EDELWEISS PUBLICATIONS
OPEN ACCESS

https://doi.org/10.33805/2765-8821.102
Volume 1 Issue 1 | PDF 102 | Pages 7

Edelweiss: Food Science and Technology

Research Article ISSN: 2765-8821

Process Optimization for Extraction of Polyphenols from Avocado Seeds (*Persea americana* Mill.) Using Response Surface Methodology

Hau Tan Vo[1*], Tha Thi Nguyen[1] and Vinh-Nghi Kim Ho[1]

Affiliation: [1]Food and Agro-materials Preservation and Processing, Food Industry Research Institute, Ho Chi Minh City, Vietnam

[*]**Corresponding author:** Hau Tan Vo, Food and Agro-materials Preservation and Processing, Food Industry Research Institute, Ho Chi Minh City, 58-Nguyen Binh Khiem, DaKao Ward, District 1, Ho Chi Minh City, Vietnam, Tel: +84-28-3299678, Email: hautanvo@gmail.com

Citation: Vo TH, Nguyen TT and Ho KVN. Process optimization for extraction of polyphenols from avocado seeds (*Persea americana* Mill.) using response surface methodology (2019) Edelweiss Food Sci Tech 1: 5-11.

Received: Nov 12, 2019
Accepted: Nov 22, 2019
Published: Nov 28, 2019

Abstract

At the present time, polyphenolic compounds have attracted great interest due to their roles in the prevention of degenerative diseases and have used as the input material for manufacturing functional foods, nutraceutical and pharmaceutical products. Previous researches have revealed the avocado seed is rich source of polyphenolic compounds with antioxidant and antimicrobial activities; which they could be used as a source of potent natural ingredients and additives. In this study, extraction factors of polyphenols were optimized for recovery yield by using response surface methodology and the obtained polyphenol rich solution was encapsulated with different coating agents (Maltodextrin-MD and Gum Arabic-GA) as well as their mixtures. A Box-behnken design was used to investigate the effects of three independent variables including ethanol concentration (X_1:35-65%, v/v), solvent to solid ratio (X_2:8-12, v/w) and extraction time (X_3:1.0-3.0 h). The result shown that the optimized extraction conditions were using ethanol concentration of 40% (v/v), ratio of solvent to solid at 12:1 and extraction time of 1.5 h. Under the conditions, the experimental recovery yield of polyphenols is 83.1% which is well matched with the predicted yield of 82.5%. Micro particle prepared by 20:80 of MD:GA ratio as coating agent can be selected for encapsulation of the polyphenolic compounds.

Keywords: Avocado seeds, extraction of polyphenols, optimization, response surface methodology.

Abbreviations: MD-Maltodetrin, GA-Gum Arabic, RSM- Response Surface Methodology, TPC- Total Polyphenol Content, GAE- Gallic Acid Equivalents, SPC- Surface Polyphenol Content, EE- Encapsulation Efficiency, RPY- Recovered Polyphenol Yield, WSI- Water Solubility Index, BBD- Box-Behnken Design, ANOVA- Analysis of Variance.

Introduction

Avocado (*Persea americana* Mill.) is an evergreen tree native to Central America that now widely cultivated in the tropical and subtropical regions of the world for edible fruits, which are very rich in oil [1]. In 2017, world avocado production was approximately 5.92 million metric tons. This tree was first introduced into the Lam Dong province of Vietnam in 1940 by the French [2,3]. Although no statistical figures are available on the area and output of avocado, the tree is widely grown in the uplands of Vietnam such as Dong Nai, Ba Ria-Vung Tau, Lam Dong, Dak Lak and Phu Tho with various local names given to them according to their fruit shape and quality.

The fruit of the plant, also called avocado (or avocado pear or alligator pear), is commercially importance. The edible part of the fruit is rich in unsaturated fatty acids, vitamins B, C and E, and other nutrients [4]. The avocados are mainly consumed as a fresh fruit but at the moment many value added products have manufactured as guacamole, avocado pulp and avocado oil [5]. Industrial processing of avocados generates a large amount of peels and seeds as waste which can cause environmental problems. The avocado seed contents up 16% of the total weight of the fruit and has a long history of ethno botanical use [6]. They are a rich source in phytochemicals, especially polyphenolic compounds such as hydroxycinnamic acid derivatives, flavonoids and proanthocyanidins with many bioactivities such as anticancer, antidiabetic effect antihypertensive effect [7-10] and antioxidant and antibacterial properties [11-16].

Polyphenols are a group of plant-derived secondary metabolites with phenolic structural features. They can be divided into at least 10 different classes depending on their basic chemical structure of aglycones such as phenolic acid derivatives, flavonoids and proanthocyanidins [17]. Currently, the compounds have attracted great interest due to their roles in the prevention of degenerative diseases, particularly cancers, cardiovascular diseases and neurodegenerative diseases [18]. They have used as the input materials for manufacturing functional foods, nutraceutical and pharmaceutical products. However, the effectiveness of polyphenols depends on preserving the stability, bioactivity and bioavailability of the active ingredients. Moreover, the unpleasant taste of most polyphenolic compounds also limits their application. Therefore, encapsulation process can be a useful method to alleviate these deficiencies.

Encapsulation may be defined as a process to entrap substances (active agents) within another substance (wall materials) [19]. Up to now, many encapsulation technologies of polyphenols have been used effectively such as spray drying, liposome entrapment and emulsion. In the food industry, the encapsulation process is a useful tool to improve delivery of bioactive molecules into foods [20].

