Polyphenolic Composition of Raisins

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The polyphenolics of raisins were extracted, separated by HPLC, and characterized by their UV−vis spectra, and their concentrations measured. Color measurements and browning indices were also determined. Samples ($n = 20$) included sun-dried, dipped, and golden raisins. Comparisons were also made with fresh and frozen Thompson Seedless grapes. Golden raisins (which are treated with SO_2) had the highest amount of hydroxycinnamic acids and the highest lightness values. In comparison with fresh grapes, percent losses of the two major hydroxycinnamics (caftaric and coutaric acids) in sun-dried, dipped, and golden raisins were on the order of 90%. Flavonols were not influenced by processing as much as hydroxycinnamics, while procyanidins and flavan-3-ols were completely degraded in all raisin samples. Formation of hydroxymethylfurfural and loss of amino acids in sun-dried and dipped raisins are ascribed to Maillard browning reactions.

Keywords: *Polyphenolics; flavonoids; procyanidins; hydroxycinnamics; raisins; Thompson Seedless grapes*

INTRODUCTION

The polyphenolic constituents of fruits, vegetables, and beverages are important contributors to color quality, sensory properties (Macheix et al., 1990), and juice stability (Beveridge, 1997). Interest in these compounds has intensified in recent years because of their possible health benefits including anticancer and antiviral activities (Hertog et al., 1992) and reduced risk of coronary heart disease and stroke (Ho, 1992). Many of these properties are attributed to their antioxidant properties, and grapes, wines, and wine pomace have been shown to be good sources of phenolic antioxidants (Larrauri et al., 1996; Teissedre et al., 1996). Ellagic acid and *trans*-resveratrol contents of grape products are of particular interest because of ellagic acid's anticarcinogenic properties (Maas et al., 1991) and *trans*-resveratrol's ability to inhibit low-density lipoprotein oxidation (Frankel et al., 1993).

The phenolic compositions of grapes (Lea et al., 1979; Cheynier and Rigaud, 1986; Jaworski and Lee, 1987; Merida et al., 1991; Souquet et al., 1996), grape juices (Spanos and Wrolstad, 1990), and wines (Baranowski and Nagel, 1981; Singleton and Trousdale, 1983; Salagoity-Auguste and Bertrand, 1984; Oszmianski et al., 1986) have been investigated by many researchers. Raisins are an important processed product in many parts of the world where grapes are grown (Dudman and Grncarevic, 1962). The USA is the third largest grape producer after Italy and France, and the world's largest raisin producer (Pollack and Perez, 1997). However, no report has been published related to the polyphenolic composition of raisins.

The brown color of raisins is a combination of pigments produced by polyphenoloxidase activity and nonenzymatic reactions (Ramshaw and Hardy, 1969). Klason lignin, a constituent of insoluble dietary fiber, contains condensed tannins and proteins as protein−tannin complexes or as Maillard reaction products. Raisins are considered a particularly important source of dietary fiber (Valentie et al., 1995). With a higher degree of tannin polymerization, the inhibitory effect on digestive enzymes is also higher, which has a beneficial influence on diet digestibility (Martin-Carron et al., 1997).

The objectives of this study were to determine the polyphenolic composition of raisins, and do qualitative and quantitative comparisons of raisins produced by different processing methods. Comparison to fresh and frozen Thompson Seedless grapes was also part of the investigation. The effect of processing on raisin color was also studied by measuring color indices of raisins and their extracts.

MATERIALS AND METHODS

Materials. Raisins and frozen Thompson Seedless grapes (*Vitis vinifera* L. cv. sultanina) were supplied by the Health and Studies Research Center, Inc., Los Altos, CA. After receipt, raisin samples were stored at 1 °C until analysis.

Sun-Dried Raisins. After being harvested, Thompson Seedless grapes grown in California's Central Valley were left to dry between vineyard rows on clean paper in the sun for 2−3 weeks. Grapes were dried to a moisture content of 9−16%, and the raisins were then mechanically destemmed and cleaned. The raisins were washed and packaged with a final moisture content of 15−18%.

Dipped Raisins. Grapes were harvested from the vine when fully ripened, washed in cold water, and then quickly dipped (15−20 s) in hot water (87−93 °C). They were then spread on stacking trays, placed in a dehydration tunnel (71 °C), and dried to a moisture content of 10−14% for from 20 to 24 h. The raisins were destemmed, cleaned, washed, and packaged as described above.

Golden Raisins. Fully ripe Thompson Seedless grapes were harvested, washed in cold water, quickly (15−30 s) dipped in hot water (87−93 °C), spread on stacking trays, and treated with sulfur dioxide (SO_2) for 5−8 h. The trays were then moved

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to a dehydration tunnel (63 °C) and dried to a moisture content of from 10% to 14% within 24 h. The final SO_2 content was approximately 2000−3000 ppm. The raisins were destemmed, cleaned, washed, and packaged as described above.

Fresh and Frozen Thompson Seedless Grapes. Thompson Seedless grapes grown in California were harvested from the vine at fully ripe maturity stage, placed in a plastic bag, frozen at −15 °C, and air-freighted to Corvallis, OR. Upon receipt, the grapes were stored at −15 °C. Fresh Thompson Seedless grapes were purchased from a local market (WINCO Foods, Corvallis, OR).

Standards. Phenolics (protocatechuic acid, rutin, kaempferol, ellagic acid, *trans*-resveratrol, catechin, and epicatechin) and amino acids (tryptophan and tyrosine) were obtained from Sigma Chemical Co. (St. Louis, MO). Hydroxymethylfurfural (HMF) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Procyanidins (dimers B1, B2, B3, and B4, trimer, and tetramer) and caftaric and coutaric acids were identified by comparing the UV spectra and peak elution order to the data reported by Spanos and Wrolstad (1990).

