European Journal of Biotechnology and Bioscience
ISSN: 2321-9122; Impact Factor: RJIF 5.44
Received: 12-02-2019; Accepted: 15-03-2019
www.biosciencejournals.com
Volume 7; Issue 3; May 2019; Page No. 94-99

Effects of spray-drying conditions on functional properties of bioactive compounds extracted from lotus leaf

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Abstract
In order to determine effects of spray-drying conditions on functional properties of lotus leaf extract, total phenolic content (TPC), total flavonoid content (TFC), alpha-amylase inhibitory, alpha-glucosidase inhibitory and DPPH scavenging activities of the extracts before and after spray-drying at different temperatures and carrier concentrations were investigated. Lotus leaf was extracted by 75% ethanol in 20 min for three times and then concentrated to 50% (v/v) by vacuum evaporator. Maltodextrin and gelatin were used as carriers in spray drying process with the ratio 7:3 (w/w) and at concentrations of 10, 20 and 30% (w/v). The mixtures of carriers and extract were dried in spray drying chamber with 400 mL/min feed rate, 1.2 – 1.3 bar air pressure, 10 rpm pump speed and air inlet temperature at 130, 150, 170 and 190 °C. TPC, TFC and bio-functional properties of the extracts were found to have strong correlation which suggested that the phenolic compounds presented in the extract were responsible for the DPPH radical scavenging capacity and alpha-amylase and alpha-glucosidase inhibition activities. Significant loss on TPC and TFC (28.74 to 40.63% and 25.52 to 36.87%, respectively) resulted in the decrement of DPPH scavenging capacity and alpha-amylase and alpha-glucosidase inhibition activities after spray-drying. In this study, the highest TPC and TFC recovery (79.24% and 81.87%, respectively) were obtained after spray-drying at 130 °C and 20% of carrier concentration.

Keywords: Lotus leaf, total phenolic compound, total flavonoid compound, antioxidant activity, alpha-amylase inhibition, alpha-glucosidase inhibition, spray-drying

1. Introduction
Nelumbo nucifera Gaertn., which is also known as Indian lotus, Chinese water lily, sacred lotus or just simply lotus, is an aquatic plant in the family of Nelumbonaceae [1]. The origin of lotus is still debated, but there was suggestion that lotus may native to India and widely spread through Persia, Egypt and China in the ancient times. It is commonly planted in Australia, China, India, Iran, Japan and Viet Nam. In Viet Nam, lotus is naturally grown mostly in Dong Thap and An Giang provinces. It is also a popular plant cultivated in delta and midland regions throughout Viet Nam.

Almost all parts of N. *nucifera* are marketed, since they can be both consumed as food and used as traditional medicine. Among them, leaves are considered as effective drugs to treat haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia and hyperlipidaemia [1, 2]. Lotus leaves are rich in bioactive compounds, especially alkaloids and flavonoids. Several studies on lotus leaf extract have shown that the leaf may contain: quercetin 3-O-α-arabinopyranosyl-(1→2)-β-galactopyranoside, quercetin-3-O-β-D-glucuronide, rutin, (+)-catechin, hyperoside, isoquercitrin and astragalin [3, 4]. According to Do *et al* [5], quercetin-3-O-β-D-glucuronide, hyperoside, and isoquercitrin were the most dominant flavonoids extracted by capillary zone electrophoresis, which are about 67.25 – 90.66% of total flavonoids.

Extracts from the lotus leaves have shown strong antioxidant ability, inhibitory capacity of diabetic complication factors, anti-hyperlipidemia effects on rodents as well as ability to lower elevated cholesterol levels in mice and reducing levels of phospholipids and triglycerides [7 – 10]. In the study of Ono *et al* [11], lotus leaf extracts were reported to have inhibition effect on the activity of digestive enzyme α-amylase and lipase. They also were found to modulate lipolysis-activity along with decreased adipogenesis in human pre-adipocytes [12]. Moreover, (+)-1(R)-coclaurine and (–)-1(S)-norcoclaurine in the 95% ethanol lotus leaf extract have been reported to show potent anti-HIV activity [3].

