

Optimization of extraction parameters on the isolation of phenolic compounds from sour cherry (*Prunus cerasus* L.) pomace

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Revised: 24 March 2014 / Accepted: 26 March 2014 / Published online: 11 April 2014
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Abstract There is an increasing interest to bio-components obtained from fruit & vegetable wastes. Anthocyanin is one of the components regained from fruits especially from red ones. Sour cherry is considered as industrial fruit since most of the grown fruit is processed into juice and hence considerable amount of pomace is removed from process. The influences of process parameters on the extraction of phenolic compounds from sour cherry pomace were investigated. Fifty-one percent ethanol concentration, 75 °C temperature and 12 mL/g solvent to solid ratio were selected as optimum process parameters. Time effect on the process efficiency was monitored at three different temperatures (25, 50 and 75 °C) and total phenolic and total anthocyanin contents were found to reach equilibrium concentrations between 80 and 100 min time intervals. Ethanol concentrations affected differently extraction yield of anthocyanins and non-anthocyanin phenolics. Various individual phenolic compounds present in sour cherry pomace were identified and quantified by HPLC equipped with UV/Vis PDA. Of the phenolic compounds, cyanidin-3-glucosylrutinoside, neochlorogenic acid and catechin were the most abundant ones found in the pomace. 14.23 ± 0.38 mg/g total phenolic, 0.41 ± 0.02 mg/g total anthocyanin, 0.19 ± 0.02 mg/g cyanidin-3-glucosylrutinoside, 0.22 ± 0.01 mg/g neochlorogenic acid and 0.22 ± 0.02 mg/g catechin contents (dry weight) were determined in the pomace at optimum extraction conditions.

Keywords Sour cherry pomace · Food byproducts · Optimization · Purification · Phenolic compound · HPLC

Introduction

Vast amount of phytochemical rich byproducts are discarded from food production plants (Wijngaard et al. 2012; Roldan et al. 2008) at different stages of processing. Fruit and vegetable byproducts can be utilized as food additives such as, antioxidant, antimicrobial, colorant, dietary fiber, thickener (O'Shea et al. 2012; Yağcı and Göğüş 2008; Ayala-Zavala et al. 2011). Evaluation or recovery of food byproducts is necessary when concerning economical and environmental issues (Aguedo et al. 2012; O'Shea et al. 2012; Bonilla et al. 1999). There are many investigations in the literature subjecting phenolic compound extraction from grape marc (Boonchu and Utama-ang 2013), apple pomace (Parmar et al. 2014), pomegranate leaves and peels (Wang et al. 2013; Çam and İcyer 2013), bayberry leaves (Yang et al. 2012) and lycopene extraction from tomato waste (Kumcuoglu et al. 2013; Baysal et al. 2000). Grape marc extract has found commercial usage in the market because of its rich coloring anthocyanin and other phenolic compound content. Similar to grape marc, sour cherry pomace became the subject of the scientific studies and its rich phenolic and anthocyanin content were reported. Kolodziejczyk et al. (2013) showed that cyanidin-3-glucosylrutinoside and neochlorogenic acid are the predominant anthocyanin and hydroxycinnamic acid, respectively, found in sour cherry pomace. Simsek et al. (2012) reported that epicatechin is the major phenolic compound in the sour cherry pomace.

Turkey is the prominent sour cherry (*Prunus cerasus* L.) producing country in the world and the tonnage increases each year. From 2005 to 2010, production rate increased by 45 %

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(FAOSTAT 2010). Sour cherry is an industrial fruit that is mostly processed to concentrates, toppings, jams, and juices (Kim et al. 2005). In 2010, 73 500 ton of sour cherry fruit was processed into juice mainly to concentrate in Turkey (MEYED 2011). During manufacturing of sour cherry juice, peel is purged with seed and stem from the system after pressing step. Currently, there is no industrial utilization of sour cherry residues. Factories consider sour cherry pomace as waste and give them away to graziers. However, fruit and vegetable pomaces are rich in food-value biocomponents such as anthocyanins, and non-anthocyanin phenolics. Anthocyanins, subgroup of phenolic compounds, substitute synthetic food colorants as consumers tend to consume natural and healthy foods and prefer non-synthetic additives (Fan et al. 2008). Anthocyanins acquired from food byproducts do not exert toxic properties and may have positive effect on human health (Bridle and Timberlake 1997). The extraction of pigments and antioxidative compounds from food industry wastes is very important when considering their low cost, waste evaluation and substitution with synthetic antioxidants.

Specifically, it has been shown that sour cherry phenolics are able to reduce allergenicity of protein β – Lactoglobulin (Tantoush et al. 2011), inhibit cancer cell line development, occurrence of cardio-vascular diseases, and able to help relief muscle pains and aches (Kang et al. 2003; Wang et al. 1999).

