Optimization of Methanolic extraction of polyphenols and betalains from *Opuntiaficus-indica* fruits

Charbel El Tawil
Department of Nutrition and Food Sciences, Holy Spirit University of Kaslik, P.O. Box 446, Jounieh, Lebanon
Nathalie Estephan
Department of Chemistry and Biochemistry, Holy Spirit University of Kaslik, P.O. Box 446, Jounieh, Lebanon
Nabil Nemer*
Department of Agriculture and Food Engineering, Holy Spirit University of Kaslik, P.O. Box 446, Jounieh, Lebanon
*Corresponding author.Email: nabilnemer@usek.edu.lb
N. NemerORCiD ID number: 0000-0002-7105-0449

Abstract

Extraction of phenolic compounds and betalains from plant matrices represent a real challenge to researchers due to the complexity and diversity of bioactive compounds. In this study we used the response surface methodology to address the effect of three important extraction factors; Temperature, time and solvent content on extraction yield from the barbary fig fruit, *Opuntiaficusindica*. Temperature range was between 0 and 80°c, Time between 0.36 and 96 h and methanol content between 0 % and 100 %. The goal was to identify the impact of each parameter on extraction yield. Our results show that pulp had the highest betalain content and peel exhibited the highest total phenolic compounds. Polyphenols and betalains from pulp are more extractable using methanol solvent and from peel using water. Optimal condition for betalain content of 19.6 mg/100 g was obtained from pulp using 1.37 °C, 100 % methanol for 0.36 h. However, Optimal condition for total phenolic compounds of 2.77 mg GAE g-1 was obtained using 2.38 °C, 0 % methanol content for 96 h.

Keywords: *Opuntiaficusindica*, polyphenols,batalain, response surface methodology

INTRODUCTION

Nowadays, synthetic antioxidants have safety concerns, however, *Opuntiaficus-indica* (OFI) fruits recently attracted a lot of investigation that could lead to the discovery of new natural compounds with antioxidant activity (Aruwa*et al.*, 2019). The barbary fig is well known for its use in traditional medicine for the treatment of different of diseases such as diabetes, asthma, bronchial, burns and indigestion in many countries around the world (Kaur *et al.,* 2012). Recently, many authors are investigating OFI pharmacological benefits such as sedative and anxiolytic effects on mice bioassay (Akkol et al., 2020), antimicrobial and antioxidant activities, anticancer, anti-viral, anti-inflammatory and anti-ulcer activities (Bargougui*et* al., 2019; El-Beltagi*et* al., 2019; Kaur*et al.*, 2012;Rasoulpour*et al*., 2018). Moreover, researchers are interested in betalain content of OFI for replacing synthetic additives in food industry because betalains are natural colorant containing natural antioxidants beneficial for health (Robert *et al.*, 2015). In addition, encapsulation of betalain pigment from OFI gained a lot of attention in modern food industry to increase their stability, due to rapid degradation of betalains when exposed to pH, water activity, light, oxygen or temperature (Carmona *et* al., 2021; Ileana *et al.*, 2019; Otálora*et al.*, 2019).

Phytochemicals are known for their wide diversity in plants, phenolic compounds are the largest group with more than 8000 identified polyphenols (Martins *et al*., 2011). Betalains are divided onto two groups, betacyanins and betaxanthins and are water soluble pigments (Fernandez-Lopez, 2001). Studies shows that profile of phenolic compounds, biological activity and yield could be affected by the extraction solvent, extraction temperature and time (El Mannoubi, 2021; Maran*et al*., 2013). Extraction efficiency of phytochemicals is mainly influenced by the polarity of solvents and the species of used plants (Ye *et al*., 2015). Different solvents were used for phytochemicals extraction such as ethanol, methanol, acetone, dimethylsulfoxide, water and their mixture (Assefa and Keum, 2017). Recently, various researchers used response surface methodology (RSM) to optimize and improve the performance of many plant based extraction processes (Los et al., 2019; Zivkovic*et al*., 2018). Highest yields could be obtained using optimal conditions of resources and without increasing expenses and time (Sarfarazi*et al*., 2015). In addition, RSM investigate the interaction between multiple parameters with a minimal number of experiments, which result in less laborious ant time consumption (Sharma *et al.*, 2016). RSM use a series of mathematical and statistical techniques to develop a mathematical relationship between responses and factors (Bezerra*et al.,* 2008).

The purpose of this study was to elaborate the combined effects of two solvents, methanol and water, extraction temperature and extraction time on the betalains and polyphenols content of OFI. Pulp and peel were tested separately and wide range of extraction time (0 – 96 h), solvent percentage (0 -100%) and extraction temperature (0 – 80 °C) was applied to fully investigate the impact of each factors on responses.

