Composition of Flavonols in Red Raspberry Juice As Influenced by Cultivar, Processing, and Environmental Factors†

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Flavonols were characterized and measured in experimental (n = 46) and commercial (n = 9) red raspberry juices by HPLC/diode array spectral techniques. Samples were prepared using minicolumns, packed with Polyamide 6. A fraction eluted with methanol contained eight or fewer quercetin glycosides, quercetin, and kaempferol. A second fraction eluted subsequently with 0.5% ammonia in methanol contained three flavonol glucuronides, two flavonol forms, aglycons, ellagic acid, and its derivatives. Quercetin 3-glucuronide was the major flavonol in experimental and commercial juices, respectively (mean of 54 and 51 ppm), and a flavonol presumed to be quercetin 3-sophoroside was the second primary compound (means of 29 and 33 ppm). In addition, 36 flavonol forms were measured in trace amounts. The mean total concentrations of quercetin and kaempferol forms, respectively, in experimental juices (n = 45) were 118 and 3.6 ppm and in commercial juices (n = 7) 121 and 3.4 ppm, respectively. The mean total flavonol concentrations in experimental and commercial juices were 122 and 125 ppm, respectively. Influences of cultivar (n = 10), processing method (standard, high-speed centrifugation, depectinization, diffusion extraction, vacuum and osmotic concentration) and environmental factors (geographic origin, maturity, harvesting method, mold contamination) were evaluated.

INTRODUCTION

Authenticity. The composition of red raspberry juice is of much interest to the food industry and to regulatory agencies in the determination of juice authenticity, in an effort to protect the consumer from fraudulent products. Raspberry juices and concentrates are targets for adulteration due to their high commercial value.

Of the constituents in raspberries, flavonoids and other phenolics (i.e., secondary plant metabolites) are particularly reliable indicators of authenticity as they are present within finite ranges of concentration, varying according to ripeness, cultivar, berry size, and growing conditions (Herrmann, 1976). Previous research has focused on the use of anthocyanins, the red pigments and major flavonoids of raspberries, as juice authenticity indicators (Spanos and Wrolstad, 1987). Anthocyanin analyses can be inconclusive in detecting adulteration; hence, additional authenticity indicators are needed. Non-anthocyanin flavonoids should be particularly suitable for detecting adulteration of red raspberry juices with, for example, relatively inexpensive nonred fruit juices such as apple or pear. Most non-anthocyanin flavonoids are not commercially available and so cannot be added to hide adulteration.

Previous Work. The non-anthocyanin flavonoids present in red raspberries are mainly flavonols (3-hydroxyflavones; Figure 1), catechins, and trace amounts of flavones (Henning, 1981; Herrmann, 1974, 1976; Mosel and Herrmann, 1974; Ryan and Coffin, 1971). Flavonols are apparently present as 3-glycosides (i.e., glucuronides, glucosides, galactosides, xylosylglucuronides, xylosylglucosides) of the flavonols quercetin and kaempferol (Henning, 1981; Herrmann, 1974, 1976; Ryan and Coffin, 1971). Only fresh raspberries and a few European cultivars have been investigated in these studies, and there is little or no information on the influences of environmental factors or processing on the concentrations of flavonols.

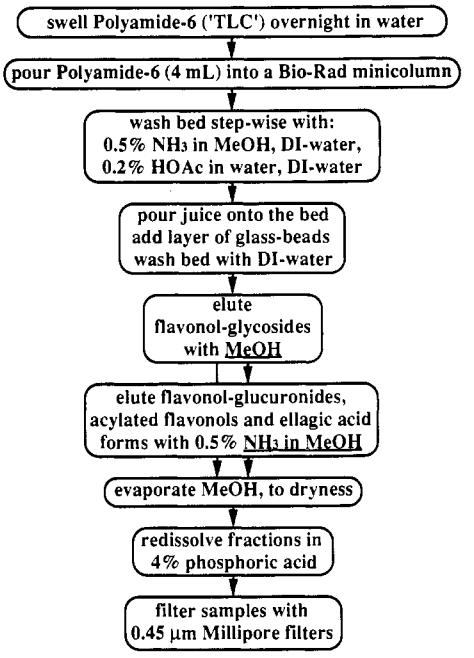

Figure 1. Flow diagram of juice sample preparation for HPLC analysis [procedure adapted from those of Wald and Galensa (1989) and Henning and Herrmann (1980); DI, deionized].

