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EFFECTS OF GRAPE (*VITIS LABRUSCA* B.) PEEL AND SEED EXTRACTS ON PHENOLICS, ANTIOXIDANTS AND ANTHOCYANINS IN GRAPE JUICE

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Abstract

Grape peel and seed are good sources of important bioactive components such as phenolics, anthocyanins and antioxidants. Recovery of these components and their proper utilization is important for the development of functional foods. We have utilized the extracts of grape peel and seed obtained by ultrasonic-assisted (UAE) and supercritical fluid extractions (SFE) for the enrichment of Campbell Early grape juice (CEJ). CEJ samples were analyzed for different functional compounds and it was observed that the addition of these extracts in CEJ significantly improved total phenolic compounds, antioxidants, antiradical activities and total anthocyanin contents. HPLC analysis of CEJ samples containing these extracts showed that the phenolic acids (benzoic and cinnamic acids) and catechins contents were also significantly improved with the addition of grape peel and seed extracts. Generally SFE extracts proved to be of superior quality for the functional enrichment in CEJ. The sensory evaluation revealed that the CEJ samples containing the extracts had good overall acceptability.

Introduction

In the last few years, an increased attention has been focused on the industrial wastes, especially those containing residual phenols from the plant raw materials. Grape is one of the largest fruit crops in Republic of Korea, with an annual production of approx. 325000 tons (FAOSTAT, 2007) and Campbell Early grape (*Vitis labrusca* B.) is the major grape cultivar grown (Lee *et al*., 2006). Grapes are antimutagenic, antineoplastic and reduce human low-density lipoprotein (LDL) oxidation and allergic inflammation (Shaker, 2006). Grape juice is a representative product of grapes and there is continued need to improve the sensory and nutritional properties of grape juice. The high demand for grape products is due to the associated health benefits for consumers and this has motivated research for formulating ways to improve its quality and health effects (Ghafoor *et al*., 2008). Tons of grape pomace is produced while processing grapes and peels and seeds constitute a major proportion of pomace (Schieber *et al*., 2002). Grape peel and seeds are rich sources of functional components such as phenolics and anthocyanins which have antioxidant and radical scavenging activities (Negro *et al*., 2003; Yilmaz & Toledo, 2004; Pinelo *et al*., 2006). Phenolics may also act selectively at very low concentrations to inhibit LDL oxidation in vitro (Frankel *et al*., 1993; Teissedre *et al*., 1996). These natural antioxidants have favorable effects on human health by decreasing the heart disease risks, and being anticarcinogenic in nature (Williams & Elliot, 1997). Antiradical and antioxidant activities of plant extracts have been confirmed by β-carotene linoleate and linoleic acid peroxidation methods (Jayaprakasha *et al*., 2001) as well as by 1, 1-diphenyl-2-picrylhydrazyl and phosphomolybdenum complex methods (Jayaprakasha *et al*., 2003). Polyphenolic compounds such as gallic acid, caffeic acid, *m*-hydroxy benzoic acid, syringic acid, *p*-coumaric acid, sinapic acid, ellagic acid, *p*-hydroxy benzoic acid and catechin are found in various fruits including grapes and have been reported to have ample health benefits (Russell *et al*., 2009). Due to the presence of important compounds in grape peel and seed, addition of their extracts may improve the functional properties of different food products (Yilmaz & Toledo 2004; Shaker, 2006). The addition of functional compounds into food matrices is an effective method in decreasing disease risks and the scientific community should develop innovative functional foods with the potential to produce physiological benefits or reduce the long-term risk of developing diseases (Elliott & Ong, 2002).

Extraction is a major step in the isolation, identification and use of phenolic and other compounds (Stevigny *et al*., 2007). Ultrasonic-assisted extraction (UAE) is a simple and efficient alternative to conventional extraction techniques and the enhancement in extraction obtained by using ultrasound is mainly attributed to the effects of acoustic cavitations produced in the solvent by the passage of an ultrasound wave (Ghafoor *et al*., 2009). Supercritical fluid extraction (SFE) with CO_2 has been used for the extraction from natural products and researchers have paid considerable attention towards various aspects of this process (Lu *et al*., 2007). CO_2 is an inert, non-toxic, environmentally friendly solvent and allows extraction at lower temperatures and relatively low pressures. The extracts obtained by SFE are of better quality than those obtained by organic solvent extraction methods (Friedrich & List, 1982).