MATERIALS AND METHODS

Sample preparation and colorimetric analysis

Opuntiaficusindica fruits were harvested during summer 2018 from a Lebanese orchard and were stored at -20°C. The fruits peeling was carried out manually using knife. A robocoup was used to crush the pulp and peel separately. The fruits paste was then stored at -80°c until use. Solid-liquid extraction was performed at the ratio of 1:3 (w/v). Different aqueous solution of methanol (Sigma Aldrich, USA) and pure water were used to extract phenolic compounds and betalains from OFI pulp and peel using different extraction temperature and time. Incubated samples were then centrifuged at 10000 x g for 5 min and were filtered using 0.45µm syringe filter. Final extracts were kept at -80°C until use.

Quantification of total phenolic compounds (TPC)

Total phenolic compounds were determined by spectrophotometry using folinciocalteu method as described by Singleton and Rossi (1965). Gallic acid (Sigma Aldrich, USA) was used as standard and TPC were expressed as mg gallic acid equivalents (GAE) per g of fresh fruit.

Quantification of betalain content (BC)

Spectrophotometry was used to calculate bethaxanthin and betacyanin content of *Opuntia* pulp and peel. Extracts were diluted with water to obtain absorption value lower than 1 at 538 and 480 nm. Bethacyanin and bethaxanthin were calculated as betanin and indicaxanthin equivalent, respectively.

$$B\ [mg/L] = \frac{A \times DF \times MW \times 1000}{\varepsilon \times 1}$$

B represent bethacyanin or bethaxanthin content. A is the value of maximal absorption (λmax = 538 for betanin and 480 nm for bethaxanthin) corrected by the absorbance of impurities at 600nm. MW is the molecular weight (550g/mol for betanin and 308g/mol for indicaxanthin). E is the molar extinction (60000 L mol-1 cm-1 for betanin and 48000 L mol -1 cm-1 for indicaxanthin). Finally,Betalain content was calculated as the sum of bethacyanin and bethaxanthin and was expressed as mg/100g of fresh fruit.

Design of experiments

Response surface methodology was applied to optimize the extraction of phenolic compounds and betalains from *Opuntia* fruits. The impact of three factors; extraction temperature (X1), extraction time (X2) and Methanol solvent percentage (X3) were evaluated using a rotatable central composite design. The design of experiment consisted of 18 runs. Variables values range was as follow: temperature between 0 °C and 80 °C, Time between 0.36 h and 96 h and solvent content between 0 % and 100%. Variable were coded at 5 levels (-α**,** -1, 0, 1**,** α) (Table 1).

$$Y= \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^{2} + \beta_{33} X_3^2$$

Where Y is the predicted response, $\beta_0$is the mean value of responses at the central point of the experiment;β_1, β_2 andβ_3, β_{11}, $\beta_{22}$andβ_{33}andβ_{12} and β_{13} are the linear, quadratic, and the interactioncoefficients, respectively. Experimental design and statistical treatment were performed using STATGRAPHICS Centurion 18.

Table 1. Coded and uncoded values of the independent variables and their actual and predicted responses

Run	Temperature °C/(coded)	Time h/(coded)	Solvent %/coded	Pulp (Pu) TPC* (mg GAE g-1)		Pulp (Pu) BC** (mg/100g)		Peel (Pe) TPC (mg GAE g-1)		Peel (Pe) BC (mg/100g)	
				Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted
1	63.9 (1)	76.6 (-1)	20.2 (-1)	1.09	1.25	1.25	0.68	2.70	2.48	0.72	0.01
2	40 (0)	48.1 (0)	50 (0)	0.89	1.01	7.5	7.64	1.97	2.10	4.45	4.86
3	40 (0)	48.1 (0)	50 (0)	0.99	1.01	8.24	7.64	2.34	2.10	5.70	4.86
4	40 (0)	48.1 (0)	50 (0)	1.05	1.01	7.99	7.64	1.98	2.10	4.56	4.86
5	63.7 (1)	19.7 (-1)	20.2	1.35	1.35	4.74	5.37	2.67	2.58	3.41	4.17
6	40 (0)	0.3 (-α)	50 (0)	1.13	1.20	14.80	13.73	2.32	2.49	10.00	9.026
7	80 (α)	48.1 (0)	50 (0)	1.86	1.56	2.14	0.88	2.30	2.57	1.39	1.09
8	16.2 (1)	19.7 (-1)	20.2 (-1)	1.30	1.14	14.40	13.67	2.85	2.70	10.90	10.73
9	16.2 (-1)	76.6 (1)	20.2 (-1)	1.27	1.14	14.18	12.40	2.78	2.77	10.31	9.63

