DOI: 10.1111/j.1745-4514.2010.00413.x

OPTIMIZATION OF ULTRASONIC-ASSISTED EXTRACTION OF TOTAL ANTHOCYANINS FROM GRAPE PEEL USING RESPONSE SURFACE METHODOLOGY

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Accepted for Publication July 21, 2009

ABSTRACT

Total anthocyanins from Campbell Early grape peel were extracted by using ultrasonic-assisted extraction technology. The experiments were performed according to a five-level, three-variable central composite rotatable design. The best possible combinations of extraction variables were obtained for the maximum extraction of total anthocyanins from grape peel using response surface methodology. The optimal extraction conditions include 52.35% ethanol, 45.14C temperature and 24.50 min time for the maximum total anthocyanins (6.26 mg/mL). Under these conditions, the experimental total anthocyanins were 6.24 mg/mL of the grape peel extract, which is well matched with the predicted values.

PRACTICAL APPLICATION

Anthocyanins are functional compounds and natural food colorants, which can replace synthetic colors. Grapes and grape residues are good sources of anthocyanins, and maximum recoveries of these compounds are possible by using optimized extraction processes. In our study, we have optimized the ultrasonic-assisted extraction of anthocyanins from grape peel by using central composite rotatable design and response surface methodology. This extraction method is an inexpensive, because of low instrumental involvement, simple and efficient alternative to conventional extraction techniques. Optimized extraction conditions predicted and validated in our study can be used for improved recovery of anthocyanins from grape peel.

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INTRODUCTION

Interest in the investigation of active components from natural sources such as fruits and vegetables has greatly increased in recent years. The reason for this increased interest is the restricted use of synthetic additives in foods because of the possible undesirable effects on human health (Jayaprakasha *et al.* 2003). Grapes are among the most widely consumed fruits, and the demand for grapes and grape products is increasing because of the associated health benefits (Ghafoor *et al.* 2008). Grapes are rich in polyphenolic compounds (Sánchez-Alonso *et al*. 2008) with approximately 75% of grape polyphenols existing in the skin and seeds. Grape skin phenols may be classified as cell-wall phenols; phenols confined in the vacuoles and phenols associated with the cell nucleus (Pinelo *et al.* 2006). Grape skin also contains anthocyanins, which are used to produce authorized colorants, nutraceuticals and drugs (Bordignon-Luiz *et al.* 2007). Anthocyanins account for 30% of the total polyphenols in grape (Yoo *et al.* 2007) and possess strong biological functions, such as anti-inflammatory and antioxidant activities (Kong *et al.* 2003). These compounds are also reported to inhibit the oxidation of human low-density lipoproteins *in vitro* (Meyer *et al.* 1997).

Extraction is a very important stage in the isolation, identification and use of functional compounds from plant materials (Lapornik *et al.* 2005). The recovery of these components is commonly performed through a solvent extraction procedure, and the concentration of solvent, temperature and time are important parameters to be optimized (Spigno *et al.* 2007). Response surface methodology (RSM) has been applied as a powerful tool for optimal extraction of functional compounds from plant materials (Meng *et al.* 2009). Ultrasound-assisted extraction (UAE) is an inexpensive, simple and efficient alternative to conventional extraction techniques. When sound energy passes to the medium, it creates a continuous wave-type motion; as a result, longitudinal waves are generated. This motion creates alternative compression and rarefaction of the medium particles, resulting in cavitation bubbles. Formation and behavior of the cavitation bubble upon the propagation of the acoustic waves constitute the essential events which induce the majority of the acoustic effects (Dahnke *et al.* 1999). The enhancement in extraction obtained by using ultrasound is mainly attributed to the effect of acoustic cavitations produced in the solvent by the passage of an ultrasound wave (Wang *et al.* 2008). Ultrasound also offers a mechanical effect, allowing greater penetration of solvent into the sample matrix and increasing the contact surface area between the solid and liquid phase, and, as a result, facilitating mass transfer of solutes to the extraction solvent (Rostagno *et al.* 2003). The efficiency of ultrasonication could be explained by the fact that sonication simultaneously enhanced the hydration and fragmentation process (Toma *et al.* 2001). The use of high-

frequency ultrasounds may increase the formation of OH radicals; however, the hydrophilic nature of anthocyanins makes them unavailable to react with free radicals (Chowdhury and Viraraghavan 2009). Instead, the use of UAE may prevent the possible chemical degradation of targeted compounds because of decreased chemical involvement and reduction in extraction time (Wang and Weller 2006).

