

Development of lemon spice powder from seedless lemon (*Citrus aurantiifolia*) juice by freeze-drying method

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Abstract

Seedless lemon (*Citrus aurantiifolia*) is valued for its high vitamin C content and refreshing flavor, making it popular in food products. Freeze-drying is an effective method to preserve the bioactive compounds of lemon juice while extending its shelf life and maintaining quality. Developing lemon spice powder from seedless lemon juice through freeze-drying offers a long-lasting and versatile product for culinary and industrial applications. In this study, the production of lemon powder from seedless lemon juice was successfully carried out using the freeze-drying method and then applied to develop lemon spice powder. Several properties of lemon juice were assessed, including total vitamin C content, Brix, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity. Firstly, survey the appropriate parameters to produce lemon base powder, including the concentration of maltodextrin used and appropriate drying conditions. The formula for lemon spice powder product includes: lemon juice powder (10%), dextrose sugar (73%), citric acid (10%), salt (5.8%), curcumin pigment (0.2%) and anti-caking agent (0.2%). The development of lemon spice powder offers a promising solution to preserve the nutritional and sensory qualities of lemons, while providing a versatile and convenient product for many applications in food products, enhancing the natural flavor without using fresh lemons.

1. Introduction

Seedless lemon (*Citrus aurantiifolia*) (Rutaceae family) is grown in tropical countries such as Vietnam, Malaysia and Thailand (Feizy *et al.*, 2012). It has a strong aroma, sweet taste with a hint of bitterness (Chaturvedi and Shrivastava Suhane, 2016). Naringin is one of the flavonoids that is responsible for the bitter taste of *Citrus* juices. This compound is the main flavonoid in seedless lemon juice (Kummer *et al.*, 2013). The major flavonoids of seedless lemon are didymin, eriocitrin, hesperidin, naringin, narirutin, neoeriocitrin, neohesperidin, poncirin, which are highly present in peels and fruit juice (Bharate, 2014). Seedless lemon juice has profitable bioactives, such as anti-inflammatory, anti-tumor, anti-fungal and blood clot inhibition activities (Aneja *et al.*, 2014). The health benefits of citrus fruit have mainly been attributed to the presence of bioactive compounds, such as phenolics (Lemes *et al.*, 2018), vitamin C (Francis and Elizabeth,

2002), and carotenoids (Agócs *et al.*, 2007). Interestingly, it has been reported that the phytochemical profile varies with the species and cultivars, which can exhibit different biological properties, especially antioxidant activity (Jayana *et al.*, 2010).

Since ancient times, spices have played an important role in the lifestyle of people around the world. A wide variety of pharmaceuticals throughout history, including colourants, flavoring agents, preservatives and food additives, have been produced (Kunnumakkara *et al.*, 2009). All have created flavors in food processing that help stimulate the organs of taste, smell and sight for people who eat food. In addition, spices contain bioactive compounds that have the ability to reduce or inhibit the risk of degenerative diseases such as diabetes, obesity, cancer and cardiovascular diseases (Chi and Wu, 2014).

Freeze-drying has as its core principle the removal of

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solvent from a liquid formulation, the first step being that the product is frozen, and then the frozen solvent is removed by sublimation, at reduced pressure, followed by a desorption process to remove unfrozen solvent. The drying process is also divided into sublimation (primary drying) and desorption (secondary drying). Sublimation methods can be applied to produce dehydrated products; sublimation is always recognized as the best drying process to preserve food quality (Assegehegn *et al.*, 2019; Ma *et al.*, 2023; Ratti, 2024). This process has found many applications for the production of high-quality food and pharmaceuticals (Nowak *et al.*, 2020; Oyinloye and Yoon, 2020).

Highlighting the feasibility and applicability of the freeze-drying method on seedless lemon, previous studies on citrus fruits were also conducted by the authors (Lee *et al.*, 2012; Papoutsis *et al.*, 2017). The study aimed to investigate the parameters of lemon spice powder products made from lemon powder using the freeze-drying method. The following product properties, including moisture content, vitamin C content, antioxidant capacity and sensory evaluation, were analyzed. The research results are expected to develop a database of new coating materials and cut production costs of plant-based lemon spice powder products.

