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# Recovery of Proanthocyanidin from Waste of Turkish Traditional Product, Pekmez (Molasses)

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**The possibility of recovering of proanthocyanidin (PA) from the by-product of Turkish traditional product *pekmez* (molasses) industry, one of the agro industries, was investigated. In order to obtain optimum extraction conditions, the effects of different solvents and their aqueous forms were studied and the highest PA concentration was achieved as 31 (±0.68) g/L with acetone/water (30: 70, v/v) solution. The correlations between phenolic contents, PA concentrations and antioxidant capacities were shown. Antioxidant capacities of extracts were determined and the comparison of them with synthetic and commercial antioxidants was performed. The antioxidant capacity of PA extract from grape seed of molasses pomace was 93 (±1.43) TEAC values, which was relatively high compared with those of the synthetic antioxidants. Silk fibroin was used as a novel adsorbent to recover the PA from the grape seed extracts. Effect of parameters such as, solid-liquid ratio, pH and initial concentration of PA on the recovery performance was investigated. All parameters were found statistically significant parameters ($P<0.005$) and the best adsorption conditions were found to be pH 5.5, initial concentration as 10 g/ml and solid-liquid ratio as 0.1 g/ml, which yielded the maximum percentage of adsorbed PA amount as 85.2%.**

Keywords: Grape seed, proanthocyanidin, extraction, antioxidant, adsorption, silk fibroin

## Introduction

The recovery of high value added substances from agro industry wastes has become important contribution in our country. In agro industry, grape pomace consisting of seeds, skins and stems, is an important by-product of winemaking and vinegar production processes. Furthermore, the grape seeds form 400–500 g/kg (dry weight) of pomace (Shrikhande, 2000). In Turkey, the grape seed is recovered considerably in large amounts during the production of molasses (pekmez), one of the important Turkish traditional foods. The grape seeds contains large amount of polyphenolic substances known as proanthocyanidin (PA), which can be used as a natural antioxidant (Fuleki *et al.*, 1997; Monogas *et al.*, 2003; Ramila *et al.*, 2005; Yilmaz, 2006). The recovery of PAs from wastes of molasses (pekmez) industry is of great economical importance, since it is possible to produce value-added natural products from these wastes.

Free radicals are generated in human body because of the occupational exposure to chemically and structurally diverse environmental pollutants including pesticides, toxic chemical wastes, air pollutants, radiation and physical stress. They contribute human diseases by causing oxidative deterioration of lipids, proteins and DNA, inhibition of cellular and antioxidant defense systems; changes in gene expression and induction of abnormal proteins (Bouhamidi *et al.*, 1998). Antioxidants are capable of preventing or delaying the rate of oxidation by scavenging these free radicals. Therefore, they have many favorable effects on human health by lowering low-density lipoprotein (LDL) and cholesterol, preventing heart disease and cancer (Yamakoshi *et al.*, 1999; Bagchi *et al.*, 2000; Buelga *et al.*, 2000; Yilmaz and Toledo, 2004).

Antioxidants can be synthetic or natural. Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) are widely used in food industry in order to prevent oxidative deteriorations. However, recent studies have shown that these antioxidants have toxicological effects (Burlow, 1998; Louli *et al.*, 2004). The use of natural antioxidants in the food industry is preferred to synthetic ones (Moure *et al.*, 2001). Thus interest in the natural antioxidants steadily increases. The most important natural antioxidants are tocopherols, ascorbic acid and recently some plant extracts including grape seed extracts. Plant polyphenols are responsible to exert protective effects by scavenging free radicals (Taubert *et al.*, 2003). PA is potent antioxidant comparing with the others because of the number of hydroxyl groups in its structures (Yilmaz and Toledo, 2006).

PA is formed via interflavanol linkages between the flavan-3-ol subunits, which are (+)-catechin, (−)-epicatechin and their gallate forms (Burlow, 1998; Gabetta *et al.*, 2000; Yanagida *et al.*, 2003). Complex PA's structure including dimmers, trimers, oligomers and polymers can be seen based on their degree of polymerization (Schofield *et al.*, 2001; Monogas *et al.*, 2003).

