Comparison of Total Phenolic Content, Antioxidant Activity and *Trans*-Resveratrol Content of Fresh Red Grapes and Raisin Ethanolic Extracts

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Abstract

The aim of this research was to determine the total phenolic content, antioxidant activity, and *trans*-resveratrol content of red grape ethanolic extracts obtained from 2 varieties from Thailand and 4 international varieties and raisin ethanolic extracts. All grape extracts showed total phenolic value range of 165.90 ± 4.49 to 272.21 ± 1.96 mg GAE/g extract. For antioxidant activities determined by DPPH and TEAC methods, all the extracts showed significant antioxidant activities at $p < 0.05$. The *trans*-resveratrol content was found at the highest value of Cardinal grape (42.47 ± 0.41 µg/g extract). Heat treatments to Cardinal grape at temperatures of 60 °C and 70 °C for 24, 48 and 72 hours resulted in a decrease of total phenolic content, antioxidant activity and trans-resveratrol content compared with unheated grape.

Keywords : Grape, Oven Drying, Total Phenolic Content, Antioxidant Activity, *Trans*-Resveratrol Content

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Introduction

The grape or table grape (*Vitis vinifera* Linn.) is a plant grown widely in many countries. Its prevalence can be attributed to its commercial versatility; it can be consumed as fresh produce and used in the production of several products such as wine, jam, grape seed oil, grape seed extract supplements and cosmetic products. Grape have a high content of phenolic compounds consisting of mostly gallic acid and flavonoids such as catechin, epicatechin, quercetin, rutin, anthocyanin and resveratrol (3,4',5-trihydroxy-trans-stilbene) (Romero-Perez et al., 2001; Roldán et al., 2003; Iacopini et al., 2008; Karakaya et al., 2001; Franco et al., 2004; Zhao & Hall, 2008; Meng et al., 2011; Moo-Huchin et al., 2015). The presence of these compounds makes grapes a common ingredient in resveratrol supplements because of their high antioxidant activity (Rocha et al., 2009; Prasad, 2012). As an antioxidant compound, resveratrol may also affect several physiological aspects of fruit during storage (Urena et al., 2003). On the other hand, many factors are related with other parameters that have a direct influence on the grape, such as the variety, climate, soil and sanitary stage. These factors can modify the concentrations of resveratrol and its derivatives in the grape during harvest and product processing of the grape (Roldán et al., 2003).

The most popular processed grape product is the dried fruit called a "raisin" (Jara-Palacios et al., 2014), which has been a favorite around the world. It has a high nutritional value and is a good source of necessary vitamins and minerals. Drying the grape into raisins is often done using solar energy, particularly in tropical areas. Alternatively, grape can be dried mechanically by an oven drying process or artificially dried using sulfur dioxide treatment (Mary & Michael, 2003; Fadhel et al., 2005). Drying methods are known to have a significant impact on the sensory characteristics of color, texture and nutritional value due to the high temperatures and long drying times required in the process (Tarhan, 2007). Furthermore, the dehydration process decreased the antioxidant activity and polyphenol content of raisin (Erenturk et al., 2005). While there have been many reports on the assessment of phytochemical compounds and their antioxidant activity of grape. However, no research has been reported on comparison of antioxidant activity and *trans*-resveratrol content in red grape varieties and raisin ethanolic extracts.

Objectives

The main objectives of this study were to determine the total phenolic content, antioxidant activity and *trans*-resveratrol content of extracts from different grape varieties extracts (2 grown in Thailand and 4 international imported) and raisin extract. The study also examined the effect of heat treatment of raisins at different on total phenolic content, antioxidant activity and *trans*-resveratrol content.

Methods

1. Chemicals and apparatus

DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-etramethylchroman-2-carboxylic acid), Folin-Ciocalteu phenol reagent, ABTS (2,2′-azinobis-(3-ethylbenz-thiaziline-6-sulfonic acid) and *trans*-resveratrol (3,4',5-trihydroxy-*trans*-stilbene) were purchased from Sigma Chemical Co. Acetonitrile for chromatographic analysis was analytical grade and purchased from Sigma Chemical Co. All other chemicals used were analytical grade.

