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Optimization of conditions of ultrasound-assisted extraction of effective compounds from apple pomace (*malus domestica*)

Abstract. Every year millions of tons of fruit peels, such as that from apples, oranges and pomegranates are disposed of as juice factory wastes in our country as well as globally. Unfortunately, Iran with a 15.2-30% green waste production, currently holds the first place in the world fruit waste production. The current study was carried out with the goal of optimizing the ultrasound-assisted extraction of antioxidant compounds from apple pomace. In this study the optimal conditions of ultrasound-assisted extraction (US) of effective compounds from the ethanolic extract of apple pomace by use of the response surface method (Box-Behnken design) with three variables consisting of ultrasound amplitude (20, 60 and 100 %), ultrasound exposure time (15, 35 and 55 min) and ultrasound temperature (35, 50 and 65 ^{0}C). According to Derringer's desired function approach, the optimal conditions based on both individual and combinations of all process variables were ultrasound amplitude 82.36%, ultrasound exposure time 35.24 min and ultrasound temperature 51.48 ^{0} C. At this optimum condition, the predicted maximum values of the total phenolic compound (TPC) 1,1-diphenyl-picrylhydrazyl free radical scavenging (DPPH) and extraction yield (EY) were 74.53% of gallic/ 100 g, 83.85% and 17.74%, respectively. The experimental values were in a good agreement with the predicted values. Also, the TPC, DPPH and EY maceration method were 11.10 mg of gallic acid/100 g, the free-radical scavenging as 2.79% and the extraction efficiency as 4.46%. The results demonstrated that US could be a very effective method for continuous extraction of natural compounds.
Key words: apple pomace extract, Box-Behnken design, total phenolic compounds, ultrasound.

Introduction

Every year millions of tons of fruit peels such as that from apples, oranges, and pomegranates are disposed of as juice factory wastes in our country all the while in the world as we know it today special attention is given to optimum use of plant wastes and extraction of bioactive compounds, namely, antioxidants and antimicrobials. Unfortunately, Iran with a 15.2-30% green waste production, currently holds the first place in the world fruit waste production. On the one hand, wastes lead to loss of national resources, on the other hand, the disposal of which causes some difficulties. For example, wastes rich in phenolic compounds which are usually buried in the ground, in addition to being expensive, cause environmental problems [1].

One of the valuable sources for the production of natural additives is apple pomace. Apple pomace is the main product of the juice and apple center industries which consists of 25% of the original fruit volume with a humidity content (wb) of 85% [2].

Pomace is rich in raw fiber, protein, vitamin, and minerals. Its main compounds are simple sugars such as glucose, fructose, and arabinose while its insignificant compounds include sucrose, galactose, and xylose. Hence, several microorganisms are able to make use of apple residue as a substrate for growth [3].

Phenolic and flavonoid compounds are often gathered in apple fruit peel, and the major of which

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are phenolic acids, flavonols, quercetin, glycoside, di-hydrochalcones and anthocyanin [4].

The extraction method of the plant extract is one of the main factors which can affect the properties of effective constituents of extracts [5]. Thermal extraction causes antioxidant degradation and reduces the antioxidative activity of the produced extract. There are different extraction methods from plants and fruits [6-8]. Although, use of novel and practical methods such as the ultrasound process for extraction of higher quality, more stable, soluble in water and remaining safe solvents extracts along with reduced related expenses (high efficiency and low energy and water consumption), seems essential [9, 10].

Ultrasound-assisted extraction (UAE) is an emerging potential technology that can accelerate heat and mass transfer and has been successively used in extraction field. UAE is a clean method that avoids the use of large quantity of solvent along with cutting down in the working time. Ultrasounds are successively employed in plant extraction field [11, 12].

Hammi *et al*. (2015) has found that the optimum operating conditions for extraction of antioxidant from *Zyzyphus lotus* fruit by ultrasound was ethanol concentration of 50 %, extraction time of 25 min, extraction temperature of 63°C. The obtained extract exhibited a high content of phenolic compounds (40.782 mg gallic acid equivalents/g dry matter) with significant antioxidant properties (the total antioxidant activity was 75.981 mg gallic acid equivalents/g dry matter and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was 0.289 mg/mL) [13]. Similarly, Luengo *et al.* (2014) reported that the use of ultrasound has been proved as a promising technology to extract carotenoids from tomato by-product (skin, seeds, and part of the pulp). Ultrasound significantly increased the extraction yield (143 %) without any degradation of carotenoids in comparison with conventional extraction [14].

The study by Sharayei *et al.* (2017) was performed with the purpose of optimum use of pomegranate peel as the residue of agriculture and pomegranate juice factories, and extraction of bioactive compounds (antioxidative and antimicrobial), employing the ultrasound-assisted method. Results showed that all aqueous extracts of pomegranate and peel, possessed the antioxidative activity and antioxidative strength of aqueous extract of pomegranate peel with a 6% concentration and was roughly the same as that of synthetic butylated hydroxytoluene antioxidants [15]. Kalamara *et al.* (2014) extracted pomegranate seed oil utilizing ultrasound and then dried it via spray drying. The results of this study demonstrated that the ultra-sound method increases production efficiency and reduces extraction time by 12 times [16]. Rosangela *et al.* (2007) examined the chemical composition of yerba mate tea (*Ilex paraguariensis* leaves) using the ultrasound-assisted method. The use of ultrasound waves led to improved caffeine and palmitic acid amounts efficiencies in methanol solvent [17].

Again, in this study, optimization ultrasound-assisted extraction of TPC from apple pomace, and later comparison between the two methods, i.e., maceration and ultrasound are dealt with. The following study was performed due to the importance of optimum use of residue of agricultural products and food industry factories (apple juice factories) and the extraction of bioactive compounds from them.

Materials and methods

Plant materials

Apple wastes were purchased from Iran Citrus Co., located in Tonekabon, Mazandaran Province in August 2016. The apple wastes, after collection at room temperature and away from sunlight, were then completely dried and then ground by using an electric grinder and passed through a mesh strainer No. 20 and kept at a dark, cold and dry place.

Chemical and reagents

All chemicals and reagents used in this study, were analytical grade consisting of Folin-Ciocalteu (FC), gallic acid, DPPH were provided from Sigma-Aldrich (St. Louis, MO), and chemical and organic solvents were purchased from Merck (Darmstadt, Germany).

Extraction procedures

In order to produce extracts from apple wastes, the maceration and ultrasound-assisted methods were used. The solvent used in both methods was 70% ethanol.

