



Microencapsulation of *Ocimum Gratissimum* L. Essential Oil Using Spray-Drying



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Abstract

This study was conducted to investigate the factors affecting on microencapsulation process of essential oils using the spray-drying method. Various parameters were clearly investigated including concentrations of maltodextrin as wall material (20% - 30 %), concentration of essential oils (0.5% - 2%), homogenization methods (rotor-stator blend, ultrasound), inlet temperature (130 - 140 °C), and feed flow rate (from 4 ml/min to 10 ml/min). The suitable conditions were the maltodextrin concentration of 25% (w/w), the concentration of essential oil of 1.5% (w/w), rotor-stator blend, an inlet air temperature of 140 °C, and feed drying rate of 6 ml/min. The microencapsulation efficiency and the microencapsulation yield were 94.68% and 80.74%, respectively.

Keywords: *Ocimum gratissimum* L.; microencapsulation; spray-drying.

1. Introduction

Ocimum gratissimum L. belongs to the Lamiaceae family which includes about 200 species and is grown mainly in the Americas, Africa and Asia. It is one of the spices and medicinal herbs used in the treatment of diarrhea, as an antipyretic and as an ingredient in antimalarial remedies, stomach and general tonics, antiseptics, etc [1]. This plant is used in traditional Brazilian medicine to treat pain conditions [2]. The essential oils are extracted from different parts of the plant, such as flowers (rose and jasmine), leave (rosemary and eucalyptus), stems (cloves), roots (ginger), fruits (anise), and peel/shell (cinnamon and orange) showed different biological and medicinal properties [3]. Essential oils are usually extracted by distillation, cold pressing or water immersion, and their biological or antibacterial activities are directly correlated with the presence of bioactive volatile components [4-5]. The *Ocimum gratissimum* L. essential oil is extracted from the

leaves of the plant *O. gratissimum* L. by steam distillation [6]. The chemical composition of essential oils of aerial parts collected from three different sites: Nepal, Tajikistan and Yemen, consisted of six components: linalool, eugenol, estragole, methyl eugenol, 1,8-cineole, and geraniol [7]. Eugenol (75-77%), 1,8-cineole, germacrene D and β -caryophyllene are the main components [8]. The chemical composition of *Ocimum gratissimum* L. essential oil also changed with the harvest season. There are many studies on the antibacterial and antioxidant activities of eugenol and caryophyllene [9-12]. Due to its volatile nature and susceptibility to decomposition (oxidation, chemical interactions, heat, sunlight...), *Ocimum gratissimum* L. essential oil is therefore protected against evaporation losses and other harmful effects. Environmental factors are essential. In addition, because of their hydrophobic nature and lower density than water, essential oils are often lipophilic, soluble in organic solvents and

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insoluble in water, making it difficult to add them to food products [13]. Therefore, microencapsulation is considered as one of the promising techniques to solve these problems. Another important application of microencapsulation in the food industry is the conversion of liquid flavors into dry powders, providing convenience with reduced volatility and can be used in a variety of finished products.

There were several technologies used to conduct microencapsulation such as simple coacervation, complex coacervation, molecular inclusion, mechanical techniques (spray-drying, spray chilling, extrusion, etc.). The spray-drying was commonly used because of high quality, high solubility, and low moisture of encapsulated powder [14-17]. Many previous studies also showed similar noticeable results when using the spray drying method to conduct the microencapsulation process [17-18]. Several works can be found in literature on microencapsulation of essential oils and flavors, such as basil [19], rosemary [20-21], mint [22], and lemon [23]. However, very few articles are available on the encapsulation of *Ocimum gratissimum* L. essential oil.

This present study aims to investigate the suitable conditions for *Ocimum gratissimum* L. microencapsulation by spray drying using maltodextrin as wall material. In particular, maltodextrin concentration, essential oils concentration, inlet temperature and feed flow rate were evaluated. The tested microencapsulation results include product moisture, microencapsulation yield (MEY), microencapsulation efficiency (MEE), and the quality of essential oil after micro-encapsulation.

