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RECOVERY OF NATURAL ANTIOXIDANTS FROM FRUIT JUICE INDUSTRY RESIDUALS BY ULTRASOUND-ASSISTED EXTRACTION AND RESPONSE SURFACE METHODOLOGY

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Fruit processing industries produce by-products that are good sources of natural antioxidants. These residuals are non-toxic and available in large quantities. A central composite design (CCD) and response surface methodology (RSM) were used to optimize experimental conditions. The processing variables were solvent type, solvent to solid ratio, ethanol concentration, temperature, and time. The responses were total phenolic content (TPC), scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and yield. The optimal conditions were 70% ethanol–water mixture as a food grade solvent, temperature of 35 °C and extraction time 60 min for obtaining extracts with maximum of total phenolic content. Predicted values for total phenolic content in pear, apricot, and peach were 24.7, 19.3, and 10.4 mg gallic acid equivalents per 100 g fruit residual, respectively.

Keywords: antioxidants, pear, peach, apricot, ultrasound extraction, response surface methodology

The oxidative stress imposed by reactive oxygen species (ROS) plays an important role in many chronic and degenerative diseases (FINKEL & HOLBROOK, 2000). High intake of fruit and vegetables can provide the antioxidants, trace minerals, and other bioactive compounds to counter oxidative stress. The growing interest in the substitution of synthetic food antioxidants by natural ones has fostered on vegetable sources.

Due to the perishable nature of fruit (such as pear, peach, and apricot) and restricted marketing chance, a large proportion of these fruit is wasted during harvesting season and the losses are as high as 29% of total fresh production. Each year, more than 1.5, 1.5, and 2.8 million tons of peach, apricot, and pear are produced in Iran, respectively (<http://dbagri.maj.ir/zrt/product.asp>). The desirable taste, high digestibility, and delightful aroma of pear (*Pyrus communis* L.), peach (*Prunus persica* L.), and apricot (*Prunus armeniaca*) make them very popular among consumers (SOLIS-SOLIS et al., 2007; SALTA et al., 2010). Phenolic compounds, such as chlorogenic acid, caffeic acid, *p*-coumaroyl quinic and *p*-coumaric acids, and procyanidin and quercetin, have been reported in pear (SCHIEBER et al., 2001). Phenolics and carotenoids are natural antioxidants of peach that possess beneficial properties for human health (OLIVEIRA et al., 2012). Apricot is a natural antioxidant source of vitamin A, vitamin C, polyphenols, flavonoids, and carotenoids.

The extraction of bioactive compounds under ultrasound irradiation (20–100 kHz) is one of the upcoming extraction techniques that can offer shorter operation times, simplified manipulation, lower energy input, and reduced solvent consumption and temperature. Hence, ultrasound-assisted extraction (UAE) can be called an “environment-friendly” or “green” technique (VIROT et al., 2010). The efficiency of the extraction process is affected by several

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factors, such as solvent type and its concentration, solvent to solid ratio, contact time, temperature, and particle size of the sample matrix. When many factors and interactions affect desired process response, response surface methodology is an effective tool for optimizing the process. RSM is a collection of statistical and mathematical techniques that has been successfully used to determine the effects of several variables and optimize processes (BEZERRA et al., 2008).

The aim of this study was to optimize experimental conditions for ultrasound-assisted extraction of natural antioxidants from pear, peach, and apricot residuals by response surface methodology. Till now, UAE has not been used for recovery of antioxidants from these residuals.

1. Materials and methods

1.1. Plant materials

Fruit (pear, peach, and apricot) were purchased from local markets in Ilam, Iran. Fruit were washed with distilled water and then cut into small pieces. Fruit pieces were introduced in an electrical juicer (Pars Khazar, Rasht, Iran) to obtain juice and the residuals were separated. The residuals were maintained at -20°C in vacuum packages.

1.2. Chemicals

2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), Folin-Ciocalteu (FC) reagent and gallic acid were purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Munich, Germany). All reagents were of analytical grade.

1.3. Extraction procedure

The process of polyphenols and antioxidants extraction from pear, peach, and apricot residuals by ultrasonic was performed in an ultrasonic bath RK103H (Bandelin Sonorex, Berlin, Germany) with a maximum capacity of 4 l (35 KHz, 140 W). Sample (5 g) was sonicated in the solvent (5 ml) for different times and at different temperatures. Then, the extract was centrifuged at 4500 r.p.m. for 10 min. The extracts were concentrated by rotary evaporation at 40°C under vacuum to dryness and the yield of extraction was determined.

