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Antioxidant Activity of Raisin Extracts in Bulk Oil, Oil in Water Emulsion, and Sunflower Butter Model Systems

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Abstract The antioxidant activities of the raisin extract (RE) in stripped corn oil, stripped corn oil emulsions, and sunflower butter stored at 60 °C for up to 15 days was evaluated. Peroxide values and hexanal content were measured on a half day, 2 or 3 day basis for the emulsion, sunflower butter, and bulk oil, respectively. The RE had the best antioxidant activity in the bulk oil system. Statistical contrasts indicated the oxidation of bulk corn oil treated with RE was significantly ($p < 0.001$ and $p = 0.044$) lower than bulk oil and bulk oil treated with tertiary-butylhydroquinone (TBHQ), respectively. No differences ($p = 0.15$) in hexanal concentrations were observed in stored bulk oils treated with RE and TBHQ. However, both these materials inhibited hexanal formation better ($p < 0.001$) when compared to the control corn oil. In contrast, 200 μg/g TBHQ had better ($p = 0.0004$) antioxidant activity than 3,000 μg/g RE in the oil in water(o/w) emulsion. No significant differences ($p = 0.1637$) in hexanal formation were observed in the emulsions treated with RE and TBHQ. However, the data indicated that the RE treated emulsion did undergo more secondary oxidation than the emulsion treated with TBHQ beyond 110 h. The 3,000 μg/g RE had antioxidant activity in sunflower butter, but was less effective than the 200 μg/g TBHQ and a lower RE concentration (200 μg/g). The observations supported the hypothesis that RE has antioxidant activity in the multiple model systems.

Keywords Antioxidant activity · Bulk oil · Hexanal · O/W emulsion · Peroxide value · Raisin extract · Sunflower butter

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Introduction

Natural antioxidants are commonly associated with spices and herbs as replacements for synthetic antioxidants in food systems. However, other natural products such as grape and grape seed extracts, and berries also have antioxidant capacity. Regardless of the antioxidant source, simple phenols, polyphenolics and phenolic acid derivatives are common compounds responsible for the antioxidant activity of the aforementioned plant materials. Flavonoids are a group of polyphenolic compounds that are ubiquitous in nature. A significant number of reports exist regarding the activities of flavonoids, such as radical scavengers, metal chelators and enzyme inhibitors [1–3].

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 [4–6]. The phenolic composition of sun dried, dipped and golden raisins were determined by Karadeniz et al. [4]. These authors reported that the phenolic acid content was approximately 46 and 53 mg/kg for sun-dried and dipped raisins, respectively. These values were statistically lower than those of the golden raisins (111 mg/kg) and fresh grapes (131 mg/kg). This reduction is not unexpected due to the fact that during the drying process the phenolic compounds can undergo oxidation and polymerization resulting in brown coloration. In contrast, the higher

phenolic acid content in golden raisins is due to the sulfur dioxide treatment, which prevents the oxidation and polymerization reaction. In general, processing did not have a significant influence on flavonol glycosides [4]. Arts et al. [5] reported a catechin concentration in raisins of approximately 37 mg/kg. Collectively, these phenolic materials possess antioxidant activity and in part could explain the antioxidant activity of raisins [6, 7]. Similar to synthetic antioxidants, phenolic antioxidants function as free radical terminators and sometimes as metal chelators [8]. Phenolic compounds have powerful inhibitory activity, which may be due to their partitioning between the water and lipid phases in emulsions [9].

In addition to phenolic compounds, non-enzymatic browning compounds have been identified and isolated from raisins [4, 10, 11]. Non-enzymatic browning products have been shown to be effective antioxidants [12, 13]. The 5-hydroxymethyl-2-furaldehyde (5-HMF) is an important product of the Maillard browning reaction and is used as an indicator for browning reaction products (BRPs) extraction [14]. Thus, non-enzymatic browning compounds, along with the phenolic compounds, identified in raisins could contribute to the antioxidant capacity of raisins.