10	16.2 (0)	76.6 (1)	79 (1)	0.83	0.88	11.38	10.36	2.56	2.39	9.34	8.28
11	40 (1)	48.1 (0)	0 (-α)	0.99	1.09	5.95	7.23	2.25	2.40	4.72	5.07
12	40 (0)	48.1 (0)	100 (α)	1.07	0.89	10.36	9.63	1.58	1.79	5.54	5.62
13	16.2 (0)	19.7 (-1)	79 (1)	0.99	0.89	15.11	15.29	2.33	2.30	9.94	10.38
14	63.7 (1)	76.6 (1)	79 (1)	1.06	1.27	1.56	1.91	2.27	2.16	1.11	0.98
15	0 (0)	48.1 (0)	50 (0)	0.84	1.06	13.15	14.96	2.77	2.86	12.01	12.74
16	40 (0)	48.1 (0)	50 (0)	1.09	1.01	6.87	7.64	2.18	2.10	4.79	4.86
17	63.7 (1)	19.7 (-1)	79 (1)	1.20	1.38	8.85	10.25	2.48	2.24	5.81	6.19
18	40 (-α)	96 (α)	50 (0)	1.25	1.11	4.04	5.65	2.30	2.48	2.31	3.72

*TPC: Total Phenolic Compounds

**BC: Betalain content

RESULTS AND DISCUSSION

Optimization of Total phenolic compounds and betalain content

The optimization of total phenolic compounds and betalain content was obtained using response surface methodology. A wide range of extraction temperature, extraction time and methanol solvent percentage was chosen to cover most extreme applicable conditions. Temperature was chosen between 0 and 80°C, time between 0 and 96 h and solvent content between 0 and 100%. As shown in Table 1 different runs were tested as elaborated by statgraphics software. Maximum experimental TPC yield of 2.85 mg GAE g-1 was obtained at 16.2°C, 19.7 h and 20.2% methanol from OFI peel. Maximum experimental BC of 15.11 mg/100g was obtained at 16.2 °C, 19.7h and 79% methanol from pulp (Table 1). These results showed that maximal TPC and BC were obtained using aqueous polar solvent containing methanol and water.

Predicted and experimental optimal conditions

Predicted yields were shown for every actual experiment (Table 1). Multiple regression equations for three independent variables were used to predict responses (Table 2). The predicted responses were analyzed to determine the coefficient of determination R2 (Table 3). The values of R2 for Pu-BC (94.74%) and Pe-BC (96.5%) are very close to 1, which indicate high correlation between actual and predicted BC. Values of R2 for Pu, TPC (60.1%) and pe, TPC (73.25%) indicate that the model explain reasonably the variability in TPC.

Table 4 shows the predicted combination of factor levels which maximize TPC and BC from pulp and peel. These predicted data were experimentally tested to compare the experimental and predicted values of the responses. Optimal experimental yields for BC were 19.6 and 15.44 mg/100g and were very close to predicted optimal responses of 20.8 and 15.98 mg/100g, respectively for pulp and peel (Table 4). These results show that prediction for BC was precise and yielded higher BC in comparison to all experimental runs in which the maximum was 15.11 (Table 1). However, for TPC experimental yields were slightly lower than optimal predicted yield. Optimal predicted TPC was 1.841 and 3.59 mg GAE g-1 and were slightly lower than optimal predicted TPC yields of 1.39 and 2.77 mg GAE g-1. These results shows that when R2 are close to 1, prediction of different variables that optimize responses will be very precise. RSM will be very useful to determine the optimal conditions from a wide range of possibilities that could not be tested experimentally due to high number of experiments.

Table 2. Regression equations of the predicted models

Regression equations
Pu-TPC = 1.45663 - 0.0118465X1 - 0.00539288X2 - 0.0050917X3 + 0.000187816X1^2 - 0.0000381386X1X2 + 0.0000986245X1X3 + 0.0000624294X2^2 - 0.00000171418X2X3 –0.00000742986X3^2
Pu-BC = 17.9696 - 0.186708X1 - 0.0661841X2 - 0.00140405X3 + 0.000173438X1^2 - 0.00126382X1X2 + 0.00115289X1X3 + 0.000895335X2^2 - 0.00108133X2X3 + 0.000313753X3^2
Pe-TPC = 3.50589 - 0.0324876X1 - 0.0143969X2 - 0.00697627X3 + 0.000383154X1^2 - 0.0000594757X1X2+ 0.0000206179X1X3 + 0.000169328X2^2 + 0.00000650185X2X3 - 0.00000255861X3^2
Pe-BC = 15.9082 - 0.234973X1 - 0.0585549X2 - 0.0330618X3 + 0.00128472X1^2 - 0.00114372X1X2 + 0.000834583X1X3 + 0.000662015X2^2 - 0.000297825X2X3 + 0.000195521X3^2

Pu: pulp; Pe: peel; TPC: total phenolic compounds; BC: betalain content; X1: Temperature (°C); X2: Time (h); X3: Solvent (%)

Table 3. Coefficient of determination R2 for thepredicted models

Source	Coefficient of determination R2
Pu, TPC	60.10%
Pu, BC	94.74%
Pe, TPC	73.25%
Pe, BC	96.5%

Pu: pulp; Pe: peel; TPC: total phenolic compounds; BC: betalain content

Table 4. Optimum conditions for maximal extraction yield of total phenoloc compounds (TPC) and betalain content (BC) of *Opuntiaficus-indica*fruits peel (Pe) and pulp (Pu).