Health Effects. There is increasing interest in red raspberry juice composition because of the possible beneficial health effects associated with their phenolic content. Phenolics present in raspberries which have been shown to have anticarcinogenic effects in mammals include quercetin, ellagic acid, and kaempferol (Deschner, 1992; Leighton, 1992; Verma, 1992; Weisburger, 1992; Yasukawa et al., 1988). Quercetin also exhibits antiviral effects *in vitro* (Vlietinck et al., 1988), and kaempferol shows antifertility activities (Anton, 1988). We have reviewed the phenolic composition of raspberries as it relates to

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† Technical Paper 9958 from the Oregon State University Agricultural Experiment Station.

0021-8561/93/1441-1941$04.00/0

Table I. Mean Concentrations of Quercetin and Kaempferol Glycosides, Glucuronides, and Other Forms in Experimental Raspberry Juices and Concentration Ranges in Commercial Samples

flavonol compound	concn in experimental juices, ppm: n	mean ± SE	% variance due to difference: among juices	% variance due to difference: between repl	concn range in commercial juices, ppm: (n = 9)
quercetin 3-glucuronide[a]	45	54.4 ± 4.48	64.2	35.8	9.72–88.5
quercetin glycoside 2 (3-sophoroside?)	44	29.2 ± 4.88	88.1	11.9	1.35–67.9
quercetin glycoside 4 (MeOH fraction)	30	7.78 ± 1.32	75.7	24.3	nd[b]–14.8
quercetin form 2 (NH_3 fraction)	43	3.86 ± 0.54	57.9	42.1	tr[c]–8.96
quercetin glycoside 6 (MeOH fraction)	8	3.41 ± 1.23	13.2	86.8	nd or tr
quercetin glycoside 5 (MeOH fraction)	10	3.33 ± 0.97	64.0	36.0	nd or tr
quercetin 3-glucoside[a]	45	2.99 ± 0.55	86.1	13.9	tr–8.94
quercetin glycoside 8 (MeOH fraction)	42	2.55 ± 0.33	58.3	41.7	tr–6.46
quercetin glycoside 1 (MeOH fraction)	26	2.48 ± 0.49	55.6	44.4	nd–6.18
quercetin 3-xylosylglucuronide[a]	39	2.43 ± 0.25	56.8	43.2	tr–5.72
kaempferol 3-glucuronide[a]	45	2.31 ± 0.48	96.4	3.6	tr–4.50
quercetin 3-galactoside[a]	43	2.03 ± 0.38	77.0	23.0	tr–6.18
quercetin form 5 (NH_3 fraction)	36	1.90 ± 0.46	93.9	6.1	nd–2.37
quercetin[a] (sum, both fractions)	45	1.75 ± 0.23	73.8	26.2	1.89–12.6
kaempferol[a] (sum, both fractions)	39	1.25 ± 0.20	3.0	97.0	tr–2.00
sum, all above quercetin forms	45	118 ± 8.47	77.1	22.9	31.2–211
sum, all above kaempferol forms	45	3.55 ± 0.64	79.2	20.8	2.00–6.00
sum, all above flavonols	45	122			

[a] Reported previously for fresh berries by Ryan and Coffin (1971) and/or Henning (1981). [b] nd, not detected. [c] tr (trace), ≤1 ppm.

health (Rommel et al., 1992), and Daniel et al. (1989) and Maas et al. (1991) have reviewed the anticarcinogenic effects of ellagic acid.

The objective of this study was to characterize and measure red raspberry flavonols so that they can be used as supplementary authenticity indicators and as a database for evaluating the effects of raspberry juice on health. The composition of ellagic acids and its derivatives in these same samples is reported in another paper (Rommel and Wrolstad, 1993). The anthocyanin composition of these same raspberry juice samples was determined by Boyles and Wrolstad (1993).

MATERIALS AND METHODS

The raspberry juice samples (n = 55), juice processing methods, environmental factors, and statistical procedures are described in another paper (Rommel and Wrolstad, 1993).

