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Spray drying of Mengkudu herbal extract: Effect of wheat flour

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Abstract. The aim of this study is to determine the antioxidant activity, total phenolic content and total flavonoid content of Mengkudu, and the mixture of Mengkudu with wheat flour. Three major processes are involved in this research such as extraction of Mengkudu, spray drying of Mengkudu extract and the mixture of Mengkudu with wheat flour at 140°C. From results, it was found that the phenolics and flavonoid compounds are present in concentrated Mengkudu extract (CME) and due to these compounds the antioxidant activity of CME was increased. The total phenolic and flavonoid content in the mixture of concentrated Mengkudu extract with wheat flour (MCMEWF) and also in the mixture of spray dried Mengkudu extract with wheat flour (MSDMEWF) are lower than in CME but antioxidant activity of these mixtures was higher compared to antioxidant activity of their individual components. This shows that antioxidant compounds in the mixture exhibit synergistically to donate the hydrogen to DPPH radicals thus increased the antioxidant activity.

1.0 Introduction

Epidemiological studies have shown that regular consumption of whole grains and fruits reduce the risks of various types of chronic diseases such as cardiovascular disease, type 2 diabetes, and cancer [1]. There are various types of phytochemicals found in fruits and grains that help to prevent these kinds of diseases. Phytochemicals are the bioactive, non-nutrient, naturally occurring plant compounds found in fruits, vegetables, and also in whole grains [2,3,4]. The major groups of phytochemicals that may contribute to the total antioxidant capacity (TAC) of plants and foods include polyphenols, carotenoids and traditional antioxidant vitamins such as vitamin C and vitamin E. These phytochemicals may be present in small amounts but are very important to the health of consumers [5]. In the whole grain, the phytochemicals that present consist of phenolic compounds containing one or more aromatic rings and one or more hydroxyl groups carotenoids, and vitamin E, amongst others. Nevertheless, the phenolic content of whole grains had been underestimated, as most of the research only determined the free phenolic content and not the content of phenolics that are bound to cell wall materials [6,7].

Mostly fruits were extracted due to the high antioxidant activities in the fruits. The examples of fruits that are commonly used are sugar beet, cherry, oranges, noni, tamarind, longan, guava and etc. There are also some fruits that can be considered as herbal plant or medicinal plant. These types of plants have medicinal value in the relief and treatment of various ailments [8]. A medicinal plant typically contains a complex mixture of hundreds or more chemical compounds, e.g. alkaloids, glycosides, and flavonoids. For analysis of such a complex mixture, analytical technique with strong separation capability is needed [9].

Due to the useful compounds contain inside the fruits and grain, the researchers have done many fruits and grain extraction to study the properties of these compounds. The correct parameters used in the extraction process definitely will give the good result for extraction. The combination of



phytochemicals in a fruit can give a significant benefits in health but the combination of phytochemicals from different fruits may give different result. Multiple phytochemicals combinations may result in synergistic activity that increases their bioavailability and their action on multiple molecular targets, thus offering advantages over treatments with single chemical [10]. Synergisms refers to phenomena where the combined effect of independent processes is significantly different from the individual effects considered separately [11]. Many reaserchs were done to determine the synergistic effect of chemical constituents that found in the fruits. The additive and synergistic effects of these biologically active components inside the fruits and grains may be responsible for the health benefits [10]. Objective of this work was to study the spray drying of herbal plant extract on synergistic effect of Mengkudu with wheat flour [13,14].

2.0 Methods

2.1 Preparation of Mengkudu extracts

Mengkudu fruits were washed with tap water followed by washing with distilled water. Then these fruits were cut into small pieces and dried below the sunlight for three days. To remove the moisture that still contained in the dried fruit, it was kept at 65°C in a hot air oven for one day. The extraction of Mengkudu fruits followed the literature procedure with slight modifications [15-17]. Then dried fruit was finely powdered using a mixer. 25 g of powdered sample and 250 ml of ethyl acetate was mixed. This mixture was put in a water shaker bath at 45°C in three days for extraction. After three days, the Mengkudu extract was filtered using filter paper which is Whatman number 1 to separate the supernatant from the residue. The supernatant was put in the oven for one day at 50°C to get concentrated Mengkudu extract. The Mengkudu extract was taken from the oven and stored in a closed container and kept at 5°C in refrigerator before being analysed [18].