1.4. Optimization of solvent and solvent to solid

In this study, several extraction solvents, such as methanol, ethanol, water, and acetone, were used to study a wide range of polarity of antioxidants. The extraction of antioxidant was performed in ultrasonic bath over a 30 min extraction period at 50°C .

A second set of tests was performed for the selection of appropriate solvent to solid ratio (ml:g) to extract the phenolic compounds from fruit residuals. The extraction was carried out using 5 ml of ethanol solution (50% ethanol:water; v/v) and different weights (1, 2, 4, 5, and 7 g) of residuals (solvent to solid ratios: 5, 2.5, 1.25, 1, and 0.7). The extraction of antioxidants was performed in ultrasonic bath over a 30 min extraction period at 50°C .

1.5. Total phenolic content

Total phenolic content (TPC) of the extracts were determined using Folin-Ciocalteu (FC) reagent (SINGLETON & ROSSI, 1965). Forty microlitres of properly diluted extract solution

were mixed with 1.8 ml of FC reagent. The reagent was pre-diluted, 10 times, with distilled water. After standing for 5 min at room temperature, 1.2 ml of (7.5%, w/v) sodium carbonate solution was added. The solution were mixed and allowed to stand for 1 h at room temperature. Then, the absorbance was measured at 765 nm using a UV-Visible spectrophotometer (Varian 300, Mulgrave, Australia). The results of total phenolic content were expressed as mg gallic acid equivalents per 100 g of residuals.

1.6. Scavenging activity of DPPH radical

DPPH radical-scavenging activity of residual extract was determined according to the method reported by BRAND-WILLIAMS and co-workers (1995), with some modification. An aliquot of 0.5 ml of sample solution was mixed with 2.5 ml of a methanolic solution of DPPH (0.5 mM). The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm against a blank, using a UV-Vis spectrophotometer. Results were expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation:

$$\% \text{ Inhibition of DPPH} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100 \quad (1)$$

where $\text{Abs}_{\text{sample}}$ and $\text{Abs}_{\text{control}}$ are the absorbances of DPPH solutions with and without extract.

1.7. Experimental design and central composite design

Three factors that can potentially affect extraction of antioxidants, such as ethanol percentage (X_1 , %), extraction temperature (X_2 , °C), and extraction time (X_3 , min), were chosen as key variables. The minimum and maximum levels to each factor were chosen based on preliminary experiments, our experience, and that of our previous works. A version of central composite design, face centre cube with the star points at the centre of each face of the factorial space ($\alpha = \pm 1$), was used to identify the relationship between three independent factors and the dependent variables or responses. The design had 16 runs and each run was performed in triplicates. Centre point (run 15 and 16) was replicated to have a measurement of reproducibility and to model lack of fit. The factors (ethanol concentration, temperature, and time) were set at three separate coded levels, -1, 0, and +1. The total phenolic content TPC (Y_1), DPPH scavenging activity (Y_2), and extraction yield (Y_3) were chosen as the dependent variables.

The complete quadratic equation used is as follows:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (2)$$

where Y is the estimated response; β_0 , β_i , β_{ii} , and β_{ij} are the regression coefficients for intercept, linear, square, and interaction terms, respectively; and X_i and X_j are the independent variables.

All the analysis was carried out in triplicates and the experimental results were expressed as mean \pm SD. Statistical analysis was performed by using the Minitab 15.1 (Minitab Inc., State College, PA, USA) software.

2. Results and discussion

2.1. Effect of solvent

Several solvents were used and results are shown in Table 1. The results show that ethanolic extracts exhibited the highest TPC, DPPH, and extraction yield. Environmentally benign and non-toxic food grade organic solvents, like water and ethanol, are also recommended by the US food and drug administration for extraction purposes (BARTNIK et al., 2006). So ethanol–water mixture was chosen as the extraction solvent for the next experiments.