Extraction of Polyphenolics. The polyphenolics were extracted using the procedure described by Giusti and Wrolstad (1996). Grapes and raisins were liquid nitrogen powdered using a stainless steel Waring blendor. Powdered samples were subsequently blended with 1 volume of acetone and filtered on a Buchner funnel using Whatmann no. 1 paper. The resulting residue was re-extracted with aqueous acetone (30:70 v/v) until a clear solution was obtained. The filtrate was placed in a separatory funnel, shaken with 2 volumes of chloroform (1:2 acetone−chloroform v/v), and allowed to stand until the phases separated. The aqueous phase was collected and residual acetone removed using a rotary evaporator (Rotavapor R., Buchi, Switzerland) at 40 °C. The aqueous extract was made up to volume with deionized water, filtered through a 0.45 μm Millipore filter, type A, and injected onto the HPLC column for the determination of phenolic acids and flavonol glycosides. The organic phase was also analyzed to check for the presence of phenolic compounds.

Procyanidin Purification. For isolation of procyanidins, the method described by Spanos and Wrolstad (1990) was used. The aqueous extract was passed through a Bio-Rad minicolumn (Bio-Rad Laboratories) containing Sephadex-LH20 (Pharmacia, Uppsala, Sweden). The bed was washed with 30 mL of 20% methanol to remove sugars, nonvolatile acids, and phenolic acids. The procyanidins were eluted from the column with 15 mL of methanol. The methanol was evaporated, and the procyanidins were redissolved in deionized water, filtered through a 0.45 μm Millipore filter, type A, and injected onto the HPLC column.

Ellagic Acid Analysis. Analysis for ellagic acid was done following the procedure described by Rommel and Wrolstad (1993). The aqueous phase was passed through a Bio-Rad minicolumn containing Polyamide-6 (particle size <100 μm, J.T. Baker, Phillipsburg, NH) as a chromatographic medium. After flavonol glycosides were eluted with methanol, flavonol glucuronides, acylated flavonols, and ellagic acid forms were eluted with 0.5% NH_3 in methanol. Methanol was evaporated to dryness, and the residue was redissolved in 4% phosphoric acid, filtered through a 0.45 μm Millipore filter, type A, and injected onto the HPLC column.

Color Measurements. Hunter *L*a*b*C*h* values were determined using a Hunter ColorQuest colorimeter (Hunter Lab, Hunter Associates Laboratories, Inc., Reston, VA), with a specular component included, an illuminant observer C, and a viewing angle of 10°.

Spectrophotometric browning measurements were also conducted using the method described by Baloch et al. (1973). Raisins (10 g) were rehydrated for 1 h in 50 mL of acetic acid−formaldehyde aqueous solution (2−1% v/v) and homogenized for 5 min, and the slurry was centrifuged. The supernatant was then collected in a volumetric flask, made up to volume with acetic acid−formaldehyde solution, and filtered through a 0.45 μm Millipore filter, type A. Absorbance measurements were taken at 420 and 600 nm by using a Shimadzu 300 UV spectrophotometer. Browning is calculated from the difference between the two absorbance values. In addition, the pellet obtained from centrifugation was spread on filter paper and air-dried; *L*a*b*C*h* measurements of the insoluble brown pigments were made as described above.

Moisture Analysis. Moisture analysis was performed in triplicate in a vacuum oven at 70 °C (AOAC, 1990).

High-Performance Liquid Chromatography. *Equipment.* A high-performance liquid chromatograph, Perkin-Elmer series 400, equipped with a Hewlett-Packard 1040A photodiode array detector, Gateway 2000 P5-90 computer with Hewlett-Packard HPLC ChemStation software, and Beckman 501 autosampler with a 50 μL loop was used. Simultaneous detection was at 260, 280, and 320 nm. The absorption spectra (from 220 to 600 nm) were recorded for all peaks.

Columns and Mobile Phase. Supelcosil LC-18 column (5 μm), 250 × 5 mm i.d. (Supelco Inc., PN), fitted with a ODS-10, 4 cm × 4.6 mm i.d., Microguard column (Bio-rad Laboratories). Solvent A: 0.07 M KH_2PO_4 adjusted to pH 2.5 with phosphoric acid. Solvent B: 100% HPLC grade methanol. Solvent C: 100% HPLC grade acetonitrile. The elution program, at a flow rate of 1 mL/min, followed a linear gradient from 95% to 65% A and from 0% to 30% C, while solvent B was isocratic (5%) in 30 min.

Quantitation. Concentrations of individual compounds were measured by the external standard method. Standard curves were constructed using three different concentrations of standards in aqueous solution. Cinnamic acid derivatives were calculated as chlorogenic acid, quercetin glycosides as rutin, kaempferol glycosides as kaempferol, and procyanidins, catechin, and epicatechin as catechin. Additional standards included tyrosine, tryptophan, and hydroxymethylfurfural.

Statistical Analysis. All analyses were replicated. Significant compositional and color differences among sun-dried, dipped, and golden raisins were determined at the 95% and 99% levels using the Tukey test of means, which was performed using SAS statistical software (SAS system for Windows, release 6.11, SAS Institute, Inc., 1996). Fresh and frozen grapes were excluded from the statistical analyses.

