

## EXTRACTION AND ENCAPSULATION OF POLYPHENOLS FROM GUAVA LEAVES

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### Abstract

*Ultrasound has been an extraction technique received much attention in recent years.*

*This technique is proven to be able to increase extraction efficiency, shorten extraction time and maintain the activity of antioxidants especially polyphenols. Liquid state of the antioxidant mixture after extraction reduces the application of extracts containing bioactive compounds. This is due to the rapid decline in activity over time and higher transport costs for the liquid extracts with a large amount of water. Therefore, this study was focused on extraction process assisted by microwave to obtain the guava extracts and encapsulated these extracts by the suitable carrier to remain the polyphenol activity. It could be seen that microwave was a potential method to extract the polyphenols in comparison with solid-liquid extraction. The cell structure of plant materials was significantly destroyed by microwave in order to release more polyphenols from inner parts of guava leaves. The total phenolic and flavonoid content were increased 12.7% and 36.5% when microwave applied in comparison with the solid-liquid extraction. The combined 20% maltodextrin and 10% arabic gum was the best way to maintain the polyphenol activity. The polyphenols were encapsulated by the combined maltose dextrin and arabic gum was 5.2% more than those of maltodextrin only. Color indices such as  $L^*$ ,  $a^*$ ,  $b^*$  were significantly different between the powders encapsulated by the maltose dextrin and the combined maltose dextrin and arabic gum.*

**Keywords:** Guava, polyphenol, encapsulation, extraction, spray drying.

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## 1. INTRODUCTION

Guava (*Psidium guajava*) has long been used as a traditional medicine with several biological activities such as antidiabetic (Oh et al., 2005), anticough, antibacterial (Jaiarj et al., 1999) and antispasmodic actions (Lozoya et al., 2002). It also has been reported to possess high potential for antioxidant activity (Guo et al., 2003) based on a couple of chemical compounds such as saponins, flavonoids, tannins, eugenol and triterpenoids.

Over the years, solid liquid extraction procedures have been accepted for obtaining a wide range of compounds including phenolics from many sources including plants, animals and microorganisms. However, this technique

has been involved to many of the problems such as increased extraction time, high solvent consumption and poor solute recovery (Fellows, 2000). Therefore, many alternative extraction procedures have been introduced to enhance extraction efficiency and reduce the burdens of environment. Among of alternative techniques, microwave assisted extraction have been paid attention to a potential method for improving the extraction of bioactive substances from natural sources. For a long time, microwave assisted extraction (MAE) have been widely studied as a modern process facilitating the phenolic extraction such as isoflavones, carvone and limonene, gallic acid, vanillic acid, catechin, p-coumaric acid, ferulic acid and caffeine (Taghvaei, 2014). Most of

these studies concluded that microwave assisted extraction reduced the extraction time, solvent usage and enhanced the amount of extractable phenolics.

Unstable compounds as polyphenols can be entrapped in a protective polymer by using microencapsulation process. Based on this procedure, polyphenols can be protected from degradation or oxidation for a longer period of time and improve the range of applications (Dehkharghanian, 2009). Spray drying has been widely used for encapsulation of bioactive substances. This technique is relatively low cost, flexible, and leads to the production of high quality and stable particles with oxidative processes. The liquid solution containing the coating agent and the phenolic active substances is transferred into dry microparticle powders. The most common wall materials for encapsulation are arabic gum, maltodextrin and modified starch. Maltodextrins have been considered as the best thermal defenders and preserved the integrity of the polyphenols during their encapsulation. In recent years, a novel technique has been introduced for encapsulation is combined maltodextrin and arabic gum for coating procyanidins (polyphenols) from grape seeds (Munin, 2011). This study aimed to compare phenolic characters collected from solid liquid extraction and microwave assisted one in order to figure out the best method to extract polyphenols from guava leaves. In addition, the effect of encapsulated process on the quality of phenolic containing powders was also evaluated.

## 2. MATERIALS AND METHODS

**Guava leaves:** Fresh guava leaves (*Psidium guajava*) were obtained from the garden in Tien Giang province, Vietnam from April to June, 2013. They were separated from their damaged leaves. The leaves were dried at 50°C in 5 hours, grinded and screened to select particles of 250-350 µm. Then, they were kept in cold storage until they were needed for the experiments.

