Effect of Sun, Oven and Freeze-Drying on Anthocyanins, Phenolic Compounds and Antioxidant Activity of Black Grape (Ekşikara) (*Vitis vinifera* L.)

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Submitted for publication: April 2017
Accepted for publication: July 2017

Key words: Ekşikara, anthocyanins, phenolic compounds, antioxidant activity, sun drying, oven drying, freeze drying

The aim of this study was to determine whether a change occurs in the phenolic compounds and antioxidant activity of grapes after drying. Grapes pre-treated with potassium hydroxide solution were dried using three different drying methods, namely freeze drying, oven drying and sun drying. The effectiveness of the drying methods was evaluated in terms of total phenolic content, antioxidant activity (ABTS, FRAP and DPPH), individual phenolics and anthocyanins. Losses in total phenolic content of the grapes were found to be 1.89, 20.26 and 46.79% for freeze-, oven- and sun-dried grapes respectively. The DPPH and ABTS antioxidant activities of the grapes decreased after drying by all three methods, while an increase was observed in the FRAP value of freeze-dried grapes compared to the fresh sample. No significant effect of drying methods was observed on the gallic acid, 2,5-dihydroxybenzoic acid and (-)-epigallocatechin gallate contents of the grapes. The highest levels of procyanidin B1, (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, chlorogenic acid, *trans*-resveratrol and rutin were determined in freeze-dried grapes. Sun and oven drying caused drastic decreases in all anthocyanins, while no loss of anthocyanin was observed in freeze-dried grapes. Sun drying was found as the most detrimental drying method for grapes in terms of phenolic compounds and antioxidant activity when compared to the other drying methods.

INTRODUCTION

Grapes are one of the most important agricultural products and are available in almost all parts of the world. World grape production in the 2014/2015 season was reported as 20 637 000 metric tons (USDA, 2016). In the same season, Turkey was ranked as the world's third largest grape producer with an amount of 3 226 000 tonnes (TSI, 2016). According to 2014/2015 data on agricultural production, 1 361 000 tonnes of dried grapes were produced worldwide. Turkey was the largest manufacturer and exporter of dried grapes in 2014, and this constituted 24% of the total world dried grape production (INC, 2016). The USA, Iran, China and India follow in descending order after Turkey in terms of dried grape production.

Drying, one of the oldest methods of food preservation, is widely used to extend the shelf life of fruit in order to keep them available throughout the season. On the other hand, drying methods have a significant effect on the quality characteristics of dried fruit, such as phenolic compounds and sensorial properties (Angulo *et al*., 2007; Tseng & Zhao, 2012). The sun-drying method has the advantage of being more cost-effective than the other drying methods. However, because of the slow drying rate it takes a long time. Grape drying takes nearly 20 days for untreated fruits and eight to 10 days for pre-treated ones (Jairaj *et al*., 2009). Sun-dried grapes have a high risk of contamination due to their direct exposure to the environment (Jairaj *et al*., 2009). To shorten the drying time and reduce the contamination risk, different methods such as solar, oven and hot-air drying could be used in raisin production. Sensorial properties, nutritional quality and cost of product are usually taken into consideration in choosing an appropriate drying method (Angulo *et al*., 2007). The grape berry contains water, sugar, minerals, polyphenolics, organic acids, vitamins, aroma and nitrogen compounds (Armstrong & Stratton, 2016). Polyphenols are one of the most important constituents of grapes and contribute to the colour, taste and aroma (Armstrong & Stratton, 2016). Grapes also possess antioxidant activity by chelating metal ions and scavenging hydroxyl radicals (.OH) and superoxide anion radicals ($.O_2^-$) (Kong *et al*., 2003; Akbulut *et al*., 2008).

Grapes contain phenolic acids (hydroxybenzoic and hydroxycinnamic acid, and their derivatives), stilbenes, flavonols, anthocyanins, flavan-3-ols and condensed tannins (Montealegre *et al*., 2006). Anthocyanins are natural plant pigments that are responsible for colours ranging from red to violet, and are located especially in the exocarp of the grape

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(Ban *et al*., 2003; Coklar, 2016; Nicoue *et al*., 2007).

A large number of studies have been carried out to determine the impact of processing on the phenolic content and antioxidant activities of different fruit and vegetables (Kamiloglu *et al*., 2016). In addition, some studies have investigated the changes in bioactive compounds (such as total phenolic, anthocyanin, total flavonol) and antiradical scavenging activity of grape skin and pomace by using different drying methods (Larrauri *et al*., 1997; De Torres *et al*., 2010; Tseng & Zhao, 2012).

The aim of this study was to evaluate the sun-, oven- and freeze-drying methods for dried grape production from the standpoint of antioxidant activity and phenolic compounds.

MATERIALS AND METHODS

Sample

In this research the Ekşikara (*Vitis vinifera L.)* grape variety from Turkey was used. Ekşikara is a cultivar and a local variety of *Vitis vinifera* originating from the south of Central Anatolia. It is grown around the towns Hadim and Taşkent under the names Keçimen and Karaoğlan. It is generally cultivated for table consumption and raisin production.

Grapes were harvested from a vineyard, located at Hadim, at commercial maturity (22°Brix) and dipped into a 1% potassium hydroxide solution for 5 min in order to remove the wax layer from the skin and accelerate the drying. Grapes (4 kg) were divided into four equal groups. One was used as a control sample and the others were dried by sun, in an oven and a freeze dryer respectively. All samples, dried by different methods, contained dry matter of 95 ± 1% after drying.

Drying methods

Oven drying

Fresh grapes (1 kg) were spread on a tray and put into the drying oven (Nuve, Turkey) at 60°C. The drying of the grapes in the oven dryer continued for 17 h.

Freeze drying

One kg of fresh grapes was kept at -18°C in a deep freezer for 24 h. Lyophilisation of samples was carried out in a freeze dryer (Labogene ScanVac Coolsafe110-4, Lynge, Denmark). Throughout the drying operation, the vacuum chamber pressure and condenser temperature were at 2.30 mbar and -110°C respectively. The drying of the grapes in the freeze dryer was completed within 48 h.