Jung *et al.* [6] reported that after methanol extraction, the total phenolic content of lotus leaf was found to be the highest among other parts: TPC (GAE, mg/g extract) of leaves (177.7) > de-embryonated seeds (92.7) > stamens (83.4) > embryos (41.0) > rhizomes (21.6). Bioactive compounds of lotus leaves were mostly extracted by solvent extraction method using various type of solvent such as ethanol, methanol, acetone, water or their mixtures. Choe *et al.* [13] reported that extraction yields, total phenolic and flavonoid contents of lotus leaves extracted by methanol were higher than those extracted by ethanol. However, several studies still chose ethanol and water as potential solvents for extraction due to it safeness and acceptability for human consumption [9, 14, 15].

Lotus leaf is an interesting source of bioactive compounds, which can be used as bioactive ingredients in foods or made into functional food. However, degradation of their constituents is the concerned to both manufactures and consumers. Therefore, microencapsulation is used to preserve the nutritional and functional characteristics of the

raw material from which it originates. Microencapsulation is a technique which encapsulates bioactive compounds by a biopolymer thereby protecting it from oxygen, water, light or other conditions in order to improve its stability and also to change liquid solutions to powders for easier handling [16]. Spray-drying is the most common and cheapest technique to microencapsulate food materials. There are four stages in microencapsulation by spray-dying: dispersion preparation; dispersion homogenization; infeed emulsion atomization; and atomized particles dehydration [17]. The efficiency of microencapsulation is affected by the wall material and spray-drying condition such as, feed temperature, air inlet temperature and air outlet temperature [18]. Therefore, this project is carried out to determine effects of microencapsulation of lotus leaf extract by spray-drying method on TPC, TFC, antioxidant capacity and alpha-amylase and alpha-glucosidase inhibition activities of the final products.

2. Materials and methods

2.1. Materials

Fresh lotus leaves obtained from Dong Thap province, Viet Nam. The chosen leaves were uniform in size (30-40 cm in diameter) and color while damaged and diseased leaves were eliminated.

Chemicals used for analytical tests (Folin-Ciocalteau reagent, p-nitrophenyl-alpha-D-glucopyranoside, dinitrosalicylic acid, DPPH reagent, ferulic acid, rutin, alpha-amylase and alpha-glucosidase were purchased from Sigma – Aldrich Co. (St.Louis, MO, USA). Maltodextringlucidex 12 D and Gelatin bloom 250 (Rousselot, France) were used in microencapsulation.

2.2. Sample preparation

Fresh lotus leaves were washed under running tap water at least three times to remove all dust and contaminant on surface. Then they were cut into small pieces, put in an aluminum tray and dried by hot air oven at 50 ºC for 24 h. After that, dried leaves were ground to fine powder by a grinder. The powder was stored in a nylon-linear package and then preserved in a desiccator at room temperature until extraction.

2.3. Extraction of bioactive compounds

Bioactive compounds were extracted according to the method of Adom and Liu [19] with some modifications. One gram of ground lotus leaves was mixed with 20 ml of 75% ethanol in an Erlenmeyer flask at room temperature and then continuously shaken at 150 rpm for 20 min. Then the supernatant was separated by centrifugation at 5,000 rpm for 10 min at 25 ºC. The supernatant was collected while the residue was re-extracted twice with 20 ml of 75% ethanol under the same condition. All supernatants (60 ml) were combined and stored at -18 ºC for analysis.

The extract was completely evaporated by vacuum oven at 35 ºC for extraction yield determination. The extraction yield was calculated by the following formula.

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of lotus leaf powder}} \times 100\%$$

2.4. Preparation of encapsulation and spray-drying process

Lotus leaf extracted solution was evaporated at 50 ºC by vacuum evaporator until the half volume remained. Maltodextrin glucidex 12 D and gelatin bloom 250 were used as carriers in spray drying process with the ratio 7:3 (w/w). They were completely dissolved in distilled water at warm temperature. Mixture of the two carriers was mixed with concentrated extract at different concentration and then homogenized by constant stirring in 15 min. Then the solution was filtered by filter papers. The mixture was then dried in spray drying chamber with feed rate of 400 mL/min, air pressure of 1.2 – 1.3 bar and pump speed of 10 rpm [20]. Spray-dried powders were collected, weighed, sealed in nylon-linear package and kept at -18ºC until analysis.