RESULTS AND DISCUSSION

Identification of Polyphenolic Compounds. The structures of selected polyphenolic compounds of interest to this investigation are shown in Figure 1. Several phenolic acid derivatives—*trans*-caftaric acid, *trans*-coutaric acid, *cis*-caftaric acid, *cis*-coutaric acid, and feruoyltartaric acid—have been reported to be present in grapes and wine (Baranowski and Nagel, 1981; Singleton and Trousdale, 1983; Lee and Jaworski, 1987). Singleton et al. (1978) assumed that the *trans*-configuration of caffeoyltartrates and coumaroyltartrates was found naturally and that the *cis*-form was the product of UV-induced isomerization. Figure 2 shows the HPLC separation of phenolic acids and flavonol glycosides in Thompson Seedless grapes. Peaks 2 and 5 had UV spectra and retention behavior identical to those previously demonstrated in our laboratory for *trans*-caftaric and *trans*-coutaric acids, respectively (Spanos and Wrolstad, 1990). Peaks 1 and 3 were tentatively identified as *cis*-caftaric and *cis*-coutaric acids, respectively. Several investigators have reported the presence of quercetin and kaempferol glycosides in white grapes (Spanos and Wrolstad, 1992). Peak 6 was identified as quercetin-3-rutinoside (rutin) by matching its UV spectra and retention time to those of a reference standard. Peaks 7 and 8 were characterized as quercetin glycosides from their UV spectra, but the glycosidic substituent was not identified. Peak 9 was tentatively identified as a kaempferol glycoside from its UV spectrum. Additional evidence for the presence of quercetin and kaempferol glycosides was the generation of quercetin and kaempfer-

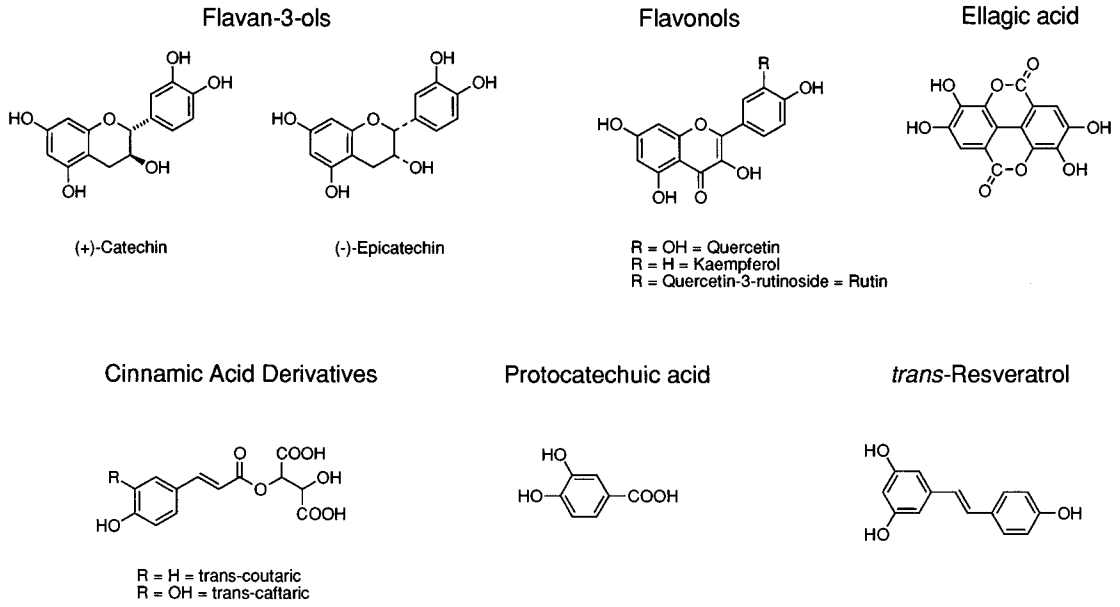

Figure 1. Structures of selected polyphenolics featured in this investigation.