Sun drying

Grapes (1 kg) were spread on a tray and placed in sunlight. Drying in the sun lasted seven days and the grapes were turned over every day by hand in order to ensure homogenous drying in all parts of the grape as far as was possible. The drying experiments were repeated twice for all drying methods.

Chemicals

The HPLC-grade acetonitrile, ethyl acetate and methanol were from Merck (Darmstadt, Germany). All chemicals were analytical grade and acquired from either Sigma Chemical Co. (St. Louis, MO) or Merck (Darmstadt, Germany). Gallic acid (PubChem CID:370), (+)-catechin (PubChem CID: 9064), (-)-epicatechin (PubChem CID: 72276), procyanidin B1 (PubChem CID: 11250133), procyanidin B2 (PubChem CID: 122738), chlorogenic acid (PubChem CID: 1794427), caffeic acid (PubChem CID: 689043), rutin (PubChem CID: 5280805), kaempferol-3-glucoside (PubChem CID: 5282102), (-)-epigallocatechin gallate (PubChem CID: 65064), (-)-epicatechin gallate (PubChem CID: 107905), 2,5-dihydroxybenzoic acid (PubChem CID: 3469), trans-resveratrol (PubChem CID: 445154), isorhamnetin-3-O-glucoside (PubChem CID: 5318645), delphinidin-3-O-glucoside (PubChem CID: 165558), cyanidin-3-O-glucoside (PubChem CID: 441667), petunidin-3-O-glucoside (PubChem CID: 176449), peonidin-3-O-glucoside (PubChem CID: 14311152) and malvidin-3-O-glucoside (PubChem CID: 443652) were purchased from Extrasynthese (Genay, France).

Analysis

Extraction of phenolics and other antioxidant-effective compounds

One gram of ground dried grape was extracted using a homogeniser (WiseMixTM HG-150; Daihan Scientific, Korea) at 10 000 rpm for 3 min in 50 ml methanol:water:formic acid (50:48.5:1.5) solvent mixture. The extract was then centrifuged (NF 800R, Nuve, Turkey) at 4 100 x g for 10 min. The supernatant was transferred into a glass jar and the residue was re-extracted with a methanol:water mixture. Supernatant from two extractions was poured into a jar glass and stored at -18°C until further analyses (Coklar & Akbulut, 2017).

Analysis of total phenolic content

The total phenolic content of the fresh and dried grapes was determined using the Folin–Ciocalteu procedure (Singleton & Rossi, 1965). The methanolic extract (0.5 mL) was mixed with 2.5 mL Folin–Ciocaleu's reagent (0.2 N) and 2.0 mL sodium carbonate (75 g/L). Absorbance of the reaction mixtures was read against the blank at 765 nm using a spectrophotometer (U-1800, Hitachi, Japan) after the mixture was left to stand in the dark for 120 min. Gallic acid standard solutions at concentrations of 12.5 to 200 ppm were used to prepare the calibration curve, and the results were expressed as mg gallic acid equivalents (GAE) per gram dried grape.

Analysis of antioxidant activity

FRAP assay

To estimate the ferric reducing antioxidant powers (FRAP) of the grapes, 50 µL of extract and 150 µl deionised water were added to 1.5 ml freshly prepared FRAP reagent (300 M acetate buffer (pH 3.6):10 M TPTZ:20 M $FeCl_3.6H_2O$ (10:1:1)). The absorbance of the reaction mixture after incubation at 37.8°C for 4 min was read at 593 nm. The results were calculated according to the calibration curve of $FeSO_4.7H_2O$ prepared in the range of 100 to 1 000 µmol/L. The results were expressed as µmol Fe^{+2} per gram dried weight of sample (Benzie & Strain, 1998).

 DOI: http://dx.doi.org/10.21548/38-2-2127

DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH•) antioxidant activities of fresh and dried grapes were determined according to Brand-Williams *et al*. (1995). Briefly, 0.1 ml of extract of the samples was added to 3.9 ml of a DPPH (6×10^{-5} M) methanolic solution. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 515 nm. The results were expressed as mmol Trolox equivalent per kilogram dried weight of sample.

ABTS assay

The protocol described by Re *et al*. (1999) was followed to determine the ABTS antioxidant activity of the extracts. To generate the ABTS• radical, 2.5 mL of potassium persulfate solution (2.45 mM) was added to 5 mL ABTS solution (7 mM). The mixture was incubated at room temperature for 16 h. The stock solution was diluted with ethanol to an absorbance of 0.700 ± 0.02 at 734 nm. The extract (10 µL) was added to 990 µL ABTS• solution. The absorbance at 734 nm was measured after 6 min and the reduction in the absorbance was noted. The results were reported as mmol Trolox equivalent per kilogram dried weight of sample.

Analysis of anthocyanin and phenolic compounds

Extracted phenolics from the grapes were purified before the HPLC analysis by using C18 SPE cartridges (Agilent, USA), preconditioned by passing through water, ethyl acetate and methanol. For this purpose, the extract (2 ml) was loaded into the cartridges. Non-anthocyanin phenolics and anthocyanins were eluted with ethyl acetate and methanol respectively. Ethyl acetate and methanol eluates were evaporated at 35°C, re-suspended in 1 mL of methanol and then filtered through a 0.45 µm pore size syringe filter (Sartorius AG, Göttingen, Germany) (Kähkönen *et al*., 2001). The analysis of the phenolic compounds of the extracts was carried out by an Agilent 1260 Infinity Series HPLC system equipped with a diode array detector. Separation was achieved by a reverse phase C18 column (5 μm, 250 × 4.6 mm i.d.). The mobile phase consisted of acetic acid:water and water:acetonitril:acetic acid, and the flow rate was 0.75 ml/min. The detector was set at 280, 306, 320 and 360 nm for non-anthocyanin phenolics and at 520 nm for the anthocyanins (Demir *et al*., 2014). The identification of phenolics was confirmed by comparing their retention times and UV spectra. The data were analysed by ChemStation software.