The objective of our study was to investigate the effects of adding the extracts from grape peel and seed obtained by the application of UAE and SFE on the polyphenolic contents, functional and sensory properties of Campbell Early grape juice (CEJ).

Material and Methods

Materials: Freshly harvested ripened Campbell Early grapes were purchased from a local farm in Kyungbuk province of Korea. Grapes were excised from the stems, sorted and washed for juice processing and extractions from peel and seed. Peels and seeds were manually removed from grape berries and oven dried at 50°C until the moisture level was constant (6.2% w/w). Dried grape peels and seeds were 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 from Sigma Chemical Co. (St. Louis, MO) and Duksan Pure Chemical Co. (Ansan, Korea).

Extraction from grape peel and seed: 2 g powdered samples of grape peel and seed were extracted either by using ultrasonic-assisted extraction (UAE) or supercritical fluid extraction (SFE). Extracts were prepared on the conditions optimized in a series of designed experiments by using regression analysis and response surface methodology for total phenols, antioxidant activities and total anthocyanins. UAE was carried out by taking the samples in a flask and volume

was made 100 mL with the ethanol. Flask was kept in a sonication water bath (JAC Ultrasonic 2010P; Jinwoo Engineering Co., Ltd., Hwasung, Gyeonggi, Korea). The working frequency and power were fixed at 40 KHz and 250 W respectively. Optimal UAE conditions for grape peel were 52, 53 and 54% ethanol at 45, 46 and 50ºC for 24, 25 and 26 min respectively for the above mentioned functional compounds. UAE extracts from grape seed were prepared by using 52, 53 and 54% ethanol at 55, 56 and 60ºC for 29, 30 and 31 min respectively (Ghafoor *et al*., 2009). After extraction the flask was immediately cooled to room temperature by using chilled water. The extract was filtered through filter paper # 5A under vacuum and the volume was made 100 mL with the extraction solvent.

SFE system consisted of a CO_2 cylinder, cool water circulator (VTR-620, Jeio Tech., Seoul, Korea), column thermostat (CO-1560, JASCO Corporation, Tokyo, Japan), HPLC pumps (PU-1580, JASCO), UV/VIS detector (UV-1575, JASCO) and back pressure regulator (880-81, JASCO). Optimal SFE conditions for total phenols, antioxidants and total anthocyanins from grape peel were 44, 45 and 46ºC temperature, 160, 161 and 164 $kgcm^{-2}$ pressure and 6.2, 6.5 and 6.6% ethanol whereas those for SFE from grape seed were 44, 45 and 46ºC temperature, 156, 160 and 166 $kgcm^{-2}$ pressure and 5.8, 6.5 and 6.7% ethanol (Ghafoor *et al*., 2010). The extracts were collected in 100 mL flasks and volume was made up to the mark.

Preparation of grape juice samples: Campbell Early grape juice (CEJ) was prepared by heating grapes at 65°C in an airtight steel vessel for 20 min and then pressed by using a cheese cloth to get the juice. Juice was kept in refrigerator at 4°C for cold settling followed by filtration. Grape peel and seed extracts obtained either by UAE and SFE were dried under reduced pressure at 40°C and 100 mg of each of the crude extracts were added in 200 mL of CEJ samples followed by stirring on magnetic stirrer, bottling and pasteurization in a water bath at 70°C for 20 min. Samples were stored at 4°C before different physicochemical and sensory analysis.