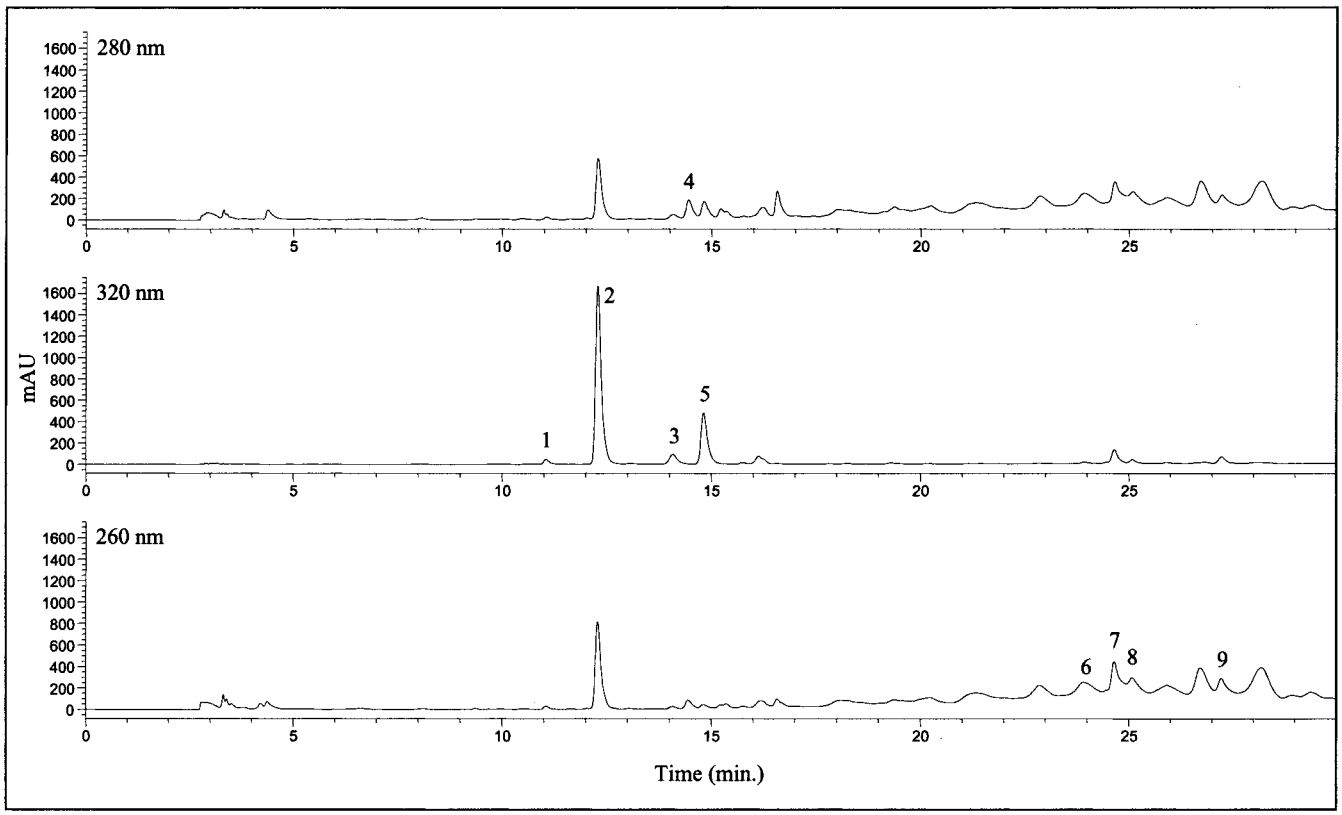

Figure 2. HPLC chromatogram of Thompson Seedless phenolic acids and flavonols. Peaks: (1) *cis*-caftaric acid; (2) caftaric acid; (3) *cis*-coutaric acid; (4) tryptophan; (5) coutaric acid; (6) rutin; (7) quercetin glycoside A; (8) quercetin glycoside B; (9) kaempferol glycoside B.

ol aglycons when the extract was subjected to acid hydrolysis (data not shown). Peak 4 was identified as tryptophan by matching its retention time (cochromatography) and UV spectrum to those of a reference standard. A number of flavan-3-ols and procyanidins have been identified in Thompson Seedless grapes by various workers (Spanos and Wrolstad, 1992). These include catechin, epicatechin, and procyanidin dimers B1, B2, B3, and B4, along with higher polymeric forms. Figure 3 shows the HPLC separation of the procyanidin fraction of Thompson Seedless grapes. Peak assignments were based on retention indices and UV spectra properties from an earlier investigation (Spanos and Wrolstad, 1990). Quantities measured were 16.4, 1.1, 9.0, 1.0, 0.3, 8.7, 16.4, 0.3, and 1.4 mg/kg for catechin, epicatechin, and procyanidin dimers B1, B2, B3, and B4, trimer, and tetramer, respectively.

The raisin polyphenolic profile (Figure 4) is strikingly different from that for Thompson Seedless grapes. This is ascribed to both enzymatic oxidation and nonenzymatic browning reactions which occur during dehydration of grapes. The spectra and elution order of peaks 1

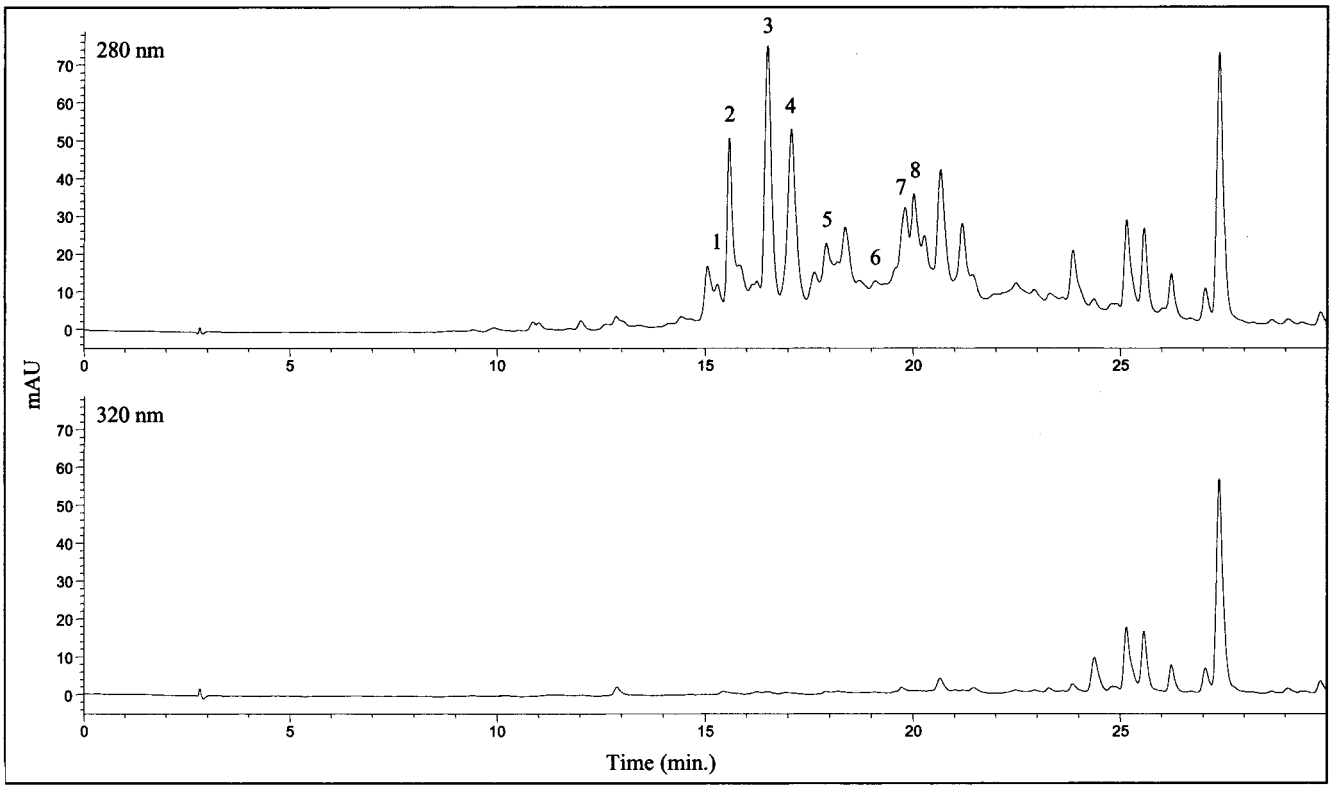

Figure 3. HPLC chromatogram of Thompson Seedless procyanidins. Peaks: (1) procyanidin B3; (2) procyanidin B1; (3) procyanidin B4; (4) catechin; (5) procyanidin B2; (6) procyanidin trimer; (7) procyanidin tetramer; (8) epicatechin.