2. Sampling

Two varieties of red grape seed were examined, namely Cardinal and seedless, namely Harmony from Thailand and four varieties of Red globe imported from the USA. Harmony imported from China, Beauty and Flame seedless varieties imported from the USA. The grape samples were purchased Thai market, and collected according to the uniformity of shape and color in the commercially berry ripening stage, during April – July 2016.

3. Raisin preparation

One hundred grams of Cardinal grape were washed with tap water, spread in a hot air drying (MEMMERT Co., Germany) and then air-dried at temperatures of 40 °C, 50 °C, 60 °C and 70 °C. The samples were removed consecutively after 24, 48 and 72 hours for analysis. Raisin moisture (%) in weight was calculated relative to the initial weight.

4. Extraction

According to the reference, the extraction was greatly modified. (Zhao & Hall, 2008). Fresh grape and Cardinal raisin (CR) (100 g) were blended and macerated with 300 mL of 95% ethanol for 24 hours at room temperature. The extract was filtered and rinsed with 20 mL of 95 % ethanol. The solvent was evaporated to dryness by the rotary vacuum evaporator (EYELA ACE CA-1100, Switzerland) at 40 °C. The extraction was carried out in triplicate. Yields were expressed in the present and calculated as the percentage of gram of extract.

5. Determination of total phenolic content (TP)

Total phenolic content was determined with the Folin-Ciocalteu reagent according to a procedure described by Singleton & Rossi (1965); Parkpoom et al. (2016). Briefly, 0.50 mL of the diluted sample with methanol was reacted with 1 mL of 10 % Folin-Ciocalteu reagent in water for 5 min, and then 2 mL saturated sodium carbonate solution (about 75 g/L) was added to the reaction mixture. The absorbance readings were taken at 760 nm (UV-VIS spectrophotometer; Shimadzu UV-2401 PC, Japan) after incubation at room temperature for 1 hour. Gallic acid was used as a reference standard, and the results were expressed as milligrams of gallic acid equivalent (mg GAE)/g extract.

6. Determination of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according to a slightly modified method proposed by Proestos et al. (2013); Waranusantigul et al. (2016); Koodkaew & Limpichotikul (2017). In this procedure, the reduction of the radical is followed by the decrease in the absorbance at 517 nm. A volume of 2 mL of the methanolic stock solution of the extracts was put into the test tube and 2 mL of 0.2 mM DPPH solution was added. The tube was covered with parafilm and placed in the dark for 1 hour. Absorbance at 517 nm was measured by the UV-Visible spectrophotometer (Shimadzu UV-2401 PC, Japan) and compared to Trolox calibration curves. The results were expressed as μM Trolox/g extract, respectively. Each assay was carried out in triplicate.

7. Determination of trolox equivalent antioxidant capacity assay (TEAC)

The TEAC assay was carried out according to the method of Re et al., (1999); Parkpoom et al. (2016), which is based on the capacity of the sample to inhibit the ABTS radical ($ABTS^{\bullet+}$) compared with a reference antioxidant standard (Trolox). Firstly, to produce the radical cation $ABTS^{\bullet+}$, 7 mmol/L ABTS salt and 2.45 mmol/L potassium persulfate were mixed in a volume ratio of 1:1; the reaction mixture was allowed to stand in the dark for 16 hours at room temperature and was used within two days of preparation. The $ABTS^{\bullet+}$ radical solution was diluted with ethanol to an absorbance of 0.7 ± 0.05 at 734 nm. All samples were diluted to provide approximately 20-80 % inhibition of the blank absorbance. 500 µL of the diluted sample was mixed with 4 mL $ABTS^{\bullet+}$ working solution, the reaction mixture was left at room temperature to react for 6 min, and then the absorbance at 734 nm was taken using the UV-visible spectrophotometer. Trolox solution was used as a reference standard, and the results were expressed as µM Trolox/g extract

