Effects of pretreatment and spray drying on the physicochemical properties and probiotics viability of Moringa (Moringa oleifera Lam) leaf juice powder

Yih Foo Looi1 | Sze Pheng Ong1 | Advina Julkifle2 | Mohd Suhaimi Alias3

Abstract
Spray drying was investigated in this study to produce Moringa leaf powder which was examined for its nutritional value and ability to support probiotics during storage with and without pretreatment. Response surface methodology with central composite rotatable design was performed to optimize the drying process for maximum yield based on two independent variables: inlet air temperature (116–144°C) and feed maltodextrin concentration (8.0%–22.5% w/w). The responding factors include drying characteristics (yield, color, bulk density, tapped density, water activity, and hygroscopicity) and phytochemical content (total phenolic content, total chlorophyll, and antioxidant activity). Each response was fitted to a model with goodness of fit determined using t test, coefficient of determination $(R^2)$, coefficient of variation (CV %), lack-of-fit, and signal-to-noise ratio. Pretreated and non-pretreated leaves powder at the optimum settings demonstrated viable (more than $10^6$ CFU/g powder) counts of both Lactobacillus acidophilus and Bifidobacterium lactis up to 28 days after inoculation.

Practical applications
Spray drying is a proven dehydration technique for heat-sensitive substance in its atomized liquid form to obtain the counterpart dry powder product without substantial thermal degradation. It is a rapid, cost-effective, and scalable process for the production of dry powders from vegetable and fruits with superior physical attributes and phytochemical properties as compared to other drying methods. Response surface methodology is a statistical tool that is very useful in the optimization and control of food processes. The information provided in this work will serve as the basis for further study on producing spray-dried Moringa leaves powder at optimal pretreatment and spray drying conditions. The result is very valuable in considering the different processing variables and product qualities at the same time as compared with single factor experiments. This potentially leads to improved product color and phytochemicals retention while increasing production yield. Positive result shown in the probiotic compatible and viability analysis has revealed that the spray-dried Moringa leaves powder may find its potential applications in the production of...
1 | INTRODUCTION

Moringa (Moringa oleifera Lam) is a nutritious but low requirement crop that is mainly cultivated in Africa. Its leaf powder is commercially valued above common subsistence crops and is a complete protein source consisting of (dry basis) 44.4% carbohydrates, 28.7% protein, and 7.1% fat along with minerals as well as fiber (Baipheithi & Jacobs, 2009; Moyo, Masika, Hugo, & Muchenje, 2013; Teixeira, Carvalho, Neves, Silva, & Arantes-Pereira, 2014). Moringa leaves are rich in natural antioxidants such as ascorbic acid, carotenoids, and phenolic substances. Among those bioactive compounds, the flavonoid groups in Moringa leaves (e.g., quercetin and kaempferol) were found to be the major contributor to its antioxidant activity (AOA) (Siddhuraju & Becker, 2003). Several studies demonstrated the antihypertensive, antibacterial, antioxidant, anticancer, and radio protective effects of Moringa leaf extracts during in vitro trials (Dangi, Jolly, & Narayanan, 2008; Luqman, Kumar, Maurya, & Chanda, 2012; Rao, Devi, & Kamath, 2001; Singh et al., 2009).

The current commercial method of producing Moringa leaf powder begins with oven or solar drying for several hours followed by grinding into powder (Bosch, 2004). These techniques would result in a partially soluble green powder and thorough processing is required before drying in order to prevent inedible small branches from entering the final product. Attempting to speed them using higher drying temperature may result in the loss of heat-sensitive nutrients which includes vitamin C and chlorophyll (Davies, Austin, & Partridge, 1991). Furthermore, color degradation would occur when the chlorophyll pigments (that released during processing) are rapidly converted into pheophytin (a green-gray pigment) during the drying process. Consequently, this gives the resulting powder the familiar color of wilting leaves which is perceived by consumers as a loss of quality (Gupte, El-Bisi, & Francis, 1964; Hörtenstein & Kräutler, 2011; NiIR Board of Consultants & Engineers, 2016).

Spray drying is an established technique for rapidly drying large quantities of liquids into fine powder while remaining gentle enough for use with heat-sensitive compounds (Bates, Morris, & Crandall, 2001). Generally, plant-derived juices possess low glass transition temperature ($T_g$) due to the low molecular weight sugars (e.g., sucrose, glucose, fructose) present in the liquid and this would cause stickiness problem when spray drying temperature goes above its $T_g$ (Bhandari, Senoussi, Dumoulin, & Lebert, 1993; Goula & Adamopoulos, 2010). Hence, adding a drying agent into the plant juice could raise its $T_g$ and enable drying at a higher temperature without sticking on the dryer wall or form unwanted agglomerates that would lead to reduction in product yield. Maltodextrin is one of the favored drying agents due to its lower cost and greater availability as compared to gum Arabic. Maltodextrins of lower dextrose equivalent (DE) were shown able to reduce product’s hygroscopicity and increase product yield for spray dried mulberry juice (Fazaeli, Emam-Djomeh, Kalbasi-Ashtari, & Omid, 2012b; McNamee, O’Riordan, & O’Sullivan, 1998). Nevertheless, determination of the optimum concentration of drying agent is crucial in ensuring the desired product quality and minimizing material cost.