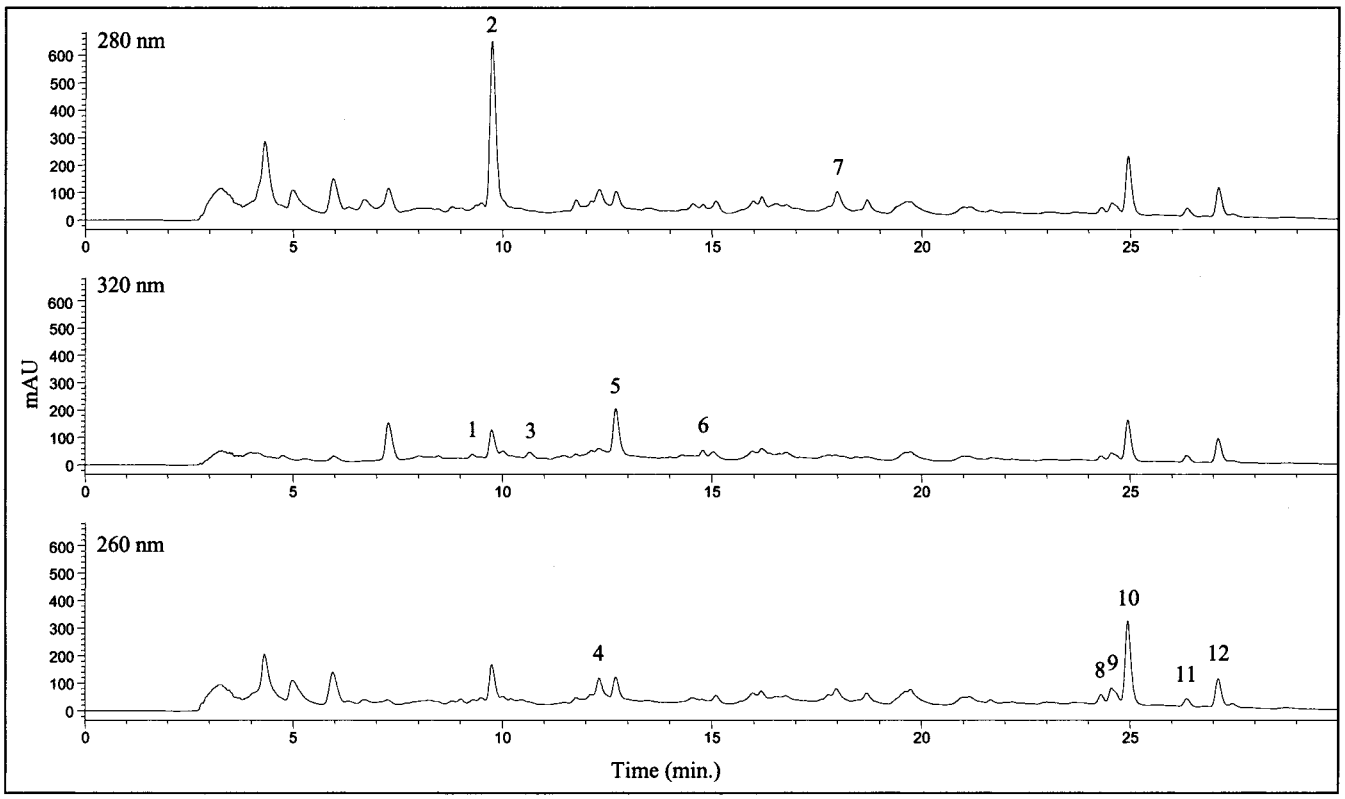

Figure 4. HPLC chromatogram of sun-dried raisin phenolic acids and flavonols. Peaks: (1) oxidized cinnamic A; (2) HMF; (3) oxidized cinnamic B; (4) protocatechuic acid; (5) caftaric acid; (6) coutaric acid; (7) unknown A; (8) rutin; (9) quercetin glycoside A; (10) quercetin glycoside B; (11) kaempferol glycoside A; (12) kaempferol glycoside B.

and 3 correspond closely to those for the oxidized cinnamics reported by Spanos and Wrolstad (1990). Peaks 2 (HMF) and 4 (procatechuic acid) were identified on the basis of comparisons of their retention times and UV spectra to those of reference standards. Protocatechuic acid has previously been reported to be present in grapes (Merida et al., 1991) and wines (Salagoity-Auguste and Bertrand, 1984). The amino acid tryp-

Table 1. Phenolic Acid Compositions of Raisins and Grapes (mg/kg of sample)[a]

sample	oxidized cinnamics[b] A	oxidized cinnamics[b] B	caftaric acid[b] cis	caftaric acid[b] trans	coutaric acid[b] cis	coutaric acid[b] trans	2-*S*-glutathionyl caftaric acid[b]	protocatechuic acid
sun-dried raisins ($n = 10$), mean	3.7[a] (2.5)	6.1[a] (2.5)	nd	39.6[A] (16.9)	nd	6.7[A] (3.1)	nd	6.8[A] (1.1)
dipped raisins ($n = 5$), mean	2.9[a] (1.2)	5.1[a] (2.8)	nd	45.2[A] (4.3)	nd	7.7[A] (2.6)	8.1 (2.4)	2.8[B] (1.7)
golden raisins ($n = 5$), mean	nd	nd	nd	84.3[B] (9.4)	nd	27.3[B] (6.0)	nd	nd
frozen grapes	nd	nd	nd	18.2	nd	1.2	nd	nd
fresh grapes	nd	nd	2.7	100.7	8.0	31.8	nd	nd

[a] Mean values in the same column not sharing superscript lowercase letters are significantly different ($p \leq 0.05$). Mean values in the same column not sharing superscript uppercase letters are significantly different ($p \leq 0.01$). [b] Quantitated as chlorogenic acid. nd = not detected.

