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Multiple response optimization to determine the suitable solvent for the extract production from defatted grape seed powder: A simplex lattice mixture design approach

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Abstract

In this study, effects of different solvents (ethanol, methanol and water) on bioactive performance of defatted grape seed powder (GSP) were investigated using simplex lattice mixture design approach. Also, multiple response optimization process was applied to determine the best solvent type for the high bioactive GSP extract production. For this purpose, the bioactive compound concentrations and their antioxidant and antiradical properties were characterized and the effect of solvent type on the processing variables was modelled. Total phenolic and flavonoid contents of GSP ranged between 0.31-7.29 mg GAE/g and 44.3-537.4 mg CE/kg sample respectively. In addition to that, DPPH and $ABTS^{\cdot+}$ radical scavenging activity of the samples were in the range of 2.11-80.5% and 0.31-4.08 µg Trolox/ g sample respectively. The effect of solvent type showed a significant effect on all studied bioactive parameters and the best solvent mixture was determined as ethanol (33.84%), methanol (20.17%) and water (45.99%) by the considering the all studied parameters.

Keywords: Grape seed powder, Solvent, Optimization, Bioactivity

Introduction

Grape (*Vitis vinifera* L.) is an important fruit especially the most basic raw material for the wine industry and grape pomace including the skin, seed, stalk etc., is the main processing waste of this fruit. The grape pomace is a reasonable and lucrative raw material for the cosmetic, pharmaceutical and food industries because it is rich in some important bioactive compounds namely fatty acids, tocopherols, proanthocyanidins, sterols, etc. (Demirtaş et al., 2013, Barba et al., 2016). Grapes are one of the mostly cultivated fruits by an approximate annual production of 58 million metric tons (FAO, 1997). Teixeira et al. (2014) reported that the grape seeds ratio in the whole pomace is 38-52% on a dry weight basis. In many researches, antioxidant, anti-inflammatory and antimicrobial characteristics of grape seeds were reported (Oliveira et al., 2013; Sofi et al., 2016; Soto et al., 2015). Saito et al. (1998) reported that the grape seeds are rich in monomeric phenolic compounds such as catechin and epicatechin and these compounds are responsible for the antimutagenic and antiviral performance of the sample. Jayaprakasha et al. (2001) reported that the grape seed is accepted as a dietary supplement because of its health benefits of catechins and procyanidins. It was reported that the most of the bioactive compounds (approximately 75%) existed in the skin and seeds of the fruit (Pinelo et al., 2006). Due to these important biological properties of grape pomace especially seeds, there is an increased interest for the extraction of polyphenols to be used as an antioxidant instead of synthetic antioxidants in foods because of the possible undesirable effects on human health (Jayaprakasha et al., 2003). Bioactivity of grape seed powder (GSP) or another food material is affected by some processing factors such as solid/liquid ratio, extraction time, temperature, type and also extraction solvent. The solvent used for the extraction of polyphenols has a significant effect on the extraction yield and also the final product bioactivity. Many solvents such as methanol, ethanol, acetone, diethyl ether, ethyl acetate (Bonilla, Mayen, Merida, & Medina, 1999; Lafka, Sinanoglou, & Lazos, 2007), and their binary or ternary mixtures of each other or combinations with distilled water have

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been widely used for the extraction of phenolics (Lafka et al., 2007). Downey and Hanlin (2010) reported that the tannins yield changed with the type of solvent (acetone or ethanol) or the percentage of water in the mixtures significantly. Similarly, Bosso et al. (2016) studied the effects of some solvents (water, ethanol, acetone, ethyl acetate), as pure or in binary and ternary mixtures and they reported that the different solvents had different effects on the composition of condensed tannins in GSP extracts. Clearly, there are different studies aiming to determine the suitable solvent type for the extraction of food sample.

The main aim of the current study was to determine the best solvent mixture for the extraction of grape seed powder (GSP). For this purpose, multiple response optimization technique was selected to see the simultaneous effect of solvent mixtures to compose the best solvent mixture to provide a highly yielded GSP extract in terms of bioactivity. So, ethanol, methanol and water were the solvent used and simplex lattice mixture design was used for the optimization of processing variables.

Material and Methods

Material

Grape seeds were provided from a local seller in Kayseri, Turkey. The seeds were ground using a grinder and exposed to oil extraction using n-hexane at the ratio of 1:2 sample/n-hexane. The sample was mixed on the magnetic stirrer for 30 min and then n-hexane was removed from the residue in a fume hood. The residue was dried at room temperature for 2 hours and then the defatted GSP was used for further extraction studies.

Extraction of bioactive compounds from GSP

For the extraction of bioactive compounds from grape seed powder (GSP), three different solvents (ethanol, methanol and water) and their mixtures at different levels as shown in Table 1 were used. To determine the best solvent or solvent mixture achieving high bioactivity, simplex lattice mixture design approach was used as shown in Table 1. A 1 g of the GSP sample was weighed and 30 mL of the solvent prepared according to the experimental design points (Table 1) was incorporated into the tubes and then the tubes were mixed by vortex for 1 min and then covered tightly and placed in a shaking water bath at 25 ºC. The samples were extracted for 1 hour and then the samples were centrifuged at 9000 *g* at 5 ºC for 3 min. Finally, the supernatants were filtrated using a filter paper (0.45 µm) and then the extracts were subjected to further bioactive analysis.

