International Food Research Journal 22(3): 1169-1179 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of extracting conditions on phenolic compounds and antioxidant activity from different grape processing byproducts

[1]Samavardhana, K., [1]Supawititpattana, P., [2]Jittrepotch, N., [2]Rojsuntornkitti, K. and [2*]Kongbangkerd, T.

[1]Division of Food Science and Technology, Faculty of Food and Agriculture Technology, Rajabhat Pibulsongkram University, Phitsanulok 65000, Thailand
[2]Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok 65000, Thailand

Article history

Received: 24 July 2014
Received in revised form: 18 September 2014
Accepted: 22 September 2014

Keywords

Grape seed
Ultrasonic-assisted extraction
Antioxidant activity
Particle size

Abstract

Grape seeds are usually byproducts from wine and juice processes and they still contain abundant of bioactive compounds which are now being consumed for health promotion. In the present study, the effects of different extracting conditions such as grape seed byproduct sources (wine and juice processing), extracting methods (shaking and ultrasonic-assisted) and particle sizes (ground, <20, 20-40 and >40 mesh) were investigated. The results showed that the defatted particle of >40 mesh of the seeds from juice process using ultrasonic-assisted extraction contained higher amounts (P<0.05) of total phenolics and total flavonoids for 362.02 mg GAE/g DM and 272.61 mg CE/g DM, respectively and phenolic compounds namely catechin, procyanidin B2 and epicatechin were 12.60, 3.29 and 12.42 mg/g DM, respectively. The antioxidant activities as DPPH, FRAP and ABTS were also higher (P<0.05) for 2,583.77, 2,450.26 and 4,806.11 mmol TE/g DM, respectively. In addition, the total phenolic contents indicated highly positive correlation with antioxidant activity.

Introduction

In recent years, research has been growing interest in the determination of phenolic compounds and antioxidant activity from agro-industrial byproducts. Several studies demonstrated different phenolic profiles and antioxidant activity from food plant byproducts such as grape pomace and seeds from winery processed (Yilmaz and Toledo, 2006; Lafka *et al.*, 2007; Bozan *et al.*, 2008), spent coffee grounds (Mussatto *et al*., 2011), pomegranate marc (Qu *et al*., 2010), soybean (Tyug *et al*., 2010) and orange peel (Khan *et al*., 2010) and there has been a significant data showing that these byproducts could be used to produce innovative foods as they might promote human health.

Grape (*Vitis vinifera* L.) is one of the most commonly consumed fruit growing worldwide. The total amount about 80% is used in wine making (Maier *et al*., 2009) and the grape byproduct consists 20% of weight from winery process (Lafka *et al.,* 2007). In Thailand, grape is usually processed into various products such as wine, juice and raisins. Black queen is one of the grape varieties that is normally processed into wine and juice and the large quantity of byproducts from both processes such as pomace (grape pulp, peels and seeds) were obtained and there has been several studies showing that these kind of by products could be a good source of antioxidants such as polyphenols and flavonoids.

Many authors have reported that the total phenolic content of grape seed was higher than that of the peel and pomace hence grape seeds could then be a valuable source of phenolics and antioxidants (Xu *et al*., 2010). Ribéreau-Gayon *et al*. (2000) reported that grape seeds contained 60-70% phenolic compounds and catechin, epicatechin and procyanidin were found to be the major antioxidants (Maier *et al*., 2009; Chedea *et al*., 2010; Katalinic´*et al*., 2010). Besides, they also contained higher concentration of monomeric, oligomeric and polymeric flavan-3-ols than those of grape skins (Da Parto *et al*., 2014). Grape has been appreciated for their rich content of phenolic compounds and the beneficial effects on human health were investigated such as inhibition of oxidation of human low-density lipoproteins (LDL) (Frankel *et al*., 1995), anti-inflammatory effect (Sakurai *et al*., 2010) and therapy of cancer (Vaid *et al*., 2012).

Extraction process is an important step in the recovery, isolation and identification of phenolic compounds. The phenolic compounds and their purity are dependent on the extraction techniques such as solid-phase extraction, shaking extraction

*Corresponding author.
Email: *teerapornk@nu.ac.th*

(Hussain *et al*., 2012), soxhlet extraction (Baydar *et al*., 2007), microwave-assisted extraction (Li *et al*., 2011), ultrasound-assisted extraction (Ghafoor and Choi, 2009) and supercritical fluid extraction (Casas *et al*., 2010). Solvent extraction such as methanol, ethanol, acetone and ethyl acetate (Yilmaz and Toledo, 2006), particle sizes (Bonilla *et al*., 1999) and solvent concentration, extraction temperature and time (Spigno *et al.*, 2007). Although, there were many reports about phenolic compounds and antioxidant activity from grape skins and seeds but there was not much data relating grape seed extracts and their antioxidant capacity of the grapes that have been planted in Thailand. The objectives of this study were to investigate the effects of extracting methods and particle sizes on phenolic compounds and to evaluate antioxidant activity from grape seed (Black queen variety) as byproducts from winery and juice processing industries.

Material and Methods

Raw materials

The grape seeds of Black queen variety (*Vitis vinifera* L.)grown in Nakornratchasima province, Thailand, were byproducts obtained from wine and juice processes.Grape seeds from wine process (GSW) were obtained after 4 weeks of maceration for the red vinification from Wangpikul wine and the seeds from juice process (GSJ) were separated from grape juice from Village Farm and Winery Company. Both seeds were separated from skin by manually coarse screened and washed with tab water. Grape seeds were dried at room temperature for 1 hour and stored at -20°C prior to use.

Chemicals and reagents

All chemicals used in the study, such as ethanol, methanol, Folin–Ciocalteu reagent and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) were purchased from Merck (Darmstadt, Germany). Sodium carbonate, potassium phosphate, hydrochloric acid and acetic acid were purchased from Fisher Scientific (Leicestershire, UK). 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma–Aldrich (St. Louis, MO). All chemicals and solvents were analytical reagent grade.

Sample preparation

The grape seeds were dried at 50°C for 3.5 hours until moisture content to 6.0-7.0%. The seeds were milled for 14 s to powder using a grinder and stopped for 15 s to avoid heating, then the milling was repeated. The obtained seed powders were packed in aluminum foil bags and stored at -20°C.