2.2 Spray drying of the mixture of Mengkudu extract with wheat flour

100 ml of Mengkudu extract (which equal to 1.5 g) was mixed with 1.5 g of wheat flour that dissolved in 15 ml ethyl acetate to form a mixture. The resulting mixture was stirred continuously before and during the spray drying process to ensure that the samples are fully mixed. A lab scale spray dryer SD-05 with co-current flow was used to spray dried the mixture and in the spray drying process the the inlet gas temperature, feed flow rate and flow rate of drying air were kept constant at 140°C, 250 ml/h and 60 m³/h respectively. The atomising air is set at a pressure of 1 bar. The same procedure was followed for Mengkude extract with ethlyacetate as solvent.

2.3 Encapsulation yield (EY)

The encapsulation yield was calculated as the ratio of the mass of the microcapsules obtained after spray drying to the mass of the mixture of Mengkudu power with wheat flour [15].

$$EY\% = \frac{\text{the weight of microparticles after spray drying}}{\text{Total weight of adjuvants and weight of Mengkudu powder added initially}} \times 100 \quad (1)$$

2.4 DPPH radical scavenging activity

DPPH is a stable free radical that reacts with compounds and able to donate hydrgen atom. Thus, the hydrogen donating ability of concentrated Mengkudu fruit extract, the mixture of Mengkudu with wheat flour and spray-dried Mengkudu fruit extract were determined from the change in absorbance at 515nm [19]. To measure the free radical scavenging activity, the samples in methanol solution were

prepared by dissolving 10 mg of spray-dried powder in 30 ml of methanol and centrifuging for 10 min using a Sartorius Sigma 3-18 K centrifuge. After that, aliquots of supernatant were added to 3 ml of 0.025 g/l DPPH in methanol. After 38 minutes the change in absorbance was determined by using a 4802 UV- VIS double-beam spectrophotometer. Here, methanol was used as a reference. Besides, DPPH (0–100 mg/l) was used to obtain a standard calibration curve. The percentage of DPPH radical scavenging activity was calculated by using the following equation.

$$\text{DPPH scavenging activity (\%)} = \left[1 - \frac{\text{Abs}_{515} \text{ sample}}{\text{Abs}_{515} \text{ DPPH solution}} \right] \times 100 \quad (2)$$

2.5 Total phenolic content

The total phenolic content was determined with Folin Ciocalteu reagent [17]. Briefly, the samples in methanol solutions were prepared by dissolving 10 mg of spray-dried powder in 30 ml of methanol and then the mixture was centrifuged for 10 min using a Sartorius Sigma 3-18 K centrifuge. Then, 2.5 ml of the 0.2 N Folin Ciocalteu reagent was mixed with the supernatant of the sample extract (0.5 ml) and let it react for 5 minutes. After 5 minutes, 2 ml of 75 g/l sodium carbonate was added to the reaction mixture and diluted to 25 ml using distilled water. Lastly, the reaction mixture was incubated for 2 hours at room temperature and then the absorbance was measured at 760 nm using a 4802 UV- VIS double-beam spec-trophotometer. Here, methanol was used as a reference. Tannic acid (0–100 mg/l) also was used to produce a standard calibration curve. In this work, the total phenolic content was expressed in mg of tannic acid equivalents (TAE/g of spray-dried powder).

