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A Comparative Study on the Extraction of Betalain Rich Phenolic Compounds in Cactus Pear

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Abstract

In recent years, consumer demands grow on non-artificial food colorants because of health concerns. Betalain is one of the most popular natural food colorant. The present study concentrated on the extraction of betalain rich bioactive compounds from cactus pear *(Opuntia ficus-indica* L.*).* For this purpose, conventional extraction (CE) and ultrasound assisted extraction (UAE) was compared in terms of some extraction parameters including extraction time (x1), extraction temperature (x2), percentage of ethanol in extraction solvent (x3), solid/solvent ratio (x4). Response surface methodology was used in conjunction with Box-Behnken design to optimize the extraction parameters. In CE, the optimum extraction conditions were found as; x1=2.05 h, x2= 50°C, x3=80% and 1/x4= 22.60; optimal conditions were determined as; x1=30 min, x2= 49.99°C, x3=40% and 1/x4= 30 in UAE. Total betalain contents were 418.829 and 471.818 mg betalain/ kg dry matter, respectively in CE and UAE in optimum extraction conditions. It was found that bioactive compounds were extracted effectively in shorter time and lower solvent in UAE. According to results, UAE was superior method for extraction of betalains compared to CE.

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Keywords: *Cactus pear, extraction, response surface methodology, optimization, betalain*

1. Introduction

Phenolic compounds are the secondary metabolites which do not have any function on growth of the plant. However phenolic compounds have role in the defence mechanism that protects the plant against adverse conditions such as UV, light, high temperature [1]. Betalains, one of the group of phenolic compounds, are water soluble pigments and consist of nitrogen containing betalamic acid molecule[2]. Betalains are divided into two groups: red-violet colored betacyanins and yellow-orange colored betaxanthins[3]. Thus, betalains could be used as natural food colorant. They also have functional effects on human health such as antioxidant, antimicrobial, anti-inflammatory[4].

Betalains are extracted from some of the betalain rich vegetables and fruits' roots, flowers or leaves [5]. Cactus pear (*Opuntia ficus-indica* L.) is one of the betalain rich fruit. It has also some essential amino acids, various vitamin and minerals, phenolic and antioxidant compounds [6]. The color of the fruit can be yellow, red, purple or orange depending on the species of cactus. The color pigments are extracted from the fruit and used as natural colorant which are commonly preferred by the consumer in these days [5].

Extraction is the most important procedure to obtain betalain rich phenolic compounds from cactus pear. There are conventional method and novel technologies in extraction procedure. In conventional method, the extraction is time and solvent consuming and low efficient [7]. Ultrasound, microwave, pulsed electric field, pressurized liquid are the novel technologies in extraction of bioactive compounds. These technologies are energy efficient with less solvent volume and short duration of extraction[1]. In literature, both conventional and novel extraction methods were tried to extract betalains from cactus pear. In a study of Sigwela et. al., the betalains were extracted from 6 different cultivars of cactus pear using microwave assisted extraction. The total phenolic, flavonoid and ascorbic acid content of extracts were also investigated [8]. Morales et al., conducted a conventional extraction (CE) of betalains from cactus pear in which the extraction solvent was 80% ethanol solution. After maceration process, the extract was concentrated using rotary evaporator[9]. In another study, ultrasound assisted extraction (UAE) was used as novel method for the extraction of betalain rich phenolic compounds from cactus pear. Some of the extraction parameters (solvent concentration, time of extraction and solid: solvet ratio) was optimized using response surface methodology [10].
This study was aimed to compare 2 different extraction methods (CE and UAE) for the extraction of betalain rich phenolic compounds from cactus pear. It was also an optimization study in which the extraction parameters were optimized using response surface methodology.

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2. Materials and Methods

2.1. Plant material and chemicals

Cactus pear (*Opuntia ficus-indica* L.) was supplied from the local producer (BECOS Agriculture, Mersin, Turkey). The fruit was separated from the shell and dried in freeze dryer (Christ, Alpha 1–4 LD plus, Osterode, Germany). Dried samples were ground by a grinder and it was stored in -20^{0}C until use.

Methanol, ethanol, gallic acid, and Folin-Ciocalteu were purchased from the Merck Chemical Reagents Company (Darmstadt, Germany). Sodium carbonate, DPPH (2,2-diphenyl- 1- picrylhydrazyl), trolox, neocuprione, $CuCl_2$, ammonium acetate were purchased from Sigma-Aldrich (Darmstadt, Germany).