Analysis for total phenolics: The total phenolic compounds were analyzed using Folin Ciocalteu method with some modification (Singleton & Rossi, 1965). A 200 µL properly diluted sample or standard solution of varying concentrations were mixed with 400 µL Folin Ciocalteu reagent. The deionized water was used for dilution and control. The solution was diluted to a total volume of 4.6 mL using deionized water followed by thorough mixing. After incubation for 10 min at room temperature, 1 mL of 10% Na_2CO_3 solution was added followed by immediate thorough mixing and incubation for 2h. The absorbance was read at 765 nm on a spectrophotometer (TU-1800; Human Corporation, Seoul, Korea). Measurements were recorded in triplicates. Gallic acid of 1 mg/mL was used as the standard and the total phenolic compounds of the samples were expressed in milligram gallic acid equivalent per 100 mL of CEJ (mg GE/100 mL).

Determination of antiradical activity: The free radical activity of the CEJ was determined by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Lee *et al*., 1998). 1 mL solution of the juice extract at a concentration of 100 µL/mL methanol was mixed with 2 mL of 10 mg/L methanolic solution of DPPH. An equal amount of methanol and DPPH served as a control. The mixtures were shaken vigorously and allowed to stand at room temperature for 5 min and optical density (OD) was recorded at 517 nm. Lower OD value of the sample indicated the higher free radical scavenging activity. The antiradical activity of the CEJ was calculated in percentage as follows:

$$\text{Antiradical activity (\%)} = \frac{\text{1 - (OD of sample}}{\text{OD of control)}} \times 100$$

Determination of antioxidant activity: The antioxidant activity of the CEJ was evaluated by phosphomolybdenum complex method (Prieto *et al*., 1999). 0.4 mL of sample solution (100 µL/mL methanol) was combined with 4 mL of phosphomolybdenum complex containing 0.6 M sulphuric aicd, 2 mM sodium phosphate and 4 mM ammonium molybdate. Test tubes were caped and placed in hot water for 90 min at 95°C. Samples were cooled to room temperature and the absorbance was measured at 695 nm. Antioxidant activity was expressed as the mg ascorbic acid equivalent per mL (mg AE/mL).

Determination of total anthocyanins: Determination of total anthocyanins in CEJ was based on the method described by Iland *et al*., (1996). In 1 mL of CEJ sample, 10 mL 50% ethanol was added and sample was centrifuged at 1800 × g for 10 min., 200 µL 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 1 M HCl as the blank. Anthocyanins were calculated as mg/mL of CEJ using the absorbance (B) of a 1% w/v solution of malvidin-3-glucoside as follows:

$$\text{Anthocyanins (mg/mL)} = A \times \text{Dilution factor} \times 1000 / B$$

Analysis of bioactives using high performance liquid chromatography: The HPLC system consisted of a Hewlett Packard 1100 series system with pump, UV detector, auto sampler and degasser. Data processing was performed by using the software HPcore chemstation (Hewlett Packard, Germany). Separation was performed on an YMC pack pro C18 RS column (250, 4.6mm ID, S-5µm. 8nm, YMC Inc. USA) at room temperature. Injection volume was 10µL, flow rate was set at 1 mL/min and UV detection was carried out at 290 nm. Solvent used were 2% acetic acid and 0.5% acetic acid / 50% acetonitrile. 1 mL of CEJ sample was diluted with 5mL of methanol and filtered through 0.45µm filter before injection into the HPLC.

Sensory evaluation: The sensory evaluation of CEJ samples for color, taste, aroma and overall acceptability was conducted by a panel of 15 judges selected from the Department of Food Science and Technology at Kyungpook National University, Daegu, Korea. The judges scored each attribute for random samples on a scale of 1 to 9 in which 1 denotes dislike extremely and 9 stands for like extremely.

Statistical analysis: All the measurements were taken in triplicates and the values were reported as mean scores ± SD. Statistical analysis was done by applying analysis of variance (ANOVA) and Duncan's multiple range (DMR) tests using Statistical Analysis System (SAS, version 9.1). Significance was defined at $p<0.05$.

