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Composition and antioxidant activity of raisin extracts obtained from various solvents

Bin Zhao [a], Clifford A. Hall III [b,*]

[a] Kraft Foods Inc., 200 DeForest Avenue, East Hanover, NJ 07936, USA
[b] Department of Cereal and Food Sciences, 210 Harris Hall, North Dakota State University, Fargo, ND 58105, USA

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Abstract

Experiments were conducted to determine if the contents of phenolics and browning reaction products and antioxidant activity of raisin extracts were closely dependent on the extraction solvent. Enhanced extraction yields were obtained from solvent containing higher water concentrations. However, total phenolic content (TPC) was highest for extracts obtained from solvent to water ratios of 60:40 (v/v), whereby the extract obtained from ethanol:water (60:40, v/v) had the highest TPC of 375 mg gallic acid equivalents (GAE)/100 g extract. HMF content was highest in extracts obtained from 60% solvent, regardless of solvent type. The extract obtained from 60% methanol had the highest HMF content at 199 μg/g extract. Although the 60% solvents provided extract with high antioxidant components, the antioxidant activity of raisin extracts obtained from 80:20 (v/v) solvent/water was significantly higher than other raisin extracts obtained from different solvent concentrations. Phenolic acids, HMF, and low-molecular-weight flavonoids were responsible for the antioxidant activity, but not the high-molecular-weight flavonoids.

Keywords: Antioxidant activity; Raisin extract; Bulk oil; Solvent extraction; HMF; Phenolic compounds

1. Introduction

Raisins have been a favorite food since 1490 BC due to their nutritive value and high micronutrients content (Witherspoon, 2000). Raisins rank among the highest in the concentration of total phenolic compounds and have the highest levels of total antioxidant activity among solid fruit products (Karakaya, El, & Tas, 2001). Yeung, Glahn, Wu, Liu, and Miller (2003) reported that the antioxidant activity of raisins produced by different processing methods was due to phenolic compounds; thus, supporting the use of raisin extracts as antioxidants in food systems. The high phenolic content of raisins is ideal as extracts produced from raisins would also have high phenolic levels, which translates into lower levels of extract required to produce an antioxidant effect without negatively impacting sensory characteristics of a food (Moore et al., 2005). The phenolic composition and antimicrobial properties of raisins have also been investigated and found to be highly correlated (Bower, Schilke, & Daeschel, 2003).

Lee (1992) reported that the most effective antioxidant fraction obtained from stored orange juice was extracted using 100% methanol (MeOH). They noted that Maillard-type browning products were also extracted using 100% alcohol. Although 100% ethanol (EtOH) resulted in lower extract yield, the extract had stronger antioxidant activity than extracts obtained from a solvent consisting of 90% EtOH (Lee, Rhee, & Kim, 1975). Naczk, Shahidi, and Sullivan (1992) reported that 70% acetone was more effective than acetone alone for extracting tannins. Extraction of berries and apples with aqueous acetone (70%) resulted in extracts with high flavanols and procyanidins contents compared with extracts obtained from MeOH, hexane and water (Kahkonen, Hopia, & Heinonen, 2001). Pegg, Amarowicz, and Naczk (2003) reported that lower molecular weight

* Corresponding author. Tel.: +1 701 231 6359; fax: +1 701 231 5171.
E-mail address: Clifford.Hall@ndsu.edu (C.A. Hall III).

