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Optimization of extraction time and temperature for natural antioxidants of öküzgözü grape pomace using various solvent ratios

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Abstract

Grape pomace, a press residue of winemaking process, has great usage potential in many fields because of its phenolic components such as anthocyanins, antioxidants and dietary fiber. In this study, it was aimed to determine most suitable parameters for production of food colorant from grape pomace (variety of öküzgözü) under different extraction conditions, thus utilising waste of wine production. The dried grape pomace was extracted at 3 various temperatures, 4 different periods and 5 different solvent ratios. 12 different anthocyanins, the free radical scavenging activity, total phenolic content and total monomeric anthocyanin of the extracts were determined and values of the samples ranged between 5.2-676.1 (mg/kg), 1.99-3.65 (IC_{50} mg/mL), 83.68-1598.57 (mg gallic acid/100 g) and 730.7-1850.3 (mg/100 g as mv-3-glc equivalents), respectively. The most suitable temperature, time and solvent ratio for the highest extraction of anthocyanins were obtained at 50 °C and 50:50, 180 min, respectively.

Keywords: grape pomace; waste; press residue; öküzgözü; anthocyanin; HPLC.

Practical Application: Grape pomace, the press residue of wine making, is a good source of phenolic components but a fairly large amount is discarded. In this study, different concentration-time-temperature combinations of ethanol-water phases were tried for maximum anthocyanin extraction from grape pomace. The most suitable parameters were determined by optimizing the parameters for grape pomace. Consequently, the potential for utilising the grape pomace waste was revealed and the parameters that could give the fastest and most effective results in industrial applications were stated.

1 Introduction

Grape (*Vitis vinifera*), the world's largest fruit crop, has been appreciated for their rich content of phenolic compounds such as gallic acid, catechin, resveratrol and anthocyanins (Xu et al., 2010). In 2018, grape production reached 77.8 million tonnes in the World and almost 57, 36 and 7% of grapes are used for wine, fresh and dried grapes, respectively (International Organisation of Vine and Wine, 2019). The yield of the grapes as juice and pomace has been stated to be approximately 80% and 20%, respectively, in winemaking process (García-Lomillo & González-SanJosé, 2017).

It was notified that seven millions tons of press residues globally occur following the wine production. Most phenolic compounds are found in berry skins and seeds. So, grape residue extract has become popular as a nutritional supplement in recent years (Xu et al., 2010; Paradelo et al., 2012; Giusti & Wrolstad, 2003). The main pigments responsible for the color of grapes and wines are anthocyanins that provide bright red color of foods and constitute one of the most important groups of plant pigments. *Vitis vinifera* varieties have 15 different anthocyanins. Malvidin, delphinidin, peonidin, petunidin and cyanidin are the five different anthocyanins according to the aromatic B-ring substitutions (mono-glucoside, acetylglucoside and p-coumaroyl-glucoside) (Castañeda-Ovando et al., 2009; Kirca et al., 2007; Kennedy & Waterhouse, 2000; Kelebek et al., 2010). As well color properties, the most important feature of anthocyanin is antioxidant function capacity, which plays an important role in human health such as protection against pathological conditions (cancer, thrombosis, arteriosclerosis and coronary heart disease etc.) (Kozminski & Oliveira-Brett, 2008). Anthocyanins contain a variety of phenolic hydroxyl groups linked to ring structures and provide antioxidant activity due to different substituents which can reduce the effect of free radicals before damage occurance (Farhadi et al., 2016; Porgalı & Büyüktuncel, 2012).

The usage of grape pomace (GP) is widely in the world. Biosurfactants (food processing), dietary fiber + polyphenols (functional foods), GP powder (supplements), pullulan (pharmaceutical/biomedical) and grapeseed oil + antioxidants (cosmetics) are the commercial products of the GP (Arvanitoyannis, 2010; Dwyer et al., 2014). Additionally, GP has a potential to be used as a colorant in foods due to the content of the rich anthocyanin.

Various extraction methods including parameters such as temperature, time and solvent are used to extract anthocyanins while obtaining food colorant from GP. When applying these parameters in industrial applications, it is necessary to determine the optimum conditions to save energy, time and solvent. In recent years, researchers use different techniques both GP and other food products for optimization of the extraction methods (Madoumier et al., 2019; Meini et al., 2019; Li et al., 2020; Kurek & Sokolova, 2020; Peng et al., 2020).

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In this study, it was aimed to determine most suitable parameters for production of food colorant from GP (variety of öküzgözü) under different extraction conditions. The main objective of the study is that is the utilisation of the GP, which is the wine production waste.

2. Materials and methods

2.1. Material

Raw material

GP of öküzgözü variety was supplied from a commercial local winery in Çal district, Denizli/Turkey during 2016 vintage. Öküzgözü grape variety was grown in vineyards at 800 m above sea level in the Çal area. Following the obtaining of the pomace from the waste of grape press, skins and seeds were separated from GP. The press (Enoveneta PPC100, Italy) pressure was 10 bar. After seperation with the sieve (mesh size 5 mm), GP was shade dried during 3 days at winery yard and transferred to the laboratory in sacks. The average weather conditions during shade drying were as follows: 15 °C temperature, 59% relative humidity and 11 km/h wind speed (tr.free.meteo.com, 2019).