Bioactivity tests for the GSP extracts

Determination of total phenolic content (TPC)

Total phenolic content analysis of the samples was determined according to the methodology described by Singleton and Rossi (1965) and Köprü et al. (2020). For this purpose, 200 µL of extract was mixed with 1800 µL of distilled water. Then, 1 mL of Folin Cioceltau reagent (1:10) was added and waited for 1 min. Finally, 2 mL of sodium carbonate (2% w/v) was added and the tubes were vortexed. At the end, the samples were incubated for 2 h at room temperature in dark conditions. After incubation, the absorbance of the samples was recorded at 765 nm using a UV-vis spectrophotometer (Shimazdu, Japan). Total phenolic content of the samples was calculated as mg gallic acid equivalent (mg GAE/g sample) and the measurements were replicated with four repetitions.

Determination of total flavonoid content (TFC)

The total amount of flavonoids in the samples was determined according to the methodology described by Zhishen et al. (1999). For this purpose, 500 µL of sample was mixed with 2 mL of water and 150 µL of $NaNO_2$ (5% w/v) was added to the samples. After 5 min, 150 µL of $AlCl_3$ (10% w/v) was placed into the samples and they were incubated for 6 min and then 1 mL of NaOH and 1.2 mL of water was added to complete the sample volume to 5 mL. After mixing with vortex, the samples were incubated for 10 minutes at room temperature and in dark environment and the absorbance values of the samples were measured by UV-vis spectrophotometer (Shimadzu, Japan) at 510 nm. Total flavonoids were calculated as mg CE (catechin equivalent) / kg sample using calibration curves prepared with catechin standard. The measurements were replicated with four repetitions.

Determination of condensed tannin (CT) level

Condensed tannin contents of the samples were performed by applying the method proposed by Sun et al. (2002). For this purpose, 1 ml of extract was mixed with 2.5 ml of vanillin (prepared with 1% methanol) and 2.5 ml of H_2SO_4 (prepared with 25% methanol) was added. Subsequently, the samples were incubated for 15 minutes in a water bath at 30 ºC and the absorbance values were recorded at 500 nm by UV-vis spectrophotometer (Shimadzu, Japan). The measurements were replicated with four replicates.

Determination of antiradical activity

DPPH radical scavenging activity

Antiradical activity of the samples was determined using DPPH as radical by the method of Köprü et al (2020). For this purpose, 100 µL of nondiluted extract was mixed with 3900 µL of DPPH solution (0.2 mM in methanol). The mixture was vortexed well and the samples were incubated for 30 min at room temperature in dark conditions. At the end of the incubation, the absorbances of the samples were recorded at 517 nm using a UV-vis spectrophotometer (Shimadzu, Japan). Antiradical activity of the samples was calculated as % inhibition as following:

$$\% \text{ Inhibition: } [(Abs_{control}-Abs_{sample})/Abs_{control})]*100 \quad (1)$$

$ABTS^{\cdot+}$ radical scavenging activity

$ABTS^{\cdot+}$ radical scavenging activity of the samples was determined by the method of Wettasinge et al. (2002) and Mathew and Abraham (2006). At the beginning, 7 mmol $ABTS^{\cdot+}$ was dissolved in some amount of water and treated with 2.45 mmol potassium persulfate. The mixture was then allowed to stand at room temperature for 16 hours until a dark blue color appeared and was ready for analysis. This dark blue solution was diluted with buffer solution (pH 7.4) until the absorbance was 0.7 at 734 nm. Then, 2 ml of this diluted solution ($ABTS^{\cdot+}$ solution) was mixed with 100 µl of the diluted sample extract in appropriate proportions (1:30) and after 6 min incubation, the absorbance values were measured at 734 nm by UV-vis spectrophotometer (Shimadzu, Japan). The

reduction in $ABTS^{\cdot+}$ radical cation was calculated in percent according to the following equation and the results are given as µg Trolox/g sample. All experiments were carried out in 4 repetitions.

$$\% \text{ Inhibition: } [(Abs_{control}-Abs_{sample})/Abs_{control})]*100 \quad (2)$$

Determination of antioxidant activity

Iron chelating activity (ICA)

Iron chelating activities of the samples were determined by Rival et al. (2001) by making partial modifications. For this purpose, 1 mL of the sample extract diluted as 1:10 was taken and 3.7 mL of ethanol (95% v/v) was added. Then, 100 µl of $FeCl_2$ was added to the samples and immediately after vortexing the samples 200 µ ferrozine (5 mM) was incorporated. The homogeneously mixed samples were allowed to incubate for 10 min at room temperature in the dark and the absorbance values of the samples were measured by UV-vis spectrophotometer (Shimadzu, Japan) at 562 nm. Iron chelating activity values of the samples were calculated as % inhibition using the following equation. All experiments were carried out in 2 repetitions with 4 replicates.

$$\% \text{ Inhibition: } [(Abs_{control}-Abs_{sample})/Abs_{control})]*100 \quad (3)$$

Antioxidant activity by phosphomolybdenum (AA)

Antioxidant activity values of the samples were determined using the phosphomolybdenum method proposed by Prieto et al. (1999). In this context, firstly the test solution (0.6 M sulfuric acid (30 mL), 28 mM sodium phosphate (28 mL) and 4 mM ammonium molybdate 40 mL) was prepared freshly by combining, and then 0.4 mL sample was mixed with 4 mL test solution and the mixture was vortexed and the test tubes were allowed to incubate in a water bath at 95 °C for 90 min. At the end of the period, absorbance values of the samples were measured by UV-vis spectrophotometer (Shimadzu, Japan) at 695 nm. Results of antioxidant activity were given in mg ascorbic acid equivalent (mg AAE / kg) using calibration curves plotted with ascorbic acid standard. All experiments were carried out in 2 repetitions with 4 replicates.

