Optimization of conventional and ultrasound assisted extraction of inulin from gembili tubers (*Dioscorea esculenta* L.) using response surface methodology (RSM)

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Optimization of conventional and ultrasound assisted extraction of inulin from gembili tubers *(Dioscorea esculenta* L.*)* using response surface methodology (RSM)

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Abstract. This study aim is to optimize the condition of conventional and ultrasound assisted extraction of inulin from Gembili (*Dioscoreaesculenta* L.). Optimization was performed using Response Surface Methodology (RSM). Inulin determination was done using UV-Vis spectrophotometry method which has validated on the preliminary study. RSM designed applied Central Composite Design (CCD) 3^3 using three-factor-three-level. Variables optimized were extraction time (X1), solvent to sample (water at temperature of 50°C) ratio (v/w) (X2) and extraction batch (X3). The quadratic model well fitted for response in both conventional and ultrasound assisted extraction method. Conventional method revealed higher inulin concentration on optimal extraction condition than ultrasound assisted extraction. The optimal extraction conditions of conventional method were achieved at the extraction time of 14.4 minutes; sample to solvent ratio of 1:18.18 (w/v); and extraction batch of 2.9. Furthermore, optimal extract contained inulin of 23.21% (w/w).

1. Introduction

Inulin is a natural polymer that can be classified as oligo- or polysaccharide based on its chain length. Inulin is also classified as fructants in carbohydrate groups [1]. Inulin consists of a D-fructose monomer that is linked to a β bond (2→1) and ends with a D-glucose residue associated with D-fructose via an α bond (1→2) (figure 1) [2]. As a functional food ingredient, inulin is widely used as a substitute for sugar and fat which has low calorific value. Some inulin physiological functions that are important for body health include reducing cholesterol and blood serum triglycerides, reducing the risk of colon cancer, maintaining blood sugar levels and maintaining the balance of intestinal microflora population [3].

Inulin is detected in several types of natural sources for example chicory roots, Jerusalem artichoke, dahliatubers, garlic wheat, onion [4] and tubers [5], one of which is gembili (*Dioscoreaesculenta* L.). Indonesia produces a lot of gembili tubers. Based on research that has been done on several tubers in Indonesia, gembili contains highest inulin. Gembili tuber contains inulinof 14.77% [5].

Inulin is attained from plant sources by extraction. Inulin extraction from Jerusalem artichoke by indirect sonication on neutral pH extraction conditions, extraction time of 20 minutes, temperature of 76.65°C, and solvent ratio: solid, 10.56:1 (v/w) gives optimal results [6]. The use of high intensity

ultrasonic can increase the results of inulin extraction from Burdock root (*Arctiumlappa*) using optimal conditions of extraction time of 25 minutes, amplitude of sonication 83.22%, and temperature of 36.76°C. Furthermore, the inulin precipitation of Jerusalem artichoke can be increased by the addition of ethanol with an ethanol / solution ratio of 13 (v/v) at 42.00°C [7]. In another study, inulin extraction from Jerusalem arthicoke tuber using hot water solvents without the use of ultrasonic gave better results. The extraction condition carried out is a solid ratio: solvent of 1:16 (w/v) and temperature of 76.00°C for 90 minutes [8]. Furthermore, research into a new model of inulin extraction from Globe artichoke heart (*Cynaracardunculus* L. subsp. *Scolymus* (L.) Hegi.) Using electromagnetic induction heating (EMIH) provides high purity inulin results. The operational conditions used were 89.49°C, extraction time of 120 min, and the mass ratio in the EMIH process of 5.01% [9].

Study on optimization of inulin extraction from Gembilitubers (*Dioscoreaesculenta L.*) by comparing conventional and ultrasonic methods has never been done. Optimization using the *Response Surface Methodology* (RSM) design is very effective in determining the optimal conditions of inulin extraction [6, 9]. RSM design could optimize several variables into a model that can determine both optimum condition and targeted condition. Extraction batch parameters have never been carried out in other studies on optimization of inulin extraction. In this study, extraction parameters were optimized in terms of solid ratio: solvent (w/v), extraction time and extraction batch. Therefore, this study conducted an optimization study of inulin extraction from Gembilitubers using two extraction methods, conventional and ultrasonic assisted extractionusing RSM optimization design.