GP extraction

Dried grape pomace (DGP) was sieved with a 5 mm sieve and its extracts were prepared using a mixture of ethanol and 0.1% sitric acid (ethanol/0.1% citric acid; 90:10, 70:30, 50:50, 30:70, 10:90). Extraction was done with a sample/solvent ratio of 1:12 (m/v) according to preliminary analysis. The suspensions were shaken (60 rpm) in a shaking water-bath (Blulab BCS30, Turkey) at temperatures of 30, 40 and 50 °C for 30, 90, 150 and 180 min. Following the extraction, samples were filtered through a Whatman No 1 filter paper and then pressed. Samples were taken into a 500 mL volumetric flask and the solvent was completely removed at 50 °C on a rotary evaporator (Büchi Rotavapor R-114). DGP was redissolved by the addition of 10 mL methanol and combined extracts kept at -18 °C until analysis.

2.2. Methods

Determination of total monomeric anthocyanin pigment

The pH differential method described by Fuleki & Francis (1968) was used for the determination of total monomeric anthocyanin (TMA) pigment. DGP extracts were diluted with buffer solution (pH 1.0) to give a maximum absorbance reading between 0.4 and 0.6. The pH values of diluted DGP extracts were 4.5 (0.4 M sodium acetate buffer) and 1.0 (0.025 M potassium chloride buffer). Absorbance values of DGP extracts were measured spectrophotometrically (Uv-vis model T80, PG Instrument-UK) at 520 and 700 nm and results were calculated as mg/100 g as mv-3-glc equivalents by using the Equation 1:

$$A = \left(A_{520} - A_{700}\right)_{pH\ 1.0} - \left(A_{520} - A_{700}\right)_{pH\ 4.5} \quad (1)$$

TMA values (mg/kg) of DGP extracts were calculated by using the Equation 2:

$$TA = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \quad (2)$$

Where;

TA: Total anthocyanin pigment (mg/kg)

A: The absorbance value of the diluted GP extracts

MW: Molecular weight of Malvidin-3-*O*-glucoside (493.5)

ε: Molar absorptivity (28000)

l: Path length

DF: Dilution factor

2.2.2. Free radical-scavenging activity on DPPH

DPPH method, modified by Brand-Williams et al. (1995) and Spranger et al. (2008), was used for the determination of free radical-scavenging activity of extracts. 0.1 mL of sample, adequately diluted with methanol, was added into freshly prepared methanol solution of DPPH (2,2-diphenyl-2-picrylhydrazyl radical) (3.9 mL, 0.06 mM). Following the addition of DPPH solution, the mixture was stirred and left to stand in a dark place at room temperature for 45 minutes. The absorbance was measured with a spectrophotometer (Uv-vis model, T80, PG Instrument-UK) using methanol as a blank at 515 nm. The free radical-scavenging activity was calculated by using the Equation 3:

$$\%DPPH = \left[\left(A_{control} - A_{sample}\right) / A_{control}\right] \times 100 \quad (3)$$

Where;

$A_{control}$: Absorbance of methanol solution of DPPH (3.9 mL)

A_{sample}: Absorbance of sample

The free-radical scevenging activity was showed as IC_{50} (mg/mL), where the time required providing 50% inhibition of the sample was calculated from the graph plotting percentage vs sample concentration.

Determination of total phenolic content

Firstly, 8.4 mL of distilled water, 0.1 mL sample and 0.5 mL of the Folin-Ciocalteu reagent (1:9, v:v) were added to the test tube, respectively. Following the stirring, the mixture was left to stand at room temperature for 3 minutes and then 1 mL of 7.5% Na_2CO_3 solution added and stirred. Following the stirring, the mixture was incubated in a dark place for an hour. The absorbance against blank was measured at 720 nm using a spectrophotometer (Uv-vis model, T80, PG Instrument-UK). Six points calibration curves, covering the range of 0-200 mg/L with the correlation coefficient of 0.995, was prepared by using stock-standard solutions of gallic acid. Total phenolic content (TPC) was determined as mg gallic acid/100 g using the method of Singleton & Rossi (1965).

