Chapter 3

Methodology

This project developed lime powder with entrapment process in use for spray-drying technology. The entrapment carrier was designed with a combination of maltodextrin and gum Arabic as a core to entrap bioactive compounds and cover on the surface of particles with the purpose of protecting its physicochemical properties.

3.1 Materials

Fresh lime (*Citrus aurantifolia*) was purchased from a local market. It was immediately processed without further storage. Maltodextrin (Dextrose Equivalent, DE=10), gum Arabic (AG), aluminum trichloride ($AlCl_3$), trolox (6-hydroxy-2,5,7,8-tetramethylch roman-2-carboxylic acid), gallic acid, and quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of analytical research grade.

3.2 Preparation of lime juice

Fresh lime (*Citrus aurantifolia*) was hand squeezed and lime juice was obtained with filtering to remove seeds. The average amount per kilogram, nutrition values, and total soluble solid of lime juice were analyzed as references. Drying materials were separately rehydrated for overnight and then gently heated at 60°C in a water bath to allow complete dissolution. The obtained lime juice was mixed with 20% blended drying agent of maltodextrin and gum Arabic as encapsulating carriers at the ratio of 4:1 (w/w). The mixture was homogenized in a high shear homogenization Ultra Turrax Model T25 basic high shear homogenizer (UT-HSH) (IKA, Works Inc., Wilmington, NC,

USA) for 10 min at 12,000 rpm until complete dispersion obtained. A control was made parallel without homogenization. Then all mixtures were spray-dried as described in the following section.

3.3 Spray drying of lime powder

The prepared juice mixtures were diluted and filtered to remove insoluble solid before spray drying. Subsequently the solutions were spray-dried through two-fluid nozzle using a Mini spray dryer B-290 (Büchi Labortechnik AG, USA). The feed flow rate (6 mL/min), air flow (580 L/min), inlet temperature (120°C), outlet temperature (65°C), pressure (0.0038 MPa), and spray percentage (90%) were kept constant in all treatments. The reconstituted powders were stored at 4°C until needed for analysis. The spray drying procedure was carried out in triplicate.

The yield of the spray drying process was calculated by taking into consideration the total solid content of the feed sample with maltodextrin/gum Arabic and weight of the final dry powder. Powder yield (%) was calculated using the equation below:

$$\text{Powder yield (\%)} = \frac{\text{Obtained spray dried powder (g)}}{\text{Lime juice (g) + drying agent (g)}} \times 100$$

3.4 Particle size and morphology characterization

The particle size of all samples was measured by a particle size analyzer (Malvern Instruments, Malvern, UK). Obtained spray-dried lime powder was placed into the analyzer and the data acquisition was reported automatically in micrometers (μm) (Tonon, Brabet, & Hubinger, 2010). The morphology of spray-dried lime powders was characterized with a Scanning Electron Microscope (SEM) (JSM-6610LV, JEOL Ltd. Japan). The sample was mounted on aluminum SEM stubs and then coated with gold: palladium (60:40) in an Edwards S150 sputter coater. Then, it was systematically

observed with 300 and 3000× magnification. All measurements were performed in triplicate.

3.5 Physical properties of lime powder

Spray dried lime powder was analyzed for moisture content (MC) following the AOAC method 930.15 (AOAC, 1999). Color of the samples determined using the chroma meter LABSCAN XE (Hunterlab, VA, USA) was reported in CIELAB color scales. L* value is the degree of lightness to darkness, a* value is the degree of redness to greenness, and b* value is degree of yellowness to blueness. Chroma and hue angle were calculated using the equation below:

$$\text{Chroma} = [(a^*)^2 + (b^*)^2]^{1/2}$$

and

$$\text{Hue} = \tan^{-1}(b^* / a^*)$$

3.6 Solubility and hygroscopicity

The solubility was determined according to the method described by Chau, Wang, and Wen (2007). Briefly, samples were mixed with distilled water (1:10 w/v), stirred for 1 h at room temperature and centrifuged at 1,500 rpm for 10 min. The supernatant was collected, dried and weighed. The solubility was calculated using the equation below:

$$\text{Solubility (\%)} = \frac{\text{weight (g) of supernatant after drying}}{\text{weight (g) of sample}} \times 100$$

The hygroscopicity property of the powder samples was determined according to Cai and Corke (2000) with some modifications. Briefly, 2 g of spray-dried powder samples were placed in pre-weighed glass vials and placed in a desiccator containing

saturated salt solution of sodium chloride (relative humidity of 75.09%) maintained at 30°C and kept for 7 days. After the incubation period, sample vials were weighed and hygroscopicity was expressed as g moisture/100 g solids.

3.7 Determination of Ascorbic acid content

L-Ascorbic acid content was measured in powder samples by AOAC method 967.21 (AOAC, 2006) and the value was expressed as mg of ascorbic acid/100 g of dry solid.

3.8 Determination of total phenolic, and total flavonoid compounds

The total phenolic compound in the sample was determined by the Folin–Ciocalteu assay described by Singleton, Orthofer, and Lamuela-Raventós (1999) with slight modifications. Briefly, 20 μL each of extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 mL of distilled water was added, followed by 100 μL of Folin–Ciocalteau reagent, mixed well and within 8 min, 300 μL of sodium carbonate was added. The samples were vortexed immediately and the tubes were incubated in the dark for 30 min at 40°C. The absorbance was then measured at 765 nm in a UV-Vis spectrophotometer (Aquarius 7400, Cecil, Cambridge, England). The results were expressed in mg gallic acid equivalent (GAE)/100 g.

The flavonoid content was determined by aluminum trichloride ($AlCl_3$) method (Chang, Yang, Wen, & Chern, 2002). Briefly, 0.5 mL of the extract was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate, and 2.8 mL of deionized water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against deionized water blank in a UV-Vis spectrophotometer (Aquarius 7400, Cecil). Results were expressed as quercetin equivalent (mg QE/100 g) of sample.

3.9 Antioxidant activity

DPPH radical scavenging assay was performed according to Ghafar, Prasad, Weng, and Ismail (2010). Spray-dried samples (500 mg) was dissolved in 4 mL of 90 mL/100 mL ethanol and stirred for 30 min. Samples (200 mL) were reacted with 2.8 mL of 100 μM DPPH (dissolved in 80% ethanol) for 30 min in the dark. A control contained only DPPH solution and 80% ethanol was used as a blank. The absorbance was recorded at 515 nm using a UV-Vis spectrophotometer (Spectronic™ GENESYS™2, Thermo Fisher Scientific, Waltham, MA). Samples were analyzed in triplicate and reported as trolox equivalents.

3.10 Data analysis

All data were analyzed using SAS software version 9.2 (SAS Institute Inc., 2008). Means and standard deviations of the data were presented at the significant level of $P < 0.05$.