2. Materials and methods

2.1 Materials

Healthy and fully ripened seedless lemons were collected from Hau Giang province, Vietnam. The collected lemon was transported to the laboratory of Nguyen Tat Thanh University, where they were thoroughly washed with tap water to remove dust. Then, the seedless lemon was squeezed for juice using a squeezer and the juice was stored at 5°C before use.

2.2 Production of seedless lemon powder by the freeze-drying method

First, maltodextrin was dissolved in water at various concentrations (from 15 to 30 g/100 g). Then, prepared lemon juice was added to the solution and stirred well. The solution was spread on a stainless steel frame with a layer of baking paper placed underneath. Then, the freeze-drying process was performed at appropriate parameters with the investigation of 3 freezing processes: slow freezing outside the drying chamber (Freeze 1), quick freezing outside the freeze-drying

chamber (Freeze 2) and direct freezing method in the chamber (Freeze 3). At the end of the drying process, select the appropriate maltodextrin content and freezing method for the product. Finally, the lemon powder was collected, ground finely and stored in a sealed bag. Three formulas were designed to produce lemon spice powder (Table 1).

2.3 Characterisation of the products

2.3.1 Determination of moisture content and total solid solubility

The moisture content of the the product was determined using the AOAC INTERNATIONAL Official Method 925.10 (AOAC INTERNATIONAL, 2007). The weight loss proportion (%) of the substance obtained at 105°C before immovable weight after oven-drying led in depth to the calculation of the moisture content (%) (Amin *et al.*, 2004). Two drops of the sample solution were added to the lower prism of the electronic refractometer using a glass rod, and the results were recorded.

2.3.2 Determination of 2,2-diphenyl-1-picrylhydrazyl scavenging activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis was performed as described previously by Baliyan *et al.* (2022). A total of 10 mL of sample was taken and titrated to 50 mL with alcohol. Then, the sample was filtered, and 0.5 mL of the sample solution and 1.5 mL of standard DPPH were drawn into a test tube and shaken well. The reaction was kept in the dark at room temperature for 30 min. Optical absorbance was measured at 517 nm on a UV-Vis spectrophotometer, with an alcohol blank.

$$P = \frac{C_x \times n \times V \times 100}{m \times (100 - X) \times 10^{-3}}$$

Where P: DPPH oxidation capacity ($\mu\text{gTAA/g}$ dry matter), C_x : concentration determined from the calibration graph ($\mu\text{g/g}$), n : dilution from the stock solution, V : volume of stock extract (mL), X : sample moisture content (%) and m : weight of sample (g).

2.3.3 Determination of ABTS scavenging activity

The method described by Munteanu and Apetrei (2021) was used. A total of 10 mL of sample was taken and titrated to 50 mL with alcohol. Then the sample will be filtered, 0.5 mL of the sample solution and 1.5 mL of

Table 1. The formulas spice lemon powder products.

Formulas	Base lemon powder	Dextrose	Acid citric	Salt	Curcumin	Anti-caking agent
Formula 1	5%	76%	15%	3.7%	0.1%	0.2%
Formula 2	10%	73%	10%	5.8%	0.2%	0.2%
Formula 3	15%	70%	4.5%	10%	0.3%	0.2%

standard ABTS will be sucked into the test tube, and shaken well. The reaction was kept in the dark at room temperature for 30 min. Optical absorbance was measured at a wavelength of 734 nm on a UV-Vis spectrophotometer, with an alcohol blank sample.

$$P = \frac{C_x \times n \times V \times 100}{m \times (100 - X) \times 10^{-3}}$$

Where P: ABTS oxidation capacity ($\mu\text{gTAA/g}$ dry matter), C_x : concentration determined from the calibration graph ($\mu\text{g/g}$), n : dilution from the stock solution, V : volume of stock extract (mL), X : sample moisture content (%), and m : weight of sample (g).