Phenolic compounds present in natural sources have varied structural and physicochemical properties and thus possess different polarities (Luthria 2008). Also there are many different interactions among phenolics and other constituents (sugars, acids, alkyl groups) in food systems that is responsible for formation of myriad of different phenolic compounds including condensed tannins and polymeric phenolics (Mitic et al. 2010). So, there is no single universal phenolic compound extraction method from plant tissues. Multivarious parameters have different effect on the extraction of phenolic compounds from natural sources according to their structural properties. Therefore, description of optimization conditions for different food sources in order to extract phenolic compounds is necessary (Luthria 2008; Silva et al. 2007).

Response surface methodology (RSM) has been widely used to analyze or to optimize the independent factors which influenced the extraction yield or extract profiles of bioactive components of herbs and natural materials (Wang et al. 2013). The aim of this study was to determine the best extraction conditions of sour cherry pomace phenolic compounds by optimizing extraction parameters. The extraction process was optimized by controlling solvent concentration, temperature, solvent to solid ratio and time to maximize phenolic compound yield.

Materials and methods

Preparation of sample

Sour cherry residue after pressing step in juice production was obtained from Limkon Food Ind. and Trade Inc. (Adana, Turkey) on July 10, 2011. Pomace was transported to laboratory at 4°C within plastic bags. Seed and stem were removed from peel by hand and pomace was dried in vacuum oven (Binder VD 23, Germany) at 40 °C up to the pomace had 90± 0.5 % dry matter. Then the dried pomace was milled (Polymix, Culatti) to diminish particle size. Finally, the dried and milled pomace was stored at 4°C in dark glass jars until extraction procedures.

Chemicals

Folin-Ciocalteu reagent and gallic acid were obtained from Merck Co. (Darmstadt, Germany). Cyanidin-3-glucoside, cyanidin-3-rutinoside, neochlorogenic acid, catechin, caffeic acid, *p*-coumaric acid, ferulic acid were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other reagents were at analytical grade.

Extraction procedure

All extractions were carried out in amber glass bottles (0.5 L) within shaker incubator (Labline, USA). A 5 g of the dried pomace was put in bottle and waited at experimental extraction temperature with extraction solvent (different proportion of EtOH:H₂O; 0.01 % HCl) before mixing them for 7–8 min. Then the bottle was filled with solvent to a final volume described in experimental design (4, 6, 9, 12 or 14 mL/g). All extraction protocols were conducted at 200 rpm agitation. The homogenate was centrifuged at 6,000 rpm for 10 minute (Hitachi CT6E, Taiwan) after extraction procedure. Then the slurry was transferred to falcon tubes and stored at –40°C until analyses.

Analyses of the response variables

Determination of total phenolic (TP) compound

The TP contents of the samples were determined by the Folin-Ciocalteu (FC) method (Singleton and Rossi 1965). Briefly, 30 μ L sample extract and 150 μ L FC reagent were consecutively transferred to test tubes including 2.37 mL dH₂O. After 8 minute, 450 μ L saturated Na₂CO₃ was added to the mixture. Absorbance was read at 750 nm using a spectrophotometer (Biochrom Libra S70 UK) against the blank after 30 minute incubation at 40 °C. Results were expressed as milligram gallic acid equivalent per gram dry weight pomace (mg GAE/g dw).

Determination of total anthocyanin (TA)

TA contents of samples were determined by using pH differential method as described by Wrolstad (1976). Extracts were diluted to relevant spectrophotometric ranges (0.3–0.6) with potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). Absorbances of the dilutions were read at both 515 nm and 700 nm. The TA content was expressed as milligrams of cyanidin-3-rutinoside per g of dry weight pomace (mg cyn-3-rut/g dw). A molar absorptivity of 28 800 L/mol.cm was used for cyanidin-3-rutinoside, which has molecular mass of 595.2 g/mol.

Purification of phenolics

The extracts obtained from extraction procedure were first dried at 40 °C in a water bath under nitrogen gas (N₂) and diluted to 2 mL with dH₂O. These dilutions were used as extracts for purification of phenolics. Anthocyanin and non-anthocyanin phenolics were purified on a C-18 Cartridge (Finisterre, Teknokroma) using a vacuum manifold. Cartridge was activated with 5 mL ethyl acetate (EtaC), 5 mL methanol (0.01 % HCl, v/v) and 2 mL acidified water (0.01 % HCl) respectively. 0.5 mL sample extract was loaded and then the cartridge was washed with 2 mL acidified water (0.01 % HCl, v/v). After that the cartridge was dried by means of N₂ and washed with 5 mL EtaC to elute non-anthocyanin phenolics; and finally washed with 2 mL MeOH (0.01 % HCl, v/v) to elute anthocyanins within separate test tubes. The EtaC and MeOH extracts were put in a water bath (40 °C) and solvents were removed under a stream of N₂. Anthocyanins and colorless phenolics were dissolved in acidified water (0.01 % HCl), filtered through 0.45 µm PVDF filter (Sartorius RC, Goettingen, Germany) and transferred to vials (Türkyılmaz and Özkan 2012).