In this study, UAE process variables such as the solvent concentration, extraction temperature and extraction time were optimized using RSM, by employing a five-level, three-variable central composite rotatable design, in order to obtain the optimal conditions for the extraction of total anthocyanins from Campbell Early grape peel.

MATERIALS AND METHODS

Materials

Grapes were purchased from a local farm in Kyungbuk, Province of Korea, and the grape cultivar was identified as "Campbell Early." Grapes were excised from the stems and washed. Grapes were manually cut into halves, and the grape peels were separated with a knife. Grape peel was oven dried at 50C until the moisture level was constant (5.4% w/w). Dried grape peel was ground to a powdered form using an electrical grinder and passed through a 0.5-mm sieve. All the chemicals used were of analytical grade, and they were purchased either from Sigma Chemical Co. (St. Louis, MO) or Duksan Pure Chemical Co. (Ansan, Korea).

Ultrasonic-Assisted Extraction from Grape Peel

A sample of 2 g of powdered grape peel was kept in a glass flask, and the volume was made up to 100 mL with the extraction solvent. Contents were dissolved by using a magnetic stirrer (KMC 130SH, Vision Scientific Co., Ltd., Daegu, Korea) for 5 min. Ultrasonic-assisted extraction (UAE) was performed in a sonication water bath (JAC Ultrasonic 2010P, Jinwoo Engineering Co., Ltd., Hwasung, Gyeonggi, Korea) with a useful volume of 10 L. The working frequency and power were fixed at 40 KHz and 250 W, respectively. The temperature and time of extraction was controlled from the panel. The power output of the ultrasonication water bath can be determined calorimetrically, which is to measure the change in temperature of a known mass of water for a known period of time. The increase in the temperature of water per unit time could be given by:

$$Q_{abs} = Q = \frac{mC_p\Delta T}{t},$$

where Q is the power output (W) and Q_{abs} is the power absorbed by the system per unit time (W), m is the mass of sample in kg, C_p is the specific heat of the sample (J/kg K), ΔT is the temperature rise in the system (C) and t is the total heating time (s). In this study, the maximum power output was recorded as 518.32 W. After extraction, the flask was immediately cooled to room temperature using chilled water. The extract was filtered through filter paper #5A under vacuum, and the solution was collected in a volumetric flask. It was then used for the determination of total anthocyanin contents.

Experimental Design

A five-level, three-variable central composite rotatable design (Cochran and Cox 1992) was applied to determine the best combination of extraction variables for the extraction of total anthocyanins from grape peel. The three independent variables selected for this study were concentration of solvent, extraction temperature and extraction time (Table 1). The factorial design consisted of eight factorial points, six axial points (two axial points on the axis of each design variable at a distance of 1.68 from the design center) and four center points, leading to 18 sets of experiments. Regression analysis was performed on the data of the response variable (total anthocyanins) obtained by triplicate observations of extracts as affected by the extraction conditions, and it was fitted into an empirical second-order polynomial model as shown in the following equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3, \quad (1)$$

where Y represents the response variable, and X_1, X_2 and X_3 correspond to the independent variables, namely, ethanol concentration, extraction temperature and extraction time, respectively. The b_n values represent corresponding regression coefficients.