2.3.4 Determination of vitamin C content

Vitamin C content was determined by the 2,6-dichlorophenol-indophenol (DCPIP) titration method, as described previously by Denre (2014). A total of 0.1 g of powdered sample was extracted with 20 mL of 4% oxalic acid. Approximately 10 mL of the sample's aliquot and 10 mL of 4% oxalic acid were taken in a conical flask and titrated against 2,6-dichlorophenol indophenol (DCPIP) dye (V_2) until the appearance of a faint pink colour that persisted for a few min. Another 5 mL of 100 ppm solution of ascorbic acid and 10 mL of 4% oxalic acid were taken and also titrated against DCPIP dye (V_1). The ascorbic acid content (mg/g) was determined using the following formula:

$$\text{Amount of ascorbic acid (mg/g)} = \frac{0.5 \times V_2 \times 20}{V_1 \times 10 \times 0.1}$$

Where V_1 and V_2 represent the volume of dye consumed by 0.5 mg ascorbic acid and by 10 mL of test solution, respectively.

2.4 Sensory evaluation

The sensory evaluation was carried out according to the TCVN 3215-79 (Vietnam Standards, 1979). Samples were taken in random order and coded with 3 digits. The participants tasted each sample from left to right and indicated their level of preference for each sample with color norms. The color, smell, taste and state have been specified in the product quality assessment manual on a scale from 0 to 5 on the sensory evaluation sheet. The quality levels increase from 0 to 5 (1 - don't like much, 2 - don't like, 3 - like, 4 - like a lot, 5 - like a lot). The number of observers was 10. Each sample corresponded to a rating card.

2.5 Statistical analysis

Each measurement was conducted at least three times. Statgraphics statistics software (version 20, Statpoint Technologies, Inc., Warrenton, VA, USA) was used to evaluate the data. Analysis of variance

(ANOVA) was performed, with $p < 0.05$ indicating a significant difference.

3. Results and discussion

3.1 Evaluation of the physicochemical composition of seedless lemon juice

Analysis results showed in Table 2 that the original lemon juice had vitamin C content and total soluble solids content ($^{\circ}\text{Brix}$) of 30.57 ± 0.84 mg/100 g and 8.97 ± 0.06 , respectively. This result was similar to previous studies by Nakilcioglu-tas and Ötleş (2020) and Liu *et al.* (2022), who reported a vitamin C content of 34.34 ± 1.21 mg/100 g and 35 mg/100 g, respectively. Besides, the corresponding DPPH antioxidant capacity was 7.68 ± 0.33 mg/100 g, and ABTS antioxidant capacity was 3.25 ± 0.26 mg/100 g, which are similar to the study by Zhang *et al.* (2018) on the processing of lemon juice from raw materials. Mcharek and Hanchi (2017) also recorded the high antioxidant capacity of lemon juice. Overall, the vitamin C content and antioxidant capacity of the prepared seedless lemon juice are promising for further research.

Table 2. The physicochemical properties of seedless lemon juice.

No.	Parameters	Value
1	TSS ($^{\circ}\text{Bx}$)	0.99 ± 0.01
2	Vitamin C (mg/100 g)	9.19 ± 0.81
3	DPPH (mg/100 g)	4.89 ± 0.03
4	ABTS (mg/100 g)	8.97 ± 0.06

Values are presented as mean \pm SD (n = 3).