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compounds were present in the EtOH fraction from bearberry leaves, while those of higher molecular weight were in the acetone fraction, which had greater antioxidant activity than the EtOH fraction. Epicatechin and procyanidin B2 were correlated to the antioxidant activity of apples (Tsao, Yang, Xie, Sockovie, & Khanizadeh, 2005). Approximately 287 and 77 μg epicatechin/g apple peel and flesh, respectively, was determined from eight apple cultivars using an extraction protocol that utilized 70% MeOH (Tsao, Yang, Young, & Zhu, 2003). Chun, Kim, Moon, Kang, and Lee (2003) reported flavonoids contents between 59 and 266 mg catechin equivalence/100 g plums using 80% EtOH as an extraction solvent. Pinelli et al. (2000) obtained an extract with 37.52 mg oleuropein/g dried weight of *Olea europaea* L. from green leaves using 70% EtOH, which efficiently scavenged free radicals. Matthaus (2002) reported the flavonoids content of 11.23 mg/g in sunflower extracts obtained from 70% acetone. This extract also had high antioxidant activity. The previous information about extraction solvents demonstrates that no one solvent was best for extraction of the antioxidant compounds.

Raisins have high contents of both reducing sugars and amino acids and are, thus, susceptible to the Maillard reaction (Sanz, Castillo, Corzo, & Olano, 2001). Browning reaction products (BRPs) have been reported to prevent or retard oxidation reactions in lipid systems (Mastrocola & Munari, 2000; Wijewickreme & Kitts, 1998). The Maillard product 5-hydroxymethyl-2-furaldehyde (5-HMF) is the most important BRP and is an indicator for BRPs in extracts (Bozkurt, Gogus, & Eren, 1999). The BRPs have similar chemical properties as phenolic acids (Gogus, Bozkurt, & Eren, 1998). 5-HMF solubility in organic solvents and chromatographic retention behavior are similar to gallic acid. Thus, raisins could be extracted to simultaneously remove phenolic compounds and BRPs.

Limited studies have been completed on the antioxidant activity of raisin extracts. Furthermore, the components responsible for the antioxidant activity are not well characterized. Thus, the objectives of this study were to evaluate the antioxidant activity and phenolic composition of raisin extracts obtained from various solvent systems having diverse polarities.

2. Materials and methods

2.1. Materials

Thompson seedless raisins were kindly provided by the California Raisin Marketing Board (Fresno, California). The raw material was freeze-dried to decrease the enzyme activity upon receipt from the California Raisin Board. The freeze dried samples were stored at −20 °C. Stripped corn oil was purchased from ACROS Organics, Inc. (Geel, Belgium). Anhydrous monobasic potassium phosphate (KH_2PO_4) was purchased from Fisher Chemicals (Fair Lawn, NJ). HPLC-grade MeOH, water, and acetonitrile (ACN) were purchased from Sigma–Aldrich (St. Louis, MO) and Burdick & Jackson (Muskegon, MI), respectively. Denatured anhydrous EtOH was obtained from Mallinckrodt (Paris, KY). Analytical grade MeOH, acetone, hexane, sodium thiosulfate, potassium iodine, acetic acid, chloroform, and soluble starch were purchased from VWR (West Chester, PA). Folin–Ciocalteu's phenol reagent, gallic acid, and sodium carbonate for the assay of total phenolics (TP) were obtained from Sigma Chemicals (St. Louis, MO). Deionized water (18 Ω) was prepared using a Millipore Milli-Q purification system (Millipore Corp.).

2.1.1. Raisin extract preparation

Raisin extracts were prepared based on the report of Bin (2007) using ground raisin. In short, ground raisins (3 g) were extracted with 15 ml solvent (MeOH, EtOH, acetone or equal mixture of the three solvents). The solvents were combined with water to make solvents containing 0, 20, 40, 60, and 80% water. Water (100%) was also included as a solvent. The raisins and extraction solvent were mixed in a 50 ml Teflon centrifuge tube using a mini vortex mixer for 1 min at room temperature and then homogenized using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) at the highest speed for 1 min to reduce the particle size. The raisin and solvent mixture was then extracted using an ultrasound assisted extraction at 20 °C and pH 5.48 for 25 min. After clarification of the suspension by centrifugation (3000*g* for 25 min and 20 °C), the pellet was re-extracted following the same extraction protocol. The solvents from the repeat extractions were combined and removed under reduced pressure at 35 °C. The remaining extract solvent, mainly water, was frozen and then freeze-dried to remove water.

