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The Optimization of Parameters for the Spray Drying Process of Wood Apple Extract using Response Surface Methodology

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Abstract: ***Wood apple is a fruit, which containing many nutrient values and bioactive compounds. In the present study, The response surface methodology (RSM) was used to optimize the input variables of the spray drying process. Three coded independent variables viz. input dry substance concentration (X_1), input flow (X_2), spray drying temperature (X_3), corresponds to the encoded variables Z_1 (from 20 to 24 %), Z_2 (from 5.35 to 6.72 mL/min.), Z_3 (from 140 to 160^{o}C). The R^2 correlation coefficient between the experimental values and the predicted values from the model up to 0.967 indicated the satisfactorily of the predicted model. Three optimal input parameters to get the highest efficiency of dry matter recovery (51.80 %) were derived at Z_1 = 24 %, Z_2 = 5.35 mL/min., Z_3 = 160^{o}C. The powder product obtained has a good sensory quality, high contents of antioxidants and nutritional components.***

Keywords: Optimization, spray drying, wood apple, response surface methodology.

I. INTRODUCTION

Wood apple from the *Rutaceae* family, a tropical fruit, is popular used as the medicinal plant due to multiple biological activities including phenolic compounds, alkaloids, coumarins carotenoids, and many other antioxidants which might protect us againsted chronic diseases [1]. The fruit was rich in nutrients [2] but it has low economic value. The analysis nutrition facts of the wood apple pulp proved that the fruit was high in the source to supply energy such as protein, carbohydrate, and the source without energy such as the soluble and insoluble fiber. The wood apple pulp also contained amount of minerals and vitamins [3] [4], [5]. The high yielding fruits are popularly grown in India Pakistan, Bangladesh, Thailand, Sri Lanka, Burma and many countries of Southeast Asian [6]. The pulp of wood apple fruits can be used to eat raw or used for processing drinks or cream [7].

Especially, jams and jellies which were added the fruit pulp extract were to become popular in Sri Lanka and India. The demands for using wood apple fruits have remarkably increased in the last decades [2].

Wood apple fruits are cheap and can be used in the research to develop of nutraceutical using for diabetes [8] [9]. Antioxidant capacities of bioactive compounds in the fruits were positive correlation with polyphenol components which had many health benefits and could be used in functional foods and pharmaceutical applications [10]. Therefore, the bioactive compounds in the fruits also exhibited a high antibacterial activity to gram-positive bacteria, astringent, antifungal, anti-inflammatory and activities of the insulin secretagogue. The antimicrobial activities might be due to the phenolic compounds presence as thymol and carvacrol, which were rich in the essential oils of the fruit [11] [12]. Thymol was the cause affecting the cellular membrane or the activity inhibition of ATPase or the intracellular ATP release [13]. That mechanism explains wood apple fruits could lead to the prevention of many diseases and the bringing benefits for health [3].

The combination of cellulase and pectinase for the hydrolysis of the wood apple pulp has studied for increasing the efficiency of the nutrients and bioactive compounds. The optimal hydrolysis conditions defined at pH of 4.2, temperature of 45^{o}C, combined cellulase-pectinase concentration of 1.6 (v/dwt), hydrolysis time of 120 mins [14].

Response surface methodology (RSM) is a reliable method for the trend prediction of the optimal variables for the surveyed processes. The statistical experimental design with the mathematical model using RSM could be effectively applied to optimize the performance of processes. RSM combines between the experiments design, regression analysis and the method of optimization to optimize the expected value of variables in the process [15].

The spray drying method is popularly used today for producing powders in foods [16]. In spite of the spray drying has advantages, but fruit juice powders usually present some handling problems which related to the presence of the components having low molecular weight like acids and sugars. These components are the cause of the low glass transition temperature affecting to the spray drying process [17].