Anthocyanin profile on HPLC

Reagents

Analytical anthocyanin standards of the 3-*O*-β-glucosides of cyanidin (Cn-3-glc), delphinidin (Dp-3-glc), malvidin (Mv-3-glc), pelargonidin (Pg-3-glc), peonidin (Pn-3-glc) and petunidin (Pt-3-glc) and malvidin-3-O-acetyl-glucoside (Mv-3-acglc), malvidin 3-O-caffeoyl-glucoside (Mv-3-cafglc), petunidin-3-O-coumaroyl-glucoside (Pt-3-cmglc), malvidin-3-O-cis-p-coumaroyl-glucoside (Mv-3-cis-cmglc), delphinidin-3-O-p-coumaryl-glucoside (Dp-3-cmglc), peonidin-3-O-p-coumaryl-glucoside (Pn-3-cmglc), malvidin-3-O-trans-p-coumaroyl-glucoside (Mv-3-trans-cmglc) were purchased from Extrasynthese Co. (Genay Cedex, France) and their stock-standard solutions were prepared in mobile phase. Calibration curve was prepared with five different concentrations of each standard. Solutions used in the study were first sonicated and stored in dark glass flasks in order to protect them from light, and then kept under refrigeration. Thus, five point calibration curves with the determination coefficients of 0.999 based on the concentration (mg/L) versus peak area (mAU) were prepared for investigated compounds mentioned above.

Equipment

A liquid chromatography (Shimadzu Corporation, Kyoto, Japan) system consisting of a UV-VIS DAD detector set at 530 nm (Model SPD-M10 AVP, Shimadzu), a column oven (Model CTO-10ASVP, Shimadzu), a quadruple liquid chromatography pump (Model LC-10AT-VP, Shimadzu), a degasser (Model DGU 14A, Shimadzu), a temperature programmable column oven to maintain the column temperature at 35 °C and a Shimadzu Software Program was used for the analysis. A syringe (Hamilton Co., Reno, NV, USA) was used for the injection of the sample (20 µL) into the HPLC. Additionally, a reversed-phase discovery C_{18} column (15 cm x 4.6 mm ID, 5µm particle size) (Cat. No: 504955) from SUPELCO (Bellefonte, PA, USA) was used in the HPLC system. Formic acid/distilled water (solvent A; 5:95) and formic acid/acetonitrile (solvent B; 5:95) were used as mobile phase. The gradient system used for the anthocyanin determination was 0 to 5 min, 5% B; 5 to 15 min, 5% to 8% B; 15 to 32 min, 8% to 15% B; and 32 to 55 min, 15% B; and 55 to 60 min, 15% to 5%.

Analysis

Analysis of investigated anthocyanins were done with some modifications of the method described by Oh et al. (2008). Prior to analysis, GP extracts were filtered using a filter (Millipore, 0.45 µm). Chromatographic data on the peaks were integrated up to 30 min. Identification of peaks were realized by comparing their retention time values and UV spectra with the standard reference compounds stored in a data bank. Integrated areas of the sample and the corresponding standards were used for the calculation of concentrations of investigated anthocyanins. As extractions and injections were done in duplicate, the final result was the arithmetic average of four analyses.

Further analysis

DGP was diluted (1:10 w/v) with a deionised water and rehydrated during one day in a refrigerator. Following the rehidration period, rehydrated pomace (RP) was filtered through a filter paper (Whatman No:4) and used for the analysis of total acidity, pH and total soluble solids (% brix). AOAC (Association of Official Analytical Chemists, 2000) methos were used for the determination of total acidity (g tartaric acid/100 mL) and total soluble solids (% brix). A pH meter (Hanna Instruments, HI 83141, Michigan, USA) equiped with an electrode and standardized by a 2 point method against pH 7 and pH 4 buffer standards was used for the pH analysis (Association of Official Analytical Chemists, 2000). The moisture content of GP and DGP was measured at 105 ± 1 °C for 4 hours in drying oven (Memmert UN 160, Schwabach, Germany) (Association of Official Analytical Chemists, 2000). The RP was grinded in a blender (Waring 8011 EB, Stamford, USA) before drying while the non-rehydrated pomace (NRP) directly.

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using the IBM SPSS statistics software version 22.0 (New York, US). Differences between means were first analyzed using the multivariate test, and the least significant differences (Tukey HSD) were calculated following significant F test ($P < 0.05$). The results are the average of three measurements.

3 Results and discussion

3.1 Characteristics of GP and DGP

Öküzgözü GP was selected for the extraction of anthocyanins because of it is the most used grape variety in winemaking process in Çal, Denizli region. In order to know the properties of the grapes which is used in the study, the selection of GP was based on the same batch production. Chemical characteristics of fresh GP and DGP are shown in Table 1. The fresh grape pomace was dried until moisture less than 10%. The final moisture of DGP was 7.80%. Results presented in Table 1 show that the total acidity of DGP decreased when the brix increased. This is due to the reduced moisture content during drying.

3.2 Optimum extraction of DGP

DGP extracts was prepared by using 5 different solvent ratios (90:10, 70:30, 50:50, 30:70 and 10:90, ethanol: 0.1% citric

Table 1. Total acidity, pH, moisture and brix values of grape pomace and dried grape pomace.