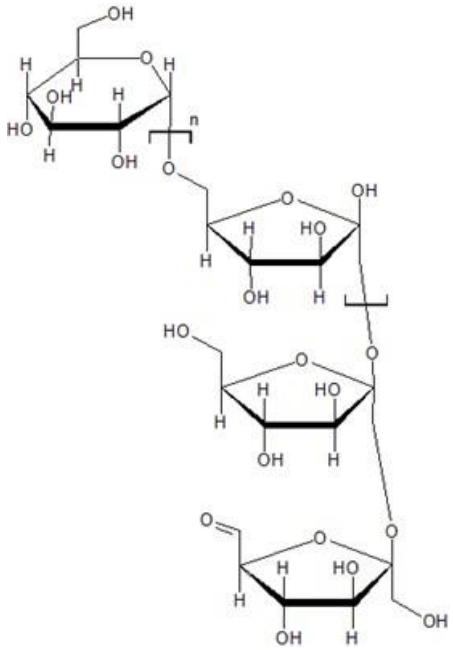

Figure 1. Inulin structure.

2. Materials and Methods

2.1. Materials

The material used in this study is gembili tubers obtained from Bringin area, Semarang Regency, Central Java, Indonesia in harvest period. The chemicals used are standard of inulin (Sigma-Aldrich, Germany) and vanillin (Merck, Germany). Reagents used are ethanol (pro analysis) and sulphuric acid (pro analysis) purchased from E-Merck, Germany.

2.2. Sample preparation

The gembili tubers, which have been cleaned and peeled, are then washed and cut into smaller pieces. Subsequent, it was dried at 50°C in the drying cabinet and then powdered using a grinder and sieved with 61 mesh particle size.

2.3. Optimization of extraction

Optimization of inulin extraction was carried out using two methods, maceration (conventional method) and ultrasonication. Both methods were using water as solvent at temperature of 50°C. Each extraction method was optimized with variation of extraction time (3.2-36.8 minutes), sample: solvent ratio (1:6.4-1:73.6, w/v), and batch (1-5 batches). Extraction batches are carried out by filtering the extract solution then accumulating the filtrate and re-extracting residue by adding the solvent. Extraction is then carried out again according to the optimized variations. In the conventional method, the sample was added with a solvent and macerated with stirring using a magnetic stirrer on a hot plate stirrer (RLABINCO L-81 model). Extraction of the ultrasonication method was carried out using sonicator (Yes Xun YX-2120) at a frequency of 40 kHz. The inulin content in the extract was determined using spectrophotometric methods

2.4. Inulin precipitation

The most optimal extraction results between the two extraction methods used are then precipitated using 95% ethanol with a ratio of extract: ethanol, 1:2 (v/v). The separation between the filtrate and precipitate is carried out by using a centrifuge at a 3000 rpm for 10 minutes. The precipitate obtained is dried at 50°C for one night. Furthermore, the inulin level is determined using spectrophotometry based on external inulin standard curved.

2.5. Determination of inulin concentration

Determination of the inulin concentration is carried out by preparing a sample solution in distilled water. Then, the sample solution was added with vanillin reagent at a ratio of 1:1 (v/v) and incubated for 15 minutes. Additionally, the absorbance of the solution was measured using a UV-Vis spectrophotometer (Shimadzu 1240) at a maximum wavelength of 520 nm. The inulin concentration was determined based on the standard curve between A_{520} and inulin concentration.

2.6. Characterization of functional groups in inulin precipitate

The result of inulin precipitation then characterized its functional group using Fourier Transform-Infrared spectroscopy (FTIRS) at a wave number of 4000–400 cm^{-1}.

2.7. Experimental design and data analysis

Extraction optimization was carried out using the Response Surface Methodology (RSM). Optimization design uses 3^3 central composite design (CCD) models with 3 variables and three factor levels. As variables are extraction time (X_1), ratio of sample: solvent (w/v) (X_2), and extraction batch (X_3). The table of independent variables and the level code factors used in optimization are presented in table 1.

Table 1. Independent variables and coded level for central composite design CCD.

Independent variable	Symbol	Coded levels				
		-1.68	-1	0	1	1.68
Extraction time (minutes)	X_1	3.2	10	20	30	36.8
solvent:solid ratio (v/w)	X_2	6.4	20	40	60	73.6
Extraction batch (times)	X_3	0.32	1	2	3	3.68

The second order polynomial modelling analysis in this study is based on the following mathematical equations [1]:

$$\gamma = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{k=1} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j + \sum_{i=1}^{3} \beta_{ii} x_i^2 \quad (1)$$

Where *y* is the response variable, *b* is the regression coefficients, and *x* is the coded level of the independent variable. Data obtained was analysed using ANOVA.

