

Spray Drying of *Morinda Citrifolia* L. and *Beta Vulgaris* L. Fruit Extract and Its Synergistic Effect

Duduku Krishnaiah, Kong Bit Hiaw, Rosalam Sarbatly, S. M. Anisuzzaman, and Rajesh Nithyanandam

Abstract—This study was to examine the antioxidant potential of *Morindacitrifolia* L. and *Beta vulgaris* L. fruits and its synergistic effect. The bioactive components of these fruits were extracted and then microencapsulated by spray drying technique. The antioxidant activities of these fruits were analyzed, before and after the spray drying process through DPPH radical scavenging activity, total phenolic content and total flavonoid content. The encapsulation yield of the spray drying process was also quantified. Among the spray drying samples encapsulation yield was found maximum 8 % for the mixed fruit extract. Similarly, synergistic effect between the two fruits showed the highest antioxidant activity of 30% and the individual fruit extract also exhibited significant antioxidant activity.

Index Terms—Antioxidant potential, *Morindacitrifolia* L., *Beta vulgaris* L., DPPH radical scavenging activity.

I. INTRODUCTION

Under stress our body produces more reactive oxygen species (ROS) than enzymatic and non-enzymatic antioxidants. This imbalance leads to cell damage. Therefore this reactive species are responsible for the generation of different diseases such as heart disease, cancer and aging problems[1]. Hence there is a need of antioxidant which can impede the ROS. Antioxidants are classified into synthetic and non-synthetic antioxidants. Even though synthetic antioxidants such as butylatedhydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used in the food processing industry, researchers found that they may be responsible for liver damage and carcinogenic [2], [3]. Hence it leads to the search of natural antioxidants which are abundantly available in natural source such as plants.

Morindacitrifolia L. is the second most popular plant used as food and medicine by the Polynesians for over 2000 years, when they migrated from Southeast Asia. They use it to treat various diseases, such as arthritis, diabetes, high blood pressure, headaches, muscle aches, etc. *Morindacitrifolia* L. fruit contains a great number of antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, flavonoids, carotene, rutin, etc.[4]. *Beta vulgaris* L. is reported to possess antioxidant properties as it contains nitrogen compounds called betalains that give the red-violet

colour to the fruit [5]. Reference [6] proved that *Beta vulgaris* L. juice displayed the highest antioxidative property among the other 23 vegetable juices studied.

Antioxidants are highly effective in synergism because of the bioactive components present and their interaction. Furthermore different antioxidants scavenge different free radicals and responsible for recovering different parts of body cell [7]. Therefore, the combination of *Morindacitrifolia* L. and *Beta vulgaris* L. may establish a greater antioxidant potential. Hence, this study was proposed to evaluate the antioxidant activity of *Morindacitrifolia* L. and *Beta vulgaris* L. and to investigate the synergistic effect of these two fruits.

II. MATERIALS AND METHODS

A. Chemicals

Methanol and ethyl acetate (HPLC grade) were obtained from J.T. Baker (USA). FolinCiocalteu reagent and tannic acid (TA) were purchased from Merck, Germany. The chemicals 2,2-diphenyl picrylhydrazyl (DPPH), and sodium carbonate were obtained from Sigma Chemicals (St. Louis, USA). All other chemicals were of reagent grade and were used without further purification.

B. Sample Preparation

Morindacitrifolia L. and *Beta vulgaris* L. were obtained from Kota Kinabalu, Sabah. The fruits were washed with tap water followed by distilled water and then peeled. The core of the fruits was cut into small pieces. After that, the fruits were dried in a hot-air oven operated at 50 °C until the mass of the fruits became constant. The dried fruits were pulverized by using a mixer grinder (mixie).

C. Solvent Extraction

15 g of pulverized fruit sample was added with 150 mL of ethyl acetate (10 wt%) in a conical flask. The mixture was shook in a water bath shaker (100 RPM) at 45 °C for three days. After three days, the mixture was filtered by using Whatman no. 1 filter paper to separate the supernatant from residue. The filtered extract was stored in a closed container and kept at 4 °C before being analyzed.