To understand this antioxidant activity, studies were previously conducted to identify and quantify the phenolic and Maillard browning compositions in RE [15]. The diverse polarities of the identified compounds indicated that the extract should be effective antioxidants in multiple oil-based systems. It is commonly observed that non-polar antioxidants such as α-tocopherol are less effective in oil but are highly effective in o/w emulsions. In contrast, polar antioxidants such as phenolic acids are more effective in oil than in an emulsion [16–20]. Thus, the effectiveness of the antioxidant will differ depending on the type of food system.

Phenolic antioxidants of grapes in liposome systems (pharmaceutical model) have been extensively studied [21–23]. However, there is little information on the antioxidant activity of raisin products in the bulk oil, emulsions, and food products. Thus, the objective of this study was to assess the antioxidant activity of a (RE) in multiple food system models.

Materials and Methods

Materials

Stripped corn oil was purchased from ACROS Organics, Inc. (Geel, Belgium); TBHQ was obtained commercially from the Eastman Chemical Co. (Rochester, NY). Hexanal standard was purchased from Sigma (St. Louis, MO). Sunflower seeds and salt were purchased from a local grocery store. Polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (65 mm) was obtained from Supelco (Bellefonte, PA). Tween 20 and dextrose were purchased from VWR (West Chester, PA). Raisin extracts were prepared according to Zhao [15] using ground raisin. In short, ground raisins (3 g) were extracted with 1:3 (w/v) of 80% EtOH. Ground raisins were mixed with solvent using a mini vortex mixer for 1 min at room temperature. The raisin and solvent mixture was then subjected to sonication (Ultrasonic Cleaner, VWR International) at 20°C and pH 5.48 for 25 min. After clarification of the suspension by centrifugation (3,000×*g* for 25 min and 20 °C), the pellet was re-extracted following the same extraction protocol. The extraction solvents were combined and the solvent removed under vacuum using a rotary evaporator at 35 °C. The remaining extract was frozen and then freeze-dried to remove water.

Bulk Oil Oxidation

The dried RE was dissolved in MeOH at a 0.1 g/mL concentration prior to addition to the oil. Stripped corn oils containing 3,000 μg/g of REs were prepared by mixing stripped corn oil (120 g) and 3.6 mL of a 0.1 g/mL RE in MeOH for 3 min in a 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 under nitrogen. This oil without RE served as the negative control. The sample containing 200 μg/g TBHQ served as the positive control. This procedure was repeated three times to give triplicate samples of oil (60 g) that were randomly placed and oxidized in the dark at 60 °C in an oven according to AOCS Recommended Practice Cg 5-97 [24]. Oxidative stability of the oils (0.5 g) was determined by measuring peroxide value and hexanal at 0, 3, 6, 9, 12, and 15 days using the methods below.

Oil-in-Water Emulsion Preparation and Oxidation

Emulsion preparation involved homogenizing the oil containing REs with water. An example preparation included homogenizing the corn oil (35%), with or without RE, and 65% water. Tween 20 (1%) was added as an emulsifying agent before homogenization. The homogenization process was completed for 3 min with a 14 speed Hamilton Beach blender (Howell, MI) at high speed. Emulsions were then transferred to 100 mL amber jar for oxidative stability testing.

The dried RE was dissolved in MeOH at 0.1 g/mL concentration prior to addition to the emulsion. O/W emulsion containing 3,000 μg/g of REs was prepared by mixing deionized water (88.5 g), 4.5 mL of a 0.1 g/mL RE in MeOH and Tween 20 (1.5 g) in a 14 speed Hamilton Beach blender (Howell, MI). Corn oil (60 g) was then added gradually under continuous blending. After all the oil had been added, samples were mixed for three additional min. The control emulsion was prepared by adding a similar level of MeOH, and served as the negative control. The sample containing 200 μ/g TBHQ (0.1 g/mL in MeOH) served as the positive control. Emulsion aliquots (60 g) from all the experimental emulsion were transferred into separate 100 mL amber glass jars. The jars were loosely covered to prevent the loss of water from the emulsions and triplicate samples of the emulsions were randomly placed and oxidized in the dark at 60 °C in an oven according to AOCS Recommended Practice Cg 5-97 [24]. Oxidative stability of the emulsions was determined by measuring peroxide value and hexanal at 0, 2, 4, 11, 26, 40, 52, 87, 111, and 135 h as discussed below.

