International Journal of Fruit Science

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/wsfr20

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To cite this article: Lady Zevallos, César Caldas, Alberto Flores, Jesús Obregón, Alberto Claudio Miano & Gabriela Barraza-Jáuregui (2020) Mixing Design for Optimizing Ultrasound-Assisted Extraction of Phenolic Components and Anthocyanins from Blue Berries and Grape Marc, International Journal of Fruit Science, 20:sup3, S1313-S1327, DOI: 10.1080/15538362.2020.1785987

To link to this article: https://doi.org/10.1080/15538362.2020.1785987

Published online: 07 Jul 2020.

INTERNATIONAL JOURNAL OF FRUIT SCIENCE
2020, VOL. 20, NO. S3, S1313–S1327
https://doi.org/10.1080/15538362.2020.1785987

Mixing Design for Optimizing Ultrasound-Assisted Extraction of Phenolic Components and Anthocyanins from Blue Berries and Grape Marc

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ABSTRACT

Waste from fruit processing can be used for obtaining additives as colorants and antioxidant compounds by extraction processes. Despite there are many works optimizing parameters as temperature and solvent concentration during this process, the mixture of solvents has not been optimized yet. Therefore, the present work aimed to find the optimum solvent mixture among water, ethanol, and acetone to obtain the maximum yields of phenolic compounds and monomeric anthocyanins from blue berry and grape marc by ultrasound-assisted extraction. For that, the Reticular Simplex Mixtures Design was used to obtain the models that can predict the optimum values of extraction yields. As a result, the quadratic model successfully fit the experimental data, demonstrating the best mixture solvents were the ones that presented a high percentage of water. This result was obtained not only for phenolic compound, but also for monomeric anthocyanin extraction from blue berry and grape marc. Further, using the optimum mixture, values of 502.2 GAE/100 g of phenolic compound and 1349.1 mg/100 g of monomeric anthocyanins from blue berry and 2642.4 GAE/100 g of phenolic compounds and 31.5 mg/100 of monomeric anthocyanins from grape marc were obtained. In conclusion, the optimum solvent demonstrated to be efficient extracting both components increasing the extraction yields and reducing the cost of extraction. Finally, by overlapping the optimization plot, a unique solvent mixture for obtaining a high yield of phenolic compound and anthocyanins at the same time from blue berry and grape marc was obtained.

KEYWORDS

Phenolic compounds; monomeric anthocyanins; optimization; blue berry; grape marc

Introduction

The phenolic compounds and the anthocyanins are important compounds from the nutritional point of view due to their antioxidant capacity (Cirkovic Velickovic and Stanic-Vucinic, 2018). They are considered as secondary

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metabolites from plants, which are presented in many tissues (Ignat et al., 2011). In fact, these components are presented in different kind of food where they can be consumed. However, they are usually lost is some residuals as marcs or discarded fruits which could be utilized. Consequently, it is important to quantify and analyze the nutrients from these raw materials in order to value them and give some alternative solutions to use them.

There are several methods for extracting phenolic compound and anthocyanins such as liquid-liquid extraction, solid-liquid extraction, using supercritical fluids, among others (Ignat et al., 2011). Regarding solid-liquid extraction method, many techniques to improve the liquid extraction yields, reducing the process time, and minimizing the use of solvents, are being studied. Further, some non-conventional technologies were used to improve mass transfer during extraction process such as ultrasound technology, high hydrostatic pressure, and pulsed electric fields (Corrales et al., 2008), being ultrasound the most used technology.

Ultrasound technology has demonstrated promising results by increasing the extraction yield of many nutrients as phenolic compounds (Carrera et al., 2012; Khan et al., 2010) and anthocyanins (D'Alessandro et al., 2014; Zou et al., 2011). Ultrasound consists of applying acoustic waves with frequencies higher than 20 kHz and high power to cause physical chemical changes on food, improving many processes (Cárcel et al., 2012). In fact, this technology increases the extraction yields due to the improvement of mass transfer by many mechanisms related to changes on the structure (rupture of cells and tissues) an on the convective mass transfer increment (Miano et al., 2017). Therefore, nowadays its use is very common for assisting extraction process.