Besides, the process can be applied for protecting the core material from degradation by the surrounding environment, preventing unwanted flavor or taste of core material as well as modifying the nature of the original material for easier handling [21].

Nowadays, there is a growing interest in finding phytochemicals as alternatives to the synthetic substances that are commonly used in the food, pharmaceutical and cosmetic industries. The research results about polyphenol rich extract of avocado seed have revealed their potential usage as they contribute to enhance the nutritional and technological value of the meat products through their antioxidant action [22-23].

Response Surface Methodology (RSM) consists of a group of mathematical and statistical techniques that are based on the fit of empirical models to the experimental data obtained in relation to experimental design [24]. It is the one of most effective tools for optimizing the process when many factors and interactions affect the desired response. RSM usually uses an experimental design such as Box-behnken or central composite to fit a second order polynomial [25-26].

The purpose of this study was

- To optimize the extraction parameters of polyphenols from the avocado seeds using RSM
- To study effect of encapsulating agents on physiochemical characteristics of polyphenol rich micro particles.

Material and Methods

Materials

Avocado fruits (named "Bơ Sáp" in Vietnamese) were purchased from the local market in Ho Chi Minh City, Vietnam and kept at room temperature until they reached ready-to-eat ripeness. MD (DE=10-13) and GA supplied by Sigma-Aldrich Chemical Co. were used as coating materials. Other chemicals either HPLC reagent grades or the highest purity were available.

Preparation of Avocado Seed Powder (ASP)

Avocado seeds were manually separated from the flesh and cleaned. The seeds were sliced with the average thickness of the sliced seed samples about 2.5 mm then they were soaked in a 1.5% w/v citric acid solution for 20 minute (the citric acid used as an inhibitor of enzymatic browning). The pretreated slices were dried in a hot air oven at 70°C for 3h. The dried slices with moisture of 7.04% were ground into powder using Grindomix machine (Retsch, GM 200, Germany) and stored at -25°C until use.

Extraction Procedure

Solid-liquid extractions were carried out at temperature 60°C. One hundred grams of ASP sample (126.02 ± 0.1 mg GAE/g) were blended with ethanol solvent at concentrations (35-65%, v/v), ratios of solvent to solid (8-12 v/w) and extraction time (1.0-3.0 h) as specified by the experimental design (**Table 1**). After the extraction, liquid extracts were separated from solids by filtration and removed ethanol in a rotary evaporator at 55°C. The recovery yield of polyphenols (RYP) from ASP was calculated according to equation 1.

$$\text{RYP} = \frac{\text{Extracted polyphenols}}{\text{Polyphenols in ASP}} \text{X100 (1)}$$

Preparation of the Polyphenol Rich Micro particles

MD and GA mixed with different ratios of 10:0, 8:2, 6:4, 4:6, 2:8 and 0:10 were used as coating materials. Polyphenolic extract after removing solid and ethanol (312.5 ml, total polyphenolic content=21 mg GAE/ml and dry matter content=2.4 g/100 ml) was previously heated at 45°C with constant stirring. 42.5 g MD:GA mixture with above ratios was added to form homogeneous solutions. Total solid content of the solutions before spray drying was corrected to 10 by adding to 137.5 ml distilled water. The obtained solutions were fed to a mini spray dryer (B-290, Büchi). The spray dryer was operated at inlet temperature 160 ± 2°C. The air flow and rate of feeding were 550L h^{-1} and 10 ml min^{-1}, respectively. The powders obtained were kept in dark container at -20°C until analysis.

Total Polyphenol Content (TPC) Determination

The Total Polyphenol Content of each extract was determined by the Folin-Ciocalteu assay with minor modifications [8]. The extracts (1 ml) were mixed with 5 ml of 1:10 diluted Folin-Ciocalteu's phenol reagent, followed by 4 ml of sodium carbonate (7.7%, w/v) and allowed to stand for 30 min in the dark at room temperature then the absorbance was read at 760 nm using a spectrophotometer (Shimadzu, Japan). The polyphenol content was calculated as mg of Gallic Acid Equivalents (GAE) per gram of dry matter from a standard curve of Gallic acid.

To extract polyphenols for TPC determination from ASP, 0.2g of samples were extracted in 10 ml acetone/water (70:30, v/v) for 30 min [15]. After the extraction, the extract was centrifuged at 3000 rpm for 15 min. The supernatant was collected and the residue was re-extracted once more. The two supernatants were combined and dried by using a rotary evaporator at 55°C. The residue was dissolved in 10ml of distilled water and kept in dark container at 5°C until analysis.

For determination of TPC_m and Surface Polyphenol Content (SPC_m) of micro particles, capsules of 1 g were dissolved in 10ml of methanol: acetic acid: water (50:8:42,v/v/v) or 10ml ethanol: methanol (50:50, v/v), respectively. The supernatants were centrifuged at 3500rpm for 15min and then filtered [27]. TPC_m and SPC_m were quantified as described above. The Encapsulation Efficiency (EE) and the Recovered Polyphenol Yield (RPY) from spray dried experiments were calculated according to equations 2-3, respectively.

$$EE(\%) = \frac{EPC}{TPC_m} \times 100 = \frac{TPC_m - SPC_m}{TPC_m} \times 100 \quad (2)$$

$$RPY(\%) = \frac{TPC_m}{\text{Theoritical total polyphenols}} \times 100 \quad (3)$$

Where EPC was the encapsulated polyphenol content which was calculated by subtracting total polyphenol content (TPC_m) of micro particles from surface polyphenol content (SPC_m) of micro particles.