Factor	Temperature (°C)	Time (h)	Solvent content (%)	Optimum predicted value	Optimum Experimental value
Pu, TPC (mg GAE g-1)	78.43	0.36	78.43	1.841	1.39
Pu, BC (mg/100g)	1.37	0.36	100.0	20.80	19.6
Pe, TPC (mg GAE g-1)	2.38	96.0	0.0056	3.59	2.77
Pe, BC (mg/100g)	1.14	96.0	0.16	15.98	15.44

Optimization of extraction temperature

The analysis of variance in Table 5 partitions the variability in TPC and BC into separate pieces for each of the effects. Variables with P-values less than 0.05 are statistically significant at the 95% confidence level. The effect of temperature is statistically significant for Pu-TPC; Pu-BC and Pe-BC (Table 5). The ascent shape of 3D surface plot shows that there was an increase in TPC with the increase of temperature from 20 to 80°C (Figure 1 a, c). However, the descent shape of 3D plot in Figure 2 (a, c, d, f) shows that the effect of temperature is negative on BC. In addition, quadratic effect of temperature is statistically significant for Pe-TPC and Pe-BC, and this effect is shown as concave 3D surface plot (Figure 1 d, f) in which a minimum number of three levels of temperature are required to quantify the behavior of 3D plot. The yield of TPC in OFI pulp decreases from 0°C until the temperature reaches 50°C and then it increases from 50°C until 100°C (Figure 1 d).

Table 5. Analysis of variance for the different factors and their interactions on total phenolic compounds (TPC) and betalain content (BC) of *Opuntiaficus-indica*fuits peel (Pe) and pulp (Pu).

Source	Pu, TPC	Pu, BC	Pe, TPC	Pe, BC
		*p-value**		
X1	0.0355	0.0000	0.2268	0.0000
X2	0.6473	0.0005	0.9561	0.0004

X3	0.3503	0.1292	0.0262	0.5551
X1X1[a]	0.1219	0.8284	0.0140	0.0306
X1X2[b]	0.7470	0.1594	0.6562	0.0574
X1X3	0.3929	0.1772	0.8711	0.1291
X2X2	0.4350	0.1371	0.0833	0.0898
X2X3	0.9855	0.1357	0.9512	0.4909
X3X3	0.9175	0.5444	0.9747	0.5506

Pu, pulp; pe, peel; TPC, total phenolic compounds; BC, Betalain content; X1, Temperature (°C); X2, Time (h); X3, Solvent (%)
*Significant ($P \leq 0.05$).
[a]quadratic effect of X1; [b]interaction between X1 and X2