HPLC analysis of phenolic compounds

Identification and quantification of phenolic compounds were performed on Waters 2795 HPLC (Waters, USA) equipped with a Waters 2996 UV/VIS photodiode array detector. Autosampler (set at 7 °C) was controlled with EMPOWER™ 2 software. Samples (mixtures of anthocyanin and non-anthocyanin, 1:1, v:v) were injected into supelcosil (Supelco) LC-18 (15 cm x 4.6 mm x 3 µm) column. The mobile phase was a mixture of 0.1 % (v/v) trifluoroacetic acid in water (A) and 0.1 % (v/v) trifluoroacetic acid in acetonitrile (B). Column temperature was 35 °C and flow rate was 1 mL min⁻¹. Anthocyanins were detected at 520 nm; neochlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid at 320 nm and catechin at 280 nm wavelengths. Quantity of each compound was calculated by peak areas and standard curves of corresponding standards. For lacking

reference standards of anthocyanins (cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside), quantification was done according to cyanidin-3-glucoside standard (Garofulic et al. 2013). The linear gradient conditions were as follows: at 0 min, 90 % solvent A and 10 % solvent B; at 50 min, 80 % solvent A and 20 % solvent B; at 56 min, 90 % solvent A and 10 % solvent B.

Experimental design

The optimization was carried out using an experimental design by RSM. Rotatable central composite design with three factors, five level design was selected. It consisted of 17 runs including three replicates at the center point. Independent variables were EtOH concentration (EtOH:H₂O, 0–100 %, v:v; X₁), extraction temperature (8–92 °C; X₂) and solvent to solid ratio (4–14, v:m; X₃). The levels of each variable were determined according to literature data and preliminary studies. The second-order polynomial model was fitted to the following equation:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i < j}}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j \quad (1)$$

where *Y* is the response variable, *X_i* and *X_j* are the independent variables (*i* ≠ *j*) affecting the response of *Y*'s, β₀, β_{*i*}, β_{*ii*} and β_{*ij*} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively.

Statistical analysis

The experimental results of the response surface design were analysed using Design-Expert 8.0.7.1. software (Trial version, Stat-Ease Inc., Minneapolis, MN, USA). Linear regression analysis was done using SPSS 10.0 software (SPSS Inc., Chicago, IL, USA). All the experiments were done triplicate and the *p* values less than 0.05 were taken into consideration.

Results and discussion

The optimization study was composed of two parts. Firstly, RSM was used to optimize EtOH concentration, temperature and solvent ratio levels. Then extraction kinetics was applied to see the effect of time on the extraction of phenolic compounds. EtOH-water mixture was used for extraction of polyphenols from sour cherry pomace. There are several drawbacks to use EtOH for extracting natural compounds from plant tissues such as its less extraction efficiency and difficulty to remove from media. However, EtOH is preferred in food processing systems as it is the least hazardous - toxic solvent (Abou-Arab et al. 2011;

Gould et al. 2009; Nakamiya et al. 2003) and considered as natural (Ferreira-Dias et al. 2003) and safe for human consumption (Pompeu et al. 2009; Dai and Mumper 2010). In all obtained extracts, individual anthocyanins and non-anthocyanin phenolics were determined by using HPLC equipped with UV/Vis PDA detector besides spectrophotometric TPC and TA analyses. The detected anthocyanins according to order of elution were cyanidin-3-sophoroside (Cy-3S), cyanidin-3-glucosylrutinoside (Cy-3GR), cyanidin-3-glucoside (Cy-3G), cyanidin-3-rutinoside (Cy-3R) and the non-anthocyanin phenolics were neochlorogenic acid (NcA), catechin (Cat), caffeic acid, ferulic acid and *p*-coumaric acid as shown in Fig. 1.

