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Effect of Microencapsulation Techniques on Physical and Chemical Characteristics of Functional Beverage Based on Red Betel Leaf Extract (*Piper crocatum*)

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Abstract

Functional drinks based on red betel leaf extract have antioxidant activity, but they still have a bitter taste. This study aims to determine the effect of microencapsulation on phenol content, antioxidant activity, and sensory quality of functional drinks based on betel leaf extract. Microencapsulation of functional drinks was made using maltodextrin coatings with concentrations of 10% and 20%. Antioxidant activity was tested by the CUPRAC method. The ready to drink (RTD) functional drink has a total phenolic content and antioxidant activity of 782.30 ± 2.54 mg GAE/g and 1660.19 ± 31.67 µmol Tr/g, respectively. These values are higher than microencapsulated functional drinks with maltodextrin (MM). The microencapsulated functional drink with 10% maltodextrin coating (MM10) is the chosen formulation since it has the smallest particle size (1.283 µm), total phenolic content of 12.90 ± 0.01 mg GAE/g and antioxidant activity of 189.41 ± 1.88 µmol Tr/g. Microencapsulated functional drinks provide sensory quality that is not significantly different ($p < 0.05$) from ready to drink (RTD) drinks.

1. Introduction

Functional drinks are included in the functional food category. One of Indonesia's herbal plants with health benefits and can be used as a functional food is red betel leaf (*Piper crocatum*) [1]. It contains alkaloids, flavonoids, and tannins, which have potential as antioxidants [2]. Antioxidants are molecules or compounds that play a role in inhibiting the oxidation of the substrate that is easily oxidized. This compound is often known as a compound that can counteract or reduce free radicals [3]. Free radicals are groups that contain one or more unpaired electrons in the outer orbitals. These free radicals can react with the molecules around them to get an electron pair to obtain new free radicals [4]. The accumulation of free radicals in the body can cause oxidation of fat compounds, damaging proteins, and DNA. Free radicals that react in mitochondria cause the production of reactive oxygen species (ROS), which are reactive, causing an aging process [5].

Red betel leaf and cinnamon leaf extract formulation are known to reduce blood glucose levels in rats because they have antihyperglycemic activity and increase the number of β-pancreatic cells to reduce blood glucose levels in mice [6]. Development of functional drink formulations based on red betel leaf extract has been carried out, with several spice extracts such as cinnamon, red ginger, and lime juice. The beverage is known to have antioxidant and anti-α-glucosidase activities at 873.2 µg/mL and 88.7%. However, sensory tests on these functional drinks have an undesirable taste on a scale of 2.7 ± 1.1 out of 1-5 [2].

Microencapsulation techniques can be used to protect active compounds against adverse environmental conditions so that the desired condition is obtained. Microencapsulation is the process of coating the core material in solid particles, liquid, or gas droplets. This technique can be applied to reduce volatile compounds, slowing lipid oxidation, and increase the stability of taste [7]. The microencapsulation process in grape seed oil

increased total phenolic content and antioxidant activity [8].

The process of making microencapsulation requires a coating to protect the core material. One of the coatings which are often used for food is maltodextrin. Maltodextrin is an excellent coating material because it has a high solubility in water, produces low viscosity, is tasteless, and is biodegradable [9]. This study aims to determine the effect of microencapsulation on antioxidant activity, total phenol, and sensory quality of functional drinks based on betel leaf extract.

2. Methodology

2.1. Tools and Materials

The tools used in the manufacture of microencapsulated beverage powder based on red betel leaf extract were homogenizer (Armfield), and spray dryer (B190). Then, the equipment used to determine the antioxidant activity of the CUPRAC method was an ELISA microplate reader (Epoch), micropipette (ThermoScientific), sonicator (B1510), vortex (BI Type 37600 mixer), and microplates. While, the instrument used to determine total phenolic levels was the UV-Vis spectrophotometer (Thermo Scientific-Genesys 20, USA). Furthermore, the instrument used for particle measurement was the Particle Size Analyzer (Beckam Coulter Delsa™ Nano and Malvern).