On the other hand, probiotics are vital for healthy intestines and their growing popularity with consumers is reflected in market demand which projected to increase from the 32.06 billion USD in 2013 to 46.55 billion USD by 2020 (Dixit, Wagle, & Vakil, 2016; Islauri, Sutas, Kankaanpaa, Arvilommi, & Salminen, 2001). Most probiotic-fortified foods in the market contain strains of either Lactobacilli or Bifidobacterium genera, each of which have different requirements to maintain a viable population over prolonged storage (Ellen, 2003; Speranza et al., 2018). In particular, Lactobacillus acidophilus and Bifidobacterium lactis have been reported in numerous clinical trials that focused on digestive and immune health. Over the course of their long use in dairy products, they have been found to enhance nutritional value by synthesizing vitamins (B and K) and enhancing the bioavailability of minerals (Gomes & Malcata, 1999). Yogurt fortified with probiotics L. acidophilus and B. lactis could reduce the total cholesterol as well as LDL-C concentrations in type 2 diabetes patients and may contribute to the improvement of cardiovascular disease risk factors (Ejtahed et al., 2011). Furthermore, supplementation of milk with B. lactis and galactooligosaccharides had shown a significant reduction of dysentery, respiratory morbidity, and febrile illness in children aged 1–4 years in India (Sazawal et al., 2010; Sazawal, Dhingra, & Sarkar, 2004). Nonetheless, ensuring their viability, which is to have more than 10^6 colony-forming units (CFU), requires controlling pH, storage temperature, and water activity (Bladon & Mahaptra, 2011).

Vanajakshi, Vijayendra, Varadaraj, Venkateswaran, and Agrawal (2015) have demonstrated that a mixture of beetroot and Moringa leaf juice could maintain a viable count of Lactobacillus plantarum for more than 30 days at pH 6.5 and 4°C. However, there is no study thus far on the ability of Moringa leaves to support Lactobacillus acidophilus and Bifidobacterium lactis.

The objective of this study is to evaluate the effect of inlet air temperature and maltodextrin concentration on the drying characteristics and phytochemical content of spray-dried Moringa oleifera leaves. Drying characteristics comprising of yield, powder density (bulk, tapped), water activity ($a_w$), and hygroscopicity as well as phytochemical content comprising of total phenolic content (TPC), total chlorophyll, and AOA will be investigated. Data on drying characteristics and phytochemical content were subsequently optimized for maximum yield using Response Surface Methodology (RSM) with Central Composite Rotatable Design (CCRD). The predicted yield, drying characteristics,
and phytochemical content was experimentally validated and the resulting powder was tested for its ability to maintain viable counts of *L. acidophilus* and *B. lactis* with and without pretreatment.

## 2 | MATERIAL AND METHODS

### 2.1 | Sample preparation

Moringa leaves were obtained from the Crops for the Future (CFF, Semenyih, Selangor, Malaysia) research center. Leaves were selected based on similar size (ca. 15–20 mm×10 mm) and color (green with *L**, *a**, and *b** values of approximately 17.2, ~4.0, and 8.1, respectively). Still attached to their stems, collected leaves were rinsed clean of dirt with tap water before being briefly soaked in 0.9% saline to remove microorganisms (Lund & Baird-Parker, 2000). The leaf samples (Figure 1) were kept in a refrigerator (Protech LR-1050) at 5°C for no more than 3 days until juicing. Moisture content of fresh sample was determined based on a method according to Bradley (2010). The initial moisture content was recorded at 80.4 ± 0.4% (wet basis).

### 2.2 | Sample pretreatment and juicing

Moringa leaf samples were pretreated with steam blanching (when pretreatment was required) according to the procedure in Agüero, Pereda, Roura, Moreira, and del Valle (2005) with slight modification. About 100 g of Moringa leaves were spread evenly in a steaming tray and placed on top a steamer (Panasonic SR-Y22 FGJ). After 100 s of blanching, the samples were immediately quenched in an ice bath (4°C) for 30 s and then drained.

Juice samples were prepared using a centrifugal juicer (Panasonic MJSG01) with a ratio of 10 g of leaves to 25 ml of distilled water. The crude juice was filtered with a strainer to remove the particle solids, resulting in filtered juice with total solids of 28.4 mg/g juice, pH of 5.05 ± 0.09, and °Brix of 3. The filtered juice was kept in the dark and frozen in a deep freezer (Sharp SJ3C315) at −16.5°C until further use.

### 2.3 | Spray drying

A pilot-scale spray dryer (Anhydro Lab S1, MARDI) as shown in Figure 2 was used to perform the spray drying. Juice was fed at 0.64 ml/s and atomized through a 3-mm pneumatic nozzle using dry compressed air at 50 kPa. Drying was performed in countercurrent where atomized juice sprayed upward encountered hot air coming down from the top of the spray dryer. Independent variables for the spray drying were determined from a CCRD using Design-Expert Version 10 (Stat-Ease, Inc).

The frozen Moringa leaf juice (pretreated or non-pretreated) was first thawed for 12 hr in dark at room temperature (ca. 24°C) and then mixed with maltodextrin of 12 DE (Roquette Glucidex 12). Once the inlet air temperature set point was achieved, water was fed at 0.49 ml/s and evaporated to even out the temperature gradient within the dryer. When the drying temperature was stabilized as indicated by a consistent ±2°C variation in outlet air temperature, the juice was pumped in at 0.64 ml/s. This flow rate was chosen as it ensured complete atomization and was fixed for all spray drying runs to avoid affecting yield (Tonon, Brabet, & Hubinger, 2008). All powder produced was packaged inside flat-pressed, heat-sealed opaque aluminum foil bags, and stored at 5°C in the presence of desiccant until further analysis.

### 2.4 | Drying characteristics

#### 2.4.1 | Yield

Yield was calculated using Equation (1) and expressed on a dry basis as g spray-dried powder/100 g dry solids fed (%) (Fazaeli et al., 2012b).
The mass of solids in the juice \( (m_{\text{solids in juice}}) \) was determined by multiplying the mass of juice with the total solids in the juice (28.4 mg/g).