2.6. Total flavonoid content

The total flavonoid content was determined using the Dowd method as adopted by Krishnaiah et al. [2]. In order to measure the total flavonoid content, a total of 5 ml of 2% aluminium trichloride (AlCl_3) in methanol was mixed with the same volume of the extract solution (0.4 mg/ml). After 10 minutes the absorption at 415 nm readings were taken by using a UV-VIS double-beam spectrophotometer against a blank sample which consists of a 5-ml extract solution with 5 ml of methanol without AlCl_3 . The total flavonoid content can be determined by using catechin (0–100 mg/l) reagent as the standard and the flavonoid content was expressed as mg of catechin equivalents (CE/g of extract).

3.0 Results and Discussion

3.1 Spray-drying of the samples

In this study, two samples were undergone spray drying process such as concentrated Mengkudu extract (CME) and also the mixture of concentrated Mengkudu extract with wheat flour (MCMEWF). During spray drying process the samples were stirred continuously with wheat flour so that the homogenous solution is formed. The inlet temperature of the spray dryer and the feed flowrate were set at 140°C , 318 ml/h respectively.

The powder at the chamber wall were taken by using brush and then the samples were analysed. Table 1 shows that the encapsulation yield (EY) value of spray-dried Mengkudu extract is low which is 2.5% compared to the EY value of the mixture of Mengkudu extract with wheat flour which is 4.5%. The EY values of both samples can be classified as low because both products of the samples were accumulated at the chamber wall due to high moisture content of particles. The wet powders easily can stick at the chamber wall thus affects the EY value.

Table 1. Encapsulation yield of the samples after spray drying

Samples, Spray dried	Weight of micro - particles, g	Weight of adjuvant, g	The weight of wheat flour, g	Mengkudu powder, g	Encapsulation yield (%)
Spray dried Mengkudu extract	0.15	3	-	3	2.5
Spray dried Mengkudu + wheat flour	0.27	3	1.5	1.5	4.5

3.2 Antioxidant activity

DPPH assay is simple and inexpensive method that involve the use of 2,2,-diphenyl-1-picrylhydrazyl (DPPH) to measure the antioxidant activity of foods. DPPH is a free radical that is used to test the ability of the compound to act as a free radical scavenger or hydrogen donors and to determine the antioxidant activity of the foods. This method is based on the decrease in absorbance of DPPH free radical at 515nm due to the action of antioxidants. In this study the DPPH scavenging activity of Mengkudu extract, and the mixture of Mengkudu with wheat flour were measured and the results of the assay were expressed in percentage (%) of inhibition of DPPH as shown in Figure 1. From the results we can see that the DPPH radical scavenging activity increased in the following order CME>MCMEWF>MSDMEWF>WF>SDME. CME has the highest radical scavenging activity which means that the antioxidant compounds in the sample has highest activity to trap the free radicals or to donate the hydrogen to the free radicals .

CMEWF and SDMEWF have slight difference in percentage of DPPH radical scavenging activity and this is same when compared to the SDMEWF with WF. SDME showed the least DPPH radical scavenging activity which is only 20.05%. By comparing the percentage of DPPH radical scavenging activity of several samples, we can deduce that more antioxidant compounds present in the CME rather than in SDME although both samples are from the same fruit. The decrease in the percentage of DPPH scavenging activity may be due to increase in amount of active components volatilization or deterioration during the spray drying process.

The hydrogen donating activity was higher in CME rather than in WF but when both samples are mixed together, it showed higher DPPH scavenging activity compared to wheat flour. MCMEWF is higher in radical scavenging activity when compared to WF. But when comparing WF with MSDMEWF the difference in radical scavenging activity is not high. MCMEWF and MSDMEWF were higher in radical scavenging activity than WF. Therefore this proves that a large variety of compounds in those mixers act synergistically to trap free radicals.