2. Materials and methods

2.1. Materials

Ocimum gratissimum L. essential oil (product of Evodia Ltd., Viet Nam) was used as the core material. Maltodextrin (DE 12) (product of Sigma-Aldrich Ltd., China) was used as the wall material. Tween 80 (99%, product of Sigma-Aldrich Ltd., China) was used as an emulsifier. n-pentane (99%, from Korea) was used to define the microencapsulation efficiency. Methanol (99%, from Vietnam), 2,2-diphenyl-1-picrylhydrazyl (99%, Sigma-Aldrich Ltd, USA) were used to determine antioxidant activities of essential oil.

2.2. Microencapsulation process

Firstly, 10 g maltodextrin (DE 12) was mixed with 300 g distilled water, stirred and placed in refrigerator for 12 hours [24]. Next, the *Ocimum gratissimum* L. essential oil was added to the mixture with different concentration from 0.5 % to 2 %. Tween 80 with an amount equal to 5 % weight of essential oil was added the mixture. The mixture was then homogenized at 6,000 rpm for 20 min using a rotor-stator homogenizer (Ultra Turrax, IKA T18 basic, Wilmington, USA). Next, 450 ml of the mixture was proceeded to spray drying in a lab-scale instrument (YC-015, Shanghai Pilottech Instrument & Equipment Co.Ltd). Operating conditions including spray drying method, inlet temperature (130 oC -140 oC), and feed flow rate (from 4 ml/min to 10 ml/min) were modified in each experiment to find the most suitable conditions. The encapsulated powder was kept in the sealed glass bottle at room temperature.

2.3. Moisture content

The moisture content of the powder was determined by an oven-drying and drying at 105 oC until the weight was constant [25].

2.4. Determination of microencapsulation yield (MEY)

Microencapsulation efficiency (MEY) is the ratio of the amount of essential oil in the product to the total essential oils in the initial mixture. The microencapsulation efficiency (MEY) was determined by dissolving 30 g of microencapsulated powder with 200 ml of water in a Clevenger steam extractor for 4 hours. The collected essential oil was weighed and the microencapsulation efficiency percentage in the grain would be calculated.

$$MEY (\%) = \frac{m_P}{m_F} * 100$$

[Eq. 1]

Where: mP: the quantity of essential oil in the sprayed-drying product (calculated on the dry basis) (g)

mF: the quantity of essential oil in the feed solution (calculated on the dry basis) (g)

2.5. Determination of microencapsulation efficiency (MEE) and surface oil (SO)

Microencapsulation efficiency (MEE) is essential oil existing inside the wall material of

microencapsulated powder. MEE was determined by mixing 150 ml n-pentane with 30 g dried microencapsulated powder for 1 h at room temperature. The mixture was then filtered through a Whatman no. 1 filter paper [26]. The filtrate solution containing the extracted oil was transferred to an oven at 60°C for 6 h in order to completely evaporate n-pentane. The essential oil powder was dissolved in 200 ml of water in a Clevenger steam extractor for 4 hours. The collected essential oil was weighed and used to determine MEE by the following formula:

$$MEE = \frac{m_E}{m_P} * 100 \quad [Eq.2]$$

where:

m_E: the quantity of encapsulated essential oil (calculated on the dry basis) (g) was determined in the same way as the determination of the m_P, m_F (MEY) value. The essential oil after the drying process will be weighed to determine the m_E value.

m_P: the quantity of essential oil in the spray-drying product (calculated on the dry basis) (g)

The surface oil was calculated as follows:

$$SO(\%) = (1 - MEE) * 100 \quad [Eq.3]$$

2.6. Solubility

Solubility was determined using the method described by Cano-Chauca *et al.* (2005) with some adjustments [27]. The powder samples (2.5 g) were dispersed to 30 ml of water (30°C) in a beaker. After stirring for 5 min, the dispersions were transferred into a centrifuge tube and centrifuged at 5000 rpm for 10 min. About 25 ml of the supernatant was transferred to the petri dishes dried and pre-weighed drying at 105 °C to constant mass. The supernatant was decanted and the water solubility was determined using the following equation:

$$W_s (\%) = (W_{ds} / W_{sa}) * 100 \quad [Eq.4]$$

Where W_s, W_{ds}, and W_{sa} are water solubility, weight of dissolved solids in the supernatant, and weight of the sample, respectively.

