

# Development of spray-dried lime juice powder with improved bioactive compound retention

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**Abstract:** Lime juice powder was developed with 20% of combined maltodextrin/gum Arabic at a ratio of 4:1. A high shear homogenization was applied to encapsulate phytochemical compounds before spray drying with the purpose of protecting bioactive compounds from thermal degradation. The effect of high shear homogenization on physicochemical properties and antioxidant activity of spray-dried lime powder was studied. The particle size, morphology, moisture content, color, solubility, hygroscopicity, ascorbic acid, total polyphenol and flavonoid contents, and antioxidant activity of the spray-dried powder undergoing the homogenization were compared with those of the non-homogenized powder. The higher yield (30%) was obtained in the homogenized powder compared with the control (28%) with no significant difference. The average particle diameter of the homogenized powder was 0.1-4 micrometers, smaller than 18-26 micrometers of the untreated powder. Morphological study revealed that the powder without homogenization was densely packed compared with the homogenized powder. Moisture content of homogenized powder and that of the particles without homogenization were 3.91% and 5.42%, respectively. Higher solubility and less hygroscopicity values and color after spray drying were observed in reconstituted lime powder with high shear homogenization. Folin-Ciocalteu and aluminum trichloride (AlCl<sub>3</sub>) assays were used to determine total phenolic and flavonoid compounds, respectively. 2,2-diphenyl-1-picrylhydrazyle-hydrate (DPPH) assay was used to determine total antioxidant activity of the powders. The retention of ascorbic acid and total phenolic contents, and antioxidant activity was significantly better in the homogenized powder, but not the flavonoid contents. The antioxidant activity derived from total phytochemical compounds in the treated powder was preserved by the homogenization before spray drying. This study indicated that the application of high shear homogenization with combined drying agent before spray drying could prevent phytochemical compounds in lime from thermal degradation.

**Keywords:** Lime powder; spray drying; physicochemical properties; bioactive retention; antioxidant activity

## 1. Introduction

Lime (*Citrus aurantifolia*) is a popular ingredient used to accent flavors of Thai foods and beverages. Several species of citrus limes were investigated in vitamin C and phenolic compounds including flavonoid compounds which are all sources of natural antioxidants (Ghafar, Prasad, Weng, & Ismail, 2010). Due to the antioxidant properties of these compounds, lime has many functional health benefits such as prevention of vitamin C deficiency, heartburn and nausea, some forms of cancer, and relief of fever, coughs, and various respiratory disorders (Attaway, 1993; Theansuwan, Triratanasirichai, & Tangchaichit, 2008). It is of great interest to transform lime juice into dried powder with desirable properties to have high nutritional value of lime juice powder available throughout the year.

In order to enhance the nutritional quality of processed lime juice, the processing techniques must be carefully selected. Of various processing methods, spray drying is a technique applied to

efficiently dehydrate sugar- and acid-rich fruit juice using different drying agents with advantages of heat short-contact time, microbial growth inhibition, and storage stability (Largo Avila, Cortes Rodríguez, & Velásquez, 2015). By contacting hot air to evaporate water from small droplets, some heat-sensitive compounds in lime juice are degraded resulting in reduced total antioxidant capacity (Fazaeli, Emam-Djomeh, Kalbasi Ashtari, & Omid, 2012).

The entrapment of antioxidant compounds inside appropriate food-grade drying agents such as maltodextrin, gum Arabic, pectin, modified starch, or cellulose etc. combined with spray drying technique has been pronounced to protect the entrapped core against caking and stickiness, temperature and enzymatic changes. As mentioned by Chuacharoen and Sabliov (2016), the stability of bioactive compounds was improved and its total antioxidant activities were enhanced when encapsulated into the particles. In previous studies, the temperature of 170°C with the addition of drying

agents (maltodextrin, gum Arabic, and sucrose syrup) of approximately 10-20% w/v was proposed to increase the product mass of the spray-dried lime juice powder (Footrakul, Mawimol, & Boonyasupa, 2003; Shihawong, 2003). Caking of dried powder was observed when using only maltodextrin (Paterson & Bröckel, 2015). Therefore, a combination of maltodextrin and gum Arabic was proposed as a good protection after spray drying (Frascareli, Silva, Tonon, & Hubinger, 2012). Gum Arabic has become an entrapment aid for essential oils due to its emulsifying properties. The addition of high shear homogenization to stabilize the droplets before spray drying is challenge to influences good re-constititional characteristics in terms of particle size, water solubility, and hygroscopicity as well as physicochemical and functional properties of the spray-dried powder. Thus, drying operations must be carefully performed to minimize the loss of the powder's properties. The aims of the present study were to develop lime powder utilizing a combination of maltodextrin/gum Arabic as drying agents processed with a high shear homogenization prior to spray drying and evaluate the physicochemical properties and functionality in terms of color, solubility, hygroscopicity, vitamin C, total phenolic and flavonoid contents, and total antioxidant capacity of lime powder.