To study the effect of spray-drying conditions, single factors experiments for air inlet temperature and carrier concentration (w/v of extract solution) were performed. One single variable was changed while others were kept constant [21]. In addition, all extractions were done in triplicate.

2.4.1. Effect of air inlet temperature

To determine the effect of feed temperature, the procedure was carried out as described above using different air inlet temperature at 130, 150, 170 and 190°C with carrier concentration was 10% (w/v of extract solution).

2.4.2. Effect of carrier concentration

To determine the effect of carrier concentration, the procedure was carried out as described above using different carrier concentration at 10%, 20% and 30% (w/v of extract solution) with optimal air inlet temperature determined in the section of 2.4.1.

2.5. Determination of total flavonoid content (TFC)

TFC of the samples was determined using aluminum chloride colorimetric method described by Chang *et al*. [22]. Standard calibration was formed using different concentration of rutin (20, 40, 60, 80, 100 µg/ml). The extract solution (0.5 ml) was mixed well with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The sample blank was prepared similarly but aluminum chloride was replaced with distilled water. The mixture was then incubated at room temperature for 30 min. After incubation, the absorbance of the mixture was measured at 415 nm by spectrophotometer. The flavonoid content was calculated based on the calibration curve and expressed as mg rutin equivalent (RE)/g of sample.

2.6. Determination of total phenolic content (TPC)

TPC of the samples was determined by folin-ciocalteau reagent based on the method described by Liyana-Pathirana and Shahidi [23]. The extract (0.5 ml) was mixed with 0.5 ml of folin-ciocateau reagent following with the addition of 1 ml of 20% sodium carbonate solution. The mixture was adjusted to 10 ml with distilled water and then thoroughly mixed by vortex. The mixture was incubated at room temperature for 45 min until the characteristic blue color developed. Then the mixture was centrifuged for 5 min at 4,000 rpm. The supernatant was collected and its absorbance was measured at 725 nm using spectrophotometer. The standard curve was prepared using different concentrations of ferulic acid (20, 40, 60, 80, 100µg/ml). The TPC of the extract were calculated based on a standard curve and expressed as mg ferulic acid equivalent

(FAE)/g of sample.

2.7. DPPH radical scavenging assay

The antioxidant activity of lotus leaf extracts were measured in terms of hydrogen donating or reducing ability of antioxidants toward DPPH radical according to the method of Huang *et al.* [24]. The extract (0.1 ml) was mixed with 3.9 ml of DPPH methanol solution (0.1mM) and kept for 30 min at room temperature. The control sample was made from 3.9 ml of DPPH and 0.1 ml of methanol.

The absorbance of the mixture was read at 515 nm using a spectrophotometer. Total antioxidant capacity of the extract was determined from the graph of equivalent amount of sample in DPPH solution and radical scavenging activity (%), which was calculated by the following equation by Liyana-Pathirana and Shahidi [23].

DPPHscavenging activity (%) = $[(A_{control} - A_{sample})/A_{control})] \times 100$

Where: $A_{control}$ was the absorbance of the control (DPPH + Methanol) and A_{sample} was the absorbance of the sample (DPPH + extract)

2.8. Alpha–amylase inhibition assay

The alpha-amylase inhibitory capacity of samples was determined as described in previous studies [25]. The extract (500 µl) was mixed with 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing alpha-amylase solution (0.5 mg/ml) and the mixture was incubated at 25ºC for 10 min. After pre-incubation, the contents were mixed with 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer and then further incubated at 25ºC for 10 min. The reaction was terminated by the addition of 1.0 ml of dinitrosalicylic acid reagent. Then the mixture was incubated in a boiling water bath for 5 min and cooled to room temperature. The mixture was diluted with 10 ml of distilled water. The control sample was prepared similarly but the extract was replaced with 500 µl of buffer solution. The absorbance of the mixture was read at 540 nm using a spectrophotometer. The results were expressed as % inhibition of enzyme activity which was calculated by the following equation:

$$\% \text{ Inhibition} = [(A^{540}_{control} - A^{540}_{extract})/A^{540}_{control}] \times 100$$

Where:

$A^{540}_{extract}$ was the absorbance at 540 nm of the extract

$A^{540}_{control}$ was the absorbance at 540 nm of the control sample.