2.2. Total phenolic content, total antioxidant capacity and total betalain content (TBC) determination

The total phenolic content (TPC) of extracts was determined using Folin–Ciocalteau. The TPC of the samples were expressed in mg gallic acid equivalence (GAE)/g dry matter in calibration curve prepared for different concentrations of gallic acid solutions [11].

Total antioxidant capacity (TAC) was measured spectrophotometrically with two methods: DPPH and CUPRAC. For TAC_{DPPH} measurement of extracts, 100 µL sample was mixed with 3.9 mL of DPPH solution (25 ppm). At the same time, for blank sample, 100 µL methanol was mixed with 3.9 mL DPPH solution (25 ppm). Blank and sample mixtures were vortexed for 5 secs and kept in dark place for 1 hour. At the end of 1 hour, the absorbance values were recorded at 517 nm. The absorbance of blank and sample were recorded as A1 and A2, respectively [12]. For CUPRAC method, 0.0075 M neocuprione solution, 10^{-2} M $CuCl_2$ solution and 1 M ammonium acetate buffer (pH= 7) were prepared. For blank, 1 mL neocuprione solution, 1 mL $CuCl_2$, 1 mL ammonium acetate buffer and 1.1 ml extraction solvent were mixed. For extract, 1 mL neocuprione solution, 1 mL $CuCl_2$, 1 mL ammonium acetate, 0.4 mL of extract solution and 0.7 mL extaction solvent were mixed. The blank and sample mixtures were mixed for 5 secs using vortex and kept in dark place for 1 hour. The absorbance values were measured at 450 nm [13]. TAC of extracts were expressed as mmol TE/kg dry matter.

In order to find total betalain content (TBC) spectrophotometer was used and it was calculated by the following equation (1). TBC was given as mg betalain/ kg dry matter.

$$\frac{A.df.M_W.V_d.1000}{\varepsilon.L.W_d} \quad (1)$$

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where A is the absorption value at 483 and 535 nm for betaxanthins and betacyanins, respectively, df is the dilution factor, M_w is the molecular weight (308 and 550 g/mol for betaxanthin and betacyanin, respectively), Vd is the the volume of volume (mL), ε is the molar extinction coefficient (48,000 and 60,000 L/(mol cm) for betaxanthin and betacyanin, respectively), W_d is the amount of the extracted sample (g) and L is the path-length (1 cm) of the cuvette.

2.3. Optimization of CE of betalains from cactus pear and statistical analysis

Betalains from cactus pear were extracted firstly by conventional method. Shaking water bath (Mikrotest, msc 30, Turkey) was used for maceration. After maceration, the extract was filtered by filter paper and the supernatant was filtered twice by 0.45 μm syringe filter.

Response surface methodology (RSM) was used for the optimization of extraction conditions. Box-Behnken design with 3 levels was used to evaluate the effects of 4 the independent variables on 4 dependent variables. Design of the experiments was done by using Design Expert 12 (Statease Inc., Minneapolis, USA). In the experimental design, the independent variables were chosen as x1; time (h), x2; temperature (°C), x3; percentage of ethanol in extraction solvent (% ethanol); x4; solid/solvent ratio (g solid sample/mL solvent). In Table 1, the coded, actual values and the levels of independent variables were given.

Experimental design was consisted of 29 conditions in which the condition of the 5 experiment was identical and called as the central point (Table 1). TPC (y1); TAC_{DPPH} (y2); TAC_{CUPRAC} (y3); TBC (y4) were chosen as dependent variables (responses).

After the experiments, the dependent variables were calculated and results were compared with the chosen model by Design Expert 12 using ANOVA. The quality of the fit of the model was checked with Fischer test value, coefficient of determination (r^2) and lack of fit. The extraction conditions were optimized using desirability function in Design expert 12.