Results and Discussion

Ultrasonic-assisted (UAE) and supercritical fluid (SFE) techniques were used for the extractions from grape peel and

seed. Extracts were prepared by using optimized conditions for each type of extraction either from grape peel or grape seed for total phenols, antioxidant and total anthocyanins (Table 1) and added into Campbell Early grape juice (CEJ) in order to evaluate their effects on the functional compounds and sensory properties of grape juice.

Total phenolics in grape juice: The effects of addition of extracts from grape peel and seeds were evaluated on the phenolic contents of CEJ and the results are presented in Table 1. Phenolic compounds react with Folin–Ciocalteu's Reagent (FCR) only under basic conditions (adjusted by aqueous sodium carbonate). Dissociation of a phenolic proton in basic medium leads to a phenolate anion, which is capable of reducing FCR in which the molybdate in testing system is reduced forming a blue colored molybdenum oxide with maximum absorption near 765 nm. The intensity of blue coloration produced is proportional to the total quantity of phenolic compounds present in the testing samples (Abdel-Hameed, 2009). Phenolic contents of CEJ were significantly (p<0.05) affected by the addition of extracts from grape peel and seed obtained by UAE and SFE. CEJ samples without any extract had lowest (3.082 mg GE/100mL) phenolic compounds where as maximum (4.447 mg GE/100mL) phenolic compounds were in the CEJ sample 11 containing SFE extract of grape seed optimized for total phenols. Negro *et al*., (2003) also found that phenolic contents of grape seed extracts are higher than that of grape peel extracts. Generally the CEJ samples containing SFE extracts from grape peel and seed had higher phenolic contents than UAE extracts. It has been reported that grapes and grape products contain large amounts of phenolic compounds, which have various biologically important functions such as radical scavenging, antioxidants and other health promoting properties (Shaker, 2006). Chronic consumption of grape phenolics is also reported to reduce obesity development and related metabolic pathways including adipokine secretion and oxidative stress (Decorde *et al*., 2009). They can also be recommended as natural food additives and preferred over synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene (Wang *et al*., 1999). The multiple mechanisms of their antioxidative activities are expressed in their abilities of radical scavenging, metal chelation, and synergism with other antioxidants (Lu & Foo, 1999). The addition of extracts containing phenolic compounds to the CEJ is an effective method to enhance the phenolic levels of juice and hence its health promoting functions.

Antiradical and antioxidant activities of grape juice: The antiradical and antioxidant activities were determined by DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging and phosphomolybdenum complex methods respectively. Antiradical assay depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine and the remaining DPPH, which showed maximum absorption at 517 nm. Phosphomolybdenum complex assay for the antioxidant activity is based on the reduction of Mo(VI) to Mo(V) by the sample and subsequent formation of a green phosphate/Mo(V) complex at acid pH (Abdel-Hameed, 2009). As presented in Table 1 the addition of UAE and SFE extracts from grape peel and seed significantly (p<0.05) affected the antiradical and antioxidant properties of CEJ samples. Higher antiradical (92.76%) and antioxidant (4.12 mg AE/mL) activities were found in sample 13 and 12 respectively, which contained SFE seed extracts optimized for the respective functional properties in the extract. CEJ samples containing SFE extracts of peel and seed had higher antioxidant activities than the samples with UAE extracts. CEJ sample without any extract showed the lowest antiradical (75.43%) and antioxidant (1.958 mg AE/mL) activities. Stresses, physical damage, viral infection, cytotoxic or carcinogenic compounds as a consequence of chemical or biological aggression may cause peroxidation of polyunsaturated fatty acids of cell membranes and liberation of toxic substances such as free radicals. These free radicals may cause aging and human degenerative diseases including, cancer, heart diseases, multiple sclerosis, Parkinson's disease, autoimmune disease and senile dementia. The consumption of products rich in natural polyphenols such as grape juice having strong antiradical and antioxidant potential can significantly decrease the risk of morbidity due to such diseases (Thériault *et al*., 2006). We can observe that there is a correlation between antiradical and antioxidant properties of CEJ samples and phenolic compounds. Such a correlation between these functional properties and phenolic compounds has also been reported by various researchers (Baydar *et al*., 2003; Su & Silva, 2006).