3.2 Effect of some technological factors on freeze-dried lemon powder

3.2.1 Effect of maltodextrin content on the quality of freeze-dried lemon powder

Results in Table 3 show that as the concentration of maltodextrin gradually increases from 15 to 30 mg/100 g, the moisture of the product also increases proportionally from $1.10 \pm 0.42\%$ to $1.56 \pm 0.78\%$. The results were consistent with those of Ray *et al.* (2017), in which the freeze-dried products had relatively low moisture from about 1 to 5%. Besides, in Figure 1, the Vitamin C content reached the highest value of 1.264 ± 0.05 $\mu\text{g/g}$ at maltodextrin content of 15 g/100 g, then gradually decreased at higher maltodextrin contents of 20 - 30 g/100 g. Vitamin C content of lemon juice was not significantly different at the maltodextrin concentrations of 25 and 30 g/100 g ($p > 0.05$). The possible explanation for this is that higher surface-active carbohydrates are able to bind to volatile compounds during the drying process (Eghbaljoo *et al.*, 2022; Nguyen *et al.*, 2022). Therefore, when the carrier

concentration is higher, the content of surface-active carbohydrates increases, leading to the retention of more volatile compounds in the core of the product and vice versa. The quality of lemon powder products is determined mainly through Vitamin C content.

Table 3. Moisture content of lemon powder after freeze-drying.

Maltodextrin content/100 g	Moisture (%)
15 g/100 g	1.10±0.42 ^b
20 g/100 g	1.26±0.83 ^a
25 g/100 g	1.29±0.96 ^a
30 g/100 g	1.56±0.78 ^c

Values are presented as mean±SD (n = 3). Values with different superscripts are statistically significantly different.

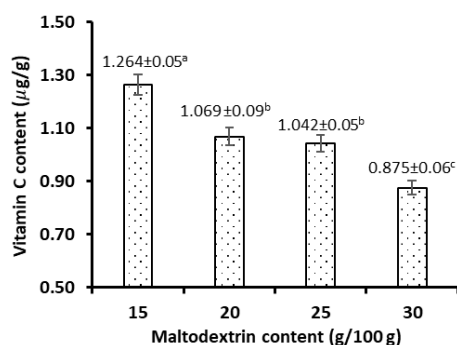


Figure 1. Maltodextrin content affects Vitamin C content. Values are presented as mean±SD (n = 3). Values with different superscripts are statistically significantly different.

Next, Figure 2 shows that antioxidant capacity tends to increase proportionally with increasing maltodextrin levels, with the highest value of DPPH and ABTS scavenging activities of 0.109±0.95 µgTAA/g and 0.071±0.53 µgTAA/g, respectively, in the presence of maltodextrin at a concentration of 25 g/100 g. ANOVA analysis results show a significant difference in the antioxidant capacity of the prepared lemon juice corresponding to various maltodextrin contents. This can be explained by the fact that different types of lemons and ripeness, when mixed with maltodextrin, affect the antioxidant activity of lemon powder, according to the authors Hsieh *et al.* (2021) and Ogundele and Bolade (2021). Therefore, the additional maltodextrin content is

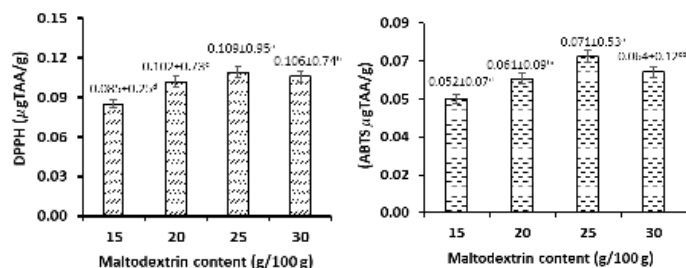


Figure 2. Maltodextrin content affects the antioxidant capacity of DPPH and ABTS. Values are presented as mean±SD (n = 3). Values with different superscripts are statistically significantly different.

suitable for mixing with lemon juice at 25 g/100 g to mix for the lemon powder drying process.

3.2.2 Effect of freezing method on the quality of lemon powder after freeze drying

Freezing method significantly affects Vitamin C content and antioxidant capacity (Harguindeguy and Fissore, 2020; Neri *et al.*, 2020). Specifically, for the freeze-drying process, freezing speed, temperature, and time affect the water distribution in the products, thus affecting the quality of frozen products as well as the kinetics of the sublimation process (Nowak and Jakubczyk, 2020). Therefore, prolonging freezing time would cause a significant loss of vitamin C content as well as DPPH and ABTS scavenging activities of the product. Meanwhile, the effect of freezing methods on moisture and recovery efficiency was not significantly affected, as reported by previous studies (Shofian *et al.*, 2011; Harguindeguy and Fissore, 2020).