TABLE 1.
INDEPENDENT VARIABLES AND THEIR LEVELS EMPLOYED IN A CENTRAL COMPOSITE ROTATABLE DESIGN FOR THE OPTIMIZATION OF GRAPE PEEL EXTRACTS

	Coded levels				
Independent variables	−α (−1.68)	−1	0	1	+α (+1.68)
	Natural levels				
Ethanol concentration (%)	33	40	50	60	67
Extraction temperature (C)	23	30	40	50	57
Extraction time (min)	11	15	20	25	29

Determination of Total Anthocyanins

Determination of total anthocyanins in grape peel extracts was based on the method described by Iland *et al.* (1996) with slight modification. In 1 mL of the sample, 10 mL of 50% ethanol was added, and the sample was centrifuged (VS 24SMTI Centrifuge, Vision Scientific Co., Ltd., Daegu, Korea) at 1,800× g for 10 min. Two hundred microliter of the centrifuged extract was mixed with 3.8 mL of 1 M HCl and incubated at room temperature for 3 h. The absorbance (A) of acidified diluted extract was measured at 520 nm using a spectrophotometer (TU-1800, Human Corporation, Seoul, Korea).One molar of HCl was used as the blank. Total anthocyanins were calculated as mg/mL of extract using the absorbance (B) of a 1% w/v solution of malvidin-3-glucoside as follows:

$$\text{Total anthocyanins (mg/mL)} = \text{A} \times \text{dilution factor} \times 1{,}000/\text{B}.$$

Statistical Analysis

All the analysis was carried out in triplicate, and the experimental results obtained were expressed as means ± standard deviation ($n = 3$). The responses obtained from the experimental design set were subjected to multiple nonlinear regression analysis to obtain the coefficients of the second polynomial model. The quality of the fit of polynomial model was expressed by the coefficient of determination R^2, and its statistical significance was checked using an F-test. The optimal extraction conditions were estimated through three-dimensional response surface analyses of the three independent variables and each dependent variable. Statistical analysis was performed by using the Statistical Analysis System (SAS, version 9.1, SAS Institute, Cary, NC). Data were analyzed by analysis of variance, and the mean values were considered significantly different when $P < 0.05$.

RESULTS AND DISCUSSION

Modeling of the Extraction Process from Grape Peel

In order to optimize the extraction process with reference to the extraction of total anthocyanins from grape peel under sonication, a central composite design was developed as represented in Table 2. Table 2 also presents the experimental values of total anthocyanins of grape peel extracts at various experimental conditions. The values of coefficients are presented in Table 3. The results of analysis of variance, goodness of fit and the adequacy of the models are summarized in Table 4. The significance of regression coefficients

TABLE 2.
EXPERIMENTAL DESIGN OF FIVE-LEVEL, THREE-VARIABLE CENTRAL COMPOSITE DESIGN AND TOTAL ANTHOCYANINS OF ULTRASONIC-ASSISTED GRAPE PEEL EXTRACTS

Test set	X_1, ethanol concentration (%)	X_2, extraction temperature (C)	X_3, extraction time (min)	Y, anthocyanins* (mg/mL)
1	40 (−1)	30 (−1)	15 (−1)	5.07 ± 0.18
2	40 (−1)	30 (−1)	25 (+1)	5.48 ± 0.12
3	40 (−1)	50 (+1)	15 (−1)	5.26 ± 0.22
4	40 (−1)	50 (+1)	25 (+1)	5.79 ± 0.16
5	60 (+1)	30 (−1)	15 (−1)	5.38 ± 0.22
6	60 (+1)	30 (−1)	25 (+1)	5.57 ± 0.33
7	60 (+1)	50 (+1)	15 (−1)	5.39 ± 0.21
8	60 (+1)	50 (+1)	25 (+1)	5.96 ± 0.08
9	33 (−1.68)	40 (0)	20 (0)	5.37 ± 0.19
10	67 (+1.68)	40 (0)	20 (0)	5.85 ± 0.07
11	50 (0)	23 (−1.68)	20 (0)	5.29 ± 0.25
12	50 (0)	57 (−1.68)	20 (0)	5.88 ± 0.36
13	50 (0)	40 (0)	11 (−1.68)	4.83 ± 0.15
14	50 (0)	40 (0)	29 (−1.68)	6.25 ± 0.23
15	50 (0)	40 (0)	20 (0)	6.13 ± 0.18
16	50 (0)	40 (0)	20 (0)	6.05 ± 0.35
17	50 (0)	40 (0)	20 (0)	5.98 ± 0.17
18	50 (0)	40 (0)	20 (0)	6.21 ± 0.41

* Analytical results are means ± standard deviation ($n = 3$).