Table 1: Conditions in each of the experiments

Investigated factors	Fixed factors	Change factors
Experiment1: Effects of wall material concentration	Essential oil concentration: 0.5% Homogenized methods: Rotor-stator blend Inlet temperature: 140 °C	Maltodextrin concentration: 20%-25%-30%

Experiment2: Effects of essential oil concentration	Feed flow rate: 4 mL Maltodextrin concentration: the result of Experiment1 Homogenized methods: Rotor-stator blend Inlet temperature: 140 °C Feed flow rate: 4 mL	Essential oil concentration: 0.5%-1.0%-1.5%-2.0%
Experiment3: Effects of homogenized methods	Maltodextrin concentration: the result of Experiment1 Essential oil concentration: the result of Experiment2 Inlet temperature: 140 °C Feed flow rate: 4 mL	Homogenized methods: Rotor-stator blend- Ultrasound
Experiment4: Effects of drying temperature	Maltodextrin concentration: the result of Experiment1 Essential oil concentration: the result of Experiment2 Homogenized methods: the result of Experiment3 Feed flow rate: 4 mL	Inlet temperature: 130 °C-140 °C-150 °C
Experiment5: Effects of feed flow rate	Maltodextrin concentration: the result of Experiment1 Essential oil concentration: the result of Experiment2 Homogenized methods: the result of Experiment3 Inlet temperature: the result of Experiment4	Feed flow rate: 4 mL-6 mL-8 mL-10 mL

2.7. Gas chromatography-mass spectrometry

The Gas Chromatography-Mass Spectrometry (GC-MS) method was used to determine the chemical composition of the essential oils. An amount of 25 µL of the sample of essential oil was blended with 1.0 ml n-hexane. The appliance employed was GC Agilent 6890 N coupled with MS 5973 inert with an HP5-MS column. The pressure of the head column was installed at 9.9 psi. The operating conditions parameters of the GC-MS system were as follows: flow rate of helium as the carrier gas was 1 ml/min in a split ratio of 1/100; injector volume of 1 µL at 250°C. The oven temperature was originally held at 50°C for 2 min, then risen by 2°C/min until 80 °C, and increased by 5°C/min to 150°C, risen to 200°C at 10°C/min and finally increased to 300°C at 20°C/min for 5 min.

2.8. Antioxidant assay

Antioxidant activity was determined by the DPPH method described by Chavan *et al.* (2013) with some modifications [28]. The DPPH stock solution was prepared by dissolving 24 mg of DPPH in 100 ml

methanol. The DPPH solution was mixed with the *Ocimum gratissimum* L. essential oil at a ratio of 3:1, v/v. The absorbance of the mixture was measured at 517 nm using a spectrophotometer (Cary 60, Agilent, Germany) after 30 min of incubation in the dark. The capability of scavenging DPPH radical was calculated using the following formula:

$$DPPH(\%) = \frac{A_{CT} - A_{SP}}{A_{CT}} \quad (5)$$

Where A_{CT} and A_{SP} are the absorbances of the sample with and without the essential oil. The half-maximal inhibitory concentration (IC_{50}) of the *Ocimum gratissimum* L. essential oil was the appropriate concentration to reach the 50 % reduction of the DPPH radical. IC_{50} was calculated using the standard essential oil curve against DPPH.

2.9. Entering text

The data was analyzed using Statgraphics Centurion XVI (Statgraphics Technologies, Inc., Virginia, USA) software. Analysis of variance (ANOVA) and the least significant difference (LSD) were carried out at significance off 95 %.

2. Results and Discussion

3.1. Chemical composition of the *Ocimum gratissimum* L. essential oil

The *Ocimum gratissimum* L. essential oil was firstly determined for chemical composition by gas chromatography-mass spectrometry (GC-MS). The GC-MS spectrum of essential oil samples is presented in Figure 1. Figure 1. shows that two peaks located at 27.07 min and 28.91 min have the greatest intensities. Mass spectrometry approximated the molecular formula and weight of the two substances as $m/z = 164$ and $m/z = 204$, respectively. In total, there were nine identified components, accounting for approximately 96.11 % of the total essential oil content. The major components were Hep-20enel (3.367%), Eucalyptol (2.2%), Linalool (16.71%), Estragole (0.98 %), α -Cubebene (1.1 %), Methylugenol (4.66%), Humulene (3.15%), and Caryophyllene oxide (0.97%).