The materials -used to make red betel extract drink powder- were red betel leaves, cinnamon, red ginger (medicinal plant gardens of the IPB Biopharmaca Study Center), and lime (Pasar Anyar Bogor) and distilled water. While the coating material used in making microencapsulation was DE 10 food-grade maltodextrins (Lihua-PRC). For the determination of the antioxidant activity of the CUPRAC method used materials such as $CuCl_2.2H_2O$ (Merck®, Germany) Neocuproine (Sigma Aldrich®, USA), ammonium acetate buffer pH 7 (Merck®, Germany) and Trolox standards (Sigma Aldrich®, USA). Materials used in the determination of total phenolic levels are the Folin-Ciocalteu reagent (Merck, Germany), Na_2CO_3 (Merck, Germany), 70% ethanol solvent CAS 64-17-5 (Merck, Germany), and gallic acid standards (Merck, Germany).

2.2. Sample preparation

Red betel leaves, red ginger, and cinnamon were sorted and washed and drained. The three ingredients were cut into small pieces and dried in an oven at 50°C for three days. Red betel leaves, red ginger, and dried cinnamon were then blended and filtered to obtain a sample with a size of 40 mesh. The sample was stored in a zip-lock plastic at room temperature [2].

2.3. Sample extraction

Red betel leaf extract was prepared by adding 10 grams of red betel leaf sample to 200 mL of distilled water in a ratio (1:20) and boiled for 15 minutes in a closed container. After that, the sample was filtered using a cloth, and the filtered volume (filtrate) was measured. The filtrate was then added with distilled water until the total volume became 100 mL. Making red ginger extract and cinnamon used the same method as making red betel leaf extract. The difference was in the addition of distilled water, where the manufacture of red ginger extract and cinnamon used a ratio of 1:10. The preparation of lime juice extract was done by squeezing the lime using a simple lime press to separate the juice from lime seeds. Lime juice was made into a stock of lime extract solution. The prepared stock solution was then stored at 8°C [2].

2.4. Ready to drink (RTD) formulation

Stock solutions of red betel leaf extract and cinnamon were mixed with a ratio of 42% (v/v) and 28% (v/v) so that the basic formula for functional drinks was obtained. The basic formula was added to the stock solution of red ginger extract and lime juice with 15% (v/v) each (Table 1). The mixture of the ingredients was stirred until homogeneous and stored in bottles at 8°C [2].

Table 1. Functional drink formula

Material	RTD	MM10	MM20
Betel red (%)	42	42	42
Cinnamon (%)	28	28	28
Ginger (%)	15	15	15
Lime (%)	15	15	15
Maltodextrin (%)	-	10	20

Note: RTD (ready to drink), MM10 (microencapsulated drink with 10% maltodextrin), MM20 (microencapsulated drink with 20% maltodextrin)

2.5. Preparation of microencapsulated drinks

The RTD drink stock solution was taken as much as 100 mL and given a maltodextrin coating material with concentrations of 10%, and 20% (w / v) (Table 1). The solution was then homogenized using a homogenizer (Armfield) at a speed of 15000 rpm for 30 minutes [10]. Furthermore, the homogenate was dried using a spray dryer (B190) with an inlet temperature of 140°C, an outlet temperature of 80°C, nozzle diameter of 0.5 mm, a feed pump of 2000 mL/hour to obtain a microencapsulated beverage powder [11].

2.6. Particle size analysis

The samples analyzed for particle size were microencapsulated functional drinks and RTD functional drinks. The difference between the preparation of the two samples was at the dissolution stage of microencapsulated beverage powder. Microencapsulated functional drinks weighed 1 gram and then dissolved with 5 mL of distilled water. After that, three drops of each sample of the microencapsulated functional beverage and RTD were then taken and then dissolved again in 5 mL of double distilled water in a beaker glass and homogenized [12]. A small amount of liquid was put into a cuvette and placed in the Particle Size Analyzer (PSA) slot. Next, object preparation slots were closed and analyzed with input data in the form of a solvent refractive index, thickness, and laser beam intensity adjustments. Laser beam shooting was carried out at 30 different sample field points. The particle size results and polydispersity index

can be seen in the cumulative method's output on the Z_D and PDI values [12].