Total anthocyanins in grape juice: The effect of addition of grape peel and seed extract was highly significant (p<0.001) on the total anthocyanin contents of CEJ. Duncan multiple range analysis (Table 1) reveals that the highest anthocyanin contents were observed in sample 4 which contained UAE extract of grape peel optimized for anthocyanin contents. Anthocyanins are natural pigments which are used to produce authorized colorants, nutraceuticals and drugs (Bordignon-Luiz *et al*., 2007). Anthocyanins account a considerable proportion of the total polyphenols in grape and possess strong biological functions such as anti-inflammatory and antioxidant activities (Kong *et al*., 2003).

Polyphenolic contents of grape juice: Phenolic acids and catechins contents were also evaluated for CEJ samples containing grape peel and seed extracts by using high performance liquid chromatography. Data of polyphenolic contents of CEJ samples is presented in Table 2. A typical chromatogram of a CEJ sample for the analyzed bioactives is shown in Fig. 1. Benzoic acids including gallic acid, *m*-hydroxy benzoic acid, syringic acid and ellagic acid; cinnamic acids including caffeic acid and sinapic acid and catechins were detected in varying amounts in the CEJ samples. Caffeic acid was not detected in samples of CEJ without any extract and those containing only UAE extract whereas samples containing SFE extracts showed considerable presence of caffeic acid. Data of polyphenolic contents of CEJ samples 8-13 containing SFE extracts of grape peel and seed (Table 2) shows considerable higher proportions of benzoic acids, cinnamic acids and catechins in these samples which is further supported by the fact that these samples showed considerably higher antiradical and antioxidant properties (Table 1). Russell *et al*., (2009) found that these bioactive compounds have ample health benefits. Presence of wide range of higher quality bioactive compounds in SFE extracts as compared to UAE extracts of *Lavandula angustifolia* was also reported by Porto *et al*., (2009).

Sensory properties of grape juice: Data of sensory evaluation of CEJ samples is presented in Table 3. CEJ was evaluated for color, aroma, taste and overall acceptability. Results of sensory evaluation revealed that the CEJ containing extracts had good acceptability. It has been reported that phenolic compounds also contribute to the sensory properties such as color, aroma besides having potential health benefits (Macheix *et al*., 1990).