Sunflower Butter Preparation and Oxidation

Sunflower butter was prepared according to Arlene [25]. Shelled sunflower seeds (188 g) were ground for 10 min with a 14 speed Hamilton Beach blender (Howell, MI) at high speed. Sunflower seeds were crushed into a paste. Dextrose (10 g) and salt (2 g) were stirred into the ground sample.

Sunflower butter aliquots (60 g), with or without antioxidants, were transferred into 100-mL amber glass jars without covers. The negative control was prepared by adding a similar level of MeOH without the extract. The positive control contained 200 μg/g TBHQ (0.1 g/mL in MeOH). Triplicate samples of sunflower butter (60 g) were randomly placed in an oven, and oxidized in the dark at 60 °C according to AOCS Recommended Practice Cg 5-97 [24]. Oxidative stability of the sunflower butter was determined by measuring peroxide value and hexanal at 0, 2, 4, 6, 8, 10, 12, and 14 days as discussed below.

Peroxide Value Determination

The primary oxidation products were measured using peroxide value according to a modified method of the AOCS official method Cd 8-53 reported by Crowe and White [26]. In short, 0.5 g oil from the bulk, emulsion or sunflower butter system was used in place of the 5 g of oil used in the standard method. Extraction of the oil from the emulsion (5 g) and sunflower butter (10 g) was completed using 50 mL of chloroform:methanol (2:1 v/v). The solvent was removed under vacuum at 30 °C. All reagents were used at 10% of the amounts recommended for the standard procedure [26]. Peroxide values were evaluated in duplicate for each triplicate sample (i.e. $n = 6$).

Hexanal Determination

The formation of the secondary oxidation products was monitored using hexanal as the indicator product by headspace solid phase microextraction (SPME) method [27]. Oil (0.5 g), emulsion (0.5 g) or sunflower butter (0.5 g) were added to a headspace vial (6 mL), and sealed with a Teflon faced silicone septa, which previously had been heated at 100 °C for 24 h prior to use. Samples were incubated at 95 °C for 10 min and then transferred to a 60 °C sonicating water bath. The filament [polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (65 mm)] was inserted into the headspace of the vial, allowed to absorb headspace gases for 15 min, removed and immediately desorbed at 250 °C onto the GC column for 10 min. An HP 5890 Series II with FID detector GC was used to separate volatiles. The GC conditions used for the separation included: helium head pressure (103.42 kPa), hydrogen head pressure (165.47 kPa), and air flow head pressure (303.37 kPa); Phenomenex Zebronwax column (60 m × 0.25 mm (ID) × 0.25 μm); and injection and detector temperatures of temp 250 °C. The oven temperature program started at an initial temp 40 °C for 5 min, then at the rate of 8.0 °C/min to 180 °C, held 1 min, then at the rate of 20 °C/min to 250 °C where it remained four min. A hexanal standard curve was used to calculate hexanal concentration in the samples. The standard concentrations of 0.50 to 1.0 mmol/kg hexanal in stripped corn oil, emulsion, and sunflower butter were used to develop the curve. Hexanal values were evaluated in duplicate for each triplicate sample (i.e. $n = 6$).

Experimental Design and Statistical Analyses

Completely Random Design (CRD) was used to evaluate the antioxidant activity difference between the positive control sample, negative control sample and sample with RE in bulk oil, o/w emulsion and sunflower butter systems. The experiments were completed in triplicate with a new batch of oil, emulsion or sunflower butter serving as the experiment unit.

Analysis of covariance (ANCOVA) was used to determine significant differences between peroxide values at every time and between treatments within each test system (i.e. oil, emulsion, or sunflower butter) using SAS

statistical program version 9.1.3. ANCOVA was also used to determine significant differences between hexanal concentrations in samples at every time and between treatments. Significant level was $p < 0.05$ unless otherwise indicated.

