

Phenolic Composition and Antioxidant Activity of Prunes and Prune Juice (*Prunus domestica*)

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Phenolic compounds in foods have been associated with reduced incidences of heart disease by acting as antioxidants for low-density lipoprotein (LDL). Commercial prune and prune juice extracts (*Prunus domestica* cv. French) were analyzed for phenolics by reversed phase HPLC with diode array detection and tested for the ability to inhibit the Cu²⁺-catalyzed oxidation of human LDL. The mean concentrations of phenolics were 1840 mg/kg, 1397 mg/kg, and 441 mg/L in pitted prunes, extra large prunes with pits, and prune juice, respectively. Hydroxycinnamates, especially neochlorogenic acid, and chlorogenic acid predominated, and these compounds, as well as the prune and prune juice extracts, inhibited the oxidation of LDL. The pitted prune extract inhibited LDL oxidation by 24, 82, and 98% at 5, 10, and 20 μ M gallic acid equivalents (GAE). The prune juice extract inhibited LDL oxidation by 3, 62, and 97% at 5, 10, and 20 μ M GAE. These data indicate that prunes and prune juice may provide a source of dietary antioxidants.

Keywords: Low-density lipoprotein (LDL); antioxidants; phenolics; neochlorogenic acid; prune; plum; *Prunus domestica*

INTRODUCTION

Phenolic compounds are important components of many fruits, vegetables, and beverages, in which they contribute to color and sensory properties such as bitterness and astringency (Macheix et al., 1990). Epidemiological studies have shown that consumption of foods and beverages rich in phenolic content is correlated with reduced incidences of heart disease (Hertog et al., 1993, 1995; Criqui and Ringel, 1994; Renaud and deLorgeril, 1992). One possible explanation is that phenolic compounds slow the progression of atherosclerosis by acting as antioxidants toward low-density lipoproteins (LDL) (Frankel et al., 1993; Kinsella et al., 1993). The oxidative modification of LDL is a critical step in the development of atherosclerosis, and prevention of this step is thought to slow the progression of the disease (Steinberg, 1993; Esterbauer et al., 1992). Studies have shown that phenolic compounds contained in fruits are potent inhibitors of the in vitro oxidation of LDL (Meyer et al., 1997; Teissedre et al., 1996; Rice-Evans et al., 1996; Frankel et al., 1995; Vinson et al., 1995; deWhalley et al., 1990). There are also studies suggesting that phenolic compounds have antioxidant activity in vivo (Fuhrman et al., 1995; Whitehead et al., 1995; Zloch, 1969; Rusznyak and Szent-Gyorgi, 1936).

Californian prunes are produced from a cultivar of *Prunus domestica* plums referred to by several names including French, Petite, d'Agen, and d'Ente (Chaney, 1981). California produces $\approx 67\%$ of the world's prune supply (California Prune Board, 1997). The primary objective of this investigation was to determine the level

of total phenolics, the concentration of each phenolic compound, and the variability of phenolic compounds in Californian prunes and prune juice. To identify changes that result from processing, freshly harvested unprocessed prune-making plums were also analyzed for phenolics. The second main objective of this investigation was to determine if prune and prune juice extracts had antioxidant activity toward human LDL.

MATERIALS AND METHODS

Phenolic Standards. (+)-Catechin, (–)-epicatechin, and *p*-coumaric acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). Rutin, (hydroxymethyl)furfural (HMF), and caffeic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Chlorogenic acid (5'-caffeoylquinic acid) was purchased from Fluka Chemical Corp. (Buchs, Switzerland). Gallic acid was obtained from MCB Manufacturing Chemists Inc. (Cincinnati, OH), sorbic acid was obtained from Eastman Kodak Co. (Rochester, NY), and malvin was purchased from Pfaltz & Bauer (Waterbury, CT). Neochlorogenic acid (3'-caffeoylquinic acid) was kindly provided by Dr. Murray Isman (Department of Plant Sciences, University of British Columbia, Vancouver).

Prunes and Prune Juice Samples. Twenty-one samples of pitted prunes, 12 samples of extra large unpitted prunes, and 9 samples of prune juice from different orchards and processing facilities throughout California were obtained from the California Prune Board (Pleasanton, CA) over a 6 month period during 1995–1996. These samples were commercially packaged and selected from lots of prunes and prune juice destined for the market. Four samples of freshly harvested unprocessed prune-making plums were also obtained from the California Prune Board.