Maceration extraction method

For this purpose, 100 g of powdered apple pomace was carefully weighed and poured into a 1000 ml in a beaker containing 70% ethanol solvent and performed on a shaker (Unimax 1010, Heidolph, Germany) for 24 hours at room temperature. The solution was then filtered under vacuum and concentrated up to the point of full dewatering using a rotary dryer (Laborota 4000 efficient model, made in Germany) at 45 ^{0}C in an oven until reaching constant weight under vacuum [18].

Ultrasound-assisted extraction method

For the purpose of extraction of bioactive compounds from apple pomace, a 100 g of powdered

apple pomace was carefully weighed and poured into a 1000 ml beaker containing 70% ethanol solvent and moved to the special ultrasound chamber. For ultrasound, the ultrasonic device model UP400S, made by Heilscher in Germany with 400 W of power and H7 type probe made from titanium and a diameter of 7 mm and a length of 100 mm, was used. For extraction, the effects of ultrasound exposure time (15, 35 and 55 min), ultrasound amplitude (20, 60 and 100%) and ultrasound temperature (35, 50 and 65 ^{0}C) with the sound frequency of 24 kHz based on the variables surfaces predicted in the Box-Behnken design, according to Table 2.

In order to investigate the effect of the Time period of the process, temperature and amplitude on the optimization of extraction conditions of phenolic, tests were performed based on an RSM design in 3 factors and 3 design surfaces and a Box-Behnken design. The software in use was Design Expert and graphs were drawn by Microsoft Excel. The coded and actual levels of each of the variables are given in Table 1.

Table 1 – Valuable codes, independence variables and actual value used in ultrasound method

Independence variables	Valuable codes	Actual value
Time (min)	-1, 0, +1	15, 35, 55
Temperature (^{0}C)	-1, 0, +1	35, 50, 65
Amplitude (%)	-1, 0, +1	20, 60, 100

Table 2 – Random treatments of the Box-Behnken design experiment

Treatment	Time (min) (A)	Temperature (^{0}C) (B)	Amplitude (%) (C)
1	35	50	60
2	35	50	60
3	35	50	60
4	35	65	20
5	55	50	100
6	15	50	20
7	15	35	60
8	55	65	60
9	15	50	100
10	55	50	20
11	35	65	100
12	35	35	100
13	35	35	20
14	15	65	60
15	35	50	60

Measurement of TPC

The TPC amount in the extract produced from the two extraction methods, maceration and ultrasound, was determined using Folin-Ciocalteu methods [19].

For 500 µl test sample (100 mg extract in 10 mL of Methanol), 6 ml of distilled water twice and 500 µl of the reagent Folin– Ciocalteau were added, after waiting for 8.8 minutes at room temperature, 1.5 ml of sodium carbonate (20% w/v) was added to the solution. The solution was mixed and allowed to remain for 30 min, and the spectrophotometric analysis was performed at 765 nm. The standard curve was obtained by plotting gallic acid adsorption data at 765 nm at a concentration of 0.04-0.4 mg/ml. The results were reported in terms of mg of gallic acid per 100 g.

Determination of antioxidant capacity

Determination of the free radical-scavenging activity was performed through the use of the DPPH test and 2, 2-diphenyl picrylhydrazyl reagent [20].

The radical-scavenging activity was calculated as a percentage of DPPH discoloration using the Eq. (1):

$$DPPH\% = [(A_{DPPH}-A_S)/A_{DPPH}] \times 100 \qquad (1)$$

where A_S is the absorbance of the solution when the sample is added at a particular level and A_{DPPH} is the absorbance of the DPPH solution.

Extraction Yield

The extraction yield is given by the following formula (Eq. 2).

$$EY = \frac{dried\ extraction\ weight}{inital\ sample\ weight} * 100 \qquad (2)$$

Results and discussion

Model fitting

After data analysis with the goal of determining the best-suggested model from the 5 existing Mean, cubic, 2FI, Linear, variance analysis, the model, for which the sum of squares had significant differences, and the Lack of fit was insignificant, was selected as the best model. Given this subject and after examining the results obtained and a comparison between the existing regression models, the results indicated that the Quadratic model for total phenolic compound, DPPH Free radical-scavenging capacity and measured extraction efficiency tests in this study, had a significant difference compared to the other models.

There models for which the Lack of fit was undefined (Table 3). Consequently, the Quadratic model was selected for examining the trend of variations of the parameters measured in this study. After selecting the best model in the desired statistical surface (1% or 5%), in order to examine the effective parameters in the study, with regard to the variance table, the parameter for which the F test insignificant (P>0.05), is eliminated and the rest of parameters which had significant differences were kept.

It is worth mentioning that in the case that the linear parameter of a variable in a model, does not have a significant effect, yet its mutual effect with one of the other variables, which has a significant effect in the model, does have a significant effect, then that parameter is kept in the model and afterward the general equation is derived for any parameter, by the given coefficients. Ultimately, from amongst the different parameters, the parameter which has the highest sum of squares is selected as the most effective parameter.

Table 3 – Regression coefficients of predicted polynomial models for the investigated responses from apple pomace extract

Source	df	EY (%)		TPC (mg GA/100 g)		DPPH (%)	
		Coefficient	Sum of Squares	Coefficient	Sum of Squares	Coefficient	Sum of Squares
Model	9	3.56	48.97	-183.29	9911.71	36.93	1732.43
Linear							
Time (A)	1	0.09	7.26	0.025	3257.05	-0.097	0.0015
Temperature (B)	1	0.01	0.80	7.87	904.83	0.366	208.28
Amplitude (C)	1	0.19	12.70	3.28	1371.83	0.774	633.86
Quadratic							
A*A	1	-0.0029	5.14	-0.0084	41.87	-0.019	228.91
B*B	1	-0.0004	0.044	-0.064	780.33	-0.0087	14.42
C*C	1	-0.0013	18.12	-0.016	2639.61	-0.0021	44.04
Interaction							
A*B	1	0.002	2.82	-0.018	121.88	0.029	320.77
A*C	1	0.00036	0.34	0.0079	160.53	-0.0002	0.198
B*C	1	-0.0014	3.06	-0.024	876.75	-0.014	305.20
Residuals	5		4.84		195.15		82.71
Lack of Fit	3		0.95		128.66		14.76
Pure Error	2		3.88		66.49		67.95
Total	14		53.80		12077.7		1815.14
Std. Dev		0.98		15.04		4.07	
Mean		8.93		63.37		45.36	
CV (%)		11.02		23.73		8.97	
R^2		0.91		0.89		0.95	
Adj R^2		0.74		0.71		0.87	