8. Determination of *trans*-resveratrol content

The *trans*-resveratrol content was carried out according to the method of Romero-Pérez et al. (2001) using an Agilent 1200 HPLC system (Waldbronn, Germany) equipped with an Agilent 1200 series DAD detector and autosampler. Separation was achieved using a ZORBAX Eclipse XDB-C18 column (150 mm × 4.6 mm id, 5 µm packing; Agilent, Santa Clara, CA, USA), with a precolumn of the same material; the column temperature was maintained at 40 °C. The HPLC conditions were described previously. The elution profile was as follows: 0 min, 83.5 % A, 16.5 % B; 13 min, 82.0 % A, 18.0 % B; 15 min, 82.0 % A, 18.0 % B; 17 min, 77.0 % A, 23.0 % B; 21 min, 75.0 % A, 25.0 % B; 27 min, 68.5 % A, 31.5 % B; 30 min, 0 % A, 100 % B, where solvent A was glacial acetic acid in water (52.6: 900 v/v) and solvent B was 20% phase A and 80 % acetonitrile at a flow rate of 1.0 mL/min. Identification of *trans*-Resveratrol was carried out by comparison of the retention time of the standard and that within the extracts. Calibration curves were plotted from 10 to 1000 µg/mL. The samples (100 µL) were directly injected after filtration through a 0.45 µm membrane filter. A photodiode array detector was used and quantification was done at 306 nm for *trans*-resveratrol.

9. Statistical analysis

Three replicates of each sample were used for statistical analysis and the result of total phenolic content, antioxidant activity and total *trans*-resveratrol presented as mean ± SD. Analysis of variance was performed by one-way ANOVA procedures (IBM statistics SPSS ver. 23). Significant differences were calculated by Duncan's multiple range test. The difference at $p < 0.05$ were considered statistically significant.

Results and Discussions

1. Appearance of raisins

The appearances of the fresh grapes compared with the raisin from heat treatment at different temperatures was shown in Fig. 1. Heat treatment at 40 ℃ and 50 ℃ (Fig. 1b and c) for 72 hours did not change the berry size and color of raisins compared to fresh grapes (Fig. 1a). However, the heat treatment at 60 ℃ for 72 hours (Fig. 1d) resulted in drastically wrinkled skin that resembled commercial raisins; the berry size was reduced and the skin changed to brown. Heat treatment at 70 ℃ for 72 hours resulted in black, hardened, color sticky skin (Fig. 1e). Heat treatment at 60 ℃ is suitable for raisin production (Zhao & Hall, 2008), and this condition showed significantly higher color intensity, indicating more phenolic compound degradation than fresh grapes (Erenturk et al., 2005; Tarhan, 2007; Pedroza et al., 2012).

The moisture content of raisins was measured by drying the grapes with temperatures of 40 ℃, 50 ℃, 60 ℃ and 70 ℃ for 24, 48 and 72 hours; the results are shown in Fig. 2. Increasing temperatures in the oven resulted in lower moisture content. Drying temperatures at 40 ℃ and 50 ℃ showed higher of moisture content because these temperatures lowered the amount of water lost. However, when the temperature increased to 60 ℃ and 70 ℃, the percentages of water lost were markedly different. The percentage of water lost increased more than 50 % of their initial weight and resulted in raisins with lower moisture content.