Table 2. Flavonol Glycoside, HMF, Tyrosine, and Tryptophan Compositions of Raisins and Grapes (mg/kg sample)[a]

sample	rutin	quercetin glycoside[b] A	quercetin glycoside[b] B	kaempferol glycoside[c] A	kaempferol glycoside[c] B	tyrosine	tryptophan	HMF	unknown[d] A	unknown[d] B
sun-dried raisins ($n = 10$), mean	5.2[ab] (1.2)	7.3[A] (3.8)	34.7[a] (17.8)	11.2[AB] (4.4)	23.7[a] (13.4)	nd	nd	53.4[A] (15.0)	17.8 (5.27)	nd
dipped raisins ($n = 5$), mean	6.5[a] (2.1)	20.6[B] (8.8)	39.0[a] (16.2)	16.7[A] (6.3)	29.5[a] (11.2)	nd	nd	85.5[B] (18.0)	nd	nd
golden raisins ($n = 5$), mean	3.5[b] (1.0)	41.5[C] (10.7)	37.1[a] (5.6)	6.5[B] (1.6)	7.6[b] (4.0)	25.6 (10.5)	nd	20.6[C] (4.6)	nd	14.8 (4.4)
frozen grapes	1.6	25.9	53.7	4.9	16.1	11.3	18.2	0.3	nd	nd
fresh grapes	0.9	21.9	3.9	nd	19.4	nd	15.7	nd	nd	nd

[a] Mean values in the same column not sharing superscript lowercase letters are significantly different ($p \leq 0.05$). Mean values in the same column not sharing superscript uppercase letters are significantly different ($p \leq 0.01$). [b] Ouantitated as rutin. [c]Quantitated as kaempferol. [d]Quantitated as tryptophan. nd = not detected.

tophan was not detected in the raisin samples; however, peak 7 (unknown A) has a UV spectrum very simliar to that of tryptophan but a longer retention time. While golden raisins did not contain this peak, they did contain a new peak which also had a UV spectrum very similar to that of tryptophan but a shorter retention time. Without any additional identification criteria, these compounds are listed in Table 2 as unknowns; however, we believe they are less polar and more polar tryptophan derivatives. An additional kaempferol glycoside (kaempferol glycoside A) was detected in raisins which elutes earlier than kaempferol B found in fresh grapes. Dipped raisins have an additional compound in their polyphenolic profile which was tentatively identified as 2-*S*-glutathionylcaftaric acid, on the basis of its spectral and chromatographic properties similar to those of the compound described by Spanos and Wrolstad (1990) in SO_2-treated Thompson Seedless grape juice. No procyanidins were detected in the raisin polyphenolic extracts. While grape procyanidins have been characterized as not being directly susceptible to enzymatic oxidation, they are readily oxidized by enzymatically generated caffeoyltartaric acid *o*-quinones (Cheynier et al., 1989; Cheynier and Ricardo da Silva, 1991).

Effect of Processing on the Polyphenolic Composition of Raisins. The phenolic acid contents of raisins and Thompson Seedless grapes are shown in Table 1. Results can be converted from a fresh weight basis to a dry weight basis from the following moisture determinations, mean (standard deviation): sun-dried raisins, 16.9 (0.56); dipped raisins, 16.4 (0.47); golden raisins, 16.3 (0.54); frozen grapes, 78.1; fresh grapes, 84.8. Significant differences ($p \leq 0.01$) in phenolic acid content were found for all raisin processing treatments. Golden raisins contained the highest amount of *trans*-caftaric and *trans*-coutaric acids. The action of sulfur dioxide resulted in less oxidation and, therefore, higher phenolic concentrations in golden raisins. *cis*-Caftaric and *cis*-coutaric acids were not detected in any of the raisin samples or in frozen grapes. The absence in frozen grapes is undoubtedly due to the action of polyphenoloxidase during freezing and/or thawing of the grapes. Fresh grapes contain 2.7 ppm *cis*-caftaric and 8.0 ppm *cis*-coutaric. Oxidized cinnamics and protocatechuic acid are only present in sun-dried and dipped raisins. 2-*S*-Glutathionycaftaric acid was only detected in dipped raisins. Aguilera et al. (1987) reported that a 2 min dip in water at 93 °C was necessary to inactivate polyphenoloxidases (PPOs) in Sultana grapes. Since the dipping time (15−30 s) and temperature (87−93 °C) were insufficient to inactivate PPOs, enzymatic oxidation reactions probably occur during drying. Furthermore, enzyme activity might be stimulated in dipped raisins by very short hot-water dips (Hussein et al., 1942). In sun-dried raisins, 2-*S*-glutathionylcaftaric acid does not normally form due to the compartmentalization of glutathione and caftaric acid (Singleton et al., 1983). A possible explanation for golden raisins not containing 2-*S*-glutathionylcaftaric acid is SO_2 inhibition of phenolic oxidation.

