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Optimum Conditions for the Extraction of Effective Substances from the Stem of *Opuntia fiscus-indica*

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Abstract The conditions for the extraction of effective substances from the stem of *Opuntia ficus-indica* were optimized by a response surface methodology. The total extract yield was maximized under the temperature of 97.77°C, at a time duration of 145.82 min and a water to sample ratio 16.59 mL/g. Moreover, the optimum conditions for the extraction of effective substances were as follows: 84.95°C, 156.50 min and a water/sample ratio of 7.46 mL/g for the phenolics content; and 97.11°C, 139.03 min and a water/sample ratio of 10.91 mL/g for the pectin content. The range of optimum extraction conditions in consideration of the physicochemical properties of the extracts were shown to be as 95-100°C as the extraction temperature, 120-180 min as extraction time and a water to sample ratio of 5-18 mL/g.

Keywords: effective substances, extraction, optimization, *Opuntia ficus-indica*, RSM

Introduction

Opuntia ficus-indica is a perennial plant derived from the tropics and belongs to the genus, *Opuntia*. It is a type of cacti that are cultivated or naturally grown on Jeju Island, Korea (1). Consuming the juice of the fruit or stem of *Opuntia ficus-indica* in an empty stomach was reported to be efficacious for constipation treatment, urination, intestinal movements activity and in improving appetite (2, 3). Moreover, the medicinal purpose of the cactus stem was known orally to be effective for the treatment of cutaneous disorder, rheumatism and burns. In the field of oriental medicine, the cactus stem has been known to treat neuralgia, mastitis and dysentery, and can be used as a nutrient, tonic, sedative, and as antipyretic and anti-inflammatory agent. The stem can also be used in detoxification, blood clarification and bloods flux treatment (4-6).

Korean scientists working on *Opuntia ficus-indica* have reported its beneficial effect on stressful gastric ulcer in rats (7), the antioxidant effect of fruit on thermostability of red pigment (8), the thermal stability of prickly pear for red pigment (9), comparative results between the substances in *Opuntia ficus-indica* and aloe (10), the antioxidative and antibacterial activities of *Opuntia ficus-indica* (3) and biological activities of the extract from the fruit and stem of *Opuntia ficus-indica* (6).

The fruit of *Opuntia ficus-indica* has been used as either a raw or a processed product after being commercially cultivated in South Africa. Korea has been trying to cultivate the cactus and to develop it as a processed product from Jeju Island. It will be feasible to cultivate the cactus as it can be easily cultivated in any type of soil under a high annual mean temperature such as those prevalent in Jeju Island. In particular, *Opuntia ficus-indica* is being cultivated in the Asan city of Chungcheongnam-do, Korea; where there are particular weather patterns which are not detrimental for the survival of the cactus even in the wintertime, and hence it is known by a general name of *Cheonnyuncho*. Therefore, *Opuntia ficus-indica* is a high-value product that can be broadly developed into a variety of processed products.

In the present study, we have monitored the extraction characteristics of the effective substances from the stem of *Opuntia ficus-indica*, through a response surface methodology so as to optimize the extraction conditions.

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Materials and Methods

Materials The stem samples of *Opuntia ficus-indica* (called *Cheonnyuncho*) produced in 2002 were taken from Asan city of Chungcheongnam-do, and were stored in a freezer at -20°C. The general composition of the raw sample was analyzed by the AOAC method (11) and is shown in Table 1. Each result indicates the mean value of thrice repeated experiments. The proximate composition of the sample was determined by the mean value acquired through the triplicate analysis.

Extraction methods To design an experiment for establishing proper extraction conditions, 10 g sample was hydrolyzed and extracted in a reflux extraction apparatus by differentiating the extraction temperature and time. Water was used as an extraction solvent because water is better than alcohol for extraction of total extracts and pectin at low concentrations. The so-obtained extract was quantified (300 mL), centrifuged at 3000 rpm for 20 min and was used for estimating quality factor. The sample was extracted three times.

Experiment design for establishing extraction conditions In the present experiment, the response surface methodology (RSM) (12) was used to predict the optimum

Table 1. Proximate composition of *Opuntia ficus-indica* stem

Composition	Content (%)
Moisture	94.00
Crude protein	0.46
Crude lipid	0.14
Crude ash	0.94
Crude fiber	0.53
Nitrogen free extract	3.93

extraction conditions and to monitor the physicochemical properties of the extract. The experimental design for the optimization of the extraction conditions was based on the central composite design (13), and the response surface analysis was conducted by using a SAS (statistical analysis system) program (14). With respect to the experiment on independent variables (X_i) by the central composite design, Table 2 shows the factors considered as principal variables in the extracting processes, like, extraction temperature (X_1), extraction time (X_2) and the water to sample ratio (X_3). The parameters were encoded into five levels as -2, -1, 0, 1, 2. Moreover, the dependent variables (Y_n) that were affected by the factor variables include; extract yield (Y_1), phenolics content (Y_2) and pectin content (Y_3). These variables were gathered from repeated experiments and the mean values were used for regression analysis. The extraction properties of the variables and the extracting conditions were shown by a 4-dimensional response surface using a Mathematica program (15).