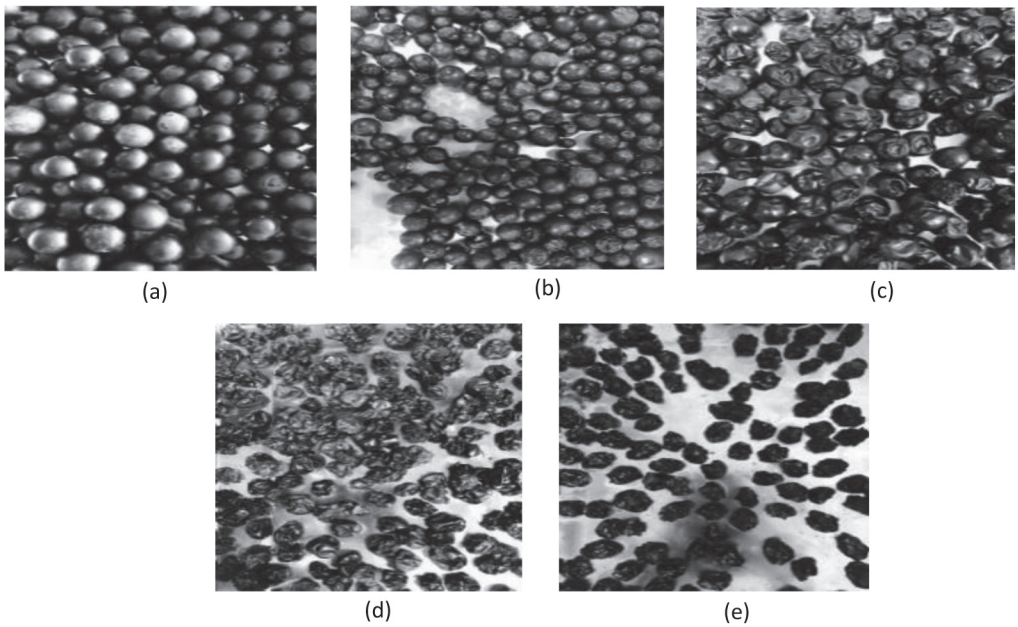

Fig. 1 Appearance of the fresh grape (a) and raisin (b-e) obtained from oven drying 72 hours at different drying temperature (b) 40 ℃, (c) 50 ℃, (d) 60 ℃ and (e) 70 ° C

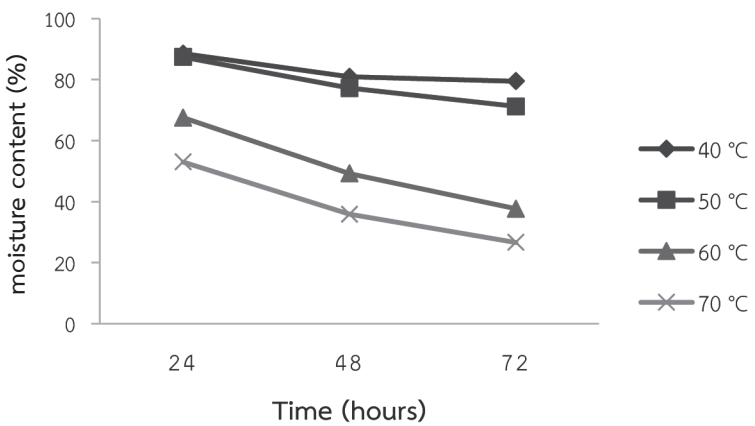

Fig. 2 Correlations of moisture content (%) between drying temperatures and drying times

2. Total phenolic content

Fig. 3 showed the total phenolic content of crude extracts. The results showed that total phenolic contents in the red grape extracts from 95% ethanol differed significantly ($p < 0.05$) in all sample extracts of the different grape varieties. However two samples of Thailand Cardinal (229.82 ± 1.76 mg GAE/g extract) and Harmony (238.28 ± 4.90 mg GAE/g extract, showed not significant differences. The total phenolic values of Harmony (China), Flame seedless and Beauty seedless were 165.90 ± 4.49, 167.60 ± 7.39 and 272.21 ± 1.96 mg GAE/g extract, respectively. Beauty seedless varieties showed the highest value and Harmony (China) showed the lowest. As expected, for all the varieties analyzed, there was greater variability than has been previously reported (Xu et al., 2010; Rockenbach et al., 2011, Derra et al., 2012); this inconstancy was attributed to multiple factors, including climate, berry size and grapevine variety (Du et al., 2012).