D. Spray Drying of Fruit Extracts

κ -Carrageenan was added with distilled water to form 0.5 wt% of carrageenan solution. The solution was well mixed by heating up to 80 °C until the carrageenan powder was fully dissolved in the distilled water. Then, the 0.5 wt% carrageenan solution was mixed with the fruit extract in a 1:1 mass ratio. The mixture was fed into a lab-plant spray-dryer SD-05 (pilot scale, Buchi, UK). The spray drying was performed co-currently, and the inlet temperature, feed flow

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rate and air flow rate were set at 140 °C, 250 ml/hr and 60 m³/hr respectively. After spray drying process, the spray-dried powders were collected and then weighed. The powders were stored in desiccators at room temperature.

E. Encapsulation Yield

The encapsulation yield (EY) was calculated as the ratio of the mass of microcapsules obtained after spray drying to the total mass of the initial substances added (carrageenan and raw powder of the fruit) before spray drying [8]. All measurements were made in triplicate.

$$\text{EY \%} = \frac{\text{the weight of microparticles after spray drying}}{\text{total weight of adjuvants and raw fruit powder added initially}} \times 100$$

F. Particle Size Analysis

The particle size of the spray-dried powder was measured by using Zetasizer Nano ZS particle size analyzer. All measurements were made in triplicate.

G. DPPH Radical Scavenging Activity

The radical scavenging activity of the samples were analyzed by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [9]. 10 mg of the fruit sample was added with 30 mL of methanol and then the mixture was centrifuged at 5000 rpm for 15 minutes. Then, 0.2 mL of the sample solution was added to 4 mL of 0.025 g/L DPPH in methanol and the mixture was left under dark condition for 30 minutes at room temperature. After 30 minutes, the absorbance of the DPPH solution was measured at 515 nm using Perkin Elmer UV-VIS lambda 25 spectrophotometer. Methanol (0.2 mL) was used as the control. The measurement was made in duplicate. The DPPH radical scavenging activity in terms of percentage was calculated according to the following equation:

$$\text{DPPH scavenging activity (\%)} = \left[\frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \right] \times 100$$

where absorbance sample was the absorbance of DPPH with the fruit sample, whereas absorbance control was the absorbance of DPPH without fruit sample. All measurements were made in triplicate.

H. Total Phenolic Content

The total phenolic content (TPC) was determined according to the FolinCiocalteu method [10]. 10 mg of the fruit sample was added with 30 mL of methanol and then the mixture was centrifuged at 5000 rpm for 15 minutes. The Folin-Ciocalteu reagent was prepared by mixing the Folin-Ciocalteu with distilled water in a volume ratio of 1:9. 1 mL of the sample solution was added into 2 mL of the Folin-Ciocalteu reagent in a tube and left for 5 minutes at room temperature. After 5 minutes, 2 mL of 75 g/L sodium carbonate was added to the mixture. The mixture was left for 2 hours at room temperature.

The absorbance of the samples was measured at 760 nm using Perkin-Elmer UV-VIS lambda 25 spectrophotometer. Tannic acid (0-100 mg/L) was used to produce a standard calibration curve. The measurement was made in duplicate. The TPC was expressed in mg of tannic acid equivalent (TAE)

per gram of extract for fruit extract and in mg of tannic acid equivalent (TAE) per gram of spray-dried powder (SDP) for spray-dried powder. All measurements were made in triplicate.

I. Total Flavonoid Content

The total flavonoid content (TFC) was determined according to the Down method as adopted by [11]. 10 mg of the fruit sample was added with 30 mL of methanol and then the mixture was centrifuged at 5000 rpm for 15 minutes. 5 mL of the sample solution was added with 5 mL of 2 % aluminiumtrichloride in methanol. The mixture was left for 10 minutes. The absorbance of the samples was measured at 415 nm using Perkin-Elmer UV-VIS lambda 25 spectrophotometer. Catechin was used to produce a standard calibration curve. The measurement was made in duplicate. The TFC was expressed as mg of catechin equivalent (CE) per gram of extract for fruit extract and as mg of catechin equivalent (CE) per gram of spray-dried powder (SDP) for spray-dried sample. All measurements were made in triplicate.