In addition, many studies of optimization for maximizing the extraction yield of phenolic compounds and anthocyanins were performed considering many parameters as temperature, time, method, solvent composition, pH, among others, using or not ultrasound technology (Cacace and Mazza, 2003; Isopencu et al., 2019; Liu et al., 2013; Liyana-Pathirana and Shahidi, 2005; Rodrigues et al., 2015; Silva et al., 2007). There are some statistical methodologies for process optimization as the Central composite rotational design Reticular simplex mixtures design (Montgomery, 2005), being the first, the most used method. All the optimization studies were performed or for phenolics compound or for anthocyanins separately. However, what happens if it is necessary to extract phenolic compounds and anthocyanins at the same time. The difficulty of this would be caused by the different functional groups as conjugated hydroxyls (with sugars, acids, or alkyl groups), whose polarities vary and not all the solvents are suitable for the extraction (Mokrani and Madani, 2016). Therefore, an optimization of the solvent composition would be interesting.

For those reasons, the present work aimed to find and optimum mixture to obtain the maximum yield of phenolic compounds and anthocyanins at the

same time from blue berry and grape marc. For that, the Reticular simplex mixtures design (Mixing design) was used considering the mixture of water, ethanol, and acetone.

Materials and Methods

Raw Material

Blue berries (*Vaccinium corymbosum*) Stella variety harvested from Motupe, Lambayeque region – Perú, and grape marc from grapes (*Vitis vinifera*) Gross Colman variety from Cascas, La Libertad region – Perú were used. Both fruits were harvested at commercial ripeness. The samples were stored in polyethylene bags at 10°C before being processed.

Sample Preparation

Approximately 500 g of sample (blue berry or grape marc) was placed into a stainless-steel recipient where liquid nitrogen was added for quick congelation (−195°C). The sample was triturated using a laboratory mill (IKA) at 5000 rpm for 5 min, for them being storage in an ultra-freezer (ARCTIKO, Lammefjordsvej, Esbjerg, Denmark) at −80°C. After that, samples were freeze dried (LabConco, Kansas, USA) at −50 C and 0.02 mBar of vacuum pressure, and pulverized (IKA® EUROSTAR 20 Digital, Königswinter, Germany; 40 rpm,). Finally, the pulverized sample was sieved using a mesh size sieve of 106 μm (ASTM Serie) for being used in the extraction process (Lou et al., 2016).

Extraction Method

For the extraction, around 50 mg of the pulverized sample was placed in a 10 ml tube with the extractive solution depending on the treatment (Table 1). The tubes were placed at the bottom of the ultrasonic bath (Branson, Danbury,

Table 1. Experimental design for optimizing the extraction yield: solvent composition.

Treatment	Water (%)	Ethanol (%)	Acetone (%)
T1	100.00	0.00	0.00
T2	0.00	100.00	0.00
T3	0.00	0.00	100.00
T4	50.00	50.00	0.00
T5	50.00	0.00	50.00
T6	0.00	50.00	50.00
T7	33.33	33.33	33.33
T8	66.67	16.67	16.67
T9	16.67	66.67	16.67
T10	16.67	16.67	66.67

USA; 40 kHz o frequency and 185 W/m^2 of intensity) with 10 l of distilled water (25°C). The volumetric power of the ultrasonic bath, measured by calorimetric method (Mason and Peters, 2004) was 7.1 W/l. The extraction process was performed for 15 min without stirring. Then, the extracts were centrifugated (Hettich EBA 20 S, Tuttlingen, Germany) at 5000 rpm for 15 min, separating and storing the supernatant at −20°C before being analyzed.

Total Phenolic Compound Determination

The Folin-Ciocalteu colorimetric method (Singleton et al., 1999) with modifications was used for determining phenolic compounds. This method is based on the phenols capacity of reacting with oxidative substances. The extracts were oxidized by Folin-Ciocalteu reagent under alkaline conditions, producing a blue chromophore quantified using a spectrophotometer UV–VIS (PLUS 250, Jena, Germany) at 750 nm of wavelength. The results were expressed as equivalent of gallic acid (mg GAE/100 g of sample) by using a calibration curve.

Monomeric Anthocyanins Determination

Total Monomeric Anthocyanins (TA) from the extract was determined using the pH differential method (Giusti et al., 2014; Lee et al., 2005). This method is based on the reversible change of monomeric anthocyanins at pH 1.0 (colored) and pH 4.5 (colorless). The absorbance difference at 515 nm and 700 nm of wavelengths is proportional to the TA concentration. The absorbances were determined using a spectrophotometer UV–VIS (PLUS 250, Jena, Germany) and the absorbance difference (A) was determined by Equation (1).

$$A = (A_{515\ nm} - A_{700\ nm})_{pH1} - (A_{515\ nm} - A_{700\ nm})_{pH4.5} \tag{1}$$

The concentration of TA was determined by Equation (2) and expressed on cyanidin-3-glucoside basis.

$$AT = \frac{A\ PM\ 100}{\varepsilon} \tag{2}$$

where A is the absorbance difference (Equation (1)), PM is the molecular mass of cyanidin-3-glucoside (449.2 g/mol) and ε is the molar absorptivity of cyanidin-3-glucoside (26,900 L/mol.cm).

