Effect of potato starch on the antioxidant properties of morinda citrifolia spray dried micro particles

Abstract
The aim of this study was to evaluate the influence of spray drying conditions on Morinda Citrifolia (Mengkudu) micro particles using potato starch (2wt%) as the encapsulation or coating agent by different ratios, Mcore/Mwall (1:1, 1:2 and 1:4) at different inlet temperatures (90, 100, 110, 120, 130 and 140°C). Evaluation on the spray dried Mengkudu powders was done using DPPH radical, total phenolic and particle size analysis. From the results, it is clear that the DPPH radical scavenging activity and total phenolic content was slightly higher at 120°C for ratio 1:1. The results indicate that the mean size of micro particles obtained was in the range of 1-25μm.

Keywords: morinda citrifolia, spray drying, antioxidant activity, potato starch.

Introduction
Antioxidants are the chemicals that neutralize reactive oxygen species. These are the secondary metabolites of phytochemicals and are preferred over synthetic antioxidants which are found to impose side effects. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases6–8 and health problems.9

According to World Health Organization,7 “traditional medicine” is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses. This study is focused on the Morinda Citrifolia because it is known as one of the best traditional medicine throughout the nations.

Morinda citrifolia is native to Southeast Asia; it is widely adapted throughout the tropics. The Morinda citrifolia trees are typically 3-6m (10-20ft) tall when it is mature. Morinda citrifolia is a small tree with avate glossy leaves 15-20cm long. The fruit technically known as a syncarp is yellowish white, fleshy, soft and pungent smell when ripe. Known as mengkudu in Malaysia, Morinda citrifolia is commercially known as “Noni”, is also called “Indian Mulberry”, “awl tree”, “Ba Ji Tian”, “Nono” or “Nonu”, “Cheese Fruit”, and “Nhau” belong to the Rubiaceae family, Morinda Citrifolia is one of the most significant sources of traditional medicines among Pacific island societies. All parts of the plants, which include fruits, leaves, bark and roots, have been shown to contain active compounds that have high medicinal values. Research has revealed the potential of Mengkudu as a source of various antioxidants in roots, fruits and leaves (Zin et al., 2004). It is used in traditional and/or modern application, including root and bark (dyes, medicine), trunks (firewood, tools), and leaves and fruits (food, medicine) although most of these have yet to be scientifically supported. Fruit, leaf and root are useful in cardiovascular diseases particularly hypertension, atherosclerosis and dyslipidemia10 while seed may have a role as a useful ingredient in cosmetics for skin-whitening and/or wrinkle prevention.11

In order to meet the demand of the market throughout the year in all areas, Morinda Citrifolia is perishable and cannot be marketed as fresh products. One of the common techniques to handle this problem is microencapsulation process by using drying method i.e. spray drying, spray-chilling, air suspension coating, extrusion, centrifugal extrusion, freeze-drying, coacervation, rotational suspension separation, co-crystallization, liposome entrapment, interfacial polymerization, molecular inclusion.

Microencapsulation is the technique by which the sensitive ingredients are packed within a coating or wall material. The wall material protects the sensitive ingredient (or core) against undesirable reaction, avoids the loss of volatile ingredient, controls release of the ingredient, enhance the productivity, to increase shelf life of food, and control moisture content so that the food becomes stable to both microbial and chemical deterioration.

Additives or coating agents are the non-active ingredients, essential in the successful production of acceptable solid dosage forms such as tablets and powders. The type of starch utilized depends on the physical and chemical characteristics of the starch, the amount used, the type of dryer used and the end use of the product. In the process of spray drying, drying aid is normally added as coating material. Effectiveness of coating material should contains functional properties, including good emulsification, film forming, high solubility, low viscosity at high concentrations and low cost properties. The coating material can be derived from any starch source such as cereal grains, i.e., corn, waxy corn, wheat, sorghum, rice; tubers or roots of such plants as cassava (tapioca), potato or arrowroot and the pith from the sago palm). The aim of this study is the evaluation on the effect of potato starch on the antioxidant properties of Morinda Citrifolia spray dried micro particles.