So far, the extraction of PA from grape seeds has been accomplished by employing solvent extraction using methanol (MeOH), ethanol (EtOH), acetone, ethyl acetate, an

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aqueous solution of solvents, or even a supercritical fluid extraction with the objective of obtaining extracts with higher yields and lower costs (Pekic *et al.,* 1998; Schofield *et al.,* 2001; Louli *et al.,* 2004). In any case, the composition of the extract depends not only on the solvent used, but also on the quality and the origin and variety of the plant material, its composition (leaves, stems, seeds, etc.), its storage conditions and its pretreatment (Monogas *et al.,* 2003; Yanagida *et al.,* 2003).

Further purification is required in order to obtain concentrated specific components selectively since many other compounds such as sugars, protein or metals may exist in the plant extracts (Yoon *et al.,* 1997; Aehle *et al.,* 2004). Since adsorption is a low cost separation technique, it is preferred for the selective recovery of target plant metabolites from the crude extracts (Yoon *et al.,* 1997). Many biopolymers, such as collagen and cellulose have been used as adsorbents for the recovery of polyphenols and the interaction between the biopolymers and antioxidative polyphenols have been widely investigated (Tang *et al.,* 2003).

Silk fibroin is an edible protein polymer which has functional amino acids in its structure, and it is preferred in many biotechnological applications such as drug delivery and tissue engineering (Park *et al.,* 1999; Altman *et al.,* 2003; Bayraktar *et al.,* 2005). Its hydrophobic character makes it favorable adsorbent used in adsorption studies since 1940's (Pauling, 1945; Kongkachuichaya *et al.,* 2002). Because of its promising health effects and bounding mechanisms, silk fibroin can be considered as a potent adsorbent for the isolation of antioxidants from the crude extracts (Bayçın *et al.,* 2007). However, to our knowledge, silk fibroin has not been used as an adsorbent for the recovery of any proanthocyanidin structures.

The objective of this study was to determine the effective solvent in extraction of PA from grape seeds obtained from molasses (pekmez) industry in Turkey by comparing the PA concentrations, total phenol contents and antioxidant activities. Furthermore, the potential use of silk fibroin as a novel adsorbent to recover proanthocyanidins from crude extracts was investigated. The antioxidant capacity of extract was determined by means of Trolox equivalent antioxidant capacity (TEAC) and comparison of the antioxidant capacity of PA with ascorbic acid, synthetic antioxidants and three different commercially available grape seed extract supplements was achieved. This is the first time that pomace of molasses industry in Turkey, is used as a source for the recovery of PA. This industry in Turkey is a good source of grape pomace since the production of molasses is considerably in large amounts.

## Experimental

*Materials* Grape pomace was obtained as by product of molasses industry from Muğla in Eagean region in Turkey. After drying of pomace at 40℃ for 24 h in static air, grape seeds were separated from its skins and stems.

HPLC-grade acetone, EtOH, MeOH, HCl, acetic acid and *n*-butanol were purchased from Merck (Darmstadt, Germany). The synthetic antioxidants, BHT, BHA, *tert*-butylhydroquinone (TBHQ), propyl gallate (PG), ascorbic acid and Trolox were obtained from Fluka. For the determination of antioxidant activity, the free radical employed ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] was purchased from Sigma (Steinheim, Germany). Hydrophobic silk fibroin from Silk Biochemical Co., Ltd. (China) was used as adsorbent.

*Extraction of PA from grape seeds* PAs from grape seeds were extracted according to the method described in the literature (Pekic *et al.,* 1998). Whole grape seeds without grinding were used in the extraction process. Grape seeds (10 g) were subjected to liquid extraction with 50 ml of pure solvent and solvent-water mixtures in a Gerhard thermo shaker at 35℃ and 180 rpm for 24 h. The solvent type is the most important factor affecting the efficiency of solid-liquid extraction. For this reason, different solvents; acetone, EtOH, MeOH and their aqueous solutions were investigated to determine the most effective extraction of PA. Deionized water was used in all experiments. After 24 h extraction, the extracts were filtrated to remove the seeds and centrifuged for 5 min at 5,000 rpm. The PA contents, total phenol contents and antioxidant capacities of all extracts were determined.

*Optimum extraction time* The time required for the optimum extraction was determined for the extraction of PA in the solutions of acetone/water (30: 70, v/v), ethanol/water (60: 40, v/v), and methanol/water (90: 10, v/v). The PA concentrations were determined according to Porter assay given below (Schofield *et al.,* 2001).