Moisture Content and Water Solubility Index

The moisture content of micro particles was determined based on the loss in weight between samples before and after drying at 105 ± 2°C.

In order to evaluate the solubility of the micro particles, the Water Solubility Index (WSI) was determined using by Anderson method with minor modifications [28]. One gram of powder samples was added to 12 ml distilled water, mixed and incubated in a water bath at 30°C for 30 min. After incubation, samples were centrifuged at 3500 rpm for 15 min. The supernatants were collected and evaporated at 105 ± 2°C until obtaining a constant weight. WSI was expressed as in equation 4.

$$\text{WSI}(\%) = \frac{\text{Dry weight of supernatant}}{\text{The Initial weight of microparticles}} X100 \text{ (4)}$$

Experimental Design and Statistical Analysis

In this study, RSM was used to predict the optimum extraction conditions of polyphenol compounds from ASP by using Design-Expert software (version 9.0, Stat-Ease Inc., Minneapolis, MN, USA). The Box-Behnken Design (BBD) with a quadratic model was

selected to investigate the combined effects of three independent variables while extraction temperature was fixed at constant rate of 60°C (determinates after several preliminary experiments, data not shown). The independent variables were ethanol concentration (35-65%, v/v), ratio of the solvent to solid (8-12, v/w) and extraction time (1.0-3.0 h). Experimental design scheme derived from Design-Expert and response value (Y, recovery yield of polyphenols) were presented in Table 1. The actual values were coded at three levels: −1, 0, and +1 according to the following equation:

$$X_i = \frac{x_i - x_0}{\Delta x} \quad (5)$$

Where X_i is the coded value, x_i is the corresponding actual value, x_0 is the actual value in the center of the domain, and Δx is the increment of x_i corresponding to a variation of 1 unit of X.

Experimental data were fitted to a quadratic polynomial model and regression coefficients obtained. The computer-generated quadratic model used in the response surface was as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (6)$$

Where Y denotes the dependent variable. The coefficients of the polynomial equation were represented by β_0 (intercept), β_i (linear effects), β_{ii} (quadratic effects), and β_{ij} (cross product effects). X_i represented the coded levels of independent variables. The terms X_iX_j and X_i^2 were expressed as the interaction and quadratic terms, respectively.

Design-Expert software was used to estimate the response of each set of experimental design and optimized conditions. The quality of the fitted model was expressed by the coefficient of determination R^2, the adjusted determination coefficient R^2_{adj} as well as the predicted determination coefficient R^2_{pred} and statistical significance of the model was determined by *F*-test. All treatments were done in triplicate and the results were expressed as a mean (± SD) for each treatment. The significant difference between treatments reported at $p \leq 0.05$.

Run	Coded variables			Uncoded variables			Y (%)
	x_1	x_2	x_3	X_1	X_2	X_3	
1	1	0	1	65	10	3	54.6
2	-1	0	-1	35	10	1	76.55
3	0	1	-1	50	12	1	76
4	0	0	0	50	10	2	68.9
5	0	-1	1	50	8	3	57.59
6	-1	1	0	35	12	2	81.88
7	1	1	0	65	12	2	77.82
8	0	0	0	50	10	2	70
9	0	-1	-1	50	8	1	56.15
10	1	-1	0	65	8	2	50.1
11	-1	0	1	35	10	3	70.01
12	0	1	1	50	12	3	71.95
13	1	0	-1	65	10	1	51.2
14	-1	-1	0	35	8	2	77.1
15	0	0	0	50	10	2	68.9

Table 1: Factors and levels for RSM and BBD with the observed values for the recovery yield of polyphenols (Y).

Results

Extraction optimization

Model fitting: The study used RSM to develop a prediction model for optimizing conditions of polyphenol extraction from ASP. The experimental conditions and experimental data of 15 runs containing 3 replicates at center point were presented in Table 1. Experimentally obtained values for polyphenol recovery varied from 50.10% to 81.88% and the highest recovery was at the point with 35% of ethanol concentration, the solvent to solid ratio of 12 and 120 min of extraction time. By performing multiple regression analysis on the experimental data, the model for the response variable (recovery yield of polyphenols from ASP) could be expressed in form of coded values by the quadratic polynomial equation as follows:

$$Y = 69.3 - 9.0X_1 1 + 8.3X_2 - 0.7X_3 + 5.7X_1X_2 + 2.5X_1X_3 - 1.4X_2X_3 + 0.06X_1^2 + 2.4X_2^2 - 6.2X_3^2 \quad (7)$$

The plot of experimental values of the recovery yield of polyphenols versus those calculated from equation (7) indicated a good fit, as shown in **Figure 1**.

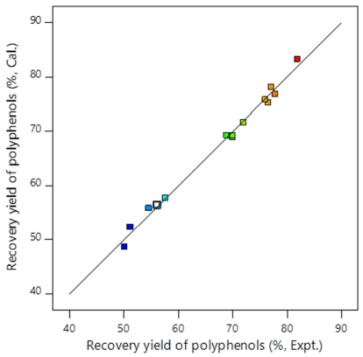


Figure 1: Correlation of calculated recovery field of polyphenols with Experimental recovery fields of polyphenols.