### 2.4.2 Bulk and tapped density

Bulk density \( (\rho_{\text{bulk}}) \) was determined by taking a known mass of spray-dried powder and then gently added into a 10-ml graduated measuring cylinder (readable to 0.1 ml) using a funnel (Jinapong, Suphantharika, & Jannong, 2008). The resulting volume was recorded. Subsequently, the same measuring cylinder was tapped 400 times from a height of 4 cm onto a thick stack of paper and the resulting volume was recorded to determine the tapped density \( (\rho_{\text{tapped}}) \). Both densities were calculated using the relation: kg of powder/m\(^3\) measured.

### 2.4.3 Water activity

Water activity, \( a_w \) (unitless), was measured using an AquaLab Pawkit water activity meter (Decagon Devices Inc.).

### 2.4.4 Hygroscopicity

Hygroscopicity of powder sample was determined according to the method described by Farimin and Nordin (2009). Spray-dried powder (1 g) was kept in a container at room temperature (ca. 24°C) and relative humidity of 83% RH (adjusted using saturated NaCl solution 36%) for 7 days. Hygroscopicity was calculated as g of moisture gained per 100-g solids.

### 2.4.5 Color

Sample color was examined using a colorimeter (Lovibond LC100). All color readings were made in triplicates with the average computed by the colorimeter. The color readings (parameters \( L^* \), \( a^* \), and \( b^* \)) were used to calculate the total color change \( (\Delta E) \) between the spray-dried sample (subscript “2”) and fresh Moringa leaf juice (the control, subscript “1”) using Equation (2) (Sharma, 2003).

\[
\Delta E = \sqrt{\left(L^*_2 - L^*_1\right)^2 + \left(a^*_2 - a^*_1\right)^2 + \left(b^*_2 - b^*_1\right)^2}
\]

The control sample possessed \( L^*_1 = 23 \), \( a^*_1 = -2.3 \), and \( b^*_1 = 20.9 \). Level of browning was determined using browning index \( (BI) \) with Equation (3) (Palou, Lopez-Malo, Barbosa-Canovas, Welti-Chanes, & Swanson, 1999).

\[
\text{Browning index } (BI) = \frac{100(x-0.31)}{0.17} \text{, where } x = \left(\frac{a^* + 1.75L^*}{5.645L^* + a^* - 0.3012b^*}\right)
\]

### 2.5 Phytochemical content

#### 2.5.1 AOA capacity

AOA capacity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay reported by Pothitirat, Chomnawang, Supabphol, and Gritsanapan (2009) with slight
modifications. First, 3 mg of spray-dried powder was dissolved in 10 ml of methanol solvent (80%) and then the mixture was centrifuged at 6,000 rcf for 10 min. Next, 6 ml of the extract supernatant was decanted and topped up with 6 ml of DPPH assay (152 μM in methanol) before being held for 30-min incubation at room temperature in dark. Lastly, the absorbance reading was measured at 517 nm using a UV–Vis spectrophotometer (Perkin Elmer Lambda 35). The % inhibition of the sample was calculated using Equation (4).

\[
\text{% Inhibition} = \left( \frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}} \right) \times 100\%
\]  

(4)

where \( A_{\text{DPPH}} \) and \( A_{\text{sample}} \) are the absorbance of DPPH and sample solution, respectively.

AOA is expressed as mg of L-ascorbic acid equivalent per g spray-dried powder required for 50% inhibition of DPPH (mg AAE/g) as shown in Equations (5) and (6).

\[
\frac{\text{mgAAE/g}}{\text{g sample}} = \left( \frac{\text{EC50}_{\text{L-ascorbic acid}}}{\text{EC50}_{\text{sample}}} \right) \times 1000 \times \frac{\text{mg sample}}{\text{g sample}}
\]

(5)

\[
\text{EC50}_{\text{sample}} = \frac{50}{\text{%Inhibition/0.15}}
\]

(6)

where \( \text{EC50}_{\text{L-ascorbic acid}} = 2.22 \mu g \text{AAE/mL} \) from a calibration curve \((y = 22727x)\) which was produced from L-ascorbic acid with concentrations ranging from 0.50 to 2.25 μg/mL. EC50sample (mg/mL) is obtained by linearly extrapolating the % Inhibition calculated using Equation (4) with Equation (6) where 0.15 refers to the concentration of the extract.

### 2.5.2 | Total phenolic content

Extraction was performed according to Siddharaju and Becker (2003). Briefly, 0.4 g of spray-dried powder was dissolved in 10 ml of acetone solvent (80%). The mixture was sonicated for 25 min at 100% power and 35 kHz (Elma TI-H-15 ultrasonicator) before being centrifuged at 6,000 rcf for 10 min. The extract supernatant was analyzed using the Folin–Ciocalteu method (Ong & Law, 2011) and the absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Perkin Elmer Lambda 35). A calibration curve was constructed with gallic acid concentrations between 0.005 and 5.000 mg/ml \((y = 0.6655x - 0.1422)\). TPC was expressed as mg gallic acid equivalents per g spray-dried powder (mg GAE/g).

### 2.5.3 | Total chlorophyll

The concentration of chlorophyll \(a\) and chlorophyll \(b\) in the spray-dried powder samples were determined according to Bojović and Stojanović (2005) using 0.1 g of spray-dried powder dissolved in 10 ml of 80% acetone. Absorbance of the extract was read using a UV–Vis spectrophotometer (Perkin Elmer Lambda 35) at 663 and 646 nm. The concentrations of both chlorophylls were calculated according to Wellburn (1994) and added to determine total chlorophyll (mg/g) according to Equation (7):

\[
\text{Total chlorophyll} \left( \frac{mg}{g} \right) = \text{Chlorophyll} \ a \left( \frac{mg}{ml} \right) + \text{Chlorophyll} \ b \left( \frac{mg}{ml} \right)
\]

\[
\times \frac{mg}{1000mg} \times \frac{10ml}{0.1g}
\]

(7)

where "10 ml" accounts for the dilution performed and "0.1 g" refers to sample size.