Statistical Analysis

In order to optimize the water:ethanol:acetone relation for maximizing the extraction of phenolic components and anthocyanins, the reticular simplex

mixtures design with expanded centroid was used. In this design, the factors represented each solvent fraction on the mixture, whose values were from 0 to 1. The complete design consisted of 10 experiments and was performed in triplicate.

Further, a variance analysis (ANOVA) for determining the significant model ($p < .05$) (Equation (3)–(5)) which described the dependent variables (Phenolic compounds extraction yield and Total monomeric anthocyanins) was performed.

$$Y = \sum \beta_i X_i \tag{3}$$

$$Y = \sum \beta_i X_i + \sum \sum \beta_{ij} X_i X_j \tag{4}$$

$$Y = \sum \beta_i X_i + \sum \sum \beta_{ij} X_i X_j + \sum \sum \sum \beta_{ijk} X_i X_j X_{jk} \tag{5}$$

where Y represents the dependent variable (response), β_i measures the effect of each component, β_{ij} measures the interactive effect of a pair of components and β_{ijk} measures the interactive effect of the three components. X_i, X_j and X_k represent the fraction of each solvent in the extraction mixture.

In addition, the determination coefficient (R^2) and the adjusted determination coefficient (R^2-adjusted) were determined to validate the models. For that, values higher than 0.85 for R^2 and 0.75 for R^2-adjusted were considered for the validation (Montgomery, 2005).

Finally, the Response Surface and the Contour Surface form the validated models were plotted for visualizing the global effect of the variables and determining the optimum region. Moreover, for obtaining the optimum extract mixture region and maximize the extraction of phenolic components and anthocyanins at the same time, the contour surfaces of both response variables were overlaid (Montgomery, 2005).

All these analyses were performed using the software Design-Expert® 7.0 version (USA).

Results and Discussion

Optimization of Phenolic Compounds Extraction

In Table 2 are presented the extraction yields of phenolic compound of all the treatments for blue berry and grape marc. Considering the pure solvents (T1, T2, and T3), ethanol showed the highest extraction yield. This preference to ethanol by the phenolic compound can be caused by the nonpolar and aliphatic fragment of ethanol (Galanakis et al., 2013; Rahmanian et al., 2014). This affinity for ethanol was also demonstrated by Do et al. (2014), who obtained the highest phenolic extraction yield from *Limnophila aromática* using ethanol, followed by methanol and acetone.

Table 2. Experimental and predicted values (Equations (6) and (7)) of total phenolic compounds extracted from blue berries and grape marc using ultrasound technology.

Raw material	Blue berry			Grape marc		
Treatment	Experimental (mg GAE/100 g)	Predicted (mg GAE/100 g)	Variation (%)	Experimental (mg GAE/100 g)	Predicted (mg GAE/100 g)	Variation (%)
T1	261 ± 27	273	4.5	1267 ± 249	1276	0.7
T2	435 ± 97	427	1.8	1653 ± 190	1581	4.4
T3	82 ± 5.80	99	20.5	137 ± 1	249	81.7
T4	440 ± 30	419	4.6	2621 ± 387	2528	3.6
T5	501 ± 78	505	0.9	2478 ± 288	2569	3.7
T6	437 ± 63	422	3.5	1748 ± 271	1758	0.6
T7	447 ± 48	510	14.0	2557 ± 76	2701	5.6
T8	492 ± 89	460	6.5	2585 ± 179	2541	1.7
T9	457 ± 86	484	5.9	2173 ± 284	2372	9.16
T10	450 ± 91	403	10.53	2296 ± 98	1942	15.44

In fact, Galanakis et al. (2013) analyzed the activity coefficient of 15 natural phenols (tyrosol, hydroxytyrosol, oleuropein, caffeic, cinnamic, *p*-coumaric, ferulic, gallic, *p*-hydroxybenzoic, *p*-hydroxyphenyl acetic, protocatechuic, rosmarinic, synaptic, syringic, and vanillic acid) in seven solvents (water, ethanol, methanol, acetone, dichloromethane, ethyl acetate, and diethyl ether) demonstrating a preference for solvents with intermediate polarity as ethanol and acetone, instead of polar solvents (water) and nonpolar solvents (dichloromethane, ethyl acetate, and diethyl ether). In addition, Galanakis et al. (2013) suggested that this affinity could be caused by the formation of hydrogen bonds between the electronegative oxygen of the solvents (ethanol, methanol, or acetone) and the hydroxyl groups of phenols or between the hydroxyl group of the solvent and the oxygen from the phenol molecule.