Extraction of Phenolic Compounds. Ten prunes were randomly selected from one freshly opened package, pitted by hand, and cut into small pieces; a 10.0 g portion was homogenized in 75 mL of methanol (HPLC grade, Fisher Scientific, Springfield, NJ) containing 50 mg/L sodium metabisulfite (Mallinckrodt Inc., Chesterfield, MO). After a 30 min extrac-

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Table 1. Conditions Used for HPLC Analysis of Phenolic Compounds in Prunes and Prune Juice

instrument	Hewlett-Packard 1090 liquid chromatograph
column	LiChrosphere C ₁₈ , 4 × 250 mm, 5 μm particle size
injection volume	25 μL
flow rate	0.5 mL/min
detection	photodiode array (190–600 nm)
mobile phase	A = 50 mM NaH ₂ HPO ₄ at pH 2.60 B = 80% acetonitrile, 20% A C = 200 mM <i>o</i> -phosphoric acid at pH 1.50
gradient	
5.0 min	%A, 100.0; %B, 0.0; %C, 0.0
8.0 min	%A, 92.0; %B, 8.0; %C, 0.0
20.0 min	%A, 0.0; %B, 14.0; %C, 86.0
25.0 min	%A, 0.0; %B, 16.5; %C, 82.0
35.0 min	%A, 0.0; %B, 21.5; %C, 78.5
70.0 min	%A, 0.0; %B, 50.0; %C, 50.0
75.0 min	%A, 100.0; %B, 0.0; %C, 0.0
80.0 min	%A, 100.0; %B, 0.0; %C, 0.0

tion period (while agitating), the sample was centrifuged (2000*g*, 5 min), the supernatant was removed, and the precipitate was re-extracted a second time with 75 mL of methanol for 30 min and then a third time with 75 mL of 80% aqueous methanol for 30 min. The supernatants were combined, rotary evaporated at 35 °C to 50 mL, and then diluted to 100 mL with water. Phenolics were extracted from prune juice by mixing 25 mL of prune juice with 75 mL of 80% aqueous methanol saturated with sodium chloride (Fisher Scientific). After a 30 min extraction period (while agitating), the solution was centrifuged to obtain two separate liquid phases. The methanol fraction was removed, and the remaining liquid was extracted two additional times with 75 mL of 80% aqueous methanol for 30 min each. The methanol-containing fractions were combined, rotary evaporated, and then diluted to 100 mL with water.

Analysis of Phenolic Compounds. Prior to analysis, the extracts were filtered through 0.45 μm poly(tetrafluoroethylene) (PTFE) syringe tip filters (Gelman Sciences, Ann Arbor, MI) into flint glass HPLC vials equipped with PTFE-lined crimp caps. The phenolic composition of the prunes and prunes juices was analyzed by high-performance liquid chromatography (HPLC) as described previously (Lamuela-Raventos and Waterhouse 1994), with slight changes in the multi-linear mobile phase gradient (Table 1). Four wavelengths were monitored for quantitation: 280 nm for catechins and benzoic acids, 316 nm for hydroxycinnamates, 365 nm for flavonols, and 520 nm for anthocyanins.

Compounds were quantified by calibration with the described standards. Compounds that were not available were quantified using the peak areas of standards with similar spectral characteristics and quantities were reported as equivalent amounts of that commercial standard.

Statistical Analysis. A *t* test was used to obtain a confidence interval for the mean level of total phenols in the population of Californian prunes and prune juice. The calculation of the confidence interval was described by Moses (1986): $\mu = \bar{x} \pm (ts)/\sqrt{n}$, where μ was the population mean (unknown), \bar{x} was the sample mean, *s* was the sample standard deviation, *n* was the number of samples, and *t* was the *t* value at *n* – 1 degrees of freedom.

Preparation of LDL. Plasma was prepared from blood collected by venipuncture from five healthy volunteers in 10 mL Vacutainer tubes (Becton Dickinson Inc., Franklin Lakes, NJ) containing ethylenediaminetetraacetic acid (K₃EDTA). LDL was prepared by sequential density preparative ultracentrifugation (Orr et al., 1991). Prior to oxidation, EDTA was removed by dialysis using Spectra/Por membrane tubing (MW cutoff of 12–14 kDa; Spectrum Medical Industries, Inc., Los Angeles, CA) into pH 7.4 deoxygenated phosphate buffered saline at 4 °C. LDL protein concentration was determined with a Lowry protein analysis kit (Sigma) and was diluted to 1000 μg/mL protein prior to oxidation.