Statistical analysis

The results were presented as means ± standard deviations (SD) and subjected to one-way analysis of variance (ANOVA), at a confidence level of 95%, to determine the effect of drying methods on total phenolic content, antioxidant activities and individual phenolic compounds. The Duncan multiple range test was used to compare differences between means. Statistical analyses were performed using the MINITAB (Released 14, Minitab Inc. USA) and Mstat C (Mstat C, 1988) programs.

RESULTS AND DISCUSSION

Total phenolic content

The results on the total phenolic contents of fresh and dried grapes are presented in Fig. 1. The highest value was found to be 20.21 mg/g DW in fresh grapes. The phenolic content of freeze-, oven- and sun-dried samples were as follows: 19.83, 16.12 and 10.76 mg/g DW respectively. There were no significant differences between fresh and freeze-dried grapes as far as the total phenolic content was concerned. Statistically, significant decreases occurred in the phenolic content of the oven-dried and sun-dried samples ($p < 0.01$). Sun drying was the most detrimental method for grape drying in terms of losses in the phenolic content.

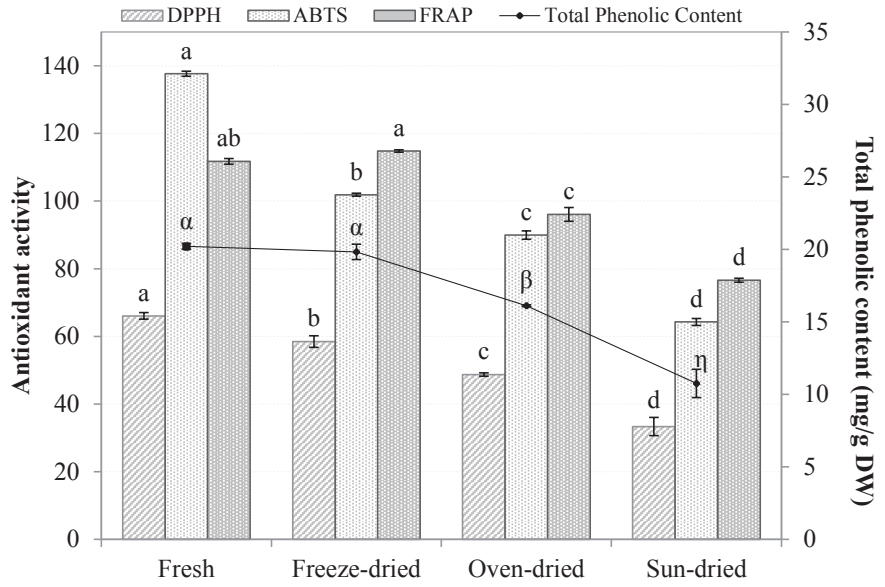

FIGURE 1

DPPH, ABTS and FRAP antioxidant activities and total phenolic content of fresh and dried grapes (DPPH and ABTS values are expressed as mmol trolox equivalent/kg DW, while the FRAP value is given as µmol Fe^{+2}/g DW. Different letters and symbols within each antioxidant activity bar and phenolic content line indicate statistically significant differences ($p < 0.05$)

 DOI: http://dx.doi.org/10.21548/38-2-2127

To the best of our knowledge, no study has been done on comparing the effect of different drying methods on grape phenolics and anthocyanins. On the other hand, researchers have reported distinct findings for phenolic content changes in plant materials owing to the drying process used (Chong *et al*., 2013; Serratosa *et al*., 2011). Serratosa *et al*. (2011) observed significant decreases in the total phenolic content of grape pomace peel after drying at 100 and 140°C. Chong *et al*. (2013) dried apple, pear, papaya and mango cubes by four different methods (hot air-cold air, hot air vacuum-microwave, heat pump and heat pump vacuum-microwave). They reported that all drying methods had a detrimental effect on the total phenolic contents of the fruit. They reported that the most appropriate drying method in terms of the retention of phenolic content was heat pump vacuum-microwave drying.

In contrast, Serratosa *et al*. (2011) investigated the phenolic changes in Merlot and Tempranillo grape musts obtained from chamber-dried grapes. They found increases in the total phenolic contents of grapes after drying, which they attributed to the increase in the dry matter concentration due to water evaporation. Similarly, Bellincontro *et al*. (2004) and Karakaya *et al*. (2001) showed that the phenolic concentration of dried grapes was higher than that of fresh grapes. However, changes in the phenolic concentration in their studies could have seemed to increase because of expressing the results on a wet weight basis.

Differences in factors like the extraction procedures and methods of analysis used in the study and in the expression of results (wet or dry weight basis) make the comparison of our results with previous studies complicated. Drastic decreases in the phenolic compounds of sun-dried and oven-dried grapes could be due to the thermal degradation and/or oxidation of these compounds (Del Caro *et al*., 2004; Figueiredo-González *et al*., 2013; Adiletta *et al*., 2016). Furthermore, pre-treatment with potassium hydroxide solution to remove the waxy layer from grape skin for improving the drying rate may also have promoted phenolic oxidation. Due to the slow drying rate, sun-dried grapes could be exposed to higher oxidation. Although grapes oven dried at 60°C had a lower total phenolic content than freeze-dried and fresh grapes, the oven-drying method compared to the sun-drying method could have protected the phenolic content against prolonged oxidative degradation.

Anthocyanins and phenolic compounds

The phenolic compounds detected in the grapes and their amounts (mg/kg DW) are presented in Table 1. As in the case of total phenolic compounds, individual phenolic compounds were also highly affected by the various drying methods used in this study.