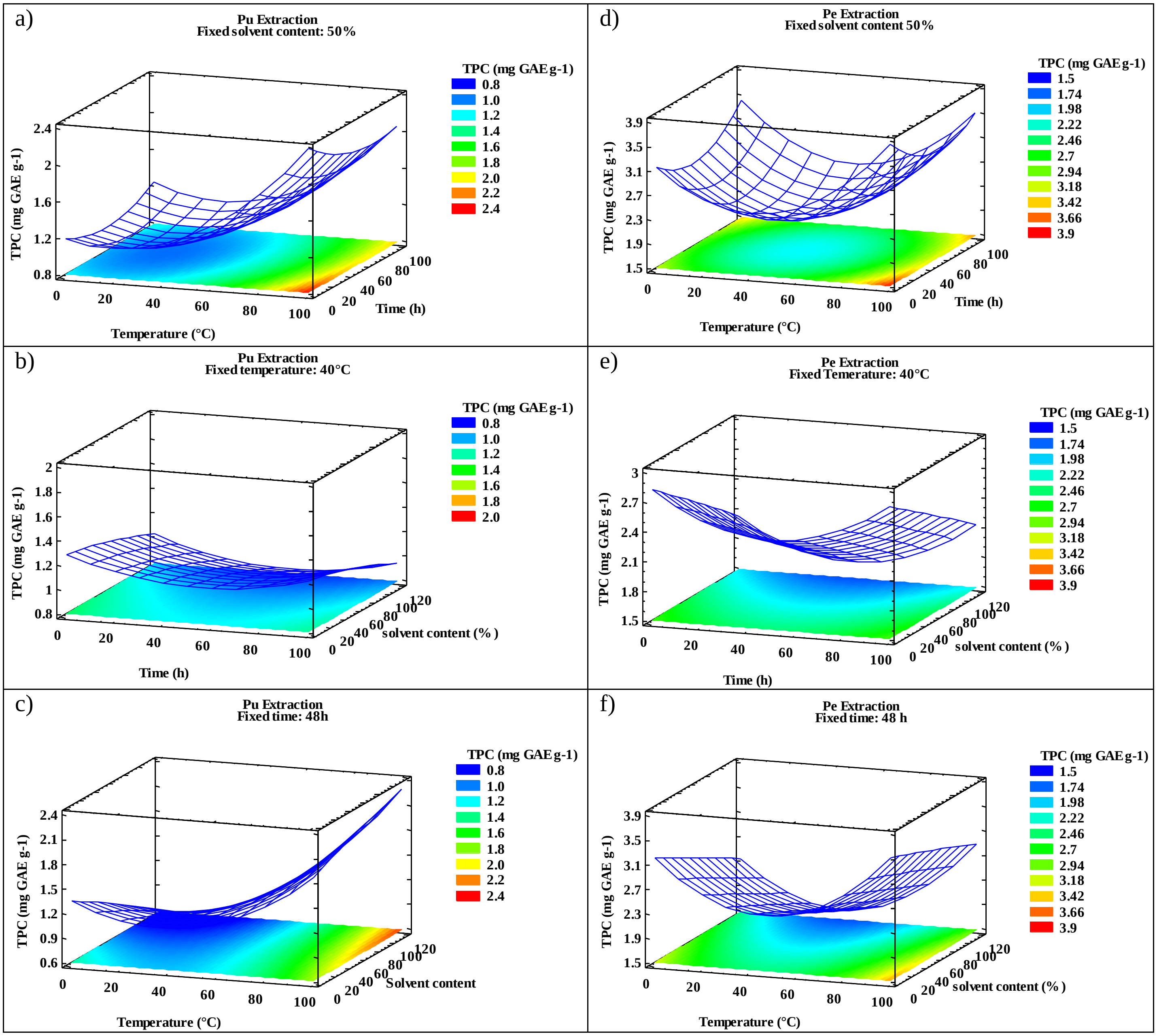

Fig.1. *3D response surface showing the effects of different variables on total phenolic compounds (TPC) for pulp (Pu) extraction (a,b and c) and for peel (Pe) extraction (d, e and f).*

Optimization of extraction time

The effect of extraction time is significant for Pu-BC and Pe- BC ($p<0.05$, Table 5) and is visible when solvent percentage is fixed to 50% (Figure 2 a, d). The effect of time was negative especially when associated with high temperature that led to a decrease in BC to 0 mg/100g (Figure 2 a, d). In addition, when extraction temperature was fixed at 40°C, BC decreased from 21 mg/100g to 6 mg/100g when time increased from 0 h to 100 h using 100% methanol. The optimal extraction time was 0.36h for pulp extraction for both BC and TPC (Table 4). However, for peel extraction optimal condition was 96h for TPC and BC (Table 4).

Optimization of extraction solvent

The effect of methanol percentage was significanton Pe-TPC extraction ($p <0.05$, Table 5). In addition, when solvent percentages increase, the contour color under 3D plot for Pe-TPC changed from green 2.7mg GAE g-1 to blue 1.5 mg GAE g-1(Figure 2 e, f), indicating a negative effect of higher percentages of methanol. This effect is also shown in Table 5where optimal conditions for maximal Pe-TPC was predicted to be 0.005% methanol content. However, in other extraction conditions, higher solvent content of 78.43% and 100% was predicted to maximize TPC and BC extraction from pulp. These results show that TPC molecules found in pulp could be different than peel.

Difference between pulp and peel yield

Opuntiaficus-indica peel yielded higher TPC (2.77mg GAE g-1) than pulp (1.39 mg GAE g-1) (Table 4). However, BC of pulp (19.6 mg/100g) was higher than peel (15.44 mg/100g) (Table 4). In addition, TPC and BC of pulp were maximized using 78.4% and 100% methanol solvent, respectively. However, TPC and BC of OFIpeel were maximized using 0 and 0.01% methanol which mean that phenolic compounds of peel are more extractable using water whereas pulp phenolic compounds and betalain are more extractable using less polar solvent such as methanol. In addition, peels are naturally rich in TPC that deteriorate when temperature increase from 0 to 50°C and then new TPC could be extracted when temperature increase from 50 to 100°C (Figure 1d) whereas Pu-TPC are almost more extracted using high temperature.