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To solve that problem, maltodextrin, a carrier agent, can be effectively used as encapsulating agents for increasing the glass transition temperature of the powder products facilitating for spray drying and storage processes [18]. Besides, maltodextrin also helps to reduce the moisture content of powder products like a physical barrier for prevention of oxygen and light (ensure protection from chemical, enzymatic destruction) resulting in preserving the nutritional powder foods. Maltodextrin also has a good solubility in water which can significantly decrease the viscosity of feed useful for the liquid feeds spray drying [19]. The aim of the present research was to optimize three parameters of the spray-drying process (Dry substance concentration (%), input flow (mL/min.), spray-drying temperature (^oC)) on the recovery performance of dry matter in the wood apple powder.

II. MATERIALS AND METHODS

Chemicals and Materials

DPPH, Trolox were provided from Sigma–Aldrich, USA. ABTS was provided from BioBasic, Canada. Potassium per sulfate ($K_2S_2O_8$) was provided from Merck, Germany. Cellulase (commercial enzyme) from *Trichoderma reesei* and pectinase from *Aspergillus aculeatus* were provided from Novozymes, Denmark. Glucidex 12 (Maltodextrin - DE 12) was provided from Roquettes Frères, France. Fully ripe wood apple fruits were purchased from the market at Tra Vinh province, Vietnam.

Methods

Experiment procedures

Wood apple pulp was diluted with pure water with a ratio 1/1. The diluted mixture was hydrolyzed in the optimal condition of pH 4.2, temperature of 45^oC, combined cellulase-pectinase concentration of 1.6 (v/dwt), hydrolysis time of 120 mins [14]. When the end of the incubation period, the inactivation of enzymes were carried out at 85^oC in 10 minutes. The mixture was then filtered by a Whatman filter paper at the vacuum pressure. The filtrate samples were determined moisture, the recovery efficiency of the dry matter, carotenoids DPPH and ABTS [20].

The Sample preparation for determining the bioactive compounds content in the fruit pulp

A mass of 25 grams sample of wood apple pulp was dried at 45^oC overnight. The sample was then milled and redissolved in 225 mL methanol. The diluted sample was then filtrated by a Whatman filter paper. The mixture was then diluted to a suitable concentration with distilled water for the following analyses [10].

Determine total phenolic compounds (TPC)

The Foline-Ciocalteu reagent method was used to determine TPC [10]. 0.1 mL the sample was dispersed in 3 mL and 2 mL of the sodium carbonate 20 % distilled water. 0.5 mL of Foline-Ciocalteu reagent continued to be added. The tubes were then incubated in a water bath for 1 min at 100 ^oC. It was then cooled. The absorbance was measured at 650 nm by a spectrophotometer. The standard curve was built in the ranges of acid Gallic from the concentration of Gallic acid from 10 ppm to 60 ppm. The results were showed by a number of milligrams of Gallic acid equivalent (GAE) in 100 grams of the sample (wood apple pulp).

Determine the radical scavenging activity by using DPPH

DPPH was used to determine radical scavenging assay the method was developed by Brand-Williams W *et al.* [21]. 0.3 mL of the sample was added with a DPPH-methanol solution 5.7 mL ($A_{515\ nm}$ of 1.1 ± 0.02). The mixture was then vortexed and put in the dark. After 20 mins, the mixture was measured the absorbance at 515 nm. The decrease percents of the absorption by DPPH were calculated by the following formula:

$$\%\ \text{of Inhibition} = [1 - \frac{\text{Asample}}{\text{Acontrol}}]\text{x}100 \quad (1)$$

The standard curve was built in the ranges of Trolox concentration from 100 µM to 700 µM. The results were showed by µM Trolox equivalent antioxidant capacity (TEAC) per 100 grams of the wood apple pulp.