3. Results and Discussion

3.1. Optimization of inulin extraction

Inulin is successfully determined by spectrophotometric method using vanillin or resorcinol reagent. Sulphuric acid contained in vanillin reagent acts to hydrolyse inulin into simple fragment or monomer. Beside, sulphuric acid also acts as modifier agent to condense chromogen and monomer of inulin reaction occurred. This complex reaction produces chromophore that absorb visible light at 520 nm [10, 11]. Inulin concentration in extracts was determined by plotting the results of absorbance on the standard curve.

Table 2. Actual and predicted results from central composite design (CCD).

Time (X_1) (minutes)	Solvent: solid ratio (X_2) (v/w)	Batch extraction (X_3) (times)	Inulin concentration (ppm)			
			Conventional		Ultrasonic	
			actual	predicted	Actual	predicted
36.80	40	2	2647.91	2414.55	1316.00	460.73
10.00	20	3	5604.89	7111.40	5876.00	6871.37
20.00	40	2	3201.40	3318.67	649.33	705.57
20.00	40	2	3144.03	3318.67	582.67	705.57
10.00	60	1	1126.98	1139.20	1004.89	416.74
30.00	20	3	6457.21	7332.15	4942.67	6353.59
30.00	60	1	3182.79	2563.44	578.22	405.63
10.00	60	3	2103.72	1859.28	1524.89	1002.40
30.00	20	1	4466.51	5598.11	3600.44	4945.71
30.00	60	3	936.28	660.85	600.44	122.34
20.00	40	2	3782.79	3318.67	653.78	705.57
03.20	40	2	2052.56	1031.28	1213.78	905.47
20.00	40	2	2956.43	3318.67	796.00	705.57
20.00	40	2	320.,4	3318.67	836.00	705.57
20.00	40	2	3410.7	3318.67	516.00	705.57
20.00	40	0.32	1615.35	1039.92	778.22	53.81
20.00	6,4	2	1287.20	10518.71	14267.1	11659.57
20.00	73.6	2	2452.56	3550.37	1462.67	2906.63
20.00	40	3.68	3782.79	3103.58	2169.33	1730.16
10.00	20	1	1592.09	2754.68	3293.67	4594.55

In this study, inulin extraction from gembili tuber was carried out using maceration and ultrasonication methods. Each extraction method was carried out using distilled water at 50°C. Water was chosen as a solvent in this study because inulin can dissolve in water as temperature increases [8]. The results of the concentration of inulin extract from gembili tuber obtained by maceration and ultrasonication methods can be seen in table 2.

The appropriate model for this research is the Quadratic model. A model is fitted and can be used if the value of P <0.05 so that it is said to be significant and has an influence. Whereas the value in the lack of fit shown is should not significant with a value of P >0.05. This insignificant lack of fit value indicates that the actual data of this study does not have significant differences with predictive data obtained from modelling.

Optimization of conventional extraction revealed significant model (P <0.05) and not significant *lack of fit* value with R^2 of 0.9786. Based on ANOVA test (table 3) it can be seen that the inulin extraction obtained by conventional method is influenced by time factor, ratio, batch, interaction between time and batch, and interaction between ratio and batch. The interaction between time and ratio is not significant because the value of P >0.05. Time and ratio interaction does not have an influence on inulin extraction by this conventional method. These results prove that batch extraction is one of the optimal parameters for extraction conditions of conventional methods. Extraction batches can extract more inulin compared to once period extraction. In contrast, time and ratio have no effect on conventional method extraction. This is because the volume of the solvent has reached the maximum extraction capacity and no longer extracts the inulin even though the time increases.