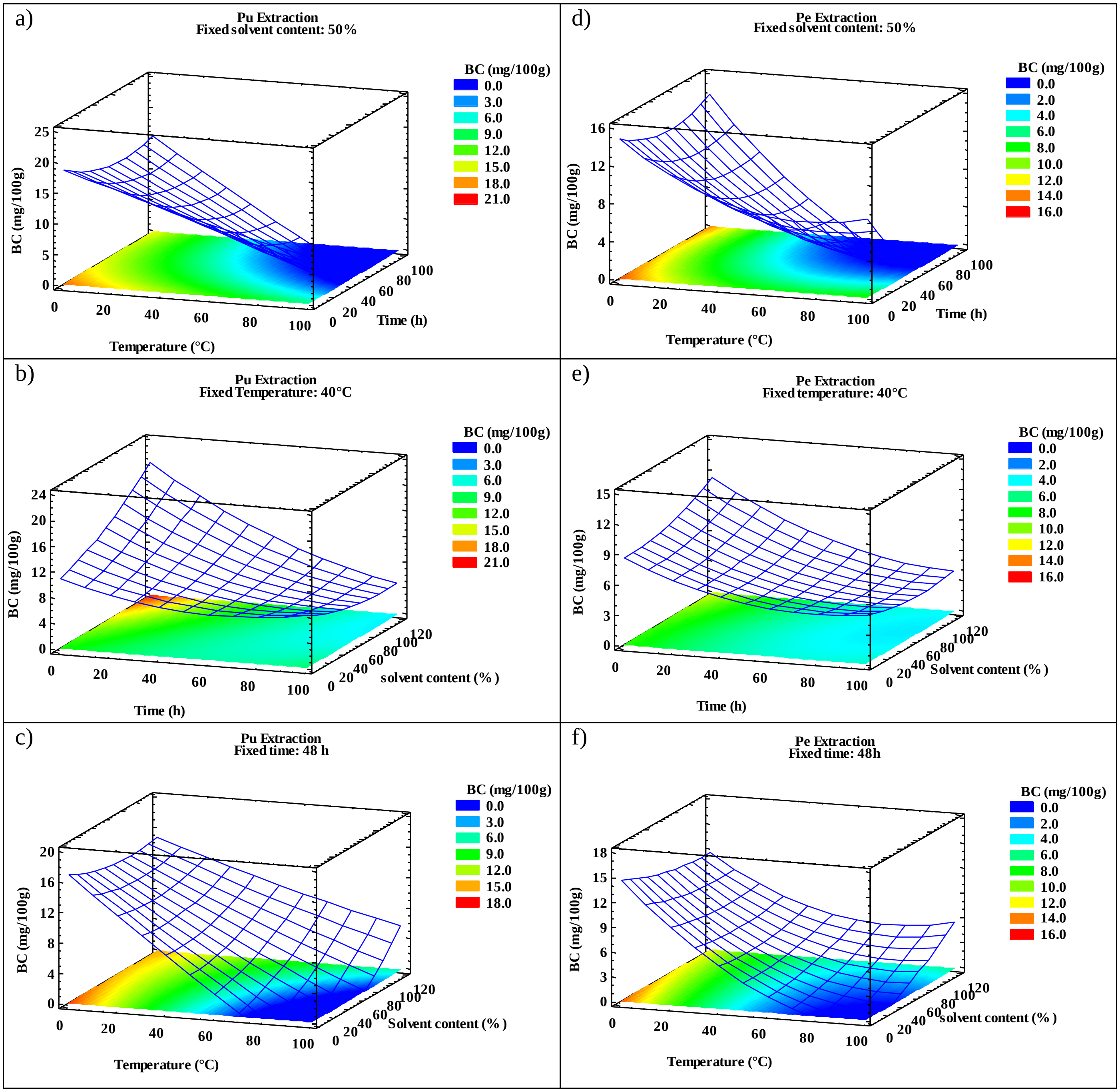

Fig. 2. *3D response surface showing the effects of different variables on betalain content (BC) for pulp (Pu) extraction (a,b and c) and for peel (Pe) extraction (d, e and f).*

Effect of solvent

Our results show that TPC and BC from pulp are more extractable using methanol/aqueous solvent (Tables 1 and 4). However, TPC and BC of peel are more extractable using pure water.Abou-Elella and Ali (2014) obtained higher BC using distilled water instead of methanol from *Opuntiaficusindica* peels. Aruwa*et al*. (2019) used acidified methanol and water, however, they obtained higher TPC for both pulp and peel using acidified methanol. Many authors reported a strong correlation between phytochemicals yields and solvent polarity (Aruwa et al., 2019; El Mannoubi, 2021; Elella and Ali, 2014). In addition, Mannoubi*et al*. (2021) obtained highest TPC from peel using more polar solvent (80% acetone) than less polar solvent (80% ethanol). As for *Opuntia*pulp, El Mannoubi (2021) obtained highest TPC using less polar solvent such as ethanol 80%. These results are in accordance with our results and show that peel contain phenolic compounds extractable with more polar solvent such as water and pulp phytochemicals are extractable with less polar solvents such as methanol.