Data modeling and optimization

Simplex lattice mixture design (SLMD) was used to determine the effects of different solvents namely ethanol (X_1), methanol (X_2) and water (X_3) on the bioactive performance of the grape seed powder extract. To determine the optimum solvent type to produce high bioactive grape seed extract having high antiradical and antioxidant capacities, multiple response optimization process was followed. As is seen in Table 1, the component proportions were expressed as the fractions of the mixture with a sum (X_1+X_2+X_3) of one. The following polynomial equation of function x_i was fitted for each factor assessed at each experimental point. This polynomial model differs from full polynomial models because it does not contain a constant term (intercept equal to zero). This polynomial model equation was:

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 \quad (4)$$

where Y is the estimated response; β_1, β_2, β_3, β_{12}, β_{13} and β_{23} are constant coefficients for each linear and nonlinear (interaction) term produced for the prediction models of processing components. The analysis was performed using uncoded units. Square root transformation was applied for the studied parameters except AA.

The computational works, mathematical modeling, preparing the ternary contour graphical presentations of the models, were performed using Design Expert statistical software. JMP statistical package software (Version 5.0.1.a, SAS Institute. Inc. Cary, NC) was used to compute the predicted equations. The correlations between the parameters were determined using XLSTAT for Windows.

Results and Discussion

Bioactive compound levels of the samples

Table 2 shows the change in total phenolic content (TPC) of the samples according to the solvent used. As is seen, the lowest TPC level was determined as 0.33 and 0.31 mg GAE/g for the sample prepared by only ethanol (runs 5 and 12) while the highest TPC (7.29 mg GAE/g) was determined for the sample extracted by using ethanol: water mixture (1:1). The change in the TPC content depending on the solvent used was statistically significant ($p<0.05$, Table 3). The ANOVA results for the studied parameters were tabulated in Table 3. As is seen, the model selected for TPC was found as significant ($p<0.05$) and the linear effects of the studied solvents (ethanol, methanol and water) were determined as to be significant ($p<0.05$). Only the interaction effects between the ethanol and methanol were not significant which means that the mixture of both two organic solvents was not effective on the extraction of bioactive compounds. The fitted second order polynomial equation for total phenolic content is as follows:

$$Y_{TPC}=0.59X_1+2.17X_2+1.32X_3+0.69X_1X_2+6.81X_1X_3+2.09X_1X_3 \quad (R^2=0.982)$$

As could be seen from the equation, the fitting ability of the model is quite good due to high coefficient of determination ($R^2>0.980$). The change in TPC depending on the solvent type was illustrated in Fig.1 as ternary plots. It was observed that TPC of the samples increased significantly ($p<0.05$) toward to the edge of ethanol:water mixture while the lowest values were determined in the vertex of the ethanol. It was concluded that the highest TPC levels could be obtained by using the organic solvent: water mixtures. Similar results were obtained by Mildner-Szkudlarz et al. (2010) for the winemaking waste extracts and they reported that the best solvent for the higher polyphenol extraction was ethanol:water. It is well known that the solvent power depends on its polarity which is related to its dielectric constant and the phenolic substance extractability is correlated by the degree of polarity (Bosso et al., 2016).

Total flavonoid content (TFC) of the samples were given in Table 2 and it was determined that the TFC of the samples ranged between 44.3-537.4 mg CE/kg sample. The highest TFC was determined in the sample obtained by binary mixture of ethanol and water at the same proportion (1:1) while the lowest one was for the sample produced by using sole water as solvent (runs 2 and 15). The effect of solvent type was found as significant and also the model selected for the description of the TFC depending on the solvent type was significant

($p<0.05$). The linear mixture of the solvents affected the TFC of the samples significantly also ($p<0.05$, Table 3). In addition to that, the interaction effect between the solvents of ethanol and water and methanol and water was significant ($p<0.05$, Table 3). The change in TFC of the samples based on the solvent type was illustrated in Fig.1. As is seen clearly, TFC was the lowest in the vertex of the ethanol and water point and it increased significantly towards to the middle edge of both these two solvents (ethanol and water) which means that the binary mixture of ethanol and water is the best solvent to extract the flavonoids from the grape seed powder. The fitted second order polynomial equation for total phenolic content is as follows

$Y_{TFC}=8.10X_1+20.19X_2+6.65X_3+3.98X_1X_2+59.95X_1X_3+16.73X_1X_3$ (R^2=0.982)

As could be seen from the equation, the fitting ability of the model is quite good due to high coefficient of determination ($R^2>0.980$). Bosso et al., (2016) stated that the TFC of the grape seed was affected by the solvent type significantly ($p<0.05$) and they concluded that the highest TFC was in the sample extracted by 75% ethanol compared to sole ethanol or water and 50% acetone compared to sole acetone or water.

As other bioactive compounds, condensed tannin (CT) presents in GSP ranged between 23.3-3346.6 mg CE/kg sample. The highest CT levels were determined for the samples extracted by only methanol (runs 6 and 9) while the lowest values were found for the sample treated with only the water (runs 2 and 15). As could seen from the Table 2, the differences between the samples were significant because there was a huge different between the lowest and highest values of CT levels depending on the extraction solvents ($p<0.05$). The linear mixture of the used solvents also affected the CT content of the samples and also the binary interactions except ethanol and methanol showed a significant effect on CT levels of the samples ($p<0.05$, Table 3). Fig 1 shows the change in CT content of the GSP samples depending on the solvent as ternary plots and it is clear from the figure that the highest CT levels were monitored in the samples extracted with only methanol because the highest CT levels placed towards to the vertex of the methanol while the lowest values were on the vertex of the ethanol and water. It could be said that the tannins in GSP are highly soluble in only methanol. To describe the effect of the processing variables on the CT level of the samples, the fitted second order polynomial equation for condensed tannin contents is as follows:

$Y_{CT}=16.12X_1+57.76X_2+3.88X_3+14.33X_1X_2+141.8X_1X_3+24.76X_1X_3$ (R^2=0.967)

As is seen from the high determination coefficient, the fitting ability of the samples is quite high. Bosso et al. (2016) investigated the effect of different solvents on the condensed tannins of grape seed powder and they reported that the acetone:water mixture was the best solvent compared to ethanol and ethyl acetate for the extraction of condensed tannins (proanthocyanidins). In another study, Downey and Hanlin (2010) reported that the best solvent for the extraction of condensed tannins from grape skin was the binary mixture of the acetone:water compared to ethanol:water. They also stated that the single solvents of ethanol and water were not effective on the extraction of tannins as similar to the current research results.

Antiradical activities of the samples

Antiradical activities of GSP extract were determined by two different methods namely DPPH and $ABTS^{\cdot+}$ radical scavenging tests. As is known, the antiradical activity of extract or antioxidant substances is related to their ability hydrogen-donating performance and DPPH or $ABTS^{\cdot+}$ radicals convert them a stable molecule by accepting an electron from the extract or other antioxidant substances (Gulcin et al., 2004). So, the most of the bioactive compounds generally are donors of hydrogen atoms (H) and the radicals of DPPH capture the H atoms and convert to the neutral form (Savitri et al., 2019). DPPH results were given as % inhibition of DPPH radical and tabulated in Table 2 for all solvent types. The % inhibitions values were in the range of 2.11-80.5 for the samples and the lowest antiradical performance was recorded for the sample extracted by only sole ethanol (runs 5 and 12) while the highest was for the sample extracted by the binary mixture of ethanol and water at 1:1 ratio (run 7). The results showed that the differences among the DPPH radical scavenging activities were influenced by the solvent type ($p<0.05$). The ANOVA results showed that the linear mixture of the solvents showed a significant effect on the DPPH radical scavenging activities and also the binary mixtures of ethanol and water or methanol and water showed a significant effect on the antiradical performance. The selected model was found as significant and constructed polynomial model to predict the antiradical activities of the sample showed a good fitting capacity with quite high coefficient of determination. The polynomial model for DPPH radical scavenging activity was given in the following;

$Y_{DPPH}=1.70X_1+7.06X_2+3.17X_3+4.35X_1X_2+24.66X_1X_3+9.76X_1X_3$ (R^2=0.956)

The change in antiradical performance of the samples was illustrated as ternary plots in Fig.2. Antiradical activity of the samples decreased dramatically towards to the vertex of ethanol while it increased towards to the edge of ethanol: water mixture. It was seen that the antiradical activity of the samples determined by the inhibition of the DPPH radical was well correlated significantly with TPC and TFC with the correlation coefficients as 0.963 and 0.944, respectively ($p<0.05$).

The another test to evaluate the antiradical performance of the samples was $ABTS^{\cdot+}$ radical scavenging test. The $ABTS^{\cdot+}$ radical scavenging values of the samples were in the range of 0.31-4.08 µg Trolox/g sample. The highest $ABTS^{\cdot+}$ radical scavenging activity value was measured for the sample extracted by the binary mixture of ethanol and water and the lowest values were for the sample extracted by only sole ethanol. The linear effects of the solvents used were determined to be significant on the radical scavenging activity and also, the influence of the binary mixtures except ethanol and methanol showed a significant effect on $ABTS^{\cdot+}$ radical scavenging activity. The selected model effect was also determined as significant ($p<0.05$, Table 3). Fig. 2 shows the change in $ABTS^{\cdot+}$ radical scavenging activity of the samples depending on the extraction solvent as ternary plots. As is seen, the lowest values were placed on the vertex of ethanol and the antiradical activity increased significantly towards to the edge of ethanol

and water ($p<0.05$). The constructed polynomial model for the ABTS radical scavenging abilities showed a good fitting ability with high coefficient of determination as following;

$Y_{ABTS^{\cdot+}}=0.65X_1+1.62X_2+1.03X_3+0.44X_1X_2+4.91X_1X_3+2.20X_1X_3$ ($R^2=0.949$)

The correlation analysis showed that there was a positive and significant correlation between DPPH and $ABTS^{\cdot+}$ radical scavenging test. And also, there was a significant and positive correlation between TPC and DPPH ($r=0.964$, $p<0.05$) and TPC and $ABTS^{\cdot+}$ ($r=0.966$, $p<0.05$). Baydar et al. (2007) reported that the grape seed extract produced by acetone:water:acetic acid (90:9.5:0.5) mixture showed a strong DPPH radical scavenging activity compared to BHA and BHT synthetic antioxidant substances and their antiradical performances is directly related to polyphenolic content. Guendez et al. (2005) investigated the low molecular weight of polyphenols in GSP extract and they reported that certain constituents of seeds are particularly responsible for strong antiradical effect. They found a lower correlation between the TPC and DPPH radical scavenging activity as $r=0.649$.