The levels of quercetin and kaempferol glycosides in raisins and grapes along with the amounts of tyrosine, tryptophan, and HMF are presented in Table 2. The concentrations of quercetin glycosides A and B were the highest among flavonols, ranging from 42 mg/kg in sun-dried raisin to 78.6 mg/kg in golden raisins. Quercetin glycosides, except for quercetin glycoside A, did not show significant differences among different raisin processing treatments. Golden raisins have the lowest amount of kaempferol glycoside B with an average value of 7.6 mg/kg. The average concentration of kaempferol B was 23.7 in sun-dried raisins and 29.5 mg/kg in dipped raisins. Mean differences of kaempferol B between golden raisins and the other raisin samples are statistically significant at a level of 5%. The content of rutin was relatively low among the flavonols determined; mean values were 5.2, 6.5, and 3.5 mg/kg in sun-dried, dipped, and golden raisins, respectively.

Bolin and Petrucci (1985) reported that raisins contain 0.067 g of tyrosine/100 g of dry matter, while no tryptophan was detected. Neither of these two amino acids were detected in sun-dried or dipped raisins; however, golden raisins contained tyrosine and a possible tryptophan derivative in average concentrations

Table 3. Color Measurements for Raisins and Grapes (CIE *L*a*b*C*h* Values)[a]

sample	*L**	*a**	*b**	*C**	h
sun-dried raisins ($n = 10$), mean	20.7^{A} (0.6)	3.6^{A} (0.5)	3.6^{A} (0.3)	5.1^{A} (0.6)	44.9^{A} (2.1)
dipped raisins ($n = 5$), mean	21.2^{A} (0.5)	5.6^{B} (0.6)	6.9^{B} (0.7)	8.9^{B} (0.9)	51.1^{B} (0.7)
golden raisins ($n = 5$), mean	34.5^{B}(1.3)	10.7^{C} (04)	27.7^{C} (1.7)	29.7^{C} (1.6)	68.7^{C} (1.7)
frozen grapes	45.4	4.9	19.3	19.9	75.8
fresh grapes	48.4	7.9	34.6	35.5	102.9

[a] Mean values in the same column not sharing superscript uppercase letters are significantly different ($p \leq 0.01$).

of 30.6 and 17.6 mg/kg of dry weight, respectively. While the frozen grape samples contained tyrosine, it was not detected in the fresh sample. This might be due to the differences in growing conditions and maturity. Spayd and Andersen-Bagge (1996) reported that the amino acid concentration of grape juices prepared from the same variety was lower in Washington samples than in California samples. While HMF is not a phenolic compound, it is extracted, separated, and quantitated by these polyphenolic analytical procedures. HMF formation is indicative of the Maillard reaction and/or acid-catalyzed sugar degradation reactions, which also contribute to the dark brown color of the product (Lee and Nagy, 1988). All of the raisin samples, as expected, contained HMF. Dipped raisins had the highest HMF content with an average value of 85.5 mg/kg, while golden raisins had the lowest level (20.6 mg/kg). Fresh grapes did not contain HMF, while frozen grapes contained 0.3 mg/kg. HMF differences between different raisin treatments are statistically significant at a level of 1% (Table 2). The acetone−chloroform phase of the raisin extracts contained even larger amounts of HMF. For example, the HMF content in the water phase of dipped raisins (sample no. 2) was 109 mg/kg, while the organic phase of the same sample contained 474 mg/kg. According to these results, 81% of the HMF was partitioned in the organic phase. Therefore, the reported HMF values in Table 3 represent an estimate of only 19% of the total HMF in the samples. The disappearance of tyrosine and tryptophan, and the higher amounts of HMF in sun-dried and dipped raisins, is attributed to Maillard browning reactions. Marked losses of tryptophan have been reported in other Maillard browning systems (Leahy and Warthesen, 1983).

Thompson Seedless grapes from the same vineyard that were the source for the raisin samples were harvested and frozen to serve as a reference with respect to polyphenolic changes in raisin manufacture. Evidently, many of the polyphenolic compounds were degraded during freezing and thawing of the grapes (Tables 1−3). For this reason, fresh Thompson Seedless grapes purchased form a local market were also analyzed; however, their source is unknown, and it is known that there can be considerable variation in phenolic concentrations in grapes from different geographic locations and different growing seasons (Lee and Jaworski, 1987). Data for these two single samples are presented in Tables 1−3 for purposes of reference comparison for gross changes. When compared with fresh grapes on a dry weight basis, there was a loss of caftaric and coutaric acids on the order of 90%. The loss of total flavonols (rutin, quercetin glycosides A and B, and kaempferol glycosides A and B) is considerably less than that for the hydroxycinnamic acids, estimated to be 67%, 56%, and 62% for sun-dried, dipped, and golden raisins, respectively. Amiot et al. (1992) has reported that flavonols are much less susceptible to enzymatic degradation than hydroxycinnamic acids and flavan-3-ols in apple tissue. Procyanidins and flavan-3-ols completely degraded during raisin formation and during freezing/thawing of grapes. The enzymically generated caftaric acid *o*-quinones have been shown to oxidize other phenolic compounds, such as 2-*S*-glutathionylcaftaric acid (Cheynier et al., 1990), catechins (Cheynier et al., 1989), and procyanidins (Cheynier and Ricardo da Silva, 1991) by coupled oxidation mechanisms with reduction of caftaric acid quinones back to caftaric acid.

Resveratrol. Resveratrol, which is found in grape berries upon infection or stress, has gained considerable interest since it can inhibit the oxidation of low-density lipoprotein and platelet aggregation, and reduce the level of triacylglycerol (Lamuela-Raventos et al., 1995). There are several studies reporting the resveratrol and piceid (the 3-β-glucoside of resveratrol) contents of grapes and wine (Siemann and Creasy, 1992; Jeandet et al., 1995; Okuda and Yokotsuka, 1996; Pezet and Cuenat, 1996; Ector et al., 1996). In this study resveratrol was not detected in either grapes or raisins. (The detection limit was determined to be 0.5 mg/L for the standard solution and <2 mg/kg for the samples.) Since grapes are known to lose their capacity to produce resveratrol during ripening, it is possible that with fully ripened grapes for raisin production, there is negligible capacity for the grape to produce resveratrol, even in the face of fungal infection (Jeandet et al., 1991; Lamuela-Raventos and Waterhouse, 1993).