### 2.6 | Optimization using RSM

Preliminary runs were performed to determine the limits of inlet air temperature (X1) and maltodextrin concentration (X2). As shown in Table 1, minimum \( X_1 \) was set to 120°C due to the minimum yield (3.6%) that could be obtained when drying the juice containing 8% maltodextrin. Meanwhile, maximum \( X_2 \) was set to 140°C to prevent outlet air temperature at the cyclone from exceeding 90°C which will clog the cyclone due to excessive sticking of the particles. Minimum \( X_2 \) was set to 8% to ensure a TSS of 0.12 g/g feed to produce powder particles of sufficient density for separation by the cyclone used (Peukert & Wadenpohl, 2001), while maximum \( X_2 \) was selected based on prior spray drying works involving maltodextrin (Fazaeli et al., 2012b; Krishnan, Bhosale, & Singhal, 2005; Kurozawa, Park, & Hubinger, 2009; Yoshih et al., 2001).

The predetermined limits of \( X_1 \) and \( X_2 \) were subjected to RSM over a two-level experimental design based on a CCRD \((\alpha = 1.41421, k < 6)\) with the aid of Design-Expert Version 10 (Stat-Ease, Inc). The selected response variables were yield \((Y_1)\), bulk density \((Y_2)\), tapped density \((Y_3)\), water activity \((Y_4)\), hygroscopicity \((Y_5)\), AOA \((Y_6)\), TPC \((Y_7)\), and total chlorophyll \((Y_8)\). The design matrix consisted of 13 trials with 5 replicates at center point to estimate experimental error. Fitting revealed that the experimental data conformed with a general quadratic polynomial model (Equation (8)) which represented the relationship between the independent variables and their responses (Montgomery, 2009).

\[
Y = \beta_0 + \sum_{i=1}^{2} \beta_i x_i + \sum_{i=1}^{2} \beta_i x_i^2 + \sum_{i=1}^{2} \sum_{j=1}^{2} \beta_{ij} x_i x_j + \epsilon
\]

(8)

where \( \beta_0, \beta_i, \beta_{ij} \) represent the intercept, linear, quadratic, and interaction coefficients, respectively. The terms \( x_i \) and \( x_j \) represent independent variables. \( Y \) is the response, while \( \epsilon \) represents the error between responses. The values of all coefficients \( \beta_i \) were determined by multiple regression and its significance by ANOVA (analysis of variance). Goodness of fit was examined by \( t \) test, coefficient of determination \((R^2)\), coefficient of variation \((CV\%)\), and lack-of-fit test. The fitted model was expressed as a response surface plot to visualize the interaction between the independent and responding variables. Subsequently, optimization was performed numerically and targeted to obtain the best product quality (minimum hygroscopicity and water activity but maximum bulk density, tapped density, AOA, TPC, and total chlorophyll) and product yield. The predicted optimum condition was validated experimentally.
2.7 | Probiotic viability in spray-dried powder

Pretreated and non-pretreated Moringa leaves were juiced and spray-dried using the optimum setting for maximum yield (13.7 wt% maltodextrin, inlet air temperature of 134°C). The powder samples (30 g each) were inoculated with 2.3 g of commercial probiotic powder (BIO-LiFE A.B. Junior PRE & PRO) rated for one billion CFUs of both *Lactobacillus acidophilus* (LA5) and *Bifidobacterium lactis* (BB12) and contained chicory-derived prebiotics. All powder produced was packaged inside flat-pressed, heat-sealed opaque aluminum foil bags, and stored in the presence of desiccant. Probiotic viability was determined by the number of CFUs of both probiotics in the powder. The CFUs were counted on the day of 0, 7, 14, 21, and 28 days of storage in the sealed aluminum foil bags which were kept in the dark at 30°C and 75% RH (Zone IVb conditions for stability testing). CFUs of *L. acidophilus* and *B. lactis* were counted after first plating on De Man, Rogosa, and Sharpe (MRS) agar and transgalactosylated oligosaccharide substrate containing mupirocin (TOS-MUP) agar, respectively, followed by incubation at 30°C for 72 hr in accordance with ISO 29981:2010 (Collins, Lyne, & Grange, 1995).

### 3 | RESULTS AND DISCUSSION

#### 3.1 | Yield

Referring to Figure 3a, increasing inlet air temperature increased yield over the range of temperatures tested. The same observation was made during the spray drying of acai fruit over inlet air temperatures of 138–202°C (10–30 wt% maltodextrin) as well as for the spray drying of black mulberry juice over inlet air temperatures of 110–150°C (maltodextrin concentration of 8–16 wt%) (Fazaeli et al., 2012b; Tonon et al., 2008). Based on these prior works and the similarity of their drying conditions with this work, it can be speculated that maximum yield lies above 144°C. However, using higher drying temperatures would risk in destroying a larger proportion of phytochemicals that vital to the powder’s nutritional value and increasing stickiness by exceeding the Tg which will decrease yield.

As for maltodextrin concentration, going beyond 14% maltodextrin reduced yield. A possible reason for this lies in the increase in feed viscosity. Since a more viscous liquid requires a greater compressed air pressure to reach the same degree of atomization, the reduction in degree of atomization with increasing maltodextrin concentration due to the same compressed air pressure being used resulted in larger droplets (Gaonkar, Vaisht, Khare, & Sobel, 2014). It is possible that these larger droplets have a greater tendency to form semi-wet deposits on the dryer wall which resulted in the observed decrease in yield (Fazaeli et al., 2012b). However, at 14% maltodextrin and below, the addition of maltodextrin increased yield by raising the feed’s total soluble solids without affecting atomization to the extent required to decrease yield.