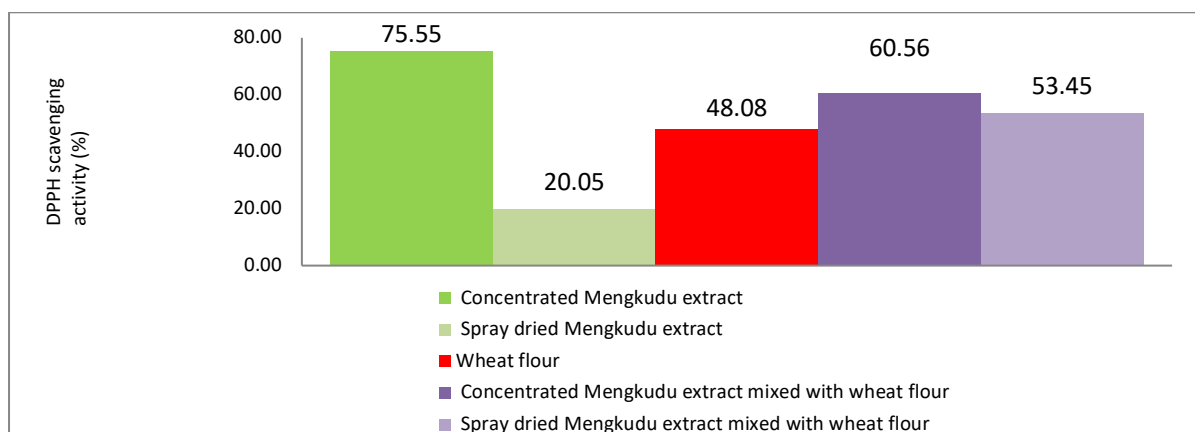


Figure 1. DPPH scavenging activity of Mengkudu extract, wheat flour and the mixtures of Mengkudu with wheat flour

3.3 Total phenolic content

Folin-Ciocalteu method was used in the determination of total phenolic content since many individual phenolic compounds which provide antioxidant activity in fruits cannot be identified and measured by HPLC methods. In this study, the quantitative estimation of the phytochemical constituents of CME, SDME, MCMEWF, MSDMEWF and WF were analyzed. Figure 2 shows that the grain product (wheat flour) and medicinal herb (Mengkudu) are rich in total phenols. In this figure CME showed the highest total phenolic content which is 91.67% followed by MCMEWF, MSDMEWF, WF at 67.7%, 45.63%, 38.97% respectively and lastly only 22 mg of TAE/g of SDP of phenolic compounds present in the SDME. There are more phenolic compounds in CME than in SDME. The low content of phenolic compounds in SDME is due to the degradation of antioxidant compounds during drying. The total phenolic content for both CME and WF were compared and it showed that the total phenolic content in CME is greater than the total phenolic content in WF. It is well known that phenolic compounds are highly effective free radical scavenging and antioxidants. The phenolic compounds also rich in MCMEWF and MSDMEWF thus increased the antioxidants activity.

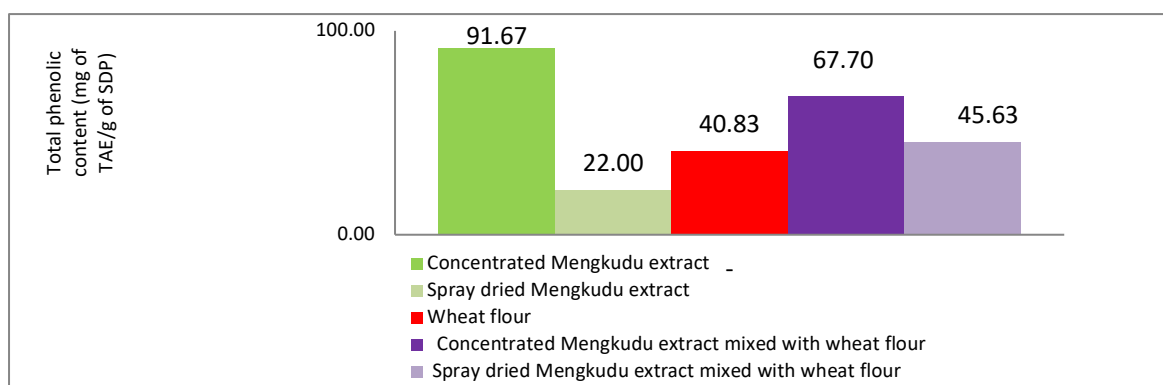


Figure 2. Total phenolic content of Mengkudu extract, wheat flour and the mixture of Mengkudu with wheat flour

3.4 Total flavonoid content

Flavonoids are highly effective free radical scavenging and antioxidants which are commonly used for the prevention and cure of various diseases that are mainly associated with free radicals. The mechanisms of action of the flavonoid compounds are through scavenging or chelating processes [20,21]. In order to quantify the total flavonoids in the samples the Dowd method has been used. In this method Catechin was used as a standard component.