Determination of extract yield For estimating the extract yield by each condition, a test solution of 20 mL was transferred into a weighing bottle and dried at 105°C to a constant weight. Subsequently, the weight was measured and is shown as percentage content for the solid matters in the extract (16).

Determination of phenolics content The content of phenolics in each extract was determined by following the Folin-Denis' method (17, 18). To a 5 mL aliquot of diluted extract, 5 mL of the Folin-Ciocalteu agent was added and after 3 min 5 mL of 10% Na_2CO_3 was added. The mixture was shaken and incuabted at room temperature for 1 h in order to measure the absorbance at 700 nm (UV-1601PC spectrophotometer, Shimadzu Co., Japan). For a control group, the sample solution was equally treated, except that distilled water was used instead of the extract. The reference curve was drawn by using tannic acid (Sigma Co., USA) as a standard (concentration range of 0.5-5 mg %).

Determination of pectin content The pectin content of each extract was quantified by the carbazole-sulfuric acid method (19, 20). To 0.1 mL of extract, 6 mL of concentrated sulfuric acid was added and heated in boiling water for 10 min and then cooled. The cooled mixture was well mixed with 0.5 mL of 0.15% carbazole reagent and 25 min of incubation, the absorbance was measured at 525 nm (UV-1601PC spectrophotometer, Shimadzu Co., Japan). The reference curve was drawn by using anhydrogalacturonic acid as a standard (concentration range of 1-10 mg%).

Prediction and experimental verification for optimum extraction conditions The range of optimum extraction conditions for the stem of *Opuntia ficus-indica*, were predicted by superimposing the 4-dimensional response surface for the extract yield, phenolics content and pectin content which are regarded as the principal quality factors of the sample extract. Moreover, the optional points were set up within the predicted ranges and were applied to the regression equations. Subsequently, the reliability was tested by comparing the predicted optimum values with the values obtained through the actual tests done under the similar conditions (21).

Results and Discussion

Total extract yield The total extract yield of the *Opuntia ficus-indica* stem by each of the extraction condition, based on the central composite design is shown in Table 2. Table 3 demonstrates the response surface regression analysis of the results. The R^2 of the regression equation for the extract yield of the *Opuntia ficus-indica* stem was 0.8994 and the level of significance was at $p<0.05$. Since a predicted stationary point was the saddle point, we performed a ridge analysis and obtained the predicted extract yield of 6.40% under the optimum extraction conditions oftemperature 97.77°C, extraction time 145.82 min and a water to sample ratio as 16.59 mL/g (Table 4). During reviewing the 4-dimensional response surface for the changes of the extract yield by extraction conditions, the higher extraction temperature influenced the increase of the yield (Fig. 1). The most influential extraction condition on the extract yield, was the extraction temperature, as confirmed in Table 5, while the least influential condition was extraction time. Jwa *et al.* (22) reported that the extract yield of the *Acanthopanax koreanum* increased

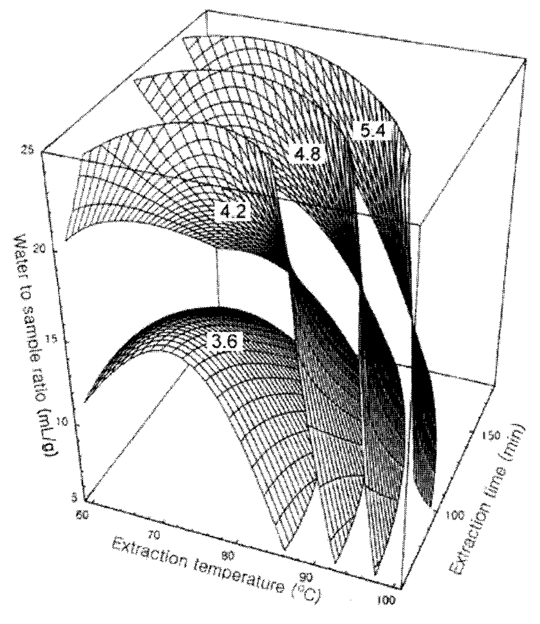

Fig. 1. Response surfaces for extract yield from *Opuntia ficus-indica* stem at constant values (extraction yield: 3.6-4.2-4.8-5.4 %) as a function of extraction temperature, extraction time and water to sample ratio.

