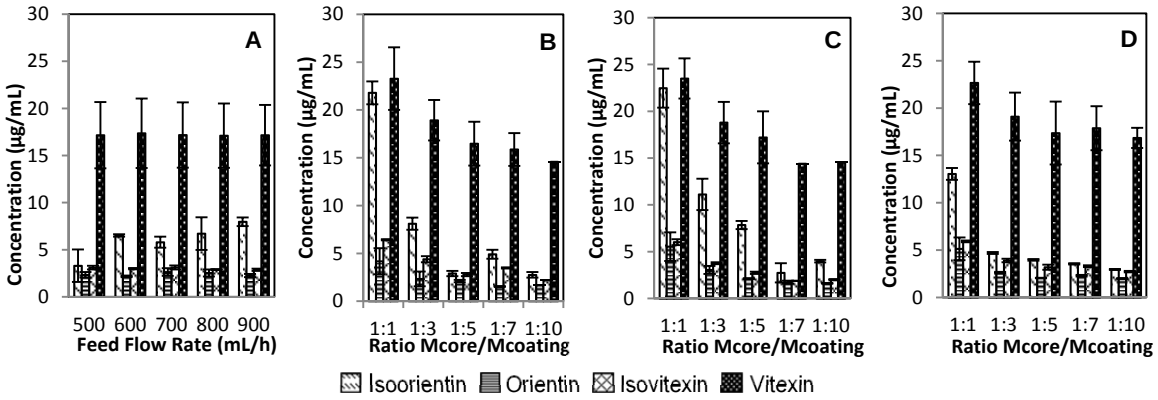

Figure 2: Effect of spray drying conditions on four C-glycoside flavones compositions in C. nutans powder on (a) feed flowrate and on inlet temperature at (b) 110°C (c) 130°C (d) 150 °C

3.3 Morphology and Particle Sizes

Figure 3 shows the FESEM micrographs of spray-dried non-encapsulated and encapsulated C. nutans powder produced at ratio 1 : 5 of extract to κC/NaCMC at inlet temperature 130 °C with feed flow rate 700 mL/h. It was observed that κC/NaCMC, is an important coating agent for promoting the formation of spherical and smooth-surfaced microparticles which encapsulated and protected the bioactive compounds. Similar findings were also reported by Krishnaih et al. (2012). Non-encapsulated C. nutans showed agglomerated particles which support by the stickiness after being exposed to air surrounding which showed hydroscopic properties (data not shown) (Mishra et al., 2014). FESEM study revealed that the average size of particles in the spray-dried powder of C. nutans for non-encapsulated (diameter of 7.33 - 25.9 µm) was larger than encapsulated (diameter of 2.71 - 5.88 µm).

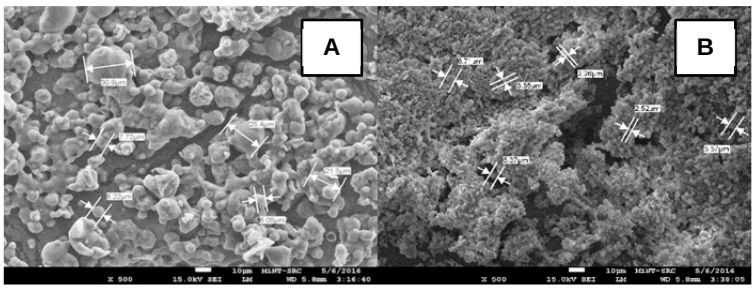

Figure 3: FESEM of spray-dried powders of C. nutans (a) non-encapsulated and (b) encapsulated using κC/NaCMC (1 : 5) at 130 °C with feed flow rate 700 mL/h at magnification 500x

4. Conclusions

The highest antioxidant activity, C-glycoside flavones and yield was observed in spray-dried C. nutans microspheres obtained at feed flow rates (700 mL/h) using 1 : 5 ratio of extract to coating agent and at 130 °C inlet temperature. The encapsulated powder presented better properties in the preservation of antioxidant capacity, powder morphology and resultant particle sizes as compared to the non-encapsulated (uncoated) C. nutans extracts. Good correlation was observed between antioxidant capacity, total phenolic content and total flavonoid of spray-dried powder of C. nutans.

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