To study the effects of particle size, the grape seed powder was separated into different particle sizes i.e. <20 mesh, 20-40 mesh, >40 mesh and ground (unsieved, served as a control) by a sieving shaker (AS 200, Retsch, Germany).The powders were extracted twice in a Soxhlet apparatus for 5 h with hexane 1:10 (w/v) at room temperature to remove oil. The defatted residues were dried overnight under fume hood to remove residual solvent. The samples were packed in aluminum foil bags and stored at -20°C.

Extraction of grape seed powders

The grape seed powders were extracted by using 2 different methods, 1) shaking method (SM) with an orbital shaker (NB-101 MH, N-Biotek, Korea) at 180 rpm for 5 h at room temperature and 2) ultrasonic-assisted extraction (UAE) with an ultrasonic bath (UL-20LDT, 40 kHz, 320W, Scilution, Thailand) with the bath size of 320 x 530 x 120 mm. at room temperature for 30 min.

5.0 g of grape seed powder was extracted with 60% ethanol solution (1:10, w/v) for each method and the extract was centrifuged for 10 min at 4,000 rpm, then the supernatant were collected. The precipitate was extracted again using the same procedure and supernatants were combined and concentrated by rotary evaporation under vacuum at 40°C and stored at -20°C until use.

An experimental study of the effects of particle size and defatting process on antioxidant activity, 5.0 g of grape seed powders from each particle size were extracted with 60% ethanol (1:10, w/v) by using UAE for 30 min at room temperature and the dried concentrates were obtained using the same procedure as described above.

Determination of total phenolic content

The total phenolic content of grape seed extract was determined using the method of Folin-Ciocalteu reagent as described by Gong *et al,* (2011). Briefly, 400 µl of the extract was mixed with 2.0 ml of 0.2mol/L Folin-Ciocalteu reagent, and 1.6 ml of sodium carbonate (7.5% w/v) and then heated at 50°C for 5 min. The mixture was allowed to keep in the dark at room temperature for 30 min. The absorbance was measured at 760 nm using UV-Vis spectrophotometer. Gallic acid was used as a standard and expressed as mg of gallic acid equivalents per gram of grape seed dry matter (mg GAE/g DM).

Determination of total flavonoid content

The total flavonoid content was determined by Yang *et al*, (2009) with some modifications. 500 µl of sample was mixed with 2.5 ml deionized water and 150 µl of 5% sodium nitrite and incubated in the dark at room temperature for 5 min. Then, 300 µl of 10% aluminum chloride solution was added and allowed to stand in the dark at room temperature for 6 min before addition of 1.0 ml 1.0 M sodium hydroxide and 1.55 ml deionized water. The absorbance was measured at 510 nm using UV-Vis spectrophotometer compared to catechin standard. Total flavonoid content of the sample was expressed as mg of catechin equivalents per gram of grape seed dry matter (mg CE/g DM).

Determination of phenolic compounds using HPLC analysis

The phenolic compounds were analyzed using HPLC (Perkin Elmer series 200, USA) equipped with a UV detector and an C18 column (250 mm × 4.6 mm) with particle size of 100A. The eluting system consisted of 2.0% (v/v) acetic acid as solvent A and acetonitrile as solvent B (A:B = 90:10) in isocratic condition. The solutions of the standards and the extract phenolics were filtered through a 0.45 µm syringe filter. The operating conditions were: column temperature, 25°C; injection volume, 10 µL; detection wavelength, 280 nm and 1.2 mL/min of flow rate. The identification and peak assignment of the phenolics were based on comparison of retention times and spectral data with those of the standards. The identified phenolics were quantified according to respective standard calibration curves. The method was a slightly modified from the protocol suggested by Hatzidimitriou *et al*., (2007).

DPPH radical scavenging assay

The DPPH method was determined as described by Yang *et al*. (2009) with some modifications. Briefly, 0.1 ml of samples were added to 3.9 ml of 0.2 mM DPPH methanolic solution. The reaction mixture was agitated and allowed to stand at room temperature in the dark for 30 min. The absorbance at 515 nm was used to measure the concentration of the remaining DPPH using a spectrophotometer. The calibration curve was performed with Trolox solution (a water soluble vitamin E analog). Total antioxidant activity was expressed as micromoles of Trolox equivalents per gram of grape seed dry matter (µmol TE/g DM).

Ferric reducing antioxidant power (FRAP)

The FRAP assay was determined using a modified method of Maier *et al.* (2009). The FRAP reagent was performed by 300 mM acetate and glacial acetic acid buffer (pH 3.6), 10 mM of TPTZ(4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM ferric chloride. The working FRAP reagent was freshly prepared by mixing three solutions together in the ratio of 10:1:1 and the reagent was incubated at 37°C in water bath. 3 ml of FRAP reagent was mixed with 400 µl of the sample and incubated at 37°C for 4 min. The absorbance was read at 593 nm and expressed as micromoles of Trolox equivalents per gram of grape seed dry matter (µmol TE/g DM).

ABTS radical scavenging assay

The ABTS assay was determined according to Re *et al.*, (1999) with some modifications. The ABTS radical cation was generated from 7.4 mM ABTS solution with 2.45 mM potassium persulfate. The mixture was allowed to stand in the dark at room temperature for 16 h before use. The ABTS•+ solution was diluted with ethanol to obtain an absorbance of 0.70±0.02 at 734 nm. Sample solutions were mixed with ABTS•+ solution and allowed to stand in a dark for 6 min at room temperature. The absorbance was then measured at 734 nm and the results were expressed as micromoles of Trolox equivalents per gram of grape seed dry matter (µmol TE/g DM).

Statistical analysis

The measurements were carried out in three replicates and the results were reported as mean±standard deviation (S.D.). Analysis of variance ($P<0.05$) and significant differences among means were tested by Tukey. SPSS version 17.0 was used for statistical analysis as well as Pearson's correlation coefficients.

Results and Discussion

Effects of extracting methods on yields, total phenolics, total flavonoids and phenolic compounds of grape seed from different sources

To obtain acceptable yields and antioxidant activity with minimal changes of functional properties of the extract required, the extraction technique is one of the most important stage (Zhu *et al.*, 2011). In this experiment, the effects of extracting methods on the yields, total phenolics and total flavonoids are summarized in Table 1.