Table 2. Experimental data on extract yield, phenolics content and pectin content in *Opuntia ficus-indica* stem extract under different conditions based on central composite design for response surface analysis

Exp. No.[1)]	Extraction conditions			Physiochemical properties		
	Extraction temperature (°C)	Extraction time (min)	Water to sample ratio (mL/g)	Extract yield (%)	Phenolics content (mg%)	Pectin content (mg%)
1	70(-1)	90(-1)	10(-1)	3.60	120.60	584.10
2	70(-1)	90(-1)	20(1)	3.90	124.80	667.50
3	70(-1)	150(1)	10(-1)	3.90	146.10	653.10
4	70(-1)	150(1)	20(1)	5.10	126.00	710.70
5	90(1)	90(-1)	10(-1)	3.90	128.10	987.30
6	90(1)	90(-1)	20(1)	4.50	171.30	822.00
7	90(1)	150(1)	10(-1)	5.10	201.30	1040.70
8	90(1)	150(1)	20(1)	5.40	159.60	789.60
9	80(1)	120(0)	15(0)	4.20	152.10	884.10
10	80(1)	120(0)	15(0)	4.20	147.60	946.20
11	60(-2)	120(0)	15(0)	3.60	96.90	389.70
12	100(2)	120(0)	15(0)	6.60	152.70	1234.20
13	80(0)	60(-2)	15(0)	3.90	81.30	514.20
14	80(0)	180(2)	15(0)	5.10	179.70	1133.10
15	80(0)	120(0)	5(-2)	3.30	185.40	740.70
16	80(0)	120(0)	25(2)	4.80	155.40	598.50

[1)]The number of experimental conditions by central composite design.

Table 3. Polynomial equations calculated by RSM program for extraction conditions of extract from *Opuntia ficus-indica* stem

Response	Second order polynomials[1)]	R^2	Significance
Extract yield	$Y_{Ey}=14.231250-0.315000X_1-0.035000X_2+0.172500X_3$ $+0.002250X_1^2+0.000250X_1X_2+0.000083333X_2^2$ $-0.001500X_1X_3+0.000500X_2X_3-0.001500X_3^2$	0.8994	0.0208
Phenolics content	$Y_{Pc}=-437.343750+9.217500X_1+2.088750X_2+0.345000X_3$ $-0.062625X_1^2+0.014500X_1X_2-0.005375X_2^2$ $+0.043500X_1X_3-0.091000X_2X_3+0.205500X_3^2$	0.9049	0.0179
Pectin content	$Y_{Pc}=-5464.931250+83.700000X_1+13.39125X_2+189.307500X_3$ $-0.258000X_1^2-0.038000X_1X_2-0.025417X_2^2$ $-1.393500X_1X_3-0.093000X_2X_3-2.455500X_3^2$	0.8659	0.0447

[1)]X_1: extraction temperature (°C), X_2: extraction time (min), X_3: solvent to sample ratio (mL/g)

Table 4. Predicted levels of extraction conditions for the maximum response of variables by the ridge analysis of *Opuntia ficus-indica* stem

Responses	Extraction temperature (°C)	Extraction time (min)	Water to sample ratio (mL/g)	Maximum	Morphology
Extract yield (%)	97.77	145.82	16.59	6.40	Saddle point
Phenolics content (mg%)	84.95	156.50	7.46	217.11	Saddle point
Pectin content (mg%)	97.11	139.03	10.91	1243.27	Maximum

Table 5. Regression analysis for regression model of physiochemical properties in extraction conditions of the extract from *Opuntia ficus-indica* stem

Extraction condition	F-Ratio		
	Extraction temperature	Extraction time	Water to sample ratio
Extract yield	7.19**	3.29*	2.62
Phenolics content	5.16**	7.38**	2.39
Pectin content	7.06**	1.78	1.66

*Significant at 10% level
**Significant at 5% level
***Significant at 1% level

with the higher temperature, which was verified to be similar to the results obtained in the presentexperiment. In addition, Lee & Hwang (23) measured the yield of *Agastache rugosa* according to the extraction conditions, and reported that the extract yield increased as the temperature increased in the hot-water extracting process, and the absolute quantity of the solid produced in the extracting process increased with an increase in the solvent amount. The solid amount, however, was shown to increase with an increase in the ratio of the solvent to sample in the case of *Rubi fructus* (24), which was a different trend from the result of the present experiment.