Table 1. Effect of solvent on the TPC, DPPH, and extraction yield

Solvent	Fruits	Responses		
		TPC	DPPH	Yield
Methanol	Pear	15.0 \pm 0.6	50.1 \pm 1.9	2.9 \pm 0.1
	Peach	6.4 \pm 0.2	66.4 \pm 2.4	5.3 \pm 0.3
	Apricot	18.0 \pm 0.8	78.8 \pm 2.7	6.8 \pm 0.4
Ethanol	Pear	18.7 \pm 0.7	59.8 \pm 2.1	4.8 \pm 0.2
	Peach	7.9 \pm 0.3	72.3 \pm 2.2	6.2 \pm 0.4
	Apricot	19.5 \pm 0.6	85.5 \pm 3.3	7.4 \pm 0.4
Water	Pear	12.0 \pm 0.6	44.9 \pm 1.4	2.1 \pm 0.1
	Peach	4.2 \pm 0.2	60.2 \pm 1.9	3.4 \pm 0.2
	Apricot	15.2 \pm 0.6	70.4 \pm 3.6	3.2 \pm 0.2
Aceton	Pear	17.2 \pm 0.7	54.0 \pm 1.8	3.8 \pm 0.3
	Peach	6.6 \pm 0.4	70.3 \pm 2.9	5.0 \pm 0.3
	Apricot	17.4 \pm 0.7	80.2 \pm 3.8	6.3 \pm 0.3

TPC (mg gallic acid equivalents/100 g pomace); DPPH (% inhibition)

2.2. Effect of solvent to solid ratio

The TPC, DPPH, and extraction yield under different solvent to solid ratios were investigated. The solvent to solid ratio varied from 0.7:1 to 5:1 (Table 2). As shown, the best results were obtained for solvent to solid ratio of 1 for all responses. Therefore, the solvent to solid ratio of 1 was used for further experiments.

Table 2. Effect of solvent to solid ratio on the TPC, DPPH, and extraction yield

Solvent to solid ratio	Fruits	Responses		
		TPC	DPPH	Yield
5	Pear	13.1±0.7	29.3±1.3	2.0±0.1
	Peach	2.5±0.1	48.5±2.3	2.8±0.1
	Apricot	8.2±0.4	61.7±3.4	3.0±0.2
2.5	Pear	15.2±0.7	34.2±1.4	3.4±0.2
	Peach	3.8±0.2	58.1±2.8	3.9±0.2
	Apricot	11.8±0.5	68.2±3.5	4.5±0.2
1.25	Pear	19.3±0.9	48.4±2.5	4.6±0.3
	Peach	5.2±0.2	62.4±3.3	5.2±0.3
	Apricot	14.1±0.6	74.8±4.2	6.4±0.4
1	Pear	19.9±1.1	49.1±1.9	4.7±0.2
	Peach	7.5±0.4	69.8±4.1	6.4±0.4
	Apricot	16.3±0.7	80.1±3.9	7.8±0.4
0.7	Pear	19.8±1.0	48.9±2.6	4.8±0.3
	Peach	7.4±0.3	69.2±3.7	6.0±0.3
	Apricot	16.2±0.7	80.2±4.4	7.27±0.3

TPC (mg gallic acid equivalents/100 g pomace); DPPH (% inhibition)

2.3. Modelling of the extraction process and effect of process variables

The responses (TPC, DPPH scavenging activity, and extraction yield) of each run of the experimental design, coded and decoded values of independent variables are presented in Table 3. The second-order polynomial equation of models for total phenolic content, antioxidant activity of extracts, and yield are summarized in Table 4. The large values of the R^2 reveal that the models adequately represent the experimental results. As shown, the regression parameters of the surface response analysis of the models, the linear, quadratic, and interaction terms have significant effects ($P \leq 0.001$, $P \leq 0.01$, or $P \leq 0.05$). The absence of any lack of fit ($P > 0.05$) also strengthened the reliability of all models.

The effects of ethanol concentration, temperature, and time on extraction yield for residuals of pear, peach, and apricot are shown in Figures 1–3, respectively.