The grape seed extract yields ranged from 4.96 to 22.24% and were significantly ($P<0.05$) affected by extracting methods. The yields of grape seed extract from juice process (GSJ) were higher than those of grape seed extract from wine process (GSW) while ultrasonic-assisted extraction (UAE) provided higher

Table 1. Yield, total phenolics, total flavonoids and antioxidant activities of grape seed extracts from different extracting methods

Extracting method	Yield (%)		Total phenolics (mg GAE/g DM)		Total flavonoids (mg CE/g DM)	
	GSW	GSJ	GSW	GSJ	GSW	GSJ
SM	4.96±0.10^{c}	21.35±0.52^{a}	18.07±0.27^{d}	151.33±0.33^{b}	12.66±0.06^{d}	107.05±0.54^{b}
UAE	7.85±0.31^{b}	22.24±0.36^{a}	28.24±0.93^{c}	159.95±0.36^{a}	18.01±0.38^{c}	111.81±0.14^{a}
Extracting method	DPPH (µmol TE/g DM)		FRAP (µmol TE/g DM)		ABTS (µmol TE/g DM)	
	GSW	GSJ	GSW	GSJ	GSW	GSJ
SM	121.71±3.10^{d}	1,221.46±2.06^{b}	119.11±0.58^{d}	1,063.35±2.39^{b}	191.22±1.71^{d}	1,940.11±3.33^{b}
UAE	160.63±0.39^{c}	1,273.57±1.45^{a}	152.64±1.85^{c}	1,153.88±2.28^{a}	313.49±3.12^{c}	2,064.23±2.49^{a}

*Different alphabetic uppercase letters indicate significant differences (P<0.05) for each measured parameter. GSW = grape seed extract from wine process; GSJ = grape seed extract from juice process; SM = shaking method and UAE = ultrasonic- assisted extraction

yields than those of shaking method (SM). Although, higher yield was observed in GSJ but there was no significant difference (P>0.05) between different extracting methods.

Polyphenols are phytochemicals from plants and are being used for prevention of various diseases mainly caused by free radicals. The higher polyphenol content would then exhibit stronger inhibition and also higher antioxidant activity (Jayaprakasha *et al.*, 2003). The total phenolics of the extracts from different sources and extracting methods varied a wide range from 18.07 to 159.95 mg GAE/g DM. GSJ with UAE revealed the highest total phenolics and the significant difference (P<0.05) suggests that GSJ with UAE (159.95 mg GAE/g DM) contained higher total phenolics than GSJ with SM (151.33 mg GAE/g DM), GSW with UAE (28.24 mg GAE/g DM) and GSW with SM (18.07 mg GAE/g DM), respectively. It could be seen that the total phenolics of GSJ was about 8 folds higher than that of GSW with SM and 5 folds than GSW with UAE. The total phenolics of Black queen seed variety from wine in this present study are lower than those obtained by other varieties as reported by Yilmaz and Toledo (2006) that total phenol contents of Chardonnay, Merlot and Muscadine seeds from wine were 52.67, 38.45 and 32.13 mg GAE/g DM, respectively while the total phenolics of Black queen seed from juice are higher than those of grape seeds from five wild grapes and two hybrids from Japan which contained 3.6 - 54.9 mg GAE/g (Poudel *et al.*, 2008) and 99.28 and 15.79 mg GAE/g DM from Cabernet Sauvignon and Purple grape, respectively. This is to be expected since the phenolic content of grape seeds is dependent on genotypes, cultural practices and extraction procedures (Xu *et al.*, 2010). Our findings are in agreement with the previous study that the significant difference of total phenolic was obtained by extracting methods comparing between ultrasonic and shaking extractions of peanut hulls (Hussain *et al.*, 2012), cherry laurel (Karabegović *et al.*, 2014) and hemp, flax and canola seeds (Teh and Birch, 2014).

Flavonoids are the most common and widely distributed group of plant phenolic compounds (Guo *et al.*, 2012) and are generally categorized as phenolics depending on their chemical structure (Sung and Lee, 2010). The result of total flavonoids was obtained as the same trend of total phenolics. GSJ with UAE contained the highest total flavonoids of 111.81 mg CE/g DM (P<0.05), followed by GSJ with SM (107.05mg CE/g DM), GSW with UAE (18.01 mg CE/g DM) and GSW with SM (12.66 mg CE/g DM), respectively, suggesting that total flavonoids from different conditions were significantly different depending again on extracting methods and sources of grape seeds.

The grape processes which provided grape seed byproducts has affected total phenolic and total flavonoid contents. GSJ showed higher yields and more total phenolic and flavonoid contents than those of GSW. This could be explained that in the winemaking process, grape pomace was immersed in the must during fermentation while ethanol was continuously increased. Although, fermentation period inevitably led to partial exhaustion of polyphenols which gradually leached from the seeds into the ethanol-enriched must, hence the lower total phenolic levels were obtained (Karvela *et al.*, 2009). Therefore, in this step, not only yeasts that convert most of sugars of the grape juice into ethanol but also phenolic compounds are extracted (Puértolas *et al.*, 2010). Gonzales-Manzano *et al.* (2004) observed that the longer time used for macerating wine, the more phenolics and flavonoids were obtained. Tolado *et al.* (2013) compared different amounts of grape seeds in maceration of grape juice and found that macerated juice with grape seeds contained significantly higher phenolic contents and antioxidant activities than that without addition of the seeds. Moreover, extracting

Table 2. Phenolic profiles of different grape seed byproducts and extracting methods

Extracting method	Phenolic compounds* (mg/g DM)					
	Catechin		Procyanidin B2		Epicatechin	
	GSW	GSJ	GSW	GSJ	GSW	GSJ
SM	0.22±0.02^{b}	5.65±0.23^{a}	0.06±0.00^{b}	1.15±0.09^{a}	0.22±0.02^{b}	5.57±0.21^{a}
UAE	0.28±0.02^{b}	5.91±0.19^{a}	0.08±0.00^{b}	1.29±0.61^{a}	0.23±0.00^{b}	5.67±0.16^{a}

*Different alphabetic uppercase letters indicate significant differences (P<0.05) for each measured parameter. GSW = grape seed extract from wine process ; GSJ = grape seed extract from juice process; SM = shaking method and UAE = ultrasonic-assisted extraction

techniques were indicated to improve the recovery extracts. SM provided extracts with less total phenolic and flavonoid contents compared with UAE for both GSW and GSJ. The higher extraction efficiency of UAE is mainly attributed to the effect of acoustic cavitations produced in the solvent by passage of ultrasonic wave. Also the mechanical destruction of cell walls offers greater penetration of solvent into the sample matrix and the solute more rapidly diffuses from solid phase into the solvent (Hossain *et al.*, 2012). However, each extracting method is prepared to estimate the desired content that is dependent upon the reaction time and complexity of kinetic reaction. Most authors found the benefits of UAE such as shortening the extraction time, increasing of yields, bioactive compounds and also antioxidant activity better than conventional solvent extraction (Khan *et al*., 2010; Teh and Birch, 2014).