TABLE 3.
REGRESSION COEFFICIENTS AND ANALYSIS OF THE MODEL FOR TOTAL ANTHOCYANINS FROM GRAPE PEEL

Coefficient	df	Estimate	Standard error	t value	P value
b_0	1	−6.610	2.536	−2.61	0.031
b_1	1	0.212	0.055	3.80	0.005
b_2	1	0.151	0.051	2.93	0.019
b_3	1	0.328	0.001	3.34	0.010
b_{11}	1	−0.002	0.001	−4.14	0.003
b_{22}	1	−0.002	0.001	−4.33	0.003
b_{33}	1	−0.007	0.002	−4.56	0.002
b_{12}	1	−0.001	0.001	−0.22	0.832
b_{13}	1	−0.001	0.001	−0.39	0.703
b_{23}	1	0.001	0.098	1.10	0.305

df, degree of freedom.

TABLE 4.
ANALYSIS OF VARIANCE OF THE SECOND-ORDER TOTAL ANTHOCYANIN CONTENTS OF THE GRAPE PEEL MODEL

	df	Sum of squares	Mean square	*F* value	*P* value
Total model	9	2.718409	0.9290	11.63	0.001
Linear	3	1.680519	0.5743	21.58	0.0003
Quadratic	3	1.001339	0.3422	12.85	0.0020
Cross-product	3	0.036550	0.0125	0.47	0.7119
Total error	8	0.207703	0.0260		
Lack of fit	5	0.178028	0.0356	3.60	1.604
Pure error	3	0.029675	0.0099		

df, degree of freedom.

was estimated by using Student's *t*-test and *P* values as presented in Table 3. The data showed a good fit with Eq. (1), which was statistically acceptable at $P < 0.05$ and adequate with satisfactory R^2 values. The full model that filled Eq. (1) was used for three-dimensional plots to predict the relationships between independent variables and the dependent variable.

Effect of Process Variables on Total Anthocyanins

Solid–liquid extraction is a mass transport phenomenon in which solids contained in a matrix migrate into solvent brought into contact with the matrix. This mass transport phenomenon can be enhanced with changes in diffusion coefficients induced by ultrasounds and extraction temperature (Corrales *et al.* 2009). Solvent concentration and extraction time also play a significant role in the extraction of functional components from plant materials (Wang *et al.* 2008). We used ethanol as the solvent, which is regarded as a dietary alcohol and preferred for use in the food industry (Ovando *et al.* 2009). Total anthocyanin contents from grape peel obtained under various conditions using ultrasonic-assisted extraction (UAE) are presented in Table 1. Multiple regression analysis was performed on the experimental data, and the coefficients of the model were evaluated for significance. The effects of all process variables on the extraction of total anthocyanins from grape peel were significant ($P < 0.05$); however, that of extraction time was highly significant ($P < 0.001$). The extraction time has this kind of effect because increasing the contact time of the solvent with solids may improve the diffusion of the compounds (Corrales *et al.* 2009). The values of the coefficients as presented in Table 3 were used for the final predictive equation, neglecting the nonsignificant cross terms as given below:

$$Y = -6.610 + 0.211X_1 + 0.151X_2 + 0.328X_3 - 0.002X_1^2 - 0.0.002X_2^2 - 0.007X_3^2. \quad (2)$$