Std. Dev: Standard Deviation

Table 4 – Box-Behnken design of three variables with their observed responses of ultrasound-assisted extract

Time (min)	Temperature (^{0}C)	Amplitude (%)	EY (%)		TPC (ml GA/100 g)		DPPH (%)	
			Real Values	Predicted Values	Real Values	Predicted Values	Real Values	Predicted Values
35	50	60	19.80	20.40	15.31	22.52	84.61	77.52
35	50	60	9.00	7.50	50.66	52.73	72.51	69.94
35	50	60	13.80	13.20	23.63	16.42	43.25	49.86
35	65	20	18.80	17.13	73.13	72.86	78.72	81.82
55	50	100	20.60	18.50	51.34	46.21	66.49	62.11
15	50	20	8.00	8.00	8.90	8.90	77.41	69.94
35	50	60	10.20	12.30	49.46	54.59	21.47	26.82
15	35	60	18.41	17.13	68.24	72.86	77.93	81.82
55	65	60	16.26	17.13	80.34	72.86	85.52	81.82
15	50	100	11.00	10.40	85.24	78.03	72.12	76.80
55	50	20	20.00	17.90	10.43	5.30	58.12	49.86
35	65	100	7.60	9.70	34.17	39.31	70.73	77.52
35	35	100	17.00	17.60	22.34	29.55	60.47	56.76
35	35	20	15.03	17.13	69.75	72.86	83.17	81.82
15	65	60	20.40	21.90	12.42	10.34	69.47	77.57

Investigating the effect of independent variables on qualitative and quantitative properties of Apple pomace

Effects of process variables on TPC extraction

The results from the variance analysis (Table 3) illustrates that the linear models of time (A) and ultrasound amplitude (C) and the square of temperature (B^2) have a significant effect on the TPC amount.

With regard to Equation 3 and Table 3, the parameter B^2 (the mutual effect of temperature), was selected as the most effective factor in the extraction of TPC.

$$TPC = -361/31926 - 0/88101A + 17/37277B + +1/06101C - 0/031319A2 - 0/19439B2 - -5/15113E\text{-}003C2 + 0/049792AB - -2/11781E\text{-}003AC - 2/36167E\text{-}003BC \quad (3)$$

In the RSM methodology, there is a step called verification. In this step, the value for TPC extraction in the extermination step should be statistically compared to the value predicted by the model. In this examination, after having carried out this step, the observed values were compared to the predicted values and the calculations may be seen in Table 4. The results are demonstrative of the good correlation between results obtained from the experimental method and the values predicted with the statistical method.

Regarding the mutual effect of temperature and ultrasound amplitude, the response surface plot (Figure 1, A), with temperature rising up to 48 ^{0}C, the TPC percentage increased, both in low and high ultrasound intensities and a trend of decrease was observed in the TPC, which was stronger at high ultrasound intensities. Additionally, at both low and high temperatures, with an increase in ultrasound amplitude up to 60%, the TPC level expanded and up to 80% this growing trend was observed gradually, and its amount had dropped thereafter. Such findings are in line with by the Pinelo *et al.* (2005) they declared that at high temperatures, drops in the phenolic compounds extraction amount has been witnessed [21]. This scholar has stated the reason behind this to be the polymerization reactions of phenolic compounds. Furthermore, in the study performed, the explanation for the TPC extraction amount decrease, according to the response surface diagram, may be solvent satura-

tion at lower temperatures and dissolution of TPC at high temperatures. In the research carried out on the extraction of polyphenolic compounds from some kind of raspberry, the highest polyphenolic compounds extraction efficiency was reported at 60 ^{0}C.

Investigation of the phenol variations against temperature and time showed that at both the start and finish of the experiment, with a rise in temperature up to 47.21^{0}C, the TPC increased and then a trend of decrease was observed in the TPC extraction amount. Considering Figure 1, B, it can be said that temperature and time are significantly effective on the amount of TPC extraction such that with an increase in temperature and time, a trend of increase has been witnessed in the TPC extraction amount. Rises in temperature lead to a growth in the solvent penetration rate and also increases the mass transfer period. Taking into account, the fact that the sample/solvent ratio was assumed to be constant in this study, the explanation for the TPC extraction amount decrease, according to the response surface diagram, may be solvent saturation at lower temperatures and dissolution of TPC at high temperatures.

The contents of TPC extracted from wheat bran at different times of sonication increase of the total phenolics content was observed up to 30 min, remaining constant until 50 min. A significant increase of the total phenolic content was observed over the extraction temperature range (25–75 ^{0}C), and the total phenolic content reached a maximum of around 2.80 mg GAE/g of wheat bran at 65 ^{0}C. At a higher temperature, the solubility of phenolic compounds in wheat bran could be enhanced, and the viscosity of wheat bran extracts was decreased, accelerating the whole extraction [19].

Spigno *et al*. (2007) investigated the relationship of extraction temperature and extraction time with TPC was demonstrate both of the factors displayed significant linear and quadratic effect (at least at p<0.05) on TPC. With regard to extraction temperature, TPC of limau purut peels extracts increased readily with increasing temperature up to 47.5^{o}C and followed by a slight decrease afterwards (22). This suggested that incubation in warm water did improve phenolics extraction, yet was gentle enough to avoid heat degradation of the target phenolic antioxidants. Mild heating might soften the plant tissue, weaken the cell wall integrity, hydrolyze the bonds of bound phenolic compounds (phenol-protein or phenol-polysaccharide) as well as enhance phenolics solubility, thus more phenolics would distribute to the solvent [23, 24]. At optimum extraction temperature (about 47.5^{o}C), higher amounts of phenolic contents were obtained with short extraction time. In other words, long extraction time may compensate the beneficial effects of moderate temperature by inducing oxidation or degradation of phenolic compounds, yielding low TPC [23, 25].

In the research carried out by Ya-Qin *et al.* (2009) for all detected phenolic acids under study, their yields depended dramatically on extraction time and temperature. At relatively lower temperature (15 and 30 ^{0}C), the yields of phenolic acids increased with increased extraction time. For example, after UAE at 15 ^{0}C for 40 min, the yields of caffeic, p-coumaric, ferulic, p-hydroxy-benzoic acid increased from 15.4 to 103.7, 36.4 to 171.3, 452.3 to 1672.3, 18.3 to 42.0 μg/g of DW, respectively (26). In agreement with these results, Sueli and Pinto (2007) reported that extraction time significantly affected the yields of phenolic compounds from coconut shell powder. Likewise, the yield of phenolic compounds increased also with extraction temperature increased. For example, after UAE from 15 to 30 ^{0}C for 20 min, the yield of caffeic acid increased from 36.9 to 86.7 lg/g DW, 80.0 to 165.3 lg/g DW in p-coumaric acid, 951.8 to 2283.3 lg/g DW in ferulic acid, 27.0 to 43.4 lg/g DW in p-hydroxy-benzoic acid, respectively [27].