Identification and quantification of individual phenolic compound of GSJ with UAE by HPLC are shown in Table 2. The identified phenolic compounds were flavan-3-ols as monomers (catechin and epicatechin) and procyanidin B2 as a dimer and these compounds are the most abundant phenolics found in grape seeds (Chedea *et al.*, 2010). Different extracting conditions were expected to generate different amount of phenolic compound concentrations.

Table 2 summarizes the chemical profile of the extracts from both extracting conditions showing that the major compounds were catechin and epicatechin and procyanidin B2 was a minor constituent. GSJ with UAE was found to generate the highest catechin, procyanidin B2 and epicatechin contents of 5.91, 1.29 and 5.67 mg/g DM, respectively, compared to those of GSJ with SM, GSW with UAE and GSW with SM. GSJ contained about 21-26 times higher phenolic compounds than that of GSW (P<0.05), while UAE provided slightly higher phenolic compounds than those of SM but there were not significantly different. Ginjom *et al.* (2011) found that during wine making process, phenolic compounds of wine determined by HPLC-DAD-MS, were increased during the fermentation and maceration was the procedure for leaching phenolic compounds from grape and residues (Peralbo-Molina *et al*., 2012). The results in this study was similar to that reported by Cheng *et al.* (2012) indicated that Pinot noir seed extract contained higher catechin than epicatechin while Pinot meunier and Shirazseed extracts contained more epicatechin than catechin (Montealegre *et al*., 2006). Catechin and epicatechin are major flavanols found in grape seeds and catechin usually displays similar level in some grape varieties (Chedea *et al.*, 2010), while procyanidin B2 was found to be the lowest phenolic compound concentration. The results from several authors suggested that different concentrations of the major phenolic compounds in grape seed extracts were dependent on extracting conditions especially extracting solvent. Cheng *et al.* (2012) reported that the best extracting solvent for extraction of Pinot noir grape seed was acetone water mixture compared to ethanol and methanol. This could be attributed to the structure of polymeric flavan-3-ols which containing several hydroxyl functions and exhibited more ability to donate hydrogen atoms and support the unpaired electron compared to the lower molecular weight phenols (Da Parto *et al.*, 2014).

Effect of extracting methods on antioxidant activities

Antioxidant activity of foods can be determined using different mechanisms of actions. In general, it is based on two major mechanisms, hydrogen atom transfer and single electron transfer, hence the effectiveness of antioxidant should not be provided by evaluating only one assay protocol. Thus, it is necessary to evaluate the potential of antioxidant activity by assaying different antioxidant mechanisms (Çelik *et al*., 2010; Xu *et al*., 2010). In this study, the antioxidant activities of the grape seed extracts was assessed by 3 different protocols namely DPPH, FRAP and ABTS methods which they have been the most common methods for determining in vitro antioxidant activity of food (Prior *et al.*, 2005; Pérez-Jiménez *et al*., 2008) and the results are shown in Table 1.

It was found that the DPPH values of the grape seed extracts ranged between 121.71 and 1,273.57 µmol TE/g DM and GSJ with UAE had the highest

Table 3. Yield, total phenolics, total flavonoids and antioxidant activity of grape seed extract determined by DPPH, FRAP and ABTS assays from different particle sizes and defatted process

Particle size	Yield (%)		Total phenolics (mg GAE/g DM)		Total flavonoids (mg CE/g DM)	
	Control	Defatted	Control	Defatted	Control	Defatted
Ground	21.69±0.74^{c}	22.70±0.14^{c}	158.14±0.18^{d}	173.31±0.28^{c}	112.75±1.05^{d}	128.31±0.54^{c}
<20 mesh	8.95±0.41^{e}	9.25±0.35^{e}	46.02±0.57^{h}	60.89±0.46^{t}	44.87±0.05^{h}	47.61±0.20^{g}
20-40 mesh	10.78±0.39^{d}	10.45±0.34^{de}	61.85±0.84^{f}	72.20±0.84^{e}	53.83±0.72^{f}	61.34±0.20^{e}
>40 mesh	41.27±0.62^{b}	46.37±0.69^{a}	306.02±0.90^{b}	362.02±0.79^{a}	228.78±0.47^{b}	272.61±0.66^{a}
Particle size	DPPH (µmol TE/g DM)		FRAP (µmol TE/g DM)		ABTS (µmol TE/g DM)	
	Control	Defatted	Control	Defatted	Control	Defatted
Ground	1,273.27±3.80^{d}	1,446.36±2.51^{c}	1,150.33±2.56^{d}	1,281.84±1.93^{c}	2,064.51±2.22^{d}	2,413.05±2.38^{c}
<20 mesh	439.10±3.00^{h}	501.41±3.26^{g}	406.66±4.30^{h}	451.01±2.13^{g}	827.32±6.26^{h}	939.15±1.85^{g}
20-40 mesh	521.26±1.98^{f}	619.78±5.46^{e}	531.90±3.68^{f}	569.35±1.85^{e}	1,012.30±4.25^{f}	1,204.35±4.42^{e}
>40 mesh	2,343.04±4.17^{b}	2,583.77±2.18^{a}	1,963.14±4.54^{b}	2,450.26±2.93^{a}	4,343.43±3.65^{b}	4,806.11±4.66^{a}

*Different alphabetic uppercase letters indicate significant differences (P<0.05) for each measured parameter

value of 1,273.57 µmol TE/g DM (P<0.05). The FRAP and ABTS values from different grape seed byproducts and extracting methods also showed significant differences with the same trends as those observed from the DPPH assay. GSJ with SM and UAE had about 8 times higher FRAP values than those of GSW, while GSJ with UAE provided the highest value of 1,153.88 µmol TE/g DM and GSW with SM had the lowest value of 119.11 µmol TE/g DM (P<0.05). It was found that FRAP values were increased by using UAE for both GSW and GSJ (P<0.05). The effects of extracting method was agreement with the finding of Hossain *et al.* (2012) that the FRAP value of marjoram extracted by UAE was higher than that of conventional solid/liquid extraction.