Effect of time

The effect of extraction time was not significant TPC extraction (Table 5). However, the effect of time was significant and negative for BC (Table 5 and Figure 2 a, b, d, e) possibly because prolonged extraction time lead to the decomposition of bioactive compounds especially at higher temperature (Maran*et al.*, 2013). This effect is shown by contours coloration change from orange to blue under 3D surface plot in Figure 2 (a) and (d) in which higher extraction time and temperature lead progressively to a decrease in BC from 16mg/100 g to 0mg/100g. Similarly, Maran*et al*. (2013) obtained significant negative effect for extraction time on betalain yield and showed a decrease of BC exposed to long extraction duration of over 115 min.

Effect of temperature

A methodology for experimenting most extreme range of temperature extraction was used, because attempts to extract bioactive compounds at temperature higher than 80°C for 96h were unsuccessful due to total evaporation of extraction solvents. Maximum temperature was chosen to be 80°C and minimum 0°C. In our study, we observed an increase of TPC when temperature was increased for both pulp and peel of *O. ficus-indica*. Many studies showed that high extraction temperature increase the solubility of bioactive compounds in solvents by decreasing surface tension (Suh *et al*., 2017). Some studies obtained highest yields of phenolic compounds using even high temperature (93°C) for a shorter time (4h) (Jorge *et al.*, 2013). However, Betalain pigment showed sensitivity and fast degradation when temperature increased above 0°c and we observed total destruction of betalain pigment starting from 70°C and above (Figure 2 a, c, d, f). Khatabi*et al*. (2016) obtained a yield of 10mg/100g BC from red *O.ficussindica* fruits at 40°C. However, in our studywe obtained higher yield of 19.6 mg/100g using lower temperature. Moreover, Martins *et al.*(2017) showed that extraction temperature affects directly betalain stability even at room temperature.

CONCLUSION

Temperature, time and solvent parameters have an important impact on phenolic compounds and betalains yield. More polar solvent are more suitable for peel extraction than less polar solvent. These observations imply that pulp bioactive compounds are different from peel. Taking into account deleterious effect of extraction temperature on betalain pigment and its positive effect on phenolic compounds, temperature has direct impact on the quality and quantity of bioactive compounds. Therefore, extraction time and temperature could be adapted accordingly to the stability of targeted compounds. Further analyses of extracts should help to define precise roles of each parameters on the extraction yield of phytochemicals.

ACKNOWLEDGEMENTS

The authors wish to express their profound gratitude the laboratory services of the Holy Spirit University of Kaslik.

Conflict of interests

The authors declare that there is no competing interest.

REFERENCES

Abou-Elella, F. M., Ali, R. F. M. (2014). Antioxidant and anticancer activities of different constituents extracted from Egyptian prickly pear Cactus (*OpuntiaFicus-Indica*) peel. *Biochem Anal Biochem, 3,* 2161-1009.1000158

Akkol, E. K., Ilhan, M., Karpuz, B., Genç, Y., Sobarzo-Sánchez, E. (2020). Sedative and anxiolytic activities of *Opuntiaficusindica* (L.) Mill.: An experimental assessment in mice. *Molecules, 25,*1844.

Aruwa, C. E, Amoo, S., Kudanga, T. (2019). Phenolic compound profile and biological activities of Southern African *Opuntiaficus-indica* fruit pulp and peels. *LWT,* 111, 337-344.

Assefa, A. D., Keum, Y. S. (2017). Effect of extraction solvent and various drying methods on polyphenol content and antioxidant activities of yuzu (*Citrus junos*Sieb ex Tanaka). *Journal of Food Measurement and Characterization, 11,* 576-585.

Bargougui, A., Tag, H. M., Bouaziz, M., Triki, S. (2019). Antimicrobial, antioxidant, total phenols and flavonoids content of four cactus (*Opuntiaficus-indica*) cultivars. *Biomedical and Pharmacology Journal, 12,*1353-1368

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta, 76,* 965-977.

Carmona, J. C., Robert, P., Vergara, C., Sáenz, C. (2021). Microparticles of yellow-orange cactus pear pulp (*Opuntiaficus-indica*) with cladode mucilage and maltodextrin as a food coloring in yogurt. *LWT, 138,* 110672.

El Mannoubi, I. (2021). Effect of extraction solvent on phenolic composition, antioxidant and antibacterial activities of skin and pulp of Tunisian red and yellow–orange *OpuntiaFicusIndica* fruits. *Journal of Food Measurement and Characterization, 15,* 643-651.