Concerning binary mixtures (T4, T5, and T6), a synergetic effect was observed compared to pure solvents, obtaining the highest extraction yield with the mixture T4 (50% water – 50% ethanol) and T5 (50% water – 50% acetone). In the case of ternary mixture, the extraction yield was increased as water concentration was increased. Consequently, T8 (66.7% water – 13.7% ethanol – 13.7% acetone) obtained the highest extraction yield of phenolic compound from both raw materials. These results were observed for blue berries and grape marc. In fact, many published works reported that by adding water in the extraction mixture (with ethanol or acetone), the extraction yield is increased (Do et al., 2014; Durling et al., 2007).

In fact, despite water is the most polar solvent, using 100% of this solvent presents lower extraction yield of phenolic compounds than ethanol. This can be attributed to the higher viscosity of water than ethanol and acetone (Dranca and Oroian, 2016). However, mixing water with acetone, ethanol, or both, the extraction yield is significantly increased. This is probably due to the reduction of viscosity and surface tension of water by adding ethanol and/or acetone, improving the mass transfer of the phenolic compounds from the raw materials. Further, the extraction of chemicals that are soluble in water and/or organic solvent may

improve their extraction using mixed solvents (polar – nonpolar) (Do et al., 2014). In addition, the high dielectric constant of water causes the polarity increment of ethanol in solution improving the extraction (Bashi et al., 2012).

Four optimization models: Linear, Quadratic, Special cubic, and Complete cubic were used to fit the extraction yield data. Table 3 shows the significance and the adjusted goodness of the four considered models. In fact, quadratic model was the best for estimating the extraction yield of phenolic compounds from blue berry and grape marc since it presented the higher significance ($p < .05$) and the highest values of determination coefficient and adjusted determination coefficient. From here, the regression analysis was performed to calculate the estimated coefficients of the model. Table 4 shows the estimated coefficients for the components water, ethanol, acetone, and binary combinations. Despite not all the coefficients were significant ($p < .05$), all the coefficient were taken into account in order to improve the predictive capacity of the model (Montgomery, 2005).

Consequently, the quadratic models for estimating the ultrasound-assisted yield of phenolic compounds from blue berries and grape marcs are presented in Equations (6) and (7) respectively. It is important to mention that the coefficients of the Equations (6) and (7) are determined in real units (0–100%).

$$FT\left(mgAGE/_{100g}\right) = 2.74 \times A + 4.28 \times B + 0.99 \times C + 0.03 \times A \times B + 0.13 \times A \times C + 0.04 \times B \times C \quad (6)$$

$$FT\left(mgAGE/_{100g}\right) = 12.76 \times A + 15.81 \times B + 2.49 \times C + 0.44 \times A \times B + 0.72 \times A \times C + 0.34 \times B \times C \quad (7)$$

Table 3. Adjustment goodness of the optimization models for phenolic compounds extraction yield (*p*-value was determined considering 95% of confidence).

	Blue berry			Grape marc		
Model	p	R^2	R^2-ajus	p	R^2	R^2-ajus
Linear	0.386	0.238	0.02	0.435	0.212	−0.013
Quadratic	**0.011**	**0.94**	**0.865**	**0.005**	**0.960**	**0.910**
Special cubic	0.442	0.952	0.857	0.859	0.960	0.881
Complete cubic	0.691	0.977	0.795	0.382	0.994	0.948

Table 4. Significance of quadratic model parameters for predicting phenolic compounds extraction yield.

	Blue berry		Grape marc	
Component	Estimated coefficient	p	Estimated coefficient	p
A-Agua	274	0.004	1276	0.005
B-Ethanol	428	0.001	1581	0.002
C-Acetone	100	0.096	249	0.336
AB	277	0.261	4398	0.014
AC	1276	0.004	7227	0.002
BC	634	0.040	3372	0.033

where FT is the extraction yield of phenolic compounds and A, B, and C represent the extraction solution components (%) of water, ethanol, and acetone, respectively.

In Table 2, is presented the comparison between the experimental values against the estimated values for the ultrasound-assisted extraction yields from blue berry and grape marc. Most of the values presented variation coefficient less than 10%, suggesting a good estimation of the models. In contrast, three treatments for blue berry and two treatment for grape marc presented higher values of variation percentage, setting them as marginally acceptable (Montgomery, 2005).