Determine the free radical scavenging ability by using ABTS

ABTS was used to determine radical scavenging assay the method was developed by Re R *et al.* [22]. ABTS was dissolved to a 7µM concentration by using distilled water. Radical cation of ABTS ($ABTS^+$) was created by the reaction between ABTS stock solution and 2.45µM potassium per sulfate. The reaction mixture was put in the dark about 12 to 16 hours at room temperature before using. The $ABTS^+$ solution samples were added with distilled water to get an absorbance at 734 nm of 0.70 ± 0.02.

After mixing 3.0 mL of the diluted solution of $ABTS^+$ ($A_{734\ nm}$ of 0.700 ± 0.02) with 30 µL of the extract samples, the absorbance was then read exactly in 6 mins by using spectrophotometer. The percents of the decrease of absorption by ABTS were calculated by the following formula:

$$\%\ \text{of Inhibition} = [1 - \frac{\text{Asample}}{\text{Acontrol}}]\text{x}100 \quad (2)$$

The standard curve was built in the ranges of Trolox concentration from 100 µM to 700 µM. The results were showed by µM Trolox equivalent antioxidant capacity (TEAC) per 100 grams of the wood apple pulp.

Determine carotenoids content

Carotenoids content was determined by using the spectrophotometric method [23]. 2 grams of the homogenized sample were placed into a conic retort, a volume of 20 mL of ethanol (96 %) was added on the sample. The sample was then stirred in 15 minutes. A volume of petrol ether (25 mL) was then added and stirred for 1 hour. After reaction time about 4 hours, the yellow layer on the upper layer was used for analyzing carotenoids. Wavelength 440 nm was used to measure the absorbance of the sample. The standard curve was built in the ranges of concentration from 10 to 60 ppm of $K_2Cr_2O_7$.

The carotenoids content (mg/100 grams) was calculated by the equation:

$$X = \frac{12.5 * 100 * KE}{36 * a} \quad (3)$$

Where coefficients 12.5 and 36 showed the relationship between carotenoids and $K_2Cr_2O_7$.

KE, equivalent carotenoids.

A, the mass of sample (g).

Methods of nutrient components analysis

Moisture content: using the drying method until to a constant weight at 105°C

Protein: the method of FAO, 14/7, 1986 page 221

Carbohydrate: the method of AOAC 986.25 (2011)

Lipid: the method of FAO, 14/7, 1986 page 222

The efficiency dry matter recovery from hydrolysis (Y) was calculated by equation.

$$Y(\%) = \frac{\text{The yield of dry matter in the filtrate after hydrolysis process}}{\text{The yield of dry matter in the fruit pulp of the sample before hydrolysis process}} x100 \quad (4)$$

Experimental design

The response surface methodology (RSM) with star distance of the Circumscribed Central Composite (CCC) design was used to optimize the parameters of the spray-drying process. The independent variables were coded: dry substance concentration (X_1), Input flow (X_2), Spray-drying temperature (X_3) corresponds to the uncoded variables Z_1, Z_2, and Z_3. The relationship of the variables were showed the following equation:

$$X_j = \frac{Z_j - Z_{0j}}{\Delta Z_j}, j = 1,2,3,..k \quad (5)$$

Where, X_j are dimensionless coded variables having only values -1, 0, +1;

Z_j are the actual value of the independent variables, Z_{0j} are the actual values of variables at the middle points,

ΔZ_j are the step change values. Investigated factors and levels tested are reported in Table 1.

Table. 1 The levels of the un coded independent variables and coded independent variables in Circumscribed Central Composite design

Independent variables	Unit	Symbol	Coded levels				
			-α	Low (-1)	Middle (0)	High (+1)	+α
Dry substance concentration	%	X_1	18.64	20	22	24	25.36
Input flow	mL/min.	X_2	4.88	5.35	6.03	6.72	7.18
Spray-drying temperature	°C	X_3	133	140	150	160	167

After the conducted experiment, the data were fitted with the equation as follows:

$$Y(\%) = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=1+1}^{4} \beta_{ij} x_i x_j \quad (6)$$

Where Y(%) is a response (the efficiency of dry matter recovery from the spray drying process); β_0 is constant coefficients; β_i, β_{ii}; β_{ij} are coefficients of the model. The analysis was performed by using Modde 5.0 software.