Phenolics content Phenolic compounds are known to be widely spread in various plants, and are generally water-soluble. These compounds are mainly flavonoids, and also include phenols, phenolic acids, phenyl propanoids and phenolic quinones. All of these kinds of compounds have been reported to have excellent antioxidative effects (25, 26). Table 2 shows the content of phenolics of each extract of *Opuntia ficus-indica* in order to obtain the extract containing qualitative phenolics. The response surface regression equation for the phenolics content according to extraction conditions, is demonstrated in Table 3, and the R^2 of the regression equation was 0.9049, which was within the 5% of level of significance. The stationary point for the responses was a saddle point. From the ridge analysis on the saddle point in the response surface, the optimum conditions of the three variables were shown to be; 84.95°C, 156.50 min and 7.46 mL/g as a water to sample ratio (Table 4). The predicted phenolics content in optimum conditions was 217.11 mg%. As shown in Fig. 2, the changes in the phenolics content by extraction conditions were demonstrated to be similar to the extraction characteristics related to the extract yield. The phenolics content increased with an increase in the extraction temperature and time. The phenolics content were affected by the temperature and time of the extraction conditions, but were not significantly affected by the water to sample ratio (Table 5). Park *et al.* (27) reported that the phenolics content increased with an increase in the ratio of solvent to sample rather than by the extraction time in the ethanolic extraction of *Chrysanthemum boreale*, which was significantly different from the results of the present experiment.

Pectin content Dietary fiber exists as a complex carbohydrate form in plant foods such as fruits, vegetables, beans and grains. Moreover, the dietary fiber's function is to clean the intestines by the process of absorption and swelling, and it also helps in preventing constipation or colon cancer. Dietary fiber is low in calories and has a digestion delaying action, which is effective in treating obesity and diabetes. These dietary fibers can be divided into water-insoluble and water-soluble types, and the physiological effects on the human body are different due to their physical and chemical properties (based on the components and structure of the fiber) (28, 29). Water-soluble and viscous dietary fibers such as pectin, soluble gum, b-glucan and alginate are able to absorb or combine cholesterol, and consequently, the products or foods containing these fibers have been reported to reduce blood cholesterol, especially, LDL-cholesterol (30, 31). *Opuntia ficus-indica* is effective in decreasing LDL-cholesterol, and this effect is known to be caused by abundant levels of pectin in the cactus (32, 33). The changes in the pectin content of the *Opuntia ficus-indica* stem according to the extraction conditions are shown in Table 2, and the response surface regression equation for the results is illustrated in Table 3. The R^2 of the regression equation was 0.8659 and the significance was admitted within 5%. As verified in Table 4, the predicted stationary point was the maximum point, and the predicted optimum conditions were 97.11°C, 139.03 min and the water to sample ratio as 10.91 mL/g, together with the maximum pectin content 1243.27 mg%. As in Fig. 3, the pectin content extracted by the extraction conditions increased with the higher extraction temperature. The increase of pectin content was dependent on extraction temperature, compared with extraction time and mix ratio of water (Table 5).

Prediction and experimental verification of optimum extraction conditions To establish the extraction conditions for the effective substances from *Opuntia ficus-indica*, we examined the extractiion characteristics for

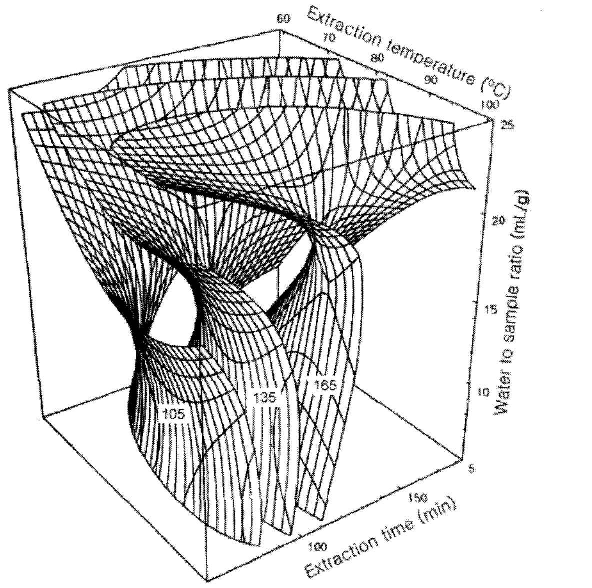

Fig. 2. Response surfaces for phenolics content from *Opuntia ficus-indica* stem at constant values (phenolics: 105-135-165 mg%) as a function of extraction temperature, extraction time and water to sample ratio.