Table 1 – The levels, coded and actual values of independent variables in CE

		Level		
Independent variable	**Symbol**	**-1**	**0**	**1**
Extraction time	x1	1	2	3
Extraction temperature	x2	30	40	50
Percentage of ethanol in extraction solvent	x3	40	60	80
1/(solid/solvent ratio)	x4	10	20	30
Unit of independent variables; x1= extraction time (h) x2= extraction temperature (°C)				

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x3= percentage of ethanol in extraction solvent (% ethanol) x4= solid/solvent ratio (g solid sample/mL solvent)				
Run number	**x1**	**x2**	**x3**	**1/x4**
1	2 (0)	50 (1)	60 (0)	30 (1)
2	3 (1)	30 (-1)	60 (0)	20 (0)
3	1 (-1)	40 (0)	40 (-1)	20 (0)
4	2 (0)	40 (0)	60 (0)	20 (0)
5	3 (1)	40 (0)	80 (1)	20 (0)
6	2 (0)	30 (-1)	60 (0)	10 (-1)
7	2 (0)	30 (-1)	80 (1)	20 (0)
8	1 (-1)	40 (0)	80 (1)	20 (0)
9	2 (0)	40 (0)	60 (0)	20 (0)
10	2 (0)	30 (-1)	40 (- 1)	20 (0)
11	3 (1)	50 (1)	60 (0)	20 (0)
12	2 (0)	40 (0)	60 (0)	20 (0)
13	2 (0)	50 (1)	80 (1)	20 (0)
14	2 (0)	40 (0)	60 (0)	20 (0)
15	1 (-1)	30 (-1)	60 (0)	20 (0)
16	3 (1)	40 (0)	60 (0)	10 (-1)
17	1 (-1)	50 (1)	60 (0)	20 (0)
18	2 (0)	50 (1)	40 (-1)	20 (0)
19	1 (-1)	40 (0)	60 (0)	30 (1)
20	2 (0)	40 (0)	80 (1)	10 (-1)
21	3 (1)	40 (0)	60 (0)	30 (1)
22	3 (1)	40 (0)	40 (-1)	20 (0)
23	2 (0)	50 (1)	60 (0)	10 (-1)
24	1 (-1)	40 (0)	60 (0)	10 (-1)
25	2 (0)	40 (0)	40 (-1)	10 (-1)
26	2 (0)	40 (0)	40 (-1)	30 (1)
27	2 (0)	30 (-1)	60 (0)	30 (1)
28	2 (0)	40 (0)	60 (0)	20 (0)
29	2 (0)	40 (0)	80 (1)	30 (1)

2.4. Optimization of ultrasound assisted extraction of betalains from cactus pear and statistical analysis

UAE was also applied for the extraction of betalain rich phenolic compounds in cactus. Ultrasounic water bath (Şimşek Laborteknik, Turkey) was used for extraction process.

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All the procedure was same with the conventional method. In the design, the independent variables were x1; time (min), x2; temperature (°C), x3; percentage of ethanol in extraction solvent (% ethanol); x4; solid/solvent ratio (g solid sample/mL solvent). In Table 2, the coded, actual values and the levels of independent variables were shown.

Experimental design was consisted of 29 conditions in which the condition of the 5 experiment was identical (Table 2). The same responses were chosen and statistical analysis were identical with the conventional method. Desirability function was also used for UAE in order to optimize the extraction parameters.

Table 2 – The levels, coded and actual values of independent variables in UAE

		Level		
Independent variable	**Symbol**	**-1**	**0**	**1**
Extraction time	x1	30	60	90
Extraction temperature	x2	30	40	50
Percentage of ethanol in extraction solvent	x3	40	60	80
1/(solid/solvent ratio)	x4	10	20	30
Unit of independent variables; x1= extraction time (min) x2= extraction temperature (°C) x3= percentage of ethanol in extraction solvent (% ethanol) x4= solid/solvent ratio (g solid sample/mL solvent)				
Run number	**x1**	**x2**	**x3**	**1/x4**
1	90 (1)	40 (0)	80 (1)	20 (0)
2	60 (0)	40 (0)	40 (-1)	30 (1)
3	60 (0)	40 (0)	60 (0)	20 (0)
4	60 (0)	40 (0)	60 (0)	20 (0)
5	90 (1)	40 (0)	60 (0)	10 (-1)
6	90 (1)	50 (1)	60 (0)	20 (0)
7	60 (0)	30 (-1)	40 (-1)	20 (0)
8	30 (-1)	30 (-1)	60 (0)	20 (0)
9	60 (0)	40 (0)	60 (0)	20 (0)
10	90 (1)	30 (-1)	60 (0)	20 (0)
11	60 (0)	30 (-1)	60 (0)	10 (-1)
12	60 (0)	40 (0)	80 (1)	30 (1)
13	30 (-1)	50 (1)	60 (0)	20 (0)
14	60 (0)	50 (1)	40 (-1)	20 (0)
15	30 (-1)	40 (0)	60 (0)	30 (1)
16	60 (0)	50 (1)	60 (0)	10 (-1)