2.7. Determination of total phenolic content

Total phenol levels can be determined using the Folin-Ciocalteu reagent. A total of 0.2 mL samples of functional drinks (RTD, MM10, MM20) were mixed into 2.5 mL of 10% Folin–Ciocâlteu reagent and 2 mL of 7.5% Na_2CO_3, then incubated for 30 minutes at 25°C. The mixture was then measured for absorbance with a UV-Vis spectrophotometer at a wavelength of 765 nm. Total phenolic red betel leaf extract is expressed as milligrams (mg) gallic acid equivalent per gram of dry extract weight (mg GAE g sample). The standard used was gallic acid at various concentrations of 0, 25, 50, 75, 100, 150, and 175 ppm [13].

2.8. Antioxidant activity test using the CUPRAC method

A total of 50 µL $CuCl_2.2H_2O$ 0.01 M, 50 µL Neocuproine 7.5×10^{-3} M, 50 µL ammonium acetate buffer pH 7, and 50 µL drinks in distilled water were put into a microplate well. The total volume of each well was 200 µL. The measurement of antioxidant capacity used a standard Trolox curve with concentrations of 50, 100, 200, 400, 600, 800, 1000 µM. Blanks were prepared with a mixed solution mixed with a solvent, instead of a beverage solution. After that, the microplate was incubated for 30 minutes at room temperature and in a dark condition. Absorbance was measured using a microplate reader ELISA at a wavelength of 450 nm [14].

2.9. Sensory analysis

Sensory analysis of functional drinks of microencapsulation of red betel leaf extract was performed with a favored test [2]. The panelists consisted of 30 students. In the sensory test, 5-10 mL of RTD and MM10 red betel functional drinks were served. Panelists were asked to taste the two functional drinks and then compare the level of color and flavor of the sample using a 5-point hedonic scale with the description: very like = 5, like = 4, little like = 3, dislike = 2, and immensely dislike = 1.

2.10. Data analysis

All data obtained were displayed in the form of mean values and standard deviations. Furthermore, the data was processed and analyzed by ANOVA analysis of a single randomized complete design system (CRD) to determine the effect of the treatment on the measured parameters. The further test used was the Duncan test to determine the difference between the mean parameters measured by the appearance of the data as mean and standard deviation (SD). Significant differences between the parameters measured were indicated by p values <0.05 [15].

3. Results and Discussion

3.1. The yield of functional drinks

A spray dryer of 300 mL of MM10 functional drink solution was obtained 12.07 grams, while 300 mL of MM20 functional beverage solution was obtained 16.76 grams. These results indicate that maltodextrin, used as a coating material for drinks with a greater concentration, produces higher drink powder. Thus, the yield of MM10 drinks (4.02%) is smaller than that of MM20 (5.59%). The results of this study are in line with the treatment of the addition of maltodextrin concentrations (5%, 10%, and 15%) that have a significant effect (α = 0.05) on the yield value of noni leaf instant drinks produced. The more amount of maltodextrin added, the higher the yield of the product. This is due to the use of maltodextrin in beverage products to increase the volume and increase the material's total solids so that the yield obtained is higher [10].

3.2. Particle Size and Polydispersity Index of Functional Drinks

Analysis of functional beverage particles based on red betel leaf extract in this study was conducted using Particle Size Analyzer (PSA). The average particle size and polydispersity index can be seen from the results of the cumulant method on the Z-average and polydispersity index (PI) (Table 2). RTD functional drinks have the largest average particle size, which is 2,384 ± 479.9 µm, while MM10 functional drinks have the smallest average particle size, which is 1,283 ± 100.4 µm. Based on this data, the value of the polydispersity index (PI) is also varied for each sample. The highest PI value was found in MM10 of 0.319, while the lowest PI value is found in RTD functional drinks, which is 0.114.

Table 2. Particle size and polydispersity index of functional drinks

Drink samples	average particle size (µm)	polydispersity index
RTD	2384 ± 479.9	0.114
MM10	1283 ± 100.4	0.319
MM20	1716 ± 149.6	0.175

Note: RTD (ready to drink), MM10 (microencapsulated drink with 10% maltodextrin), MM20 (microencapsulated drink with 20% maltodextrin)

The average diameter of the particle size of MM10 and MM20 drinks is smaller than that of RTD drinks. This is when making microencapsulation solutions; there; there are stages of sizing, electrostatic interactions, and ionic gelation. This process produces dots of nanopolysaccharide C, which are ready to absorb the active compound from the beverage extract to produce microparticles with a smaller size compared to the particle size of RTD drinks [16].