High-Performance Liquid Chromatography (HPLC). *Preparation of Samples.* A clean separation of the phenolics into classes proved to be impossible using conventional separation materials (e.g., C_{18} cartridges, Sephadex LH-20, PVPP, various cation and anion exchangers) and solvents. We attributed this to the numerous flavonols and ellagic acid derivatives present in raspberries.

We obtained a clean separation by adapting large-column fractionation procedures developed by Wald and Galensa (1989) and Henning and Herrmann (1980) to minicolumn techniques using TLC grade Polyamide 6 (Figure 1). The compositions of the two fractions recovered by our adapted technique were (1) a methanol fraction containing flavonol glycosides and aglycons and (2) an ammonia/methanol fraction containing flavonol glucuronides, acylated flavonol glycosides, and aglycons as well as ellagic acid and ellagic acid derivatives. Sample preparation was replicated for each of the 55 juice samples analyzed.

Materials Used: TLC grade Polyamide 6, particle size <100 μm (J. T. Baker Inc., Phillipsburg, NH); Bio-Rad minicolumns, 10-cm length (Bio-Rad Laboratories, Richmond, CA); glass beads, 212–300-μm size (Sigma Chemical Co., St. Louis, MO); 0.45-μm pore size filters, type HA (Millipore Corp., Bedford, MA).

HPLC Analysis. Many HPLC columns and solvent systems were tested before a procedure to separate all of the components in the two juice fractions was established. Columns commonly used for flavonoid separation such as polymer columns or end-capped C_{18} columns with high C_{18} carbon load were not effective for our red raspberry juices. A C_8 column, used specifically for separating flavonol glycosides by Harborne and Boardley (1984), Harborne et al. (1985) and Hostettmann et al. (1984), also proved to be unsuccessful. However, a C_{18} column, not end-capped and with low carbon load, gave a good separation (Spherisorb ODS-1, 5 μm, 250-mm length, 4.6 mm i.d.; Alltech Associates, Inc., Deerfield, IL). A C_{18} guard column (5 μm, 10-mm length; Supelco, Inc., Bellefonte, PA) was attached before the ODS-1 column.

HPLC Separation Conditions: solvent A, 100% acetonitrile; solvent B, 1% acetic acid in deionized water; gradient elution program (for both fractions), 5 min at 16% A, to 19% A in 30 min, 5 min at 19% A, to 30% A in 7 min, to 50% A in 10 min, to 100% A in 5 min, 5 min at 100% A, return to initial conditions in 5 min (total run time: 77 min); flow rate, 0.6 mL/min; injection volume, 50 μL. Ellagic acid and ellagic acid derivatives were detected at 260 nm and flavonols at 360 nm.

Instrumentation: Perkin-Elmer liquid chromatograph, Series 400 (Perkin-Elmer Corp., Norwalk, CT), equipped with a Hewlett-Packard diode array detector, Model 1040A, a data station, Series 9000 (Hewlett-Packard Co., Palo Alto, CA), and a Beckman autosampler, Model 501 (Beckman Instruments, Inc., San Ramon, CA).

Peak Characterization. Flavonols and ellagic acid compounds were characterized by (1) their UV spectra, which characterize different classes of compounds (e.g., flavonols, ellagic acid, cinnamic acids, benzoic acids, anthocyanins) and also in some cases compounds within classes, e.g., quercetin and kaempferol, which have slightly different absorption maxima; (2) comparison to standards (standards were separated by HPLC either by themselves or mixed with juices); (3) comparison of raspberry flavonol chromatograms to those of other authentic fruits of known composition (e.g., blackberry, cherry, currants; Macheix et al., 1990); (4) relative retention times of known and unknown compounds; (5) the elution order of glycosides of the same aglycon (e.g., flavonol 3-rutinoside followed by 3-galactoside and then 3-glucoside), which was used in the cases when standards were unavailable.

Standards for Peak Characterization: quercetin 3-glucoside, querctin 3-galactoside, quercetin 3-xylosylglucuronide, kaempferol 3-glucoside, kaempferol 3-xylosylglucoside, and kaempferol 3-glucuronide (provided by Prof. Dr. Herrmann, University of Hannover, Germany); quercetin 3-glucoside and quercetin 3-arabinoside (Carl Roth GmbH & Co., Karlsruhe, Germany); quercetin 3-rutinoside (rutin), kaempferol, quercetin, and quercetin 3-L-rhamnoside (quercitrin) (Sigma); ellagic acid (Sigma).