Antioxidant Activity for LDL. To assess the antioxidant activity of the prunes and prune juice, the extracts were

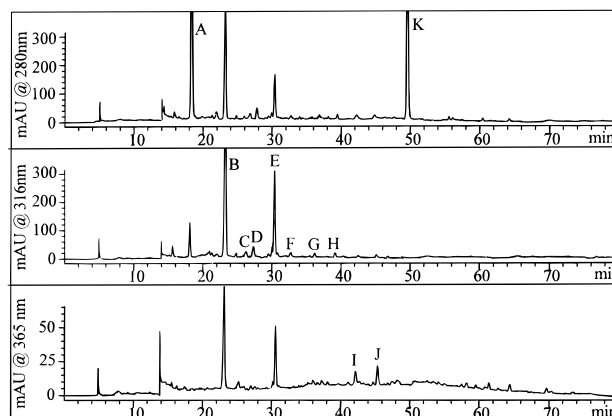


Figure 1. HPLC chromatogram of pitted prune extract: (A) HMF; (B) neochlorogenic acid; (C) cinnamate; (D) 3'-*p*-coumaroylquinic acid (tentative); (E) chlorogenic acid; (F) caffeic acid; (G) cinnamate; (H) coumaric acid; (I) flavonol; (J) rutin; (K) sorbic acid. The signal at 520 nm was also monitored; however, it is not shown here because no signals were obtained at that wavelength.

prepared without the addition of sodium metabisulfite. After the methanol was completely removed by rotary evaporation, total phenols were measured using the Folin–Ciocalteu (FC) method (Singleton and Rossi, 1965). The extracts were stored at 4 °C and used before any measurable degradation of phenolics had occurred (12–24 h).

Inhibition of LDL oxidation was determined by monitoring hexanal production by static headspace gas chromatography from copper-catalyzed LDL oxidation (2 h at 37 °C, 80 μM CuSO₄) as previously described (Frankel et al., 1992). Results were calculated after replicate analyses and expressed as percent inhibition of the control LDL: $[(C - S)/C] \times 100$, where *C* was hexanal formed from the LDL with no extract added and *S* was hexanal formed from the LDL with the prune or prune juice extract added.

RESULTS AND DISCUSSION

Extraction and Analysis Procedure. The extraction procedures were sufficient to extract phenolic compounds from prunes and prune juice. The extent of extraction was investigated by performing two extractions with 100% methanol in addition to the described procedure. For both prunes and prune juice, less than an additional 4% of chlorogenic and neochlorogenic acid (the two predominant phenolics in prunes) was obtained in these extracts. The HPLC method described here provided separation of the major peaks in these extracts. The peaks that were specifically identified generally made up 90% of the total area at 280 nm. The peaks that were tentatively identified by class (that is, cinnamates and flavonols) made up only 1–2% of the total amount of phenols identified in prunes. The peaks that were not identified generally made up <5% of the area at 280 nm. A chromatogram of a pitted prune extract is shown in Figure 1. The extraction and HPLC analysis were also very reproducible. Reproducibility was assessed by separate extractions and HPLC analyses of six homogeneous samples of prunes and prune juice. The coefficients of variation were <3% for neochlorogenic and chlorogenic acid in both prunes and prune juice.

Phenolic Composition of Prunes. The phenolic composition and the amount of total phenols as determined by HPLC were similar in the pitted prunes and the extra large prunes with pits (Table 2). The confi-