From the Equations (6) and (7), the respond surfaces and the response contours that explain the effect of the solvent mixture composition on the extraction yield of phenolic compounds were plotted (Figure 1). The surface response plots also evidenced that the extraction yield increased as more quantity of water is mixed with ethanol and/or acetone, demonstrating a suitable agreement with the experimental data. Further, the contour surfaces (Figure 1b,d) estimated the optimum mixture for obtaining the maximum yield during the ultrasound-assisted extraction of phenolic compounds: for blue berry the optimum mixture was 40.5% water – 16.1% ethanol – 37.4% acetone to obtain 514.29 mg GAE/100 g of yield and for grape marc was 44.9% water – 28.3% ethanol – 26.8% acetone to obtain 2771.43 mg GAE/100 g of yield. For both raw materials, to obtain the maximum extraction yield, the concentration of water was similar and represented the highest value of the mixture. As discussed above, the increase of water concentration in the extraction solvent was beneficial for increasing the extraction yield. On the other hand, the phenolic compounds from blue berry and grape marc presented a slight difference regarding their extraction by ethanol and acetone. This was probably due to the different composition of the phenolic compounds from each raw material. In fact, some compounds would have more affinity to ethanol and other to acetone.

Optimization of Monomeric Anthocyanins Extraction

Concerning monomeric anthocyanins extraction, Table 5 shows the extraction yield from blue berry and grape marc. For both raw materials, ethanol as pure solvent (T2) was more efficient than water and acetone. Further, all the binary and ternary mixtures present similar values of extraction yields, like the extraction yield of pure ethanol. In fact, anthocyanins have more affinity to organic-water mixtures as solvents (Barnes et al., 2009) due to the presence of a phenolic ring, several hydroxyl groups, and the charge of flavylium ion in its chemical structure (Badui Dergal and Cejudo Gómez, 2006).

Similar results were found on extraction of anthocyanins from purple potato, where a mixture of ethanol-water (70:30 v/v) was the optimum solvent (Mane et al., 2015). In addition, Oancea et al. (2012) stated that the best extraction yield from blue berries was obtained using ethanol-water (50:50 v/v) and the worst