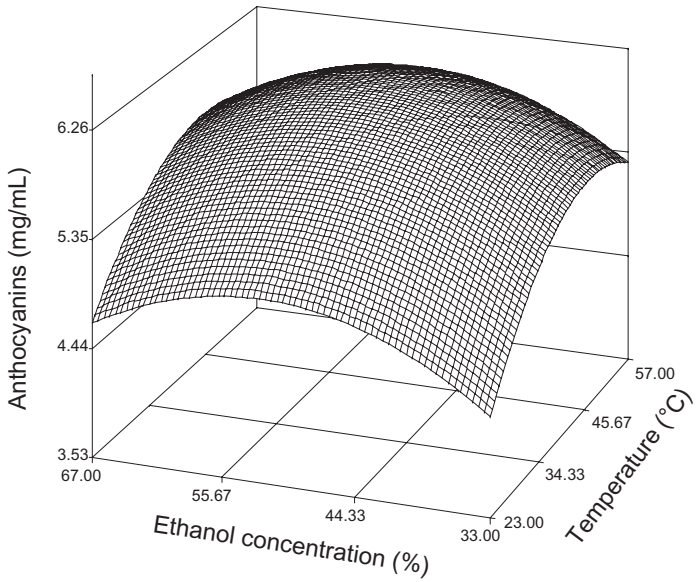

FIG. 1. THE RESPONSE SURFACE PLOTS OF TOTAL ANTHOCYANIN CONTENTS OF GRAPE PEEL EXTRACT AS AFFECTED BY ETHANOL CONCENTRATION AND TEMPERATURE (TIME 20 MIN)

Based on Eq. (2), three-dimensional plots that represent the effects of extraction process variables on the anthocyanins from grape peel are presented in Figs. 1–3. Figure 1 shows the effects of ethanol concentration and extraction temperature on the anthocyanin contents. There was a linear increase in total anthocyanins with an increase in ethanol concentration at a constant temperature. A similar effect of extraction temperature on grape peel anthocyanins at a fixed ethanol concentration was observed. Figure 2 represents that total anthocyanins increased linearly with the increase of ethanol concentration at a fixed extraction time, while a rise in extraction time at a fixed ethanol concentration also led to a marked increase in total anthocyanins. The effect of extraction temperature and extraction time shown in Fig. 3 demonstrated that there was a linear increase in total anthocyanins with the increase of extraction temperature at a fixed extraction time, while an obvious quadratic effect of extraction time was observed. Table 3 represents that anthocyanin contents were significantly effected ($P < 0.05$) only by the linear and quadratic terms of extraction variables. Similar linear and quadratic effects of extraction variables in UAE from wheat bran were observed by Wang *et al.* (2008). In another study, Lapornik *et al.* (2005) found a statistically significant influence of extraction variables on the yields of anthocyanins from red grape marc. They

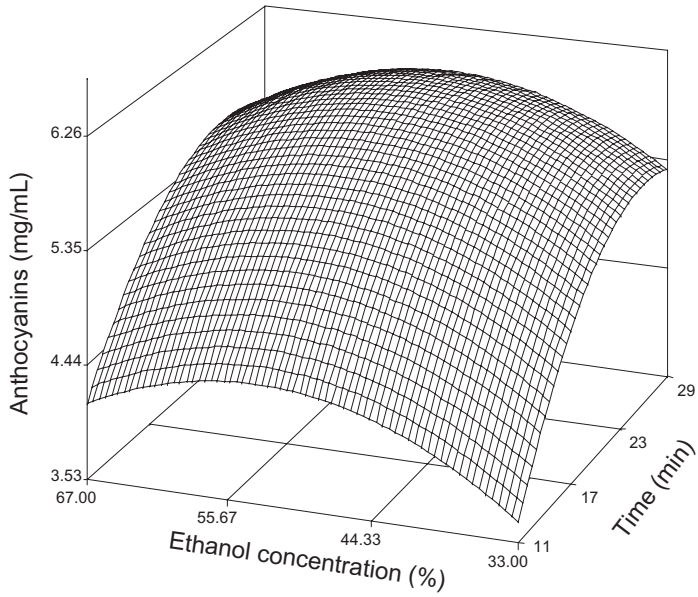

FIG. 2. THE RESPONSE SURFACE PLOTS OF TOTAL ANTHOCYANIN CONTENTS OF GRAPE PEEL EXTRACT AS AFFECTED BY ETHANOL CONCENTRATION AND TIME (TEMPERATURE 40C)