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17	30 (-1)	40 (0)	40 (-1)	20 (0)
18	60 (0)	50 (1)	60 (0)	30 (1)
19	60 (0)	40 (0)	40 (-1)	10 (-1)
20	90 (1)	40 (0)	40 (-1)	20 (0)
21	60 (0)	40 (0)	80 (1)	10 (-1)
22	90 (1)	40 (0)	60 (0)	30 (1)
23	30 (-1)	40 (0)	60 (0)	10 (-1)
24	60 (0)	40 (0)	60 (0)	20 (0)
25	60 (0)	40 (0)	60 (0)	20 (0)
26	60 (0)	50 (1)	80 (1)	20 (0)
27	60 (0)	30 (-1)	60 (0)	30 (1)
28	60 (0)	30 (-1)	80 (1)	20 (0)
29	30 (-1)	40 (0)	80 (1)	20 (0)

3. Results

According to experimental responses, multiple regression analysis was applied by the Design expert 12 program. Bioactive components of cactus pear were extracted more efficiently in UAE than CE. In literature, UAE was found effective method in order to extract bioactive components comparing with CE. Moreover, UAE gave higher TPC, TAC and TBC for shorter extraction time [14]. The results of this study supported the literature in a way of higher responses in UAE than CE. In figure 1, the response surface plots for the binary effects some of the independent variables on TBC were illustrated. In CE, the higher percentage of ethanol and longer extraction duration had significant effect on TBC (Figure 1a). In addition, in Figure 1b, the TBC was predicted as maxima in solid to solvent ratio was between 1:20 and 1:25 in 2 to 3 hours of extraction duration. As can be seen from Figure 1c and 1d, in UAE, binary effects of x1, x3 and x3, x4 had considerable effect on TBC. The response surface plots confirmed that shorter and effective extraction with lower solvent was applicable for higher TBC in UAE [15]. In Figure 2, the predicted and actual (experimental) values of each response were represented in CE and UAE. The actual responses were found nearly the same with the experimental ones. Thus, coefficient of determinations (r^2) of models gave satisfactory values which were higher than 0.9. It was also understood from second order polynomial equations of each extraction method, all independent variables, their binary and quadratic effects affected the responses. According to ANOVA results, the suitability of the model was checked by finding the model was significant ($p<0.05$) with insignificant ($p>0.05$) lack of fit. It was found that for each response and method the model was found as significant and the lack of fit was insignificant.

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In optimization part of this study, desirability function was used to find optimum extraction conditions for both extraction methods. The solutions whose desirability functions were 0.841 and 0.943 in CE and UAE, respectively, were selected as optimal extraction conditions. Although, in CE, the optimum conditions were proposed as; x1=2.05 h, x2= 50°c, x3=80% and 1/x4= 22.60; in UAE, the program suggested the optimal conditions as; x1=30 min, x2= 49.99°C, x3=40% and 1/x4= 30. The experimental and estimated responses were given in Table 3. The experimental responses were nearly same with the estimated responses which were given in Design expert 12. These findings supported the optimization of extraction parameters for each method.