Table 2. Phenolic Composition of Pitted Prunes, Extra Large Prunes with Pits, Prune Juice, and Fresh Unprocessed Prunes^a

compound	pitted prunes (mg/kg) (<i>n</i> = 21)	extra large prunes with pits (mg/kg) (<i>n</i> = 12)	prune juice (mg/L) (<i>n</i> = 9)	fresh prune-making plums (<i>n</i> = 4)
neochlorogenic acid	1306 ± 629	928 ± 219	225 ± 34	807 ± 103
3'-coumaroylquinic acid ^b	15 ± 13	10 ± 4	4 ± 1	10 ± 1
catechin	nd ^c	nd	nd	54 ± 14
chlorogenic acid	436 ± 201	411 ± 126	193 ± 26	144 ± 23
caffeic acid	9 ± 8	10 ± 5	3 ± 1	nd
coumaric acid	10 ± 5	10 ± 5	4 ± 1	nd
other cinnamates ^b	24 ± 54	4 ± 6	7 ± 2	nd
rutin	33 ± 25	14 ± 6	4 ± 1	25 ± 5
other flavonols ^b	9 ± 13	14 ± 3	2 ± 1	2 ± 2
anthocyanins ^b	nd	nd	nd	76 ± 14
HMF ^d	220 ± 189	291 ± 205	528 ± 91	nd
sorbic acid ^d	818 ± 310	425 ± 177	nd	nd
total phenolics	1840 ± 855	1397 ± 299	441 ± 59	1107 ± 114

^a Values are expressed as mean concentrations ± the standard deviation. ^b Other cinnamates are reported in caffeic acid equivalents, other flavonols in rutin equivalents, and anthocyanins in malvin equivalents; the identification of 3'-coumaroylquinic acid is tentative and reported in coumaric acid equivalents. ^c nd, not detected (<3 mg/kg of prune). ^d HMF and sorbic acid are not phenolic compounds.

dence intervals for the level of total phenolics were 1840 ± 390 mg/kg in the population of Californian pitted prunes and 1397 ± 190 mg/kg in the population of Californian extra large prunes with pits (*t* test, *p* < 0.05, mass does not include the pit).

Hydroxycinnamates made up 98% of the phenolic material, and neochlorogenic acid generally accounted for >65% of the total phenolics. Flavonols accounted for ≈2% of total phenolics and were the only flavonoids identified in the prune samples.

Flavan-3-ols and anthocyanins were absent in the samples (although there was 54 mg/kg catechin in the fresh prune-making plums, as will be discussed below). The lack of flavan-3-ols is in contrast to a previously published report that water extracts of Californian prunes, normalized to 18.5% solids, contained 126–179 mg/L catechin (van Gorsel et al., 1992). If catechin was present at these levels, it would have been detected in this study, so the reason for this inconsistency is not apparent. However, other researchers have reported that fresh prune-making plums (that is, not dehydrated) contain low amounts of catechin, that it predominates in the skin of the plum, and that levels significantly decrease during the dehydration process (Raynal et al., 1989). The present results agree with previous reports that neochlorogenic acid is the principal phenolic compound in prunes, followed by its isomer chlorogenic acid. Rutin has also been previously identified as a predominant flavonol, and prunes have been characterized by the absence of anthocyanins (Raynal and Moutounet, 1989; van Gorsel et al., 1992).

Levels of phenolics in prunes are difficult to compare with other commercial fruits because most previous studies did not analyze large, representative samples of commercial products. Many factors can affect phenolic levels (Macheix et al., 1990) so the amount found in one sample may not reflect the average level on the market. However, the level reported here for prunes surpasses the levels reported for many other popular fruits. Red Flame seedless table grapes were recently reported to contain <250 mg of phenolics/kg of grape and white table varieties <50 mg/kg of grape (Meyer et al., 1997), although a mild extraction procedure was used during this study. Apples were reported to contain 1200 mg/kg (Kühnau, 1976; Herrmann, 1989), oranges 830 mg/kg (Kühnau, 1976; Bilyk, 1986), pears 265 mg/

kg (Kühnau, 1976; Herrmann, 1989), and cherries 850 mg/kg of fruit (Kühnau, 1976; Herrmann, 1989), while blueberries were exceptionally high in phenolics and surpassed the amount found in prunes, containing 4500 mg of phenolics/kg of fruit (Kühnau, 1976; Peleg, 1991; Bilyk, 1986).