Mathematical equation resulting from the analysis of second-order polynomial modelling on conventional methods is:

$$Y = -4265{,}14743 + 329{,}90707X_1 - 996{,}36658X_2 + 3223{,}97111X_3 + 17{,}04730X_1X_2 - 83{,}99921X_1X_3 + 1093{,}48048X_2X_3 - 3{,}28953X_1^2 + 618{,}59931X_2^2 - 205{,}62032X_3^2 \quad (2)$$

Table 3. ANOVA of conventional extraction optimization model

Source	Df	Sum of square	Mean of square	F value	P value	Info
Model	9	2.86×10^7	3.18×10^6	40.60	<0.0001	Significant
Lack of Fit	3	2.21×10^5	73607.48	0.91	0.4997	Not significant

Optimization of ultrasonic extraction revealed significant model (P <0.05) and not significant lack of fit value with R^2 of 0.9926. ANOVA assay performed that the inulin extraction using ultrasonication method is influenced by the ratio factor, batch, interaction between time & ratio, and the interaction between ratio &batch. For time factor and interaction between time &batch do not have an effect on inulin extraction by ultrasonication method (P >0.05) (table 4). The interaction of time and ratio influence the extraction. These results indicate that ultrasonic waves affect the extraction time at difference ratios. These results are consistent with Lingyun *et al.* [6] which shows that the addition of ultrasonic waves can make extraction more efficient. In addition, the extraction batch is also an important parameter in the extraction optimization using the ultrasonic method shown from the ANOVA results to have a significant good value including its interaction with the time and ratio factor.

Table 4. ANOVA of ultrasonic extraction optimization model.

Source	Df	Sum of square	Mean of square	F value	P value	Information
Model	9	$2{,}55\times10^7$	$2{,}83\times10^6$	117,92	<0,0001	Significant
Lack of Fit	3	$1{,}22\times10^5$	40705,53	3,53	0,1273	Not significant

Mathematical equation resulting from the analysis of second-order polynomial modelling on ultrasonic methods is:

$$Y = +1822{,}17275 - 83{,}63914X_1 + 591{,}74661X_2 - 682{,}75936X_3 + 24{,}55407X_1X_2 - 6{,}22649X_1X_3 + 267{,}82044X_2X_3 + 2{,}29870X_1^2 + 1281{,}39711X_2^2 + 303{,}72291X_3^2 \quad (3)$$

Surface plot of both extraction methods influenced by interaction of solvent: solid ratio batch extraction is shown in figure 2.

Model validation is performed by comparing the actual values and predictions of the 5 optimal treatment combinations based on the highest desirability values of each method. Deviation between actual and predictive values is expressed as percent of root mean square error prediction (% RSEP). The treatment combination was used to determine the optimal extraction conditions (table 5). Through the calculation of the actual value and% RSEP, the most optimal extraction conditions were extraction by conventional method. Optimal conditions achieved were combination of extraction time of 14.4 minutes; solvent: solid ratio 18,18 (v/w); and batch extraction of 2.9 which were revealed highest inulin content in extract of 6907.11 ppm.

Conventional extraction methods provide better results compared to ultrasonic extraction methods. Inulin levels contained in extracts produced by conventional extraction methods are higher than ultrasound extraction. On the other hand, conventional extraction optimization models achieved with RSM provide lower RSEP values. This result is in line with the research of Rubel *et al.* [8] which states that the inulin extraction from Jerusalem Artichoke Tuber using water solvents without the addition of ultrasonic give better results than the addition of ultrasonic. Ultrasonic waves can break the degree of polymerization of inulin [8].

Table 5. Validation of optimal extraction using conventional and ultrasonic method.

Conventional method					
Time (minutes)	Solvent: solid ratio	Batch (times)	Predicted (ppm)	Actual (ppm)	% RSEP
27.1	19.96	2.8	6459.37	6209.33 6538.22	2.91
11.8	19.72	2.7	6469.62	5609.33 4889.33	11.56
10.0	20.00	3.0	7079.47	4991.56 4991.56	41.83
14.4	18.18	2.9	6589.35	6907.11 6662.67	3.39
27.7	19.84	2.9	6485.46	6511.56 5849.33	7.27
Ultrasonic method					
Time (minutes)	Solvent: solid ratio	Batch (times)	Predicted (ppm)	Actual (ppm)	% RSEP
30	20.0	3	4919.56	5129.33 4609.33	5.43
29.8	19.98	3	4899.97	3929.33 4862.67	15.54
29.3	20.0	3	4879.71	3538.22 4062.67	29.16
30.0	20.0	2.9	4874.50	5489.33 4698.22	8.85
30.0	19.76	3	4863.25	4391.56 4151.56	14.13