To test the significance and adequacy of the model, the Analysis of Variance (ANOVA) for the quadratic model was performed. The lack of fit test measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression, which determines whether the selected model is adequate to explain the experimental data or another model should be reselected [29]. As shown in Table 2, the lack of fit test was not significant relative to the pure error ($p>0.05$) and implied that the model equation was available. The model F-value of 66.1 ($p \sim 0.0001$) revealed the model was significant. There was only a 0.01% chance that and this large F-value could occur due to noise.

The results of the analysis of variance also generated the determination coefficients for the model as shown in Table 2. For the fitted model, the coefficient of determination (R^2), which is a measure of degree of fit, was 0.9917. This indicated that only 0.83% of the total variations were not explained by the fitted model as well as revealed the satisfactory correlation between actual values and predicted ones. Lundstedt, et al. suggested that, for a good fit of a model, R^2 should be at least 0.80 [30].

Moreover, the adjusted determination coefficient (R^2_{adj}, 0.9767) was high and very close to R^2. The higher the value of R^2_{adj} is, the deeper the correlation between the observed and predicted values performs [31]. R^2_{prep} (0.9738) was in reasonable agreement with R^2_{adj}. CV (coefficient of variation), which indicates the degree of precision with that the experiments are compared, was 2.39. A relatively low value of CV disclosed a better precision and reliability of quadratic polynomial model adequate precision compares the range of the predicted values at the design points to the average prediction error and a ratio greater than 4 is desirable. The value of adequate precision was 26.3372 as shown in **Table 2**. Therefore, the model is adequate for prediction in the range of experimental variables and could be used to navigate the design space.

Source	Sum of Squares	DF	Mean Square	*F*-value	p-value (Prob>*F*)	Significant
Model	1543.86	9	171.54	66.14	0.0001	**
X_1	644.76	1	644.76	248.59	<0.0001	**
X_2	556.28	1	556.28	214.47	<0.0001	**
X_3	4.13	1	4.13	1.59	0.2625	Insignificant
X_1X_2	131.56	1	131.56	50.72	0.0008	**
X_1X_3	24.7	1	24.7	9.52	0.0272	*
X_2X_3	7.54	1	7.54	2.91	0.149	Insignificant
X_1^2	0.015	1	0.015	0.0006	0.9431	Insignificant
X_2^2	21.19	1	21.19	8.17	0.0355	*
X_3^2	143.75	1	143.75	55.42	0.0007	**
Residual	12.97	5	2.59			
Lack of fit	12.16	3	4.05	10.05	0.0918	Insignificant
Pure error	0.81	2	0.4			
Cor. total	1556.82	14				
R^2	0.9917		Adeq. precision		26.3372	
R^2_{adj}	0.9767		C.V.%		2.39	
R^2_{prep}	0.9738		PRESS		196.4	

Note: *Significant at p<0.05; **Significant at p<0.001.

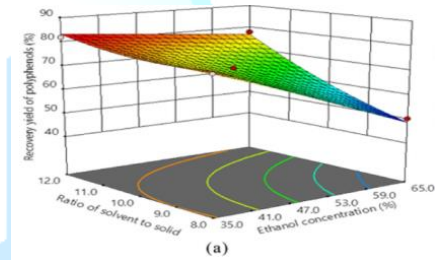
Table 2: The analysis of variance (ANOVA) table for response surface quadratic model.

Effects of Extraction Conditions on Recovery Yield of Polyphenols: The effects of extraction conditions of ASP on polyphenol recovery yield by the regression coefficients of fitted second-order polynomial are presented in Table 2 and the significance of each coefficient was determined using *F*-value and p-value. It could be seen that the effects of ethanol concentration and the solvent to solid ratio (X_1, and X_2; $p<0.05$) were the major contributing factors to the recovery yield of polyphenols, while extraction time had no significant effect (X_3; $p>0.05$) within the experimental range. In addition, it was evident that coefficients (X_1X_2, X_1X_3, X_2^2, and X_3^2) were significant at the level of $p<0.05$, whereas the other coefficients were insignificant (X_1X_3, X_1^2; $p>0.05$).

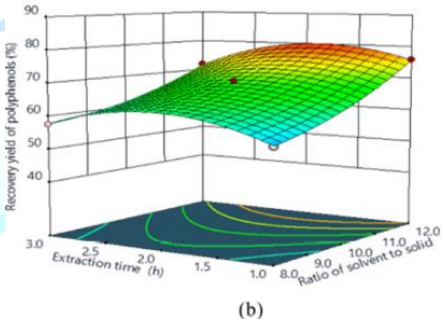
To aid visualization, the relationship between independent variables and response variable of polyphenolic extraction from ASP was graphically represented by 3D response surfaces generated by the model as in **Figure 2** and the relationships could be intuitively conveyed as two variables were depicted in same plots while the other variable was kept at level 0. The interactions of ethanol concentration (X_1) with solvent to solid ratio (X_2) and the extraction time (X_3) on the recovery yield of polyphenols shown in Figures 2(a) and 2(b), respectively. The yield of polyphenols increased rapidly with the increment of solvent to solid ratio and reduction of ethanol concentration. The results demonstrated that the interaction between ethanol concentration (X_1) and solvent to solid ratio (X_2) was very remarkable but this interaction was against each other. The yield also increased lightly along with the increment of extraction time and diminution of ethanol concentration, while it declined lightly with higher extraction time after a critical value of 2 h.