	Grape Pomace	Dried Grape Pomace
Total Acidity (%)	4.38	2.40
pH	3.33	3.54
Moisture content (%)	62.62	7.80
Brix (%)	4.10	9.00

acid), 4 different periods (30, 90, 150, 180 min) and 3 different temperatures (30, 40 and 50 °C). The parameters giving the highest amount of TMA for each temperature and time were determined as optimum (Table 2, Figure 1, Figure 2, Figure 3). As shown in Table 2, 12 different anthocyanins were determined with the above mentioned extraction method. It was determined that mv-3-glc is the highest one within all anthocyanins while cn-3-glc is the lowest. In addition, the most efficient extraction of all anthocyanins was achieved by using ethanol: 0.1% citric acid (50:50) at all application times while the lowest extraction was obtained with ethanol: 0.1% citric acid acid (10:90). It was determined that all anthocyanins were statistically significant at $p < 0.05$ level in all applications of time and solvents.

The highest values for the maximum extraction of anthocyanins were found at 50 °C. Although the highest anthocyanin content with the solvent ratio of 50:50 (ethanol: 0.1% citric acid) were the same in all samples, the times varied depending on the temperature. The highest anthocyanin concentration was reached in 180 min at 30 and 50 °C and in 90 min at 40 °C. However, similar results were obtained at 40 °C for 150 and 180 min. Although, the highest antocyanin concentration was found at 40 °C for 90 min, a slight decrement of antocyanin content at 40 °C for 150 and 180 min was observed because of the degradation of anthocyanin during application and the formation of new compouds. In addition, the chemical components and anthocyanin values of winery waste products vary according to the type of grape, growing conditions, extraction parameters and analysis method (Ben Aziz et al., 2019; Costa et al., 2019).

When all these results were examined thoroughly, it can be said that extracts applied with ethanol: 0.1% citric acid (50:50) solution at 50 °C for 180 min have the highest anthocyanin content. Highest values of all anthocyanins were found to be at ethanol: 0.1% citric acid (50:50). It is determined that the selected optimum parameters for this study, are similar to other studies (Mikulic-Petkovsek et al., 2017; Cebrian et al., 2017; Karacabey et al., 2013; Riquelme et al., 2019; Çetin et al., 2011). In addition, similar results were obtained by Corrales et al. (2008) who have reported that 50:50 ethanol concentration were chosen for the extraction. Moreover, Spigno et al. (2007) and Pinelo et al. (2005) also have pointed out that the using of ethanol/water mixture was more effective than water alone for anthocyanin extraction from grape pomace. Additionally, Santos et al. (2011) prepared extracts by using ethyl acetate, butanol, methanol and hexane as the solvent in different parts of grapes and found the highest antioxidant activity in ethyl acetate extract, while the lowest antioxidant activity in methanol extract.

3.3. Total monomeric anthocyanin, phenolics content and free radical-scavenging activity of DGP

Table 3 shows total monomeric anthocyanin, phenolic and DPPH free radical-scevenging activity of DGP extracts at optimum extraction conditions.

TMA was found to range from 730.7 to 1850.3 mg/100 g as mv-3-glc equivalents. Concerning the amounts of TMA of extracts, 50:50 ethanol: 0.1% citric acid solvent ratio in 180 min at 50 °C had the highest TMA, while 70:30 ethanol: 0.1% citric acid solvent ratio in 30 min at 30 °C the lowest. Nevertheless, no statistically significant difference ($p < 0.05$) was found between the extracts except for the extract of 70:30 ethanol: 0.1% citric

Table 2. Highest anthocyanin values at various temperature and time parameters.