Quantification Method. Flavonol glycosides, aglycons, and ellagic acid and its derivatives were quantified via internal and external standards.

Internal Standards. Naringin was used for the methanol

fraction and 4-methylumbelliferyl β-D-glucuronide (MUG in this discussion) for the ammonia/methanol fraction (both obtained from Sigma). Half a milliliter of a naringin stock solution (500 ppm of naringin in deionized water) and a MUG stock solution (250 ppm in deionized water), respectively, were added to 11 mL of single-strength (unconcentrated) red raspberry juice. Because juices were concentrated during sample preparation, less internal standard was added to juice samples than to external standards (see below). Juices spiked with internal standards were separated into fractions as described in Figure 1. Flavonols and ellagic acid compounds were quantified by normalizing peak areas to the appropriate internal standards.

External Standards. Rutin was used for the methanol fraction and ellagic acid for the ammonia/methanol fraction (both obtained from Sigma). For each fraction a set of external standards (consisting of the external standard at four different concentrations) was run alternately with the juice sample fractions throughout HPLC analysis. Separate sets of standards had to be used for flavonols and ellagic acid compounds as ellagic acid is only soluble in ≥80% ethanol in water. Set 1: rutin at 1.5, 25, 50, and 150 ppm (in deionized water); 100 ppm of naringin and 50 ppm of MUG were added to each standard. Set 2: ellagic acid at 0.6, 10, 20, and 60 ppm (in 80% ethanol); 100 ppm of rutin was added to each standard. Peak areas were normalized to the appropriate external standards. In the case of each set of standards a standard curve was fitted by linear regression (peak area vs concentration in parts per million). The concentration (C) of each individual flavonol or ellagic acid derivative was calculated from measured peak area (A) using the equation $C = I + SA$, where I and S were the intercept and slope of the fitted line for the corresponding external standard. Adjustments were made for differences in the concentrations of the internal standards in the juice samples and the sets of standards. The concentrations of flavonols and ellagic acid and derivatives were normalized to a standard single-strength juice soluble solids concentration of 10 °Brix.

Limitations of Quantification. At the data reduction stage of this project it became apparent that the frozen internal standard naringin had undergone some degradation during storage. A cubic function was determined to apply a correction factor to the naringin peak area for each juice sample analyzed. The external standard ellagic acid showed varying peak sharpness because of its low solubility. These factors would contribute to the substantial variation between sample replications (Table I). While the analytical variation limits the conclusions that can be drawn from treatment effects, the quantitative estimates are still useful for evaluating trends and providing a perspective of the amounts of flavonols in red raspberry juice.

RESULTS AND DISCUSSION

HPLC chromatographic profiles of the methanol fractions of juices made from different cultivars by the standard process were qualitatively quite similar, yet quantitatively very different. For example, the concentrations of glycosides (e.g., quercetin glycosides 1 and 2, quercetin 3-galactoside, and quercetin 3-glucoside) varied greatly between Willamette (Figure 2a) and Norna (Figure 2b) cultivars. For flavonols present in low concentrations, however, there was also a qualitative difference between these two cultivars in that three kaempferol glycosides (kaempferol 3-glucoside and two unknown glycosides) were detected in the cultivar Norna but not in Willamette; these glycosides may have been present in Willamette below the detection limit.

In the case of ammonia/methanol fractions the differences in compositional profiles were quantitative, except for a few compounds present in trace amounts. Examples of chromatograms of the ammonia/methanol fractions of the above Willamette and Norna juices are shown in Figure 3. The composition of ellagic acid and its derivatives in these samples is described in another paper (Rommel and Wrolstad, 1993).

The concentrations of quercetin and kaempferol glycosides, glucuronides, and other forms in experimental and commercial samples are given in Table II. Eight of these flavonols were identified as quercetin 3-glucuronide, quercetin 3-glucoside, quercetin 3-xylosylglucuronide, kaempferol 3-glucuronide, quercetin 3-galactoside, kaempferol 3-glucoside, quercetin, and kaempferol. The presence of these six flavonol glycosides and glucuronides confirms reports in the literature (Henning, 1981; Ryan and Coffin, 1971). Five flavonols were characterized as quercetin glycosides; however, the specific sugars attached could not be identified, as standards were unavailable. Quercetin forms 2 and 5 (ammonia fraction) may have been quercetin glucuronides or acylated quercetin glycosides (Wald and Galensa, 1989).