Freeze-dried extracts were weighed to determine the extraction yield and then stored under nitrogen at −18 °C to reduce the phenolic degradation by temperature and light. Each extraction process was done in triplicate.

2.2. Total phenolics

Total phenolics were evaluated using the spectrophotometric analysis with Folin–Ciocalteu's phenol reagent according to Kim, Chun, Kim, Moon, and Lee (2003). The standard curve for total phenolics was made using gallic acid standard solution (0–100 mg/L) and total phenolics were expressed as mg of gallic acid equivalents (GAE) per 100 g of raisin extract.

2.3. Phenolic and HMF profile analysis by HPLC

The concentration of phenolic and HMF compounds in the extracts was measured using a Waters 2695 (Separations Module, Waters, Milford) high-performance liquid chromatograph (Waters, Milford, MA) system equipped with Gemini C18 110A column (5 μm, 250 × 4.6 mm; Phenomenex Inc. Torrance, CA) set at 35 °C, a photodiode array detector (Waters 996, Waters, Milford, MA) at 280 nm, and an autosampler set to inject 70 μl. The elution

of the phenolic compounds was completed using a gradient profile. Solvent A was 0.07 M mono potassium phosphate (KH_2PO_4) adjusted to pH 2.5 with phosphoric acid. Solvents B and C were 100% HPLC-grade MeOH and acetonitrile, respectively. The elution program, at a flow rate of 0.6 ml/min, was solvent A (99%) and C (1%) for 25 min; solvent A (90%), B (5%), and C (5%) between 26 min and 45 min; followed by a linear gradient from 90% to 30% A, C from 5% to 0%, and from 5% to 70% B in 1 min; and finally, a linear gradient from 30% to 90% A, C from 0% to 1%, and from 70% to 0% B in 19 min. The total run time was 100 min. Individual phenolics were confirmed by retention time and quantified on a dry basis against standard plots of known phenolic standards (0.10–15 μg/g). The HMF content was measured using the same HPLC system and conditions. HMF was confirmed by its retention time and quantified on a dry basis against standard plots of known 5-HMF concentrations (0.12–30.72 μg/g).

2.4. Antioxidant activity evaluation

The dried raisin extract was dissolved in MeOH at a 0.1 g/ml concentration prior to addition to the oil. Stripped corn oil (120 g) and 3.6 ml of a 0.1 g/ml raisin extract in MeOH were mixed thoroughly for 3 min in the blender. The MeOH was removed under nitrogen flow at room temperature. Oil aliquots (60 g) from all the experimental oils were transferred into separate 100 ml amber glass jar without covers. Control oil was prepared by adding a similar level of MeOH followed by MeOH evaporation. This oil without raisin extract served as the negative control. Triplicate samples of oil (60 g) were randomly placed and oxidized in the dark at 60 °C according to AOCS Recommended Practice Cg 5-97 (AOCS, 1997). After 16 h oxidation, the oil samples were evaluated for peroxide values according to the modified AOCS official method Cd 8-53 (AOCS, 1997; Crowe & White, 2001). The modified method included using 0.5 g oil and 10% of all reagents. Oil samples were titrated with a 0.001 N sodium thiosulfate solution until the solution color changed from purple to clear.

2.5. Statistical analysis

The phenolic and HMF results are the mean values of three replicates and data were analyzed for significance at the $p < 0.5$ level. The three separate extracts obtained from the same solvent system served as the replicate in the oil oxidation studies. All data were analyzed by one-way analysis of variances ($p \leqslant 0.05$) using SAS statistical program version 9.1.3. Duncan multiple range test was used for mean discrimination. A confidence level superior to 95% ($p < 0.05$) were considered as significant.