Temperature (°C)	30				40				50			
Time (min)	30	90	150	180	30	90	150	180	30	90	150	180
Solvent (Ethanol:%0.1 sitric acid)	70:30:00	70:30:00	50:50:00	50:50:00	70:30:00	50:50:00	70:30:00	50:50:00	70:30:00	70:30:00	70:30:00	50:50:00
Mv-3-glc*	271.3 ± 5.4Ce	504.9 ± 4.2Bc	572.0 ± 3.1Ab	569.2 ± 13.2Ab	487.1 ± 1.9Bcd	586.8 ± 4.7Ab	476.5 ± 2.5Bd	482.1 ± 3.3Bd	474.1 ± 0.8Dd	571.1 ± 2.3Cb	586.7 ± 2.9Bb	676.1 ± 1.8Aa
Dp-3-glc*	6.4 ± 0.2Ce	11.3 ± 0.4Bcd	11.9 ± 0.4Bcd	13.3 ± 0.2Ab	11.4 ± 0.4ABcd	12.4 ± 0.3Abc	10.9 ± 0.3Bd	10.9 ± 0.4Bd	10.8 ± 0.1Cd	13.3 ± 0.2Bb	13.3 ± 0.1Bb	15.5 ± 0.2Aa
Pn-3-glc*	25.5 ± 0.6Af	46.7 ± 0.6Bd	52.9 ± 0.8Ac	54.0 ± 0.4Abc	46.1 ± 0.4Bd	53.8 ± 0.3Abc	44.1 ± 0.3Ce	45.2 ± 0.4BCde	44.3 ± 0.4De	53.2 ± 0.4Cc	55.5 ± 0.3Bb	63.3 ± 0.1Aa
Pt-3-glc*	10.0 ± 0.4Cg	19.6 ± 0.4Be	20.7 ± 0.4Bd	23.0 ± 0.1Ab	18.1 ± 0.2Bf	22.1 ± 0.1Abc	18.2 ± 0.3Bf	18.4 ± 0.1Bf	18.7 ± 0.2Def	21.3 ± 0.2Ccd	22.6 ± 0.1Bb	25.8 ± 0.1Aa
Cn-3-glc*	5.2 ± 0.1Cf	8.3 ± 0.2Be	9.4 ± 0.4Acd	10.0 ± 0.1Abc	8.6 ± 0.3Bde	10.0 ± 0.3Abc	8.0 ± 0.1Be	7.9 ± 0.1Be	8.2 ± 0.1Ce	10.5 ± 0.1Bab	10.3 ± 0.1Bb	11.4 ± 0.1Aa
Mv-3-acglc*	192.1 ± 3.5Cg	360.2 ± 4.6Be	401.7 ± 4.5Ad	406.7 ± 2.4Acd	346.6 ± 1.7Bf	419.6 ± 2.5Ab	341.5 ± 1.4Bf	342.3 ± 2.8Bf	335.4 ± 1.5Df	406.1 ± 1.7Ccd	416.7 ± 2.4Bbc	479.7 ± 3.1Aa
Mv-3-cafglc*	26.3 ± 0.4Cd	47.3 ± 0.9Bc	54.9 ± 0.7Ab	54.7 ± 0.6Ab	47.0 ± 0.3Bc	55.9 ± 0.5Ab	44.8 ± 1.6Bc	46.0 ± 0.4Bc	45.7 ± 0.5Dc	55.2 ± 0.4Cb	56.7 ± 0.2Bb	65.2 ± 0.4Aa
Pt-3-cmglc*	9.3 ± 0.2Ce	18.5 ± 0.2Bc	21.8 ± 1.3Ab	20.4 ± 0.4ABb	16.0 ± 0.3Bd	21.2 ± 0.6Ab	17.2 ± 0.4Bcd	17.1 ± 0.3Bcd	17.6 ± 0.2Dcd	20.6 ± 0.3Cb	21.9 ± 0.1Bb	24.4 ± 0.2Aa
Mv-3-cis-cmglc*	18.4 ± 0.5Ce	33.5 ± 0.4Bd	36.7 ± 1.1Ac	37.9 ± 0.3Abc	32.0 ± 0.4Bd	38.9 ± 0.4Ab	32.1 ± 0.4Bd	31.5 ± 0.7Bd	31.5 ± 0.6Cd	37.9 ± 0.4Bbc	39.4 ± 0.2Bb	44.4 ± 0.1Aa
Dp-3-cmglc*	22.9 ± 0.6Cg	43.6 ± 0.9Be	52.5 ± 0.9Acd	51.1 ± 0.4Ad	39.9 ± 0.4Cf	51.8 ± 0.4Ad	42.1 ± 0.6Be	39.1 ± 0.1Cf	43.4 ± 0.6De	54.4 ± 0.4Cbc	56.0 ± 0.2Bb	62.5 ± 0.1Aa
Pn-3-cmglc*	6.5 ± 0.3Ce	11.8 ± 0.6Ac	9.0 ± 0.3Bd	12.3 ± 0.2Abc	9.3 ± 0.3Bd	11.4 ± 0.3Ac	9.9 ± 0.2Bd	9.2 ± 0.2Bd	9.5 ± 0.1Dd	13.4 ± 0.1Bb	12.1 ± 0.1Cc	14.7 ± 0.1Aa
Mv-3-trans-cmglc*	65.9 ± 0.6Cf	122.8 ± 1.2Bd	138.7 ± 2.6Ac	140.9 ± 0.9Abc	119.6 ± 1.1Bde	145.5 ± 1.1Ab	115.6 ± 2.0Be	118.9 ± 0.5Bde	115.4 ± 1.1De	140.9 ± 1.2Cbc	144.9 ± 1.3Bb	166.6 ± 1.3Aa

*Abbreviations: Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; glc, glucoside; ac, acetyl; caf, caffeoyl; cm, coumaroyl; TMA, total monomeric anthocyanin. Values are on the basis of dry matter (mg/kg); $^{a-g}$Small letters within rows denote significant differences at $p < 0.05$; $^{A-D}$Capital letters within rows denote significant differences in 30, 40 and 50 °C at $p < 0.05$.