Results and Discussion

Antioxidant Activity of Raisin Extracts in Bulk Oil

The antioxidant activity of REs and TBHQ in stripped corn oil was assessed by the peroxide value, the primary oxidation products indicator, and by hexanal, a secondary oxidation products indicator. The slopes of the three regression lines in Fig. 1 indicated that the slopes were significantly ($p < 0.0001$) different. Further contrasts determined that the slopes for the TBHQ and RE were significantly different ($p = 0.0444$). The slope for control bulk corn oil was also different from the other two ($p < 0.0001$ for RE and $p = 0.0001$ for TBHQ). This indicates that 3,000 μg/g RE inhibited the formation of hydroperoxide better than the 200 μg/g TBHQ in the bulk oil system. The 3,000 μg/g RE showed antioxidant activity after 3 days of oil oxidation and was more active than 200 μg/g TBHQ in bulk corn oxidation at 60 °C.

The relative antioxidant activities, as measured by hexanal formation, of 3,000 μg/g RE and 200 μg/g TBHQ followed a similar pattern to hydroperoxide formation. The slopes of the three regression lines in Fig. 2 indicate that the slopes were significantly ($p < 0.0001$) different. Further contrasts determined that the slopes for TBHQ and RE were not significantly different ($p = 0.1547$); however, the slope of the line for corn oil without antioxidants was different from the other two ($p = 0.0131$ for TBHQ and $p = 0.0012$ for RE). This indicates that 3,000 μg/g RE could inhibit the hexanal production to the same degree as the 200 μg/g TBHQ.

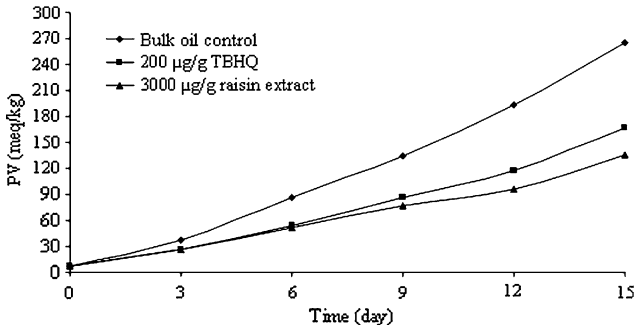

Fig. 1 Peroxide values (PV) of stripped corn oils containing raisin extract or TBHQ over 15 days

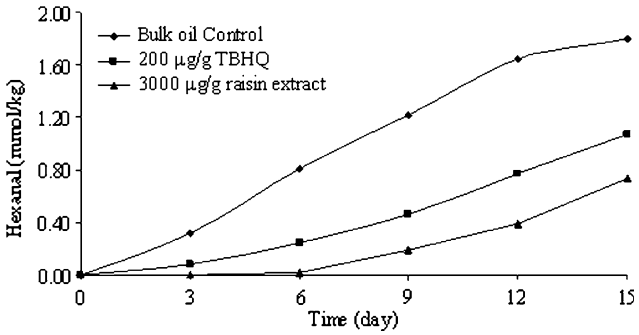

Fig. 2 Hexanal content of stripped corn oils containing raisin extract or TBHQ over 15 days