### **Conventional extraction**

The ground guava leaves was extracted with

water in a hot water bath at a temperature of 65°C for 120 min. Solid-liquid ratio of 1:20 (w/v), ethanol concentration of 50% were used to extract polyphenols from guava leaves in this case. The slurry was filtered to get a clear extract that was used for quantitative analysis.

### **Microwave-assisted extraction (MAE)**

Phenolic compounds were extracted from leaf powders of guava, using a microwave extraction system with solid-liquid ratio of 1:25 (w/v), ethanol concentration of 50%, microwave power of 385W and extraction time of 13 min. For analysis, the crude extract was filtered through Whatman paper.

### **Microcapsule spray drying**

The maltodextrins and mixtures of arabic gum – maltodextrins (1:3 w/w) were prepared at 30% in comparison with guava extract from microwave assisted extraction procedure. The emulsions were prepared in a mixer until completely homogeneous. Drying was performed using a spray dryer (Switzerland), fed by a peristaltic pump with flow feed of 3.5 L/h and an atomizing pressure of 2 bar. The input and output temperatures were fixed at 150 and 80°C, respectively. The control samples were only prepared with maltodextrin and guava leaf extracts.

### **Analytical methods**

#### **Total polyphenols**

Total polyphenols content (TPC) of the extracts was determined by using Follin-Ciocalteu (FC) reagent. 0.1 ml of the samples was completely mixed with FC reagent (0.5 ml) and saturated sodium carbonate solution (7.5 ml). The sample solution was made up to 10 ml with distilled water and the absorbance was measured at 765 nm. Total polyphenol content was expressed as gallic acid equivalents (Swain & Hillis, 1959).

#### **DPPH radical-scavenging activity**

The radical scavenging activity (RSA) of different extracts was evaluated according to the procedure described by Blois (1958). Sample solution (1 ml) at various concentrations (25, 50, 100 and 200 ppm) were mixed with 0.1 mM methanolic solution (4 ml) of DPPH and allowed to stand at 27°C for 20 min. The absorbance of the samples was

measured at 517 nm. DPPH scavenging effect (%) was calculated using the formula:  $RSA\% = [(A_0 - A_1/A_0) \times 100]$ . Where  $A_0$  was the absorbance of the control reaction and  $A_1$  the absorbance in the presence of the sample.

#### **Reducing power assay**

Method reported by Qi et al. (2005) was used to determine the reducing power of guava leaf extract. Guava extracts solution (1 mL) was mixed with 2.5 mL phosphate buffered saline (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1%, w/v). The mixture was incubated at 50°C for 20 min, followed by addition of trichloroacetic acid (2 mL, 10%, w/v) and centrifugation at 2000 rpm for 15 min. A 2.5 mL aliquot of the supernatant was mixed with 2.5 mL pure water and 0.5 mL ferric chloride (0.1%, w/v), and the absorbance was measured at 700 nm.

#### **Determination of flavonoids**

Aluminum chloride colorimetric assay was applied to measure the total flavonoid content. 5ml of dilute solution was added to a 10ml volumetric flask, containing 4ml of distilled water. Then, 0.3ml  $NaNO_2$  5% was added to this flask. After 5min, 0.3ml  $AlCl_3$  10% was added. Absorbance was measured at 446 nm. Standard curves were established using various concentrations of rutin (20, 40, 60, 80 and 100 mg/l). Flavonoid content was expressed as rutin equivalent (mg rutin/g sample) (Atanassova, 2011).

#### **Scanning electron microscopy**

The morphology of the dry powders after treated with solvent or microwave irradiation were observed using a scanning electron microscope with electron gun tungsten filament, in the low vacuum operating mode ( $2 \times 10^{-2}$  torr) with auxiliary nitrogen gas ( $N_2$ ). The sectioned particles were fixed on a

specimen holder with aluminium tape and then sputtered with gold in a JEOL JEC-1200 sputter-coater. All the specimens were examined with a JEOL JSM-5600 LV scanning electron microscopy under high vacuum condition and at an accelerating voltage of 5.0 kV.