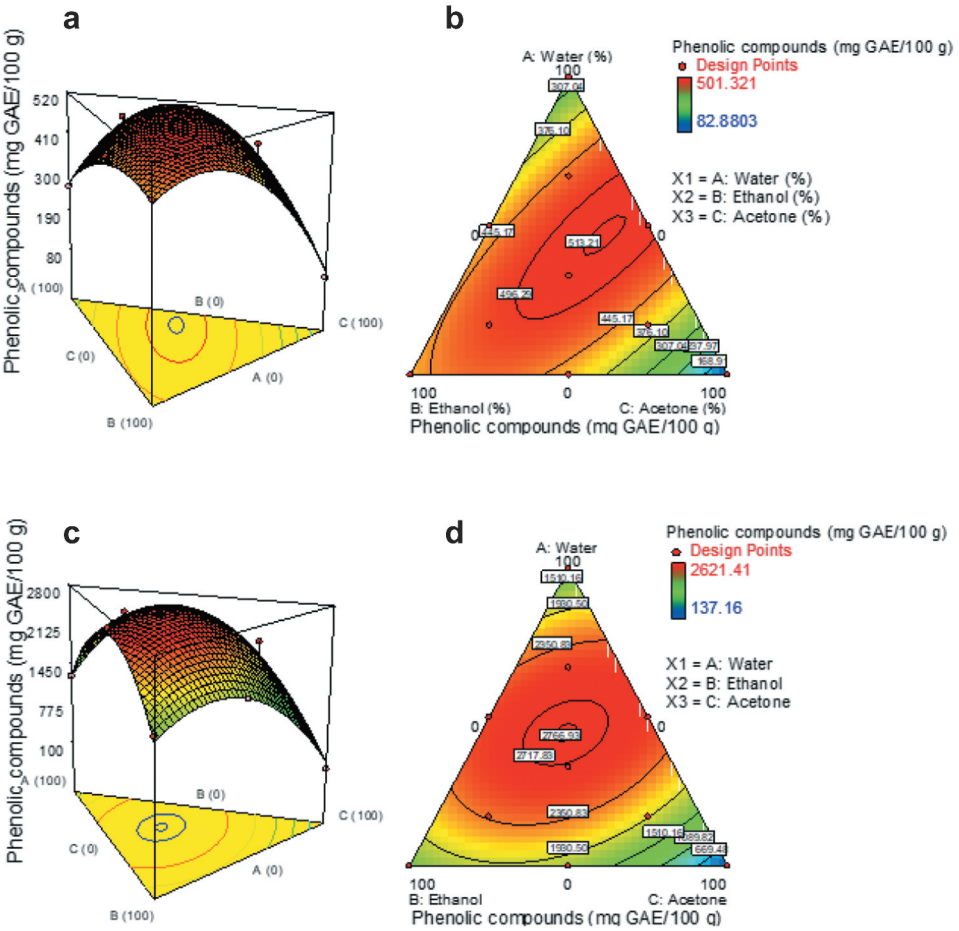

Figure 1. Response Surface for maximizing the yield of ultrasound-assisted extraction of Phenolic compounds of blue berry (a) and grape marc (c). Contour surface for maximizing the yield of ultrasound-assisted extraction of Phenolic components of blue berry (b) and grape marc (d).

Table 5. Experimental and predicted values (Equations (8) and (9)) of Monomeric Anthocyanins extracted from blue berries and grape marc.

Raw material	Blue berry			Grape marc		
Treatment	Experimental (mg/100 g)	Predicted (mg/100 g)	Variation (%)	Experimental (mg/100 g)	Predicted (mg/100 g)	Variation (%)
T1	207 ± 77	238	15.1	18 ± 3	18	0.02
T2	1304 ± 90	1259	3.5	31 ± 7	30	2.6
T3	137 ± 11.38	187	36.9	2 ± 1	3	79.2
T4	1043 ± 63	1041	0.2	30 ± 7	28	6.4
T5	1117 ± 78	1211	8.5	28 ± 7	29	1.1
T6	1157 ± 25	1175	1.6	26 ± 6	25	1.9
T7	1227 ± 107	1336	8.9	29 ± 6	31	6.4
T8	1167 ± 104	1024	12.3	28 ± 7	29	1.8
T9	1265 ± 127	1352	6.9	29 ± 6	32	10.3
T10	1253 ± 43	1052	16.1	26 ± 5	23	14.5

extraction yield was using pure water. Consequently, besides pure ethanol, the mixture of ethanol, especially with water, presents high extraction yields of anthocyanins. This would be desirable since the addition of water to ethanol would reduce the cost of the solvent without affecting the process efficiency.

Similar to the phenolic compounds section, the variance analysis of four optimization models was evaluated (linear, quadratic, special cubic, and complete cubic). Table 6 shows that the best model was the quadratic not only for blue berry, but also for grape marc. This model was significant ($p < .05$) and presents the highest determination coefficient and adjusted determination coefficient. Therefore, by regression analysis the estimated coefficients for each component (water, ethanol, acetone, and binary combinations) of quadratic model were obtained (Table 7), obtaining Equation (8) for blue berry and Equation (9) for grape marc (coefficients determined in real units (0–100%)).

$$MA\left({}^{mg}/_{100g}\right) = 2.39 \times A + 12.59 \times B + 1.88 \times C + 0.117 \times A \times B + 0.399 \times A \times C + 0.181 \times B \times C \quad (8)$$

$$MA\left({}^{mg}/_{100g}\right) = 0.187 \times A + 0.307 \times B + 0.033 \times C + 0.002 \times A \times B + 0.007 \times A \times C + 0.003 \times B \times C \quad (9)$$

where MA is the extraction yield of monomeric anthocyanins and A, B, and C represent the extraction solution components (%) of water, ethanol, and acetone, respectively.

From Equations (8) and (9), the response surface and response contours for estimating monomeric anthocyanin yield from blue berry and grape marc were

Table 6. Adjustment goodness of the optimization models for Monomeric Anthocyanins extraction yield (*p*-value was determined considering 95% of confidence).

	Blue berry			Grape marc		
Model	p	R^2	R^2-ajus	p	R^2	R^2-ajus
Linear	0.23	0.343	0.155	0.094	0.492	0.346
Quadratic	**0.013**	**0.944**	**0.875**	**0.017**	**0.951**	**0.891**
Special cubic	0.908	0.945	0.834	0.568	0.957	0.872
Complete cubic	0.634	0.978	0.8	0.195	0.998	0.985

Table 7. Significance of quadratic model parameters for predicting Monomeric Anthocyanins extraction yield.