Antioxidant capacities of the samples

Antioxidant characteristics of the GSP extracts were evaluated by two important methods namely iron chelating ability (ICA) and antioxidant activity (AA) by phosphomolybdenum approach. Table 2 shows the ICA values ranged between 2.53-87.80% and the lowest chelating activity was determined for the sample extracted by only ethanol (runs 5 and 12). The highest ICA values were determined for the samples extracted by the methanol. Similar to the other bioactive parameters, the differences among the ICA values depending on the solvent were significant ($p<0.05$). As could be seen from the Table 3, linear effects of the solvents were also showed a significant effect on the chelating ability values. Also, the interaction effects between the aqueous binary mixtures of the solvents were determined as significant. The change in the ICA values of the GSP extracts was illustrated in Fig. 3 as ternary plot and it is seen clearly from the figure that the ICA values increased towards to the methanol-water edge while the lowest values were recorded towards to the vertex of ethanol and water. The constructed polynomial model for the ICA values showed a good fitting ability with high coefficient of determination as following;

$Y_{ICA}=2.18X_1+9.04X_2+6.56X_3+6.62X_1X_2+8.89X_1X_3+5.00X_1X_3$ ($R^2=0.882$)

To evaluate the antioxidant performance of the samples, the samples were exposed to phosphomolybdenum assay. The results of the AA values were given in Table 2 and as it is seen, the lowest AA value was determined as 3.65 mg AAE/g for the sample extracted by sole ethanol similar to ICA values. The highest AA value (14.77 mg AAE/g) was recorded for the sample extracted by the binary mixtures of methanol and water. Antioxidant activity determined by phosphomolybdenum approach was affected by the solvent type significantly similar to the other bioactive parameters ($p<0.05$). As could be seen in Table 3, the selected model was found as significant and the processing variables showed a significant effect on the studied parameter ($p<0.05$). The change in AA depending on the solvent type was also showed in Fig.3 as ternary plots and it is clear from the figure that the AA values increased towards to the edge of ethanol:water or methanol:water while the lowest values were placed on the vertex of ethanol and then methanol (Fig.3). The constructed polynomial model for the AA values showed a good fitting ability with high coefficient of determination higher than 0.907 as following;

$Y_{AA}=4.16X_1+9.13X_2+8.76X_3-2.12X_1X_2+29.45X_1X_3+20.57X_1X_3$ ($R^2=0.907$)

Multiple response optimization to determine the best solvent mixture

The best solvent or solvent mixture was studied using the all characterized bioactive parameters and so, multiple response optimization was performed using the desirability functions. Optimization was conducted on squared values of real measurements because of calculating the ratio of max to min was higher than 10. Both minimization and maximization procedure were followed to calculate the limit values considering the all bioactive parameter values and the calculated results were tabulated in Table 4. Minimization process showed that the minimum values for the bioactive parameters would be at 100% ethanol usage for the extraction of GSP (Fig.4). By using this solvent, TPC and TFC would be 0.59 mg GAE/g and 8.09 mg CE/kg. Also, the lowest antiradical activities characterized by DPPH and $ABTS^{\cdot+}$ radical scavenging tests would be at 1.71% and 0.65 µg Trolox/g sample, respectively. It was resulted that the sole ethanol usage as a solvent in the extraction of bioactive compounds from defatted GSP is not suggested due to very low bioactivity. To suggest a good solvent system a maximization process was applied using desirability functions and the calculated results for the studied parameters were tabulated in Table 4. As is seen from the Fig.4, the optimized solvent mixture is not a sole organic solvent, on the contrary the ternary mixture of ethanol (33.84%), methanol (20.17%) and water (45.99%) is suggested. Using that solvent mixture, the maximum TPC and TFC would be 2.54 mg GAE/g and 21.02 mg CE/kg sample, respectively. Also, the maximum antioxidant parameter values would be 8.92% and 13.59 mg AA/kg for ICA and AA, respectively. Desirability function values for both process were also acceptable. It was concluded that by the multiple response optimization for more than one parameter having important effect on the sample bioactivity showed good optimization result. So, by the determination of good solvent mixture determined by multiple response optimization, a GSP extract having bioactivity could be produced.

Conclusions

Grape seed is a food waste of winemaking industry but recently its powder form can be evaluated as a functional food matrix due to its good bioactive properties. It is rich in many phytochemical compounds and shows good antioxidant, antiradical and also antimicrobial activities. To produce an extract from GSP, selection of correct solvent or solvent mixture has a critical role because the extract yield and its bioactivity are affected from the solvent polarity. In this study, an optimized ternary solvent mixture was suggested to provide a high bioactive GSP extract. In this regard, the highest bioactivity based on

the concentrations of the total bioactive compounds and their antiradical and antioxidant activities, could be obtained by the solvents of ethanol, water and methanol at the determined ratios using multiple response optimization process. The results of the current work could be evaluated by the industries which process the GSP extracts.

Table 1. Simplex lattice mixture design showing the solvent levels used for the extraction

	Coded levels			Uncoded levels		
Runs	X_1	X_2	X_3	X_1	X_2	X_3
1	0.50	0.50	0.00	50.0	50.0	0.0
2	0.00	0.00	1.00	0.0	0.0	100.0
3	0.50	0.50	0.00	50.0	50.0	0.0
4	0.33	0.33	0.33	33.3	33.3	33.3
5	1.00	0.00	0.00	100.0	0.0	0.0
6	0.00	1.00	0.00	0.0	100.0	0.0
7	0.50	0.00	0.50	50.0	0.0	50.0
8	0.17	0.67	0.17	16.7	66.6	16.7
9	0.00	1.00	0.00	0.0	100.0	0.0
10	0.67	0.17	0.17	66.6	16.7	16.7
11	0.50	0.00	0.50	50.0	0.0	50.0
12	1.00	0.00	0.00	100.0	0.0	0.0
13	0.00	0.50	0.50	0.0	50.0	50.0
14	0.17	0.17	0.67	16.7	16.7	66.6
15	0.00	0.00	1.00	0.0	0.0	100.0