Experimental
Preparation of plant extracts
The Morinda Citrifolia fruits are obtained at ripe stage as shown in Figure 1. The fruits were washed with distilled water. The fruits were separated into pulp and seed. Then they were sun dried for 2days.
then oven-dried at 60°C for 1 day to remove all moisture content and subsequently ground into finely powdered by using mixer. The powder is kept for storage at 40°C. Pulverized sample of 20g was added with 200ml of ethyl acetate inside a 250ml conical flask and left in shaking incubator for 3 days at the temperature of 37°C. The solution was filtered using Whatman filter paper and the filtrate portion was kept at 4°C for further analysis.

**Figure 1 Ripe Mengkudu fruits.**

The drying was carried out in a spray dryer model SD-05 (Labplant, UK), with con-current flow regime. The drying chamber has diameter of 215mm and height of 500mm. The main components of the system were the feed system of the mengkudu extract, comprised of a peristaltic pump, a two fluid atomizer (inlet orifice diameter of 0.5mm) and an air compressor; a feed system of the drying gas, comprised of a blower and a air filter; a temperature control system of the drying gas and a product collect system (cyclone) with diameter 0.085m. The pump speed was set to fairly slow rate, between 15 to 30ml/h and the compressor air pressure of 1.2bar. After spray drying, the powders were collected through a high efficiency cyclone in a glass container, transferred in to a glass vial and stored in the desiccators at ambient temperature. The spray drier was operated at six different temperatures between 90 to 140°C, the yield of mengkudu powder was 6%.

**Preparation of emulsion and spray drying**

The concentration of potato starch is set at 2w/v%. The concentration of potato starch was chosen to be 2% w/v% due to its viscosity in the solution. If the solution of feed is not adequately viscous, the spray drying powder may not come out at the collector bottle. The starch was mixed evenly with distilled water based on the ratio of M<sub>core</sub>/M<sub>wall</sub> (M<sub>core</sub>=mass of core, M<sub>wall</sub>=mass of wall) makes the volume of the solution 400ml. During the addition of potato starch, 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 the starch was set to 400ml with selected of M<sub>core</sub>/M<sub>wall</sub> ratio: 1:1,1:2, 1:4 at temperature of 40 to 50°C.  

**DPPH radical scavenging activity**

DPPH is a stable free radical that reacts with compounds, which are able to donate a hydrogen atom. The effect of the microcapsules extract as an antioxidant source was estimated using free-radical scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) radical. The determination was based on the reported method. An aliquot (10µl) of powder extract solution was mixed with 90µl of distilled water and 3.9µl of 25mM DPPH methanolic solution. The mixture was shaken vigorously and left in the dark at room temperature for 30min. Then, the hydrogen donating ability of the microcapsules to DPPH was determined by the decrease absorbance of the DPPH solution was evenly mixed measured at 515nm against blank methanol with spectrophotometer. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity.

The different in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical. The results were expressed as percentage of inhibition of the DPPH according to Eq 1:

\[
DPPH (\%) = \frac{Abs \; control - Abs \; sample}{Abs \; control} \times 100
\]

Where, DPPH is the radical scavenging activity of antioxidant, 
Abs control is the absorbance of DPPH solution without extract and 
Abs sample is the absorbance of DPPH with sample taken after 60min.

**Total phenolic content**

Total Phenolic Content (TPC) was determined according to the Folin Ciocalteau (FC) method. Sample in methanol solution was prepared by dissolving 10mg of spray dried powder in 30ml of methanol and centrifuge for 10min using Sartorius Sigma 3-18K Centrifuge. The supernatant of sample extract (0.5mL) was added to 1.8mL of FC reagents (1:9) and allowed to react for 5min. Later 1.2mL of 7.5gL<sup>-1</sup> of sodium carbonate was added to the reaction mixture. Finally, the reaction mixture was incubated for 2h at room temperature and adsorbance was measured at 765nm using 4802 UV-VIS double beam spectrophotometer. Methanol was used as reference. Gallic acid (0-1.0mgL<sup>-1</sup>) was used to produce standard calibration curve. The total phenolic content was expressed in mg of Gallic acid equivalents (GAE/g of spray dried powder). A 4802 UV-VIS double beam spectrophotometer is used to measure the total phenolic content of Mengkudu powder in the form of mixture/solution. In order to read the absorbance, the wavelength was set at 765nm. To measure the absorbance, firstly distilled water is measured as blank, which is taken as zero absorbance. Each of the diluted extraction samples is measured within a glass cuvette and the absorbance is measured in triplicate to ensure precision. The average readings are thus taken as the absorbance value.