3. Results and discussion

Bulk oil and emulsions represent diverse model systems (Frankel, Gracia, Meyer, & German, 2002). However, one system can provide a rapid comparison of antioxidant activity obtained from the same material but with different solvents. Thus, peroxide value determination in bulk oil, which was treated with extracts obtained from various extractions, was evaluated in this study. Solvent type (EtOH, MeOH, acetone, and mixed solvent) and concentration had a significant ($p < 0.001$) influence on the extraction yield, total phenolic and HMF contents, and antioxidant activity of the raisin extract.

Similar data (e.g. extraction yield, Table 1) between solvent systems were not statistically analyzed due to the complexity of the experimental design. Instead, extract characteristics within a solvent type were first analyzed to determine the best condition within the solvent type that produced the best antioxidant activity. The extract within each solvent having the best antioxidant activity were later compared to establish significant differences among extracts from different solvents.

3.1. Extraction yield

Solvents containing EtOH concentrations between 0 and 80% produced extraction yields between 88 and 82% (Table 1), respectively. Only the 100% EtOH extraction solvent did not achieve a high extraction yield. The 100% water produced the highest yield, which was significantly higher ($p < 0.05$) than the other treatments. Similar trends were observed using MeOH as the extraction solvent (Table 1). The main difference in extraction yield from that observed with EtOH was that no significant differences were observed in extract yields for extraction solvents with a 0–40% MeOH concentration. Again, the 80% and 100% MeOH produced the lowest extract yield. However, the extraction yield obtained by 100% MeOH was 50% points above that observed for the 100% EtOH extraction solvent. Acetone at concentrations between 0 and 60% produced extraction yields similar to the yield obtained from the alcohol solvents, although the 80 and 100% acetone solutions produced yields that were substantially lower than yields obtained from the comparable alcohol solvents (Table 1).

Interestingly, the mixed solvent system containing EtOH, MeOH and acetone resulted in yields similar to those obtained by alcohol solvents (Table 1). This suggests that alcohols play a major role in the extraction efficiency of the mixed solvent. The general trend was that increased water concentration enhanced extract yield. The extract yield represents the amount of material extracted from the raisins. Thus, components other than phenolics could be extracted and contribute to yield. For example, Bin (2007) found that fructose and glucose contents were as high as 362 mg/g (36.2%) and 361 mg/g (36.1%) of extracts obtained from alcohol extractions, respectively. Thus, the combined sugar of 72.3% was observed in the alcohol extracts of raisins. This suggests that the extract is still very crude.

Table 1
Characteristics of raisin extract obtained from different solvents[A,B]