X_1: Ethanol, X_2: Methanol, X_3: Water

Table 2. Bioactive performance of the defatted grape seed powder

Runs	TPC (mg GAE/g)	TFC (mg CE/kg)	CT (mg CE/kg)	DPPH (%)	$ABTS^{+}$ (µg Trolox/g)	ICA (%)	AA (mg AA/g)
1	2.23	217.4	1558.0	30.01	1.33	39.88	5.39
2	1.82	44.3	25.0	14.11	0.93	47.74	9.28
3	2.33	234.4	1526.7	27.96	1.39	48.24	5.73
4	5.38	365.8	1699.5	64.40	3.94	78.76	12.67
5	0.33	62.0	211.3	2.11	0.31	2.53	3.65
6	4.58	387.1	3163.8	48.17	2.77	83.04	9.45
7	6.88	492.4	2093.5	80.50	4.08	35.60	14.00
8	5.81	451.6	3002.8	58.67	3.21	77.91	11.79
9	4.61	408.3	3346.6	51.19	2.56	87.80	8.63
10	4.26	338.7	2140.0	55.00	3.43	67.59	11.92
11	7.29	537.4	2130.2	71.20	4.00	33.37	13.70
12	0.31	63.3	239.2	2.37	0.43	5.55	3.86
13	5.31	328.3	1508.0	66.60	3.50	71.18	14.77
14	4.72	272.8	786.9	39.64	3.02	68.94	10.72
15	1.85	49.3	23.3	8.63	1.35	41.62	9.23

TPC: Total phenolic content, TFC: Total flavonoid content, CT: Condensed tannin, ICA: Iron chelating activity, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, $ABTS^{+}$: 2,2-Azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity, AA: Antioxidant activity

Table 3. ANOVA results showing the significance of the studied solvent effect on the bioactive parameters

Source	df	TPC	TFC	CT	DPPH	$ABTS^{+}$	ICA	AA
Model	5	96.02*	100.9*	53.97*	38.92*	33.74*	13.53*	17.45*
Linear mixture	2	61.80*	55.87*	74.42*	20.72*	16.22*	27.44*	14.42*
X_1X_2	1	3.39	1.63	1.20	4.18	0.94	4.14	0.22
X_1X_3	1	330.5*	369.3*	117.6*	134.6*	117.7*	7.45*	42.12*
X_2X_3	1	21.95*	20.33*	2.53*	14.98*	16.67*	1.67*	14.52*
Residual	9							
Lack of Fit	4	23.92*	10.68	94.36*	7.89*	6.88	16.0	46.3*
Pure Error	5							
Cor Total	14							
R^2		0.982	0.982	0.967	0.956	0.949	0.882	0.907
Adj R^2		0.971	0.973	0.950	0.931	0.921	0.817	0.855

$^{\phi}$ X_1: Ethanol, X_2: Methanol, X_3: Water , TPC: Total phenolic content, TFC: Total flavonoid content, CT: Condensed tannin content, ICA: Iron chelating activity, $ABTS^{+}$: 2,2-Azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, AA: Antioxidant activity (mg AA/kg) *p<0.05

Table 4. Multiple response optimization values for the extraction of defatted grape seed powder

	Minimization process				Maximization process			
Response parameters	X_1	X_2	X_3	*Desirability*	X_1	X_2	X_3	*Desirability*
	100	0	0		33.84	20.17	45.99	
TPC (mg GAE/g)		0.59				2.54		
TFC (mg CE/kg)		8.09				21.02		
CT (mg CE/kg)		16.12				44.37		
ICA (% Inh.)		2.24		0.921		8.92		0.898
DPPH (% Inh.)		1.71				8.50		
$ABTS^{+}$ (µg Trolox/g)		0.65				2.01		
AA (mg AA/kg)		4.16				13.59		

$^{\phi}$ X_1: Ethanol, X_2: Methanol, X_3: Water , TPC: Total phenolic content, TFC: Total flavonoid content, CT: Condensed tannin content, ICA: Iron chelating activity, $ABTS^{+}$: 2,2-Azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, AA: Antioxidant activity (mg AA/kg). Square root transformation was applied for the studied parameters except AA.