*PA content* The Porter method is widely used for measurement of extractable condensed tannins (PA) in foods and feeds. This colorimetric reaction uses an acid-catalyzed oxidative depolymerization of condensed tannins to yield red anthocyanidins. PA structure is depolymerized in acidic environment with butanol and ferric reagent at 100℃. Under these conditions, fraction of depolymerized proanthocyanidins gives pinkreddish coloured anthocyanidins, which determined by a Shimadzu UV-visible spectrophotometer at 550 nm. In brief, 0.5 mL aliquots of prepared extracts were transferred into test tubes. After addition of 3 mL butanol-HCl reagent (butanol/HCl, 95: 5, v/v) and 0.1 mL 2% ferric reagent (2% ferric ammonium sulfate in 2 M HCl), test tubes were vortexed and put into a boiling water bath for 60 min. After cooling, absorbance values were recorded at 550 nm against blank, containing 0.5 mL of solvent without extract. Required dilutions were done in order to obtain absorbance below 0.6. PA concentration was determined by the calibration of the PAs produced by lyophilized grape seed extract solution.

*Total phenol content* Total phenol content (TPC) of extracts was determined by the method Folin- Ciocalteu assay (Makkar *et al.,* 1993). An aliquot (0.5 mL) of each extract was reacted with the freshly prepared 1.25 mL of 20% sodium carbonate and 0.5 mL of 1 N Folin reagent in a screw-capped test tube. Required dilutions were prepared with distilled water. Test tubes were vortexed and after 40 min, absorbance readings were recorded at 725 nm. The phenol content was expressed as mg tannic acid per g of dry seeds.

*Antioxidant activity* The TEAC assessment was performed in terms of radical scavenging ability according to the ABTS/$K_2S_8O_2$ method (Re *et al.*, 1999). This is based on the ability of an antioxidant to scavenge the preformed radical cation $ABTS^+$ relative to that of the standard antioxidant Trolox. The $ABTS^+$ radical was generated by a reaction between 7 mM ABTS and activated with 2.45 mM $K_2S_8O_2$. The $ABTS^+$ solution was diluted withethanol to an absorbance of 0.70 (±0.03) at 734 nm and equilibrated at 30℃. Twenty μL of sample was added to $ABTS^+$ solution and absorbance decrease was recorded during 6 min. Series of dilutions were prepared such that they produced between 20–80% inhibition of the blank absorbance. TEAC calculation was performed by the gradient of the plot of the percentage inhibition of absorbance versus concentration plot for the antioxidant in analysis. It is divided by the gradient of the plot for standard; Trolox. The values of TEAC were given as mmol Trolox for 1 g of grape seed. Results were expressed as mean±standard error. Correlation between polyphenol contents, PA concentration and antioxidant activity was established by regression analysis.

*Adsorption of proanthocyanidin on silk fibroin* Crude grape seed extract was dissolved in proper solvent and adsorption studies were performed in a batch system in order to investigate the effects of solid- liquid ratio, pH and initial concentration on adsorption performance. The parameters were optimized by 23 factorial experimental design. Adsorption process was performed in the thermo shaker at 250 rpm at 30℃ for 2 h. In order to investigate the statistically significant parameters effective on adsorption process, the parameters and levels wereb selected as; solid-liquid ratio as 0.025 g/mL (level: −1) and 0.1 g/mL (level: +1); pH as 2.5 (level: −1) and 5.5 (level: +1), adjusted with citric acid-phosphate buffer solutions; and initial concentrations as 10 g/L(level: −1) and 30 g/L (level: +1). After centrifuging the crude grape seed extract-silk fibroin solution at 5,000 rpm for 5 min, the adsorbed amount was determined by analyzing the total phenol content of the supernatant.

## Results and Discussion

*Extraction of phenolic compounds* The results of the phenolic compound recovery from grape pomace with different solvents and their aqueous forms are presented in Fig. 1. The acetone solvent recovered more phenolic compounds from grape pomace than the MeOH and EtOH solvents. The solvents with water content between 40 to 70% extracted approximately the same amounts of phenolic compounds. However acetone/water (30: 70, v/v) extracts showed the highest TPC. The selection of proper solvent was important for the efficient recovery of active substances, contained in grape seeds of pomace.