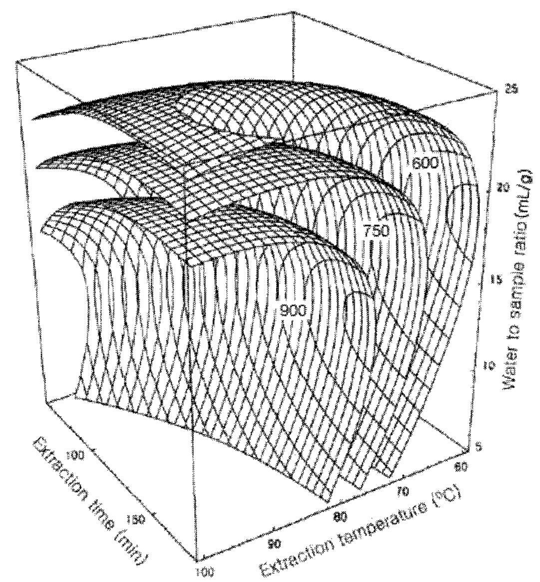

Fig. 3. Response surfaces for pectin content from *Opuntia ficus-indica* stem at constant values (pectin: 600-750-900 mg%) as a function of extraction temperature, extraction time and water to sample ratio.

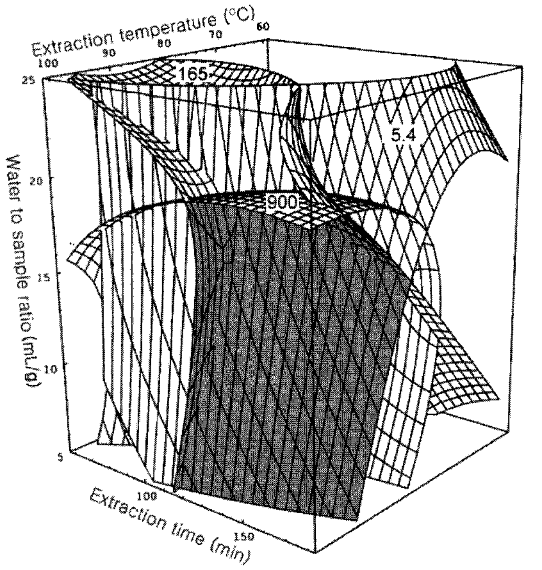

Fig. 4. Superimposed response surfaces for the optimization of extract yield (5.4%), phenolics content (165 mg%) and pectin content (900 mg%) of extract from *Opuntia ficus-indica* stem.

extract yield, phenolics content and pectin content. We predicted the range of the optimum extraction conditions by superimposing the response surface of the representative quality factors such as extract yield, phenolics content and pectin content. The optimum range satisfied the extract yield, phenolics content and pectin content as shown in Fig. 4. The conditions were verified as 95-100°C, 120-180 min and a water to sample ratio of 5-18 mL/g as found in the dark area of Fig. 4. Accordingly, we predicted the physiochemical properties by substituting the following optional conditions of 98°C, 150 min and a water to sample ratio of 15 mL/g from the predicted optimum condition range for each response surface regression equation. From the test the following predicted values were obtained; extract yield 6.44%, phenolics content 180.65 mg% and pectin content 1167.85 mg%.

The reliability of the regression equation was examined by comparing the predicted values for the extraction of the substances (related to the quality of *Opuntia ficus-indica*) with the experimented values obtained through the actual experiment carried out under the similar conditions. Based on the results, the optional extraction conditions were selected to be the following; temperature 98°C, time 150 min and a water to sample ratio of 15 mL/g. We verified the reliability of the derived regression equation, because the predicted values obtained through the response surface analysis exhibited similar results to the experimental actual values of the quality factors. Similar results were observed for extract yield, phenolics content and pectin content, which were obtained through the actual experiments conducted under the optional conditions, as shown in Table 6. The range of optimum extraction conditions in consideration of the physicochemical properties of the extracts were shown to be as 95-100°C as the extraction temperature, 120-180 min as extraction time and a water to sample ratio of 5-18 mL/g.

Table 6. Comparison between predicted and observed values for physiochemical qualities of their extracts

Physiochemical qualities	Predicted value[1)]	Observed [2)]
Extract yield (%)	6.44	6.04
Phenolics content (mg%)	180.65	178.48
Pectin content (mg%)	1167.85	1099.11

[1)]Extraction condition was calculated using the predicted equations for response variables on given values of independent variables: extraction temperature 98°C, extraction time 150 min, water to sample ratio 15 mL/g.
[2)]Mean values of triplicates determinations.

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