No statistically significant changes in the concentrations of gallic acid, 2,5-dihydroxybenzoic acid and epigallocatechin gallate were found after all the drying methods used. Although freeze drying did not affect the amount of procyanidin B1 in the grapes, the oven- and sun-drying methods showed a destructive effect on the compound. At the same time, procyanidin B1 was maintained better by the oven-drying method compared to sun drying. Similarly, all drying methods reduced the procyanidin B2 content in the grapes. However, decreases in the freeze- and oven-dried samples were found to be statistically insignificant. In sun-dried grapes, procyanidin B2 decreased by 83.6% compared to fresh grapes. The epicatechin content in freeze-, oven- and sun-dried grapes decreased by 25.3%, 61.8% and 85.7% respectively. Decreases in the epicatechin content of sun-dried and oven-dried grapes were 61.8% and 85.7% respectively. As in the case of many other phenolic compounds, the lowest amount of epicatechin was found in sun-dried grapes. However, the differences in epicatechin concentration in

TABLE 1
Phenolic compounds of fresh and dried grapes

	Fresh	Freeze dried	Oven dried	Sun dried
Gallic acid	6.09 ± 0.15	5.99 ± 0.35	7.41 ± 2.03	5.23 ± 0.59
Procyanidin B1	179.19 ± 20.60a*	158.55 ± 12.44a	104.07 ± 20.04b	29.14 ± 13.68c
(+)-Catechin	1340.43 ± 103.98a	965.73 ± 60.55b	505.60 ± 59.19c	184.43 ± 27.47d
Procyanidin B2	227.54 ± 16.45a	167.83 ± 13.22a	174.96 ± 43.32a	37.35 ± 4.89b
(-)-Epicatechin	1 148.76 ± 144.44a	858.03 ± 52.88b	439.14 ± 83.27c	163.83 ± 48.27c
(−)-Epigallocatechin gallate	25.66 ± 4.47	19.48±7.88	23.97 ± 4.64	32.17 ± 1.83
(-)-Epicatechin gallate	431.83 ± 15.82a	316.94 ± 34.82b	116.29 ± 16.90c	34.88 ± 1.34d
2,5-Dihydroxybenzoic acid	10.57 ± 1.37	9.58 ± 2.59	12.07 ± 2.18	17.56 ± 3.24
Chlorogenic acid	32.50 ± 0.36b	119.68 ± 6.37a	22.72 ± 4.00b	20.63 ± 4.49b
Caffeic acid	2.06 ± 0.04b	1.89 ± 0.17b	2.86 ± 0.48b	4.78 ± 0.43a
trans-Resveratrol	4.24 ± 0.04a	4.14 ± 0.20a	0.41 ± 0.07b	nd**
Rutin	56.68 ± 2.98a	58.39 ± 2.50a	28.77 ± 7.19b	22.51 ± 1.86b
Kaempferol-3-glucoside	4.80 ± 1.05bc	3.48 ± 0.28c	14.14 ± 1.01a	13.64 ± 5.60ab
Isorhamnetin-3-*O*-glucoside	12.65 ± 1.45b	12.40 ± 0.34b	13.04 ± 2.87b	20.97 ± 1.69a

* Results are given as mean ± standard deviation (n = 4) and expressed as mg/kg DW. Different letters in the same row indicate statistically significant differences between drying methods ($p < 0.05$)
** non-detectable

 DOI: http://dx.doi.org/10.21548/38-2-2127

oven- and sun-dried samples were statistically insignificant.

Although all three drying methods had a destructive effect on both catechin and epicatechin gallate, the highest amounts of these compounds were found in freeze-dried grapes. At the same time, catechin and epicatechin gallate were maintained better by oven drying compared to sun drying. Even though there was a small increase (nearly 2.9%) in the rutin content of freeze-dried grapes compared to the fresh sample, this increase was not found to be statistically significant. The rutin concentrations in the oven- and sun-dried grapes were lower than in fresh grapes. Similar to our results, Kamiloglu and Capanoglu (2015) reported that the sun-drying method reduced the rutin content of figs by up to 59% compared to fresh figs.

Oven-dried grapes contained 90.4% less resveratrol than the fresh sample, while *trans*-resveratrol was not detected in sun-dried grapes. The loss of *trans*-resveratrol in the freeze-drying method was only 2.3%.

The resveratrol concentration in grapes differs according to factors such as variety and ripening. In addition, fungal infection and UV light exposure induce the stimulation of resveratrol synthesis (Cantos *et al*., 2001). *Cis*-resveratrol is rarely found in grape berries in its free form. The occurrence in free form is attributed to the hydrolysis of glycosides of *cis*-resveratrol and the isomerisation of the *trans* form via UV radiation (Versari *et al*., 2001; Montsko *et al*., 2008). *Trans*-resveratrol transforms to its *cis* isomer when exposed to UV light (Montsko *et al*., 2008). In our study, the trans-resveratrol levels in sun-dried grapes were lower than the threshold level that can be detected by the HPLC. This might have been caused by UV radiation during drying.

The chlorogenic acid concentration in freeze-dried grapes was considerably higher than that in both the fresh and other dried grapes. When compared to many other drying methods, highly porous materials with a higher capacity for rehydration are obtained via freeze drying (Krokida & Maroulis, 1997; Voda *et al*., 2012). The observed increase in the chlorogenic acid content of the freeze-dried grapes could be interpreted as being a result of extraction efficiency.

Caffeic acid and isorhamnetin-3-glucoside were not affected, except by sun drying. Increases of 39.66% and 56.98% were observed in the caffeic acid and isorhamnetin-3-*O*-glucoside contents respectively of sun-dried grapes compared to fresh grapes. One of the other phenolic compounds of which the concentration seemed to increase after drying was kaempferol-3-glucoside. Oven- and sun-dried samples contained 66.06% and 64.81% more kaempferol-3-glucoside than fresh grapes. In contrast to the oven- and sun-drying methods, freeze drying recorded a reduced amount of kaempferol-3-glucoside, at 27.6%. This finding is in complete agreement with the results of Slatnar *et al*. (2011) and Kamiloglu and Capanoglu (2015), who reported an increase in the kaempferol-3-rutinoside content of figs after sun drying.