	Blue berry		Grape marc	
Component	Estimated coefficient	p	Estimated coefficient	p
A-Agua	239	0.184	19	0.003
B-Etanol	1259	0.001	31	0.000
C-Acetona	188	0.276	3	0.309
AB	1171	0.163	15	0.306
AC	3994	0.004	71	0.005
BC	1809	0.058	34	0.059

plotted (Figure 2). All the surfaces agree with the experimental results, indicating that anthocyanins are more extracted when pure ethanol and the mixtures are used. Furthermore, the optimum solvent mixture was obtained for reaching the maximum extraction yield of monomeric anthocyanins. For blue berry, the solvent should have the composition 16.88% water – 57.28% ethanol – 25.24% acetone in order to obtain an approximately yield of 1365.3 mg/100 g. For grape marc, the solvent composition should be 22.70% water – 60.94% ethanol – 16.36% in order to obtain an approximately yield of 31.61 mg/100 g.

Table 5 shows the comparison between the experimental and estimated values (Equations (8) and (9)) of the yield of ultrasound-assisted extraction of monomeric anthocyanins from blue berry and grape marc. Most of the estimated values present variation coefficients less than 10%, suggesting a successful goodness degree. Further, few of the treatments had variation coefficients higher than 10%, considering them as marginally acceptable values (Montgomery, 2005).

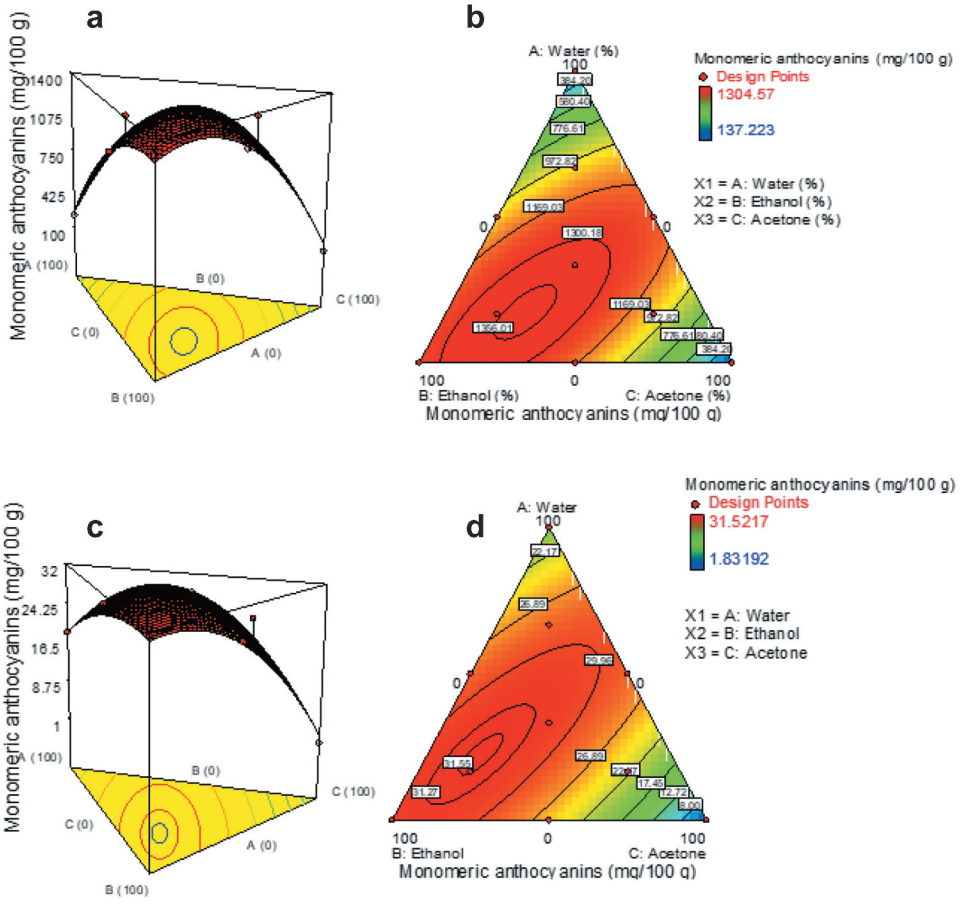

Figure 2. Response Surface for maximizing the yield of ultrasound-assisted extraction of Monomeric anthocyanins of blue berry (a) and grape marc (c). Contour surface for maximizing the yield of ultrasound-assisted extraction of Phenolic components of blue berry (b) and grape marc (d).