Antioxidant Activity of Raisin Extracts in Oil-in-Water Emulsion

The slopes of the three regression lines in Fig. 3 indicated that the slopes were significantly ($p < 0.0001$) different. Further contrasts determined that the slopes for the TBHQ and 3,000 μg/g RE were significantly different ($p = 0.0004$). The slope for the control emulsion was also different from the other two ($p < 0.0001$ for 3,000 μg/g RE and 200 μg/g TBHQ). This indicates that 3,000 μg/g RE inhibited the formation of hydroperoxide less than the 200 μg/g TBHQ, but still had antioxidant activity based on the lower peroxide value compared with control o/w emulsion. As shown in the Fig. 3, the 3,000 μg/g RE had antioxidant activity and was more active than the 200 μg/g TBHQ in the oil-in-water emulsion during the first 40 h of oxidation at 60 °C. However, as time progressed the RE became less efficient at controlling oxidation compared to the TBHQ treatment. These data support the antioxidant activity of 3,000 μg/g RE. However, the lower antioxidant activity of the extract compared to TBHQ suggests that the composition of the extract may be more hydrophilic than TBHQ, resulting in a lower activity in the emulsion. The basis for this suggestion is the hydrophilic nature of an antioxidant is important for stabilizing oil oxidation. In bulk oils, the more hydrophilic antioxidants function best, whereas the more lipophilic antioxidants function best in emulsions [17–19]. Another reason could be that the extract components might hydrogen bond to the aqueous phase resulting in reduced activity. This reduction might be based on ineffective hydrogen donation to lipid radicals in cases where hydrogen bonds complex with aqueous components [18].

The relative antioxidant activities were measured by hexanal formation in o/w emulsion. The slopes of the three regression lines in Fig. 4 indicated that the slopes were significantly ($p < 0.0001$) different. Further contrasts determined the slope for the control oil-in-water emulsion was different from the other two ($p = 0.0002$ for TBHQ

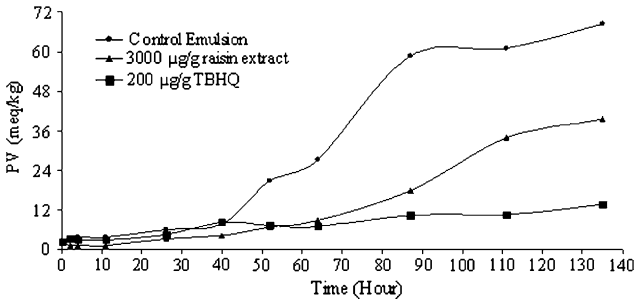

Fig. 3 Peroxide values (PV) of oil-in-water emulsions containing raisin extract or TBHQ over 135 h

and $p = 0.0092$ for RE). In addition, the slopes for TBHQ and RE were not significantly different ($p = 0.1637$); however, the variability in the data for the hexanal content in the RE treated emulsion may have accounted for the lack of significant differences between the slopes. The data indicates that the RE treated emulsion did undergo more secondary oxidation than the emulsion treated with TBHQ (Fig. 4). This indicates that 3,000 µg/g RE could inhibit the hexanal production to the similar degree as the 200 µg/g TBHQ in o/w emulsion system up to 110 h; however, beyond that time TBHQ was more effective. In addition, the difference in hexanal formation may have been further extenuated if the oxidation time had been lengthened.

The above data showed that RE had less antioxidant activity in o/w emulsions than in bulk oil. As mention previously, the hydrophilic nature of the extract might be the reason. The phenolics of RE are hydrophilic compounds, and thus, are likely to be responsible for the antioxidant activities in bulk oils. In bulk oil systems, the oxidation occurs at the air–oil interface, thus less oxidation occurs in the bulk phase. The distribution of the hydrophilic antioxidant at the oil–air interface causes a concentration effect. Thus, the concentration of hydrophilic antioxidant at the oil–air interface helps to stabilize the bulk oil [19]. In contrast, a dilution effect caused by the solubility of the phenolics into the water phase reduces the antioxidant activity of the phenolics compounds in the emulsion [19]. Thus, the observed data are in agreement with proposed antioxidant mechanisms.

In previous research [15], the phenolics of raisin extracts included gallic acid, protocatechuic acid, hydroxymethylfurfural, caftaric acid, (+)-catechin, chlorogenic acid, epicatechin, ferulic acid, rutin, resveratrol, and kaempferol. The phenolics of the RE are hydrophilic compounds, and thus, are likely to be responsible for the antioxidant activities reported above. The RE also contained 5-HMF [15], which is more lipophilic than the phenolic compounds. Thus, the antioxidant activity of the RE in the emulsion may have been partly due to the Maillard browning products.