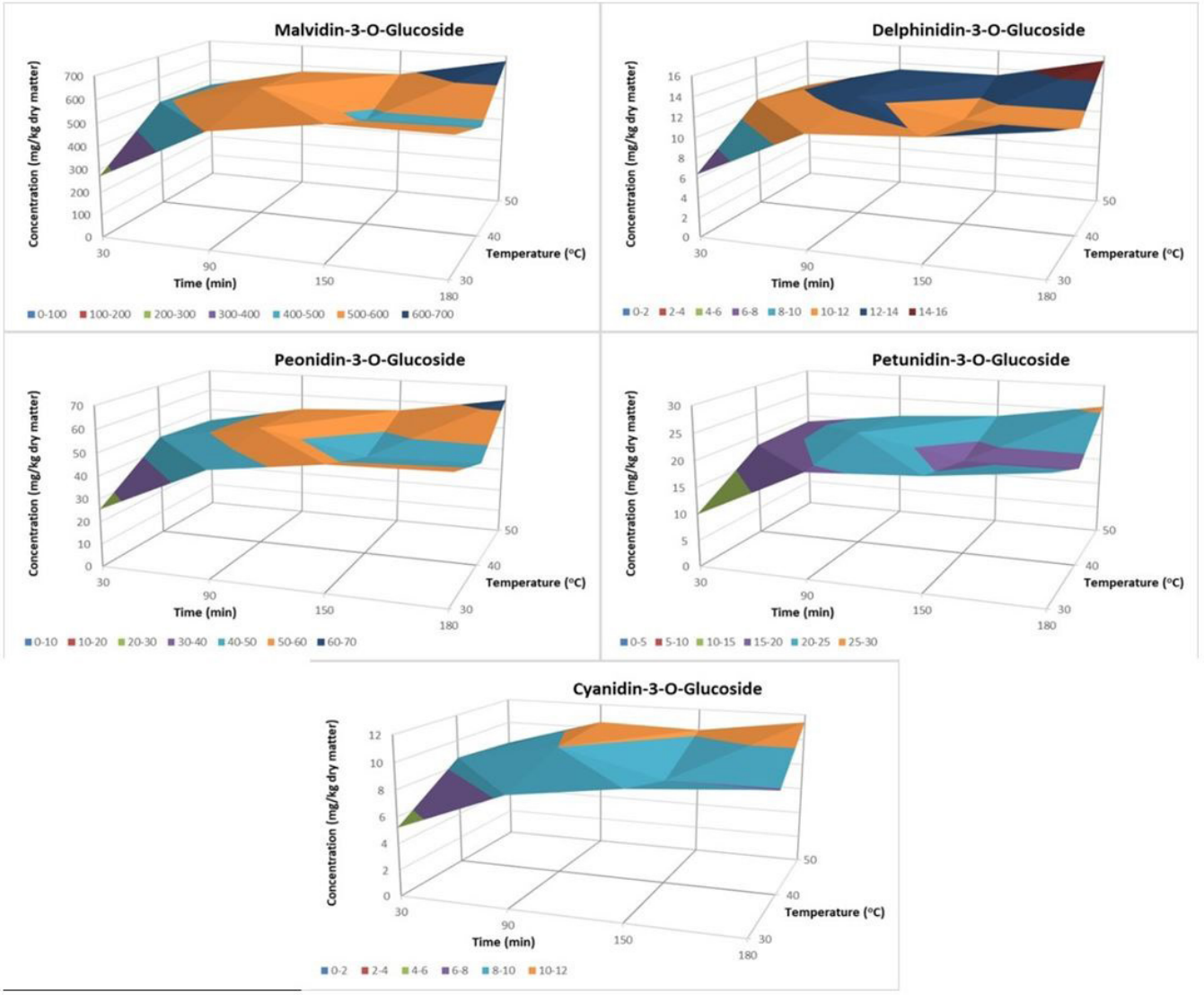

Figure 1. Three dimension graph of highest values of mono glucosides with various solvent extraction ratio and periods on the anthocyanin profile of dried grape pomace at 30, 40 and 50 °C.

acid solvent ratio in 30 min at 30 °C. When compared with other similar studies, the appropriate parameters were found to be the same as in this study (Benmeziane et al., 2016; Caldas et al., 2018; MohdMaidin et al., 2019; Pereira et al., 2019). Farhadi et al. (2016) found the TMA values of different cultivars of grapes between 7-6310 mg/100g as cy-3-glc equivalents. In addition, Orak (2007) has studied on extracts of 16 different grape varieties in 2007 and found the values of TMA between 40.3-990.8 mg/100 g as mv-3-glc equivalents. The same researcher reported the TMA values of Öküzgözü variety as 938.5 mg/100 g as mv-3-glc equivalents.

TPC content of DGP extracts was determined as mg gallic acid/100 g using the method of Singleton & Rossi (1965). TPC was found to range from 360.55 to 1598.57 mg gallic acid/100 g. The results show that 50:50 ethanol: 0.1% citric acid solvent ratio in 180 min at 50 °C had the highest value. TPC differed significant differences ($p < 0.05$) depending on the time at 30 °C. On the other hand there were no significant differences statistically at 40 °C and 50 °C. Only 180 min at 50 °C was the statistically different from other extraction parameters. The results obtained from TPC are in accordance with other studies (Orak, 2007; Brazinha et al., 2014; Karasu et al., 2016). In contrast, Çetin et al. (2011) have found greater TPC value in (36.56 mg gallic acid/100 g) and Shiri et al. (2013), Ünal & Şener (2016), Corrales et al. (2008) and Pezzini et al. (2019) have found lower TPC values than our study results. The variation between the results may be caused by the differences in soil and climate conditions of the grown region as well varieties of grapes. Additionally, different grape species and extraction parameters used in the studies may have caused this change.