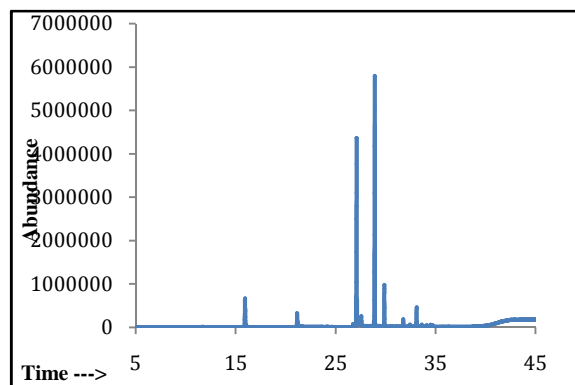


Fig. 1. The spectrum of *Ocimum gratissimum* L. essential oil

3.2. Effects of wall material concentration

Figure 2 shows that the maltodextrin concentration increased from 20 % to 25 %, the MEE increased from 80.51 % to 88.48 %. However, when increasing maltodextrin concentration to 30 %, MEE decreased. One-way ANOVA revealed that the effects of maltodextrin concentration on MEY, MEE, and SO was statistically significant ($p < 0.05$). The least significant difference (LSD) of the MEY index indicated that there was a difference between the 25 % and 30 % concentrations compared to the 20 % of concentration, while there was no difference between the above concentrations. For MEE, multiple range tests indicated that there is a clear difference between treatments at three concentrations. The highest MEE (88.48 %) was achieved at the 25 % concentration of maltodextrin. This result tended to Sanchez-Reinoso and Gutiérrez (2017) indicating that the MEE index increases (from 90.3 % to 96.3 %) when maltodextrin concentration varied from 20 % to 30 % [29]. These results were in line with those of Xu *et al.* (2020) when the concentration of maltodextrin increased from 0 % to 30 %, MEE was increased from 76.45 % to 88.80 % [30].

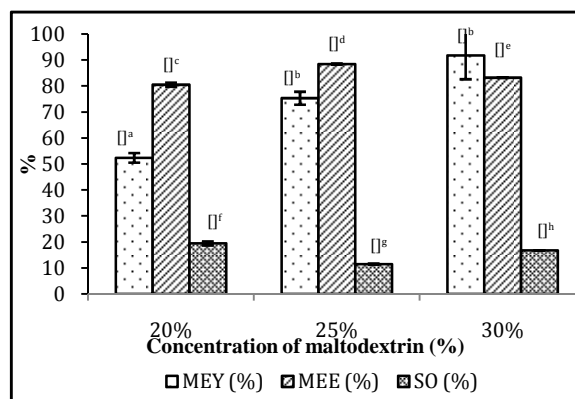


Fig. 2. Effect of concentration of maltodextrin on MEE, MEY and SO

3.3. Effects of essential oil concentration

Figure 3 presents the effect of different concentrations of *Ocimum gratissimum* L. essential oil on MEY, MEE and SO. The results show that when increasing the concentration of essential oils from 0.5 % to 2 %, the MEY decreases. The MEE did not change significantly when increasing the concentration of essential oil from 0.5 % to 2 % (MEE increased from 88.40 % to 92.82 %). One-way ANOVA revealed that the concentration of *Ocimum gratissimum* L. essential oil had significant effects ($p < 0.05$) on the MEY. The MEY was highest (79.78 %) at the oil concentration of 1.5 %, and decreased thereafter to 61.97 % when rising the concentration to 2 %. However, there was no difference observed between MEE values when the essential oil concentration changed from 0.5 % to 2 %. These results are in line with the study of Hogan *et al.* (2001). Belong to Hogan *et al.*, there is a decrease of MEE from 89.2 % to 18.8 % when increasing the soy oil/sodium casein ratio from 0.25 to 3.0 % [24]. These results can be caused by the removal of the -OH group from hydrophobic compounds from the linkage network of maltodextrin under a high essential oil concentration [31].