Phenolic Composition of Prune Juice. The mean concentration of total phenolic compounds, identified by HPLC, in prune juice was 441 mg/L. The confidence interval for the average concentration of total phenolics in the population of Californian prune juice was 441 ± 48 mg/L (*t* test, *p* < 0.05). The specific phenolic compounds in the prune juice were the same as the phenolics in the prunes (Table 2). Hydroxycinnamates made up almost 99% of the phenolics in prune juice. Neochlorogenic acid accounted for 51% of the phenolics, and chlorogenic acid accounted for 44% of the phenolics. The ratio of these two compounds is in contrast to the prune samples, in which chlorogenic acid was found at less than half of the level of neochlorogenic acid. The present investigation does not, however, permit us to determine the reason for this difference. Flavonols were found at 6 mg/L and accounted for 1% of phenolics. Like the prunes, flavan-3-ols and anthocyanins were absent in all of the samples tested.

The presence of large quantities of neochlorogenic acid in prune juice sets it apart from other popular juices such as apple, orange, pear, and grape (Macheix et al., 1990). Flavonoids and phloridzin in apple juice (Spanos et al., 1990), tartaric acid esters of hydroxycinnamates in grape juice, anthocyanins in red grape juice (Spanos and Wrolstad, 1990a; Mazza and Miniati, 1993; Fernández de Simon et al., 1992), and catechins in pear juice (Spanos and Wrolstad, 1990b) are components that make these juices different from prune juice. Also, prune juice contained higher levels of phenolic compounds than the levels reported for many other commercial juices. White grape juices were reported to contain <100 mg/L (Fernández de Simon et al., 1992; Spanos and Wrolstad, 1990a), but a comprehensive analysis of phenolics in commercial red grape juice has not been reported. White wines usually contain <200 mg/L phenolic compounds, whereas red wines typically contain between 400 and 700 mg/L monomeric phenolic compounds in addition to 1000 mg/L polymeric polyphenols (Singleton, 1980). Pear juice has been reported to

Table 3. Total Amount of Phenolic Compounds Identified by HPLC in Prune Products Expressed on the Basis of Total Mass and Total Soluble Solids

sample	soluble solids ^b (%)	total phenols (mg/kg)	total phenols (mg/kg of soluble solids)
pitted prunes	62	1840	2698
extra large prunes with pits ^a	62	1397	2253
prune juice ^a	18.5	441	2384
fresh unprocessed plums	24	1107	4613

^a Values do not include the mass of the pit; phenolics in prune juice are expressed as mg/L. ^b Soluble solids contents are based on previously reported levels (Miller, 1981; California Prune Advisory Board, 1990).

contain up to 300 mg/L and apple juice up to 330 mg/L, but some processing procedures reduced the levels in both these juices to <1 mg/L (Spanos and Wrolstad, 1990b; Spanos et al., 1990).

Phenolic Composition of Prune-making Plums.

The amount of total phenolics, identified by HPLC, in prune-making plums ranged from 948 to 1219 mg/kg with an average of 1107 mg/kg (Table 2). Hydroxycinnamates were found only in their esterified form and accounted for 84–90% of total phenols, with neochlorogenic acid predominating. The free cinnamates found in the prunes and prune juice were probably formed during processing by ester hydrolysis, which may have been acid-catalyzed or perhaps due to an esterase naturally present in the plums. Flavonols accounted for 2–3% of phenolics. The flavan-3-ol, catechin, accounted for 4–8% of total phenolics; however, epicatechin was not detected in any of the samples, and this result agrees with previous reports that this cultivar of plums does not contain epicatechin (Raynal et al., 1989; van Gorsel et al., 1992). Anthocyanins were also found in the fresh plums and accounted for 4–9% of phenolics.

The edible portion of fresh prune-making plums contains ≈24% soluble solids, whereas commercial prunes contain ≈62% soluble solids. Prune juice is an aqueous extract of dehydrated prunes normalized to 18.5% solids (Miller, 1981; California Prune Board, 1990). The water content and mass of each product change during processing. This must be considered when the phenolic levels of these products are compared because levels are reported on the basis of final mass or volume. During processing, the total amount of soluble solids remains constant, and when phenolic levels are expressed on the basis of soluble solids, it is clear that the processing of prunes from fresh plums degrades approximately half of the phenolic compounds. Because prunes and prune juice had similar levels of phenolics on the basis of soluble solids, the processing of prune juice from prunes does not appear to significantly degrade phenolic compounds. Phenolic levels in each product are expressed on the basis of mass or volume and soluble solids content in Table 3.

HMF and Sorbic Acid Levels in Prune Products.