GSJ with UAE also showed the most efficient scavenger of radicals as the ABTS value was the highest of 2,064.23 µmol TE/g DM, while GSW with SM had about 11 times lower value, suggesting that extracting methods and different sources of grape seeds had significantly affected their antioxidant activity. The increasing values of ABTS were as the same trend as those of DPPH and FRAP values. Regarding the antioxidant activity assays, FRAP measures the ability of samples that can reduce metals, while DPPH and ABTS measure free radical scavenging capacity of sample. From a mechanical standpoint, there is a single electron transfer reaction in FRAP and ABTS assays, while DPPH combines both single electron transfer reaction and hydrogen atom transfer (Pérez-Jiménez *et al*., 2008). Considering the results from the determination of total phenolics, total flavonoids and flavan-3-ol together with antioxidant activities of the extracts, the antioxidant activities depended on the amount of these compounds. In the flavan-3-ol fraction, it was shown that catechin exhibited the highest phenolic content, followed by epicatechin and procyanidin B2. Since catechin molecule contains three hydroxyl groups in the B ring, the antioxidant activity responds broadly to the tenet that the structures with the most hydroxyl groups exert the greatest antioxidant activity (Rice-Evans *et al*., 1996). The obtained results were in accordance with Bonilla *et al*. (1999) and Hatzidimitriou *et al.* (2007) that catechin was the most effective antioxidant, followed by epicatechin since they were the major phenolic compounds in the seed extracts (Cheng *et al*., 2012). GSJ with UAE indicated the strongest radical scavenging of both the DPPH and ABTS assays as well as the highest ferric reducing ability as measured by FRAP. In general, most phenolics and flavonoids usually exhibit some degree of antioxidant activity. According to the differences of total phenolics, total flavonoids and phenolic compounds found in each grape seed byproducts, the antioxidant activities were then different. In this study, GSJ with UAE gave the highest total phenolics and total flavonoids, hence the highest antioxidant activities were obtained so that it was selected to be used in the next experiments.

Effects of particle size and defatting process on yields, total phenolics, total flavonoids and phenolic compounds of grape seed extracts

The yields of defatted GSJ with UAE and the control (non-defatted) of grape seed extracts from different particle sizes are given in Table 3. The yields of the control and the defatted GSJ with different particle sizes obtained values between 8.95 to 41.27% and 9.25 to 46.37%, respectively. It could be seen that the yields of grape seed extracts

Table 4. Phenolic profile of grape seed extract from GSJ with different particle size and defatting process

Particle size	Phenolic compound*(mg/g DM)					
	Catechin		Procyanidin B2		Epicatechin	
	Control	Defatted	Control	Defatted	Control	Defatted
Ground	5.98±0.46^{d}	7.16±0.06^{c}	1.36±0.04^{c}	1.61±0.12^{c}	5.56±0.61^{d}	6.58±0.26^{c}
<20 mesh	3.28±0.09^{e}	3.30±0.12^{e}	0.59±0.10^{d}	0.60±0.13^{d}	2.58±0.10^{e}	2.77±0.12^{e}
20-40 mesh	3.39±0.15^{e}	4.06±0.10^{e}	0.61±0.02^{d}	0.62±0.02^{d}	2.75±0.12^{e}	3.06±0.11^{e}
>40 mesh	10.97±0.34^{b}	12.60±0.86^{a}	2.84±0.15^{b}	3.29±0.24^{a}	10.89±0.06^{b}	12.42±0.51^{a}

*Different alphabetic uppercase letters indicate significant differences (P<0.05) for each measured parameter.

were increased with decreasing particle size for both control and defatted GSJ (P<0.05) but the statistical analysis results indicated there were not significantly different (P>0.05) between the control and defatted GSJ. The results revealed that defatting process did not affect the extraction yields, while the yield of defatted grape seed powder with particle size >40 mesh was the highest (P<0.05) and it was also higher than that of the control (P<0.05). The yields of grounds were higher than those of <20 and 20-40 mesh (P<0.05) since the grounds might contain higher proportion of >40 mesh, while the yields of <20 and 20-40 mesh were more or less the same.

The total phenolics were significantly increased with decreasing particle size. The highest total phenolic content was obtained from the smallest defatted particle (>40 mesh) of 362.02 mg GAE/g DM and the lowest value was from the control of <20 mesh with the amount of 46.02 mg GAE/g DM. This indicated that not only defatting process but also particle size affected the total phenolics. Similar results were observed by Guo *et al.* (2012) reported that among different milling fractions, the finer wheat bran had higher phenolics than those of the coarse ones and Landbo and Meyer (2001) also found that total phenolics of blackcurrant juice press residues were increased with decreasing particle size on the extraction process. Defatting process provided higher total phenolics since phenolics were concentrated, hence the contents became more than that of control. The result of this study was similar to Bravo *et al*. (2013) that defatted coffee beans showed higher total phenolics than that of the control.

The effects of the different particle sizes and defatting process on the total flavonoids are shown in Table 3. The particle size had significant influenced on the total flavonoids which the controls and the defatted particles, from coarse to fine particles, contained in the range 44.87 to 228.78 mg CE/g DM and 47.61 to 272.61 mg CE/g DM, respectively. The smaller the particles, the higher total flavonoids were obtained, while the defatting process resulted higher total flavonoids than those of the controls. The resulting trend was similar to those determined for the total phenolics that the values were significantly different among different particle sizes (P<0.05) and the defatted particles of >40 mesh provided the highest total flavonoids of 272.61 mg CE/g DM (P<0.05).

The obtained results clearly demonstrated that the defatted GSJ with particle size of >40 mesh increased the yields, total phenolics and total flavonoids of the extract. This indicated that diffusion of hydroalcoholic solvent into particles and solvent-solute diffusion out of particles may limit the extracting process (Durling *et al*., 2007). It is obvious that reducing the size of vegetal material particles will increase the number of cell directly exposed to extraction by solvent and thus exposed to ultrasonically induced cavitation (Vinatoru, 2001). Therefore, at the same time, the smaller particle size means a shorter mass transfer distance and more surface area available for molecular transport which contributed to a more extensive mass transfer of solute between phase (Bucić-Kojić, *et al*., 2007; Qu *et al*., 2010). The total phenolics and total flavonoids of defatted GSJ were significantly higher than those of the controls (P<0.05), eventhough, the solubility of phenolic compounds in the oil is poor (Maier *et al*., 2009), the controls had then lower amounts of phenolic compounds than those of the defatted ones.