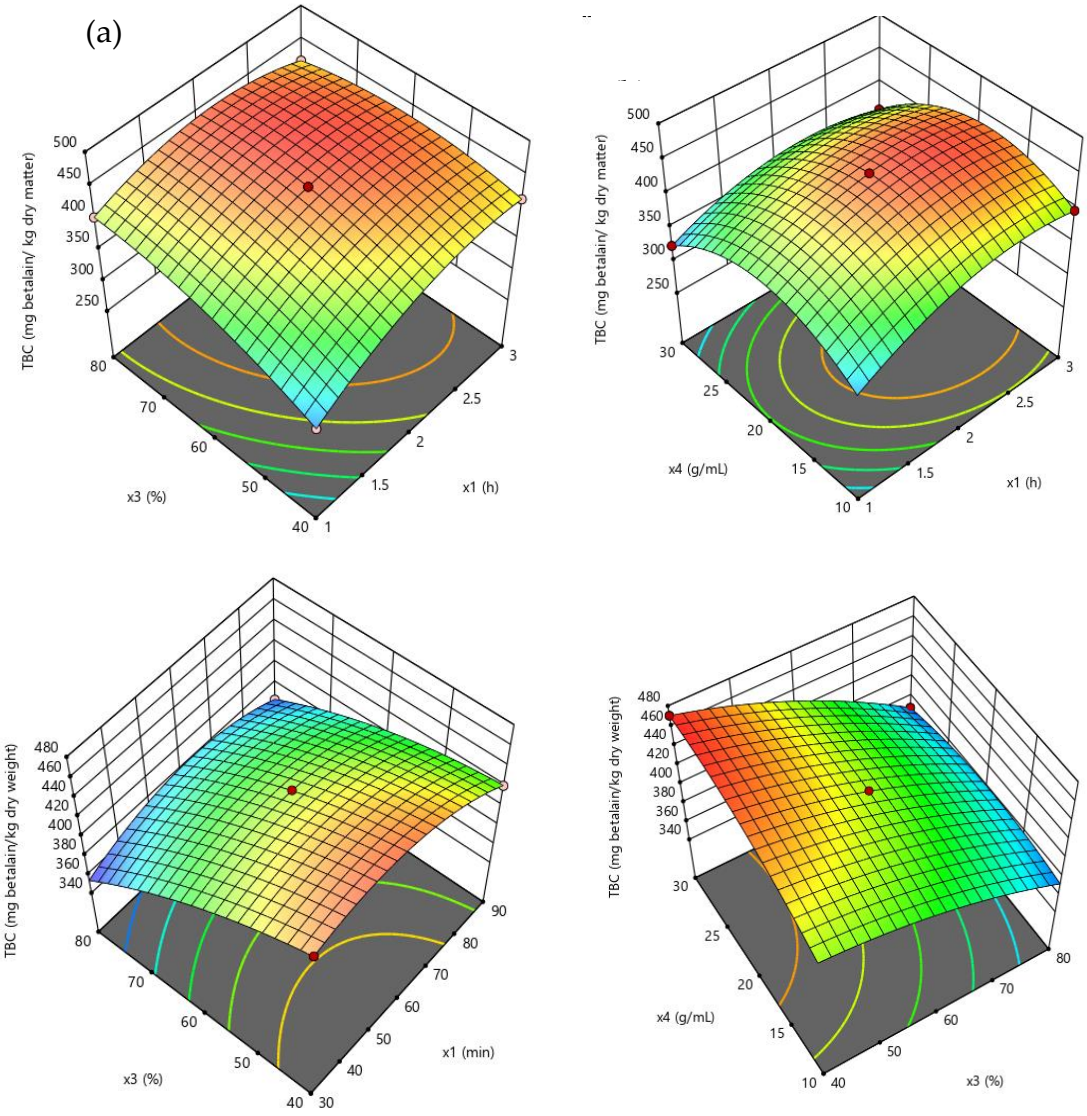

Figure1: Response surface plots for the effects of independent variables on TBC, (a) and (b) in CE ; (c) and (d) in UAE