HMF is not a phenolic compound; however, because it was extracted and separated using the described analytical procedure for phenolics, it is reported in this study. HMF forms from the dehydration of sugars in the presence of acid and heat and also during the Maillard browning reaction. This compound is found in many common foods and other thermally processed fruit juices (Lang, 1970). As expected, all of the prune samples contained HMF (Table 2). However, on average, the amount of HMF in the prune samples was

Table 4. Inhibition of Cu²⁺-Catalyzed LDL Oxidation in Vitro by Prune and Prune Juice Extracts and Selected Compounds Found in These Products

sample	% inhibition at		
	5 μ M ^a	10 μ M ^a	20 μ M ^a
prune extract	24 ± 3	82 ± 6	98 ± 1
prune juice extract	3 ± 3	62 ± 1	97 ± 1
neochlorogenic acid	87 ± 3	99 ± 1	99 ± 1
chlorogenic acid	91 ± 6	99 ± 1	99 ± 1
HMF	3 ± 4	5 ± 2	0 ± 6
sorbic acid	nd ^b	nd	15 ± 7

^a Inhibition data are given as mean values ± the standard deviation of replicate results; the prune and prune juice extracts were tested at levels reported in gallic acid equivalents (GAE).

^b nd, not determined.

<10% of the amount of total phenolics. Prune juice, on the other hand, contained approximately twice as much HMF as the prune samples, and the amount surpassed the level of phenolics in the juice. The increased level of HMF in the prune juice should be expected because of the additional exposure to heat during the extraction procedure. HMF was not detected in any samples of fresh unprocessed prunes. Although little information is available on the nutritional importance of this compound, no adverse effects were observed when rats were fed levels of 450 mg/kg of diet (Lang, 1970).

Sorbic acid is a preservative, used to prevent unwanted microbial growth, which is routinely used in prune processing (California Prune Board, 1990). The average concentrations of sorbic acid were 818 mg/kg in pitted prunes and 425 mg/kg in the extra large prunes with pits (Table 2). As expected, sorbic acid was not detected in any of the samples of fresh unprocessed prunes or prune juice. Because prune juice is heat treated prior to bottling (Jackson, 1981), there is no need to add this preservative.

Inhibition of LDL Oxidation in Vitro. The prune and the prune juice extracts significantly inhibited the oxidation of lipids in isolated human LDL when tested at micromolar phenol concentrations (Table 4). At equivalent total phenol levels according to the FC assay, the prune extract was a more powerful inhibitor of LDL oxidation than the prune juice extract. Total phenols by the FC assay in the prune juice extract were ≈4 times the amount identified by HPLC [1640 mg/L gallic acid equivalents (GAE)]. Conversely, total phenols by the FC assay in the prune extract were only 1.3 times the amount identified by HPLC (2370 mg/kg GAE). The increased FC response of the prune juice extract may be due to interferences which increased the apparent phenol concentration and thus the dilution factor of the juice extract for the antioxidant test. If so, this may explain the decreased antioxidant activity of the juice extract compared with the prune extract. Reductones formed from Maillard browning reactions may have been interferences in these assays. HMF, however, is not thought to be an interference because it did not have a response in the FC assay or appreciable antioxidant activity toward LDL.

The antioxidant activity of the prune extract is similar to that of other foods that contain phenolic phytochemicals such as grapes, wine, and chocolate. These foods were shown to significantly inhibit LDL oxidation at 5–10 μ M (Frankel et al., 1993, 1995; Teissedre et al., 1996; Waterhouse et al., 1996; Meyer et al., 1997).

The predominant phenolic compounds in prunes also inhibited LDL oxidation (Table 4). The antioxidant

activity of neochlorogenic acid toward LDL has not been previously reported; however, the strong antioxidant activity of chlorogenic acid toward LDL is similar to previously described reports (Rice-Evans et al., 1996; Nardini et al., 1995). Other phenolic components in prunes such as rutin and caffeic acid have also been reported to be active inhibitors of human LDL oxidation (Teissedre et al., 1996; Rice-Evans et al., 1996; Nardini et al., 1995).

Relatively little is known about the absorption and metabolism of hydroxycinnamic acids, and no information is available on the existence of these compounds in human blood or tissues. However, both caffeic and ferulic acid are thought to be absorbed, at least in part, by humans because their metabolites have been detected in human urine (Jacobson et al., 1983).