observed that increasing the extraction time from 12 to 24 h was found to have statistically significant effects on the yields of total anthocyanins. This indicated that the use of UAE for the extraction of anthocyanins was as effective as any other high-temperature long-time extraction process, and it could greatly decrease the extraction time, which also reduces the chances of denaturation of the targeted compounds (Spigno *et al.* 2007). The contents of total phenolic compounds in grape peel extracts ranged from 5.74 to 6.58 mg GAE/100 mL, which shows a good recovery of phenolics from grape peel besides the optimal extraction of total anthocyanins.

Optimum Extraction Conditions for Maximum Total Anthocyanins from Grape Peel

Based on our findings, the predicted UAE conditions were 52.35% ethanol concentration, 50.65C temperature and 25.58 min time for the maximum total anthocyanins (6.26 mg/mL). The R^2 value of the model was 0.929, R^2-adjusted value was 0.879, F value was 11.63 and P value was 0.001, which represent that the model had adequately represented the real relation between the parameters chosen. Using the same predicted optimum condition,

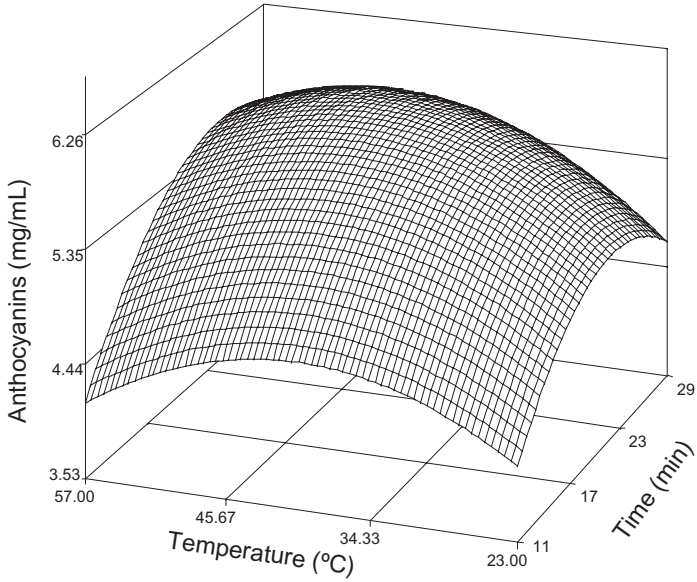

FIG. 3. THE RESPONSE SURFACE PLOTS OF TOTAL ANTHOCYANIN CONTENTS OF GRAPE PEEL EXTRACT AS AFFECTED BY TEMPERATURE AND TIME (ETHANOL CONCENTRATION 50%)

an extraction experiment was performed without the application of sonication, and it was found that the total anthocyanins in the grape peel extract were 3.46 mg/mL, which was much lower than that obtained by using UAE. In order to compare the predicted results with experimental values, experimental rechecking was performed using the optimum UAE conditions, and the mean values of 6.24 $\pm$ 0.08 mg/mL of total anthocyanins were obtained. The comparison of experimental values with predicted values was done by using Student's *t*-test, which showed nonsignificant differences. The good correlation between these results validated that the response model was adequate in reflecting the expected optimization.

ACKNOWLEDGMENT

This study was supported by the Grape Research Project Group of the Agriculture R&D Promotion Center, Ministry of Agriculture and Forestry, South Korea.

REFERENCES

BORDIGNON-LUIZ, M.T., GAUCHE, C., GRIS, E.F. and FALCÃO, L.D. 2007. Colour stability of anthocyanins from Isabel grapes (*Vitis labrusca* L.) in model systems. Lebensm. Wiss. Technol. *40*(4), 594–599.

CHOWDHURY, P. and VIRARAGHAVAN, T. 2009. Sonochemical degradation of chlorinated organic compounds, phenolic compounds and organic dyes: A review. Sci. Total Environ. *407*(8), 2474–2492.