III. RESULTS AND DISCUSSION

A. Microencapsulation of Fruit Extracts by Spray Drying

The fruit extracts obtained from the conventional solvent extraction by using ethyl acetate as the solvent were spray-dried so as to get the bioactive components encapsulated within a wall material. κ-Carrageenan was used as the wall material in this study. The inlet air temperature for this study was set at 140 °C. This temperature (140 °C) was fixed for the spray drying of the fruit extracts. Wet powders were produced from the spray drying process. This condition was more obvious in the spray drying process. The wet powders stuck on the walls of the chamber and hence it reduced the encapsulation yield. To overcome this issue κ-Carrageenan was added as drying aid. Only samples collected in the collection bottle were considered as effective yield.

B. Encapsulation Yield

The mixture of *Morindacitriifolia* L. and *Beta vulgaris* L. fruit extract produced the highest EY of 8.02 % from the spray drying process (Fig. 1). The lowest EY of 5.67 % was obtained by the sole *Beta vulgaris* L. fruit extract, whereas the sole *Morindacitriifolia* L. fruit extract produced the intermediate EY of 7.21 %.

The EY obtained from the spray drying processes of these three sample extracts fluctuated from the actual amount. This may be due to active component volatilization. And also because of not all of the spray-dried powders produced were collected for weighing due to the production of wet powders. The wet powders mainly stuck to the upper part of the chamber wall and thus were not collected.

The lower EY of sole *Beta vulgaris* L. fruit extract than sole *Morindacitriifolia* L. fruit extract might indicate that the κ-Carrageenan has lower efficiency as a binding agent to the *Beta vulgaris* L. than the *Morindacitriifolia* L.. The highest EY for the mixture of *Morindacitriifolia* L. and *Beta vulgaris* L. fruit extract might be due to presence of more bioactive components in the given volume of sample. It was also

noticeable in the spray dryer during the process of mixed fruit extract there is a very little amount of wet powders produced. This may be due to interaction of bioactive components of different fruit extracts and their effective binding towards κ -carrageenan. This is in agreement with the researcher [12] who obtained the encapsulation yield around 14-39% for the fruit extract.

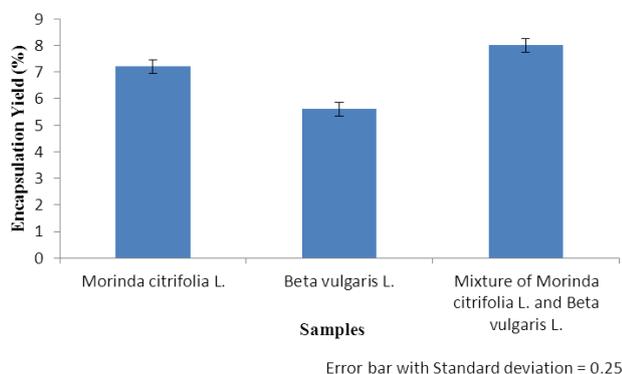


Fig. 1. Encapsulation yield of samples.

C. Particle Size Analysis

The three different spray-dried powders had spherical shapes. The sole *Beta vulgaris* L. spray-dried powder was looked fine than the other two spray-dried powders. From the particle size analysis (Fig. 2), the sole *Beta vulgaris* L. spray-dried powder had the smallest particle size which was 737.2 nm. The sole *Morindacitrifolia* L. spray-dried powder was found to be 945.8 nm whereas the spray-dried powder of the mixture (*Morindacitrifolia* L. with *Beta vulgaris* L.) was reported with the largest particle size of 1040 nm. Particle size analysis reveals that the significant difference between the microparticle size of these three extracts are less. This is in agreement with [12] who claimed that there is no significant difference between particle size for different inlet air temperature and ratio of Mcore/Mwall.