#### 3.2 | Bulk and tapped density

Bulk and tapped densities are two of the many parameters that are important in measuring powder flow characteristics, especially in the evaluation of apparent volume and compressibility index of pharmaceutical powders. High bulk and tapped densities are very
FIGURE 3 Effects of inlet air temperature and maltodextrin concentration on the chosen responses. (a) Yield, (b) Bulk density, (c) Tapped density, (d) Water activity, (e) Hygroscopicity, (f) AOA, (g) TPC, and (h) Total chlorophyll
important in packaging and transportation of powder product as it can significantly reduce the production costs via maximum packing density of powder, which can be achieved with or without externally applied forces (Augustin, Clarke, & Craven, 2003; Shah, Tawakkul, & Khan, 2008). In the present study, Figure 3b,c show that both bulk and tapped densities were increasing with increasing maltodextrin concentration. The results were in agreement with observations made for the spray drying of pitaya and soy milk (Jinapong et al., 2008; Tze et al., 2012). However, it was observed that the bulk density was decreasing with increasing inlet air temperature regardless of maltodextrin concentration. This could be due to the increasing inlet air temperature that encourages the production of larger powder particles due to fast drying (Korhonen et al., 2002) and hence increasing the intergranular air space per unit volume. In addition, the powder particles become progressively lighter due to greater removal of moisture by evaporation (Tze et al., 2012). By contrast, the tapped density was increasing with increasing inlet air temperature when above 14% maltodextrin concentration. This is likely due to the individual powder particles having a fragile structure when dried at higher temperature, which upon tapping easily collapses into smaller particles that rearrange into a more compact form (Li, Rudolph, Weigl, & Earl, 2004).

### 3.3 Water activity ($a_w$)

Water activity is a vital water sorption property in ensuring the end quality of a dehydrated product and its stability during storage. In the present study, Figure 3d shows that increasing both the concentration of maltodextrin and inlet air temperature led to reduced $a_w$ with inlet air temperature having a greater effect. Tze et al. (2012) credited the decrease in $a_w$ with increasing inlet air temperature to decreasing moisture content. Maltodextrin reduces $a_w$ owing to its low hygroscopicity which reduces the amount of free water molecules available for chemical and biological reactions (Aramouni & Deschenes, 2014; Rockland & Nishi, 1982). Furthermore, the entire response surface of $a_w$ in Figure 3d is below the threshold for spoilage ($a_w < 0.6$) which could prevent microbial spoilage and some deteriorative reactions (Campbell-Platt, 2011).

### 3.4 Hygroscopicity

Hygroscopicity is a measure of the ability of a powder to take up moisture from the atmosphere. It is an important adsorption isotherm in the case of spray dried powder particularly in determining the alteration of physical, chemical, and biochemical properties of the powder during the production and storage. As shown by Figure 3e, hygroscopicity increased with increasing inlet air temperature. This could be due to the corresponding decrease in moisture content (Tonon et al., 2008). However, increased maltodextrin concentration had the opposite effect of decreasing hygroscopicity and this effect is more pronounced at higher inlet air temperatures. This is due to the low hygroscopicity of maltodextrin and the same effect was observed during the spray drying of betacyanin pigments with maltodextrin (Cai & Corke, 2000).

### 3.5 Total color change ($\Delta E$) and browning index ($BI$)

Color parameters for fresh Moringa juice was determined to be $L^* = 23$, $a^* = -2.3$, and $b^* = 20.9$. It can be observed from Table 2 that all the spray dried powders displayed a noticeable total color change compared to freshly made Moringa leaf juice ($\Delta E > 2.3$). However, the total color change decreased sharply for all samples upon reconstitution in a ratio of 1 g of powder to 100 ml of distilled water. This could be due to the changes in $L^*$, $a^*$, and $b^*$ after reconstitution with $L^*$ being reduced considerably for all samples, while $a^*$ and $b^*$ increased significantly.

![Table 2](image)

**Table 2** Effect of drying temperature and maltodextrin concentration on physicochemical properties of spray-dried powder and its reconstitution

| Sample | Powder | Reconstitution |
|--------|--------|----------------|
|        | $L^*$  | $a^*$ | $b^*$ | $\Delta E$ | $BI$ | $L^*$  | $a^*$ | $b^*$ | $\Delta E$ | $BI$ |
| 1      | 62     | -1    | 31    | 40.1      | 3.82 | 42     | 0     | 33    | 22.2      | 8.12 |
| 2      | 64     | 0     | 28    | 41.6      | 4.74 | 28     | 2     | 19    | 7.1       | 13.08|
| 3      | 70     | 0     | 26    | 47.1      | 3.56 | 48     | 0     | 29    | 26.6      | 5.87 |
| 4      | 66     | 3     | 26    | 43.3      | 7.59 | 41     | 5     | 38    | 25.9      | 18.23|
| 5      | 66     | 1     | 29    | 43.3      | 5.38 | 41     | 2     | 34    | 22.8      | 12.41|
| 6      | 59     | -1    | 31    | 37.7      | 4.23 | 27     | 1     | 28    | 9.0       | 13.21|
| 7      | 73     | 0     | 22    | 49.6      | 3.25 | 51     | 0     | 22    | 28.1      | 3.83 |
| 8      | 74     | 0     | 21    | 51.0      | 3.16 | 52     | 0     | 20    | 29.1      | 3.37 |
| 9      | 57     | -1    | 34    | 36.1      | 4.20 | 24     | -1    | 23    | 2.6       | 8.33 |
| 10     | 70     | -1    | 25    | 47.2      | 2.35 | 49     | -1    | 26    | 26.2      | 4.20 |
| 11     | 60     | -1    | 29    | 37.8      | 3.94 | 44     | 0     | 33    | 24.2      | 8.21 |
| 12     | 63     | -1    | 30    | 40.7      | 3.32 | 46     | 0     | 29    | 24.8      | 5.90 |
| 13     | 67     | 0     | 26    | 44.1      | 3.70 | 43     | 1     | 31    | 22.5      | 8.29 |

Note. Color parameters of control fresh juice: $L^*_1 = 23$, $a^*_1 = -2.3$, and $b^*_1 = 20.9$. 