#### **Color measurement**

Color analyses were carried out by using a Chroma Meter CR-300 (Minolta Camera Co. Ltd., Osaka, Japan).  $L^*$ ,  $a^*$ ,  $b^*$  values were recorded. A white standard plate was used to calibrate the instrument before each set of determinations. The reported data are the mean of ten determinations.

### **3. RESULTS AND DISCUSSIONS**

#### **Evaluation of extracts from microwave and conventional extraction procedures**

Microwave has been used widely for obtaining the biologically active compounds in recent years (Pan 2003). This technique has some definite advantages over the conventional one – the solid-liquid extraction method – including quick, relative cheap and in keeping with the dictates of modern environment concerns (Li 2011). The results from table 1 showed that microwave extraction releases more 12.7% of polyphenols than the conventional one. The reason can be explained to this result that microwave destroys the cellular structure more effective than the classical conductive heating because the localized temperature and pressure rise produces by dipole rotation and ionic conduction. These phenomena would be disrupted the weak hydrogen bounds between polyphenols and cell walls (Baiano 2014). That is a reason why a greater amount of boundedly phenolic compounds migrates to surrounding solvent.

**Table 1. The IC<sub>50</sub> and EC<sub>50</sub> of the control samples and the samples treated with microwave in comparison with BHT**

| Samples                        | TPC (mgGAE/100g)            | Flavonoids (mg/100g)      | IC <sub>50</sub> (ppb) -DPPH | EC <sub>50</sub> (ppb) – Reducing ferric |
|--------------------------------|-----------------------------|---------------------------|------------------------------|--|
| Control samples                | 17163.8±77.3 <sup>a</sup>   | 516.8 ± 5.52 <sup>A</sup> | 1259.7 ± 23.5 <sup>c</sup>   | 1125.7 ± 7.8 <sup>f</sup>                |
| Samples treated with microwave | 19351.9 ± 52.9 <sup>b</sup> | 705.5 ± 1.14 <sup>B</sup> | 997.2 ± 54.5 <sup>d</sup>    | 791.3 ± 5 <sup>g</sup>                   |
| BHT                            | -                           | -                         | 1140.3 ± 8.1 <sup>e</sup>    | 806.7 ± 19.3 <sup>h</sup>                |

Flavonoids belong to a group of phenolic substances with some health benefits such as free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Pourmorad, 2006). The results showed that microwave extraction was released more 36.5% of flavonoids in comparison with the traditional way. It can be seen that the microwave also enhances the flavonoids releases from plant cells in comparison with the traditional method.

DPPH is the stable free radical can be accepted electrons or hydrogen radicals. It is widely used for determining the total antioxidant capacity of bioactive-chemicals. IC<sub>50</sub> is the dose of polyphenols that half maximally inhibits of the DPPH free radicals. The lower IC<sub>50</sub> value is shown, the higher antioxidant activity is archived. IC<sub>50</sub> of guava leaf extracts obtained by traditional extraction was 20.8% and 9.5% lower than that of novel extraction and BHT, respectively.

The reducing power of crude extract can be evaluated by the direct reduction of Fe<sup>3+</sup>/ferricyanide complex to the ferrous form. Fe<sup>2+</sup> formed can be estimated by measuring the formation of Perl's Prussian blue at 700 nm. An increase of absorbance showed that reducing power was enhanced because of the formation of the complex. The ferric ion reducing antioxidant power assay takes advantage of an electron transfer reaction in which a ferric salt is used as an oxidant (Gülçin et al., 2010). It could be seen that the samples treated with microwave have the lowest in EC<sub>50</sub> values. This indicated that the

antioxidant capacity of these samples were the highest in compare with other ones.

#### *Analysis of plant cell wall structures*

Scanning electron microscopy was applied to study the structure alteration of materials treated by different extraction techniques and to understand the extraction mechanism. Distinguishable physical changes of guava leaf powders could be seen in fig 1 when different extraction techniques were used to obtain polyphenol from these materials. Microwave disrupts the surface of guava leaf powders better than solvent extraction. In the case of microwave, both ionic conduction and dipole rotation occur when transformation of electromagnetic energy takes place. Ionic conduction generates heat because of the resistance of medium to ion flow (Tripti Jain et al, 2009). The dipole rotation is the alternative movement of polar molecular depends on electric field. The friction force forms during the alternative movement can be considered as the causes of increasing temperature suddenly. This phenomenon expands the liquid and releases channels in cell wall due to high vapor rate of solvent and high internal pressure. Both sudden temperature rise and internal pressure increase were caused to damage the structure of cells for releasing more phenolics (Yan et al, 2010). In addition, microwave energy penetrated into the samples and improved the extraction efficiency. However, the little destruction of the microstructure of sample occurred in the case of the traditional extraction.