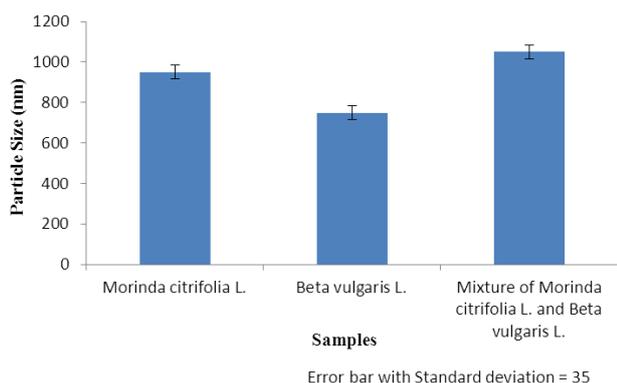


Fig. 2. Mean particle size of spray-dried powders.

D. DPPH Radical Scavenging Activity

Three different fruit extracts and spray dried powder (before and after spray drying) were analyzed for radical scavenging activity (Fig. 3). The DPPH radical scavenging activity of sole *Morindacitrifolia* L. fruit extract was much higher than the sole *Beta vulgaris* L. fruit extract, with 74.61 % and 43.33 % respectively. However, the DPPH radical scavenging activities of their spray-dried powders were not much different. The sole *Morindacitrifolia* L.

spray-dried powder was found to have 9.85 % whereas the sole *Beta vulgaris* L. spray-dried powder was reported with 6.93% of DPPH radical scavenging activity. For both the fruit extracts and spray-dried powders, the sole *Morindacitrifolia* L. was found to possess the highest antioxidant activity than the sole *Beta vulgaris* L.. This suggests that the antioxidant compounds of the *Morindacitrifolia* L. are readily to donate hydrogen atoms to the DPPH radicals than of the *Beta vulgaris* L..

Significant lost in antioxidant activity was occurred between fruit extract (sole as well as mixed) and spray dried powder. This is because of high temperature (140°C) maintaining in the spray dryer where all the bioactive components might be volatilized. This is in agreement with [13] who claimed that retention of volatile components in durian fruit was significantly affected by the type of additives and inlet drying temperature. This is also in agreement with [2] who suggest loss of bioactive components can be minimized by reducing the temperature in the spray drying process.

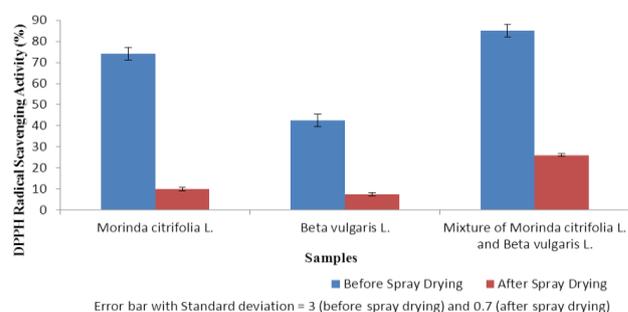


Fig. 3. DPPH radical scavenging activity of samples before and after spray drying process.

The mixture of *Morindacitrifolia* L. and *Beta vulgaris* L. exhibited the highest DPPH scavenging activity of 84.74 % and 26.58 % for the fruit extract and spray-dried powder respectively. The synergism between the antioxidants of *Morindacitrifolia* L. and *Beta vulgaris* L. might contribute to the highest DPPH scavenging activity to this sample. Therefore we claim that the amount of the antioxidant effects was increased by mixing these two fruits, this is because the antioxidants assist one another in scavenging the free radicals [7].

E. Total Phenolic Content

The total phenolic content (TPC) of fruit extracts (Fig. 4) and spray-dried powders (Fig. 5) of the three samples were analyzed. The fruit extract of sole *Beta vulgaris* L. displayed the smallest TPC among the three samples, which was reported as 0.0455 mg of TAE/g of extract only. The TPC of sole *Morindacitrifolia* L. fruit extract, which was found as 0.2104 mg of TAE/g of extract, was much higher than sole *Beta vulgaris* L. fruit extract. This may suggest that the antioxidant activity of *Morindacitrifolia* L. is higher than the *Beta vulgaris* L. in terms of phenolic content. The higher TPC of *Morindacitrifolia* L. than *Beta vulgaris* L. may be attributable to the greater performance of phenolic compounds of *Morindacitrifolia* L. in the Folin-Ciocalteu method as different phenolic compounds have different response to the reagent [14].