Solvent	Extraction yield (%)	Total phenolics (mg GAE/100 g extract)[C]	HMF (μg/g extract)[D]	Peroxide value (meq/kg)
Ethanol concentration				
0%	87.89 ± 0.34a	339.24 ± 0.40c	173.00 ± 1.73c	40.1 ± 0.99b
20%	84.68 ± 1.20b	375.03 ± 4.30a	179.67 ± 2.52b,c	35.6 ± 1.11c
40%	83.77 ± 1.20b,c	363.09 ± 0.87b	186.67 ± 3.51b	28.8 ± 1.05d
60%	82.61 ± 0.80c,d	367.37 ± 2.36b	199.00 ± 6.08a	26.3 ± 0.83e
80%	81.72 ± 0.78d	318.55 ± 6.59d	198.67 ± 8.14a	20.2 ± 1.29f
100%	29.69 ± 1.88e	345.55 ± 4.05c	178.00 ± 7.21b,c	23.0 ± 0.29g
Stripped oil[E]				44.2a
Methanol				
0%	85.67 ± 0.81a,b	309.69 ± 7.14d	161.33 ± 0.58d	31.3 ± 1.49b
20%	86.40 ± 1.38a	311.47 ± 5.01d	182.67 ± 2.08b	29.5 ± 0.63c
40%	85.36 ± 1.66a,b	344.93 ± 2.42b	184.00 ± 3.61b	29.4 ± 0.40c
60%	83.98 ± 1.11b	375.27 ± 3.49a	189.67 ± 2.08a	26.6 ± 0.59d
80%	81.67 ± 0.99c	324.02 ± 2.27c	169.00 ± 3.61c	22.1 ± 0.98e
100%	79.93 ± 1.59c	283.19 ± 2.08e	170.00 ± 1.73c	26.9 ± 1.00d
Stripped oil[E]				40.4a
Acetone				
0%	83.79 ± 0.80a	271.63 ± 5.39b	184.00 ± 1.00c	34.4 ± 0.42b
20%	83.57 ± 1.92a	316.87 ± 3.11a	189.00 ± 1.00c	31.3 ± 2.24c
40%	83.48 ± 2.40a	327.64 ± 1.09a	191.33 ± 5.69c	30.0 ± 0.84c
60%	85.09 ± 3.16a	316.88 ± 2.83a	253.67 ± 24.70b	26.4 ± 0.52d
80%	45.28 ± 3.16b	245.03 ± 12.79c	443.67 ± 50.54a	23.8 ± 1.16e
100%	3.25 ± 0.02c	4.84 ± 0.25d	64.00 ± 38.69d	22.5 ± 0.75e
Stripped oil[E]				47.1a
Mixed Solvent[F]				
0%	87.40 ± 1.60a	243.65 ± 2.46d	185.33 ± 5.86a	27.5 ± 0.92d
20%	86.18 ± 1.90a,b	270.72 ± 1.60c	189.33 ± 4.51a	30.6 ± 0.20b,c
40%	84.95 ± 1.33a,b	285.21 ± 5.60b	194.67 ± 4.04a	32.7 ± 0.75b
60%	84.52 ± 0.65b	304.46 ± 6.18a	193.67 ± 2.52a	28.6 ± 0.75c,d
80%	81.05 ± 2.19c	266.19 ± 5.22c	194.33 ± 2.08a	19.8 ± 1.31e
100%	56.41 ± 0.42d	183.46 ± 12.97e	25.80 ± 10.71b	21.8 ± 2.19e
Stripped oil[E]				51.2a

[A] Values within a solvent type with different letters are significantly different at $p < 0.05$; $n = 3$.
[B] Values are expressed as mean ± SD.
[C] GAE: gallic acid equivalent.
[D] HMF: hydroxymethylfurfural.
[E] The peroxide value in the control (i.e., stripped corn oil without extract).
[F] Mixed solvent included equal parts of ethanol, methanol and acetone (1:1:1, v/v/v), then diluted with appropriate concentration of water.

3.2. Total phenolic content

Total phenolic content was highest for extraction solvents containing 20–60% EtOH, which produced extracts with 375–367 mg GAE/100 g (Table 1), respectively. The 100% water, 80 and 100% EtOH extraction solvents produced extracts that had significantly ($p < 0.05$) less total phenolic content than other extraction solvents. The extracts obtained from 20% EtOH extraction solvent had significantly ($p < 0.05$) higher yield than the other treatments. Significant differences were observed in total phenolic content of the extracts obtained from the solvent containing 40–100% MeOH concentrations (Table 1). The 100% water, 80 and 100% MeOH solvents produced extracts with the lowest total phenolic content. Furthermore, the 100% MeOH had significantly lower total phenolic (283 mg GAE/100 g extract) content compared with concentrations observed for the 100% EtOH solvent. Similar trends were observed using acetone as the extraction solvent (Table 1). The main differences were that total phenolic content for almost every acetone concentration was substantially lower than the comparable alcohol solvent. In particular, the extract obtained from 100% acetone had a total phenolic content of approximately 5 mg GAE/100 g. This observation could be due to the limited solubility of the ground raisin in 100% acetone. Also, sugars are not soluble in acetone, thus, phenolic glycosides and sugar might not be extracted. Finally, extracts obtained from the mixed solvent system containing EtOH, MeOH, and acetone resulted in total phenolic contents that were lower than in single solvent extraction (Table 1). The general trend was that 40–60% solvent concentrations produced extracts with high total phenolic content. Based on total phenolic content, the best solvents were those containing only MeOH or EtOH. A similar trend was reported for phenolics extraction from black cohosh (Mukhopadhyay, Luthria, & Robbins, 2006).