Table 3. Central composite design of three variables with their observed responses

Exp No	Independent variables			Responses								
				TPC			DPPH			Yield		
	X ₁	X ₂	X ₃	Pear	Peach	Apricot	Pear	Peach	Apricot	Pear	Peach	Apricot
1	-1	-1	-1	14.2±0.7	6.2±0.4	11.3±0.2	48.8±1.2	55.4±1.3	74.0±2.5	5.7±0.2	7.7±0.2	7.0±0.2
2	-1	-1	1	17.4±0.5	9.0±0.2	16.4±0.5	70.7±1.5	64.8±1.7	81.8±3.1	7.8±0.5	8.5±0.2	7.6±0.3
3	-1	1	-1	18.7±1.2	7.1±0.1	12.9±0.4	55.8±1.7	57.1±0.9	75.9±2.2	6.2±0.2	4.9±0.4	6.4±0.3
4	-1	1	1	20.4±0.9	8.1±0.3	14.8±0.6	63.7±0.9	59.3±1.2	83.1±2.9	7.2±0.4	5.2±0.2	7.1±0.2
5	1	-1	-1	16.0±0.6	8.8±0.2	16.1±0.6	69.7±1.9	51.5±2.2	79.3±2.4	4.8±0.2	7.8±0.5	9.0±0.2
6	1	-1	1	21.3±1.3	10.5±0.4	19.2±0.7	82.9±0.9	51.8±1.6	88.0±3.6	4.2±0.2	7.3±0.3	8.5±0.1
7	1	1	-1	23.9±1.4	9.5±0.2	17.3±0.7	74.9±0.8	56.4±1.3	79.8±2.6	5.9±0.2	6.4±0.1	7.2±0.2
8	1	1	1	24.6±1.1	10.7±0.1	19.7±0.8	85.0±2.6	58.8±2.1	84.4±2.1	6.6±0.3	7.5±0.2	7.3±0.4
9	-1	0	0	17.8±0.6	4.1±0.3	7.5±0.2	32.3±1.2	62.9±3.2	80.2±1.9	7.3±0.1	6.9±0.6	5.1±0.3
10	1	0	0	20.7±0.9	6.4±0.2	11.8±0.3	50.9±2.2	53.4±1.5	83.2±2.4	5.3±0.3	6.7±0.3	7.8±0.2
11	0	-1	0	18.1±0.9	6.2±0.4	11.6±0.4	50.0±0.8	63.8±2.4	83.1±3.1	7.6±0.4	7.5±0.2	9.2±0.3
12	0	1	0	22.8±1.2	6.8±0.6	12.4±0.5	53.5±2.3	68.4±1.8	83.2±2.8	8.2±0.4	6.2±0.4	7.7±0.2
13	0	0	-1	19.2±0.8	7.4±0.1	13.5±0.4	58.2±3.3	69.2±2.5	79.6±1.7	8.1±0.3	6.6±0.1	7.1±0.3
14	0	0	1	20.8±0.8	9.2±0.3	16.8±0.6	72.4±0.9	69.7±1.1	83.4±0.2	8.4±0.1	8.0±0.4	8.2±0.2
15	0	0	0	20.2±1.1	5.9±0.2	10.6±0.3	45.8±0.7	71.5±1.3	83.9±2.5	10.4±0.2	7.9±0.2	7.9±0.4
16	0	0	0	20.2±0.6	5.8±0.1	10.6±0.2	46.0±0.9	70.7±1.7	83.4±3.2	10.5±0.6	7.8±0.3	7.7±0.3

X₁: Ethanol concentration (%), coded levels [-1 0 1]=[30 50 70].

X₂: Temperature (°C), coded levels [-1 0 1]=[35 50 65].

X₃: Time (min), coded levels [-1 0 1]=[30 45 60] for pear and peach; coded levels [-1 0 1] = [15 30 45] for apricot.

TPC (mg gallic acid equivalents/100 g pomace); DPPH % inhibition; Yield (%).