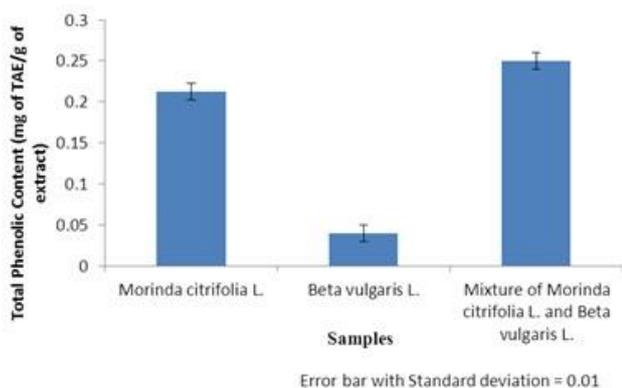


Fig. 4. Total phenolic content of samples before spray drying process.

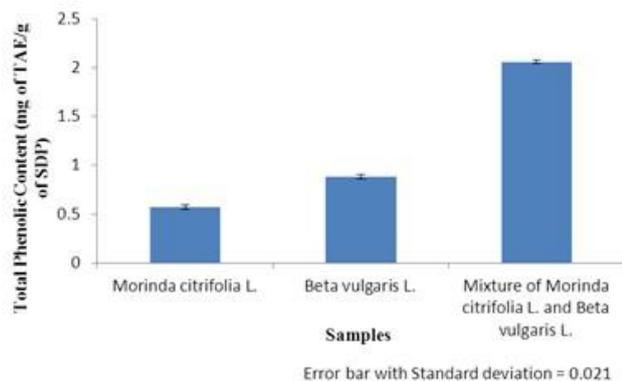


Fig. 5. Total phenolic content of samples after spray drying process.

The TPC of the mixture of *Morindacitrifolia* L. and *Beta vulgaris* L. fruit extract was the highest among the three samples, which was 0.2534 mg of TAE/g of extract. The additional effect of the phenolic compounds of *Morindacitrifolia* L. and *Beta vulgaris* L. may have contributed to the highest TPC of this sample. Hence, this sample may have significant antioxidant activity than the sole samples.

After the three fruit extracts were spray dried, the mixture of *Morindacitrifolia* L. and *Beta vulgaris* L. powder was still found to have the highest TPC among the three samples (Fig. 5). The TPC of this spray-dried powder was 2.0834 mg of TAE/g of spray-dried powder. This again showed the additional effect between the phenolic compounds of *Morindacitrifolia* L. and *Beta vulgaris* L. even after the spray drying process. The TPC of sole *Beta vulgaris* L. spray-dried powder (0.8733 mg of TAE/g of spray-dried powder) was higher than sole *Morindacitrifolia* L. spray-dried powder (0.5794 mg of TAE/g of spray-dried powder). This may suggest that the phenolic compounds of the *Morindacitrifolia* L. might be more volatile than *Beta vulgaris* L..

F. Total Flavonoid Content

The total flavonoid content (TFC) of fruit extracts (Fig. 6) and spray-dried powders (Fig. 7) of the three samples were analyzed. The mixture of *Morindacitrifolia* L. and *Beta vulgaris* L. fruit extract had the highest TFC (0.0111 mg of CE/g of extract) among the three samples, followed by the sole sample of *Morindacitrifolia* L. fruit extract (0.0077 mg of CE/g of extract) and the sole sample of *Beta vulgaris* L. fruit extract had the least TFC (0.004 mg of CE/g of extract). The highest TFC of the mixture of *Morindacitrifolia* L. and *Beta vulgaris* L. may due to the contribution of flavonoid

compounds from both fruit extracts, as the TFC of the two sole samples were not differ in a large extent.