3.3. HMF (hydroxymethylfufural)

In contrast to the total phenolic content, HMF was highest in extracts obtained from 60% to 80% acetone (Table 1). In fact, the HMF level in the extract was significantly higher than extracts obtained from other solvents. The extracts obtained from 60% to 80% EtOH (Table 1) had the highest HMF contents of the extracts obtained from other alcohol based solvents. The HMF concentration of raisin extracts obtained from 60% to 80% EtOH was significantly different from extracts obtained from other alcohol concentrations, excluding the mixed solvent (Table 1). There was no significant difference in the HMF concentration of the extracts obtained from the 0% to 80% mixed solvent system (Table 1). However, a significantly lower HMF content (~25 μg/g extract) was observed in the 100% mixed solvent concentration. This observation follows a trend among the 100% solvents tested as the extracts obtained from the 100% solvents had the lowest HMF content. This indicated that some water may be required for HMF extraction; however, excess water (i.e. 100% water) also negatively impacts HMF extraction. Generally, 80% solvent concentrations produced extracts with the highest HMF contents.

3.4. Antioxidant activity

The primary lipid oxidation products can be determined by peroxide value (PV) in oil. A high PV indicates a high degree of lipid oxidation. Thus, low PV would be expected upon addition of an antioxidant to oil. The lower the PV, the better is the antioxidant activity of an extract. The antioxidant activity of raisin extracts obtained from 80% EtOH was significantly higher than other raisin extracts obtained from different EtOH concentrations (Table 1). This suggests that yield and total phenolic content may not be good indicators of potential antioxidant activity of the extracts based on the fact that total phenolic compounds were lowest in the 80% EtOH extracts, but had high antioxidant activity in corn oil (low PV, Table 1). This may be due to the type of phenolics extracted or some unidentified antioxidants such as condensed tannins. However, HMF may provide a weak indication of potential antioxidant activity, as the extracts with the highest HMF concentration also had the best antioxidant activity. The antioxidant activity of raisin extracts obtained from 80% MeOH was higher than other raisin extracts obtained from different MeOH concentrations (Table 1). This observation agrees with the antioxidant activity of the extracts obtained from 80% EtOH. The antioxidant activities of raisin extracts obtained from 80% and 100% acetone were higher than other raisin extracts obtained from different acetone concentrations (Table 1). This observation agrees with the antioxidant activity of the extracts obtained from 80% EtOH and MeOH extractions. The antioxidant activities of raisin extracts obtained from 80% to 100% mixed solvent were higher than other raisin extracts obtained from different acetone concentrations (Table 1). This observation also agrees with the antioxidant activity of the extracts obtained from 80% EtOH and MeOH extractions. The general trend was that increased solvent concentration enhanced antioxidant activity. The observation that extracts obtained from 80% solvent generally had lower total phenolic contents, but higher antioxidant activity suggests that specific components may be responsible for the antioxidant activity.

All the extracts obtained from the 80% solvent (including 100% Acetone) had the best antioxidant activity based on the corn oil having the lowest PV. In order to compare the extracts having the best antioxidant activity, 80% solvent extracts were obtained, and then antioxidant activity of the raisin extracts were evaluated in stripped corn oil. The results (Table 2) showed that the 80% extraction solvent type had no significant ($p > 0.05$) influence on the antioxidant activity of raisin extracts. Safety concerns associated with acetone and methanol, including solvent residue in the product, disposal of waste solvent, and pollution of the environment combined with approved for food use status of EtOH favors the use of EtOH as an extraction solvent for obtaining raisin extracts.