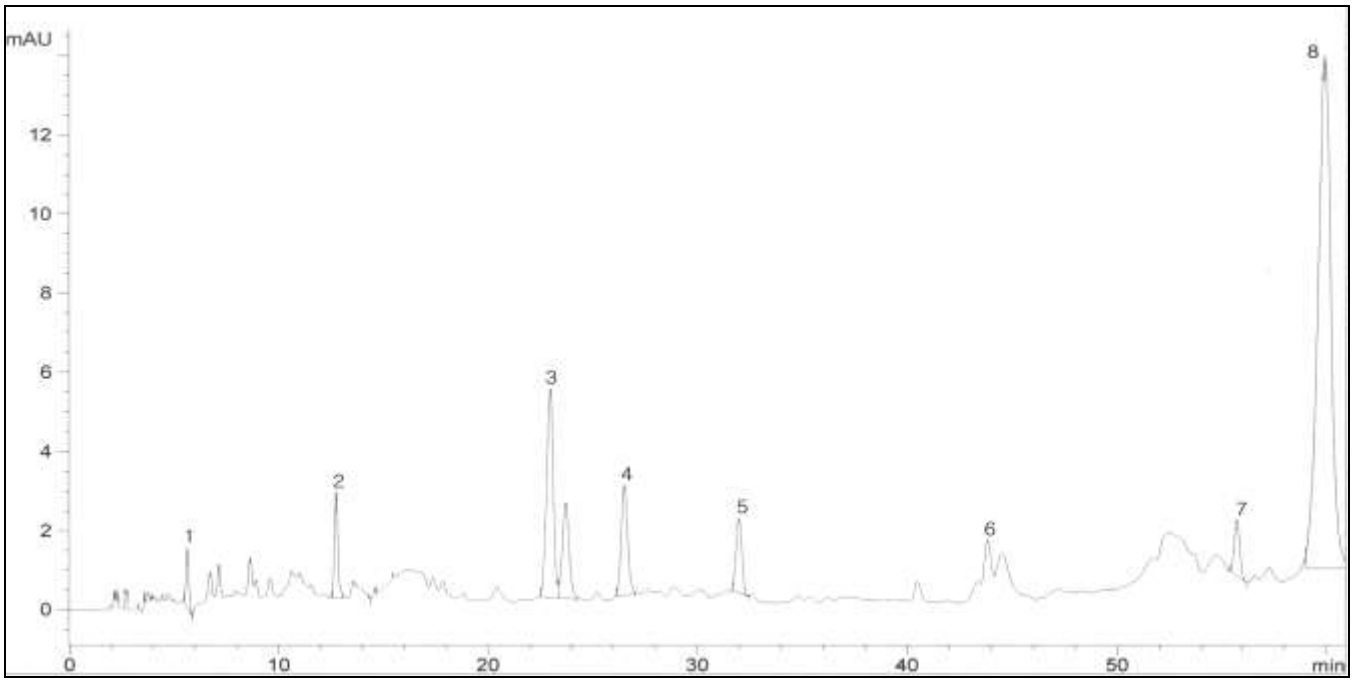

Fig. 1. A typical chromatogram of grape juice sample with extract. Gallic acid (1); catechin (2); caffeic acid (3); *m*-hydroxy benzoic acid (4); syringic acid (5); *p*-coumaric acid (6); sinapic acid (7); ellagic acid (8).

Table 1. Effects of various grape peel and seed extracts on the functional properties of grape juice.

Treatment	Sample	Total phenols * (mg·ml^{-1})	Antiradical activity (%)	Antioxidants ** (mg·ml^{-1})	Anthocyanins (mg.ml^{-1})
Control (juice)	1	3.082 ± 0.114^{g}	75.43 ± 2.85^{g}	1.958 ± 0.26^{h}	4.966 ± 0.15^{h}
UAE peel extracts & juice	2	3.854 ± 0.114^{ef}	87.13 ± 1.24^{cd}	2.218 ± 0.28^{f}	5.446 ± 0.07^{f}
	3	3.790 ± 0.153^{ef}	85.63 ± 0.54^{ef}	2.155 ± 0.06^{fg}	5.926 ± 0.25^{de}
	4	3.755 ± 0.152^{f}	86.23 ± 0.24^{de}	2.125 ± 0.47^{fg}	8.206 ± 0.24^{a}
UAE seed extracts & juice	5	3.923 ± 0.125^{ef}	87.03 ± 1.45^{cd}	2.045± 0.08^{gh}	4.326 ± 0.072^{i}
	6	3.770 ± 0.141^{ef}	87.73 ± 1.24 ^{c}	2.405 ± 0.14^{e}	5.126 ± 0.08^{g}
	7	3.814 ± 0.213^{ef}	86.53 ± 1.8^{cde}	1.980 ± 0.11^{h}	5.526 ± 0.18^{f}
SFE peel extracts & juice	8	4.283 ± 0.075^{bc}	84.61 ± 1.04^{f}	3.437 ± 0.82^{b}	5.937 ± 0.03^{de}
	9	4.090 ± 0.158^{d}	87.81 ± 0.41^{c}	3.392 ± 0.76^{b}	6.057 ±0.12^{c}
	10	3.932 ± 0.024^{e}	87.61 ± 0.24^{c}	3.475 ± 0.27^{b}	6.697 ± 0.08^{b}
SFE seed extracts & juice	11	4.447 ± 0.024^{a}	91.11 ± 1.28^{b}	3.127 ± 0.35^{d}	4.097 ± 0.32^{j}
	12	4.417 ± 0.128^{ab}	90.81 ± 0.64^{b}	4.120 ± 0.21^{a}	5.857±0.15^{e}
	13	4.145 ± 0.172^{cd}	92.76 ± 0.72^{a}	3.260 ± 0.14^{c}	6.017 ±0.22^{cd}

Analytical results are means ± SD (n=3)
Means within same column which have no common letters are significantly different (p<0.05)
Each category of the juice sample contained the extracts optimized for total phenols, antioxidants and total anthocyanins respectively
* Expressed as gallic acid equivalent per 100 ml CEJ, ** Expressed as ascorbic acid equivalent

Table 2. Polyphenolic contents of grape juice containing grape peel and seed extracts.