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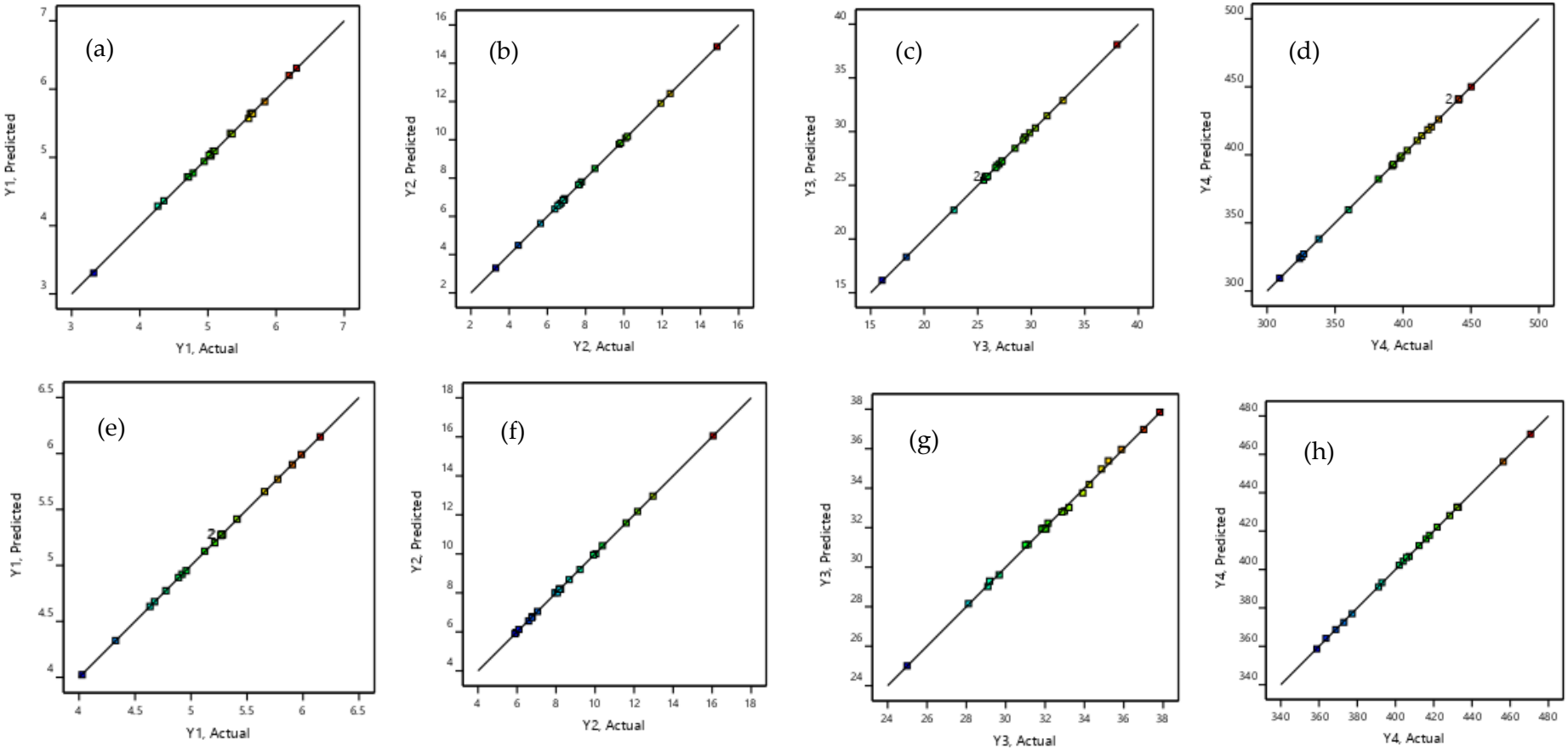

Figure 2: Predicted vs actual responses (a), (b), (c), (d) in CE and (e), (f), (g), (h) in UAE

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Table 3- Experimental and estimated responses in optimum conditions of CE and UAE

	CE		UAE	
	Experimental response	Estimated response	Experimental response	Estimated response
y1	6.125	6.048	6.384	6.486
y2	13.963	14.343	13.560	13.603
y3	34.873	32.587	49.383	49.210
y4	418.829	419.449	471.818	472.883

y1= TPC (mg GAE/g dry matter)
y2= TAC_{DPPH} (mmol TE/ kg dry matter)
y3= TAC_{CUPRAC} (mmol TE/ kg dry matter)
y4= TBC (mg betalain/ kg dry matter)

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4. Discussion and Conclusion

In this study, two extraction methods; CE and UAE, were compared with respect to extraction of betalain rich phenolic compounds in cactus pear. It was determined that UAE was superior method in terms of getting higher values of bioactive components than CE. UAE was also time, solvent and energy saving process comparing with CE[16]. Since UAE was more advantegous in terms of lower solvent usage, shorter extraction time and lower solid to solvent ratio, it could be more applicable method to extract betalain rich phenolic components in cactus pear. In UAE, in same optimum extraction temperature, which was found as 50 °C, 30 min extraction and 40% ethanol was sufficient to extract higher total betalain than CE.

Color is the important property of a food material that affects the consumer acceptability of products. Nowadays, artificial food colorants were disflavored by the consumer due to health concerns [17]. Betalains were gained consumer attention in terms of natural food colorants having functional properties like antioxidant character. According to findings of this study, UAE of betalains is highly recommended in order to get natural food colorant from cactus pear.