Figure (2c) suggested that the interaction between solvent to solid ratio (X_2) and time extraction (X_3) was not significant. Curvature of the response surface in this Figure may be due to quadratic effects of solvent to solid ratio (X_2) and time extraction (X_3) on the response.

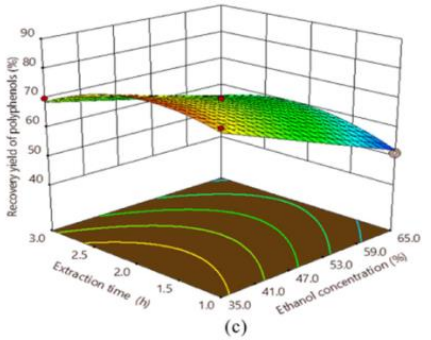
Determination of Optimum Conditions and Model Verification: From the model, the optimum conditions for polyphenolic extraction from ASP were obtained by using Design-Expert software was presented as in **Table 3**. Under optimum conditions, recovered yield of 82.5% polyphenols was predicted. The suitability of the model equation for predicting the optimum response value was tested by additional independent experiments (triplicate) using the recommended optimum conditions in Table 3. The result has shown the experimental recovery yield of polyphenols (83.1%) was not significantly difference from the predicted value (82.5%).



(a) Ethanol concentration and ratio of solvent to solid.



(b) Time extraction and ratio of solvent to solid.



(c) Time extraction and ethanol concentration.

Figure 2: Response surface plots for the effects of studied factors on recovery yield of polyphenols.

Fixed condition	Optimum condition			Recovered polyphenol yield (%)	
Temperature (°C)	Ethanol concentration (%)	Solvent to solid ratio	Extraction time (h)	Predicted value	Experimental value [a]
60	40	12	1.5	82.5	83.1 ± 0.3

Note: [a]Mean ± standard deviation of three replicate.

Table 3: Optimum conditions for extraction of polyphenols from ASP, predicted and experimental values from RMS.

Physicochemical Properties of Micro Particles

The polyphenol rich extract from ASP at optimum conditions as in Table 3 was used for preparing spray dried solution with different mixtures of MD and GA as coating agents. After spray drying, the results of physiochemical evaluation are shown as in Table 4.

As can be seen in **Table 4**, the moisture content of the micro particles ranges from 1.9% to 3.7% which decreases with the increment of the MD fraction, reaching the lowest value when only MD (100%) was used as coating agent, while the decreasing of the GA fraction rises WSI, from 87.3% to 99.8% when MD:GA ratio ranges from 0:100 to 100:0 due to solubility of GA in water is lower than that of MD. Although EE was almost unchanged at any MD:GA ratio but the total polyphenol content in the micro particle at 20:80 of MD:GA ratio had the highest value (149.5 mg GAE/g). RPY at all experimental points varies from 78.0% to 98.6%.

MD:GA	Moisture (%)	WSI (%)	TPC_m (mg GAE/g dw)	EE (%)	RPY (%)
0:100	3.7 ± 0.2^a	87.3 ± 1.3^a	119.9 ± 0.8^a	98.8 ± 0.8^a	78.0 ± 1.1^a
20:80	2.7 ± 0.1^b	88.0 ± 1.0^a	149.5 ± 1.2^b	99.1 ± 0.3^a	98.6 ± 1.0^b
40:60	2.3 ± 0.1^c	90.8 ± 1.6^b	132.0 ± 1.7^c	99.0 ± 0.2^a	87.4 ± 1.4^c
60:40	2.2 ± 0.3^c	92.7 ± 1.1^b	116.3 ± 0.9^d	98.8 ± 0.5^a	76.9 ± 1.3^d
80:20	2.0 ± 0.1^d	95.1 ± 1.3^c	122.6 ± 2.1^e	98.9 ± 0.9^a	86.5 ± 1.1^e
100:0	1.9 ± 0.2^d	99.8 ± 1.2^d	119.0 ± 1.9^f	98.8 ± 0.8^a	79.2 ± 1.1^f

Note: WSI: Water solubility index; TPC_m: Total polyphenol content of Micro particles: EE: Encapsulation efficiency; RPY: Recovered polyphenol yield. Different letters within a column are significantly different ($p<0.05$).

Table 4: The moisture contents, WSI, TPC_m, EE and RPY of spray dried polyphenols rich micro particles.

Discussion

The exploitation of waste from fruit and vegetable processing as a source of bioactive compounds is a promising field and it offers a new avenue for industrial growth and waste management. Extraction is the first and the most important step in the recovery and purification of bioactive compounds from plant materials and it was significantly influenced by many process factors such as solvent, temperature and solvent to solid ratio [32-34]. Therefore, optimizing the extraction process in order to improve recovery of added-value compounds represents a necessary technological innovation for the benefit of related industries.

Despite several disadvantages such low recovery yield and use of high solvent volumes but solvent extraction techniques have been mostly used for the recovery of polyphenolic compounds from plant materials due to their simple operation, wide range of applicability and low outlay [35]. Many solvents can be selected to extract these compounds such as ethyl acetate, acetone, propanol, ethanol, methanol and water but the selection of ethanol and water as extraction solvent throughout the study because they are safer for human consumption and less toxic as compared to other organic solvents [36].