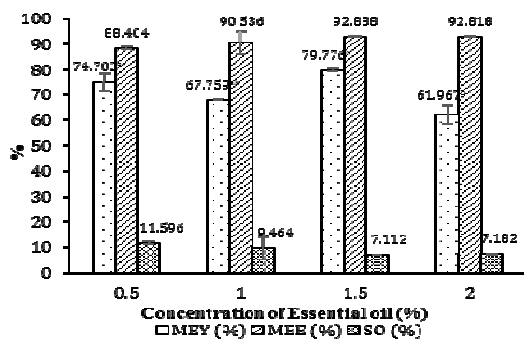


Fig. 3. Effect of concentration of essential oils on MEE, MEY and SO

3.4. Effects of homogenized methods

Figure 4 presents that the MEY and MEE reached 74.22 %, 92.22 %, respectively when using rotor-stator homogenizer. The MEE and MEY was 81.54 % and 52.57 %, respectively when using the ultrasound method. ANOVA analysis's results show that the effect of homogenizer methods on microencapsulation process is significant at 95 % of confidence. Test of LSD indicates that the difference in MEE index between rotor-stator homogenizer and ultrasound. These results are similar to Koç, *et al.* (2015) [32].

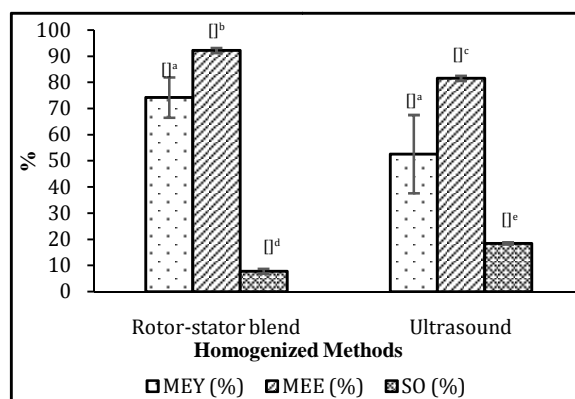


Fig. 4. Effect of homogenized methods on MEE, MEY and SO

3.5. Effects of drying temperature

The influence of inlet temperature on microencapsulation is shown in Figure 5. The MEY, MEE and SO value change with different the spray drying temperature. When the temperature increases from 130°C to 140°C, MEE increased (from 82.48% to 90.76%). At 140°C, the highest microencapsulation efficiency was obtained (90.76%). One-way ANOVA revealed that inlet temperature had a significant on the MEY, MEE and SO indexes at 95 % confidence. Multiple range test and the least significant difference (LSD) of MEY and MEE showed that there was a difference between 140 °C and 150 °C compared to 130°C, while there was not a difference between the above concentrations. The MEY (80.75 %) and MEE (90.76 %) were the highest value when using the the spray drying temperature of 140 °C. In drying process, the water evaporation rate increase with temperature leading to the loss of essential oil, which reduces the process efficiency [33-34].

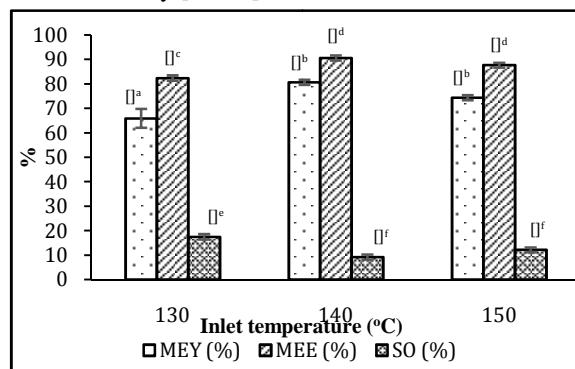


Fig. 5. Effect of inlet temperature on MEE, MEY and SO

3.6. Effects of feed flow rate in spray-drying

Figure 6 shows that MEY, MEE and SO value change with the feed flow rate of spray drying process. The MEE value changed from 92.23% to 94.68% when rising the feed flow rate from 4 ml/min to 6 ml/min. However, when increasing the feed flow rate to 8 ml/min and 10 ml/min, the MEE value tends to decrease. The highest MEE (94.68%) was obtained at the feed flow rate of 6 ml/min. The ANOVA analysis revealed that the effect of feed flow rate on MEY, MEE and SO were statistically significant ($p < 0.05$).