With regard to anthocyanins, fresh grapes contained fewer individual anthocyanins than the freeze-dried sample (Table 2). However, except for delphinidin-3-glucoside, the increases in petunidin-3-glucoside (24.0%), malvidin-3-glucoside (16.8%), cyanidin-3-glucoside (19.2%) and peonidin-3-glucoside (13.81%) concentrations were found to be statistically insignificant. The concentration of delphinidin-3-glucoside increased by up to 37.5% after freeze drying of the grapes. On the other hand, both the oven- and sun-drying methods reduced the amount of all anthocyanins detected in the grapes dramatically, with no differences between these two drying methods. Decreases in grape anthocyanins after drying can also be seen in the HPLC chromatogram detected at 520 nm (Fig. 2). Unfortunately, as can be seen in Fig. 2, it was not possible to provide the changes in acylated anthocyanin amounts of the grapes after drying due to a lack of pure standards for them in our study.

Wojdyło *et al*. (2009) reported a statistically insignificant increase in the pelargonidin-3-O-glucoside and pelargonidin-3-O-malonyl-glucoside amounts of two different strawberry cultivars (Kent and Elsanta) after freeze drying. They also pointed out that freeze-dried Kent strawberries contained higher amount of cyanidin-3-O-glucoside than the fresh sample.

Our results are consistent with previous studies evaluating the effect of hot air-, oven- and sun-drying methods on individual anthocyanins in fruit (Slatnar *et al*., 2011; Nora *et al*., 2014; Wojdyło *et al*., 2014; Kamiloglu & Capanoglu, 2015). For instance, the drying of guabiju and red guava at 70°C resulted in losses of cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside (48% to 100%) (Nora *et al*., 2014). Similarly, as reported by Wojdyło *et al*. (2014), sour cherry dried at 50°C, 60°C and 70°C had 13% to 38% less cyanidin-3-O-glucoside compared to fresh ones. According to the results of Kamiloglu and Capanoglu (2015), sun drying of yellow and purple figs led to a drastic reduction in the amounts of cyanidin-3-glucoside and cyanidin-3-rutinoside (98%).

TABLE 2
Anthocyanins of fresh and dried grapes

	Delp-3-glu*	**Cyn-3-glu**	**Pet-3-glu**	**Peo-3-glu**	**Malv-3-glu**
Fresh	202.37 ± 13.37b**	122.27 ± 6.54a	189.30 ± 10.11a	478.51 ± 42.06a	760.49 ± 94.70a
Freeze-dried	278.31 ± 16.63a	145.68 ± 20.03a	234.79 ± 40.78a	544.63 ± 56.22a	888.36 ± 123.97a
Oven-dried	5.67 ± 1.22c	0.41 ± 0.08b	4.59 ± 1.10b	6.07 ± 1.96b	11.76 ± 2.55b
Sun-dried	7.04 ± 3.46c	0.88 ± 0.43b	6.52 ± 3.21b	10.60 ± 1.72b	19.77 ± 3.86b

***Dl-3-glu** (delphinidin-3-*O*-glucoside), **Cy-3-glu** (cyanidin-3-*O*-glucoside), **Pt-3-glu** (petunidin-3-*O*-glucoside), **Pn-3-glu** (peonidin-3-*O*-glucoside), **Mv-3-glu** (malvidin-3-*O*-glucoside)

** Different letters in the same column indicate statistically significant differences between drying methods ($p < 0.05$), and the results, given as mean ± standard deviation (n = 4), are expressed as mg/kg DW

 DOI: http://dx.doi.org/10.21548/38-2-2127

All drying methods, except for freeze drying, led to a reduction in each of the anthocyanins of more than 95%. Anthocyanins in grapes were retained better by the freeze-drying method when compared to the other drying methods.

The efficiency of phenolic extraction is affected by various factors, such as product structure, particle size, solvent, solvent-sample ratio and extraction temperature (Haminiuk *et al*., 2012; Coklar & Akbulut, 2016). Higher efficiency could be observed in phenolic extraction by the reduction in particle size, choosing the correct solvent and increasing the porosity of the dried sample. Solvent diffusion through dried tissue accelerates when the sample has higher porosity, which in turn increases the transfer of phenolic compounds to the solvent. The freeze-drying method produces dried products that have greater porosity (80% to 90%) than those produced by convective-, microwave- and