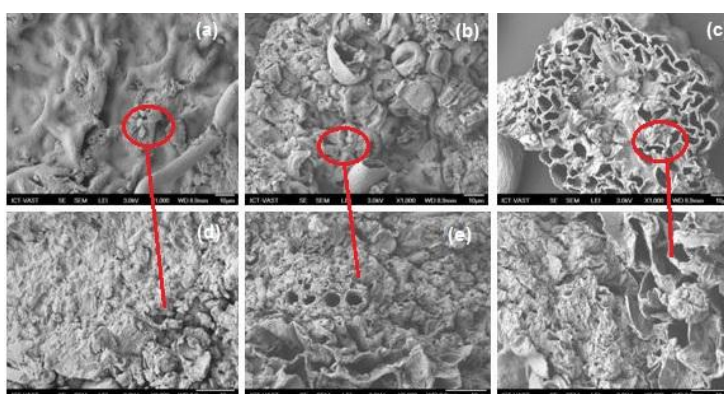


Fig 1. Structure of (a) - raw materials, (b) - the control samples and (c) - the samples of microwave extraction



**Physical characters of encapsulated products**

The wall matrixes with 20% maltodextrin and 10% arabic gum were remained 48% polyphenols in compare with these of mixture before encapsulation. However, the maltodextrin was only remained 42.8% polyphenols in their matrixes. The comparison between the maltose dextrin and the combined maltose dextrin and arabic gum for wall matrixes showed that both phenolic content and antioxidant activities were independent each other. The significant decrease of antioxidant capacity was recorded with the combined maltose dextrin and arabic gum. It can be these matrixes were tightly bounded the phenolics. Therefore, the release efficiency of polyphenols was lower than these encapsulating by maltose dextrin only. The L\* is the lightness of encapsulated powders. The results show that powders encapsulate by the combined maltose dextrin

and arabic gum were darker than those of maltose dextrin. It might be that arabic gum using to encapsulate guava leaf extract pronounced the darkness of powders. The b\* was reflected the color from blue to yellow. Positive b\* values were obtained from the samples indicating the yellow tint. The results showed that the combined maltose dextrin and arabic gum increased the yellowness of powders. For the a\* (red-green) values, the range from each samples was very narrow, close to zero. The a\* does not appear to be a particularly useful parameter for color measurement of the extracted guava leave powders. The differences of color were indicated by the ΔE values. The ΔE of the powders encapsulated by the combined maltodextrin and arabic gum were significantly different from those of maltodextrin only.

**Table 2 Total phenolic content and antioxidant capacity before and after spray drying**

| Characteristics   | 30% Maltose dextrin (MD)   | 20% maltose dextrin + 10% arabic gum (MDA) |
|---|----------------------------|--|
| Moisture (%)  | 4.56 ± 0.07 <sup>a</sup>   | 6.12 ± 0.2 <sup>b</sup>                    |
| Total phenolic content before encapsulation (mg GAE/100g dried basis) | 19289 ± 159.2 <sup>A</sup> | 19240 ± 172.8 <sup>A</sup>                 |
| Total phenolic content after encapsulation (mg GAE/100g dried basis)  | 8250.6 ± 68.5 <sup>c</sup> | 9237.3 ± 76.2 <sup>d</sup>                 |
| IC50 before encapsulation (ppb)                                       | 974.7 ± 26.9 <sup>e</sup>  | 982.6 ± 18.6 <sup>c</sup>                  |
| IC50 after encapsulation (ppb)  | 3381.9 ± 34.0 <sup>g</sup> | 5592.9 ± 54.2 <sup>h</sup>                 |