El-Beltagi, H. S., Mohamed, H. I., Elmelegy, A. A., Eldesoky, S. E., Safwat, G. (2019). Phytochemical screening, antimicrobial, antiaxidant, anticancer activities and nutritional values of cactus (opuntiaficus indicia) pulp and peel. *FreseniusEnvironmental Bulletin, 28*(2A), 1534-1551

Fernandez-Lopez, J. A., Almela, L. (2001). Application of high-performance liquid chromatography to the characterization of the betalain pigments in prickly pear fruits. *Journal of Chromatography A, 913,* 415-420.

Ileana, T. K., Guadalupe, G. T., Francisco, T., Ismael, O. G. (2019). Spray drying microencapsulation of purple cactus pear (Opuntiaficusindica) peel extract and storage stability of bioactive compounds. *Emirates Journal of Food and Agriculture,* 958-968.

Jorge, A. J., De La Garza, T. H., Alejandro, Z. C., Ruth, B. C., Noé,A. C. (2013). The optimization of phenolic compounds extraction from cactus pear (*Opuntiaficus-indica*) skin in a reflux system using response surface methodology. *Asian Pacific Journal of Tropical Biomedicine, 3,*436-442.

Kaur, M., Kaur, A., Sharma, R. (2012). Pharmacological actions of *Opuntiaficusindica*: A Review. *J.Appl.Pharm.Sci.*, 2, 15-18.

Khatabi, O., Hanine, H., Elothmani, D., Hasib, A. (2016). Extraction and determination of polyphenols and betalain pigments in the Moroccan prickly pear fruits (*Opuntiaficusindica*). *Arabian Journal of Chemistry, 9,* S278-S281.

Los, F. G. B., Zielinski, A. A. F., Wojeicchowski, J. P., Nogueira, A., Demiate, I. M. (2019). Extraction optimization of phenolic extracts from carioca bean (*Phaseolus vulgaris* L.) using response surface methodology. *Food Analytical Methods, 12,* 148-159.

Maran, J. P., Manikandan, S., Mekala, V. (2013). Modeling and optimization of betalain extraction from Opuntiaficus-indica using Box–Behnken design with desirability function. *Industrial crops and products, 49,* 304-311.

Martins, N., Roriz, C. L., Morales, P., Barros, L., Ferreira, I. C. (2017). Coloring attributes of betalains: a key emphasis on stability and future applications. *Food & function, 8,* 1357-1372.

Martins, S., Mussatto, S. I., Martínez-Avila, G., Montañez-Saenz, J., Aguilar, C. N., Teixeira, J. A. (2011). Bioactive phenolic compounds: production and extraction by solid-state fermentation. A review. *BiotechnolAdv, 29,* 365-373.

Otálora, M. C., de Jesús Barbosa, H., Perilla, J. E., Osorio, C., Nazareno, M.A. (2019). Encapsulated betalains (Opuntiaficus-indica) as natural colorants. Case study: Gummy candies. *LWT, 103*, 222-227.

Rasoulpour, R., Afsharifar, A., Izadpanah, K. (2018). Antiviral activity of prickly pear (*Opuntiaficus-indica* (L.) Miller) extract: Opuntin B, a second antiviral protein. *Crop Protection*, *112*, 1-9

Robert, P., Torres, V., García, P., Vergara, C., Sáenz, C. (2015). The encapsulation of purple cactus pear (*Opuntiaficus-indica*) pulp by using polysaccharide-proteins as encapsulating agents. *LWT-Food Science and Technology,* 60, 1039-1045.

Sarfarazi, M., Jafari, S. M., Rajabzadeh, G. (2015). Extraction optimization of saffron nutraceuticals through response surface methodology. *Food analytical methods*, *8,* 2273-2285.

Sharma, S., Saxena, D. C., Riar, C. S. (2016).Analysing the effect of germination on phenolics, dietary fibres, minerals and γ-amino butyric acid contents of barnyard millet (*Echinochloafrumentaceae*). *Food Bioscience, 13,* 60-68.

Singleton, V. L., Rossi, J. A. (1965).Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*, *16,* 144-158.

Suh, S., Kim, Y. E., Yang, H., Ko, S., Hong, G. (2017). Influence of autoclave treatment and enzymatic hydrolysis on the antioxidant activity of *Opuntiaficus-indica* fruit extract. *ood science and biotechnology*,26, 581-590.

Ye, F., Liang, Q., Li, H., Zhao, G. (2015). Solvent effects on phenolic content, composition, and antioxidant activity of extracts from florets of sunflower (*Helianthus annuus* L.). *Industrial Crops and Products*, *76*, 574-581.

Živković, J., Šavikin, K., Janković, T., Ćujić, N., Menković, N. (2018). Optimization of ultrasound-assisted extraction of polyphenolic compounds from pomegranate peel using response surface methodology. *Separation and Purification Technology, 194,* 40-47.