Final Considerations

The optimization of the extraction solvent mixture will allow to obtain high extraction yields, reducing the cost of the solvent. In fact, for both phenolic compounds and monomeric anthocyanins, the optimum mixtures present a considerable quantity of water, which would reduce the solvent cost. In other words, thanks to the studied optimization, the maximum quantity of water to reduce the cost of the solvent and to obtain the highest yield can be known.

Furthermore, another final consideration is the validation of the models. For that, a new experiment considering the optimum mixture was developed obtaining the results of Table 8. The new results demonstrate that the models have a successful prediction capacity since the predicted values are very close to the experimental values. In addition, the prediction values agree with the confidence intervals with 95% of confidence.

On the other hand, another interesting analysis to be performed is to overlap the contour surfaces from Figures 1 and 2. This method allows to know the optimum mixture for extracting the highest quantity of phenolic compound and monomeric anthocyanin at the same time in one process. Therefore, Figure 3 shows the contour surfaces overlapping for blue berry and grape marc. This figure shows that the optimum mixing for blue berry is 28.5% water – 47.4% ethanol – 24.1% acetone to obtain 502.2 GAE/100 g of phenolic compounds and 1349.1 mg/100 g of monomeric anthocyanins. On the other hand, the optimum mixture for grape marc is 28.7% of water – 49.8% of ethanol – 21.5% of acetone to obtain 2642.4 GAE/100 g of phenolic compounds and 31.5 mg/100 g of monomeric anthocyanins.

Conclusion

As conclusion, optimizing the solvent for obtaining the maximum yields of ultrasound-assisted extraction of phenolic compound and monomeric anthocyanins was possible using the Reticular simplex mixtures design (Mixing design). Not only for phenolic compound, but also for monomeric anthocyanins, the addition of water in the mixture with ethanol or acetone increased the extraction yield. Therefore, the solvent cost would be reduced. In addition, by overlapping

Table 8. Validation of the prediction models for estimating Total phenolic compounds and Monomeric Anthocyanins from blue berry and grape marcs.

Raw Material	Component	Predicted value	Experimental value	Confidence value (95%)	
				Inferior	Superior
Blue berry	Total phenolic compounds (GAE/100 g of sample)	502	517	435	569
	Monomeric Anthocyanins (mg/100 g)	1349	1353	1131	1567
Grape marc	Total phenolic compounds (GAE/100 g of sample)	32	31	27	36
	Monomeric Anthocyanins (mg/100 g)	2642	2529	2306	2979

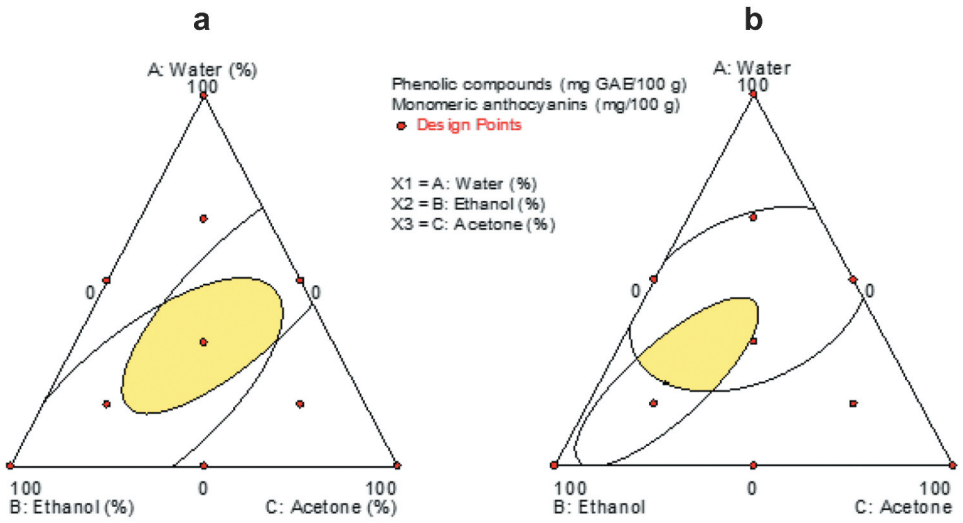

Figure 3. Optimum solvent mixture for obtaining the maximum extraction yield (ultrasound-assisted extraction) of phenolic compounds and monomeric anthocyanins at the same time for (a) blue berry and (b) grape marc. The shaded region represents the optimum region.

the contour plots a unique mixture for obtaining a high yield of phenolic compound and anthocyanins at the same time from blue berry and grape marc was obtained.

Acknowledgments

No conflicts of interest nor financial disclosure to declare.

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