## 2. Materials and Methods

### 2.1 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<sub>3</sub>), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, and quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of analytical research grade.

### 2.2 Preparation of lime juice

Fresh lime (*Citrus aurantifolia*) was hand squeezed, and lime juice was filtered to remove seeds. The average amount of lime juice per kilogram, nutrition values, and total soluble solids of the lime juice were analyzed for references. Drying materials were separately rehydrated overnight and then gently heated at 60°C in a water bath to allow complete dissolution. The obtained 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 an 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 in parallel without homogenization. Then all mixtures were spray-dried as described in the following section.

### 2.3 Spray drying of lime powder

The prepared mixtures were diluted and filtered to remove insoluble solids 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 for all treatments. The reconstituted powders were stored at 4°C for further analyses. The spray drying experiment was carried out in triplicate. The yield of the spray drying process was calculated by considering the total solid content of the feed sample with maltodextrin/gum Arabic and weight of the final dried powder followed the equation below:

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

### 2.4 Particle size characterization and morphological study

The particle size of sample was measured using a laser diffraction particle size analyzer, Mastersizer 3000 (Malvern Instruments, Malvern, UK) equipped with a wet sample unit. A small amount of obtained spray-dried lime powder was suspended in ethanol under agitation, and the particle size was measured successively. The data acquisition was presented in micrometers (µm) (Tonon, Brabet, & Hubinger, 2010). The morphological study was done 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 observed with 1000× magnification. All measurements were performed in triplicate.

### 2.5 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 a 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 angles were calculated using the equation below:

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

and

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

## 2.6 Solubility and hygroscopicity

The solubility of the powder was evaluated according to the method described by Chau, Wang, and Wen (2007). Briefly, sample was added with distilled water (1:10 w/v) and stirred for 1 hour at room temperature and centrifuged at 1,500 rpm for 10 min. Then, 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 samples were placed in pre-weighed glass vials and placed in a desiccator containing saturated salt solution of sodium chloride (relative humidity of 75%) maintained at 30°C and kept for 7 days. After the incubation, sample vials were weighed, and hygroscopicity was presented as g absorbed moisture/100 g solids.

## 2.7 Determination of ascorbic acid, total phenolic, and flavonoid contents

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. Total phenolic compounds in the sample were 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 then 1.58 mL of distilled water was added, followed by 100 µL of Folin–Ciocalteu reagent, mixed well, and set for 8 min, 300 µL of sodium carbonate was added. The samples were vortexed immediately, and 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 as gallic acid equivalent (mg GAE/100 g). The flavonoid content was determined by aluminum trichloride (AlCl<sub>3</sub>) 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<sub>3</sub>, 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 spectrophotome-

ter (Aquarius 7400, Cecil). Results were expressed as quercetin equivalent (mg QE/100 g).

## 2.8 Antioxidant activity

DPPH radical scavenging assay was performed according to Ghafar et al. (2010). Spray-dried samples (500 mg) were dissolved in 4 mL of 90% ethanol solution and stirred for 30 min. Samples (200 µL) were reacted with 2.8 mL of 100 µM DPPH (dissolved in 80% ethanol) for 30 min in the dark. A control containing 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, Thermo Fisher Scientific, Waltham, MA). Samples were analyzed in triplicate and reported as trolox equivalents.

## 2.9 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  $P < 0.05$ .

## 3. Results and discussion

### 3.1 Lime juice composition

The average amount of lime juice per kg of lime was 621.8 mL or 0.59 kg. Vitamin C of lime juice per 100 g was 29.1 mg. The total soluble solids (TSS) of lime juice measured using a digital Refractometer (AR 200, Reichert, USA) was 11.7% Brix. A pH of lime juice was 2.1 which is sufficient to inhibit microbial growth due to its high acidity.