Results acquired from the Ahmadian and Niazmand study (2016) illustrate the specific effect of the mean temperature (43˚C) on the extraction of polyphenolic compounds from the saffron petal [28]. The effect of temperature on increasing extraction efficiency is most probably due to the improvement of mass transfer at a higher temperature and therefore increasing the solubility of phenolic compounds, increasing the penetration rate and decreasing the coefficient of viscosity, of the solvent. In addition, the rise in extraction temperature, bringing about the dissociation of the bonds of the phenolic compounds with the rest of the compounds and affecting the membrane structure of plant cells can be responsible for extraction procedure facilitation.

In agreement with this research, also researchers showed that the extraction yield increased with increasing temperature. When extraction temperature increased from 30 to 50 °C, the extraction yield increased from 17.6±0.4 to 30±0.8%. Similarly, the total phenolic content also increased from 21±0.6 to 23± 0.3 mg/g DW. A rise in extraction temperature can break the phenolic matrix bonds and influence the membrane structure of plant cells making them less selective by coagulation of lipoproteins [29]. The dielectric constant of water decreases and solvent property and capacity change at higher temperature, resulting in a better extraction of phenolic [5].

However, further increase in extraction temperature could degrade phenolic compounds [30, 31].

On the mutual effect of time and ultrasound amplitude in the response surface diagram (Figure 1, C), by raising the ultrasound amplitude up to 60%, the amount of TPC increases and then until 87.93% of amplitude a marginally rising trend was observed and extraction declined thereafter. Yet, by increasing time at low ultrasound intensities, the extraction amount, until 25 minutes, had a slowly increasing trend and then a sensible declining trend in the amount of TPC extraction was observed. In addition, at higher sound intensities, at times higher than 35 minutes, the declining trend occurred more steeply. The principal mechanism of ultrasound-assisted extraction is due to the cavitation phenomenon which leads to the generation of micro bubbles and subsequently an implosion within the liquid mass. The implosion of these bubbles is often accompanied by release vast amounts of energy which is exerted on the nearby environment in the form of shear stress [32]. The decline in the effective constituent extraction amount, caused by increasing the ultrasound amplitude from 80% to 100%, is probably a result of the destruction of some active natural compounds because of the high amplitude of the waves [33]. Moreover, it has been reported that application of ultrasonic waves with high intensities and time periods, having brought about the enhanced cell wall disruption, leads to the extraction of insoluble compounds within the solvent, which results in the lower solvent penetration of cells [34, 35].

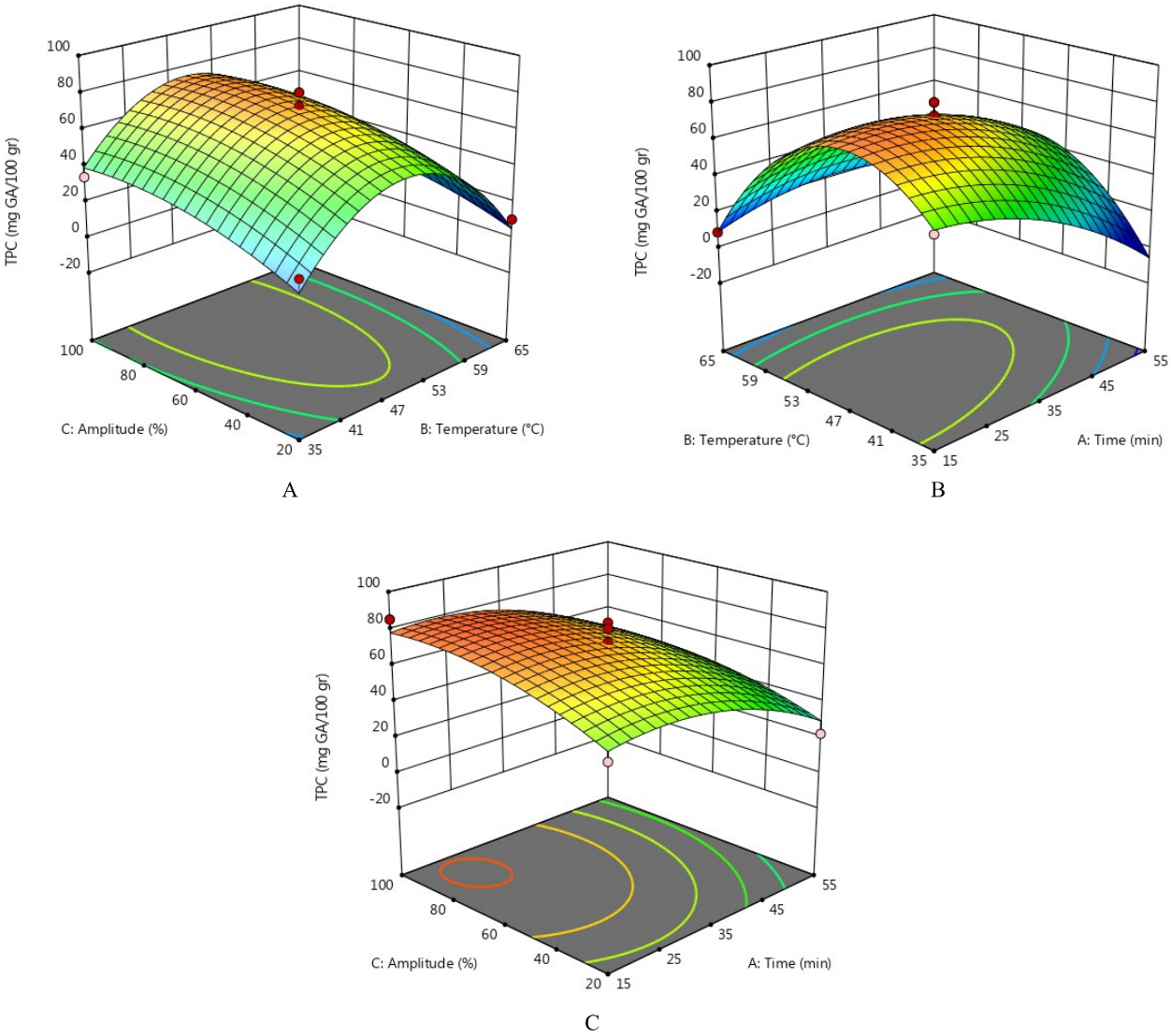

Figure 1 – Response surface plots of the total phenolic contents (TPC) of apple pomace extract as affected by A: ultrasound amplitude and ultrasound exposure time, B: temperature and time C: ultrasound amplitude and time

Effects of process variables on antioxidant activity

The results of the variance analysis (Table 3) showed that the linear amplitude model, the square of the amplitude (C^2) and the mutual effect of time and amplitude have a significant effect on the free-radical scavenging capacity.