Antioxidant Activity of Raisin Extracts in Sunflower Butter

An ANCOVA comparing the slope of the four regression lines in Fig. 5 determined that the slopes were significantly different. The slope for the sunflower butter control was significantly ($p < 0.0001$) different from 200 µg/g TBHQ, 200 µg/g RE, and 3,000 µg/g RE. This indicates that 200 µg/g TBHQ, 200 µg/g RE, and 3,000 µg/g RE had antioxidant activity. The slope for 200 µg/g RE was also significant different ($p < 0.0001$) from the slope of the line for the 3,000 µg/g RE.

The sunflower butter stability parallels that of the emulsions and bulk oil. The peroxide values of samples to which 3,000 or 200 µg/g RE had been added were higher ($p = 0.0001$ and 0.027, respectively) compared with the positive control (200 µg/g TBHQ) but lower than the negative control (without RE) for up to 14 days (Fig. 5). An interesting observation occurred in that the 200 µg/g extracts inhibit the oxidation better ($p = 0.0062$) than the 3,000 µg /g extract on day 12 and 14. In fact, the oxidation in the sunflower butter containing 3,000 µg/g extracts increased 10 mequiv/kg from day 12 to 14, whereas a

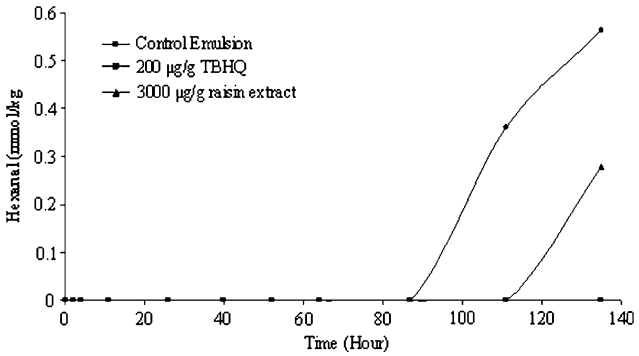

Fig. 4 Hexanal content of oil-in-water emulsions containing raisin extract or TBHQ over 135 h

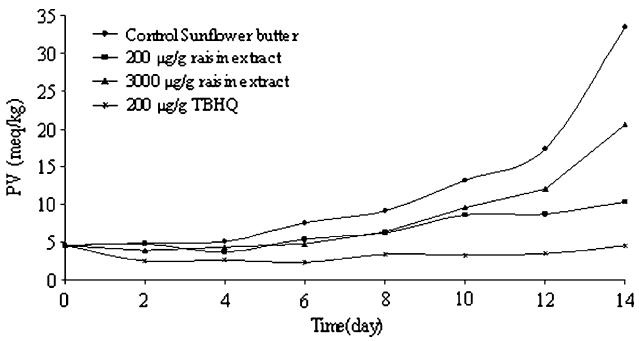

Fig. 5 Oxidative stability (PV) of sunflower butter containing 200 or 3,000 µg/g raisin extract or TBHQ over 14 days

slight increase was observed in the sunflower butter containing 200 μg/g RE. This observation suggests that the high RE levels may contain high concentration of non-antioxidant components such as iron, which enhance the oxidation or diminish the effectiveness of the extract. Unlike the other systems, no hexanal was observed in none of the oxidized sunflower butter, including the control.

The antioxidant activity of RE in sunflower butter was predicted to be the same as in the bulk oil, due to the expected limited oxygen diffusion into the sunflower butter. The oxidation was anticipated to occur at the surface. In fact, the above data showed that RE had less antioxidant activity in sunflower butter than in bulk oil. This is possible because the sunflower butter was very sticky, and that RE can disperse during sunflower butter processing, but the movement of phenolic compound is restricted, thus the phenolics cannot migrate to the surface where much of the oxidation occurs. In turn, the phenolics cannot prevent free-radical formation.

The RE did have antioxidant activity in multiple systems suggesting that the extract has a diverse chemical composition. The extract also prevented both primary and secondary oxidation product formation thus proving to be an effective antioxidant. No attempt was made to create highly purified RE, which may enhance the antioxidant activity in the appropriate food system.

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