*Extraction of proanthocyanidin* Figure 2 illustrates the recovery of PA from grape seed with three different solvents. The highest PA concentration was obtained as 31 (±0.68) g/L with acetone/water (30: 70, v/v) solution. On the other hand, ethanol/water (30: 70, v/v) solution

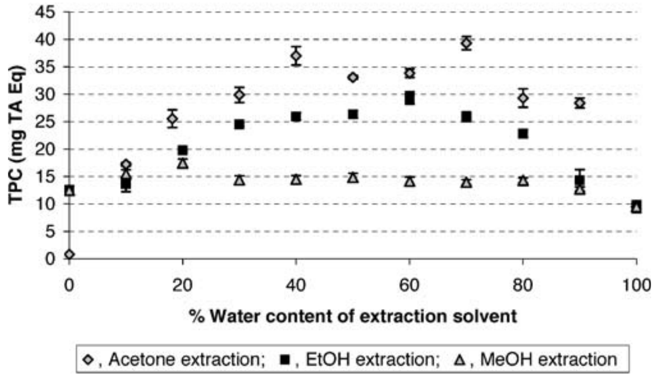


**Fig. 1.** Effect of solvent types and their water contents on the extraction performance of phenolic compounds.

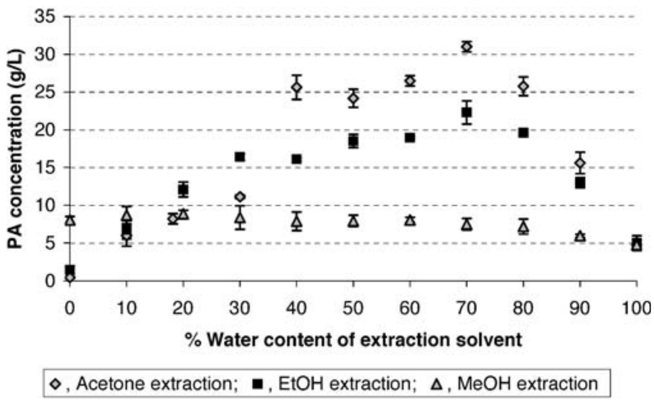


**Fig. 2.** Comparison of extractable proanthocyanidin (PA) by using different solvents (extraction time: 24 h).

and methanol/water (90: 10, v/v) solution gave the PA concentrations of 22.3 (±0.76) g/L and 8.9 (±1.03) g/L, respectively. The amount of extracted PA with MeOH was lower compared with ethanol/water (30: 70, v/v) solution and acetone/water (30: 70, v/v) solution. Acetone/water (30: 70, v/v) solution was found to be the best solvent for extracting the PA from grape seeds.

It could be concluded that the presence of water increased the permeability of grape seeds and thus enabled a better mass transport by diffusion. However, the extraction with water alone was not effective to extract PA with high yield. Only 5 g/L PAs were extracted with water. Similarly, EtOH, acetone and MeOH alone was ineffective as a solvent for the extraction of PAs from grape seeds of molassesμ pomace. As it can be observed from Figs 1 and 2 , Both the highest TPC and PA concentrations were obtained from the solvent of acetone/water (30: 70, v/v). For all the solvent-water systems, similar observations were made.

The correlation between the PA concentrations and the TPC should be noted. PA concentrations increased as TPC increased (Fig. 3). A linear relationship between PA concentration and TPC was established. High correlation coefficient values were obtained for all extraction processes. Regression coefficients were found as 0.9354 for acetone extraction, 0.8419 for EtOH extraction and 0.8929

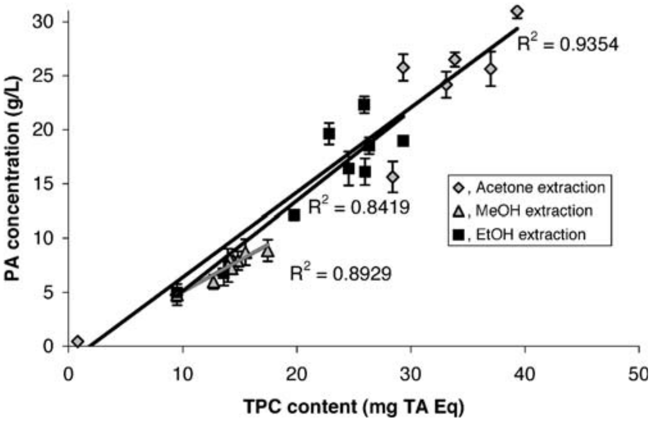


**Fig. 3.** Correlation between the proanthocyanidin (PA) concentration and total phenol content (TPC) for acetone, MeOH and EtOH extraction.