This table provides the effect of drying temperature and maltodextrin concentration on the physicochemical properties of spray-dried powder and its reconstitution.
b" experiencing either a relatively small increase or decrease. Higher L* value was observed in the spray-dried powder as compared to its counterpart reconstitution and fresh juice. This could be due to the added maltodextrin which usually appears in white color when in its powder form making the spray dried product look brighter. On the other hand, it was found that both a* (~1–5) and b* (~19–38) values had increased marginally (toward positive axes of red and yellow color) in the spray-dried powder and its reconstitution when compared to the fresh juice. These findings showed the occurrence of browning after the spray drying process.

Samples 2, 6, and 9 possessed the lowest ΔΕ (7.1, 9.0, and 2.6, respectively) after reconstitution while ranking among the top five for TPC (237.5, 152.4, and 221.9 mgGAE/g, respectively) and total chlorophyll content (0.73, 0.90, and 1.25 mg/g, respectively) as shown in Table 1. This revealed the importance of retaining original phenolics and chlorophyll in Moringa leaf juice to minimize ΔΕ. Nevertheless, it was not possible to fit a response surface over the ΔΕ as a function of inlet air temperature and maltodextrin concentration due to the insignificant correlation. It can also be observed that samples 1 and 11 did not achieve low ΔΕ (22.2 and 24.2, respectively) despite having high retention in TPC (161.3 and 232.6 mgGAE/g, respectively) and total chlorophyll content (0.78 and 0.93 mg/g, respectively), possibly due to their higher browning index. This could possibly be due to a lower degree of oxidation of chlorophyll and phenolics in these two samples (Chaturvedula & Prakash, 2011; Pirie, 1987). In addition, Table 2 shows that browning indices generally decreased with increasing maltodextrin concentration. Similar findings were reported by Fazaeli, Emam-Djomeh, Kalbasi-Ashtari, and Omid (2012a) in the spray drying of black mulberry juice, where increasing the concentration of maltodextrin causes lightness (L’ value) to increase but a* and b* values to decrease. Fazaeli et al. (2012a) also revealed that maltodextrin concentration could affect the rate of browning reactions if spray drying is performed at an elevated temperature. They found the browning index of black mulberry powders were significantly increased when the inlet air temperature rose. This could be due to the fact that when drying temperature increased, the lightness (L’ value) of black mulberry powders decreased owing to more browning reaction in the final powder with higher sugar content (high maltodextrin concentration). On the other hand, it was found in the present study that the rehydrated powder always showed a higher browning index compared to its counterpart in the dried powder form. All samples experienced a decrease in lightness (L’ value) and an increase in the redness (a* value) after reconstitution thus becoming darker. Yousefi, Emam-Djomeh, and Mousavi (2011) observed the same when reconstituting pomegranate powder produced from spray drying with maltodextrin.

3.6 | Phytochemical analysis

3.6.1 | AOA and TPC

Figure 3f shows that increasing maltodextrin concentration would result in reducing AOA, possibly due to the added maltodextrin having “diluted” the amount of antioxidants in the powder sample (Mishra, Mishra, & Mahanta, 2014). In contrast, higher inlet air temperature had resulted in increased AOA at the same maltodextrin concentration. This observation ran contrary to the expected decrement in AOA after heat treatment (Vallejo, Tomás-Barberán, & García-Viguera, 2002). The results revealed that the main antioxidant capacity of the spray dried powder was most likely contributed by phenolic compounds, which have a higher resistance to the thermal processing, instead of vitamin C or other antioxidants (Dewanto, Wu, Adom, & Liu, 2002; Kikugawa, Kunugi, & Kurechi, 1990; Ranilla, Genovese, & Lajolo, 2009). A few studies reported higher AOA could correspond to higher TPC (Bourekoua et al., 2018; Sowndhararajan & Kang, 2013).

As shown in Figure 3g, increasing the concentration of maltodextrin would result in a large decrease in TPC. Similar to AOA mentioned above, Mishra et al. (2014) found this phenomenon could be due to the added maltodextrin having a “diluting” effect on the phenolics concentration. Furthermore, it was observed that TPC was increasing with inlet air temperature, which was consistent with the increasing trend in AOA. It can be hypothesized that drying may have resulted in partial degradation of cell wall that could lead to release of phenolic derivatives and hence enhance extractability of the hydroxyl phenol groups in the dried sample (Maillard & Berset, 1995). Thus far, the identified phenolic compounds in Moringa leaves consisted of kaempferol, queratin, chlorogenic, caffeic, coumaric, ferulic, and gallic acid (Castillo-López et al., 2017; Manguro & Lemmen, 2007). Some of these phenolic compounds were reported to be thermostable, for instance, the queratin that could be retained up to 120°C (start to decrease at 150°C) and kaempferol that could be retained up to 160°C (start to decrease at 180°C) (Oliveira et al., 2017; Sharma et al., 2015).

3.6.2 | Total chlorophyll

It can be observed from Figure 3h that the total chlorophyll content was decreasing with increasing maltodextrin concentration, due to the same dilution effect mentioned above for both AOA and TPC (Mishra et al., 2014). However, there was a slight increase in the chlorophyll content when there was an increment in drying temperature. A possible explanation for this observation could be that the detected “additional chlorophyll” (or color pigment) is the product of thermal breakdown during drying, which more than compensates for the loss of chlorophylls a and b measured at 646 and 663 nm, respectively. Furthermore, a study on drying of Welsh onion leaves had shown that small increase in chlorophyll content at elevated temperature could be due to the concentration of remaining chlorophylls owing to higher moisture content removal at the higher drying temperature (Mongpraneet, Abe, & Tsurusaki, 2002).