Moreover, binary solvent system was found superior to the mono-solvent system due to the compositions as well as the structure and physicochemical properties of polyphenolic compounds from different plant sources [37]. For food and pharmaceutical industries, target compound recovery from plant materials by extraction is very significant when higher yield means lower production cost. In this study, the results obtained from analysis of experimental model have indicated that the effects of ethanol concentration and the solvent to solid ratio were the major contributing factors to the recovery yield of polyphenol from ASP as former reports and their effects depend on the polyphenol composition of the plant materials using for extraction [38-42]. Shi, et al. reported the polyphenol content extracted from grape seed increased when ethanol concentration decreased and the best the ethanol concentration obtained at 50% [43]. Polyphenol extraction was highly dependent on the solvent to solid ratio reported by Pompeu, et al and the ratio was at 40:1 (v/w) in extraction polyphenols from *Euterpe oleracea* fruits [44].

Our optimized extraction conditions were similar with Boyadzhieva, et al. wherein their conditions obtained by using one factor at a time experiments with results ethanol concentration of 30% (v/v), ratio of solvent to solid at 8 and extraction time of 60 min from the avocado seed material but the polyphenol recovery yield has not reported [45]. The recovery yield of polyphenols increased when ethanol concentration was decreased due to polyphenolic composition of ASP containing lots of polarity compounds such as procyanidins. Moreover, water increases the contact surface area between plant matrix and solvent, increasing the swelling capability of plant material which results in increasing extraction efficiency. Increase in the recovery yield of polyphenols under higher solvent to solid ratio is based on the mass transfer principles where the driving force for mass transfer is considered to be the concentration gradient between the solid and the solvent. At a lower ratio, the solvent can attain saturation state soon during extraction.

Polyphenolic compounds originate from plant have recently obtained a great attention due to their bioactive roles. However, they are sensitive and they can be easily affected by physicochemical factors that create a great challenge to incorporate them into the food products [19]. Hence, the encapsulation process becomes an effective strategy to overcome this problem. There are many different encapsulation methods for bioactive compounds but spray drying is an industrial and economical method which is commonly used to transform the liquid products into dry powders [46]. In this method, the sensitive compounds are covered within the carrier material, which leads to their protection against environmental disadvantages. Previous studies have revealed that the type and characterization of carrier materials influence on many properties of encapsulated micro particles [47-50]. Therefore, choosing the correct wall material is the important step to produce efficient encapsulated powders. MD and GA have been frequently used as carrier materials for encapsulation of plant polyphenols due to their high solubility, good biocompatibility, optimum viscosity and safety. Our results have shown that physicochemical properties of micro particles were influenced by the carrier type as well as their mixture ratio, which were similar to previous reports [51-52]. RPY and EE are the most important indicators which shows the efficiency of the spray dried process and they gained the best values at 20:80 of MD:GA ratio in this study (Table 4). Micro particles produced with GA presented higher moisture contents in compared with MD. This may be due to higher water holding capacity of GA than MD. This similar behavior also observed by Akhavan Mahdavi, et al. when studying microencapsulation of natural anthocyanin's from barberry fruits [53]. Solubility of micro particles (evaluated by WSI) is an important physiochemical property that influences functional

characteristics of micro particles in food system. Our data shown that high solubility of all samples has been noticed.

Conclusions

Avocado seeds can be used as a raw material to extract bioactive polyphenols. The RSM based on the BBD was successfully used to optimize process parameters for polyphenolic extraction from ASP. The optimum conditions in the polyphenolic extraction were using ethanol concentration of 40% (v/v), solid to solvent ratio at 1:12 and extraction time of 1.5 h. Under the conditions, the experimental yield of polyphenols is 83.1%. Besides, the characteristics of polyphenol rich micro particles were also determined. Micro particle prepared by 20:80 of MD:GA ratio as coating agent can be selected for encapsulation of polyphenolic compounds from ASP.