5. Acknowledge

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References

[1] O. R. Alara, N. H. Abdurahman, and C. I. Ukaegbu, "Extraction of phenolic compounds: a review," *Current Research in Food Science,* vol. 4, pp. 200-214, 2021.

[2] I. Belhadj Slimen, T. Najar, and M. Abderrabba, "Chemical and antioxidant properties of betalains," *Journal of Agricultural and Food Chemistry,* vol. 65, no. 4, pp. 675-689, 2017.

[3] C. M. Moreno-Ley, G. Osorio-Revilla, D. M. Hernández-Martínez, O. A. Ramos-Monroy, and T. Gallardo-Velázquez, "Anti-inflammatory activity of betalains: A comprehensive review," *Human Nutrition & Metabolism,* vol. 25, p. 200126, 2021.

[4] E. Madadi, S. Mazloum-Ravasan, J. S. Yu, J. W. Ha, H. Hamishehkar, and K. H. Kim, "Therapeutic application of betalains: A review," *Plants,* vol. 9, no. 9, p. 1219, 2020.

[5] O. Zannou *et al.*, "A comprehensive review of recent development in extraction and encapsulation techniques of betalains," *Critical Reviews in Food Science and Nutrition,* pp. 1-18, 2023.

[6] F. J. Barba *et al.*, "*Opuntia Ficus Indica* Edible Parts: A Food and Nutritional Security Perspective," *Food Reviews International,* pp. 1-23, 2020.

[7] C. Bitwell, I. S. Sen, C. Luke, and M. K. Kakoma, "A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants," *Scientific African,* p. e01585, 2023.

[8] V. Sigwela, M. De Wit, A. du Toit, G. Osthoff, and A. Hugo, "Bioactive betalain extracts from cactus pear fruit pulp, beetroot tubers, and amaranth leaves," *Molecules,* vol. 26, no. 16, p. 5012, 2021.

[9] N. X. C. Morales, K. Y. V. Gómez, R. M. Schweiggert, and G. T. C. Delgado, "Stabilisation of betalains and phenolic compounds extracted from red cactus pear (*Opuntia ficus-indica*) by spray and freeze-drying using oca (*Oxalis tuberosa*) starch as drying aid," *Food Science and Technology International,* vol. 27, no. 5, pp. 456-469, 2021.

[10] V. Espinosa-Muñoz, C. Roldán-Cruz, A. Hernández-Fuentes, A. Quintero-Lira, I. Almaraz-Buendía, and R. Campos-Montiel, "Ultrasonic-assisted extraction of phenols, flavonoids, and biocompounds with inhibitory effect against *Salmonella typhimurium* and *Staphylococcus aureus* from Cactus pear," *Journal of Food Process Engineering,* vol. 40, no. 2, p. e12358, 2017.

[11] V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventós, "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent," in *Methods in Enzymology*, vol. 299: Elsevier, 1999, pp. 152-178.

[12] B. Akdeniz, G. Sumnu, and S. Sahin, "Microencapsulation of phenolic compounds extracted from onion (*Allium cepa*) skin," *Journal of Food Processing and Preservation,* vol. 42, no. 7, p. e13648, 2018.

[13] R. Apak *et al.*, "Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay," *Molecules,* vol. 12, no. 7, pp. 1496-1547, 2007.

[14] I. Savic Gajic, I. Savic, I. Boskov, S. Žerajić, I. Markovic, and D. Gajic, "Optimization of ultrasound-assisted extraction of phenolic compounds from black locust (*Robiniae pseudoacaciae)* flowers and comparison with conventional methods," *Antioxidants,* vol. 8, no. 8, p. 248, 2019.

[15] M. Singla and N. Sit, "Application of ultrasound in combination with other technologies in food processing: A review," *Ultrasonics Sonochemistry,* vol. 73, p. 105506, 2021.

[16] C. Picot-Allain, M. F. Mahomoodally, G. Ak, and G. Zengin, "Conventional versus green extraction techniques—A comparative perspective," *Current Opinion in Food Science,* vol. 40, pp. 144-156, 2021.

[17] B. G. Nabi *et al.*, "Natural pigments: Anthocyanins, carotenoids, chlorophylls, and betalains as food colorants in food products," *Food Bioscience,* p. 102403, 2023.