In the microencapsulation drinks obtained, the particle size was analyzed using a particle size analyzer (PSA). The principle of measuring PSA is based on the principle of scattering light. Particles that scatter the light will form the angle of light scattering, which is inversely proportional to the size of the particles [17]. The results of particle measurements using PSA are interpreted in the form of particle distribution to describe the overall sample conditions [18]. Table 2 shows that the average diameter of the functional beverage particles MM10 and MM20 obtained were included in the microparticles. This

is because the particle size is above 1000 nm. Particles resulting from the microencapsulation process have sizes between 1-5000 µm [19].

In addition to particle size, the microencapsulation polydispersity index value can also be determined by PSA. The polydispersity index (PI) shows the particle size distribution parameters. The range of polydispersity index (PI) of a particle is at 0.0 to 1.0. The smaller the polydispersity index value indicates the increasingly homogeneous particle size distribution [20]. The polydispersity index (PI) higher than 0.5 indicate heterogeneous particle distribution, whereas the polydispersity index (PI) closer to 0 indicate uniform (homogeneous) particle distribution [21]. The results showed that the distribution of functional drinks based on red betel leaf extract had a relatively uniform (homogeneous) particle distribution. This is indicated by a polydispersity index of less than 0.5.

3.3. Total Phenolic and Antioxidant Activities of Functional Drinks

Measurement of total phenolic content was carried out by the Folin–Ciocâlteu method, which used gallic acid as the standard. Based on the measurement of the absorbance of the gallic acid solution, the line equation y = 0.005x - 0.0005 is obtained with an R^2 value of 0.9915. The total phenolic content in functional drinks based on red betel leaf extract showed significantly different results ($p < 0.05$). RTD functional drinks have the highest total phenolic content, amounting to 782.30 ± 2.54 mg GAE/g, while MM20 functional drinks obtained the smallest total phenolic content, which is 9.68 ± 0.06 mg GAE/g (Table 3). In vitro, the release of active compounds in the encapsulation material was slow compared to the non-encapsulated active substances [22]. This causes the measured bioactive compounds to be lower than those that are not encapsulated. The release of bioactive compounds is slow due to having to pass through a homogeneous system of microencapsulation.

Table 3. Total phenolics of functional drinks

Drink samples	Average total phenolic (mg GAE/g)
RTD	782.30 ± 2.54 [c]
MM10	12.90 ± 0.01 [b]
MM20	9.68 ± 0.06 [a]

Note: RTD (ready to drink), MM10 (microencapsulated drink with 10% maltodextrin), MM20 (microencapsulated drink with 20% maltodextrin). The same letters in each column indicate that the value is not significantly different at the real level of 95% ($p > 0.05$).

This functional drink contains several phenolic compounds, including flavonoids, tannins, and alkaloids found in red betel leaves, the highest component of these drinks. The addition of spices to the functional drink composition based on red betel leaf extract can increase the total phenolic content [2]. Analysis of the total phenolic content of RTD functional drinks in previous studies has been carried out and obtained results of 1385.25 ± 0.96 µg/mL. The difference in results is due to the addition of stevia sweetener to RTD functional drinks in previous studies [2]. Stevia contains several active compounds such as alkaloids, flavonoids, tannins, and phenol compounds so that the total phenolic content of the RTD functional drinks is higher than the RTD functional drinks in this study [23].

The treatment of variations in the amount of maltodextrin in the manufacture of microencapsulated functional drinks showed significantly different results ($p < 0.05$) in total phenolic content. As maltodextrin is added, the total phenolic content decreases. This is caused by the increasing number of total solids contained in drinks in the form of maltodextrin as a coating material so that the total phenol measured is less [11]. Also, the encapsulant material (maltodextrin) causes the phenol compound's bioactive compounds to be coated, so that not all bioactive components of the beverage can react with reagents measuring the total phenol content [16].