With regard to Equation 4 and Table 3, the amplitude and the temperature parameters were reported as to possessing the most and least effective parameter upon the scavenging of free radicals, respectively.

$$DPPH = -40.38433 + 2.43910A + 2.19379C - 0.020167A^2 - 0.011332C^2 - 0.013948AC \quad (4)$$

On the mutual effects of time and amplitude in the response surface diagram (Figure 2), by increasing the amplitude to 60%, the free-radical scavenging amount is increased and thereafter until 76.94% of amplitude, a marginally increasing trend was observed and the scavenging capacity was reduced. At low sound intensities, through increasing time, at first, the scavenging amount grew until 35.91 minutes and then was diminished. More on the matter, a trend of decrease was observed at higher sound intensities.

The cause behind the aforementioned findings may either be oxidization of phenolic compounds due to prolonged extraction time or increase in impurities as a result of ultra-sonication time [36]. Heidari *et al.* (2011) investigated the antioxidantal properties of *Flomidoschema parviflora* [37]. These researchers reported that by means of increasing the time and temperature, the free-radical scavenging has risen which is because of the growth of TPC extraction with the increase in time and their inhibition effect on the free radicals. Yet, with increasing time (times greater than 46 minutes) this trend has become very gradual and the free-radical scavenging amount declines, with which study, the results from the relation by increasing time to 36 minutes, agree.

In the research on grape peels conducted by Ghafoor (2009) in order to optimize the conditions of ultrasound-assisted extraction, by means of the two extraction temperature and ultrasound amplitude parameters, it was determined that with rises in the extraction temperature and ultrasound amplitude the free-radical scavenging capacity increases [38].

Researchers demonstrated that when the results for fruits were omitted from the regression analysis, the relationship between the phenolic contents and antioxidant activities was significant ($p < 0.05$). This indicates that factors other than total phenolic can play a major role in the antioxidant activity of plant materials such as sea buckthorn [39].

In the research carried out by Ahmadian and Niazmand (2016) the results showed that with an increase in the amplitude, the free-radical scavenging amount has grown, in such a way that at 100% of amplitude, the highest inhibition percentage was observed. In fact, this is a result of the higher poly phenolic compounds extraction at this amplitude and therefore increasing cell wall destruction and expanded exit and access of such materials. Additionally, by raising the extraction time up to the maximum (15 minutes), a significant increase of inhibition percentage was witnessed. According to the results obtained, an increase in poly phenolic compounds extraction was also observed by increasing time. Therefore, one may relate the growth in inhibition percentage to the enhanced extraction of such compounds by increasing time and in the current study, by means of increasing the amplitude up to 76%, the highest free-radical scavenging capacity was observed as well [28].

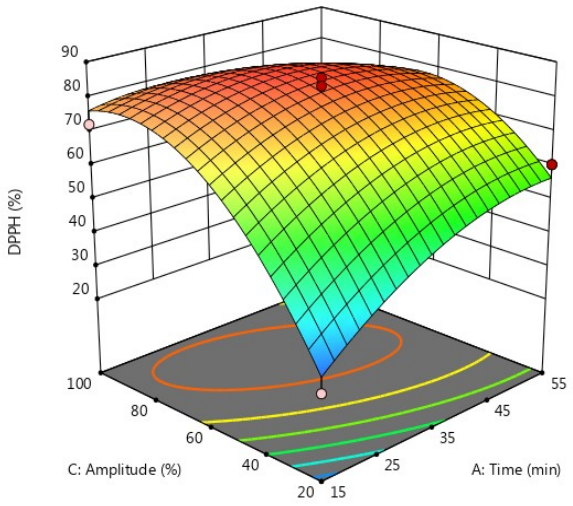

Figure 2 – Response surface plots of the DPPH of pomace extract as affected by ultrasound amplitude and ultrasound exposure time

Effects of process variables on Extraction yield

The variance analysis table (Table 3) shows that the linear models of time (A) and temperature (B)

have a significant effect on the amount of extraction efficiency.

With regard to the data from the sum of squares column, the effect of temperature was introduced as the most significant.

$$\text{yield} = -9.14488 + 0.094688A + 0.89222B - 0.22844C - 8.28125E\text{-}003A^2 - 0.012056B^2 + 5.54688E\text{-}004C^2 + 0.012000AB + 8.75000E\text{-}004AC + 2.50000E\text{-}003BC \quad (5)$$

On the mutual effect of time and temperature, taking the response surface diagram (Figure 2, A) into account, at low times, with an increase in temperature up to 60 ^{0}C, the extraction efficiency is increased and then was diminished. Yet at high times, with a rise in temperature up to 65 ^{0}C, the extraction efficiency ratio grew and also at low temperatures, with a time increase up to 51.97 minutes, the extraction efficiency ratio was enhanced and then it was lowered, and at high temperatures up to 55 minutes of time, it was added on the extraction amount, as well. Temperature is one of the key factors in the extraction efficiency amount, in a way that the rise in temperature causes an increase in the solvent penetration rate. The drop in the effective constituents' extraction amount with rises in temperature from 60 to 65 ^{0}C and time from 51 to 55 minutes is probably due to the destruction of some active natural compounds.

On the mutual effects of time and amplitude in the response surface (Figure 2, B), at low sound intensities, with an increase in time up to 44 minutes, the extraction efficiency ratio is enhanced and then declines with a steep slope. Moreover, also at high sound intensities, the extraction efficiency amount rises with a sharp incline and reduced with a very gradual incline and in which case of the extraction time is constant, with a rise in the amplitude amount, the extraction efficiency amount increases. The extraction amount growth by an increase in amplitude is probably because of the cavitation phenomenon resultant of the ultrasound; the cavitation phenomenon leads to increased plant tissue [40]. The decline in the effective constituent's extraction amount, caused by increasing the ultrasound amplitude from 80% to 100%, is probably a result of the destruction of some active natural compounds because of the high amplitude of the waves [33].