2.9. Alpha–glucosidase inhibition assay

The alpha-glucosidase enzyme inhibition assay was modified according to the method described by Girish *et al.* [26]. A solution (50µl) of p-nitrophenyl-α-D-glucopyranoside (10 mg in 2 mL of phosphate buffer) and 10 µl of different concentrations of extracts were mixed and made up to 2.8 ml with phosphate buffer (pH 6.8). A solution (20 µl) of alpha-glucosidase enzyme (2 mg in 1 mL of phosphate buffer; 5.7 U/mg; Sigma Aldrich, USA) was added into the content. Then the reaction mixtures were incubated at 37 °C for 60 min. The enzyme reaction was stopped by incubated 90 ºC for 5 min. The reaction was monitored by increasing in absorbance at 405 nm and compared with the enzyme reaction without the extract (the control sample). The results are expressed as % inhibition of enzyme activity calculated by the following equation:

$$\% \text{ Inhibition} = [(A^{405}_{control} - A^{405}_{extract})/A^{405}_{control}] \times 100$$

Where: $A^{405}_{extract}$ is the absorbance at 405 nm of the extract and

$A^{405}_{control}$ is the absorbance at 405 nm of the control sample.

2.10. Statistical analysis

All tests were performing in triplicate. Analysis of variance (ANOVA) was performed using Tukey's test to compare treatment means at $P<0.05$ using SPSS 16.0 software.

3. Results and discussion

3.1. Concentration of bioactive compounds and functional properties of lotus leaf extract

In this study, ethanol and water were used as solvent for extraction because they are non-toxic solvents compared to other organic solvents such as methanol and acetone. The results of the TPC, TFC, DPPH scavenging capacity and alpha-amylase and alpha-glucosidase inhibition activities of lotus leaf extract using these solvent are shown in Table 1. The extraction yield of lotus leaf extract was determined as 14.9 ± 1.23%. TPC of lotus leaf extract was 179.79 ± 1.99 mg FAE/g lotus leaf powder. This result was similar to that reported by Jung *et al* [6].

Table 1: TPC, TFC, DPPH scavenging capacity and alpha-amylase and alpha-glucosidase inhibition activities of lotus leaf extract[1]

Functional properties	Extracted sample
Extraction yield (%)	14.9 ± 1.23%
TPC (mg FAE/g lotus leaf powder)	179.79 ± 1.99
TFC (mg RE/g lotus leaf powder)	34.84 ± 0.81
Alpha-amylase inhibition % (275µg/ml)	86.42 ± 0.85
Alpha-glucosidase inhibition % (275µg/ml)	65.41 ± 1.26
DPPH scavenging activity % (275µg/ml)	57.07 ± 0.36

[1]The data are presented as mean ± SD ($p<0.05$)

TFC of the extract was 34.84 ± 0.81 mg RE/g lotus leaf powder which was much lower than that previously reported [6, 13]. It may due to the differences in extraction solvent and procedures between this study and others. DPPH scavenging activity was used to evaluate antioxidant activity of lotus leaf extract. At the concentration of 275 µg/ml, DPPH scavenging activity of lotus leaf extract was 57.07 ± 0.36%. The percentage of DPPH scavenging increased with increment of phenolic concentration. The result indicated that lotus leaf extract possessed strong antioxidant activity. Two amylase inhibition assays were served to determine the ability to inhibit alpha-amylase and alpha-glucosidase activity of lotus leaf extract. At TPC of 275 µg FAE/ml, the alpha-amylase inhibitory capacity was 86.42 ± 0.85% and the alpha-glucosidase inhibitory capacity was 65.41 ± 1.26%. These results indicated that lotus leaf extract could be a potential therapeutic agent in diabetes.

3.2. Effects of air inlet temperature on concentration of bioactive compounds and functional properties of lotus leaf extract in spray-drying process

As mention above, carrier or wall material, air inlet temperature and outlet temperature are important factors that must be optimized to obtain good microencapsulation efficiency. After spray drying, concentration of bioactive

compound and functional properties of spray-dried powder were evaluated in order to determine the effect of air inlet temperature on spray-drying condition.

The differences of TPC and TFC at four different temperatures and the TPC and TFC recovery after spray-drying are presented in **Table 2**. After spray-drying, TPC and TFC reduced in a range of 28.74 to 40.63% and 25.52 to 36.87%, respectively. However, there was no significant change of TPC and TFC when the temperature rose from 130 to 150ºC. TPC and TFC decreased when air inlet temperature increased from 150ºC to 190ºC. The decrease in TPC and TFC was due to thermal degradation of heat-sensitive compounds in the extract.