The DPPH free radical-scavenging activity of DGP extracts were calculated as the IC_{50} (mg/mL) value. Since the IC_{50} value is the concentration required to remove 50% of the DPPH radicals, the lower IC_{50} value expresses higher antioxidant activity. The IC_{50} values of DGP extracts ranged from 1.99 to 3.65 mg/mL. There was significant difference ($p < 0.05$) between all extracts but not with the time. Concerning the extracts, 50:50 ethanol: 0.1% citric acid solvent ratio in 180 min

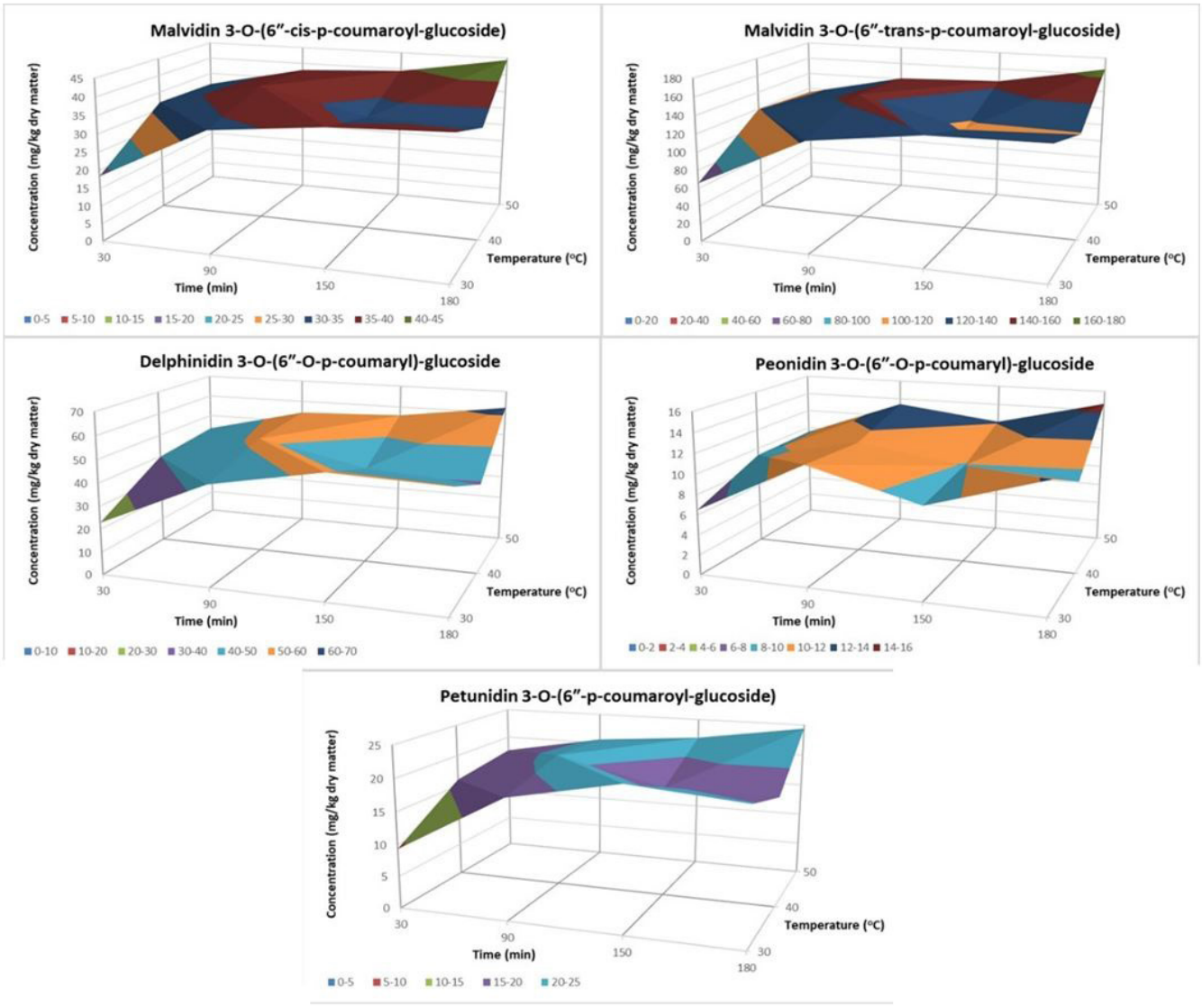

Figure 2. Three dimension graph of highest values of coumaryl glucosides with various solvent extraction ratio and periods on the anthocyanin profile of dried grape pomace at 30, 40 and 50 °C.