Considering the response surface diagram (Figure 2, C), at low sound intensities, with a rise in temperature up to 57.51 ^{0}C, the extraction efficiency amount increases and was reduced thereafter with a smooth slope and also at high sound intensities up to 65 ^{0}C, it grew. In addition, with increases in temperature at low sound intensities, the extraction efficiency grew but with more rapid incline at high sound intensities.

A study on the carambola or star fruit, and lemon peels in order to find the optimal extraction conditions approves of our result [25].

Optimization comparison ultrasound-assisted extraction with conventional extraction

The measurement of TPC, free-radical scavenging, and extraction efficiency amount through maceration and ultrasound methods demonstrated the method of extraction has a profound effect on the total amount of TPC in terms of gallic acid. As apparent, the ultrasound method has extracted more TPC compared to the maceration method (for the ultrasound method, the TPC amount in terms of mg of gallic acid/ 100 g was obtained as 74.52 mg, the free-radical scavenging amount as 73.85% and the extraction efficiency as 17.75% and for the maceration method the TPC amount was obtained as 11.10 mg of gallic acid/ 100 g, the free-radical scavenging as 2.79% and the extraction efficiency as 4.46%). It can be said the shear stress exerted by ultrasonic waves lead to the breakdown of large polymer molecules which in turn results in the better extraction of TPC compared to the maceration method. These results are in accordance with the report by Albu *et al.* (2004) and they reported utilization of the ultrasound method has brought about the added extraction of carnosic acid from rosemary [41].

Hemwimol *et al.* (2006) investigated the use of ultrasound-assisted extraction for improvement of anthraquinones from roots *morinda citrifolia* with solvent [42]. Ultrasound-assisted extraction in a system (water-ethanol), led to a 75% decrease in time and extraction efficiency as compared to samples not treated with ultrasonic waves.

In agreement with this research, also Heidari majd *et al.* (2012) demonstrate that yield extraction of phenolic compounds in ultrasound method is more than maceration method [37].

C´ujic. N *et al.* (2016) showed that extract obtained by maceration contained higher yield of TP (27.6 mg GAE/g) than extracts obtained by ultrasonic-assisted extraction for 30 min (25.4 mg GAE/g). These preliminary results indicate that maceration could be preferable method for the extraction of polyphenols from chokeberry fruit under tested conditions [43].

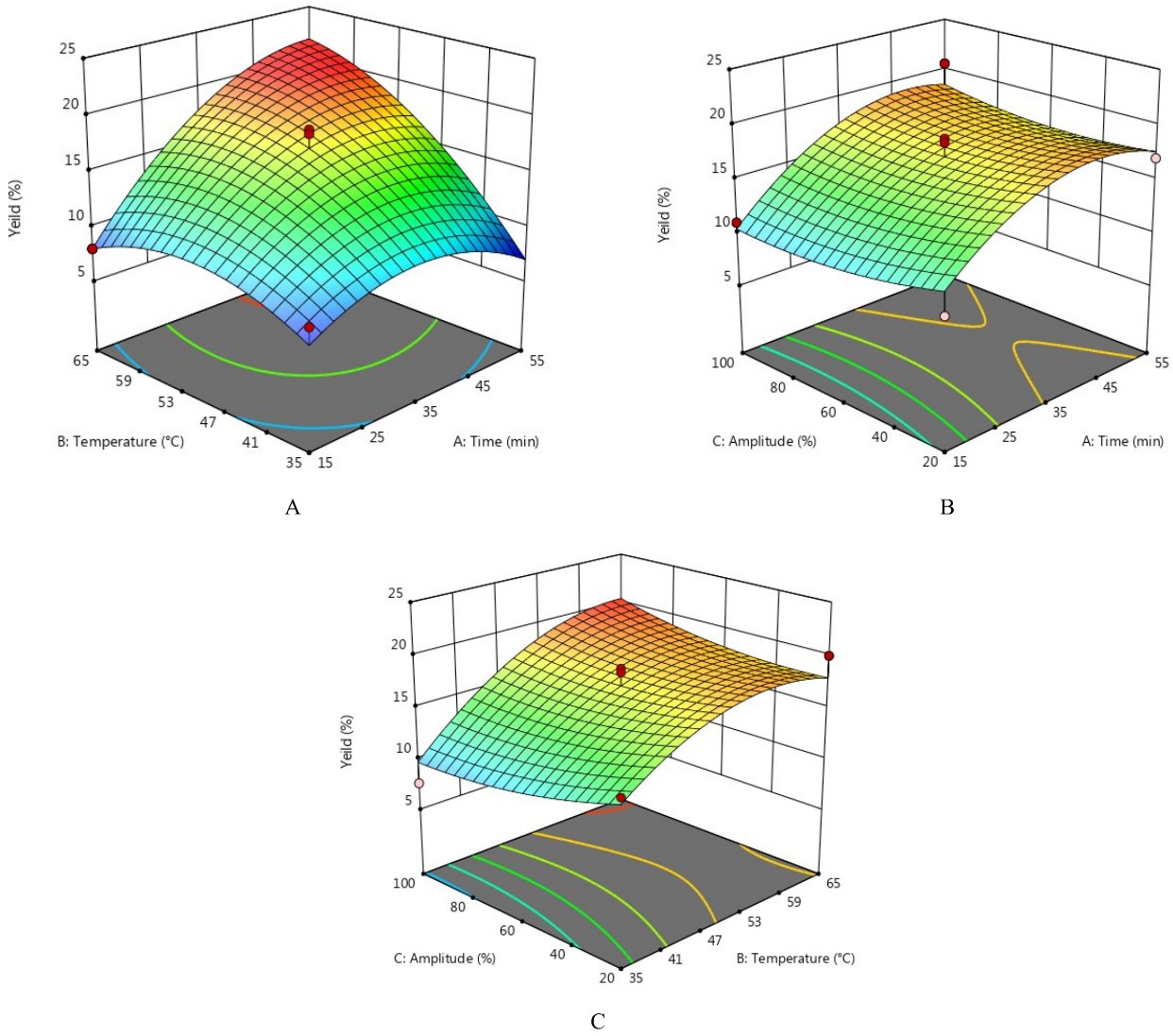

Figure 3 – Response surface plots of the extraction yield (Yield) of apple pomace extract as affected by A: ultrasound amplitude and ultrasound exposure time, B: temperature and time C: ultrasound amplitude and time

Table 5 – Comparison of the maceration and ultrasound methods

Ultrasound	Maceration	
74.53	11.70± 0.28	TPC (mg GA/100 g)
83.85	2.79± 0.38	DPPH (%)
17.74	4.49± 0.14	YIELD (%)

Mean ± standard deviation

Conclusion

Apple pomace has agreeable antioxidantive activity due to possessing high amounts of polyphenolic compounds and flavonoids. Therefore, it is possible to have it introduced as a natural antioxidant source within the food industry. Analysis of the Box-Behnken design response surface with 3 independent variables consisting of time, temperature and device ultrasound amplitude serving as effective parameters on the extraction of antimicrobial compounds from apple pomace was carried out. All three extraction temperature, extraction time and ultrasound amplitude led to the increasing antioxidantal activity of the treatments. In this study, a model which its sum of squares amount had a significant difference and its lack of fit was insignificant was selected as the best model. On the one hand, by utilizing the models, adjustment of the extraction conditions is made possible, and on the other, considering the utilized extraction conditions, predict and rectify the desired properties.