Quercetin glycosides 1 and 2 had much shorter retention times (Figure 2) than any of the identified flavonol glycosides, glucuronides, or aglycons; such early-eluting flavonols have not been reported previously in raspberries. We speculate that quercetin glycoside 2, which was present in much higher concentrations than quercetin glycoside 1, was quercetin 3-sophoroside for the following reasons: (1) Diglycosides have earlier retention times than monoglycosides. (2) Anthocyanidins and flavonols, closely related products of phenylpropanoid metabolism (Bilyk and Sapers, 1986), are glycosylated by the same enzymes. Because the early-eluting cyanidin 3-sophoroside is the primary anthocyanin in raspberries (Rommel et al., 1990; Spanos and Wrolstad, 1987), it is very likely that the 3-sophoroside of the corresponding flavonol (quercetin) is also present in relatively high concentrations. (3) The only two anthocyanidins produced by raspberries are cyanidin and pelargonidin (Rommel et al., 1990; Spanos and Wrolstad, 1987); because the hydroxylation pattern of anthocyanidins is the same as that of flavonols (Wildanger and Herrmann, 1973), quercetin and kaempferol must be the corresponding and only two flavonol aglycons present in raspberries. Furthermore, (4) glycosides of cyanidin and pelargonidin appeared to be present in our raspberry juices (Boyles and Wrolstad, 1993) in about the same ratio as those of quercetin and kaempferol. In strawberries the ratio of kaempferol to quercetin corresponds to that of pelargonidin to cyanidin (Wildanger and Herrmann, 1973), and in black currants the ratio of myricetin to quercetin corresponds to that of delphinidin to cyanidin (Herrmann, 1976). Quercetin glycoside 1 may have been a similar diglycoside or even a triglycoside, because it eluted several minutes before quercetin glycoside 2.

Two of the commercial samples (COM-G-65, COM-H-66) had considerably lower concentrations of quercetin 3-glucuronide (9.7, 14 ppm), total quercetin forms (31, 36 ppm), and total flavonol forms (35, 39 ppm) than the other commercial samples (Table II). In addition, sample COM-H-66 contained 44.4 ppm of flavonol glycoside 4 (methanol fraction), a glycoside that was either not detected in the other commercial and experimental juices or present only in trace amounts (Table III). Kaempferol glycoside 1 was also only detected in sample COM-H-66 (in trace amounts; Table III). Boyles and Wrolstad (1993), who analyzed the same raspberry juice samples investigated in this study for their anthocyanin composition, found that sample COM-G-65 contained considerable amounts of delphinidin, an anthocyanidin which is not synthesized by red raspberries. In addition, both sample COM-G-65 and sample COM-H-66 had much higher concentrations of polymeric color than the other commercial and experimental samples (Boyles and Wrolstad, 1993). These two samples also had much lower concentrations of ellagic acid

Table II. Concentrations of Flavonol Glycosides, Glucuronides, and Other Forms in Experimental and Commercial Juices