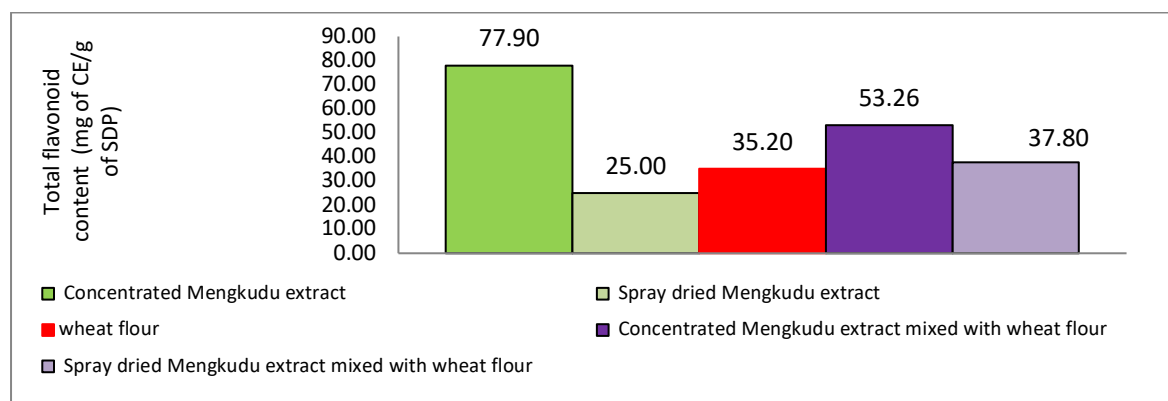


Figure 3. Total flavonoid content of Mengkudu extract, wheat flour and the mixture of Mengkudu with wheat flour

From the Figure 3, the samples that contain flavonoids content are in the following order CMF> MCMEWF> MSDMEWF> WF> SDME. Total flavonoid content was significantly higher in CME and less amount of flavonoid compound in SDME. MCMEWF has higher flavonoid compounds than WF but lower when compared to CME. This is same when we compare MSDMEWF with WF and CME.

3.5 Particle Size

The particle size of the samples were determined by using the particle size analyzer. The samples are dissolved in distilled water before we put into that equipment. From Table 2, it can be seen that the mixture of spray-dried Mengkudu extract mixed with wheat flour has large particle size which is 280.8nm compared to other samples. The smaller particle size of samples is wheat flour which is 113.1nm.

Table 2. Particle size of samples

Samples	Particle size, radius (nm)
Mengkudu powder	138.1
Wheat flour	113.1
The mixture of Mengkudu powder before spray drying mixed with wheat flour	199.3
The mixture of spray-dried Mengkudu extract mixed with wheat flour	280.8

4.0 Conclusion

It can be concluded that more phenolics and flavonoids compound present in CME and due to these compounds the antioxidant activity of CME was increased. The total phenolic and flavonoid content

in MCMEWF and MSDMEWF are lower than the CME but antioxidant activity of these mixture were higher compared to their individual components. This showed that antioxidant compounds in the mixture exhibit synergistically to donate the hydrogen to DPPH radicals. In this study, we can deduce that the antioxidant activity increases as the phenolics and flavonoids increase. From the result, the encapsulation yield of the mixture of Mengkudu powder with wheat flour is higher compared to individual components which is 4.5% and the larger particle size of the samples is the particle size of the mixture of spray dried Mengkudu extract with wheat flour which is 280.8 nm.

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