**Particle size distribution**

The microcapsule was observed by a microscope with image analyzer (MIA). To get a mean size and size distribution, individual microcapsules were selected randomly and accurate measurements were taken for statistics analysis.
Results and discussion

Antioxidant activity

In this study, ratio $\frac{M_{\text{core}}}{M_{\text{wall}}}$ is defined as the volume of ratio of Mengkudu extract against wall material (2% potato starch). Figure 2 shows a similar trend was obtained at these three ratios $\frac{M_{\text{core}}}{M_{\text{wall}}}$. At temperature of 90°C, the inhibition percentage is high and then it will decreased at temperature of 100°C and at 110°C it will increased until temperature of 120°C, and lastly it will fall until the temperature reach 140°C. Among all temperature the highest antioxidant activity was measured at 120°C and 90°C followed by 130°C and 140°C respectively.

![Figure 2 DPPH (%) vs. temperature (°C) at different ratios.](image)

To compare among $\frac{M_{\text{core}}}{M_{\text{wall}}}$ ratio, the percentage of DPPH inhibition is significantly higher at ratio 1:1 followed by 1:2 and 1:4 respectively. During the drying at higher temperature leads to degradation of antioxidative compounds and also possibility of losses of volatile substance.16 However, as mentioned by Krishnaiah et al.,15 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.

Total phenolic content

The contents of total phenolic compounds in Mengkudu powder is shown in Table 1. As shown in Figure 3, the ratio of $\frac{M_{\text{core}}}{M_{\text{wall}}}$ 1:1 exhibits the highest total phenolic content compared to 1:2 and 1:4. The ratio of $\frac{M_{\text{core}}}{M_{\text{wall}}}$ at 120°C is the highest total phenolic content at 54.575mg GAE/100g extract. TPC shows the lowest content when dried at higher temperature. At this condition, there are possible losses or degradation of certain types of phenolic compounds.17 As the temperature increases, the value for total phenolic content also decreases, may due to some components which are ready to get volatilized may attach to the binding material.

Particle size analysis

Table 1 show the mean diameter at different temperatures and ratio of microparticles. The increase on inlet air temperature results in larger particle size. Higher temperature results in faster drying rates due to the swelling of droplets produces larger particles than drying under lower temperature in which slower drying rate could be found. This is due to the fact that when inlet temperature is high, very fast drying sets up a structure early and does not permit the particles to shrink during drying. When inlet air temperature is low, the particle remains more shrunk and thus, results in smaller diameter.

Higher starch concentration also leads to the production of larger particles. This correlation maybe due to the feed viscosity used, which increased with wall concentration. Similar results obtained by Krishnaiah et al.,9 in the production of Mengkudu powder a range of temperature between 110 to 150 degree Celsius. This result also similar to the result obtained by Souza et al.18

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<th>Table 1 DPPH (%), TPC (mg GAE/100 g powder) and diameter of micro particle (µm) obtained at different spray drying conditions</th>
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<td>Ratio ($\frac{M_{\text{core}}}{M_{\text{wall}}}$)</td>
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Figure 3 TPC at different ratio with different temperatures.

Figure 4 shows that the mean particle size decreases with increasing core/wall ratio from 1:1 to 1:4. Similar results were obtained. Increasing $M_{\text{core}}/M_{\text{wall}}$ ratio means that the concentration of the potato starch is higher thus obtaining higher feed viscosity. Therefore, it leads to the larger the droplets formation during atomization and thus the larger the particles are obtained during spray drying.

Figure 4 Mean particle diameter (µm) vs. Temperature (°C).

Conclusion

The work presented in this paper can be concluded that potato starch can be used as encapsulation in the Mengkudu micro particles. Different ratio were used to prepare the fine particles and showed a significant effect on all the responses studied. For the extracts containing volatile components the major issue affecting the antioxidant content is drying temperature, as well as in sample handling and storage. Drying at higher temperature has decreased the antioxidant contents of extract.

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None.

Conflict of interest

The author declares no conflict of interest.

References