	flavonol compound,[b] ppm																		
cultivar[a] (sample no.)	Q3Guu	Q3So	QG4M	QF2A	QG6M	QG5M	Q3Glu	QG8M	QG1M	Q3XGuu	K3Guu	Q3Gal	QF5A	K3Glu	QU	KA	QUsum	KAsum	FlavSum
Meeker UR (8)	65.5	10.8	nd	4.7	nd	nd	tr	nd	8.2	3.1	tr	tr	tr	tr	tr	tr	100	3.5	104
Meeker UR (16)	19.6	2.8	nd	tr	nd	nd	tr	tr	1.1	tr	tr	tr	nd	nd	tr	tr	32.3	3.0	35.3
Meeker R (11)	14.0	5.7	nd	tr	nd	nd	tr	tr	2.9	tr	tr	tr	tr	nd	tr	nd	30.6	2.0	32.6
Meeker OR (5)	33.4	6.2	nd	1.5	nd	nd	tr	tr	3.4	tr	tr	1.6	1.1	tr	tr	tr	53.9	3.0	56.9
Meeker OR (3)	10.1	13.9	tr	tr	nd	nd	tr	nd	3.3	nd	tr	2.0	nd	tr	tr	2.0	34.1	3.0	37.1
Meeker OR (10)	17.9	4.4	nd	tr	nd	nd	tr	tr	tr	tr	tr	1.2	2.2	tr	tr	tr	31.6	3.0	34.6
Meeker OR (15)	19.8	9.7	tr	tr	nd	nd	tr	tr	2.8	tr	tr	1.9	nd	tr	tr	tr	42.6	4.0	46.6
Meeker R moldy (17)	tr	tr	nd	tr	nd	nd	tr	tr	2.7	tr	tr	tr	tr	nd	tr	tr	13.7	2.0	15.7
Willamette UR (18)	9.1	8.2	tr	tr	nd	nd	tr	tr	nd	tr	tr	tr	tr	nd	tr	nd	29.3	tr	30.3
Willamette UR (20)	76.5	48.3	5.5	1.1	nd	nd	4.5	3.8	tr	3.3	2.0	tr	tr	nd	tr	tr	150	2.5	153
Willamette R (34A)	63.2	18.4	7.8	3.6	nd	nd	tr	2.0	nd	3.4	tr	tr	nd	nd	tr	tr	107	3.5	111
Willamette R (34B)	82.3	23.5	5.5	5.7	nd	nd	tr	1.5	tr	4.9	tr	tr	nd	nd	tr	tr	131	3.0	134
Willamette R LP (34C)	59.7	49.5	12.3	3.2	nd	nd	2.1	4.7	tr	2.5	tr	tr	tr	nd	1.6	8.6	142	11.1	153
Willamette R LP (34D)	80.8	34.8	10.5	5.4	nd	nd	1.5	4.4	tr	5.2	tr	tr	nd	nd	4.6	tr	153	3.0	156
Willamette OR (7)	40.1	28.9	3.0	3.1	nd	nd	tr	tr	nd	1.8	tr	tr	tr	nd	tr	tr	83.8	2.5	86.3
Willamette OR (4)	53.4	87.1	15.4	13.2	nd	nd	2.7	3.1	nd	1.9	tr	tr	nd	nd	2.2	tr	181	2.5	184
Willamette OR (6)	53.6	59.5	8.5	4.8	nd	nd	4.5	2.9	nd	2.6	tr	tr	tr	nd	1.6	tr	142	3.0	145
Willamette OR (9)	46.3	30.1	7.4	4.5	nd	nd	1.5	1.3	nd	2.6	tr	tr	tr	nd	tr	tr	99.3	2.0	101
Willamette OR (14)	23.4	67.4	13.4	3.5	nd	nd	2.1	1.7	nd	tr	tr	tr	tr	nd	tr	tr	119	2.0	121
Willamette OR (19)	3.0	111.0	8.5	5.8	11.5	nd	7.6	5.2	tr	3.0	tr	tr	tr	nd	3.4	nd	164	tr	165
Chilcotin R (1)	18.8	1.6	nd	tr	nd	nd	tr	tr	tr	nd	tr	tr	tr	nd	tr	tr	29.4	2.0	31.4
Skeena R (2)	1.2	3.6	nd	nd	nd	nd	tr	nd	nd	nd	tr	tr	tr	tr	tr	nd	10.8	2.0	12.8
Heritage R (32)	104	4.4	nd	9.4	nd	nd	3.3	tr	nd	nd	13.0	nd	nd	nd	tr	tr	127	16.0	143
Heritage OR (31)	127	7.0	nd	12.6	nd	nd	5.0	tr	nd	nd	15.6	nd	nd	nd	tr	tr	158	20.6	179
Malling Promise 1 (30)	107	6.9	tr	1.3	nd	nd	3.0	4.3	nd	7.0	1.9	tr	tr	nd	tr	tr	135	3.4	138
Malling Promise 2 (29)	62.6	1.4	tr	tr	nd	nd	tr	tr	nd	2.2	tr	tr	tr	nd	tr	tr	77.2	3.0	80.2
Malling Promise 3 (24)	80.2	4.8	tr	tr	nd	nd	2.7	3.2	nd	3.8	1.1	tr	tr	nd	tr	tr	102	2.6	104
Malling seedling 1 (13)	79.4	2.3	tr	tr	nd	nd	tr	1.5	nd	3.0	tr	tr	tr	nd	tr	nd	95.3	1.5	96.8
Malling seedling 2 (28)	72.2	4.8	tr	tr	nd	nd	tr	1.5	nd	1.5	tr	tr	tr	nd	tr	tr	89.5	2.5	92.0
Malling seedling	27.1	7.7	tr	tr	nd	nd	1.6	1.9	nd	1.2	tr	tr	tr	nd	tr	tr	49.0	3.0	52.0
Norna 1 (26)	104	1.8	nd	nd	3.5	9.7	1.3	3.0	4.5	1.4	12.3	6.7	12.8	tr	2.6	tr	155	17.3	172
Norna 2 (22)	66.7	tr	nd	tr	3.1	5.8	1.1	3.3	2.7	tr	5.9	8.6	9.7	tr	tr	2.0	110	9.9	120
Norna 3 (21)	61.5	3.7	nd	tr	3.9	6.8	1.7	3.7	10.3	tr	4.3	9.9	9.6	tr	tr	tr	117	7.3	124
Veten 1 (12)	49.3	tr	nd	tr	tr	2.3	tr	tr	1.2	tr	2.5	8.8	tr	tr	tr	nd	72.1	3.5	75.6
Veten 2 (23)	38.3	1.5	nd	tr	1.9	1.9	1.4	tr	3.3	tr	1.3	8.8	tr	tr	tr	tr	64.7	5.0	69.7
Veten 3 (25)	33.2	3.8	nd	tr	1.3	2.8	tr	tr	6.8	tr	1.6	3.6	tr	tr	tr	tr	60.6	5.1	65.7
Golden R (33)	88.4	nd	tr	tr	tr	nd	1.3	tr	tr	nd	1.1	tr	tr	nd	tr	tr	112	5.1	117
Willamette STD (37D)	81.4	60.6	9.4	8.2	nd	tr	4.8	4.1	tr	3.6	1.5	tr	tr	nd	4.8	tr	182	3.5	186
Willamette VC (37E)	61.9	58.4	9.1	4.3	nd	nd	4.1	2.3	tr	2.8	tr	tr	2.4	nd	2.2	tr	152	3.0	155
Willamette OS8A (37A)	54.7	54.7	8.1	4.9	nd	tr	3.8	2.0	tr	1.9	tr	tr	tr	nd	2.1	tr	139	1.5	141
Willamette OS26A (37B)	48.7	48.1	6.7	5.3	nd	tr	3.1	1.2	tr	2.4	tr	tr	tr	nd	tr	tr	122	1.5	123
Willamette OS26B (37C)	78.2	55.1	7.9	6.9	nd	tr	3.9	1.7	tr	2.2	2.0	tr	1.5	nd	tr	tr	167	2.5	170
Willamette CT (35A)	78.5	123	30.9	9.9	nd	nd	10.7	9.5	tr	5.8	3.0	1.9	tr	nd	3.9	tr	279	7.0	286
Willamette CT ESP (35B)	63.7	61.0	21.4	8.5	nd	nd	19.2	6.8	nd	5.6	4.3	tr	tr	nd	7.8	tr	198	9.2	207