Fitting the models

The dependent variables TP, TA, Cy-3GR, NcA and Cat contents depending on independent variables were demonstrated in (Table 1). The experimental data were used to

determine the coefficients of the second order polynomial equation (Eq. (1)) and Table 2 lists regression coefficients and the summary of analysis of variance (ANOVA) results. The larger regression coefficient with smaller *p*-value indicates a more significant effect on the respective response variables (Yang et al. 2009; Yim et al. 2012). ANOVA showed that the model adequately represented the data with the coefficients of multiple determinations ($0.950 < R^2 < 0.988$). The lack of fit to verify the adequacy of the fit were insignificant for all the responses ($p > 0.05$) indicating that the model could adequately fit the experimental data. The results showed that all the process variables (% EtOH, temperature and solvent ratio) displayed significant linear effects on all the responses with varying confidence intervals and % EtOH had significant negative quadratic effect on all responses ($p < 0.001$). No significant interaction effect was found among independent variables for any of the responses.

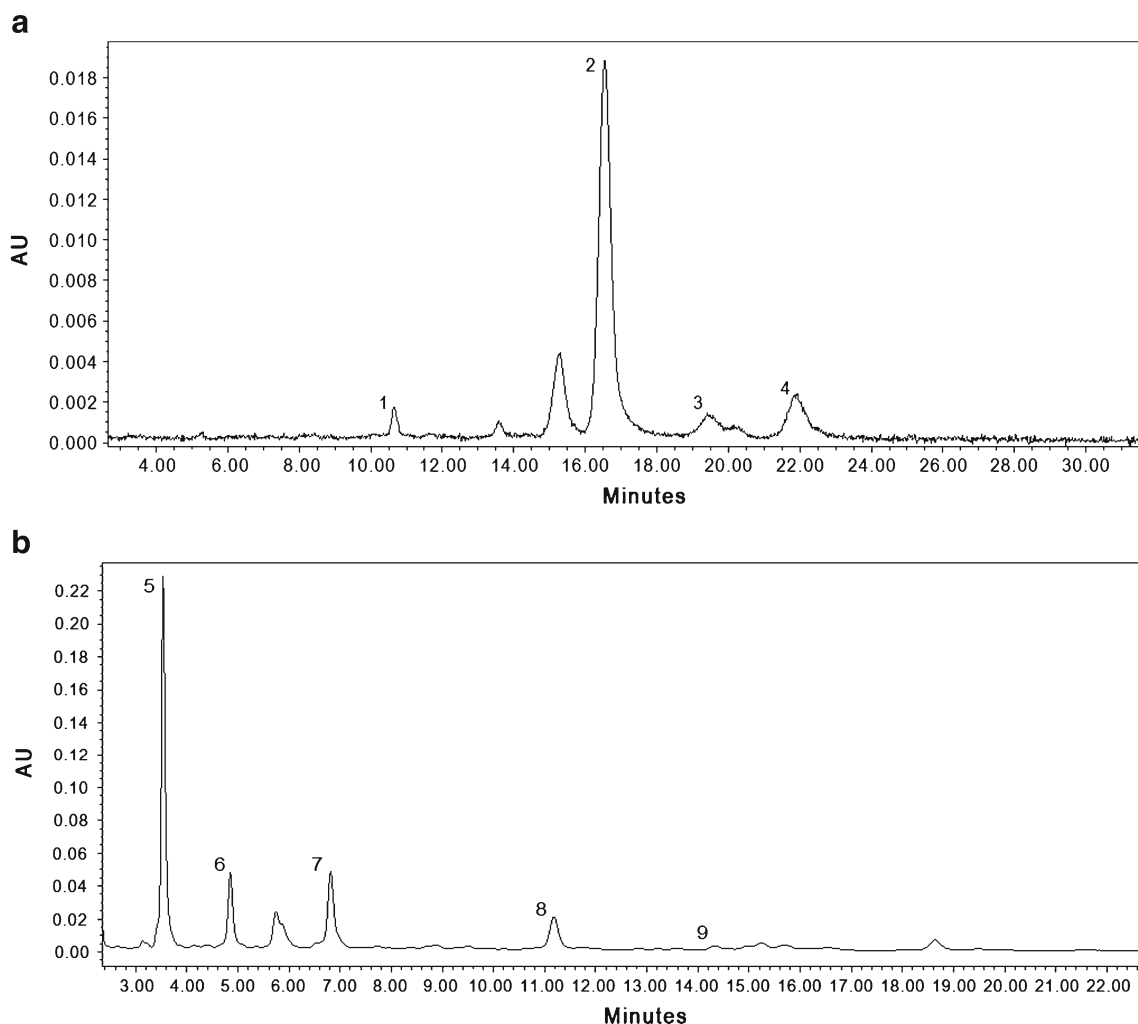


Fig. 1 Typical HPLC chromatograms of sour cherry pomace extract (**a**, anthocyanins, 520 nm; **b**, non-anthocyanins, 320 nm) obtained from optimized conditions (1: cyanidin-3-sophoroside, 2: cyanidin-3-

glucosylrutinoside, 3: cyanidin-3-glucoside, 4: cyanidin-3-rutinoside, 5: neochlorogenic acid, 6: catechin, 7: caffeic acid, 8: *p*-coumaric acid, 9: ferulic acid)