Table 2: TPC and TFC of lotus leaf extract before and after spray-drying at different air inlet temperature (T_{inlet}) [1, 2]

	T_{inlet} (ºC)	130	150	170	190
TPC	Before (mg FAE/g powder)	54.33±0.60*			
	After (mg FAE/g powder)	38.72±0.26	39.02±0.34	35.97±0.20	32.25±0.21
	Recovery (%)	71.26±0.47^{c}	71.81±0.63^{c}	66.21±0.36^{b}	59.37±0.38^{a}
TFC	Before (mg RE/g powder)	10.53±0.25*			
	After (mg RE/g powder)	7.84±0.13	7.90±0.13	7.27±0.09	6.65 ± 0.35
	Recovery (%)	74.48±1.24^{c}	75.02±1.24^{c}	69.07±0.81^{b}	63.13±0.92^{a}

[1]The values are presented as mean ± SD.

[2]Values within each column marked by different lower case letters (a-c) are significant different ($p<0.05$).

The results indicated that alpha-amylase inhibition and alpha-glucosidase inhibition capacity and DPPH scavenging activity reduced respectively from 64.33 ± 0.35% to 48.76 ± 0.27%, 48.32 ± 0.21% to 28.53 ± 0.36% and 47.17 ± 1.58% to 31.66 ± 0.96% with the increment of air inlet temperature from 130 ºC to 190 ºC (Figure 1). As discussed above, high temperature caused the thermal degradation of bioactive compounds of encapsulated lotus leaf extract resulting in the decrease in alpha-amylase inhibition and alpha-glucosidase inhibition capacity and DPPH scavenging activity. The activities of the extracts spray-drying at 130 ºC and 150 ºC were also not significantly.

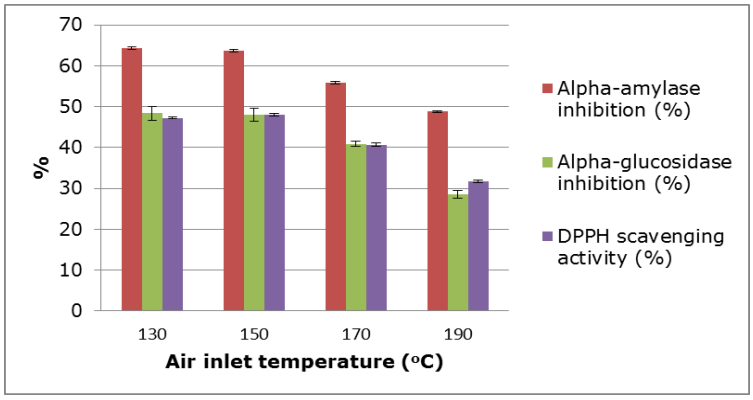

Fig 2: DPPH scavenging activity and alpha-amylase and alpha-glucosidase inhibition capacities of lotus leaf extract after spray-drying at different air inlet temperature.

There were strong positive correlations among TPC, TFC and functional properties of the lotus leaf extract (Table 3). This correlation suggested that the phenolic compounds presented in the extracts were responsible for the DPPH radical scavenging ability, alpha-amylase and alpha-glucosidase inhibition capacities and the air inlet temperature had significant effect on concentration of bioactive compounds as well as functional properties of the extracts from lotus leaf. As a result, 130 ºC or 150ºC is the acceptable air inlet temperature to obtain spray-dried powder with highest functional properties recovery.