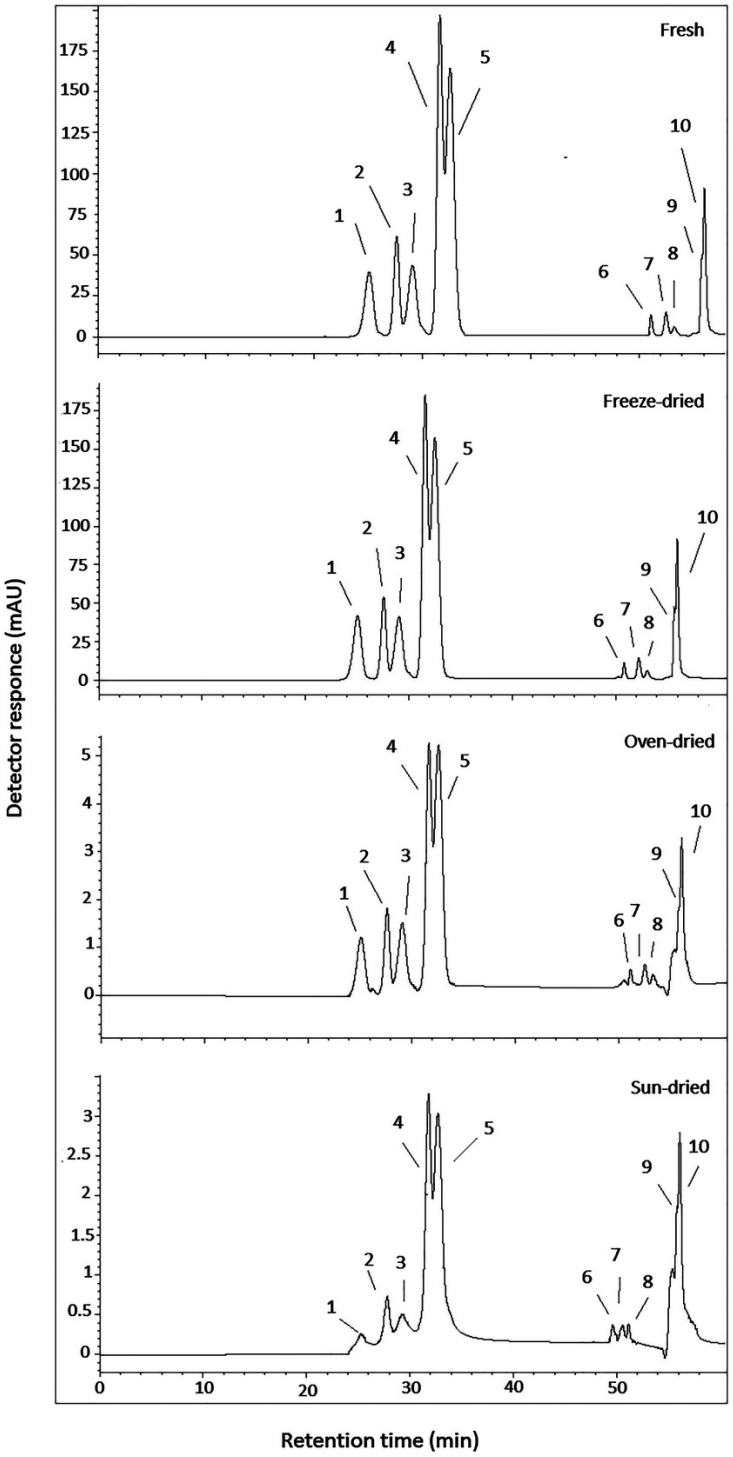

FIGURE 2

HPLC chromatogram of anthocyanins in fresh and dried grapes at 520 nm (1: delphinidin-3-O-glucoside, 2: cyanidin-3-O-glucoside, 3: petunidin-3-O-glucoside, 4: peonidin-3-O-glucoside, 5: malvidin-3-O-glucoside, 6-10: Undefined anthocyanins)

 DOI: http://dx.doi.org/10.21548/38-2-2127

vacuum-drying methods (Joardder *et al*., 2015). The higher concentration of some phenolics in freeze-dried grapes compared to a fresh sample could be attributed to the higher extraction efficiency.

Cell integrity and cell membranes might be damaged during drying, depending on the drying method and pre-treatments (Lewicki, 1998; Ahmad *et al*., 2006), and therefore polyphenols in the plant undergoing drying could be oxidised by polyphenol oxidase (PPO). The optimum temperature of grape PPO differs among grape varieties and ranges from 25°C to 45°C (Zheng *et al*., 2012; Ünal & Şener, 2014). The temperature of the sun-drying method is close to optimum temperature of grape PPO. Polyphenol oxidase activity during long drying might have led to decreases in phenolic compounds.

Antioxidant activity

To determine the changes in antioxidant activity in grapes after drying, three common assays (DPPH, ABTS and FRAP) were used in our research (Fig. 1). The value of DPPH antioxidant activity in fresh grapes was found to be 66.07 mmol TE/kg DW. The value decreased by 12%, 26% and 50% in freeze-, oven- and sun-dried grapes respectively. Statistically significant decreases for the DPPH antioxidant activity value ($p < 0.01$) were observed in all the drying methods. The lowest value was determined in sun-dried samples (33.36 mmol TE/kg DW). The DPPH results for dried grapes were confirmed by the ABTS results in terms of the destructive effect of drying. As with DPPH antioxidant activity, the highest ABTS value was determined in fresh grapes (137.65 mmol TE/kg DW). The ABTS results of the products prepared by freeze-, oven- and sun-drying methods showed statistically significant decreases ($p < 0.01$). In contrast to the DPPH and ABTS antioxidant activity values, the FRAP value of freeze-dried grapes (114.81 µmol Fe^{+2}/g DW) was higher than that of the fresh sample (111.74 µmol Fe^{+2}/g DW). However, the FRAP value of fresh grapes decreased from 111.74 to 96.06 and 76.60 µmol Fe^{+2}/g DW in the oven-dried and sun-dried samples respectively. All differences in FRAP results between the fresh and dried grapes were statistically significant ($p < 0.01$).

Polyphenolics are the primary antioxidants in grapes. There is a close correlation between the amount of phenolics and the antioxidant activity of grapes and grape fractions (Coklar, 2016). The lower antioxidant activities determined in the sun- and oven-dried grapes compared to those of the freeze-dried and fresh grapes could be due to the loss of phenolic compounds.

Previous studies on the impact of different drying techniques on the antioxidant activity of fruit confirm our results (Gao *et al*., 2012; Chong *et al*., 2013; Annegowda *et al*., 2014). Significant reductions in the antioxidant activity of jujube fruit in ABTS from oven- and sun-drying methods were reported in a previous study (Gao *et al*., 2012). Chong *et al*. (2013) reported that the heat pump, heat pump vacuum-microwave, hot air vacuum-microwave and hot air-cold air drying methods significantly reduced the DPPH antioxidant activities of apple, papaya and mango. Another study on the sun drying of yellow and purple figs showed lower values of ABTS, FRAP and DPPH after drying, except for the ABTS value of yellow figs. The authors reported that the decrease in ABTS antioxidant activity of yellow figs was statistically insignificant. In addition, Nunes *et al*. (2016) stated that there were statistically significant decreases in the FRAP and ORAC antioxidant activities of guava fruit after both freeze drying and forced air circulation drying (at 55°C for 22 h).