Table 1 Observed concentrations of total phenolic, total anthocyanin, cyanidin-3-glucosylrutinoside, neochlorogenic acid and catechin contents at different combinations of ethanol concentration, temperature and solvent ratio used in the experimental design

Experiment No ^a	Independent variables			Dependent variables ^b				
	Ethanol concentration (%)	Temperature (° C)	Solvent ratio (v/m)	TP	TA	Cy-3GR	NcA	Cat
1	50	8	9	9.08	0.28	0.12	0.17	0.16
2	50	50	9	13.05	0.36	0.17	0.18	0.18
3	50	92	9	14.46	0.41	0.18	0.21	0.22
4	80	25	12	7.45	0.15	0.08	0.15	0.17
5	80	75	12	10.89	0.27	0.10	0.21	0.20
6	0	50	9	0.36	0.14	0.08	0.01	0.00
7	80	75	6	8.11	0.24	0.09	0.13	0.17
8	50	50	4	9.94	0.32	0.11	0.16	0.14
9	20	75	12	6.89	0.35	0.16	0.15	0.12
10	50	50	14	13.59	0.38	0.19	0.22	0.23
11	80	25	6	5.66	0.13	0.07	0.12	0.14
12	50	50	9	12.38	0.39	0.18	0.19	0.20
13	50	50	9	12.52	0.37	0.17	0.18	0.20
14	20	25	12	4.12	0.23	0.14	0.13	0.07
15	20	25	6	3.54	0.21	0.11	0.08	0.04
16	20	75	6	5.50	0.27	0.12	0.09	0.05
17	100	50	9	1.63	0.02	0.00	0.08	0.07

^a Randomized order

^b Total phenolic (TP), total anthocyanin (TA), cyanidin-3-glucosylrutinoside (Cy-3GR), neochlorogenic acid (NcA), catechin (Cat). Results were expressed as mg/g (dw)

Effect of % EtOH, temperature and solvent ratio

EtOH concentrations ranging from 0 to 100 % were used to investigate the effect of ethanol concentration. This range was

selected to see the contents of responses at margin points. The linear effect of % EtOH was verified to be statistically significant for TP, TA, Cy-3GR, NcA and Cat contents. Negative quadratic effect of % EtOH was observed for all the responses