The concentrations of individual phenolic compound analyzed by HPLC are shown in Table 4. The individual phenolic compound of grape seed extracts from defatted GSJ with different particle sizes and the control (non-defatted) were compared namely catechin, procyanidin B2 and epicatechin. It is no surprise that catechin was the major phenolic compound in all extracts determined. Large difference was found among different extractions in relation to the flavan-3-ol content. The main compound was catechin, while epicatechin was slightly lower and procyanidin B2 was a minor constituent in all extraction conditions. The amounts of flavan-3-ol present in smaller particle was higher than those of larger particles and defatted particles provided

Table 5. Pearson's correlation coefficients of total phenolics (TP), total flavonoids (TF), phenolic compounds and antioxidant activities of GSJ with different particle sizes and defatting process

	TP	TF	DPPH	FRAP	ABTS	CT	Pro B2	ECT
TP	1							
TF	0.999**	1						
DPPH	0.997**	0.994**	1					
FRAP	0.998**	0.996**	0.995**	1				
ABTS	0.998**	0.998**	0.996**	0.992**	1			
CT	0.994**	0.995**	0.992**	0.991**	0.994**	1		
Pro B2	0.993**	0.993**	0.991**	0.989**	0.993**	0.993**	1	
ECT	0.995**	0.996**	0.993**	0.992**	0.995**	0.997**	0.993**	1

** Correlation is significant at the 0.01 level (2-tailed)
(catechin;CT, procyanidin B2;Pro B2 and epicatechin; ECT)

higher concentrations than those of the control. The data shows significant differences of the means for each compound. The higher contents of flavan-3-ols of defatted grape seed was higher than those of the control. The particle sizes had influenced the flavan-3-ol contents, where the fine particle provided more flavan-3-ol content than that of the coarse one. Our results are in good agreement with Bonilla *et al.* (1999) that grape pomace extract obtained from crushed pomace had higher phenolic compound than that of the uncrushed one. Since Flavan-3-ol is a water soluble phenolic compound, it was found with higher content in defatted grape seed powder than that of the control. The results may be explained that the removal fat process not only facilitate extracting water soluble antioxidants but also preventing fat rancidity and radical formation during long storage of samples (Bravo *et al.*, 2013). The flavan-3-ol contents corresponded with the maximum value measured as total phenolics and total flavonoids. Therefore, the difference observed in the phenolic profiles was not only attributed to the grape particle size, but also the defatting process which influenced phenolic compound contents.

Effects of particle sizes and defatting process on antioxidant activity of grape seed extracts

The antioxidant activities namely DPPH, ABTS and FRAP values of GSJ with UAE which the influences of particle sizes and defatting process were studied and the results are presented in Table 3. It was found that the antioxidant activity values from the three assays increased with decreasing particle sizes and the defatting process provided better activities. Similar phenomenon was also observed in the total phenolics and total flavonoids. The defatted grape seed particle size of >40 mesh had the highest antioxidant activity values of 2,583.77, 2,450.26 and 4,806.11 µmol TE/g DM for DPPH, FRAP and ABTS assays, respectively, followed by their controls (particle size of >40 mesh), while the lowest activities were found in the control with particle size of <20 mesh. Chan *et al.* (2012) reported antioxidant properties of ground and shredded leaves of *Morusalba* extract and supported the general consensus that particle size of the sample was an important parameter that influenced extraction and smaller particle size increased the extraction surface and enhanced extraction efficiency. The finer particle size of the food would release better the bound antioxidants, hence reducing the distance of the compounds to reach the surface (Pérez-Jiménez *et al.*, 2008). Our results were in accordance with some authors i.e. Bonilla *et al.* (1999) and Moure *et al***.** (2001) reported that reduced grape marc particle size showed the increase of antioxidant activity. Consequently, the diffusion of solute to the solvent phase is better with the finer particles, hence the extracts showed greater antioxidant activities.

Interestingly, the antioxidant activity values from the defatted grape seed were significantly higher than those of their controls and the possible explanation could be due to the higher amount of total phenolics and total flavonoids obtained after defatting process (Xu *et al*., 2010) while Pérez-Jiménez *et al.* (2008) found that total antioxidant capacity of the non-defatted sample was much lower than that of defatted fraction and Yue *et al.* (2008) also found that the defatted soybean flour extract showed higher scavenging capacity than that of the soybean oil and gum since more hydrophilic compounds might be highly concentrated in defatted soy flour extract. Another possible reason to explain the higher antioxidant activity of the defatted grape seed, is that the DPPH, FRAP and ABTS assays are usually used to measure the antioxidant activity of hydrophilic compounds so that the observed activities of the control were lower.

Correlation between bioactive compounds and antioxidant activity of GSJ with different particle sizes and defatting process

The correlation analysis between total phenolics, total flavonoids and antioxidant activities obtained from the grape seed extract are given in Table 5. The total phenolics and total flavonoids of the defatted

grape seed extracts with different particle sizes also exhibited a significant correlation (P<0.01) with antioxidant activities. Strong correlation between total phenolics and antioxidant activity values were found in various conditions. The results presented in this study demonstrated that total phenolics played an important role in the antioxidant activity and total flavonoids and antioxidant activity assays (DPPH, FRAP and ABTS) were highly correlated with total phenolics. The correlation data between total flavonoids, antioxidant activities (DPPH, FRAP and ABTS) and total phenolics were 0.999, 0.997, 0.998 and 0.998, respectively. The correlation study confirms that the total phenolics are likely to contribute to the radical scavenging activity of the grape seed extract. The strong correlation between total phenolics and antioxidant activity are in agreement with other reports (Xu *et al.*, 2010; Cheng *et al*., 2012).

Conclusion

Several factors including different sources of grape seed byproducts, extracting methods, particle sizes and defatting process had influenced the extraction efficiency and the source of grape seed byproduct had significant affected total phenolics, total flavonoids, phenolic compound contents and antioxidant activities. The defatted grape seed juice with particle size of >40 mesh with ultrasonic-assisted extraction was the most suitable extracting method which provided higher total phenolics, total flavonoids, phenolic compound contents and antioxidant activities. Catechin, procyanidin B2 and epicatechin were the major phenolic compounds in the grape seed extracts and a significantly positive correlation between these bioactive compounds and antioxidant activities were observed.