3.7 | Optimization with RSM

Eight responses were considered in the present study. Values of each response variable and its corresponding independent variables
are shown in Table 1. Standard deviations shown for results obtained in triplicate were not factored into the optimization. All models developed in accordance with the independent variables are significant (p < 0.05), which means they can model the response variables accurately. Some models include variables outside the standard models (linear, 2FI, quadratic) to improve their fit with response data. However, the relatively low \(R^2\) and relatively large CV % of AOA (\(Y_6\)), TPC (\(Y_7\)), and total chlorophyll (\(Y_8\)) suggests that the experimental data of these responses were affected by experimental error (Tonon et al., 2008). Despite this, the models for \(Y_6\), \(Y_7\), and \(Y_8\) and the other responses have an adequate lack-of-fit (more than 10%) and a sufficient large signal-to-noise ratio (more than 4) to navigate the design space (Montgomery, 2009; Suhag & Nanda, 2016). The regression coefficients \(\beta_{ij}\) for each model are shown in Table 3.

It was found that inlet air temperature (\(X_1\)) is statistically significant for models developed for yield (\(Y_1\)), tapped density (\(Y_3\)), and water activity (\(Y_4\)). Meanwhile, maltodextrin concentration (\(X_2\)) is statistically significant for models developed for bulk density (\(Y_2\)), tapped density (\(Y_3\)), hygroscopicity (\(Y_5\)), AOA (\(Y_6\)), TPC (\(Y_7\)), and total chlorophyll (\(Y_8\)).

### 3.8 Validation of optimum settings

Response surface models were developed for each of the response variables (\(Y_1\)–\(Y_8\)) using the data in Table 1 by fitting them to the generalized quadratic polynomial model. None of the variables were subjected to transformation and equal weights were used for lower and upper limits. Upon the completion of numerical optimization using Design-Expert, one solution was obtained with a desirability of 0.588 at an inlet air temperature of 134°C and maltodextrin concentration of 13.7%. The predicted responding variables were validated experimentally and their values are shown in Table 4. The difference in yield was within the expected range of 20–70% for...
the size of spray dryer used (Sosnik & Seremeta, 2015). The concentration of all phytochemicals measured had the greatest differences between predicted and experimental values while drying characteristics, especially tapped density and $a_w$, had the smallest differences.

Two types of Moringa powder were investigated made from nonblanched (non-pretreated powder) and blanched (pretreated powder) Moringa leaves. Blanching was applied to deactivate enzymes present which would result in oxidation (Agüero et al., 2005). Both powders have a water activity, $a_w$ below 0.6 which is a safe level to prevent microorganism growth (Teixeira, Castro, Malcata, & Kirby, 1995). Referring to Table 5, blanching had the greatest effect on phytochemical content (difference % >20) but not on the powder drying characteristics (difference % <20). The increase in TPC in the pretreated powder can be attributed to the release of phenolics due to collapses in the leaf cell structure (Kidmose & Martens, 1999). However, blanching also resulted in a slight decrease in AOA possibly due to leaching (e.g., antioxidant compounds like vitamin C, polyphenols) and thermal degradation of heat sensitive compounds (e.g., degradation of chlorophyll to pheophytin) (Agüero et al., 2005). Overall, pretreatment with blanching could improve the TPC retention while maintaining the drying characteristics. It could be further optimized in order to reduce the leaching problem.

### Table 5 Difference in properties of spray dried powder produced from pretreated and unbalanced non-pretreated Moringa leaves

| Property                        | Non-pretreated | Pretreated | Difference after treatment (%) |
|---------------------------------|----------------|------------|--------------------------------|
| Drying characteristics          |                |            |                                |
| Yield (%)                       | 27.4a          | 28.3a      | 3                              |
| $\Delta E$                      | 50.2a          | 46.9a      | 7                              |
| $\rho_{\text{bulk}}$ (kg/m$^3$) | 389a           | 422b       | 8                              |
| $\rho_{\text{tapped}}$ (kg/m$^3$) | 638a           | 681b       | 7                              |
| $a_w$                           | 0.32a          | 0.33b      | 3                              |
| Hygroscopicity ($g H_2O/100 g$) | 11a            | 13a        | 18                             |
| Phytochemical content           |                |            |                                |
| AOA (mgAAE/g)                   | 3.95a          | 2.72b      | 31                             |
| TPC (mgGAE/g)                   | 0.12a          | 0.16b      | 42                             |
| Total chlorophyll (mg/g)        | 0.51a          | 0.40b      | 22                             |

Note. Data in the same row marked with same letter show no significant difference ($p > 0.05$).

**Figure 4** Change in CFU per gram powder (CFUg) over storage time with pretreated and non-pretreated samples
3.9  Viability of probiotics in inoculated powder

Moringa leaf juice powder produced from spray drying as per the optimum settings listed in Table 4 was investigated for their ability to support probiotics. Figure 4 shows the decrease in CFUs over the entire duration of storage. Despite the pretreatment applied, all powders eventually reach almost the same CFU/g by 14 days of storage and continued to remain so until 28 days. Viable counts of both L. acidophilus (LA5) and B. lactis (BB12) of more than 1 million CFU/gram were retained during the entire 28 days of storage, thus suggesting that Moringa powder could be a suitable medium to deliver probiotics to consumers (Bhadoria & Mahapatra, 2011). Under the storage conditions used, the temperature (30°C) was within growth limits and there was no oxygen exposure which will affect the survival of both probiotics (Anal & Singh, 2007). The same observation of probiotics CFUs declining in growth conditions in the presence of prebiotics was also made by Corcoran, Ross, Fitzgerald, and Stanton (2004) with Lactobacillus rhamnosus spray-dried with inulin. As for the effect of blanching on probiotic survival, the rate of reduction of CFUs of BB12 was slightly faster in the pre-treated powder while for LA5 it was slightly faster in the non-pretreated powder, particularly during the first 7 days of storage. This is likely due to the different compositions of both powders shown in Table 5 which is in line with the observations of López de Lacey, Pérez-Santín, López-Caballero, and Montero (2014) regarding the survival of probiotics L. acidophilus, L. paracasei, and B. animalis in six different green teas.