References

1. Giffoni J, Salles E, Aguiar R, Nogueira R, Costa J, et al. Chemical composition, toxicity and larvicidal and antifungal activities of *Persea americana* (avocado) seed extracts (2009) Rev Soc Bras Med Trop 42: 110-113. http://dx.doi.org/10.1590/S0037-86822009000200003
2. http://www.fao.org/faostat/en/#data/QC Accessed 19/11/2019.
3. Chau NM and Truyen VT. Avocado Production in Vietnam, in Avocado Production in Asia and the Pacific, Bruce A. Schaffer, B. Nigel (Ed) (2000) RAP Publication, Bangkok, 65-72.
4. Dreher ML and Davenport AJ. Hass avocado composition and potential health effects (2013) Crit Rev Food Sci Nutr 53: 738-750. http://dx.doi.org/10.1080/10408398.2011.556759
5. Duarte PF, Chaves MA, Borges CD and Mendonca CRB. Avocado: characteristics, health benefits and uses (2016) Cienc Rural 46: 747-754. http://dx.doi.org/10.1590/0103-8478cr20141516
6. Dabas D, Shegog RM, Ziegler GR and Lambert JD. Avocado (Persea americana) seed as a source of bioactive phytochemicals (2013) Curr Pharm Design 19: 6133-6140. http://dx.doi.org/10.2174/1381612811319340007
7. Rosero CJ, Cruz S, Osorio C and Hurtado N. Analysis of phenolic composition of byproducts (seeds and peels) of avocado (*Persea americana* Mill.) cultivated in Colombia (2019) Molecules 24: 3209-3226. http://dx.doi.org/10.3390/molecules24173209
8. Lee SG, Yu MH, Lee SP and Lee IS. Antioxidant activities and induction of apoptosis by methanol extracts from avocado (2008) J Korean Soc Food Sci Nutr 37: 269-275. https://doi.org/10.3746/jkfn.2008.37.3.269
9. Edem DO. Hypoglycemic effects of ethanolic extracts of alligator pear seed (*Persea americana* Mill.) in rats (2009) European J Sci Res 33: 669-678.
10. Kate IE and Lucky OO. Biochemical evaluation of the ethnomedicinal uses of the seeds of *Persea americana* Mill. (Family Lauraceae) (2009) World J Med Sci 4: 146-146.
11. Wang W, Bostic TR and Gu L. Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars (2010) Food Chem 122: 1193-1198. https://doi.org/10.1016/j.foodchem.2010.03.114
12. Kosíska A, Karamać M, Estrella I, Hernández T, Bartolomé B, et al. Phenolic compound profiles and antioxidant capacity of *Persea americana* Mill. Peels and seeds of two varieties (2012) J Agric Food Chem 60: 4613-4619. https://doi.org/10.1021/jf300090p
13. Segovia FJ, Hidalgo GI, Villasante J, Ramis X and Almajano MP. Avocado Seed: A Comparative Study of Antioxidant Content and Capacity in Protecting Oil Models from Oxidation (2018) Molecules 23: 2421-2434. https://doi.org/10.3390/molecules23102421
14. Widsten P, Cruz CD, Fletcher GC, Pajak MA and McGhie TK. Tannins and extracts of fruit byproducts: Antibacterial activity against foodborne bacteria and antioxidant capacity (2014) J Agric Food Chem 62: 11146-11156. https://doi.org/10.1021/jf503819t
15. Rodríguez-Carpena JG, Morcuende D, Andrade MJ, Kylli P and Estévez M. Avocado (*Persea americana* Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties (2011) J Agric Food Chem 59: 5625-5635. https://doi.org/10.1021/jf1048832
16. Araújo RG, Rodriguez-Jasso RM, Ruiz HA, Pintado MME and Aguilar CN. Avocado by-products: nutritional and functional properties (2018) Trends Food Sci Technol 80: 51-60. https://doi.org/10.1016/j.tifs.2018.07.027
17. Martin KR and Appel CL. Polyphenols as dietary supplements: A double-edged sword (2010) Nutr Diet Suppl 2: 1-12. https://doi.org/10.2147/NDS.S6422
18. Tsao R. Chemistry and biochemistry of dietary polyphenols (2010) Nutrients 2: 1231-1246.
19. Fang Z and Bhandari B. Encapsulation of polyphenols- a review (2010) Trends Food Sci Technol 21: 510-523. https://doi.org/10.1016/j.tifs.2010.08.003
20. Jeyakumari A, Zynudbeen AA and Parvathy U. Microencapsulation of bioactive food ingredients and controlled release-a review (2016) MOJFPT 2: 00059-00067. https://doi.org/10.15406/mojfpt.2016.02.00059
21. Desai KDH and Park HJ. Recent developments in microencapsulation of food ingredients (2005) Dry Technol 23: 1361-1394. https://doi.org/10.1081/DRT-200063478
22. Utrera M, Rodríguez-Carpena JG, Morcuende D and Estévez M. Formation of lysine-derived oxidation products and loss of tryptophan during processing of porcine patties with added avocado byproducts (2011) J Agric Food Chem 60: 3917-3926. https://doi.org/10.1021/jf3001313
23. Rodríguez-Carpena JG, Morcuende D and Estévez M. Avocado by-products as inhibitors of color deterioration and lipid and protein oxidation in raw porcine patties subjected to chilled storage (2011) Meat Sci 89: 166-173. https://doi.org/10.1016/j.meatsci.2011.04.013
24. Bezerra MA, Santelli RE, Oliveira EP, Villar LS and Escaleira LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry (2008) Talanta 76: 964-977. https://doi.org/10.1016/j.talanta.2008.05.019
25. Ferreira SLC, Bruns RE, Ferreira HS, Matos GD, David JM, et al. Box-Behnken design: an alternative for the optimization of analytical methods (2007) Anal Chim Acta 597: 179-186. https://doi.org/10.1016/j.aca.2007.07.011
26. Bas D and Boyaci IH. Modeling and optimization I: Usability of response resurfaces methodology (2007) J Food Eng 78: 836-845. https://doi.org/10.1016/j.jfoodeng.2005.11.024
27. Saénz C, Tapia S, Chávez J and Robert P. Microencapsulation by spray drying of bioactive compounds from cactus pear (Opuntia ficus-indica) (2009) Food Chem 114: 616-622. https://doi.org/10.1016/j.foodchem.2008.09.095
28. Anderson RA. Water absorption and solubility and amylograph characteristics of roll-cooked small grain products (1982) Cereal Chem 59: 265-269.
29. Zhong K and Wang Q. Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology (2010) Carbohydr Polym 80: 19-25. https://doi.org/10.1016/j.carbpol.2009.10.066
30. Lundstedt T, Seifert E, Abramo L, Nyström BTA, Pettersen J and Bergman R. Experimental design and optimization (1998) Chemometr Intell Lab 42: 3-40. https://doi.org/10.1016/s0169-7439(98)00065-3
31. Ravilumar K, Ramalingam S, Krishnan S and Balu K. Application of response surface methodology to optimize the

process variables for reactive red and acid brown dye removal using a novel adsorbent (2006) Dyes and Pigm 70: 18-26. https://doi.org/10.1016/j.dyepig.2005.02.004