Acknowledgement

This work was financially supported by the Doctoral Research Scholarship, Faculty of Agriculture, Natural Resources and Environment, Naresuan University. The authors would like to thank all staffs of the faculty for maintenance and operation of the scientific laboratory and equipment.

References

Baydar, N.G., Özkan, G. and Yasar, S. 2007. Evaluation of the antiradical and antioxidant potential of grape extracts. Food Control 18: 1131-1136.

Bozan, B., Tosun, G. and ÖZcan, D. 2008. Study of polyphenol content in the seeds of red grape (*Vitis vinifera* L.) varieties cultivated in Turkey and their antiradical activity. Food Chemistry 109: 426-430.

Bonilla, F., Mayen, M., Merida, J. and Medina, M. 1999.Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. Food Chemistry 66: 209-215.

Bravo, J., Monente, C., Juániz, I., Paz De Peña, M. and Cid, C. 2013. Influence of extraction process on antioxidant capacity of spent coffee. Food Research International 50: 610-616.

Bucić-Kojić, A., Planinić, M., Tomas, S., Bilić, M and Velić, D. 2007. Study of solid-liquid extraction kinetics of total polyphenols from grape seeds. Journal of Food Engineering 81: 236-242.

Casas, L., Mantell, C., Rodriguez, M., Martinez, D.L.O., Roldan, E.J., De ory, I., et al. 2010.Extraction of resveratrol from the pomace of *Palomino fino* grapes by supercritical carbon dioxide. Journal of Food Engineering 96: 304-308.

Çelik, S.E., Özyürek, M., Güçlü, K. and Apak, R. 2010. Solvent effects on the antioxidant capacity of lipophilic and hydrophilic antioxidants measured by CUPRAC, ABTS/persulphate and FRAP methods. Talanta 81: 1300-1309.

Chan, E.W.C., Lye, P.Y., Tan, L.N., Eng, S.Y., Tan, Y.P. and Wong, Z.C. 2012. Effects of drying method and particle size on the antioxidant properties of leaves and teas of *Morusalba*, *Lagerstroemia speciosa* and *Thunbergia laurifolia*. Chemical Industry and Chemical Engineering Quarterly 18: 45-472.

Chedea, V. S., Braicu, C. and Socaciu, C. 2010. Antioxidant/prooxidant activity of a polyphenolic grape seed extract. Food Chemistry 121: 132–139.

Cheng, V.J., Bekhit, A.E.A., McConnell, M., Mros, S. and Zhou, J. 2012. Effect of extraction solvent, waste fraction and grape variety on the antimicrobial and antioxidant activities of extracts from wine residue from cool climate. Food Chemistry 143: 474-482.

Da Porto, C., Decorti, D. and Natolino, A. 2014. Water and ethanol as co-solvent in supercritical fluid extraction of proanthocyanidins from grape marc: A comparison and proposal. Journal of Supercritical Fluids 87: 1-8.

Durling, N.E., Catchpole, O.J., Grey, J.B., Webby, R.F., Mitchell, K.A., Foo, L.Y. and Perry, N.B. 2007. Extraction of phenolics and essential oil from dried sage *(Salvia officinalis)* using ethanol-water mixtures. Food Chemistry 101: 1417-1424.

Frankel, E. N., Waterhouse, A. L. andTeissedre, P. L. 1995. Principel phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. Journal of Agricultural and Food Chemistry 43: 890-894.

Ghafoor, K. and Choi, Y.H. 2009. Optimization of ultrasound assisted extraction of phenolic compounds and antioxidants from grape peel through response surface methodology. Journal of the Korean Society for Applied Biological Chemistry 52 (3): 295-300.

Ginjom, I., D'Arcy, B., Caffin, N. and Gidley, M. 2011. Phenolic compound profiles in selected Queensland

red wines at all stages of the wine-making process. Food Chemistry 125: 823-834.

Gong, Y., Liu, X., He, W-H., Xu, H-G., Yuan, F and Gao, Y.X. 2012. Investigation into the antioxidant activity and chemical composition of alcoholic extracts from defatted marigold (*Tagetes ereata* L.) residue. Fitoterapia 83 (3): 481-189.

Gonzalez-Manzano, S., Rivas-Gomzalo, J.C. and Santos-Buelag, C. 2004. Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. Analytical Chimica Acta 513: 283-289.

Guo, X.D., Wu, C.S., Ma, Y.J., Parry, J., Xu, Y.Y., Liu, H. and Wang M. 2012. Comparison of milling fraction of tartary buckwheat for their phenolic and antioxidant properties. Food Research International 49: 53-59.

Hatzidimitriou, E., Nenadis, N. and Tsimidou, M.Z. 2007. Changes in the catechin and epicatechin content of grape seeds on storage under different water activity (aw) conditions. Food Chemistry 105: 1504-1511.

Hossain, M.B., Brunton, N., Patras, A., Tiwari, B., O'Donnell, C.P., Martin-Diana, A.B. and Barry-Ryan, C. 2012. Optimization of ultrasound assisted extraction of antioxidant compounds from marjoram (*Origanum majorana* L.) using response surface methodology. Ultrasonics Sonochemistry 19: 582-590.

Hussain, A.I., Chatha, S.A.S., Noor, S., Arshad, M.U., Khan, Z.A., Rathore, H.A. and Sattar, M.Z.A. 2012. Effect of extraction techniques and solvent systems on the extraction of antioxidant components from peanut (*Arachishypogaea L.)* hulls. Food Analytical Methods 5: 890-896.

Jayaprakasha, G.J., Selvi, T. and Sakariah, K.K. 2003. Antibacterial and antioxidant activities of grape *(Vitis vinifera)* seed extracts. Food Research International 36: 117-122.

Karabegivić, I.T., Stojičević, S.S., Veličković, D.T., Todorović, Z.B., Nikolić, N.C. and Lazić, M.L. 2014. The effect of different extraction techniques on the composition and antioxidant activity of cherry laurel *(Prunus laurocerasus)* leaf and fruit extracts. Industrial Crops Production 54: 142-148.

Karvela, E., Markis, D.P., Kalogeropoulos, N., Karathanos, V.T. and Kefalas, P. 2009. Factorial design optimization of grape *(Vitis vinifera)* seed polyphenol extraction. European Food Research Technology 229: 731-742.