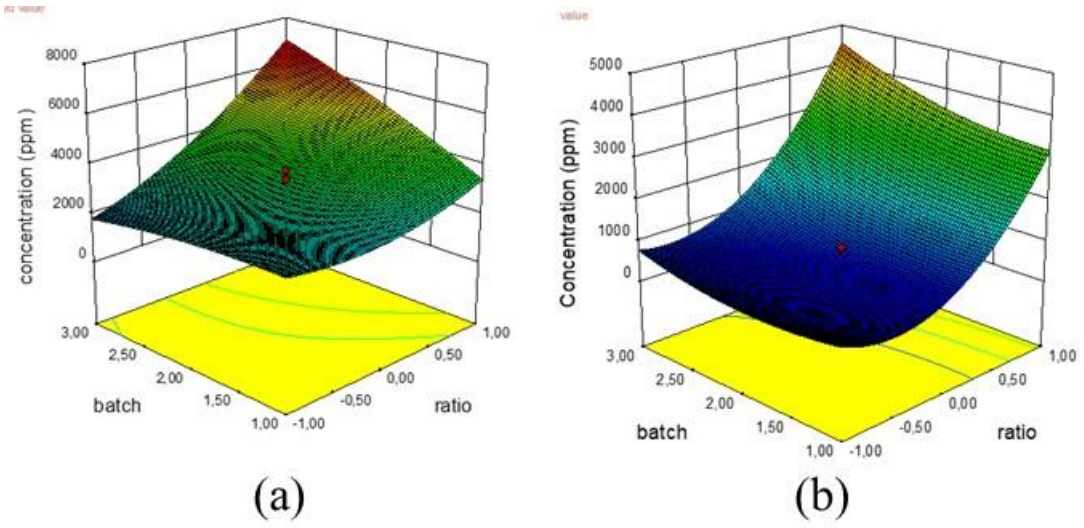

Figure 2. The response surface plot of inulin extraction yield as a function of solvent: solid ratio (X_2) and batch (X_3) for (a) conventional and (b) ultrasonic extraction.

3.2. Precipitation inulin from optimal extract using ethanol

Addition of ethanol to a water extract containing inulin can precipitate inulin [7]. The yield obtained from precipitation process was 3.44%. Inulin concentration in precipitate was 23.21%. The inulin content in this precipitate is higher than that of Winarti *et al.* [5] which stated that inulin concentration in gembili tubers were 14.4%. Extraction method developed is proven to be able to extract inulin more optimally.

3.3. Inulin Functional Groups Characterization

Characterization using FT-IR (figure 3) revealed peaks at some specific wave number that can be used for identification of inulin. These typical absorption bands are vibration stretching O-H at wave number 3418.97 cm^{-1}, vibration of the C-O-C stretching ring at wave number 1146.73 cm^{-1}, residual α-D-Glcp in the carbohydrate chain at wave number 922.98 cm^{-1} followed by 2-ketofuranose at wave numbers 892.12 and 832.32 cm^{-1} [2]. Peak of 1700 cm^{-1} is a specific character which indicates valence vibrations of carbonyl group in carbohydrate [12].

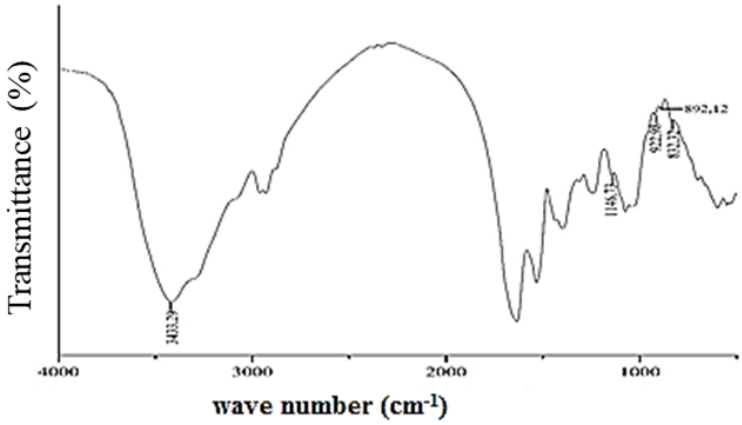

Figure 3. FTIR spectra of inulin precipitate.

4. Conclusion

Response Surface Methodology is very useful for optimizing extraction both conventional and ultrasonic. Conventional extraction generated in higher inulin concentration than ultrasonic extraction. Optimal extraction conditions were obtained by conventional extraction method. Optimal conditions are a combination of 14.4 minutes' extraction time treatment; solvent: solid ratio of 18.18 (v/w); and 2.9 batches extraction. The inulin concentration of precipitation using ethanol obtained is 23.21% per gram extract.

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