Table 2 Analysis of variance and coefficients for the responses

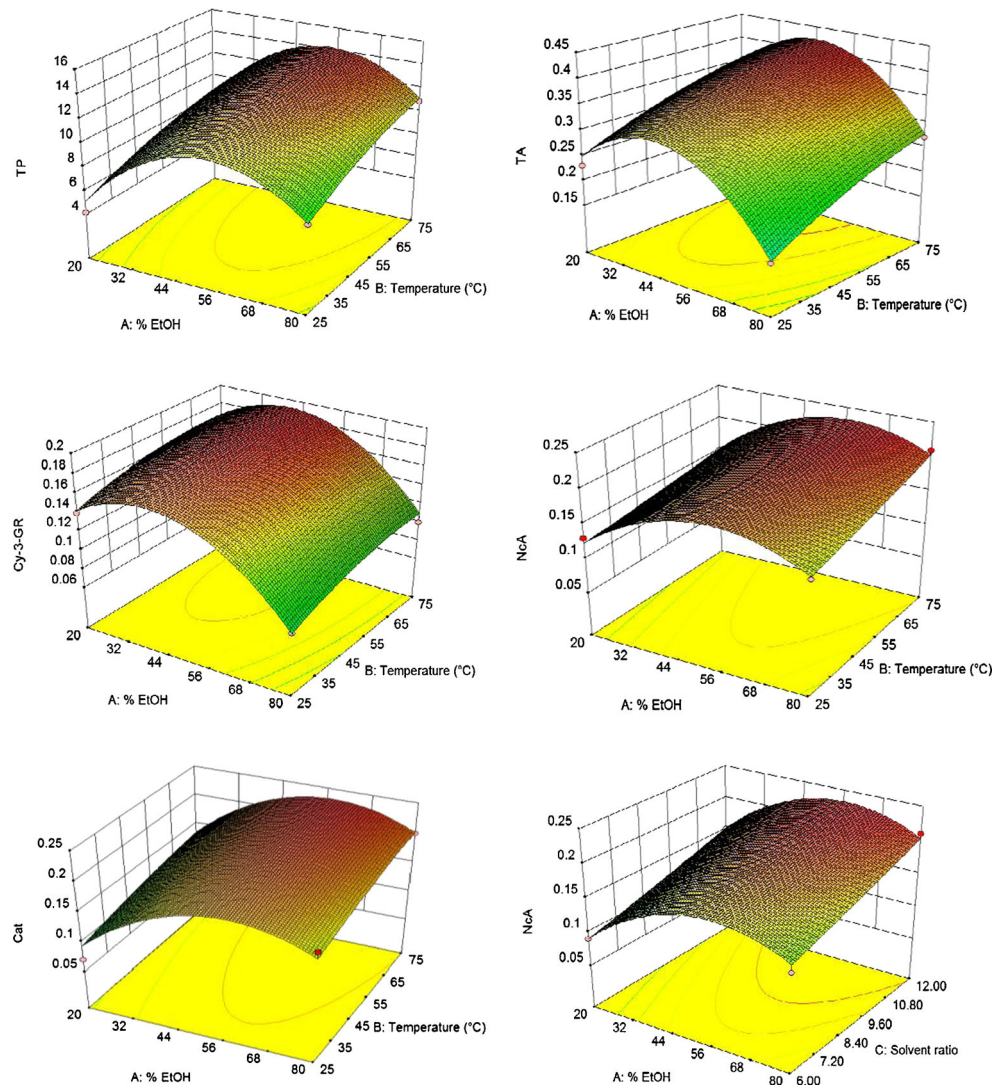
Model parameters	Regression coefficient	TP	TA	Cy-3GR	NcA	Cat
Intercept	β_0	12.75 ^{***}	0.37 ^{***}	0.17 ^{***}	0.18 ^{***}	0.19 ^{***}
Linear						
% Ethanol	β_1	1.05 [*]	-0.035 ^{***}	-0.024 ^{***}	0.02 ^{***}	0.038 ^{***}
Temperature	β_2	1.44 ^{**}	0.046 ^{***}	0.013 ^{**}	0.012 ^{**}	0.016 [*]
Solvent ratio	β_3	0.93 [*]	0.018 ^{**}	0.016 ^{**}	0.024 ^{***}	0.023 ^{**}
Interaction						
% Ethanol × Temperature	β_{12}	0.14	0.00625	0.00125	0.005	0.000
% Ethanol × Solvent Ratio	β_{13}	0.33	-0.00625	-0.00625	0.000	-0.005
Temperature × Solvent ratio	β_{23}	0.23	0.00875	0.00125	0.0075	0.005
Quadratic						
% Ethanol × % Ethanol	β_{11}	-4.50 ^{***}	-0.11 ^{***}	-0.048 ^{***}	-0.051 ^{***}	-0.060 ^{***}
Temperature × Temperature	β_{22}	-0.60	-0.014 [*]	-0.008248 [*]	0.001063	-0.003905
Solvent ratio × Solvent ratio	β_{33}	-0.62	-0.013	-0.008381 [*]	0.001062	-0.005806
R ²		0.966	0.988	0.978	0.987	0.950
Adjusted R ²		0.921	0.972	0.950	0.969	0.885
p-value		0.0003	<0.0001	<0.0001	<0.0001	0.0009
F-value		21.85	63.18	34.87	57.33	14.68
Lack of fit		0.0599	0.4266	0.1775	0.2298	0.1633

Significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

($p < 0.001$) implying that there is a maximum point for each response at a certain EtOH proportion (Silva et al. 2007). The TP, TA, Cy-3GR, NcA and Cat yields initially increased with increasing ethanol concentration until reaching maximum levels for each response and then started to decrease above this proportion. This situation may arise from coagulation of proteins at high levels of EtOH concentrations (Yang et al. 2009). The findings obtained in this study are in agreement with the data proposed in the other studies reporting that yields increase up to a certain point then start to decrease (Wang et al. 2013; Pompeu et al. 2009; Yang et al. 2009). The maximum predicted TP, NcA and Cat yields were obtained at 55, 56 and 59 % EtOH concentrations and for TA and Cy-3GR contents at 44, 43 % EtOH concentrations. As it can be seen in the results; anthocyanins have different and lower maximum % EtOH points than those of phenolics. The reason might be related to the polarity of ethanol and varying solubilities of polyphenols (Sun et al. 2011). Higher water composition in the solvent can aid in the extraction of glycosides of phenolic