**Table 3 Color indices in different formulas of encapsulated guava extract after spray drying**

| Samples   | L*                          | a*                         | b*                        | C*                        | H*                         | ΔE                       |
|---|-----------------------------|----------------------------|---------------------------|---------------------------|----------------------------|--------------------------|
| Maltodextrin before encapsulation                     | 100.47 ± 0.029 <sup>a</sup> | 0.14 ± 0.004 <sup>c</sup>  | -0.31 ± 0.01 <sup>f</sup> | 0.342 ± 0.02 <sup>k</sup> | -65.62 ± 0.06 <sup>m</sup> | 7.54 ± 0.02 <sup>r</sup> |
| Maltodextrin + arabic gum before encapsulation        | 99.8 ± 0.32 <sup>a</sup>    | 0.157 ± 0.008 <sup>c</sup> | 0.263 ± 0.01 <sup>f</sup> | 0.306 ± 0.01 <sup>k</sup> | -59.28 ± 0.07 <sup>n</sup> | 6.97 ± 0.05 <sup>r</sup> |
| 30% maltodextrin after encapsulation                  | 94.6 ± 0.68 <sup>b</sup>    | -1.75 ± 0.12 <sup>d</sup>  | 10.28 ± 0.16 <sup>g</sup> | 10.43 ± 0.46 <sup>l</sup> | -80.38 ± 0.59 <sup>p</sup> | 6.51 ± 0.45 <sup>t</sup> |
| 20% maltodextrin + 10% arabic gum after encapsulation | 90.63 ± 0.82 <sup>c</sup>   | -0.41 ± 0.01 <sup>e</sup>  | 11.53 ± 0.19 <sup>g</sup> | 11.54 ± 0.19 <sup>l</sup> | -88.03 ± 0.09 <sup>q</sup> | 8.41 ± 0.53 <sup>u</sup> |

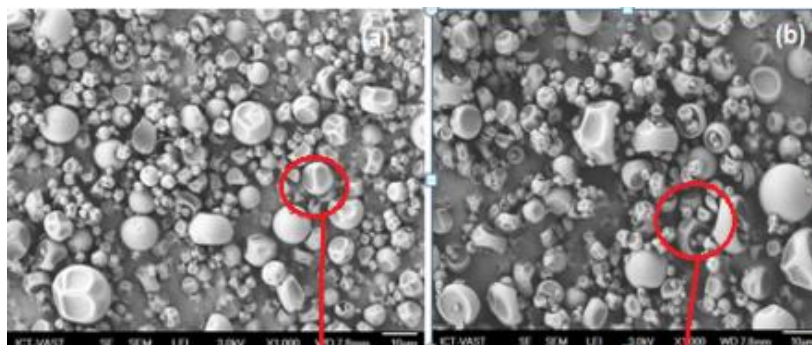


Fig 2. Morphology of the dry powder encapsulated with (a) – maltose dextrin and (b) – maltose dextrin and arabic gum

### *Morphology of the dry powders*

A diversity of sizes is observed in the final powders after spray drying at 150°C. A round external surface with non-rupture walls and no fissures has been seen in some particles. The rest of particles were concave and shriveled. The formation of hollow particles is considered as the typical phenomenon of spray drying. It can be explained by formation of “vacuole” inside the particles, immediately after crust development (Roccia et al, 2014). The particle temperature over the local ambient boiling point and the vapor pressure of vacuole above the local ambient pressure are driven forces to inflate the crusts of particles. Good encapsulation efficiency is shown by the lack of spores on the microcapsule surface (fig 2). The surfaces of powder particles with concave and shriveled shape are typical of microcapsules when using the spray drying process to encapsulate bio-substances. Bertolini et al (2001) was also recorded this type of morphology when using the gum arabic to microencapsulate monoterpenes.

### 4. CONCLUSIONS

Microwave was considered as the potential method to obtain the phenolic compounds from guava leaves. This method enhanced the polyphenols and flavonoids released from guava leaves about 12.7% and 36.5%, respectively. It could be seen that the antioxidant capacity of extracts were increased when microwave was applied. The plant cells were damaged more significant in the case of microwave extraction. In this study, the

combined maltose dextrin and arabic gum was the best choice to encapsulate the polyphenols and to maintain the antioxidant activity of guava leaves extracts. There was no different between the shape of powders obtained to the combined maltose dextrin and arabic gum and the maltose dextrin only.

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