COCHRAN, W.G. and COX, G.M. 1992. Some methods for the study of response surfaces. In *Experimental Designs* (W.G. Cochran and G.M. Cox, eds.) pp. 335–375, Wiley, New York, NY.

CORRALES, M., GARCIA, A.F., BUTZ, P. and TAUSCHER, B. 2009. Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure. J. Food Eng. *90*(4), 415–421.

DAHNKE, S.W., SWAMY, K.M. and KEIL, F.J. 1999. A comparative study on the modeling of sound pressure field distributions in a sonoreactor with experimental investigation. Ultrason. Sonochem. *6*, 221–226.

GHAFOOR, K., JUNG, J.E. and CHOI, Y.H. 2008. Effects of gellan, xanthan, and λ-carrageenan on ellagic acid sedimentation, viscosity, and turbidity of "Campbell Early" grape juice. Food Sci. Biotechnol. *17*(1), 80–84.

ILAND, P.G., CYNKAR, W., FRANCIS, I.L., WILLIAMS, P.J. and COOMBE, B.G. 1996. Optimization of methods for the determination of total and red free glycosyl-glucose in black grape berries of *Vitis vinifera*. Aust. J. Grape Wine Res. *2*(3), 171–178.

JAYAPRAKASHA, G.K., SELVI, T. and SAKARIAH, K.K. 2003. Antibacterial and antioxidant activities of grape seed extracts. Food Res. Int. *36*, 117–122.

KONG, J.M., CHIA, L.S., GOH, N.K., CHIA, T.F. and BROUILLARD, R. 2003. Analysis and biological activities of anthocyanins. Phytochemistry *64*, 923–933.

LAPORNIK, B., PROSEK, M. and WONDRA, A.G. 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. J. Food Eng. *71*(2), 214–222.

MENG, G.J., LU, J., FAN, W., DONG, J.J., ZHANG, J., KONG, W.B., LIN, Y. and SHAN, L.J. 2009. Optimization of extraction conditions for antioxidant phenolic compounds from malt rootlets using response surface methodology. J. Food Biochem. *33*, 291–305.

MEYER, A.S., YI, O.S., PEARSON, D.A., WATERHOUSE, A.L. and FRANKEL, E.N. 1997. Inhibition of low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). J. Agric. Food Chem. *45*, 1638–1643.

OVANDO, A.C., HERNANDEZ, M.L.P., HERNANDEZ, M.E.P., RODRIGUEZ, J.A. and VIDAL, C.A.G. 2009. Chemical studies of anthocyanins: A review. Food Chem. *113*, 859–871.

PINELO, M., ARNOUS, A. and MEYER, A.S. 2006. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. Trends Food Sci. Technol. *17*, 579–590.

ROSTAGNO, M.A., PALMA, M. and BARROSO, C.G. 2003. Ultrasound-assisted extraction of soy isoflavones. J. Chromatogr. A *1012*, 119–128.

SÁNCHEZ-ALONSO, I., JIMÉNEZ-ESCRIG, A., SAURA-CALIXTO, F. and BORDERÍAS, A.J. 2008. Antioxidant protection of white grape pomace on restructured fish products during frozen storage. Lebensm. Wiss. Technol. *41*(1), 42–50.

SPIGNO, G., TRAMELLI, L. and DE-FAVERI, D.M. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. J. Food Eng. *81*, 200–208.

TOMA, M., VINATORU, M., PANIWNYK, L. and MASOM, T.J. 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. Ultrason. Sonochem. *8*, 137–142.

WANG, J., SUN, B., CAO, Y., TIAN, Y. and LI, X. 2008. Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. Food Chem. *106*, 804–810.

WANG, L. and WELLER, C.L. 2006. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol. *17*, 300–312.

YOO, M.A., KIM, J.S., CHUNG, H.K., PARK, W.J. and KANG, M.H. 2007. The antioxidant activity of various cultivars of grape skin extract. Food Sci. Biotechnol. *16*(6), 884–888.