CONCLUSIONS

Phenolics are biologically active compounds that have positive effects on human health. Grapes are a good source of phenolic compounds, both in terms of their profile and concentration. However, some changes in phenolic compounds could occur through processing. Different drying methods affect the sensorial properties (flavour, appearance, taste) and bioactive compounds (phenolics, anthocyanins, etc.) of fruit. In this study we investigated the impact of freeze-, oven- and sun-drying methods on the phenolic compounds, anthocyanins and antioxidant activity of grapes. The results indicate that freeze drying is the best method to maintain the phenolics and anthocyanins in dried grapes, whereas the lowest levels were seen in sun-dried grapes. However, the sun-drying method has been used more commonly than freeze drying when it comes to dried grape production in the industry. This is because the freeze-drying method is considered expensive. Considering phenolic compounds, antioxidant activity and production costs, the oven-drying method could be an alternative to sun drying. Nevertheless, further experimental investigations are needed to improve product quality in the oven-drying method.

LITERATURE CITED

Adiletta, G., Russo, P., Senadeera, W. & Di Matteo, M., 2016. Drying characteristics and quality of grape under physical pretreatment. J. Food Eng. 172, 9-18.

Ahmad, I., Aqil, F. & Owais, M., 2006. Modern phytomedicine: Turning medicinal plants into drugs. Wiley-VCH, Weinheim, Germany.

Akbulut, M., Calisir, S., Marakoglu, T. & Coklar, H., 2008. Chemical and technological properties of European cranberrybush (*Viburnum opulus* L.) fruits. Asian J. Chem. 20, 1875.

Angulo, O., Fidelibus, M.W. & Heymann, H., 2007. Grape cultivar and drying method affect sensory characteristics and consumer preference of raisins. J. Sci. Food Agric. 87, 865-870.

Annegowda, H., Bhat, R., Yeong, K.J., Liong, M.T., Karim, A. & Mansor, S., 2014. Influence of drying treatments on polyphenolic contents and antioxidant properties of raw and ripe papaya (*Carica papaya* L.). Int. J. Food Prop. 17, 283-292.

Armstrong, D. & Stratton, R.D., 2016. Oxidative stress and antioxidant protection: The science of free radical biology and disease. Hoboken, NJ, USA.

Ban, T., Ishimaru, M., Kobayashi, S., Goto-Yamamoto, N. & Horiuchi, S., 2003. Abscisic acid and 2,4-dichlorophenoxyacetic acid affect the expression of anthocyanin biosynthetic pathway genes in 'Kyoho' grape berries. J. Hortic. Sci. Biotechnol. 78, 586-589.

Bellincontro, A., De Santis, D., Botondi, R., Villa, I. & Mencarelli, F., 2004. Different postharvest dehydration rates affect quality characteristics and volatile compounds of Malvasia, Trebbiano and Sangiovese grapes for wine production. J. Sci. Food Agric. 84, 1791-1800.

Benzie, I. & Strain, J., 1998. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 299, 15-27.

Brand-Williams, W., Cuvelier, M.E. & Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28, 25-30.

Cantos, E., Espín, J.C. &Tomás-Barberán, F.A., 2001. Postharvest induction modeling method using UV irradiation pulses for obtaining resveratrol-enriched table grapes: A new "functional" fruit? J. Agric. Food Chem. 49, 5052-5058.

Chong, C.H., Law, C.L., Figiel, A., Wojdyło, A. & Oziembłowski, M., 2013. Colour, phenolic content and antioxidant capacity of some fruits dehydrated by a combination of different methods. Food Chem. 141, 3889-3896.

Coklar, H., 2016. Antioxidant capacity and phenolic profile of berry, seed and skin of Ekşikara (*Vitis vinifera* L) grape: Influence of harvest year and altitude. Int. J. Food Prop. doi:10.1080/10942912.2016.1230870

Coklar, H. & Akbulut, M., 2016. Effect of different solvents on extraction of phenolic compounds and antioxidant activity of hawthorn (*Crataegus orientalis*) fruits. Derim. 33, 237-248.

Coklar, H. & Akbulut, M., 2017. Anthocyanins and phenolic compounds of *Mahonia aquifolium* berries and their contributions to antioxidant activity. J. Funct. Foods 35, 166-174.

Del Caro, A., Piga, A., Pinna, I., Fenu, P.M. & Agabbio, M., 2004. Effect of drying conditions and storage period on polyphenolic content, antioxidant capacity, and ascorbic acid of prunes. J. Agric. Food Chem. 52, 4780-4784.

Demir, N., Yildiz, O., Alpaslan, M. & Hayaloglu, A., 2014. Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (Rosa L.) fruits in Turkey. Lebensm. Wiss. Technol. 57, 126-133.

De Torres, C., Díaz-Maroto, M.C., Hermosín-Gutiérrez, I. & Pérez-Coello, M.S., 2010. Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. Anal. Chim. Acta 660, 177-182.

Figueiredo-González, M., Cancho-Grande, B. & Simal-Gándara, J., 2013. Evolution of colour and phenolic compounds during Garnacha Tintorera grape raisining. Food Chem. 141, 3230-3240.

Gao, Q.H., Wu, C.S., Wang, M., Xu, B.N. & Du, L.J., 2012. Effect of drying of jujubes (*Ziziphus jujuba* Mill.) on the contents of sugars, organic acids, α-tocopherol, β-carotene, and phenolic compounds. J. Agric. Food Chem. 60, 9642-9648.

Haminiuk, C.W., Maciel, G.M., Plata□Oviedo, M.S. & Peralta, R.M., 2012. Phenolic compounds in fruits – An overview. Int. J. Food Sci. Tech. 47, 2023-2044.

INC, 2016. Global statistical review 2014-2015 [Online]: https://www.nutfruit.org/wp-continguts/uploads/2015/11/global-statistical-review-2014-2015_101779.pdf [accessed on 10 December 2016].