Data analysis

The analysis of ANOVA (p-value < 0.05) was used to evaluate the compatibility of the model.

III. RESULT AND DISCUSSION

The effect of the input parameters of the spray drying designed by CCC on the values of the observed response

RSM was used to determine the effect of the input dry substance concentration, input flow and spray drying temperature on the dry matter recovery efficiency.

The encapsulation of the nutrient into micro particles has great significance in the properties and quality of the powder. In the present research, maltodextrin DE 12 was used like a carrier agent for the inlet material of the spray drying process. The results from Table 2 show the concentrations of the carrier agent are low, it leads to the low efficiency dry matter recovery of the powder. That may be explained the particles were produced very sticky and deposited onto the wall of the drying chamber and cyclone resulting in the difficult recovery of the powder product [19]. However, when the concentration of added maltodextrin was higher the necessary concentration for covering the dry matter of the fruit extracts, it would be wasteful.

When the input flow was high, the inlet drying material entered the drying chamber of the equipment too much resulting in a slow evaporation rate and the high moisture content in the powder product. The powder product was heavily adhered to the device walls and recovered cyclones. That was also the cause leading to adhesion of the powder to the drying chamber wall and the cyclone recovery of the drying equipment. The effect negative signals of the carrier agent concentration and input flow on the powder recovery efficiency are found in the agreement with Muzaffar & Kumar [24], that could explained the slower transfers of heat and mass occurred as the spray drying process was performed at a higher rates of the input flow.

The results of the experiments in Table 2 show when the temperatures of spray-drying process were unfavorable (too low or too high temperatures), the recovery efficiency of the dry matter was significantly lower than the recovery efficiency achieved from the other conditions and the recovery process is more difficult due to adhesion of the powder into the of the drying chamber and the cyclone wall as shown Fig. 4 (b). When drying at low temperatures, the moisture from the surface of the drying material evaporated slowly, resulting in a slow diffusion of moisture inside the drying material, resulting in the product of powder had been high the content of moisture.

Then the spray drying temperature was too high, the color of the powder tended to be darkly, the nutritional quality and heat-sensitive components (antioxidations and vitamins) in the powder were more lost. The effects of this trend are in agreement with the previous study by Milton C. C. *et al.* for the spray drying process of mango using maltodextrin as a carrier at the inlet spray drying air temperature of 160°C. The result showed the powder product had a high degree of solubility [25].

Table. 2 The model of experiment and the values of the considered response

Run	(x_1) Coded dry substance concentration	(x_2) Coded input flow	(x_3) Coded spray drying temperature	The efficiency dry matter recovery (%)
N1	-	-	-	47.45± 0.36
N2	+	-	-	49.39± 0.29
N3	-	+	-	42.35± 0.45
N4	+	+	-	43.74± 0.43
N5	-	-	+	49.48± 0.50
N6	+	-	+	51.69± 0.61
N7	-	+	+	45.72± 0.65
N8	+	+	+	47.57± 0.53
N9	-α	0	0	44.75± 0.49
N10	+α	0	0	48.86± 0.37
N11	0	-α	0	50.08± 0.74
N12	0	+α	0	40.57± 0.48
N13	0	0	-α	43.93± 0.37
N14	0	0	+α	50.03± 0.69
N15	0	0	0	48.69± 0.30
N16	0	0	0	49.25± 0.46
N17	0	0	0	48.31± 0.42
N18	0	0	0	48.46± 0.23
N19	0	0	0	48.81± 0.30
N20	0	0	0	49.35± 0.47

Fitting the model

The coefficients of regression collected for the coded independent variables and the results of Anova analysis for the observed responses are displayed at the table 3 and the table 4, distributively.