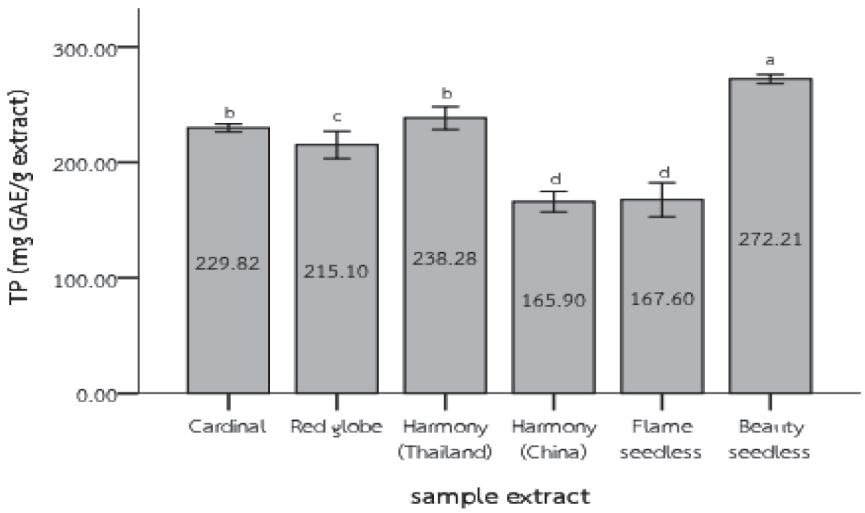

Fig. 3 Total phenolic content of grape varieties extract

Remark: $^{a-d}$, Significant different at $p < 0.05$ by Duncan's test

Heat treatment on Cardinal grape at temperatures of 60 °C and 70 °C for 24, 48 and 72 hours (Fig. 4), resulted in significant decreases in total phenolic content. When grapes were heated at temperatures of 60 °C and 70 °C for 24 hours, the results showed a fast degradation of phenolic compounds (Sólyom et al., 2014). This is because, the heating temperature of the experiment give rise to polyphenol oxidase enzyme activity in the material (Larrauri et al., 1997; Pascariu et al., 2014).

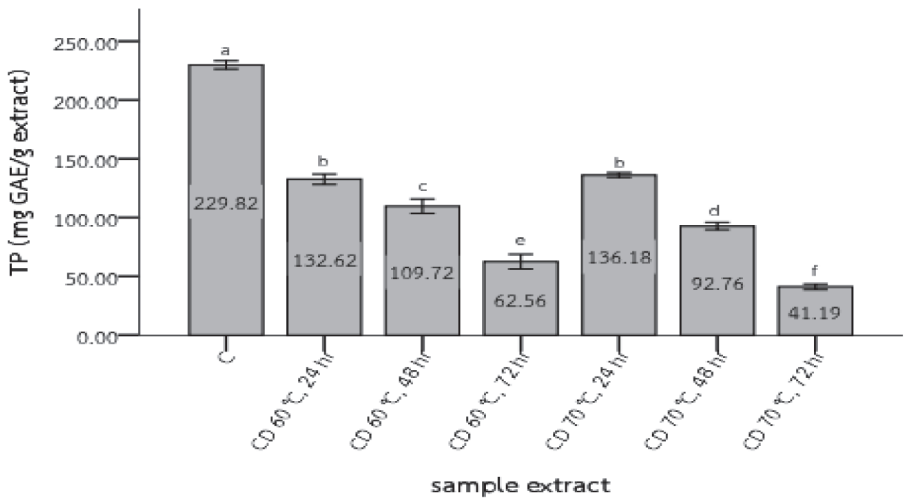

Fig. 4 Total phenolic content of Cardinal grape (C) and dehydrated grape extract (CD) heated at temperatures of 60 °C and 70 °C for 24, 48 and 72 hours

Remark: $^{a\text{-}f}$, Significant different at $p < 0.05$ by Duncan's test

3. Antioxidant activity

As shown in Table 1, the highest antioxidant activity obtained from DPPH and TEAC methods was found in 'Cardinal grape' extract with values of 42.29 ± 0.30 µM Trolox /g extract and 76.00 ± 0.46 µM Trolox /g extract, respectively. The lowest antioxidant activity was found in 'Harmony seedless grapes' (China) and showed activity values of 19.38 ± 0.94 µM Trolox /g extract and 23.40 ± 0.81 µM Trolox /g extract, respectively. Similar research has shown that the distribution and composition of phenolic compounds and their antioxidant activity are affected by geographic origin, growing season, post-

harvest storage conditions, processing and plant growth regulators (Kim et al., 2003; Jiang et al., 2006; Hulya-Orak, 2007). These are limitations of this study; previous studies have shown influence of these factors on the chemical compounds and antioxidant activity.