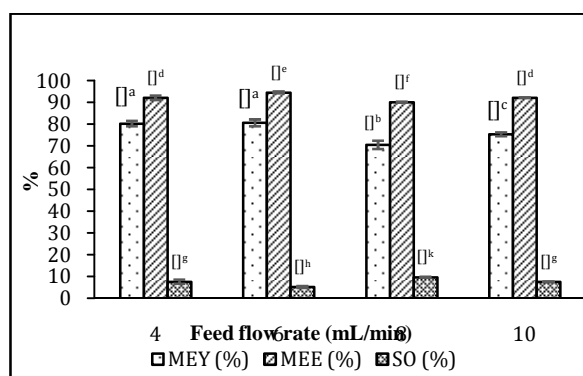


Fig. 6. Effect of feed flow rate on MEE, MEY and SO

3.7. Chemical composition of encapsulated essential oils

Analysis results by gas chromatography-mass spectrometry (GC-MS) indicated that there were 3 peaks at 15.941 min, 27.067 min, and 38.306 min with the greatest intensity. Compounds with a retention time of 15.941 min were linalool (molecular mass of $C_{10}H_{18}O$: $m/z = 154$), eugenol at 27.067 min (molecular mass of $C_{10}H_{12}O_2$: $m/z = 164$), and caryophyllene at 28.907 min (molecular mass of $C_{15}H_{22}$: $m/z = 204$) was determined by mass spectrometry. There were about 11 components and these occupied about 96.113 % by weight of the essential oil. The main ingredients were caryophyllene (36.306 %), eugenol (22.827 %), linalool (16.709 %), methyl eugenol (4.66 %), hept-2-ene (3.367 %), humulene (3.15 %), eucalyptol (2.202 %). The chemical compositions of essential oils are reliant on factors as seasons, soil structure, texture, and geographical location. Several previous studies demonstrated that Caryophyllene and Eugenol

had antioxidant and antimicrobial capabilities. That was why *Ocimum gratissimum* L. essential oil contains high levels of caryophyllene and eugenol, which made it have these abilities. Comparing GC-MS of *Ocimum gratissimum* L. essential oil before and after spray drying showed that components of the essential oil were almost unchanged after spray drying (Table 1). The main components such as caryophyllene (37.974 %), eugenol (22.674 %), linalool (15.276 %) had no difference compared to the value before spray drying. Thus, the spray drying process helped preserve volatile components and unchanged the chemical composition of the core material [35].

Table 1. Chemical composition of initial and microencapsulated essential oil

Compounds	Initial essential oils (%)	Microencapsulated essential oils (%)
Hep-2-enel	3.367	2.671
Eucalyptol	2.202	1.59
Linalool	16.709	15.276
Estragole	0.976	1.038
α -Cubebene	1.098	1.616
Eugenol	22.827	22.674
α -Copene	1.85	1.762
Methyl eugenol	4.66	6.274
Caryophyllene	38.306	37.974
Humulene	3.15	3.144
Caryophyllene oxide	0.968	1.447
Total	96.113	95.466

3.8. Solubility

Solubility is useful for the determination of its application in food ingredients [36], which is dependent on the affinity of the powders to water and hydrophilic components. The micro-encapsulated powder had the solubility of 91.92 ± 0.503 %. This value is within the range of solubility for food powders (i.e., 67.05-99.98 %) [37].

3.9. Antioxidant activity

The IC_{50} value of initial essential oils was 46.02 ± 1.34 . The IC_{50} value of microencapsulated essential oils was 48.66 ± 1.49 . LSD analysis shows that there is no statistical difference at 95 % confidence. The

microencapsulation process of essential oils using spray drying method might not change the antioxidant activity of essential oils.

4. Conclusion

Microcapsules containing *Ocimum gratissimum* L. essential oil were produced by spray drying using maltodextrin as a coating agent. Spray drying conditions significantly affected the characteristics of *Ocimum gratissimum* L. essential oil microcapsules. The suitable conditions for spray-drying process are as below: maltodextrin concentration of 25 % (w/w), essential oil concentration of 1.5 % (w/w), inlet air temperature of 140 °C, and the feed drying rate of 6 ml/min. The optimal MEE and MEY was 94.68 % and 79.72 %, respectively. The results revealed that microencapsulation by spray-drying did not change the chemical composition and antioxidant activity of essential oils. However, it is necessary to expand the study by investigating the amount of essential oil that can be lost during spray drying; the factors of the emulsion assimilation process such as the rate, time; storage time and conditions of microencapsulated powder as well as the stability of powder under environmental conditions are different. Find out factors and rates for mixing powder to create products with high commercial and applicability (increasing taste, nutritional value, easy to store and use).

5. Conflict of interest

There are no conflicts to declare.

6. Acknowledgments

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