CONCLUSIONS

Prunes and prune juice were characterized by high concentrations of hydroxycinnamic acids, especially neochlorogenic acid. The processing of prunes from fresh plums degraded phenolic compounds. Approximately half of the flavonols and half of the hydroxycinnamates were degraded after commercial processing. Furthermore, anthocyanins and flavan-3-ols had been completely degraded after processing. Conversely, the processing of prune juice from prunes did not appear to significantly degrade phenolic compounds.

The amount of phenolics in one serving of prune juice (240 mL) is 106 mg. This value is higher than the amount in one serving of prunes (42 g), which is 73 mg. Prunes and prune juice contain higher levels of phenolic compounds than many other fruits, and commercial juices and daily consumption of either of these products would increase the dietary intake of hydroxycinnamates.

The prune extracts, as well as neochlorogenic acid and chlorogenic acid, the two predominant phenolic compounds contained in prunes, were antioxidants toward isolated human LDL. If these compounds have similar activities in vivo, consumption of prunes would be a good source of dietary antioxidants. Hydroxycinnamates are abundant in many foods, and the role of these compounds in human nutrition requires further research.

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LITERATURE CITED

- Bilyk, A.; Sapers, G. M. Varietal differences in the quercetin, kaempferol and myricetin contents of highbush blueberry, cranberry and thornless blackberry fruits. *J. Agric. Food Chem.* **1986**, *34*, 585.
- California Prune Board. *The California Prune Buyer's Guide*; Pleasanton, CA, 1990.
- California Prune Board. *California Prune News Annual Report 100*; Pleasanton, CA, Jan 1997.
- Chaney, D. H. The Tree. In *Prune Orchard Management*; Ramos, D. E., Ed.; The Regents of the University of California Special Publication 3269; University of California: Davis, CA, 1981; Vol. 1, Chapter 2.
- Criqui, M. H.; Ringel, B. L. Does diet or alcohol explain the French paradox? *Lancet* **1994**, *344*, 1719–1723.
- deWhalley, C. V.; Rankin, S. M.; Houlst, J. R. S.; Jessup, W.; Leake, D. S. Flavonoids inhibit the oxidative modification of low-density lipoproteins by macrophages. *Biochem. Pharmacol.* **1990**, *39*, 1743–1750.
- Esterbauer, H.; Gebicki, J.; Puhl, H.; Jürgens, G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Res. Commun.* **1992**, *13*, 34–390.
- Fernández de Simon, B.; Pérez-Ilzarbe, J.; Hernández, T.; Gómez-Cordovés, C.; Estrella, I. Importance of phenolic composition for the characterization of fruit juices. *J. Agric. Food Chem.* **1992**, *40*, 1531–1535.
- Frankel, E. N.; German, J. B.; Davis, P. A. Headspace gas chromatography to determine human low-density lipoprotein oxidation. *Lipids* **1992**, *27*, 1047–1051.
- Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454–457.
- Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoprotein. *J. Agric. Food Chem.* **1995**, *43*, 890–894.
- Fuhrman, B.; Lavy, A.; Aviram, M. Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. *Am. J. Clin. Nutr.* **1995**, *61*, 549–554.
- Herrmann, K. Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 315–347.
- Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, J. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet* **1993**, *342*, 1007–1011.
- Hertog, M. G. L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Giampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; Pekkarinen, M.; Simic, B. S.; Toshima, H.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Internal Med.* **1995**, *155*, 381–386.
- Jackson, H. W. Harvesting and Handling the Crop. In *Prune Orchard Management*; Ramos, D. E., Ed.; The Regents of the University of California Special Publication 3269; University of California: Davis, CA, 1981; Vol. 1, Chapter 8.
- Jacobson, E. A.; Newmark, H.; Baptista, J.; Bruce, W. R. A preliminary investigation of the metabolism of dietary phenolics in human. *Nutr. Rep. Int.* **1983**, *28*, 1409–1417.
- Kinsella, J. E.; Frankel, E. N.; German, J. B.; Kanner, J. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol.* **1993**, *47*, 85–89.
- Kühnau, J. The flavonoids, a class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet.* **1976**, *24*, 117–191.
- Lamuela-Raventós, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994**, *45*, 1–5.
- Lang, K. Influence of cooking on foodstuffs. In *World Review Nutrition and Diet*; Bourne, G. F., Ed.; Karger: New York, 1970; Vol. 12, pp 266–317.
- Macheix, J. J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990; pp 24–31, 295–342.
- Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables, and Grains*; CRC Press: Boca Raton, FL, 1993; pp 184–185.
- Meyer, A. S.; Yi, O.; Pearson, D. A.; Waterhouse, A. L.; Frankel, E. N. Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). *J. Agric. Food Chem.* **1997**, *45*, 1638–1643.
- Miller, M. W. Harvesting and Handling the Crop. In *Prune Orchard Management*; Ramos, D. E., Ed.; The Regents of the University of California Special Publication 3269; University of California: Davis, CA, 1981; Vol. 1, Chapter 8.