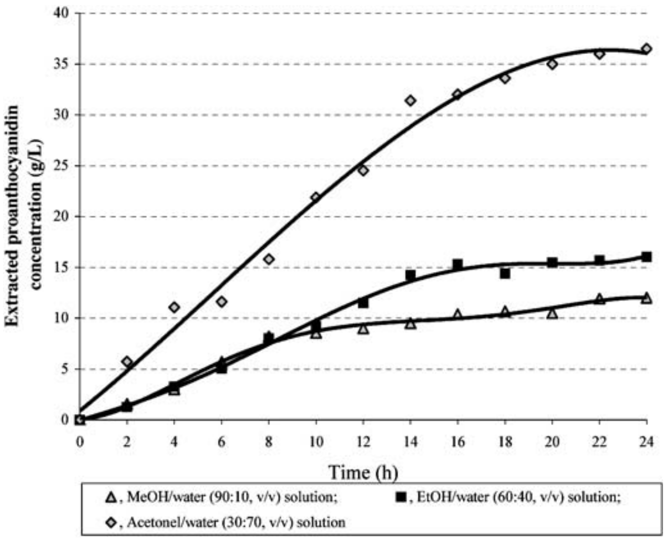


**Fig. 4.** Extraction profile of proanthocyanidin (PA).

for MeOH extraction.

*Optimum extraction time* The extraction profile on time of PA was investigated for optimizing the process as shown in Fig. 4. The equilibrium was reached within 16 h when MeOH and EtOH were used as extraction solvents. In case of acetone extraction of PAs, the equilibrium has not been attained with this period. The equilibrium time for PA-acetone extraction system was 24 h. Within this period, the maximum PA content extracted was around 35 g/L, where as it was approximately 16 and 10 g/L in the case of EtOH and MeOH, respectively.

*Antioxidant activity* Figure 5 revealed TEAC values which expressed antioxidant activity. TEAC values of all extraction systems were calculated in terms of the reference antioxidant, trolox. The extraction efficiency was significantly affected by the solvent used. Therefore the changes in the extract content directly caused the differences in TEAC values of grape seed extracts. Among aqueous acetone solvents, the extracts obtained by using acetone/water (30: 70, v/v) solution showed the highest antioxidant capacity as 93 (±1.43) mmol Trolox/g seeds.

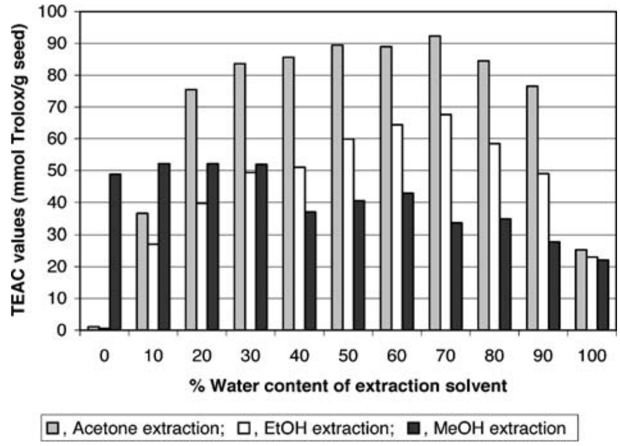


**Fig. 5.** Antioxidant capacities of grape seed extracts obtained by using acetone, MeOH and EtOH and their aqueous mixtures.

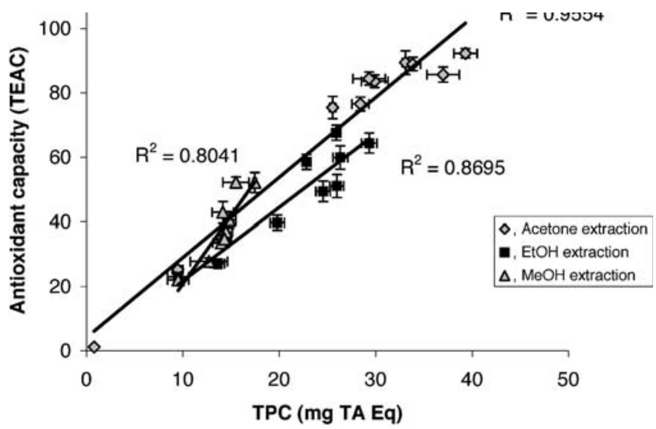


**Fig. 6.** The relationship between antioxidant capacity and total phenol content (TPC).

Furthermore, antioxidant capacities of extracts obtained by using ethanol/water (30: 70, v/v) solution and methanol/water (90: 10, v/v) solution were found to be 67 (±2.34) mmol Trolox/g seeds and 52 (±1.67) mmol Trolox/g seeds, respectively. As seen from Fig. 5, relatively lower antioxidant capacities of extracts obtained by using pure solvents were expected result, since mono-component solvent systems of water, acetone, EtOH and MeOH were less effective for extracting PAs from grape seeds of molasses $\mu$ pomace when compared to their aqueous mixtures.