The most antioxidant activity of functional drinks based on red betel leaf extract is obtained in RTD functional drinks, which is 1636.38 ± 31.64 µmol Tr/g extract. At the same time, the lowest is found in MM20 functional drinks, which is 125.96 ± 1.54 µmol Tr/g extract. The results obtained are significantly different ($p < 0.05$) as well as the total phenolic content (Table 4). Decreased antioxidant activity in microencapsulated drinks is caused by the coating material so that the release of bioactive components becomes slower. This results in a decreasing number of bioactive components that can be active [22]. Another factor that causes a decrease in antioxidant activity is the content of phenolic compounds in MM10, and MM20 functional drinks are smaller than RTD drinks (Table 2).

Table 4. Antioxidant capacity of functional drinks

Drink samples	Average antioxidant capacity (µmol Tr/g extract)
RTD	1636.38 ± 31.64 [c]
MM10	189.41 ± 1.88 [b]
MM20	125.87 ± 1.54 [a]

Note: RTD (ready to drink), MM10 (microencapsulated drink with 10% maltodextrin), MM20 (microencapsulated drink with 20% maltodextrin). The same letters in each column indicate that the value is not significantly different at the real level of 95% ($p > 0.05$).

The antioxidant activity of functional RTD based red betel leaf extract with the addition of sweetener stevia obtained the antioxidant capacity to reduce DPPH free radicals of 873.21 µg/mL [2]. These results are different from the results obtained in this study. The difference in the antioxidant activity results in the two methods is caused by differences in the mechanism of free radical reduction. The DPPH method reduces free radicals by hydrogen capture, while the mechanism of the free radical reduction in the CUPRAC method is done by electron donation [24]. The synergistic effect of the phenolic component in the sample can also affect the antioxidant capacity [25].

The treatment of maltodextrin concentration also gave the same results as the total phenolic content. The

functional drink MM10 has the highest antioxidant activity of 189.50 ± 1.88 µmol Tr/g extract. Microencapsulation drinks with the treatment of maltodextrin variations gave significantly different results ($p < 0.05$) on antioxidant activity. The addition of maltodextrin causes the total solids in drinks to be higher, resulting in less measured antioxidant activity. Increasing the concentration of maltodextrin causes a decrease in antioxidant activity [11]. Maltodextrin causes bioactive compounds of phenols to be coated so that not all bioactive components of drinks can function as antioxidants [16]. Maltodextrin has many hydroxyl groups that bind O groups to phenol compounds through hydrogen bonds [26]. Maltodextrin has a reliable binding power with coated compounds to protect bioactive components and sensitive components (taste, color, and vitamins) [27]. Maltodextrin can form a hydrogel matrix and adhesion. The microcapsules produced by maltodextrin are dry, uniform in size, and not sticky [28]. The characteristic of maltodextrin is that it is soluble in water and can protect active compounds encapsulated from oxidation and is a safe coating material. The use of maltodextrin is usually used as a binder, coating, crusher, and filler [29]. Thus, to test the bioactivity of microencapsulated drinks is more appropriate to be done in vivo, as encapsulants can be released in specific organs. Bioactive micro-size can quickly enter the cell and carry out their functional activities [30].

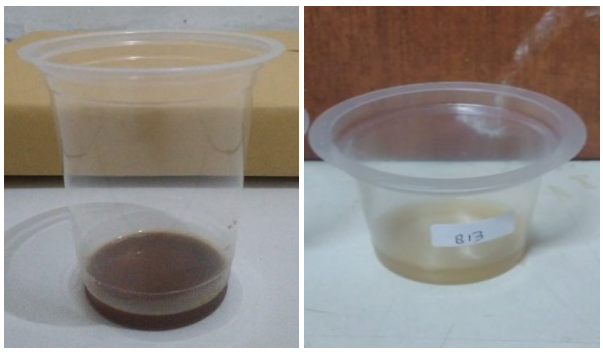
3.4. Sensory Quality of Functional Drinks

MMM 10 was sensory analyzed and compared with RTD drinks. Sensory analysis or organoleptic analysis is a test based on human sensing to assess a food product's quality and safety [31]. The results of sensory testing of MMM 10 functional drinks on RTD functional drinks (Table 5) show that the overall taste, aroma, and acceptance parameters are not significantly different ($p < 0.05$) but are significantly different ($p < 0.05$) in color parameters. Color is one of the essential food products' parameters, which is the first assessment of consumer preferences [32]. Based on the results of the sensory analysis show that the color of functional drinks MMM 10 (scale 3.07 ± 0.87) is significantly different ($p < 0.05$) from functional drinks (RTD) (scale 2.50 ± 0.86) (Figure 1). MM10 functional drinks have a brighter color compared to RTD functional drinks. This is because the encapsulation process can protect the active compound from the functional drink so that it is not immediately split in the water matrix [16].