During the freezing process, the lemon solution goes through a number of physical changes, including a supercooled state. The degree of supercooling of the solution determines the state of the coagulants (size, quantity, and morphology) during the solution's solidification, affecting the subsequent drying process, such as the ability to resist vapor stream. Therefore, the degree of freezing is not uniform, leading to uneven ice layers that can prevent the dried sample from drying. This poses significant challenges in the development, optimization, and scale-up of freeze-drying processes. Additionally, modifications to the freezing process and various techniques are used to actively control the degree of supercooling during freezing. Their impact on the performance of the freeze-drying process will also be addressed (Assegehegn *et al.*, 2019). As shown in Figures 3 and 4, the powder produced from direct freezing in the drying chamber (Freeze 3) had the highest value of vitamin C content (1.181±0.67 µg/g), DPPH (0.132±0.75 µgTAA/g) and ABTS scavenging activity (0.072±0.23 µgTAA/g). Therefore, using the direct

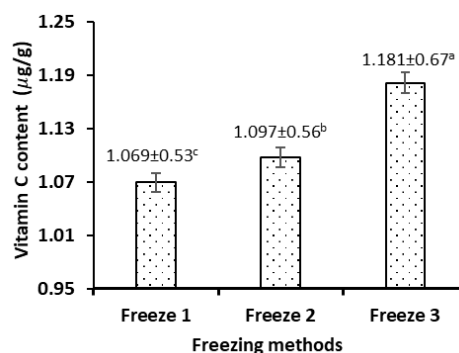


Figure 3. Freezing methods affect Vitamin C content. Values are presented as mean±SD (n = 3). Values with different superscripts are statistically significantly different.

freezing method in the chamber (Freeze 3) was reasonable for the production of lemon powder products.

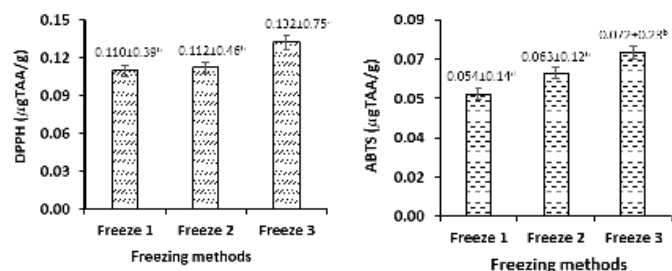





Figure 4. Freezing methods affect the antioxidant capacity of DPPH and ABTS. Values are presented as mean±SD (n = 3). Values with different superscripts are statistically significantly different.

3.3 Survey on the recipe for mixing lemon spice powder

The appearance of lemon spice powder products with different mixing formulas is presented in Table 4. In general, when changing the mixing formulas, the moisture content of the lemon spice powder product tends to increase insignificantly. As shown in Figures 5 and 6, the vitamin C content as well as the antioxidant capacity showed a difference between product formulas (p<0.05). This can be explained by the different amounts of lemon powder mixed into the product in each formula. Therefore, to select the appropriate product formula, it is necessary to conduct a sensory evaluation for the product.

The guide to evaluating the quality of lemon spice powder products according to the scoring method ISO 3215 - 79 gives the following results: color - golden white, smell - fragrant, typical lemon scent, taste - harmony between sweet, sour and salty and status - Table 4. Appearance characteristics through lemon spice powder mixing formulas.

No.	Formula	Figure	Moisture (%)
1	Formula 1		1.42±0.07
2	Formula 2		1.54±0.09
3	Formula 3		1.61±0.08

Values are presented as mean±SD (n = 3). Values with different superscripts are statistically significantly different.

homogeneous, porous product.