Linear correlation was observed between the TPC and antioxidant capacities as shown in Fig. 6. Antioxidant activities increased proportionally with increasing TPC. The correlation coefficients were determined as 0.9554, 0.8695 and 0.8041 for the acetone, EtOH and MeOH extractions, respectively. Similar correlation was also achieved between the antioxidant capacities and PA concentrations as shown in Fig. 7. The correlation coefficients were found as 0.9234, 0.9571 and 0.8618 for acetone, MeOH and EtOH extraction processes.

The antioxidant activities of ascorbic acid, synthetic antioxidants and three commercial samples were given in

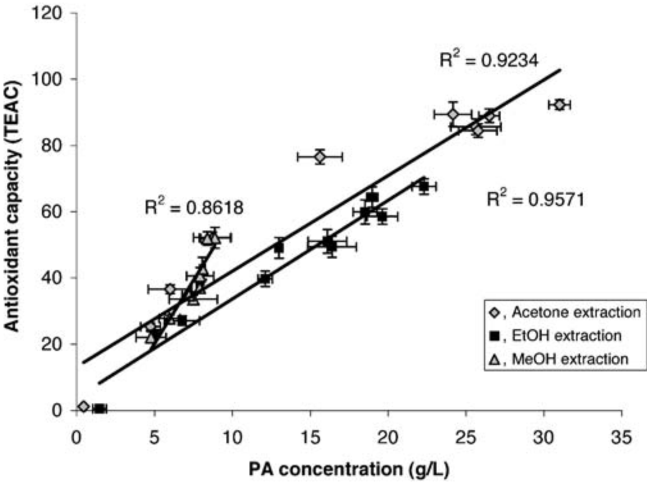


**Fig. 7.** The relationship between antioxidant capacity and proanthocyanidin (PA) concentrations.

**Table 1.** Comparison of Antioxidant Activities of Different Commercially Available Antioxidants and Grape Seed Extracts.

| Samples | | TEAC values (mmol Trolox/g antioxidant) |
|---|---|---|
| Commercial Products | 1 | 59 |
| | 2 | 100 |
| | 3 | 88 |
| PA extract from grape seed of molasses pomace | | 93 |
| (+) Catechin | | 8.01 |
| BHA | | 6.66 |
| BHT | | 0.59 |
| TBHQ | | 1.17 |
| PG | | 12.62 |
| Ascorbic acid | | 6.45 |

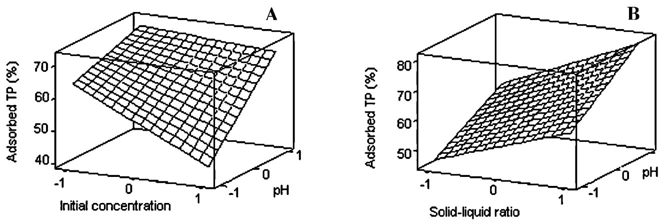


**Fig. 8.** Surface response plot for parameters, which are effective in phenol adsorption performance (A) between initial concentration and pH; (B) between solid-liquid ratio and pH. Initial concentration levels of −1 (10 g/L) and +1 (30 g/L), pH levels of −1 (pH 2.5) and +1 (pH 5.5) and solid-liquid ratio levels of −1 (0.025 g/mL), +1 (0.10 g/mL) were used.

Table 1. The PA extract obtained from grape seeds in molasses industry has considerably higher antioxidant activity when compared with other natural and synthetic antioxidants; 13 times higher antioxidant capacity than those of BHA and ascorbic acid. Since these synthetic and natural antioxidants are widely used in pharmaceutical, food and chemical industries, it should be very beneficial if they are replaced with the natural antioxidant as PAs. The antioxidant activities of commercial products vary since the quality of extract depends on various factors including origin of the seeds, pretreatments etc. By comparing the antioxidant activity of grape seed obtained from the molasses industry in Turkey with those of other commercial products, the value of this agro industry waste for economic natural antioxidant production was evaluated.