Statistical analysis in Table 4 shows that the regression model had a well-fitting and large meaning (P-value < 0.001, a high F-value of 32.8263). That indicated the predicted values close to the observed data values. The R2 value need be 0.80 to this model has the fit well [19]. In the present study, coefficient of determination R2 = 0.967 indicating the sample conformity. The fitted determining coefficient R^2 (adj) was 0.938 confirming that the sample was meaningful great [19] [11].

Table. 3 The multinomial regression coefficients of the response surface for the coded independent variables.

The efficiency of dry matter recovery	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	48.7811	0.298127	1.78589e-018	0.664262
X1	1.04721	0.197789	0.00035025	0.440697
X2	-2.53516	0.197789	1.56821e-007	0.440697
X3	1.59539	0.197789	1.09551e-005	0.440697
X1*X1	-0.508639	0.192517	0.0246475	0.428952
X2*X2	-1.03176	0.192516	0.000319318	0.428949
X3*X3	-0.446776	0.192516	0.0427207	0.428949
X1*X2	-0.11375	0.258437	0.669192	0.575827
X1*X3	0.0912496	0.258437	0.731356	0.575827
X2*X3	0.358751	0.258437	0.195234	0.575827
N = 20	Q^2 =	0.785	Cond. no. =	3.5909
DF = 10	R^2 =	0.967	Y-miss =	0

R^2 Adj. =	0.938	RSD =	0.7310
		Conf. lev. =	0.95

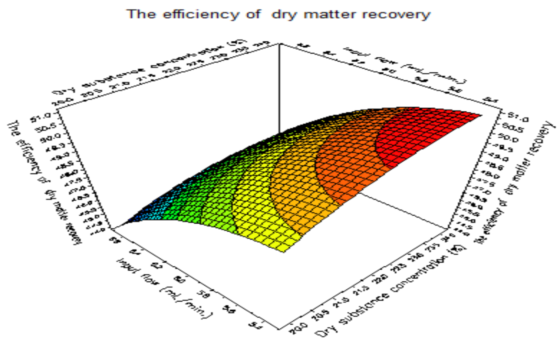
The predictive quadratic equation in relation to the independent variables was provided in equation (7).

$$Y(\%) = -206.7859007 + 6.118513*Z_1 + 22.9525*Z_2 + 1.499867*Z_3 - 0.12716Z_1^2 - 2.2096*Z_2^2 - 0.00447*Z_3^2 \quad (7)$$

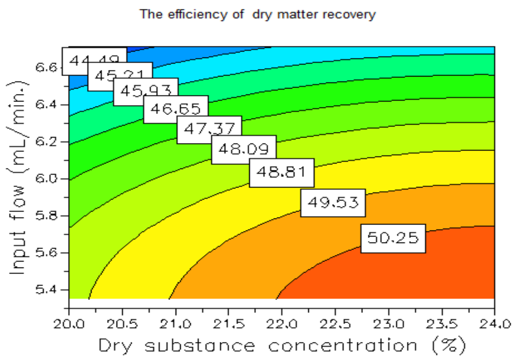
Table. 4 Analysis of variance (ANOVA) for the response surface quadratic model for the coded independent variables

The efficiency of dry matter recovery	DF	SS	MS (variance)	F	p	SD
Total	20	45143.9	2257.2			
Constant	1	44980.7	44980.7			
Total Corrected	19	163.203	8.58964			2.93081
Regression	9	157.86	17.54	32.8263	0.000	4.18808
Residual	10	5.34327	0.534327			0.730977
N = 20	Q2 =	0.785	Cond. no. =	3.5909		
DF = 10	R2 =	0.967	Y-miss =	0		
	R2 Adj. =	0.938	RSD =	0.7310		

The effects of the input variables of the spray drying on the efficiency of dry matter recovery is observed on the response surface and contour plots as indicated in Fig .1, Fig. 2 and Fig. 3.