Table II (Continued)

Willamette CT EBE (35C)	50.9	82.2	20.1	5.6	nd	nd	14.8	9.8	nd	3.6	2.6	1.5	tr	nd	5.1	tr	198	7.1	205
Willamette DE (36)	37.3	64.9	11.9	11.1	nd	nd	3.0	1.8	nd	tr	tr	tr	tr	nd	4.6	tr	138	3.0	140
A (38)	40.9	28.2	1.7	1.2	nd	tr	8.4	tr	4.7	1.9	1.7	tr	1.9	nd	12.6	2.0	106	4.2	110
B (39)	28.3	12.8	nd	3.2	nd	nd	1.7	tr	6.2	tr	tr	3.2	tr	nd	5.2	tr	66.0	2.0	68.0
C (40)	88.5	64.3	14.8	9.0	tr	nd	6.1	6.5	2.0	5.7	4.5	4.8	2.4	nd	3.8	2.0	211	6.0	217
D (41)	71.9	67.9	14.0	6.3	tr	nd	6.7	3.3	tr	2.1	2.4	1.2	tr	nd	11.5	tr	189	3.9	193
E (42)	25.3	8.0	nd	1.1	nd	nd	tr	nd	5.1	tr	tr	1.4	2.4	nd	1.9	tr	48.2	2.5	50.7
F (43)	57.6	51.2	7.7	5.2	nd	nd	8.9	3.7	nd	2.4	1.9	6.2	1.8	nd	6.1	tr	155	2.9	158
G (44)	9.7	1.4	4.1	tr	tr	nd	tr	tr	2.5	tr	tr	tr	tr	nd	5.8	2.0	31.2	4.0	35.2
H (45)	13.9	3.7	1.3	tr	nd	nd	6.3	2.0	tr	tr	tr	tr	nd	nd	1.9	tr	36.1	2.5	38.6
I (46)	46.4	1.8	tr	2.4	tr	tr	2.7	5.3	tr	2.2	tr	tr	tr	nd	6.7	tr	75.9	2.0	77.9