3.5. Phenolic compounds

As previously mentioned, the total phenolic content did not correspond well with the antioxidant activity of the extracts. Thus, individual phenolic compounds may provide a better indication of the antioxidant activity of the extract in bulk oil. The ferulic acid content was the highest in raisin extracts obtained from 80% EtOH extraction solvent. Considering that 80% raisin extracts also had the highest antioxidant activity, ferulic acid may play an important role in the antioxidant activity of raisin extracts (Table 3). This observation is supported by the hypothesis that ferulic acid was responsible for the antioxidant activity of wheat (Moore et al., 2005). The raisin extracts obtained from 80% MeOH contain more gallic acid, protocatechuic acid, catechin, and resveratrol than any other raisin extracts obtained from MeOH (Table 4). The raisin extracts obtained from 80% acetone also contained the highest level of phenolic compounds compared to other acetone concentrations, except epicatechin and ferulic acid

Table 2
The antioxidant activity of the raisin extracts, obtained from different solvents, in stripped corn oil[A,B]

Solvent used to obtain the raisin extract, which was then tested in stripped oil	Peroxide value (meq/kg) of treated stripped corn oil
80% MeOH	26.6 ± 1.43a
80% EtOH	28.1 ± 0.56a
80% acetone	28.2 ± 1.01a
80% mixed solvent	26.3 ± 1.26a
Stripped corn oil control	34.9 ± 0.89b

[A] Values within a column with different letters are significantly different at $p < 0.05$; $n = 3$.
[B] Values are expressed as mean ± SD.

Table 3
The phenolic content (μg/g) in raisin extracts obtained from various ethanol (EtOH) concentrations

EtOH concentration (%)	Gallic acid	Protocatechuic acid	Caftaric acid	(+)-Catechin	Chlorogenic acid	Ferulic acid	Rutin	Resveratrol	Kaempferol
0	77.32	64.92	20.83	320.20	60.01	1.88	1565.27	40.61	191.42
20	54.35	75.38	46.61	320.71	50.43	2.30	1731.01	56.41	213.08
40	61.81	79.88	37.62	344.81	57.80	2.77	1755.83	93.77	233.58
60	76.56	107.61	42.45	350.33	57.05	3.21	2413.35	828.77	222.60
80	64.84	85.82	41.30	337.57	55.91	7.45	1735.73	210.49	231.99
100	43.19	74.08	32.58	406.15	57.75	0.00	2428.95	112.36	265.77

Table 4
The phenolic content (μg/g) in raisin extracts obtained from various methanol (MeOH) concentrations

MeOH concentration (%)	Gallic acid	Protocatechuic acid	Caftaric acid	(+)-Catechin	Chlorogenic acid	Ferulic acid	Rutin	Resveratrol	Kaempferol
0	77.32	64.92	20.83	320.20	60.01	1.88	1565.27	40.61	191.42
20	55.29	75.20	33.90	343.46	53.89	8.04	2038.01	53.68	148.62
40	54.24	76.36	45.90	346.45	55.30	9.02	1989.41	66.80	127.15
60	56.35	79.31	46.52	349.23	57.44	9.79	1972.82	144.32	129.78
80	78.48	107.21	44.05	354.92	52.55	5.55	2864.00	918.96	244.30
100	62.29	85.15	82.01	346.43	68.39	3.30	2880.50	743.19	316.53

(Table 5). Although tannins have been reported to be extractable with 70% acetone Naczk et al. (1992), we did not observe proanthocyanidins in our acetone extract of raisins. Karadeniz et al. (2000) reported the presence of proanthocyanidins in grapes but after drying the grapes into raisins these compounds disappeared. Tannins were also lost during the sun drying of *Uapaca kirkiana* fruit (Muchuweti, Ndhlala, & Kasiamhuru, 2006). Thus, some of the tannins in the raisins we utilized may have undergone degradation/polymerization resulting in undetectable tannin levels in the extracts.