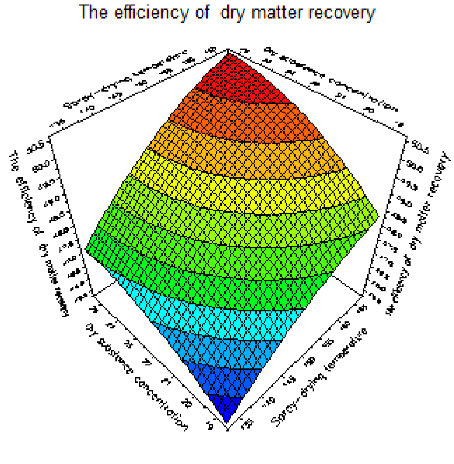


(a)

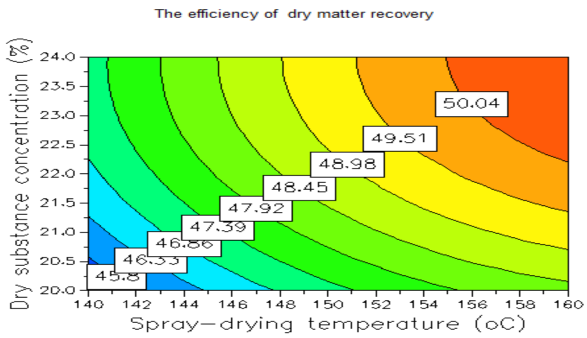


(b)

Fig. 1 The interactive effect of dry substance concentration (a) and input flow of the spray-drying process on the efficiency of the dry matter recovery (b)

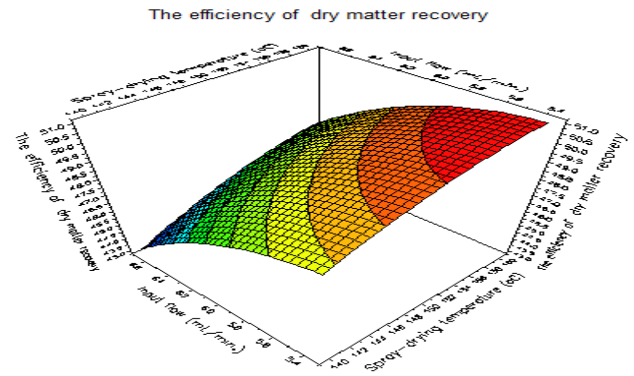


(a)

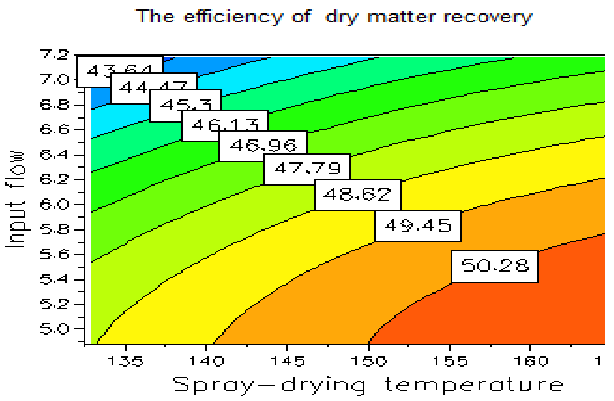


(b)

Fig. 2 The interactional effect of dry substance concentration (a) and temperature of the spray-drying process on the efficiency of the dry matter recovery (b)



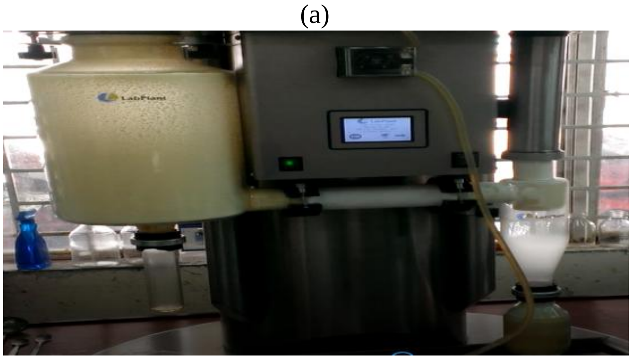
(a)