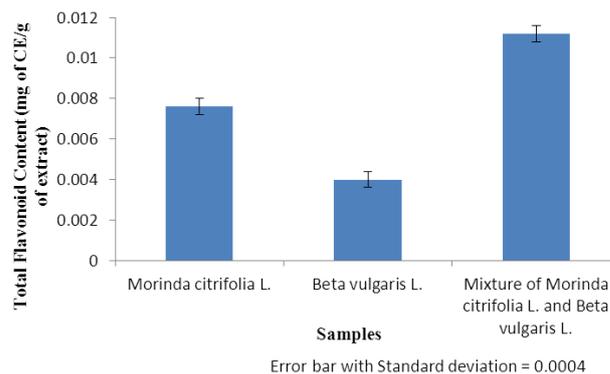


Fig. 6. Total flavonoid content of samples before spray drying process.

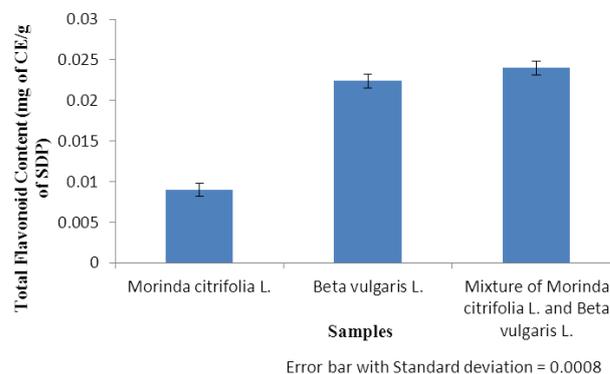


Fig. 7. Total flavonoid content of samples after spray drying process.

The TFC of the sole *Beta vulgaris* L. spray-dried powder and the mixture of *Morindacitrifolia* L. and *Beta vulgaris* L. spray-dried powder were quite close to each other, although the spray-dried powder of the mixture was still the highest TFC among the three samples (Fig. 7). The TFC of the mixture was 0.0245 mg of CE/g of spray-dried powder, whereas the TFC of the sole *Beta vulgaris* L. spray-dried powder was 0.0225 mg of CE/g of spray-dried powder. The highest TFC of the mixture may be contributed mostly by the flavonoid compounds of *Beta vulgaris* L. after the spray drying process. The sole *Morindacitrifolia* L. spray-dried powder had the least TFC, which was 0.0089 mg of CE/g of spray-dried powder only. Again as similar to the TPC, the sole *Morindacitrifolia* L. spray-dried powder possessed the least TFC may indicate that the flavonoid compounds of the *Morindacitrifolia* L. are highly volatile than the *Beta vulgaris* L..

Due to different morphologies in the formation of encapsulated powder such as mononuclear capsule and aggregate in the spray drying process may attribute to the variation in antioxidant activity, TPC and TF between the fruits extract [15].

IV. CONCLUSION

The mixture of the *Morindacitrifolia* L. and *Beta vulgaris* L. was concluded as natural source for high antioxidant. Bioactive compounds from these two fruits co-operated well with each other and established a stronger antioxidant system

through the synergistic action. The combination of these two fruits may create excellent protection mechanism against the reactive oxygen species.

The sole sample of *Morindacitrifolia* L. displayed greater DPPH radical scavenging activity than the sole sample of *Beta vulgaris* L. for both fruit extract and spray-dried powder. The same behaviour was also found in the TPC and TFC for the fruit extracts of the two sole samples. However, the spray-dried powder of *Beta vulgaris* L. displayed higher TPC and TFC than the *Morindacitrifolia* L. spray-dried sample. Hence conclusion is made that the antioxidant activity of *Morindacitrifolia* L. was higher than *Beta vulgaris* L. Further, synergistic effect of these two fruits showed highest antioxidant activity and so further investigation is needed in terms of pharmaceutical applications. Hence the future study extends to the optimization of spray drying operating conditions using response surface methodology.

NOMENCLATURE

DPPH	2,2-diphenyl picrylhydrazyl
TA	Tannic Acid
TPC	Total Phenolic Content
TF	Total Flavonoid
UV-VIS	Ultraviolet – Visible
κ-carrageenan	Kappa- carrageenan

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