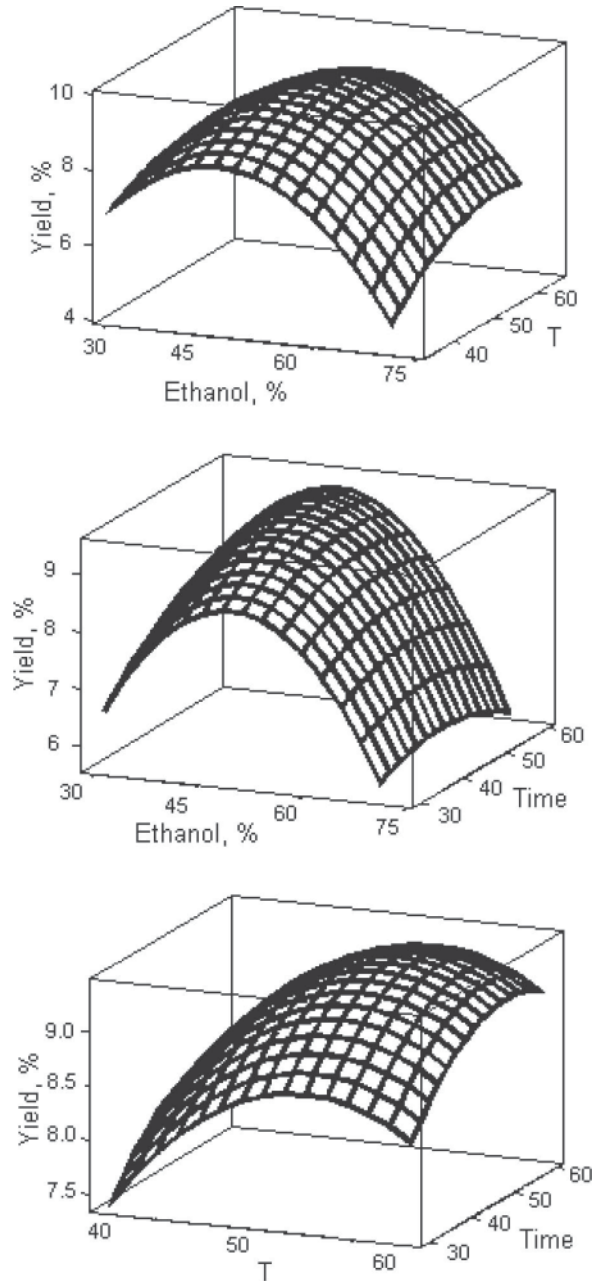


Fig. 1. Response surface plots showing the effects of ethanol percentage, temperature ($^{\circ}\text{C}$), and time (min) on yield of pear pomace

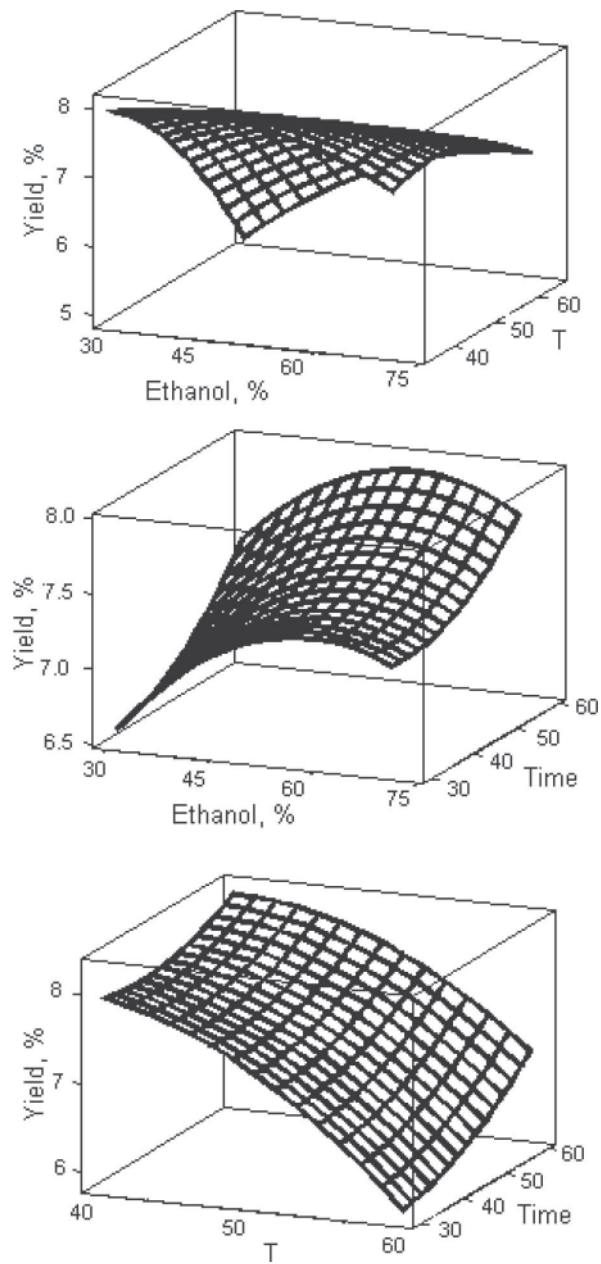


Fig. 2. Response surface plots showing the effects of ethanol percentage, temperature (°C) and time (min) on yield of peach pomace