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References

1 Negro C., Tommasi L., Miceli A. (2003) Phenolic compounds and antioxidant activity from red grape marce extracts. Bioresource Technology., no. 87, pp. 41- 44. https://doi.org/10.1016/S0960-8524 (02)00202-X.

2 Sun H., Ge X., Hao Z., Peng M. (2010) Biotechnology Cellulase production by Trichoderma sp. on apple pomace under solid state fermentation. African Journal of Biotechnology. no. 9, pp. 163-166.

3 Kosseva, M.R. (2011) Management and Processing of Food Wastes, in: Moo-Young, M. (Ed.), Comprehensive Biotechnology. Environmental Biotechnology and Safety. no. 6.

4 Takos A., Jaffé F., Jacob S., Bogs J., Robinson S., Walker A. (2006) Light-Induced Expression of a MYB Gene Regulates Anthocyanin Biosynthesis in Red Apples. Plant Physiol., vol. 3, no. 142, pp. 1216–1232.

5 Corrales M., Garcia A., Butz P., Tauscher B. (2009) Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure. Journal of Food Engineering. no. 90, pp. 415–421.

6 Adamez J., Samino E.G., Sanchez E.V., Gomez D.G. (2012) In vitro estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape-seeds (Vitis vinifera L.). Food Control. no. 24, pp.136- 141.

7 Martin J., Porto E., Correa C., Alencar M., Gloria E., Cabral I., Aquino L. (2012) Antimicrobial potential and chemical composition of agro-industrial wastes. Journal of Natural Products., no. 5, pp. 27- 36.

8 Sagdic O., Ozturk I., Kisi O. (2012) Modeling antimicrobial effect of different grape pomace and extracts on S. aureus and E. coli in vegetable soup using artificial neural network and fuzzy logic system. Expert Systems with Applications., vol. 39, vo. 8, pp. 6792- 6798. https://doi.org/10.1016/j.eswa.2011.12.047

9 Porto C.D., Porretto E., Decorti D. (2013) Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (Vitis vinifera L.) seeds. Ultrasonics Sonochemistry., no. 20, pp. 1076- 1080. https://doi.org/10.1016/j.ultsonch.2012.12.002.

10 Wijngaard H., Hossain M.B., Rai D.K., Brunton N. (2012) Techniques to extract bioactive compounds from food by-products of plant origin. Food Research International., vol. 46, no. 2, pp. 505-513. https://doi.org/10.1016/j.foodres.2011.09.027.

11 Luque-Garci´a J.L., Luque De Castro M.D. (2003) Ultrasound: a powerful tool for Leaching. TrAC Trends in Analytical Chemistry., vol. 22, no. 1, pp. 41–47. https://doi.org/10.1016/S0165-9936(03)00102-X.

12 Lopez-Avila V., Young R., Teplitsky N. (1996) Microwave-assisted extraction as an Alternative to soxhlet, sonication, and supercritical fluid extraction. Journal of AOAC Int., no. 79, pp. 142–156. https://doi.org/10.1093/jaoac/79.1.142.

13 Hammi K. M., Jdey A., Abdelly C., Majdoub H., Ksouri R. (2015) Optimization of ultrasound-assisted extraction of antioxidant compounds from Tunisian Zizyphus lotus fruits using response surface methodology. Food Chemistry., vol. 184, pp. 80-89. https://doi.org/10.1016/j.foodchem.2015.03.047.

14 Luengo E., Condon-Abanto S., Condón S., Alvarez I., Raso J. (2014) Improving the extraction of carotenoids from tomato waste by application of ultrasound under pressure. Separation and Purification Technology., vol. 136, pp. 130-136. https://doi.org/10.1016/j.seppur.2014.09.008.

15 Sharayei P., Azarpazhouh A. (2017) Investigating the effect of ultrasound on antioxidant and antifungal properties of pomegranate peel. Agricultural and Natural Resources Research and Education Center, AREEO, Mashhad, Iran, 2017.

16 Kalamara E., Goula A. M., Adamopoulos K.G. (2014) An integrated process for utilization of pomegranate wastes–Seeds. Innovative Food Science and Emerging Technologies., vol. 27, pp.144-153. https://doi.org/10.1016/j.ifset.2014.12.001.

17 Rosangela J., Lisiane F., Valeria P., Claudio D., Ana Paula O., Jose O. (2007) The use of ultrasound in the extraction of Ilex paraguariensis leaves: a comparison with maceration. Ultrasonics Sonochemistry., vol. 14, no. 1, pp. 6-12. https://doi.org/10.1016/j.ultsonch.2005.11.007.

18 Harborne J.B. (1973) Phytochemical methods: a guide to modern techniques of plant analysis. London: Chapman & Hall; New York: [Distributed in the USA by Halsted Press, a Division of John Wiley & Sons].

19 Wang J., Sun B., Cao Y., Tian Y., Li X. (2008) Optimization of ultrasound-assisted extraction of phenolic compounds from wheat bran. Food

Chem., vol. 136, no. 2, pp. 804-810. https://doi.org/10.1016/j.foodchem.2007.06.062.

20 Von Gadow A., Joubert E., Hansmann C.F. (1997) Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (Aspalathus linearis), α-tocopherol, BHT, and BHA. J. Agric. Food. Chem., vol. 3, no. 45, pp. 632-638. https://doi.org/10.1021/jf960281n.

21 Pinelo M., Rubilar M., Jerez M., Sineiro J., Núñez M.J. (2005) Effect of Solvent, Temperature, and Solvent-to-Solid Ratio on the Total Phenolic Content and Antiradical Activity of Extracts from Different Components of Grape Pomace. Journal of Agricultural and Food Chemistry., vol. 53, no. 6, pp. 2111-2117. https://doi.org/10.1021/jf0488110.