Ellagic Acid. The raisin and grape samples were analyzed for the possible presence of ellagic acid or any ellagic acid derivatives. Their presence was not detected in any of the samples. (The detection limit was 0.3 mg/L for the standard solution and <1 mg/kg for the samples.) Recovery of an ellagic acid standard solution added to raisins was determined to be 89%. Ellagic acid is associated with seeds and commonly found in nuts (Daniel et al., 1990) and several fruits such as strawberries and raspberries (Maas et al., 1991). It has been reported to be present in *Vitis rotundifolia* (Boyle and Hsu, 1990), but not in other grape species. The absence of ellagic acid in raisins and Thompson Seedless grapes is thus not unexpected.

Raisin Color. The color attributes of raisins and grapes are summarized in Table 3. As expected, sulfur dioxide inhibited browning in golden raisins, resulting in the highest lightness values (average 34.5), which were significantly different ($p \leq 0.01$) from those of the other two raisin processing treatments. Bolin et al. (1975) reported *L** values of 31.3 for sulfur dioxide treated raisins and 22.5 for unsulfited raisins, which are similar to our results. Sun-dried and dipped raisins had similar lightness values with average values of 20.7 and 21.2, respectively. The following CIE *L*a*b** indices were all significantly different ($p \leq 0.01$) among sun-dried, dipped, and golden raisins: *a**, *b**, hue angle (*h*), and chroma (*C**). Hue angle and chroma were clearly associated with visual observations. Browning of raisins is caused by both polyphenoloxidase, which is mainly located in the skin, and nonenzymic browning reactions (Grncarevic and Hawker, 1971). Increases in the drying

Table 4. Browning of Raisins and Grapes[a]

sample	water-soluble A_{420}/g of dry matter	insoluble (pellets) L*	a*	b*	C*	h
sun-dried raisins ($n = 10$), mean	0.69^{A} (0.2)	20.3^{A} (3.1)	10.9^{a} (1.1)	20.2^{A} (2.4)	23.0^{a} (2.6)	61.7^{A} (1.2)
dipped raisins ($n = 5$), mean	0.80^{A} (0.2)	26.4^{B} (3.1)	10.1^{a} (1.2)	25.7^{B} (1.8)	27.7^{b} (2.2)	68.2^{B} (1.1)
golden raisins ($n = 5$), mean	0.22^{B} (0.04)	49.9^{C} (2.9)	11.9^{a} (2.2)	39.5^{C} (3.7)	41.3^{c} (4.1)	73.5^{C} (1.8)
frozen grapes	0.012	20.8	8.5	20.9	22.6	67.9
fresh grapes	0.015	42.9	8.3	33.8	34.8	76.2

[a] Mean values in the same column not sharing superscript lowercase letters are significantly different ($p \leq 0.05$). Mean values in the same column not sharing superscript uppercase letters are significantly different ($p \leq 0.01$).

rate reduce browning since *o*-diphenoloxidase is inhibited by high concentrations of sugars (Radler, 1964). A high correlation between residual polyphenoloxidase activity and raisin darkness has been reported by Aquilera et al. (1987). Since sun-dried grapes dry much slower than mechanically dried grapes, the time for enzymatic browning is greater with more extensive brown color developed (Gee, 1980). The frozen and fresh grape samples had similar lightness values, but very different hue angles and chroma. Fresh grapes had hue coordinates in the yellow-green region, while the frozen grapes were in the yellow-red regions, substantiating the occurrence of enzymatic browning.

Color indices for the water-soluble and insoluble brown pigments from raisins and grapes are presented in Table 4. The absorbance values for the water-soluble brown pigments of raisins had the following ranges: sun-dried, 0.48−1.13; dipped, 0.54−0.99; golden, 0.19−0.28. Differences between sun-dried and dipped raisins were not significant; however, there was a visual difference. Goupy et al. (1995) explained that absorbance measurements only slightly correlated with visual evaluation of browning because oxidized, insoluble polymerized pigments bound to cell wall membranes were omitted. The following raisin pellet (insoluble filter residue) color attributes were significantly different among all three treatments ($p \leq 0.01$): L^*, b^*, chroma, and hue angle. Amiot et al. (1992) reported that most of the brown pigments present in the supernatant are derived from hydroxycinnamics, while the insoluble brown pigments of the pellets were mainly formed from flavan-3-ols. Since polymerized flavan-3-ols have been reported to be membrane-bound, they presumably contribute mainly to the pigmentation of the pellets (Macheix et al., 1991).

CONCLUSIONS

Measurement of raisin polyphenolic content shows that raisins are a good dietary source of flavonol glycosides and phenolic acids. Both HPLC analysis and color measurements revealed a number of significant differences between the raisin processing treatments. Both enzymatic and nonenzymatic browning reactions were inhibited by sulfur dioxide. Golden raisins had the highest amount of caftaric and coutaric acids and low amounts of HMF. Sun-dried and dipped raisins, on the other hand, contained oxidized cinnamics, while tyrosine and tryptophan disappeared because of the Maillard reaction. Flavonol glycosides were not as sensitive as hydroxycinnamics to enzymatic oxidation, the hydroxycinnamics being partially oxidized. The most labile polyhenolics were procyanidins and flavan-3-ols, since they were completely degraded in all raisin samples as well as in frozen grapes. Raisins are considered to be a desirable source of dietary fiber, with polymerized phenolics contributing to that fiber.

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