Table 5. Sensory quality of functional drinks

Drink samples	Aroma	Taste	Color	Overall reception
RTD	3.20 ± 1.01 [a]	1.60 ± 0.77 [a]	2.50 ± 0.86 [a]	2.17 ± 0.91 [a]
MM10	3.27 ± 0.83 [a]	1.70 ± 0.70 [a]	3.07 ± 0.87 [b]	2.30 ± 0.75 [a]

Note: RTD (ready to drink), MM10 (microencapsulated drink with 10% maltodextrin). The same letters in each column indicate the value is not significantly different at the real level of 95% ($p > 0.05$)



a b

Figure 1. Color of RTD drinks (a) and MM10 drinks (b)

RTD functional drinks have a bitter taste arising from red betel leaf extract as the most significant component of these functional drinks [2]. Based on the results of sensory analysis, it is found that the taste of MM10 functional drinks (1.70 ± 0.77) and RTD functional drinks (1.60 ± 0.87) are not significantly different ($p < 0.05$). The results of the sensory analysis of RTD drinks in this study are much lower than the functional drinks of RTD in previous studies (2.7 ± 1.1) [2]. This is because in making RTD functional drinks in this study, do not use food additives sweetener (stevia) to see the effect of microencapsulation techniques with unsweetened maltodextrin coating ingredients on a bitter taste. Thus, this study's results indicate that microencapsulation of red betel RTD drinks using a coating agent maltodextrin has no significant effect ($p < 0.05$) on the taste of the drink.

Table 6. Overall Recapitulation of Drink Test Results

Drink samples	RTD	MM10	MM20
Average particle size (µm)	2384 ± 479.9	1283 ± 100.4	1716 ± 149.6
Polydispersity index	0.114	0.319	0.175
Average total phenolic (mg GAE/g)	782.30 ± 2.54 [c]	12.90 ± 0.01 [b]	9.68 ± 0.06 [a]
Average antioxidant capacity (µmol Tr/g extract)	1636.38 ± 31.64 [c]	189.41 ± 1.88 [b]	125.87 ± 1,54 [a]
Sensory Quality			
Aroma	3.20 ± 1.01 [a]	3.27 ± 0.83 [a]	
Taste	1.60 ± 0.77 [a]	1.70 ± 0.70 [a]	
Color	2.50 ± 0.86 [a]	3.07 ± 0.87 [b]	
Overall reception	2.17 ± 0.91 [a]	2.30 ± 0.75 [a]	

Note: RTD (ready to drink), MM10 (microencapsulated drink with 10% maltodextrin), MM20 (microencapsulated drink with 20% maltodextrin). The same letters in each column indicate the value is not significantly different at the real level of 95% ($p > 0.05$)

The aroma is also an essential parameter in sensory analysis. The sensory analysis results show that the two

functional drinks are not significantly different ($p < 0.05$). They are located on a scale of 3.27 ± 0.83 for MM10 and 3.20 ± 1.01 for RTD functional drinks. Another sensory analysis parameter is overall acceptance. The sensory results of the two functional drinks have a level of acceptance that is not significantly different ($p < 0.05$), where MM10 functional drinks are on a scale of 2.30 ± 0.75, while the functional drinks RTD are on a scale of 2.17 ± 0.91. Previous research stated that the sensory quality of functional drinks based on cat whiskers leaf extract had a preference value of microencapsulated drinks of 5.55, while RTD functional drinks were 3.31 on a scale of 1-9 [16].

4. Conclusions

Functional drinks based on red betel leaf extract are successfully microencapsulated with the best coating concentration of 10% maltodextrin. Microencapsulation using the maltodextrin coating material reduced the particle size of functional drinks and decreased the total number of phenolic compounds and antioxidant activity (CUPRAC method). The sensory quality of MM10 functional drinks is not significantly different ($p < 0.05$) with RTD functional drinks.

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