22 Spigno G., Tramelli L., Faveri D. M. D. (2007) Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering., vol. 81, no.1, pp. 200-208. https://doi.org/10.1016/j.jfoodeng.2006.10.021.

23 Juntachote T., Berghofer E., Bauer F., Siebenhandl S. (2006) The application of response surface methodology to the production of phenolic extracts of lemon grass, galangal, holy basil and rosemary. International Journal of Food Science and Technology., vol. 41, no. 2, pp. 121-133. https://doi.org/10.1111/j.1365-2621.2005.00987.x.

24 Li B.B., Smith B., Hossain M.M. (2006) Extraction of phenolic from citrus peels II. Enzyme-assisted extraction method. Separation and Purification Technology., vol. 48, no. 2, pp. 189-196. https://doi.org/10.1016/j.seppur.2005.07.019.

25 Chan S., Lee C., Yap C., Mustapha W.A.W., Ho C. (2009) Optimization of extraction conditions for phenolic compounds from limau purut (Citrus hystrix) peels. Int. Food Res. J., vol. 2, no. 16, pp. 203-213.

26 Ya-Qin Ma., Jian-Chu Chen. (2009) Simultaneous extraction of phenolic compound of citrus peel extracts: Effect of ultrasound. Journal of Ultrasonics Sonochemistry., vol.16, no. 1, pp. 57-62. https://doi.org/10.1016/j.ultsonch.2008.04.012.

27 Sueli R., Pinto G.A.S. (2007) Ultrasound extraction of phenolic compounds from coconut (Cocos nucifera) shell powder. Journal of Food Engineering., vol. 80, no. 3, pp. 869–872. https://doi.org/10.1016/j.jfoodeng.2006.08.009.

28 Ahmadian koochaksarayee Z., Niazmand R. (2016) Optimization of Extraction of effective compounds from saffron petals by ultrasound-assay method. Novel food technology., no. 13, pp. 121-135.

29 Fernandez D. P., Goodwin A. R. H., Lemmon E. W., Levelt-sengers J. M. H., Williams R. C. (1997) A formulation for the static permitivity of water and steam at temperature from 238 K to 873 K at pressures up to 1200 MPa, including derivatives and Deby–Hückel coefficients. Journal of Physical Chemistry., vol. 26, pp. 1125−1166.

30 Durling N. E., Catchpole O. J., Grey J. B., Webby R. F., Mitchell K. A., Foo L. Y., Perry, N.B. (2007) Extraction of phenolics and essential oil from dried sage (Salvia officianlis) using ethanol-water mixtures. Food Chemistry., vol. 101, no. 4, pp. 1417−1424. https://doi.org/10.1016/j.foodchem.2006.03.050.

31 Prasad N. K., Yang B., Zhao M., Wang B., Chen F., Jiang Y. (2008) Effects of high pressure treatment on the extraction yield, phenolic content and antioxidant activity of litchi (Litchi chinensis Sonn.) fruit pericarp. International Journal of Food Science and Technology.doi:10.1111/j.1365-2621.2008.01768.x

32 Ji J.B., Lu X.H., Cai M.Q., Xu Z.C. (2006) Improvement of leaching process of Geniposide with ultrasound. Ultrasonics sonochemistry., vol. 13, no. 5, pp. 455-462. https://doi.org/10.1016/j.ultsonch.2005.08.003.

33 Lianfu Z., Zelong L. (2008) Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. Ultrasonics sonochemistry., vol. 15, no. 5, pp. 731-737. https://doi.org/10.1016/j.ultsonch.2007.12.001.

34 Liu S., Yang F., Zhang C., Ji H., Hong P., Deng C (2009) Optimization of process parameters for supercritical carbon dioxide extraction of Passiflora seed oil by response surface methodology. The Journal of Supercritical Fluids., vol. 48, no. 1, pp. 9-14. https://doi.org/10.1016/j.supflu.2008.09.013.

35 Tian Y., Xu Z., Zheng B., Lo Y.M. (2013) Optimization of ultrasonic-assisted extraction of pomegranate (Punica granatum L.) Seed oil. Ultrasonics Sonochemistry., vol. 20, no.1, pp. 202–208. https://doi.org/10.1016/j.ultsonch.2012.07.010.

36 Zhang L. (2011) Estimating covariation: Epps effect and microstructure noise. Journal of Econometrics., vol. 160, no. 1, pp. 33–47. https://doi.org/10.1016/j.jeconom.2010.03.012.

37 Heydari Majd M., Mortazavi A., Asili J., Bolouriyan Sh. (2012) Optimization of Extraction of Phenolic compounds from Flomidoschema parviflora by ultrasound-assay method Herbal Medicine., no 1, pp. 7-13.

38 Ghafoor K,. Choi Y., Yeong Jeon, Ju., Hee Jo. (2009) Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds, Antioxidants, and Anthocyanins from Grape (Vitis vinifera) Seeds. Agric. Food Chem., vol. 11, no. 57, pp. 4988–4994. https://doi.org/10.1021/jf9001439.

39 Velioglu Y.S., Oomah B.D. (1998) Antioxidant activity and total phenolic in selected fruits, vegetables and grain products. Journal of agricultural and food chemistry., vol. 46, pp. 4113-4117. https://doi.org/10.1021/jf9801973.

40 Vilkhu K., Mawson R., Simons L., Bates D. (2008) Applications and opportunities for ultrasound assisted extraction in the food industry – A review. Innovative Food Science & Emerging Technologies., vol. 9, no. 2, pp. 161-169. https://doi.org/10.1016/j.ifset.2007.04.014.

41 Albu S., Joyce E., Paniwnyk L., Lorimer J., Mason T. (2004) Potential for the use of ultrasound in the extraction of antioxidants from Rosmarinus officinalis for the food and pharmaceutical industry. Ultrasonic Sonochemistry., vol. 11, no. 3-4, pp. 261-265. https://doi.org/10.1016/j.ultsonch.2004.01.015.

42 Hemwimol S., Pavasant P., Shotipruk A. (2006) Ultrasonic-assisted extraction of anthraquinones from roots of Morinda citrifolia. Ultrasonics Sonochemistry., vol. 13, no. 6, pp. 543-548. https://doi.org/10.1016/j.ultsonch.2005.09.009.

43 Ujic N., Šavikin K., Jankovic T., Pljevljakušic D., Zdunic G., Ibric S. (2016) Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. Food Chemistry., vol. 194, pp. 135–142. https://doi.org/10.1016/j.foodchem.2015.08.008.