Table 3: Correlation coefficients between concentration of bioactive compounds and functional properties of lotus leaf extracts

	TFC (%)	TPC (%)	Alpha-amylase inhibitory (%)	Alpha-glucosidase inhibitory (%)	DPPH (%)
TFC (%)	1				
TPC (%)	0.948	1			
Alpha-amylase inhibitory (%)	0.981	0.989	1		
Alpha-glucosidase inhibitory (%)	0.995	0.918	0.958	1	
DPPH (%)	0.981	0.987	0.992	0.966	1

3.3. Effects of carrier concentration on concentration of bioactive compounds and functional properties of lotus leaf extract in spray-drying process

Carrier agent creates a protective barrier between the core compounds and environments. For microencapsulation efficiency, wall material concentration must be optimized due to the effect of wall solids concentration on the formation of surface core prior to the formation of crust around the drying droplets. In this study, maltodextrin and gelatin were used as carrier materials. TPC and TFC of spray-dried lotus leaf powders with different concentrations of carriers were presented in Table 4. There were increments in both TPC and TFC recovery when carrier concentration increased from 10 to 30% and no significant

difference was found between carrier concentration of 20% and 30%. According to Nguyen and Pham [20], microencapsulation efficiency increased by increasing carrier concentration since thicker and stronger crust is created to protect the core.

Table 4: TPC and TFC of lotus leaf extract before and after spray-drying with different carrier concentration (% w/v) [1, 2]

	Carrier concentration (%)	10	20	30
TPC	Before (mg FAE/g powder)*	54.33 ± 0.60	27.17 ± 0.30	18.11 ± 0.20
	After (mg FAE/g powder)	39.02 ± 0.34	21.53 ± 0.30	14.50 ± 0.35
	Recovery (%)	71.81 ± 0.63[a]	79.24 ± 1.11[b]	80.08 ± 1.92[b]
TFC	Before (mg RE/g powder)*	10.53 ± 0.25	5.27 ± 0.12	3.51 ± 0.58
	After (mg RE/g powder)	7.90 ± 0.13	4.31 ± 0.05	2.86 ± 0.05
	Recovery (%)	75.02 ± 1.24[a]	81.87 ± 0.95[b]	81.49 ± 1.43[b]

[1]The data are presented as mean ± SD.
[2]Values within each column marked by different lower case letters (a-b) are significant different ($p<0.05$).

As TPC (mg FAE/g powder) decreased when carrier concentration increased (Table 4), alpha-amylase inhibition and alpha-glucosidase inhibition capacities and DPPH scavenging activity also decreased (Figure 2). With carrier concentrations 10, 20 and 30%, alpha-amylase inhibition capacities were 63.75 ± 0.31%, 29.16 ± 0.31% and 21.15 ± 0.81%; alpha-glucosidase inhibition capacities were 48.01 ± 1.58, 5.03 ± 0.63 and 1.89 ± 0.63; DPPH scavenging activity were 48.10 ± 0.36, 24.65 ± 0.35 and 8.38 ± 0.42, respectively. The results indicated that carrier concentration has evident effect on functional properties of bioactive compounds extracted from lotus leaf. In addition, 20% is the acceptable concentration of carriers for obtaining spray-dried powder with highest TPC and TFC recovery.

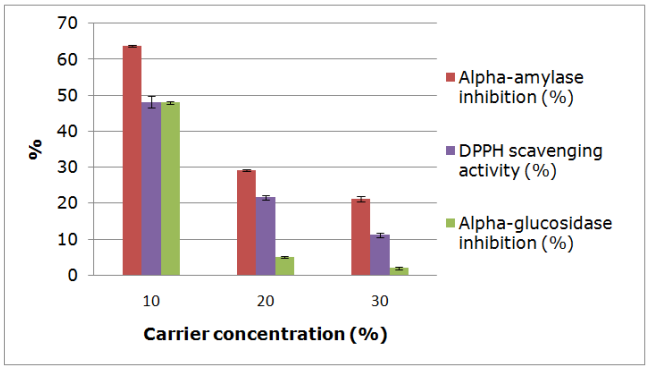

Fig 2: Alpha-amylase inhibition and alpha-glucosidase inhibition capacities and DPPH scavenging activity of lotus leaf extract after spray-drying with different carriers concentration

4. Conclusion

TPC, TFC, alpha-amylase inhibition and alpha-glucosidase inhibition capacities and antioxidant activity of lotus leaf extract were investigated in this study. Air inlet temperature and carrier concentration generated significant effects on concentration of bioactive compounds and functional properties of lotus leaf extract in spray-drying process. Higher air inlet temperature caused larger lost, while higher carrier concentration could recover higher percentage of bioactive compounds. As a result, spray-drying at 130 °C and 20% carrier concentration resulted in the highest TPC and TFC recoveries (79.24% and 81.87%, respectively) which could be applied for industrial production.

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