compounds (Tsao and Deng 2004) and anthocyanins found in plants are glycosylated form of anthocyanidins. Cy-3GR couldn't be detected at absolute ethanol concentration, while pure water (0 %) could extract certain amount of Cy-3GR (0.08 mg/g) as other independent variables were at the center points. The situation was opposite for the non-anthocyanin phenolics; Cat couldn't be detected at 100 % water concentration while some (0.07 mg/g) could be extracted by pure EtOH extraction (Table 1). Extraction temperature displayed significant effect on the contents of all responses and quadratic temperature had significant negative effect on TA and Cy-3GR contents ($p < 0.05$). Elevated temperature degrees were also studied to see the temperature effect on the responses. As it was shown in the Fig. 2, the yields increased as temperature increased and then a constant plateau formed. The maximum predicted Cy-3GR was obtained at 71 °C, NcA and Cat at 74 °C according to optimized extraction results. Some previous studies concluded that higher temperatures damage phenolic compounds and cause a loss in the yield (Sun et al. 2011;

Fig. 2 Response surface plots for total phenolic (TP), total anthocyanin (TA), cyanidin-3-glucosylrutinoside (Cy-3GR), neochlorogenic acid (NcA) and catechin (Cat) in function of ethanol concentration, temperature and solvent ratio



Ballard et al. 2009; Cacace and Mazza 2003). Silva et al. (2007) and Pinelo et al. (2005) kept the temperature degrees at certain levels by considering higher temperature may cause degradation of phenolics. Yang et al. (2009) reported that 72.9 °C is the optimum extraction temperature for crocin, geniposide and total phenolic compound extraction from gardenia fruits. The increased extraction yield was observed with an increased solvent ratio from 4 to 12 then reached a plateau. Temperature and solvent to solid ratio had significant quadratic effects on the Cy-3GR yield. Figure 2 represents the response surface plots of various interactions on the responses. As seen in Fig. 2, yields of all responses increased up to specific maximum % EtOH points with increasing temperature. However it almost reached equilibrium and then started to decrease.

Optimum extraction temperatures for TA and Cy-3GR are slightly lower than non-anthocyanin phenolics and TP content. Optimum EtOH concentrations for extracting anthocyanins are lower than those of TP and non-anthocyanin phenolics. The predicted yield of TP content at optimized conditions was 14.23 mg/g which is similar to the findings of Simsek et al. (2012). They found 14.14 mg GAE and 13.78 mg GAE per gram sour cherry pomace with microwave assisted and conventional extraction, respectively. Adil et al. (2008) found 3.80 mg GAE and 2.92 mg GAE per gram pomace with high pressure extraction and soxhlet extraction, respectively, which are much lower in comparison to our results.

Extraction kinetics

Selection of the appropriate extraction time is an important parameter while considering efficiency, labor and energy cost. Therefore a second set of experiment was performed at lowest (-1), middle (0) and the highest (+1) temperatures of the experimental design after optimization of three independent variables. EtOH concentration and solvent to solid ratio were kept

constant as 51 % and 12, respectively. As expected, concentrations of both TP and TA contents increased as temperature increased (Fig. 3). The increase in temperature may cause the increase in diffusion coefficient, decrease in viscosity coefficient and hence increase in the extraction yield (Pompeu et al. 2009). The equilibrium concentrations were reached at between 80–100 min. time intervals for both TP and TA at all temperatures examined. Nonlinear regressions were applied to the data using exponential model. Good fits were achieved using the exponential model for three temperatures of both TP (Eqs (2–4)) and TA (Eqs (5–7)) changes in the range of time used, as expressed by the coefficients of determination R^2 .