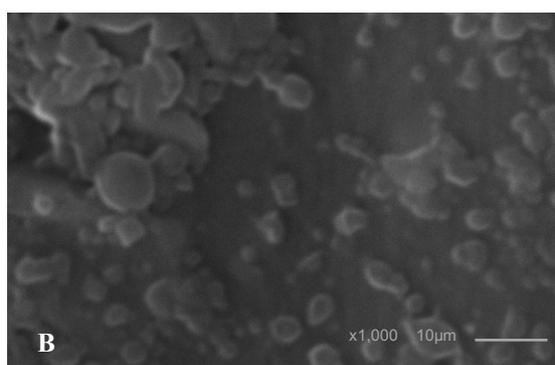
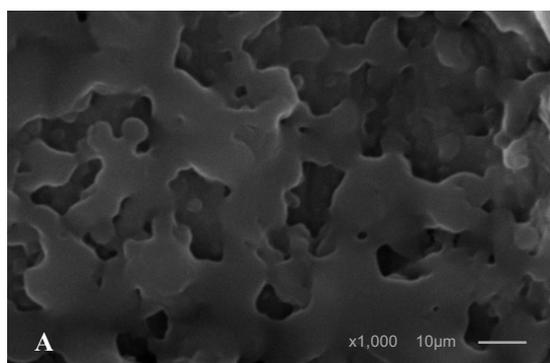
### 3.2 Morphology and particle size analyses

The spray drying conditions was kept constant for both systems. The average particle size (Table 1) of spray-dried lime powder obtained from homogenization ranged from 0.1 to 4 µm which was smaller than that of the non-homogenized powder (18-26 µm). It can be assumed that the homogenization of lime juice applied before spray-drying led to the formation of smaller and stable droplets due to the increased pressure (McClements, Decker, & Weiss, 2007) and the effect of gum Arabic by forming a thin film over the surface of the particles to consequently stabilize and reduce the surface adhesiveness among the droplets (Janiszewska, Jedlińska, & Witrowa-Rajchert, 2015).

**Table 1. Particle size of spray-dried lime powder with and without homogenization**

Samples	Average diameter (µm)
Non-homogenized lime powder	18-26
Homogenized lime powder	0.1-4

The morphological characteristics of spray-dried lime powder with and without high shear homogenization are shown in Figure 1. The surface morphology study showed different shape and sizes between two systems. In non-homogenized powder, the particles were clumped together and no individual particle was formed. While small and large spherical particles were observed in homogenized lime powder at 1,000x magnification, which is due to the effect of high shear homogenization on smooth surface, small size, and spherical shape made a difference from non-homogenized powders (Janiszewska et al., 2015).

**Figure 1. SEM images of (A) non-homogenized lime powders and (B) homogenized lime powders**

### 3.3 Lime powder analyses

The same conditions of spray drying were performed for all treatments. There was not statistically significant difference between yields in the homogenized and untreated powders (Table 2). Moisture contents regardless of drying agent are affected by the air flow rate, drying air temperature of the spray dryer (Goula & Adamopoulos, 2005), and the evaporation rate of the droplet (Chranioti,

& Tzia, 2016). When the small droplet is contacted with hot drying air, the evaporation of moisture occurs more rapidly compared with the bigger one (Bhattarai, Tran, & Duke, 2001). This caused low moisture content reported in homogenized powder (Table 2). It is consistent with Finney, Buffo, and Reineccius (2002) stated that larger particles lead to higher moisture content because it can block the spray-disc and form insufficiently dried large droplet.

**Table 2. Physical analyses of spray-dried lime powder with and without homogenization**

Analyses	Non-homogenized lime powder	Homogenized lime powder
Yield (%)	28.72±2.31 <sup>a</sup>	30.11±0.29 <sup>a</sup>
Moisture content (g/100g powder)	5.42±0.61 <sup>a</sup>	3.91±0.14 <sup>b</sup>
Color variables		
<i>L</i> <sup>*</sup>	79.5±0.01 <sup>a</sup>	84.1±0.01 <sup>a</sup>
Chroma	12.55±0.01 <sup>a</sup>	8.91±0.008 <sup>b</sup>
Hue angle	74.5±0.01 <sup>a</sup>	78.8±0.07 <sup>b</sup>
Solubility (%)	59.54±0.24 <sup>a</sup>	76.84±0.32 <sup>b</sup>
Hygroscopicity (g/100g powder)	12.99±0.11 <sup>a</sup>	11.68±0.27 <sup>b</sup>

Mean ± standard deviation of triplicate analysis. <sup>a,b</sup> means with same letters in each row are not significantly different ( $p < 0.05$ ).

Both types of lime powder had a whitish color, in which the color of homogenized lime powder was significantly lighter than that of the non-homogenized powder (Table 2). Chroma measurement indicates the vividness of color and the value of homogenized lime powder is lower. A hue angle indicates the color shade based on a degree angle of 0°, 90°, 120°, and 240° representing red, yellow, green, and blue colors, respectively. The homogenized lime powder had significantly ( $p < 0.05$ ) higher hue angle values as seen with stronger yellow color.