Jairaj, K., Singh, S. & Srikant, K., 2009. A review of solar dryers developed for grape drying. Solar Energy 83, 1698-1712.

Joardder, M.U., Karim, A., Kumar, C. & Brown, R.J., 2015. Porosity: Establishing the relationship between drying parameters and dried food quality. Springer, Switzerland.

Kähkönen, M.P., Hopia, A.I. & Heinonen, M., 2001. Berry phenolics and their antioxidant activity. J. Agric. Food Chem. 49, 4076-4082.

Kamiloglu, S. & Capanoglu, E., 2015. Polyphenol content in figs (*Ficus carica* L.): Effect of sun-drying. Int. J. Food Prop. 18, 521-535.

Kamiloglu, S., Toydemir, G., Boyacioglu, D., Beekwilder, J., Hall, R.D. & Capanoglu, E., 2016. A review on the effect of drying on antioxidant potential of fruits and vegetables. Crit. Rev. Food Sci. Nutr. 56, 110-129.

Karakaya, S., El, S.N. & Taş, A.A., 2001. Antioxidant activity of some foods containing phenolic compounds. Int. J. Food Sci. Nutr. 52, 501-508.

Kong, J.M., Chia, L.S., Goh, N.K., Chia, T.F. & Brouillard, R., 2003. Analysis and biological activities of anthocyanins. Phytochemistry 64, 923-933.

Krokida, M. & Maroulis, Z., 1997. Effect of drying method on shrinkage and porosity. Dry Technol. 15, 2441-2458.

Larrauri, J.A., Rupérez, P. & Saura-Calixto, F., 1997. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. J. Agric. Food Chem. 45(4), 1390-1393.

Lewicki, P.P., 1998. Effect of pre-drying treatment, drying and rehydration on plant tissue properties: A review. Int. J. Food Prop. 1, 1-22.

Montealegre, R.R., Peces, R.R., Vozmediano, J.C., Gascueña, J.M. & Romero, E.G., 2006. Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. J. Food Comp. Anal. 19, 687-693.

Montsko, G., Nikfardjam, M.S.P., Szabo, Z., Boddi, K., Lorand, T., Ohmacht, R. & Mark, L., 2008. Determination of products derived from trans-resveratrol UV photoisomerisation by means of HPLC–APCI-MS. J. Photochem. Photobiol. A. 196, 44-50.

Nicoue, E.E., Savard, S. & Belkacemi, K., 2007. Anthocyanins in wild blueberries of Quebec: Extraction and identification. J. Agric. Food Chem. 55, 5626-5635.

Nora, D., Cleice, M., Dal-Ri, C., De Bona, G.S., De Oliveira Rios, A., Hertz, P.F., Jablonski, A., De Jong, E.V. & Flôres, S.H., 2014. Effect of processing on the stability of bioactive compounds from red guava (*Psidium cattleyanum* Sabine) and guabiju (*Myrcianthes pungens*). J. Food Comp. Anal. 34, 18-25.

Nunes, J.C., Lago, M.G., Castelo-Branco, V.N., Oliveira, F.R., Torres, A.G., Perrone, D. & Monteiro, M., 2016. Effect of drying method on volatile compounds, phenolic profile and antioxidant capacity of guava powders. Food Chem. 197, 881-890.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. 26, 1231-1237.

Serratosa, M.P., Marquez, A., Lopez-Toledano, A., Medina, M. & Merida, J., 2011. Changes in hydrophilic and lipophilic antioxidant activity in relation to their phenolic composition during the chamber drying of red grapes at a controlled temperature. J. Agric. Food Chem. 59, 1882-1892.

Singleton, V. & Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144-158.

Slatnar, A., Klancar, U., Stampar, F. & Veberic, R., 2011. Effect of drying of figs (*Ficus carica* L.) on the contents of sugars, organic acids, and phenolic compounds. J. Agric Food Chem. 59, 11696-11702.

Tseng, A. & Zhao, Y., 2012. Effect of different drying methods and storage time on the retention of bioactive compounds and antibacterial activity of wine grape pomace (Pinot Noir and Merlot). J. Food Sci. 77(9), 192-201.

TSI, 2016. Crop production statistics [Online]: https://biruni.tuik.gov.tr/bitkiselapp/bitkisel_ing.zul [accessed on 10 December 2016].

Ünal, M.Ü. & Şener, A., 2014. Effect of harvest year on biochemical properties of Narince grape (*Vitis vinifera* L. cv. Narince) polyphenol oxidase. Eur. Food Res. Technol. 238, 613-619.

USDA, 2016. Fresh deciduous fruit: World markets and trade (apples, grapes, & pears) [Online]: https://apps.fas.usda.gov/psdonline/circulars/fruit.pdf [accessed on 10 December 2016].

Versari, A., Parpinello, G.P., Tornielli, G.B., Ferrarini, R. & Giulivo, C., 2001. Stilbene compounds and stilbene synthase expression during ripening, wilting, and UV treatment in grape cv. Corvina. J. Agric. Food Chem. 49, 5531-5536.

Voda, A, Homan, N., Witek, M., Duijster, A., Van Dalen, G., Van der Sman, R., Nijsse, J., Van Vliet, L., Van As, H. & Van Duynhoven, J., 2012. The impact of freeze-drying on microstructure and rehydration properties of carrot. Food Res. Int. 49: 687-693.

Wojdyło, A., Figiel, A., Lech, K., Nowicka, P. & Oszmiański, J., 2014. Effect of convective and vacuum–microwave drying on the bioactive compounds, color, and antioxidant capacity of sour cherries. Food Bioprocess Tech. 7, 829-841.

Zheng, Y., Shi, J. & Pan, Z., 2012. Biochemical characteristics and thermal inhibition kinetics of polyphenol oxidase extracted from Thompson seedless grape. Eur. Food Res. Technol. 234, 607-616.