$$TP(25^\circ C) = 4.60(1-\exp(-119.90t)) + 6.49(1-\exp(-0.03t))$$

$$(R^2 = 0.996) \tag{2}$$

$$TP(50^\circ C) = 5.11(1-\exp(-1.52t)) + 8.60(1-\exp(-0.03t))$$

$$(R^2 = 0.997) \tag{3}$$

$$TP(75^\circ C) = 5.18(1-\exp(-1.12t)) + 9.36(1-\exp(-0.04t))$$

$$(R^2 = 0.998) \tag{4}$$

$$TA(25^\circ C) = 0.21(1-\exp(-1.45t)) + 0.11(1-\exp(-0.04t))$$

$$(R^2 = 0.999) \tag{5}$$

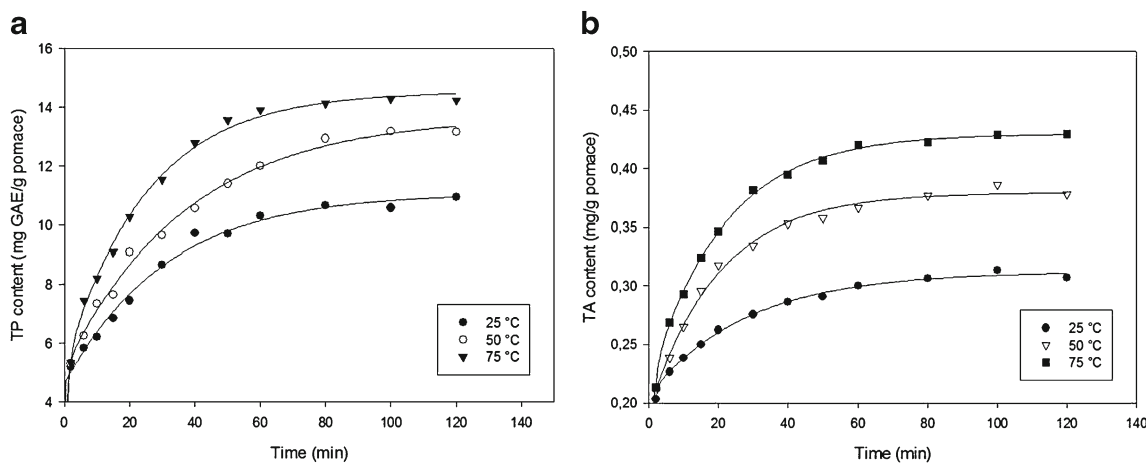


Fig. 3 Extraction kinetics of total phenolic compound a and total anthocyanin b at 25 °C, 50 °C, 75 °C at 51 % ethanol concentration and 12 mL/g solvent ratio

Table 3 Overall optimum values of dependent variables for extraction of total phenolic, total anthocyanin, cyanidin-3-glucosylrutinoside, neochlorogenic acid and catechin with their predicted and experimental verification results (\pm SD)

Extraction variables				Parameter	Predicted	Experimental
% EtOH	Temperature	Solvent ratio	Desirability			
51	75 °C	12 mL/g	0.991	TP	14.18	14.23 \pm 0.38
				TA	0.42	0.41 \pm 0.02
				Cy-3GR	0.19	0.19 \pm 0.02
				NcA	0.23	0.22 \pm 0.01
				Cat	0.23	0.22 \pm 0.02

$$TA(50^\circ C) = 0.19(1 - \exp(-3.09t)) + 0.19(1 - \exp(-0.05t))$$

$$(R^2 = 0.998) \quad (6)$$

$$TA(75^\circ C) = 0.21(1 - \exp(-1.17t)) + 0.22(1 - \exp(-0.05t))$$

$$(R^2 = 0.999) \quad (7)$$

Silva et al. (2007) showed the exponential model for the better fit in their kinetic study. Continuous extraction period until 180 minutes did not cause a loss in the yield, but when considering labor, cost and energy saving, extraction should be terminated at equilibrium points. Sun et al. (2011) and Pompeu et al. (2009) concluded that degradation started after reaching maximum yield at a certain time. Kitanovic et al. (2008) reported similar findings and graph types with our results in their time-kinetic studies. Amendola et al. (2010) found that 120 min. is the optimum time to reach equilibrium concentration for grape marc phenolics extracted at 60 °C.

Verification of the model

An overall optimization study was performed depending on experimental results of all the responses. To verify the model, three individual experiments were carried out. Table 3 shows the overall optimum extraction conditions with predicted and experimental results. No significant difference ($p > 0.05$) was found between the predicted and experimental values of the responses.

Conclusions

The present study describes an optimized extraction procedure for the sour cherry pomace which is a byproduct of juice processing. Sour cherry pomace is a food-value byproduct when we consider its total phenolic, total anthocyanin, cyaniding 3-glucosylrutinoside, neochlorogenic acid and catechin contents. Overall optimum extraction parameters were 51 % EtOH concentration, 75 °C temperature, 12 mL/g

solvent to solid ratio and circa. 100 minute operation time to maximize all of the responses. To the our best knowledge, this is the first report showing the effects of EtOH concentration, temperature, solvent to solid ratio and time all together on the optimization of different responses (including and comparing anthocyanin and non-anthocyanins). As an estimation, 600 kg anthocyanin/year can be produced from sour cherry wastes in Turkey when considering annual juice processing rate and the results presented in this study. According to the results, it can be concluded that sour cherry pomace is a candidate source of food additives as considering its high content of bio-active compounds.

Acknowledgments This study was supported with a fund from Harran University Scientific Research Committee (Project no: 1180). The authors would like to give special thanks to Prof. Dr. Mehmet ÖZKAN (Ankara University, Food Engineering Dept), Dr. Betül ERKAN KOÇ and spec. Çiğdem GÖVER (Harran University, HUMEL) for their kindest technical supports.

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