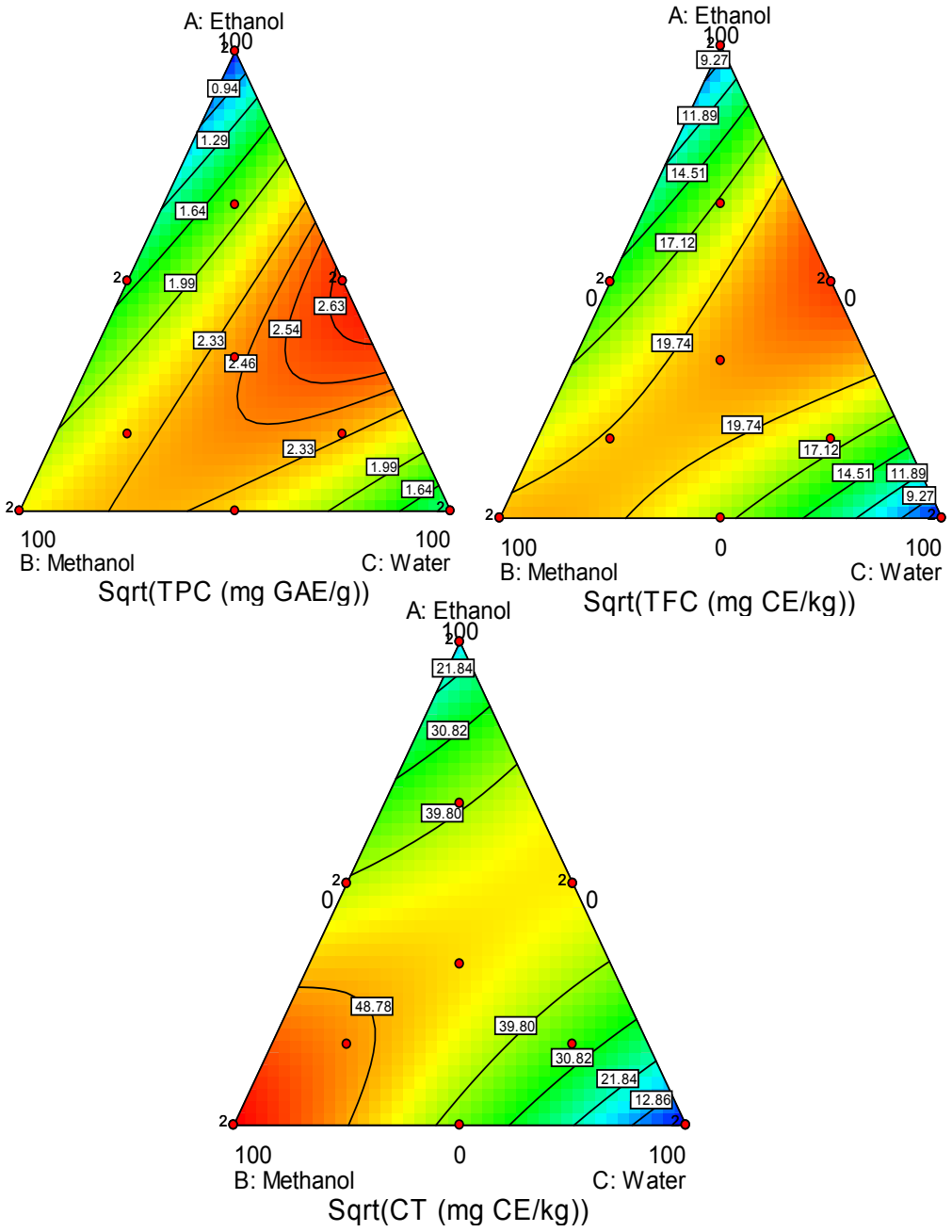

Figure. 1 Ternary contour plots showing the change in total phenolic content (TPC), total flavonoid content (TFC) and condensed tannin (CT) levels of defatted grape seed powder

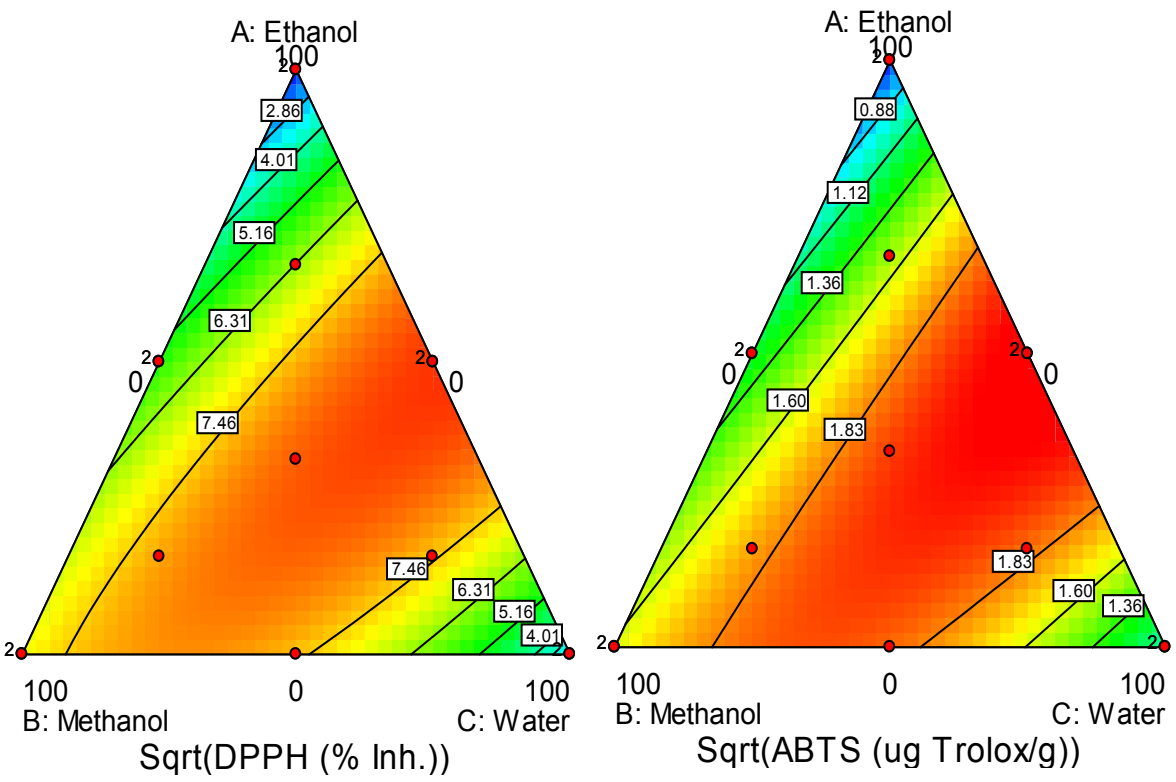

Figure 2. Ternary contour plots showing the change in DPPH radical scavenging activity and $ABTS^{\cdot+}$ radical scavenging activity of defatted grape seed powder