According to TCVN 3215 - 79 on sensory analysis of products, color, odor, taste and status of lemon spice powder are assessed, and the maximum score for each indicator is 5. As shown in Table 5, the lemon spice powder product with formula 2 scores 15.20 points; specifically, the taste indicator has an unweighted average score of 4.5 (<4.8). Therefore, formula 2, which includes lemon powder (10%), dextrose (73%), citric acid (10%), salt (5.8%), curcumin powder (0.2%), and anti-caking agent (0.2%), is classified as a suitable formula for lemon spice powder products.

Table 5. Sensory evaluation through lemon spice powder formulas.

Formula 1				
No.	Criteria	Average score	Coefficient important	Points with weight
1	Color	3.17	1	3.17
2	Odor	2.83	1	2.83
3	Taste	3.5	1.2	4.2
4	Status	3.17	0.8	2.54
Total				12.74
Formula 2				
No.	Criteria	Average score	Coefficient important	Points with weight
1	Color	3.83	1	3.83
2	Odor	3.17	1	3.17
3	Taste	4.5	1.2	5.4
4	Status	3.5	0.8	2.8
Total				15.2
Formula 3				
No.	Criteria	Average score	Coefficient important	Points with weight
1	Color	3.5	1	3.5
2	Odor	3	1	3
3	Taste	3	1.2	3.6
4	Status	4.5	0.8	2.8
Total				13.9

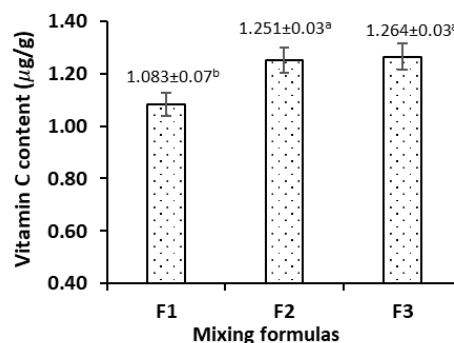


Figure 5. Lemon spice powder mixing formulas affects Vitamin C content. Values are presented as mean±SD (n = 3). Values with different superscripts are statistically significantly different.

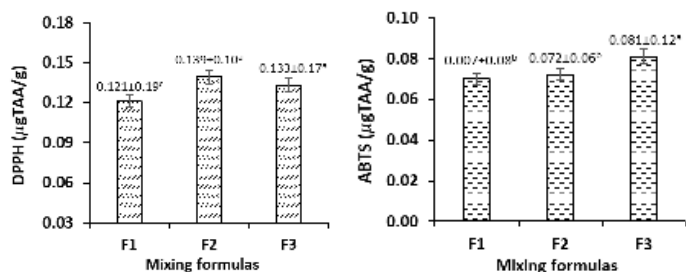


Figure 6. Lemon spice powder mixing formula affects DPPH and ABTS. Values are presented as mean±SD (n = 3). Values with different superscripts are statistically significantly different.

4. Conclusion

In this study, the results were presented with appropriate parameters for lime powder production, including maltodextrin carrier at the concentration of 25% (w/w), lemon juice concentration of 20% (w/w) and direct freezing in the drying chamber (Freeze 3). Suitable formula for lemon spice powder product: includes: lemon powder concentration of 10%, dextrose sugar concentration of 73%, citric acid concentration of 10%, salt concentration of 5.8 %, curcumin colorant concentration of 0.2%, and anti-caking agent concentration of 0.2%. The physicochemical properties of the obtained spice lemon powder are as follows: the moisture 1.54±0.09%, vitamin C content 1.251±0.03 µg/g dry matter, ABTS antioxidant capacity 0.072±0.06 µgTAA/g, and DPPH antioxidant capacity 0.137±0.10 µgTAA/g dry matter. Moreover, the freeze-drying process for seedless lemon juice not only helps to preserve the beneficial biological compounds but also provides a convenient lemon powder product, easy to preserve and has a long shelf life. This product can be used as a natural spice in cuisine or as an ingredient for the food and pharmaceutical industries, creating added value for seedless lemons.

Conflict of interest

The authors declare no conflict of interest.

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