Treatment	Sample	Gallic acid (µg.ml^{-1})	*m*-Hydroxy benzoic acid (µg.ml^{-1})	Syringic acid (µg.ml^{-1})	Ellagic acid (µg.ml^{-1})	Caffeic acid (µg.ml^{-1})	Sinapic acid (µg.ml^{-1})	Catechins (µg.ml^{-1})
Control (juice)	1	0.49±0.025^{h}	3.77±0.25^{b}	0.91±0.081^{h}	83.08±2.36^{f}	-	-	4.17±0.72^{e}
UAE peel extracts & juice	2	0.60±0.014^{g}	3.89±0.18^{b}	1.08±0.073^{fg}	94.99±3.18^{e}	-	2.78±0.06^{d}	6.19±0.68^{d}
	3	0.53±0.024^{h}	4.24±0.09^{b}	1.07±0.342^{g}	92.29±2.17^{e}	-	-	0.03±0.82^{f}
	4	0.53±0.041^{h}	4.14±0.28^{b}	1.21±0.084^{e}	96.18±1.39^{e}	-	-	6.55±0.65^{d}
UAE seed extracts & juice	5	0.67±0.033^{f}	4.98±0.36^{b}	1.19±0.027^{ef}	113.7±3.72^{cd}	-	6.08±0.07^{c}	7.26±0.77^{d}
	6	0.63±0.008^{fg}	4.62±0.08^{b}	1.27±0.036^{de}	122.07±2.96^{c}	-	-	9.59±0.71^{c}
	7	0.72±0.071^{e}	4.38±0.39^{b}	1.18±0.07^{efg}	111.95±6.15^{d}	-	-	4.85±0.67^{e}
SFE peel extracts & juice	8	1.22±0.092^{c}	8.18±0.08^{a}	1.98±0.102^{a}	159.82±4.32^{a}	19.57±2.27^{b}	7.92±0.06^{b}	11.21±0.48^{a}
	9	1.24±0.054^{c}	8.08±1.08^{a}	1.91±0.092^{a}	166.25±2.99^{a}	19.56±1.89^{b}	9.23±1.05^{a}	11.21±1.08^{a}
	10	0.74±0.071^{e}	6.97±0.44^{a}	1.68±0.086^{b}	141.16±2.83^{b}	18.49±2.61^{c}	7.74±0.03^{a}	9.83±1.24^{cb}
SFE seed extracts & juice	11	1.81±0.037^{a}	4.87±0.32^{b}	1.33±0.092^{d}	94.57±1.97^{e}	-	-	7.02±1.31^{d}
	12	0.78±0.061^{d}	7.63±0.95^{a}	2.01±0.037^{a}	167.31±3.72^{a}	21.99±2.52^{a}	9.60±0.73^{a}	10.98±0.86^{ab}
	13	1.66±0.039^{b}	7.57±1.27^{a}	1.53±0.028^{c}	39.79±1.09^{b}	17.85±1.35^{d}	-	10.4±0.92^{abc}

Results are means ± SD (n=3)
Means within same column which have no common letters are significantly different (p<0.05)
Each category of the juice sample contained the extracts optimized for total phenols, antioxidants and total anthocyanins respectively

Table 3. Effects of various grape peel and seed extracts on the sensory properties of grape juice.