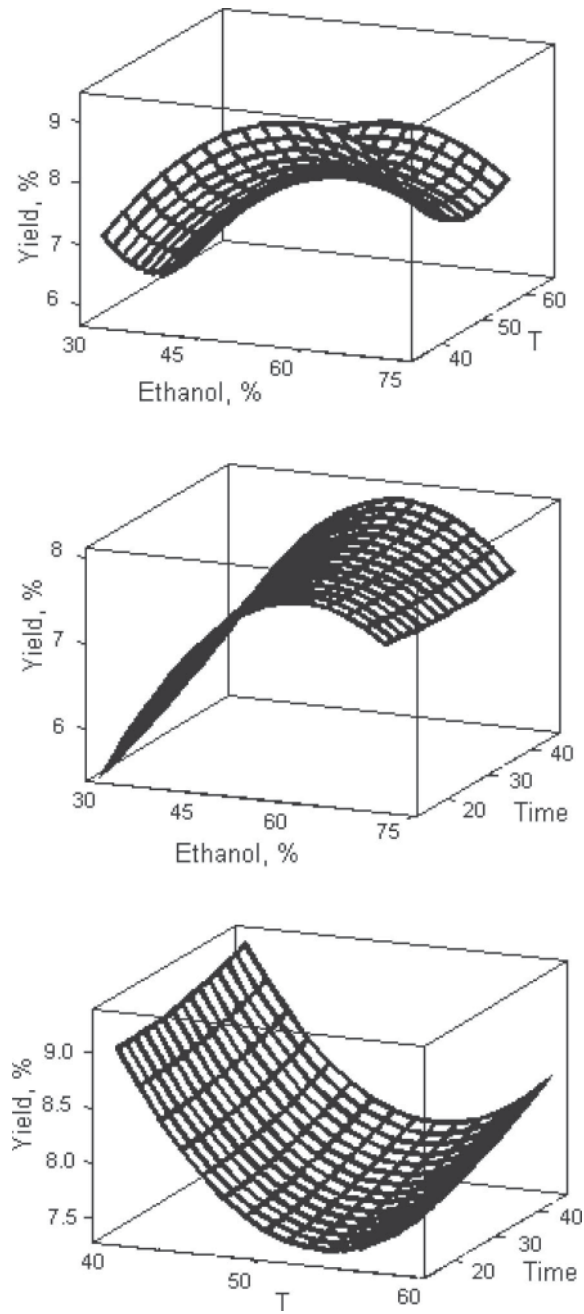


Fig. 3. Response surface plots showing the effects of ethanol percentage, temperature (°C) and time (min) on yield of apricot pomace