32. Mota M, Pinto PCR, Novo G, Sousa G, Guerreiro O, et al. Extraction of polyphenolic compounds from Eucalyptus globulus bark: process optimization and screening for biological activity (2012) Ind Eng Chem Res 51: 6991-7000. https://doi.org/10.1021/ie300103z
33. Çam M and İçyer NC. Phenolics of pomegranate peels: extraction optimization by central composite design and alpha glucosidase inhibition potentials (2015) J Food Technol 52: 1489-1497. https://doi.org/10.1007/s13197-013-1148-y
34. Chan SW, Lee CY, Yap CF, Mustapha WAW and Ho CW. Optimisation of extraction conditions for phenolic compounds from limau purut (Citrus hystrix) peels (2009) Int Food Res J 16: 203-213.
35. Brglez Mojzer E, Knez Hrnčič M, Škerget M, Knez Ž and Bren U. Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects (2016) Molecules 21: 1-38. https://doi.org/10.3390/molecules21070901
36. Spigno G, Tramelli L and De Faveri DM. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics (2007) J Food Eng 81: 200-208. https://doi.org/10.1016/j.jfoodeng.2006.10.021
37. Thoo YY, Ho SK, Liang JY, Ho CW and Tan CP. Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (Morinda citrifolia) (2010) Food Chem 120: 290-295. https://doi.org/10.1016/j.foodchem.2009.09.064
38. Uysal S, Cvetanović A, Zengin G, Đurović S and Aktumsek A. Optimization of the extraction process of antioxidants from loquat leaves using response surface methodology (2017) J Food Process Preserv 41: 1-8. https://doi.org/10.1111/jfpp.13185
39. Chu B, Shi Y, Li Z, Tian H, Li W and Wang Y. Optimization of gentisides extraction from Gentiana rigescens Franch ex Hemsl by response surface methodology (2015) J Anal Methods Chem 2015: 1-8. https://doi.org/10.1016/j.foodchem.2015.08.008
40. Ćujić N, Šavikin K, Janković T, Pljevljakušić D, Zdunić G and Ibrić S. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique (2016) Food Chem 194: 135-142. https://doi.org/10.1016/j.foodchem.2015.08.008
41. Singh A, Kuila A, Yadav G and Banerjee R. Process optimization for the extraction of polyphenols from okara (2011) Food Technol Biotechnol 49: 322-328.
42. Prasad KN, Kong KW, Ramanan RN, Azlan A and Ismail A. Selection of experimental domain using two-level factorial design to determine extract yield, antioxidant capacity, phenolics, and flavonoids from Mangifera pajang Kosterm (2012) Sep Sci Technol 47: 2417-2423.
43. Shi J, Yu J, Pohorly J, Young JC, Bryan M and Wu Y. Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution (2003) JFAE 1: 42-47.
44. Pompeu DR, Silva EM and Rogez H. Optimisation of the solvent extraction of phenolic antioxidants from fruits of Euterpe oleracea using response surface methodology (2009) Biores Technol 100: 6076-6082. https://doi.org/10.1016/j.biortech.2009.03.083
45. Boyadzhieva SS, Georgieva SS and Angelov G. Optimization of the extraction of natural antioxidants from avocado seeds (2018) Bulg Chem Commun 50: 80-84.
46. Orsat V and Murugesan R. Spray drying for the production of nutraceutical ingredients-A review (2012) Food Bioprocess Technol 5: 3-14. https://doi.org/10.1007/s11947-011-0638-z
47. Boonchu T and Utama-Ang N. Optimization of extraction and microencapsulation of bioactive compounds from red grape (Vitis vinifera L.) pomace (2015) J Food Sci Technol 52: 783-792. https://doi.org/10.1007/s13197-013-1079-7
48. Akhavan Mahdavi S, Jafari SM, Assadpoor E and Dehnad D. Microencapsulation optimization of natural anthocyanins with maltodextrin, gum arabic and gelatin (2016) Int J Biol Macromol 85: 379-385. https://doi.org/10.1016/j.ijbiomac.2016.01.011
49. Saénz C, Tapia S, Chávez J and Robert P. Microencapsulation by spray drying of bioactive compounds from cactus pear (Opuntia ficus-indica) (2009) Food Chem 114: 616-622. https://doi.org/10.1016/j.foodchem.2008.09.095
50. Kalušević A, Lević S, Čalija B, Pantić M, Belović M, et al. Microencapsulation of anthocyanin-rich black soybean coat extract by spray drying using maltodextrin, gum Arabic and skimmed milk powder (2017) J Microencapsul 34: 475-487. https://doi.org/10.1080/02652048.2017.1354939
51. Ballesteros LF, Ramirez MJ, Orrego CE, Teixeira JA and Mussatto SI. Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials (2017) Food Chem 237: 623-631. https://doi.org/10.1016/j.foodchem.2017.05.142
52. Cilek B, Luca A, Hasirci V, Sahin S and Sumnu G. Microencapsulation of phenolic compounds extracted from sour cherry pomace: effect of formulation, ultrasonication time and core to coating ratio (2012) Eur Food Res Technol 135: 587-596. https://doi.org/10.1007/s00217-012-1786-8
53. Akhavan Mahdavi S, Jafari SM, Assadpoor E and Dehnad D. Microencapsulation optimization of natural anthocyanins with maltodextrin, gum arabic and gelatin (2016) Int J Biol Macromol 85: 379-385. https://doi.org/10.1016/j.ijbiomac.2016.01.011