### 3.4 Solubility and hygroscopicity

The size of particles affects not only its physical properties, but may also contribute to solubility and hygroscopicity of the spray-dried powder. The sample treated with homogenization treatment showed the significantly higher solubility (76.84%), whereas the non-homogenized system showed the lower solubility (59.54%) (Table 2). The results indicated that the solubility of spray-dried powder increased with reducing size of particles, hence increasing the total surface area available for water binding (Fazaeli et al., 2012).

Hygroscopicity was decreased from 12.99 to 11.68 g absorbed water/100 g dried sample with reducing particle size of the powder. Such that the small particle powder containing low moisture content

indicated lower hygroscopicity. It is clear that the moisture content considerably influenced the powder's hygroscopicity (Santhalakshmy, Don Bosco, Francis, & Sabeena, 2015). Thus, the small particles with low moisture content contributed to increased solubility and less hygroscopicity which was observed in the system with homogenization.

### 3.5 Phytochemical contents and antioxidant activity analyses

Fresh lime juice contained 7.74 mg Gallic acid/g solid, 2.67 mg Quercetin/g solid, and 19.85  $\mu$ mol Trolox/g solid. The antioxidant activity derived from total phytochemical compounds in the powders was reported in Table 3. Maltodextrin and gum Arabic created a strong impermeable film around the droplets (Frascareli et al., 2012) that helps protect phytochemical compounds from hot drying air. Phytochemical contents and antioxidant activity of lime powders were decreased after the hot-air spray drying operation.

**Table 3. Ascorbic acid, gallic acid, quercetin, and trolox equivalents of spray-dried lime powder with and without homogenization**

Analyses	Non-homogenized lime powder	Homogenized lime powder
Ascorbic acid (mg/g solid)	0.19 $\pm$ 0.01 <sup>a</sup>	0.41 $\pm$ 0.02 <sup>b</sup>
Gallic acid equivalents (mg/g solid)	1.49 $\pm$ 0.12 <sup>a</sup>	2.51 $\pm$ 0.34 <sup>b</sup>
Quercetin equivalents (mg/g solid)	0.32 $\pm$ 0.01 <sup>a</sup>	0.37 $\pm$ 0.02 <sup>a</sup>
Trolox equivalents ( $\mu$ mol/g solid)	7.93 $\pm$ 0.12 <sup>a</sup>	13.48 $\pm$ 0.22 <sup>b</sup>

Mean  $\pm$  standard deviation of triplicate analysis. <sup>a,b</sup> means with same letters in each row are not significantly different ( $p < 0.05$ ).

The initial ascorbic acid in fresh lime juice (29.1 mg/100 g fresh lime juice) was degraded approximately 77% (0.19 mg/g dry solid calculated based on 30.11% yield) and 48% (0.41 mg/g dry solid calculated based on 28.72% yield) in lime powder without and with homogenization, respectively. The results showed that the entrapment process with combined drying agents and homogenization before spray drying help prevent some ascorbic acid loss. The similar results were observed with total phenolic content, which showed the higher amounts in homogenized powder, but total flavonoid content did not significantly differ between the two powders. In addition, the antioxidant activity measured by DPPH assay was significantly higher in the homogenized lime powder than the untreated powder

The variation in the phytochemical levels preserved by the homogenization may be due to the different degree of tolerance to heat of individual phenolic compounds and the difference in structural degradation during the hot drying process diversely affects the phenolic content and antioxidant activity of lime powder. Thus, the change in phenolic and flavonoid contents and antioxidant activity depends upon individual phenolic acid constituents and their susceptibility to heat and conformational changes. In summary, this study indicated that application of drying agents with the high shear homogenization helps protect some phytochemical compounds from heat during spray drying.

### 4. Conclusion

The homogenization of lime juice prior to spray drying leads to formation of smaller powder particles. The surface morphology between the powders with and without homogenization showed differences in shapes and sizes, which was smaller in the homogenized powder than the untreated powder. A reduced size of particles resulted in low moisture content due to faster diffusion rates. Both lime powders had a whitish color, in which the color of homogenized lime powder was significantly lighter than that of non-homogenized powder. The smaller particles with low moisture content resulted in increased solubility and less hygroscopicity of the powder, which implies the better powder quality during storage. The antioxidant activity derived from total phytochemical compounds was higher when the sample was homogenized before spray drying. Thus, application of encapsulating agents combined with high shear homogenization was a promising process to improve retention of phytochemical compounds in spray-dried powder during spray drying processing.

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