(b)

Fig. 3 The interactive effect of input flow and temperature of the spray-drying process on the efficiency of the dry matter recovery

(a)



(b)

Fig. 4 The comparison the at the different spray drying conditions, (a) the process from the optimal condition, (b) the process from the other unsuitable condition

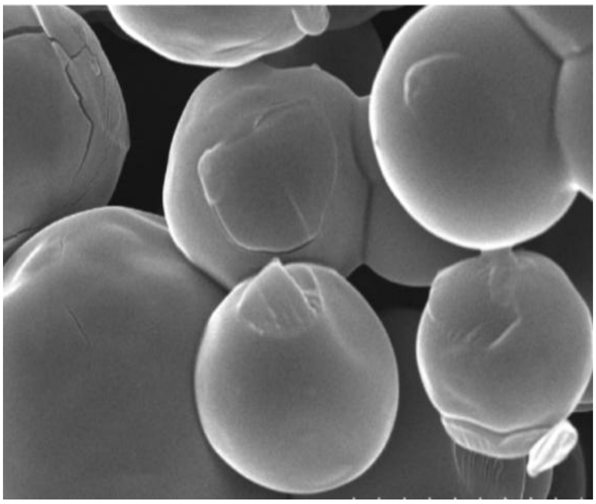


Fig. 5 SEM – typical micrograph of the wood apple powder produced at inlet conditions of dry substance concentration 24 %, input flow 5.35 mL/mins, spray-drying temperature 160°C

Table. 5 The comparison of the content of some components in the inlet and outlet samples of the spray drying process from 100 grams of the filtrate

Samples	The content of some components from 100 grams of the filtrate			
	Phenolic (mgGAE)	DPPH (mgTEAC)	ABTS (mgTEAC)	Carotene (mg)
The filtrate before spray drying	106.7±2.6	67.1±0.6	102.1±0.6	86.6±15.0
The diluted powder at the same dilution concentration as the filtrate	104.7±1.0	61.4±2.9	99.5±2.9	78.3±16.9

Table. 6 Some nutrient components in the wood apple powder

	Moisture (%)	Carbohydrate (%)	Protein (%)	Lipid (%)
The content of components in 100 grams of the wood apple powder	4.67	85.8	3.0	0.35

The optimal spray drying condition derived from the optimal model (dry substance concentration 24 %, input flow 5.35 mL/min, spray-drying temperature of 160°C not only produced the powder product having the highest efficiency of dry matter recovery (51.80 %) and the good sensory quality, but also retained high levels of antioxidants and nutrients ingredients in the final product as showing in Fig. 4 (a), Fig. 5, Table 5 and Table 6. At this condition, the powder had the uniform particle sizes and the surface them was covered well. Besides, moisture content takes an essential role of determination its fluidity, adhesion and preservation, which is related to the quality and preservation ability of the powder product [26]. The moisture of the final product reached 4.67 % and is suitable for more product preservation. A similar report of Milton, C. C. *et al.*, showed that when conducting the spray drying process of mango juice at the temperature of 160°C, the product was less sticky and more soluble [25].

IV. CONCLUSION

The spray drying process is effective use of the optimization parameters when is done by Response surface methodology. Achievements of the experiment were supported in the predicted model showing the adequacy of the suitable form. The high correlation of the mathematical model showed that the quadratic multinomial model would have used to optimize the efficiency of dry matter recovery and the quality of the wood apple powder. The optimal conditions were achieved with an input dry substance concentration 24.0%, input flow 5.35 mL/min, spray-drying temperature 160^{o}C, the highest efficiency of dry matter recovery of 51.80%. This study result will be of considerable value to the commercial producers to produce many products with adding wood apple powder.

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