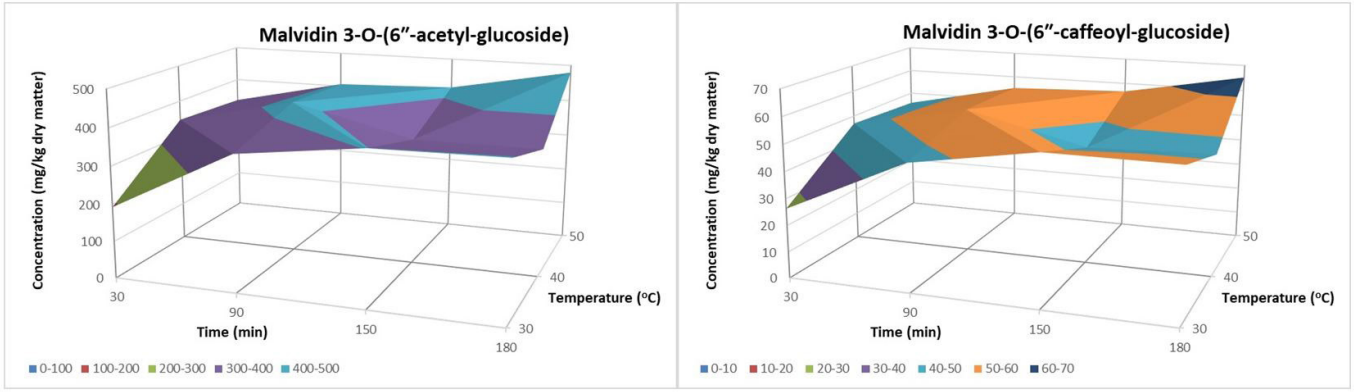

Figure 3. Three dimension graph of highest values of acetyl and caffeoyl glucosides with various solvent extraction ratio and periods on the anthocyanin profile of dried grape pomace at 30, 40 and 50 °C.

Table 3. Total monomeric anthocyanin, DPPH free-radical scevenging activity and total phenolic content of of DGP extracts at optimum extraction conditions.

Temperature (°C)	Time (min)	Solvent (Ethanol:%0.1 sitric acid)	TMA (mg/100 g as mv-3-glc equivalents)	DPPH (IC_{50} mg/mL)	TPC (mg gallic acid/100 g)
	30	70:30	730.7 ± 110.2Bc	3.65 ± 0.1Aa	360.55 ± 37.6Ce
	90	70:30	1369.3 ± 7.5Aab	3.52 ± 0.1Aab	759.50 ± 37.6Bbcde
30	150	50:50	1554.0 ± 12.2Aab	3.23 ± 0.0Bcd	865.33 ± 112.7ABbcde
	180	50:50	1578.7 ± 100.1Aab	3.11 ± 0.0Bd	1089.65 ± 93.8Aabc
	30	70:30	1334.1 ± 242.4Ab	3.38 ± 0.1Abc	934.88 ± 300.0Abcde
	90	50:50	1621.9 ± 49.0Aab	3.23 ± 0.1Acd	1039.97 ± 150.3Abcd
40	150	70:30	1316.3 ± 108.4Ab	3.3 ± 0.1Abcd	962.15 ± 0.0Abcd
	180	50:50	1323.8 ± 100.2Ab	3.35 ± 0.1Abcd	1251.78 ± 225.3Aab
	30	70:30	1293.3 ± 87.8Ab	3.4 ± 0.0Abc	508.77 ± 0.0Bde
	90	70:30	1564.8 ± 164.5Aab	2.6 ± 0.0Ae	881.04 ± 0.0Bbcde
50	150	70:30	1607.1 ± 229.7Aab	2.56 ± 0.0Ae	774.13 ± 0.0Bbcde
	180	50:50	1850.3 ± 99.4Aa	1.99 ± 0.0Af	1598.57 ± 0.0Aa

[a-f]Small letters within columns denote significant differences at $p < 0.05$; [A-C]Capital letters within columns denote significant differences in 30, 40 and 50 °C at $p < 0.05$.

at 50 °C had the highest antioxidant activity, while 70:30 ethanol: 0.1% citric acid solvent ratio in 30 min at 30 °C had the lowest. There was a positive trend between antioxidant activity and TPC, but no rational relationship between their amount was observed. Concerning the other studies, some authors (Rockenbach et al., 2011; Xu et al., 2010) remarked a positive trend between antioxidant activity and TPC however others expressed that antioxidant activity was dependent on the phenolic profile (Karasu et al., 2016; Lutz et al., 2011; Baiano & Terracone, 2011).

4 Conclusion

It is seen that the most efficient parameters in laboratory and industrial applications are by 50:50 solvent extraction (ethanol: 0.1% citric acid) at 180 min. Grape pomace, that contains anthocyanins, has great potential to be used as an additive in foods. The optimization of parameters of food colorant production is the potential for utilising the grape pomace waste. Thus the parameters that could give the fastest and most effective results in industrial applications were determined. In this extraction study, the utilising of press residue of wine making process in different fields of food industry was aimed.

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