4  CONCLUSION

The present study demonstrated the utility of spray drying for rapidly preserving Moringa leaves as a powder free of inedible fibers. The spray-dried powder had a noticeably different color from the fresh Moringa leaf juice but this difference decreased sharply upon reconstitution in distilled water. Increasing inlet air temperature would increase powder yield and hygroscopicity but at the same time decrease the bulk density, tapped density, and water activity. On the other hand, increasing maltodextrin concentration would increase the yield (below 14% maltodextrin), bulk density, and tapped density while decreasing the water activity and hygroscopicity. All Moringa powder samples produced had a water activity of less than 0.6 which is below the threshold for spoilage. For phytochemical retention, increasing inlet temperature resulted in an increase in AOA, TPC, and total chlorophyll content. However, increasing concentration of maltodextrin reduced the total concentration of all phytochemicals by acting as a diluent. Optimum drying condition for maximum yield occurred at inlet air temperature of 134°C and 13.7% maltodextrin. Viable counts of more than $1 \times 10^6$ CFUs of L. acidophilus (LA5) and B. lactis (BB12) were detected up to 28 days of storage in Moringa leaf powders, both with and without pretreatment. Further studies are necessary to determine the relationship between blanching and probiotic survival in Moringa leaf powder. Spray-dried Moringa leaves powder may find its potential applications in the production of probiotic-fortified Moringa health products (e.g., food, drink, supplements) and incorporation into existing food products as a flavoring or coloring targeted at the health food sector.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the CFF Crops for the Future (Ms. Advina Julkifle, Mr. Fadhil, Mr. Ooi Gin Teng, and Mr. Vincent Arokiam) for the provision of Moringa leaves, expertise, processing equipment and facilities as well as MARDI (Mr. Md Suhaimi Alias and Mr. Md Zin Bin Ahmad) for their facilities and equipment. The authors also gratefully acknowledge the grant provided by the University of Nottingham Malaysia Campus (UNR20005) and the assistance of the faculty laboratory staffs (Mr. Md Asyraf and Mr. Ahmad Fareez Md Ravi).

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

ORCID

Sze Pheng Ong https://orcid.org/0000-0001-8965-8711

REFERENCES

Agüero, M. V., Pereda, J., Roura, S. I., Moreira, M. R., & del Valle, C. E. (2005). Sensory and biochemical changes in Swiss chard (Beta vulgaris) during blanching. LWT - Food Science and Technology, 38(7), 772–777. https://doi.org/10.1016/j.lwt.2004.07.018

Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends in Food Science and Technology, 18(5), 240–251. https://doi.org/10.1016/j.tifs.2007.01.004

Aramouni, F., & Deschenes, K. (2014). Methods for developing new food products: An Instructional Guide. Pennsylvania: DEStech Publications Inc.

Augustin, M. A., Clarke, P. T., & Craven, H. (2003). POWDERED MILK | Characteristics of milk powders. In B. Caballero (Ed.), Encyclopedia of food sciences and nutrition (2nd ed., pp. 4703–4711). Oxford, England: Academic Press.

Baipheti, M. N., & Jacobs, P. T. (2009). The contribution of subsistence farming to food security in South Africa. Agrekon, 48(4), 459–482. https://doi.org/10.1080/03031853.2009.9523836

Bates, R. P., Morris, J. R., & Crandall, P. G. (2001). Principles and practices of small- and medium-scale fruit juice processing, Issue 146. Rome: Food & Agriculture Org.

Bhadoria, P. B. S., & Mahapatra, S. C. (2011). Prospects, technological aspects and limitations of probiotics—A worldwide review. European Journal of Food Research & Review, 1(2), 23–42.

Bhandari, B. R., Senoussi, A., Dumoulin, E. D., & Lebert, A. (1993). Spray drying of concentrated fruit juices. Drying Technology, 11(5), 1081–1092. https://doi.org/10.1080/073739930916884

Bojović, B. M., & Stojanović, J. (2005). Chlorophyll and carotenoid content in wheat cultivars as a function of mineral nutrition. Archives
Vanajakshi, V., Vijayendra, S., Varadaraj, M., Venkateswaran, G., & Agrawal, R. (2015). Optimization of a probiotic beverage based on Moringa leaves and beetroot. LWT - Food Science and Technology, 63(2), 1268–1273. https://doi.org/10.1016/j.lwt.2015.04.023

Wellburn, A. R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectro-photometers of different resolution. Journal of Plant Physiology, 144, 307–313. https://doi.org/10.1016/S0176-1617(11)81192-2

Yoshii, H., Soottitantawat, A., Liu, X.-D., Atarashi, T., Furuta, T., Aishima, S., … Linko, P. (2001). Flavor release from spray-dried maltodextrin/gum arabic or soy matrices as a function of storage relative humidity. Innovative Food Science & Emerging Technologies, 2(1), 55–61. https://doi.org/10.1016/S1466-8564(01)00019-4

Yousefi, S., Emam-Djomeh, Z., & Mousavi, S. M. (2011). Effect of carrier type and spray drying on the physicochemical properties of powdered and reconstituted pomegranate juice (Punica Granatum L.). Journal of Food Science and Technology, 48(6), 677–684. https://doi.org/10.1007/s13197-010-0195-x

How to cite this article: Looi YF, Ong SP, Julkifle A, Alias MS. Effects of pretreatment and spray drying on the physicochemical properties and probiotics viability of Moringa (Moringa oleifera Lam) leaf juice powder. J Food Process Preserv. 2019;43:e13915. https://doi.org/10.1111/jfpp.13915