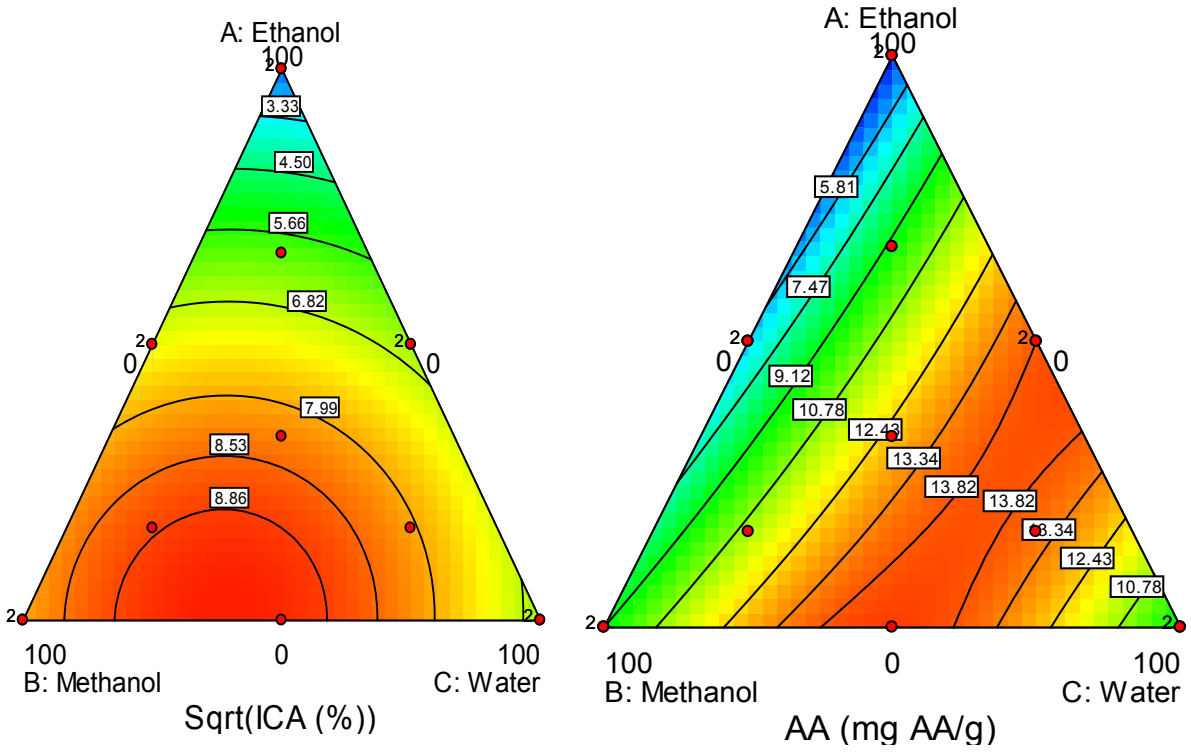

Figure 3. Ternary contour plots showing the change in iron chelating activity (ICA) and antioxidant activity (AA) of defatted grape seed powder

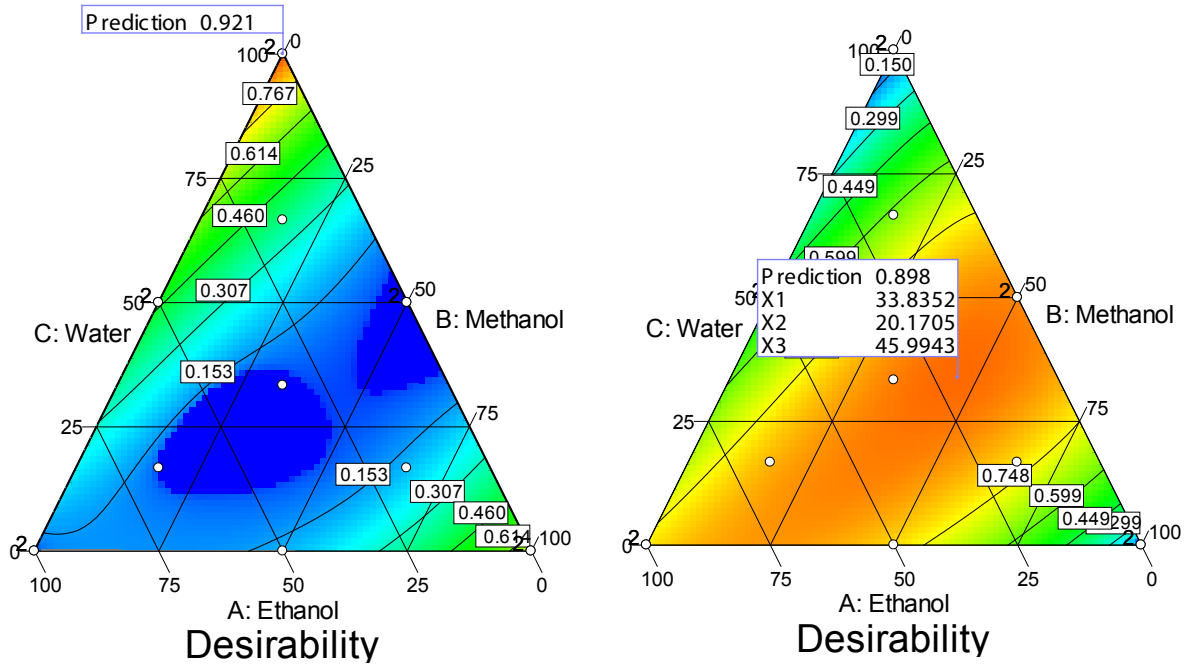

Figure 4. Ternary contour plots showing the desirability function values for the maximum and minimum response values according to the solvent mixture types. Maximum values at the right ternary plots and minimum values at the left ternary plots.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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