Table 1 Antioxidant activities of Grape extract from different variety determined

Grapes extract	DPPH activity (μM Trolox /g extract)	TEAC activity (μM Trolox /g extract)
Cardinal	42.29 ± 0.30[a]	76.00 ± 0.46[a]
Red globe	27.85 ± 0.41[d]	34.77 ± 0.30[d]
Harmony (Thailand)	33.50 ± 0.98[c]	39.73 ± 0.70[c]
Harmony (China)	19.38 ± 0.94[e]	23.40 ± 0.81[f]
Flame seedless	26.61 ± 0.91[d]	25.65 ± 0.52[e]
Beauty seedless	36.68 ± 0.27[b]	51.26 ± 0.33[b]

Remark: [a-f], Significant different at $p < 0.05$ by Duncan's test with in the same column

Table 2 Antioxidant activity of raisin extracts obtained from different drying temperature and time

Extracts	DPPH activity (μM Trolox /g extract)	TEAC activity (μM Trolox /g extract)
Cardinal	42.29 ± 0.30[a]	76.00 ± 0.46[a]
CD 60 °C 24 hours	30.65 ± 0.12[c]	54.95 ± 0.25[b]
CD 60 °C 48 hours	21.93 ± 0.38[d]	42.69 ± 0.78[c]
CD 60 °C 72 hours	13.92 ± 0.28[f]	29.69 ± 0.66[e]
CD 70 °C 24 hours	33.65 ± 0.51[b]	55.49 ± 0.19[b]
CD 70 °C 48 hours	21.29 ± 0.06[e]	41.65 ± 0.35[d]
CD 70 °C 72 hours	10.66 ± 0.56[g]	24.17 ± 0.72[f]

Remark: [a-f], Significant different at $p < 0.05$ by Duncan's test with in the same column

Antioxidant was analyzed by DPPH and TEAC activity methods. Antioxidant activity of raisin extracts compared with grape extracts (Table 2) showed behaviors to those observed for total phenolic content. Heat treatment at 60 ℃ and 70 ℃ for 24 hours caused a decrease at the beginning of the process. Our results indicate that of drying temperatures and time are important for keeping the antioxidant activity in fresh grape.

4. *trans*-Resveratrol content

The *trans*-resveratrol content of both grape extracts were measured using a wavelengths monitor; a peak was observed at 306 nm and the retention time of the expected *trans*-resveratrol peak was the same as that of the standard compound. The mean *trans*-resveratrol content was shown in Fig. 5. Resveratrol was found at the highest values in Cardinal grapes (42.47 ± 0.41 μg/g extract) and *trans*-resveratrol content of Harmony (Thailand), Flame seedless, Beauty seedless and Harmony (Chaina) were 30.07 ± 0.40, 28.09 ± 0.66, 27.14 ± 0.55 and 26.67 ± 1.08 μg/g extract, respectively. Red Globe variety showed the lowest value (22.58 ± 0.70 μg/g extract). The latter confirmed that resveratrol content is largely dependent on grape variety (Iacopini et al., 2008; Lee et al., 2014). Fig. 6 shows the effect of temperature on *trans*-resveratrol content. Heat treatment at 60 °C and 70 °C for 24 hours resulted in rapid decreases of *trans*-resveratrol content from 42.47 ± 0.41 μg/g extract of fresh grape to 10.06 ± 0.12 and 11.82 ± 0.03 μg/g extract, respectively. On the other hand, heating at 60 ℃ and 70 ℃ for 72 hours reduced the concentration to 2.32 ± 0.07 and 2.88 ± 0.09 μg/g extract, respectively.

This study observed rapid degradation after heat treatment of grape, in terms of total phenolic content, antioxidant activities (Larrauri et al., 1997) and *trans*-resveratrol content (Zupančič et al., 2015).