Similar trends in the phenolic compounds were observed using the mixed solvent as the extraction solvent (Table 6). The raisin extracts obtained from 80% mixed solvent had the highest phenolic acid content compared to other acetone concentrations. However, the polyphenolic compounds such as rutin, resveratrol, and kaempferol contents were highest for the raisin extracts obtained from 60% mixed sol-

Table 5
The phenolic content (μg/g) in raisin extracts obtained from various acetone concentrations

Acetone concentration (%)	Gallic acid	Protocatechuic acid	Caftaric acid	(+)-Catechin	Chlorogenic acid	Ferulic acid	Rutin	Resveratrol	Kaempferol
0	77.32	64.92	20.83	320.20	60.01	1.88	1565.27	40.61	191.42
20	69.12	89.02	37.63	353.43	60.32	2.20	2472.06	1032.28	283.05
40	70.60	99.33	41.44	348.95	59.03	1.94	2101.57	1088.48	247.34
60	73.00	94.61	48.61	339.03	59.42	2.37	1245.71	776.59	147.30
80	123.04	150.26	74.65	649.35	107.25	0.00	3806.49	1000.33	566.79
100	2.24	5.81	0.60	42.47	5.78	0.00	225.93	19.28	31.16

Table 6
The phenolic content (μg/g) in raisin extracts obtained from various mixed solvent concentrations[a]

Mixed solvent concentration (%)	Gallic acid	Protocatechuic acid	Caftaric acid	(+)-Catechin	Chlorogenic acid	Ferulic acid	Rutin	Resveratrol	Kaempferol
0	77.32	64.92	20.83	320.20	60.01	1.88	1565.27	40.61	191.42
20	62.46	78.30	35.88	336.59	55.22	1.38	4638.89	312.45	106.29
40	59.68	74.29	32.30	328.65	53.13	1.78	4448.11	302.31	92.18
60	76.05	107.85	38.81	346.19	57.97	2.23	3696.54	485.89	268.25
80	83.24	84.66	45.91	356.88	59.18	2.26	2214.38	230.40	90.29
100	7.79	11.23	8.45	51.31	8.59	0.18	700.10	108.44	52.77

[a] Mixed solvent means methanol:ethanol:acetone = 1:1:1, v/v/v combined with a specified concentration of water. For example, 80% mixed solvent indicates 80% of the methanol:ethanol:acetone (1:1:1, v/v/v) solvent and 20% water.

vent. From the above analysis, phenolic acids, HMF, and low-molecular-weight flavonoids (catechin and epicatechin) appear to be responsible for the antioxidant activity and not the high-molecular-weight flavonoids (rutin, resveratrol, and kaempferol). However, it was not possible to build a correlation between phenolic compositions and antioxidant activities. In contrast, Parker, Wang, Pazmino, and Engeseth (2007) reported that the ORAC values correlated to the phenolic content and *in vitro* antioxidant activity. Kahkonen et al. (2001) studied the extraction methods for berries and apples to produce phenolic extracts with high antioxidant activity. They found that the extraction method affected both the phenolic composition and the antioxidant activity, but with statistical analysis the observed activity could not be well explained with the content of individual phenolic subgroups.

4. Conclusion

The low molecular weight phenolic acids such as gallic acid, protocatechuic acid and caftaric acid, and catechin content, were highest in the extracts obtained from a pH 5 extraction solution (data not reported). Whereas, resveratrol and kaempferol in the extracts obtained from extraction with pH 3 solutions were very high and the previous components in low levels (Bin, 2007). The poor antioxidant activity of the extracts obtained from pH 3 suggested that resveratrol and kaempferol may not be important for antioxidant activity of the raisin extracts in bulk oil. Thus, supporting our conclusion that phenolic acids, HMF, and low-molecular-weight flavonoids (catechin and epicatechin) appear to be responsible for the antioxidant activity and not the high-molecular-weight flavonoids (rutin, resveratrol, and kaempferol). However, condensed tannins may also play a role in the antioxidant activity of the raisin extracts, specifically in the acetone extracts.

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