- Moses, L. E. A First Look at Statistical Inference. In *Think and Explain with Statistics*; Addison-Wesley Publishing: Reading, MA, 1986; pp 111–150.
- Nardini, M.; D'Aquino, M.; Tomassi, G.; Gentili, V.; Di Felice, M.; Scaccini, C. Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. *Free Radical Biol. Med.* **1995**, *19*, 541–552.
- Orr, J. R.; Adamson, G. L.; Lindgren, F. T. Preparative ultracentrifugation of plasma lipoproteins. In *Analysis of Fats Oils and Lipoproteins*; Perkins, E. G., Ed.; American Oil Chemists' Society: Champaign, IL, 1991; pp 524–554.
- Peleg, H.; Naim, M.; Rouseff, R. L.; Zehavi, U. Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). *J. Sci. Food Agric.* **1991**, *57*, 416–426.
- Raynal, J.; Moutounet, M. Intervention of phenolic compounds in plum technology. 2. Mechanisms of anthocyanin degradation. *J. Agric. Food Chem.* **1989**, *37*, 1051–1053.
- Raynal, J.; Moutounet, M.; Souquet, J. M. Intervention of phenolic compounds during drying. *J. Agric. Food Chem.* **1989**, *37*, 1046–1050.
- Renaud, S.; de Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523–1526.
- Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-Antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20* (7), 933–956.
- Rusznayak, I.; Szent-Gyorgi, A. Vitamin P: flavanols as vitamins. *Nature* **1936**, *138*, 27.
- Singleton, V. L. Grape and Wine Phenolics; Background and Prospects. *C. R. Collect. Centenaire Paris* **1980**, 215.
- Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.
- Spanos, G. A.; Wrolstad, R. E. Influence of processing and storage on the phenolic composition of Thomson Seedless grape juice. *J. Agric. Food Chem.* **1990a**, *38*, 1565–1571.
- Spanos, G. A.; Wrolstad, R. E. Influence of variety, maturity processing, and storage on the phenolic composition of pear juice. *J. Agric. Food Chem.* **1990b**, *38*, 817–824.
- Spanos, G. A.; Wrolstad, R. E.; Heatherbell, D. A. Influence of processing and storage on the phenolic composition of apple juice. *J. Agric. Food Chem.* **1990**, *38*, 1572–1579.
- Steinberg, D. Modified forms of low-density lipoprotein and atherosclerosis. *J. Int. Med.* **1993**, *233*, 227–232.
- Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wine. *J. Sci. Food Agric.* **1996**, *90*, 55–61.
- van Gorsel, H.; Li, C.; Kerbel, E. L.; Smits, M.; Kadar, A. A. Compositional Characterization of Prune Juice. *J. Agric. Food Chem.* **1992**, *40*, 784–789.
- Vinson, J. A.; Dabbagh, Y. A.; Serry, M. M.; Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *J. Agric. Food Chem.* **1995**, *43*, 2800–2802.
- Waterhouse, A. L.; Shirley, J. R.; Donovan, J. L. Antioxidants in chocolate. *Lancet* **1996**, *348*, 834.
- Whitehead, T. P.; Robinson, D.; Allaway, S.; Syms, J.; Hale, A. Effect of red wine ingestion on the antioxidant capacity of serum. *Clin. Chem.* **1995**, *41*, 32–35.
- Zloch, Z. Influence of quercetin and epicatechol on biochemical changes in guinea pigs during experimental C-hypovitaminosis. *Int. Z. Vitaminforsch.* **1969**, *39*, 269–280.

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