Table 4. Polynomial models for the investigated responses from pomace extracts

Polynomial model	R ²	R ² _{adj}	SE
Pear			
Y ₁ (TPC)= -0.42+0.19 E+7.80×10 ⁻² T+2.60×10 ⁻¹ t-1.99×10 ⁻³ E ² +1.54×10 ⁻³ T ² -3.68×10 ⁻⁴ t ² +1.53×10 ⁻³ ET+5.25×10 ⁻⁴ E t-3.41×10 ⁻³ T t*	0.983	0.956	0.571
Y ₂ (DPPH)= -214.29***+1.3 E** -2.66 T*** -7.04 t*** -9.0×10 ⁻³ E ² **+3.0×10 ⁻³ T ² ***+9.0×10 ⁻³ t ² ***+3.0×10 ⁻³ ET-3.0×10 ⁻³ E t-1.0×10 ⁻² T t**	0.995	0.987	1.692
Y ₃ (Yield)= -15.43+5.25 ×10 ⁻¹ E+2.47×10 ⁻¹ T+2.25×10 ⁻¹ t+2.25×10 ⁻¹ T ² -1.6×10 ⁻³ t ² +1.5×10 ⁻³ ET-1.2×10 ⁻³ E t+1.0×10 ⁻⁴ T t	0.867	0.668	1.046
Apricot			
Y ₁ (TPC)= 29.85+2.78×10 ⁻¹ E** -6.44×10 ⁻¹ T*** -1.00 t*** -2.0×10 ⁻³ E ² +6.9×10 ⁻³ T ² *** -2.08×10 ⁻² t ² ***+0.07×10 ⁻² ET-0.06×10 ⁻² E t-0.22×10 ⁻² T t*	0.995	0.987	0.391
Y ₂ (DPPH)= -69.61*** -2.77E**+1.2700T+1.47t-10.74E ² *** -2.77T ² +0.59t+1.95ET-1.09Et -0.64Tt	0.960	0.900	1.083
Y ₃ (Yield)= 7.41***+0.24E-0.86T**+0.32t-0.377E ² -0.34207T ² +0.16293t ² +0.60500Et*-0.06500Et+0.15250Tt	0.878	0.696	0.545
Peach			
Y ₁ (TPC)= 26.72***+1.65×10 ⁻¹ E*** -3.17×10 ⁻¹ T-8.95×10 ⁻¹ t*** -1.10×10 ⁻³ E ² +3.70×10 ⁻⁴ T ² ***+1.15×10 ⁻² t ² ***+3.00×10 ⁻⁴ ET-4.00×10 ⁻⁴ E t-1.3×10 ⁻³ T t**	0.994	0.986	0.222
Y ₂ (DPPH)= -23.04***+2.38E**+1.12T+1.87×10 ⁻¹ t-2.69×10 ⁻² E ² *** -1.23×10 ⁻² T ² +2.6×10 ⁻³ t ² +6.5×10 ⁻³ ET-3.6×10 ⁻³ E t-2.9×10 ⁻³ T t	0.951	0.878	2.438
Y ₃ (Yield)= 10.13***+1.42×10 ⁻² E-3.66×10 ⁻² T***-6.7×10 ⁻³ t-9.0×10 ⁻⁴ E ² -1.5×10 ⁻³ T ² +7×10 ⁻⁴ t ² +2.0×10 ⁻³ ET*-2.0×10 ⁻⁴ E t+7×10 ⁻⁴ T t	0.862	0.656	0.594

*: significant at P≤0.05; **: significant at P≤0.01; ***: significant at P≤0.001; SE: standard error; E: ethanol concentration, %; T: temperature, °C; t: time, min; TPC: mg gallic acid equivalents/100 g pomace; DPPH(% inhibition); Y: yield, (%)

2.4. Optimal conditions

The optimal conditions were obtained from the first derivatives of the second-order polynomial equations (Table 4). The optimum UAE conditions for the response variables from extracts are presented in Table 5. The predictive ability of the models was examined by extractions at optimal conditions. Based on results in Tables 3 and 5, experimental conditions for obtaining the extracts with the highest total phenolic contents for industrial applications were solvent percentage of 70% ethanol, temperature of 35 °C, and extraction time of 60 min for all residuals.

Table 5. Optimal conditions, predicted and experimental responses for extraction of antioxidants

Responses	Optimal conditions			Maximal values	
	Ethanol (%)	T (°C)	t (min)	Predicted	Actual
Pear					
TPC	70	65	60	24.7	24.6±0.3
DPPH	68	35	60	80.1	82.8±1.3
Yield	46	54	53	9.3	9.6±0.3
Apricot					
TPC	70	35	45	19.3	19.2±0.4
DPPH	70	35	45	87.4	88.0±2.1
Yield	56	35	45	9.4	9.3±0.1
Peach					
TPC	69	35	60	10.4	10.4±0.3
DPPH	46	50	60	71.8	70.0±1.9
Yield	40	35	60	8.4	8.3±0.2

TPC (mg gallic acid equivalents/100 g pomace); DPPH (% inhibition); Yield (%)

In this work, TPC results were reported as mg gallic acid equivalents per 100 g fresh pomaces. Extracts had total phenolic contents (pear, 24.6; apricot, 19.2; and peach, 10.2 mg gallic acid equivalents per 100 g fresh pomaces) comparable to some fresh fruit such as avocado (21.86), banana (25.55), green grape (23.20), muskmelon (white pulp, 20.36), olive (21.68), peach (27.58), pear (fragrant, 18.65; honey, 11.88; royal, 34.84), and watermelon (red pulp, 24.66; yellow pulp, 18.62 mg gallic acid equivalents per 100 g fresh weight) (Fu et al., 2011).

3. Conclusions

Increased concern over the safety of synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has led to an increased interest in exploration of effective and economical natural antioxidants. Pear, peach, and apricot by-products could be a good commercial source of chlorogenic acid, caffeic acid, quercetin, flavonoids, and carotenoids and they can be separated and concentrated through extraction process.

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