*Adsorption of proanthocyanidin on silk fibroin* For the adsorption of proanthocyanidin on silk fibroin, the importance of parameters was investigated by calculating the decrease in total phenol content of supernatant as the percentage adsorbed amount with three replicates. In statistical variance analysis, pH, initial concentrations and solid-liquid ratio were found as significant parameters ($P < 0.005$).

Figure 8 (A) shows that maximum percentage adsorbed TPC was obtained at pH 5.5 for the initial concentration as the 0.025 g/mL. By increasing the initial concentration, it was slightly decreased. On the other hand, it was significantly affected by pH change and minimum percentage adsorbed amount was achieved for low pH and high initial concentration values. The pH and solid liquid ratio relations can be seen from Fig. 8 (B). Overall maximum adsorption of proanthocyanidin to silk fibroin was achieved at high pH and high solid-liquid ratio. Solid-liquid ratio was important parameter, because lowering solid-liquid ratio significantly reduced percentage of adsorbed phenol amount. The best conditions for the maximum adsorption were found as pH 5.5, initial concentration as 10 g/mL and solid liquid ratio as 0.1 g/mL, which yielded the maximum percentage of adsorbed phenol amount as 85.2%.

## Conclusions

This study shows that the pomace, which is waste of the molasses industry in Turkey can be used effectively to obtain high value added antioxidant product; proanthocyanidin. Thus, grape pomace extract has potential use as a natural antioxidant for dietary supplement and food additives purposes. The optimum extraction conditions of PA from grape seeds separated from pomace was achieved with the acetone/water (30: 70, v/v) solution for 24 h. The correlations between PA, TPC and antioxidant activity was also investigated and high correlation coefficients were observed. The highest antioxidant capacity was achieved as 93 (±1.43) mmol Trolox/g seeds by using acetone/water (30: 70, v/v) solution. Antioxidant capacity of PA extracted from the byproduct of molasses industry was found to be considerably higher than those of the widely used commercial natural and synthetic antioxidants. As a conclusion, it could be possible to produce natural antioxidants economically by extracting PAs from wastes of Turkish traditional product molasses. The use of silk fibroin as an adsorbent is preferable since it is a natural polymer. Adsorption mechanism can be explained by hydrophobic interactions since adsorption of non-polar proanthocyanidin was achieved with hydrophobic silk fibroin. However, further studies should be performed in order to investigate the adsorption-desorption mechanisms and isotherms.

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## References

Aehle, E., Grandic, S.R., Ralainirina, R., Baltora-Rosset, S., Mesnard, F., Prouillet, C.,Maziere, J. and Fliniaux, M. (2004). Development and evaluation of an enriched natural antioxidant preparation obtained from aqueous spinach (*Spinacia oleracea*) extracts by an adsorption procedure. *Food Chem.,* **86**, 579–585.

Altman, G.H., Diaz, F., Jakuba, C., Calabro, T., Horan, R.L., Chen, J., Lu, H., Richmond, J. and Kaplan, D.L. (2003). Silk-based biomaterials. *Biomaterials,* **24**, 401–416.

Bagchi, D., Bagchi, M., Stohs, S.J., Das, D.K., Ray, S.D., Kuszynski, A.C., Joshi, S.S. and Pruess, H.G. (2000). Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology,* **148**, 187–197.

Baycın, D., Altıok, E., Ulku, S. and Bayraktar, O. (2007). Adsorption of olive leaf (*olea europaea* L.) antioxidants on silk fibroin. *J. Agric. Food Chem.* (in press).

Bayraktar, O., Malay, O., Ozgarip, Y. And Batıgn, A. (2005). Silk fibroin as a novel coating material for controlled release of theophylline. *Eur. J. Pharm. Biopharm.,* **60**, 373–381.

Bouhamidi, R., Prevost, V. and Nouvelot, A. (1998). High protection by grape seed proanthocyanidins (GSPC) of polyunsaturated fatty acids against UV-C induced peroxidation. *Plant Biology and Pathalogy: Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie,* **321**, 31–38.

Buelga, C.S. and Scalbert, A. (2000). Proanthocyanidins and tannin-like compoundsnature, occurance, dietary intake and effects on nutrition and health. *J. Sci. Food Agric.,* **80**, 1094–1117.

Burlow, S.M. (1998). Toxicological aspects of antioxidants used as food additives. In "Food Antioxidants", ed. by B.J.H. Hudson, Elsevier, Amsterdam; pp. 253–268.

Fuleki, T. and Ricardo da Silva, J.M. (1997). Catechin and procyanidin composition of seeds from grape cultivars grown in Ontario. *J. Agric. Food Chem.,* **45**, 1156–1160.