Treatment	Sample	Color	Aroma	Taste	Overall acceptability
Control (juice)	1	6.011 ± 0.24^{g}	6.277 ± 0.36^{e}	5.777 ± 0.31^{e}	6.197 ± 0.15^{c}
UAE peel extracts & juice	2	6.511 ± 0.51^{f}	6.697 ± 0.29^{d}	6.343 ± 0.46^{c}	6.843 ± 0.61^{a}
	3	6.843 ± 0.22^{de}	6.447 ± 0.33^{e}	5.511 ± 0.18^{f}	6.179 ± 0.38^{cd}
	4	6.843 ± 0.35^{de}	6.027 ± 0.25^{g}	5.843 ± 0.51^{e}	6.011 ± 0.37^{d}
UAE seed extracts & juice	5	6.697 ± 0.43^{e}	6.121 ± 0.82^{f}	5.863 ± 0.71^{g}	5.536 ± 0.58^{e}
	6	6.527 ± 0.38^{f}	6.011 ± 0.45^{g}	6.343 ± 0.36^{c}	6.511 ± 0.52^{b}
	7	6.363 ± 0.54^{f}	6.343 ± 0.63^{e}	6.343 ± 0.72^{c}	6.179 ± 0.39^{cd}
SFE peel extracts & juice	8	7.113 ± 0.36^{bc}	7.511 ± 0.18^{a}	6.197 ± 0.45^{cd}	6.777 ± 0.34^{a}
	9	7.011 ± 0.38^{cd}	6.843 ± 0.38^{d}	6.113 ± 0.33^{d}	6.197 ± 0.44^{c}
	10	7.511 ± 0.19^{a}	7.011 ± 0.42^{c}	6.679 ± 0.35^{b}	6.679 ± 0.43^{a}
SFE seed extracts & juice	11	7.511 ± 0.28^{a}	7.343 ± 0.39^{b}	7.011 ± 0.57^{a}	7.011 ± 0.57^{a}
	12	6.863 ± 0.72^{de}	6.363 ± 0.41^{e}	6.777 ± 0.51^{b}	6.863 ± 0.26^{a}
	13	7.197 ± 0.38^{b}	6.679 ± 0.31^{d}	6.613 ± 0.28^{b}	6.697 ± 0.33^{a}

Results are means ± SD (n=12)

Means within same column which have no common letters are significantly different ($p<0.05$)

Each category of the juice sample contained the extracts optimized for total phenols, antioxidants and total anthocyanins respectively

Extractions from plant materials are reported to affect the chemical and physical structure of bioactive compounds such as phenolic compounds which may either increase or decrease their bioavailability (Cermak *et al*., 2009). In case of UAE, sonication waves are used along with heating and higher concentration of solvents. The use of higher ultrasonic frequency may enhance OH radical formation; which may react and denature phenolic compounds (Chowdhury & Viraraghavan, 2009). SFE is an environmental friendly extraction technique which is selective and the extracts obtained are of superior quality as compared to the other techniques. It also allows extraction at lower temperatures and the solvent can be separated easily from the extract. Extraction under the CO_2 environment also reduces the chances of oxidation reactions (Pereda *et al*., 2008). Due to the use of very low concentrations of organic solvents SFE extracts are also generally recognized as safe to be used in food and pharmaceutical products (King, 2000). We have also found that grape juice can be effectively used as a vehicle for the delivery of bioactive compounds and micronutrients that provide health benefits for increased wellbeing. Consumers demand for healthy nutritious foods having not only balanced calorific content, but also carrying additional health-promoting functions, i.e., functional foods (Bech-Larsen *et al*., 2002). The addition of grape peel and seed extracts was found to have profound effects on CEJ quality with significant improvements in its bioactive contents and functional properties.

Conclusions

Grape peel and seed are good sources of valuable bioactive components which may be used for improving the functional quality of fruit juices such as grape juice without reducing the sensory acceptability. Extracts of grape seed and peel obtained by SFE were of higher quality as compared to UAE extracts for improving the bioactive constituents and functional properties of grape juice. Enrichment of fruit juices with natural extracts obtained by improved and environmentally friendly extraction techniques can also be effectively used to raise their health promoting effects.

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