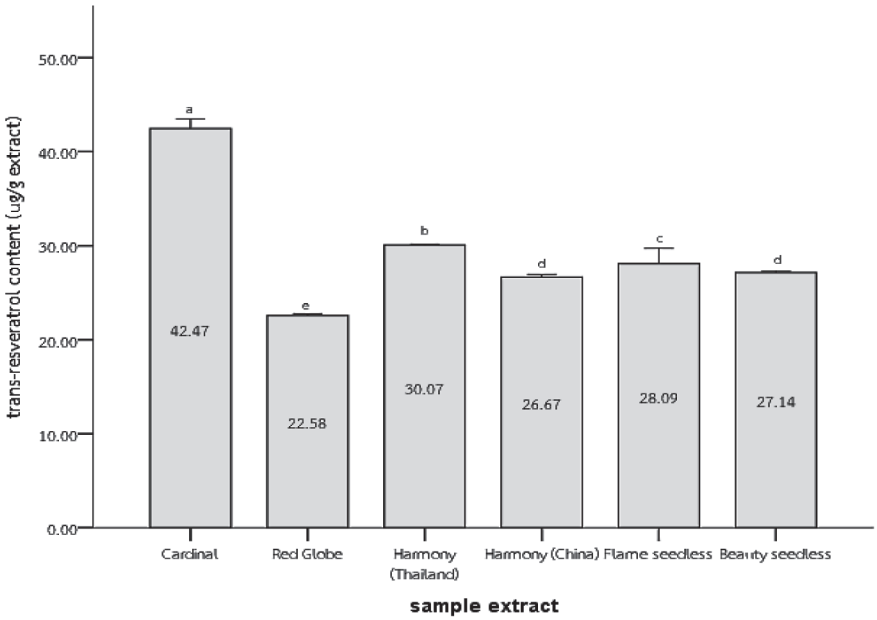

Fig. 5 *Trans*-resveratrol content of grape extracts obtained from different varieties

Remark: [a-e,] Significant different at $p < 0.05$ by Duncan's test with each bar mean

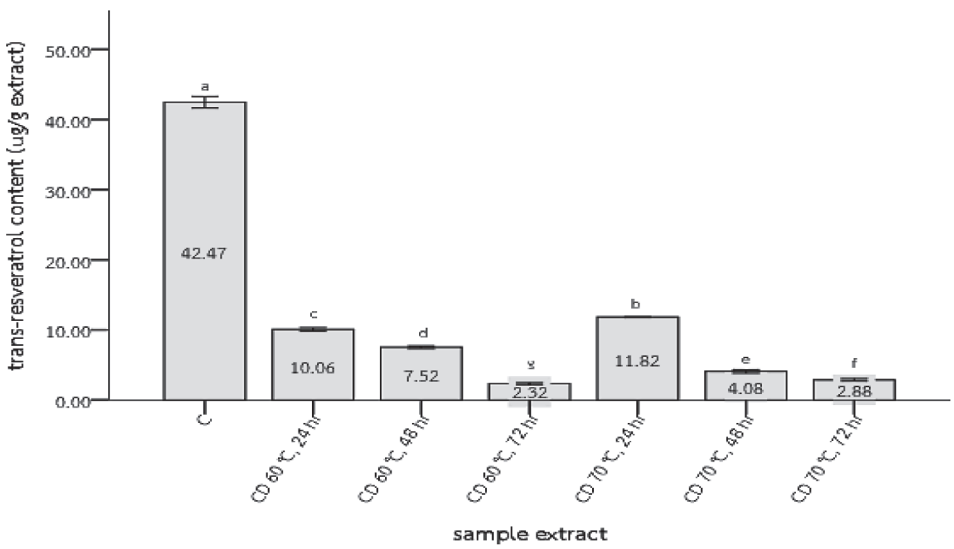

Fig. 6 *Trans*-resveratrol content of Cardinal grape (C) and raisin extracts (CD) heated at 60 °C and 70 °C for 24, 48 and 72 hours

Remark: [a-f,] Significant different at $p < 0.05$ by Duncan's test

Conclusion

The extracts obtained from the grapes of 2 Thailand varieties (Cardinal and Harmony seedless), 4 international varieties (Harmony from China, Red globe, Flame, and Beauty seedless from the USA) and raisin were evaluated for their total phenolic content, antioxidant activity, and trans-resveratrol content. All of the samples showed the total phenolic values ranged from 165.9 to 272.21 mg GAE/g extract. Cardinal grapes showed the highest DPPH and TEAC activity values of 42.29 ± 0.30 and 76.00 ± 0.46 μM Trolox /g extract, respectively. *Trans*-resveratrol content was found at highest value in Cardinal grapes (42.47 ± 0.41 μg/g extract). The effect of heat treatments to Cardinal grapes at temperature 60 °C and 70 °C for 24, 48 and 72 hours showed decreases of total phenolic content, antioxidant activity and *trans*-resveratrol content compared with the fresh grapes.

Acknowledgement

The authors wish to thanks the Department of Chemistry Technology and the Environmental Center, Faculty of Science and Technology, Suan Dusit University, Thailand for support.

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