Katalinic, V., Mozina, S.S., Skroza, D., Generalic, I., Abramovic, H., Milos, M., Ljubenkov, I., Piskernik, S., Pezo, I., Terpinc, P. and Boban, M. 2010. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in dalmatia (Croatia). Food Chemistry 119: 715-723.

Khan, M.K., Abert-Vian, M., Fabiano-Tixier, A.S., Dangles, O. and Chemat, F. 2010. Ultrasonic-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel. Food Chemistry 119: 851-858.

Lafka, T.I., Sinanoglou, V. and Lazos, E.S. 2007. On the extraction and antioxidant activity of phenolic compounds from winery waste. Food Chemistry 104: 1206-1214.

Landbo, A.K. and Meyer, A.S. 2001. Enzyme-assisted extraction of antioxidative phenols from blackcurrant juice press residues *(Ribes nigrum)*. Journal of Agricultural and Food Chemistry 49 : 3169–3177.

Li, Y., Skouroumounis, G.K., Elsey, G.M. and Taylor, D. 2011. Microwave-assistance provides very rapid and efficient extraction of grape seed polyphenols. Food Chemistry 129: 570-576.

Maier, T., Schieber, A., Kammerer, D.R. and Carle, R. 2009. Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants. Food Chemistry 112: 551-559.

Montealegre, R.R., Peces, R.R., Vozmediano, J.I.C., Gascuena, J.M. and Romero, G. 2006. Phenolic compounds in skin and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. Journal of Food Composition and Analysis 19: 687-693.

Moure, A., Cruz, J. M., Franco, D., Domnguez, J. M., Sineiro, J., Dominguez, H., Nunez, M.J. and Parajo, J.C. 2001.Natural antioxidants from residual sources. Food Chemistry 72: 145–171.

Mussatto, S.I., Ballesterons, L.F., Martins, S. and Teixeira, J.A. 2011. Extraction of antioxidant phenolic compounds from spent coffee grounds. Separation and Purificaton Technology 83: 173-179.

Peralbo-Molina, Á.,Priego-Capote, F. and Laque de Castro, M.D. 2012. Comparison of extraction methods for exploitation of grape skin residues from ethanol distrillation. Talanta 101: 292-298.

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Diaz-Rubio, M.E., Serrano, J., Goñi and Saura-Calixto, F. 2008. Update methodology to determine antioxidant capacity in plant foods, oil and beverages: Extraction, measurement and expression of results. Food Research International. 41: 274-285

Poudel, P.R., Tamura, H., Kataoka, I. and Mochioka, R. 2008. Phenolic compounds and antioxidant activities of skins and seeds of five wild grapes and two hybrids native to Japan. Journal of Food Composition and Analysis 21: 622-625.

Prior, R. L., Wu, X. and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53 (10): 4290–4302.

Puértolas, E., Hemández-Orte, P., Sladańa, G., Álvarez, I. and Raso, J. 2010. Improvement of winemaking process using pulsed electric fields at pilot-plant scale, Evolution of chromatic parameters and phenolic content of *Cabenet Sauvignon* red wines. Food Research International 43: 761-766.

Qu, W., Pan, Z. and Ma, H. 2010. Extraction modeling and activities of antioxidants from pomegranate marc. Journal of Food Engineering 99 : 16-23.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yanh, M. and Rice-Evans, C.1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26: 1231–1237.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. and Dubourdieu, D. 2000. The chemistry of wine stabilization and treatments. In: Handbook of enology. Vol. 2. 2nded. John Wiley & Sons, Chichester.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. 1996. Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Radical Biology and Medicine 20: 933-956.

Sakurai, T., Kitadate, K., Nishioka, H., Fujii, H., Kizaki, T., Kondoh, Y., Izawa, T., Ishida, H., Radák, Z and Ohno, H. 2010.Oligomerized grape seed polyphenols attenuate inflammatory changes due to antioxidative properties in coculture of adipocytes and macrophages. Journal of Nutritional Biochemistry 21: 47–54.

Spigno, G., Tramelli, L. and Faveri, D.M.D. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering 81: 200-208.

Sung, J. and Lee, J. 2010. Antioxidant and antiproliferative of grape seeds from different cultivars. Food Science Biotechnology 19 (2): 321-326.

Teh, S.S. and Birch, E.W. 2014. Effect of ultrasonic treatment on the polyphenol content and antioxidant capacity of extract from defatted hemp, flax and canola seed cakes. Ultrasonics Sonochemistry 21 (1): 346-353.

Toaldo, I.M., Fogolari, O., Pimental, G.C., Gois, J.S., Borges, D.L.G., Caliari, V. and Bordignon-Luis, M. 2013.Effect of grape seeds on the polyphenol bioactive content and element composition by ICP-MS of grape juices from *Vitis labrusca* L. Food Science and Technology 53: 1-8.

Tyug, T.S., Prasad, K.N. and Ismail, A. 2010. Antioxidant capacity, phenolics and isoflavones in soybean by-products. Food Chemistry 123: 583-589.

Vaid, M., Prasad, R., Singh, T., Jones, V. and Katiyar, K.S. 2012. Grape seed proanthocyanidins reactivate silenced tumor suppressor genes in human skin cancer cells by targeting epigenetic regulators. Toxicology and Applied Pharmacology 263: 122-130.

Vinatoru, M. 2001. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrasonics Sonochemistry 8: 303-313.

Xu, C., Zhang, Y., Wang, J. and Lu, J. 2010. Extraction, distribution and characterization of phenolic compounds and oils in grape seeds. Food Chemistry 122 (3): 688-94.

Yang, J., Martinson, T.E. and Liu, R.H. 2009. Phytochemical profiles and antioxidant activities of wine grapes. Food Chemistry 116: 332-339.

Yilmaz, Y. and Toledo, R.T. 2006. Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. Journal of Food Composition and Analysis 19: 41-48.

Yue, X., Xu, Z., Prinyawiwatkul, W., Losso, J.N., King, J.M. and Godber, J.S. 2008. Comparison of soybean oils, gum and defatted soy flour extract in stabilizing menhaden oil during heating. Journal of Food Science 73: c19-c23.

Zhu, K.X., Lian, C.X., Guo, X.A., Peng, W. and Zhou, H.M. 2011. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. Food Chemistry 126: 1122-1126.