Gabetta, B., Fuzzati, N., Griffini, A., Lolla, E., Pace, R., Ruffilli, T. and Peterlongo, F. (2000). Characterization of proanthocyanidins from grape seeds. *Fitoterapia,* **171**, 162–175.

Kongkachuichaya, P., Shitangkoonb, A. And Chinwongamorn, N. (2002). Thermodynamics of adsorption of laccaic acid on silk. *Dyes and Pigments,* **53**, 179–185.

Louli, V., Ragoussis, N. and Magoulas, K. (2004). Recovery of phenolic antioxidants from wine industry by-products. *Bioresour. Technol.,* **92**, 201–208.

Makkar, H.P.S., Bluemmel, M., Borowy, N.K., Becker, K. (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *J. Sci. Food Agric.,* **61**, 161–165.

Monogas, M., Cordoves, C.G., Bartolome, B., Laureano, O. and DaSilva, R. (2003). Monomeric, oligomeric and polymeric Flavan-3-ol composition of wines and grapes from vitis vinifera L. Cv. Graciano, Tempranillo and cabernet Sauvignon. *J. Agric. Food Chem.,* **51**, 6475–6481.

Moure, A., Cruz, J.M., Franco, D., Dominguez, J.M., Sinerio, J., Dominguez, H., Nunez, M.J. and Prajo, J.C. (2001). Natural antioxidants from residual sources. *Food Chem.,* **72**, 145–171.

Park, S.J., Lee, K.Y., Ha, W.S. and Park, S.Y. (1999). Structural changes and their effect on mechanical properties of silk fibroin/chitosan blends. *J. Appl. Polym. Sci.,* **74**, 2571–2575.

Pauling, L. (1945). The adsorption of water by proteins. *J. Amer. Chem. Soc.,* **67**, 555–557.

Pekic, B., Kovac, V., Alonso, E. and Revilla, E. (1998). Study of extraction of proanthocyanidins from grape seeds. *Food Chem.,* **61**, 201–206.

Ramila, G., Stamatina, K., Dimitris, P.M. and Panagiotis, K. (2005). An analytical survey of the polyphenols of seeds of varieties of grape (*Vitis vinifera*) cultivated in Greece: implications for exploitation as a source of value-added phytochemicals. *Phytochem. Anal.,* **16**, 17–23.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M.and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.,* **26**, 1231–1237.

Schofield, P., Mbugua, D.M. and Pell, A.N. (2001). Analysis of condensed tannins: a review. *Anim. Feed Sci. Tech.,* **91**, 21–40.

Shrikhande, A.J., 2000. Wine by-products with health benefits. *Food Res. Int.,* **33**, 469–474.

Tang, H.R., Covington, A.D. and Hancock, R.A. (2003). Structure-activity relationships in the hydrophobic interactions of polyphenols with cellulose and collagen. *Biopolymers,* **70**, 403–413.

Taubert, D., Breitenbach, T., Lazar, A., Censarek, P., Harlfinger, S., Berkels, R., Klaus, W. and Roesen, R. (2003). Reaction rate constants of superoxide scavenging by plant antioxidants. *Free Radical Biol. Med.,* **35**, 1599–1607.

Yamakoshi, J., Kataoka, S., Koga, T. and Ariga, T. (1999). Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis,* **142**, 139–149.

Yanagida, A., Shoji, T. and Shibusawa, Y. (2003). Separation of proanthocyanidins by degree of polymerization by means of size-exclusion chromatography and related techniques. *J. Biochem. Biophy. Meth.,* **56**, 311–322.

Yilmaz, Y. and Toledo, R.T. (2004). Health aspects of functional grape seed constituents. *Trends Food Sci. Technol.,* **15**, 422–433.

Yilmaz, Y. and Toledo, R.T. (2006). Oxygen radical absorbance capacities of grape/wine industry by products and effect of solvent type on extraction of grape seed polyphenols. *J. Food Comp. Anal.,* **19**, 41–48.

Yilmaz, Y. (2006). Novel uses of catechins in foods. *Trends Food Sci. Technol.,* **17**, 64–71.

Yoon, S.Y., Choi, W.J., Park, J.M. and Yang, J.W. (1997). Selective adsorption of flavonoid compounds from the leaf extract of *Ginkgo biloba* L. *Biotechnol. Tech.,* **11**, 553–556.