[a] UR, underripe; R, ripe; OR, overripe; LP, laboratory process; STD, standard process; VC, vacuum concentration; OS8A, OS26A, osmotic concentrations at 8 and 26 °C, respectively, using membrane A; OS26B; osmotic concentration at 26 °C using membrane B; CT, centrifugation; ESP, Pectinex Ultra SP enzyme; EBE, Pectinex BE enzyme; DE, diffusion extraction. [b] Q3Guu, quercetin 3-glucuronide; Q3So, quercetin 3-sophoroside; QG4M, quercetin glycoside 4 (MeOH fraction); QF2A, quercetin form 2 (ammonia fraction); QG6M, quercetin glycoside 6 (MeOH fraction); QG5M, querecetin glycoside 5 (MeOH fraction); Q3Glu, quercetin 3-glucoside; QC8M, quercetin glycoside 8 (MeOH fraction); QG1M, quercetin glycoside 1 (MeOH fraction); Q3XGuu, quercetin 3-xylosylglucuronide; K3Guu, kaempferol 3-glucuronide; Q3Gal, quercetin 3-galactoside; QF5A, quercetin form 5 (ammonia fraction); QG1M, quercetin glycoside 1 (MeOH fraction); K3Glu, kaempferol 3-glucoside; QU, quercetin; KA, kaempferol; QUsum, sum of all quercetin compounds; KAsum, sum of all kaempferol compounds; FlavSum, sum of all flavonol compounds; tr, trace (≤1 ppm); nd, not detected.

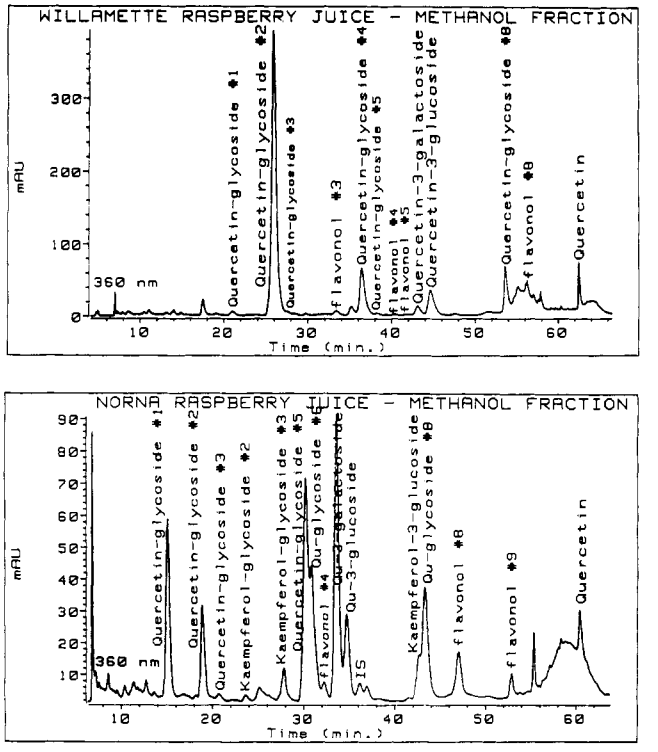

Figure 2. HPLC chromatogram of the methanol fraction (separated on Polyamide 6) of red raspberry juice made from (a, top) ripe Willamette cultivar and (b, bottom) ripe Norna cultivar. Flavonol glycosides and aglycons were detected at 360 nm.

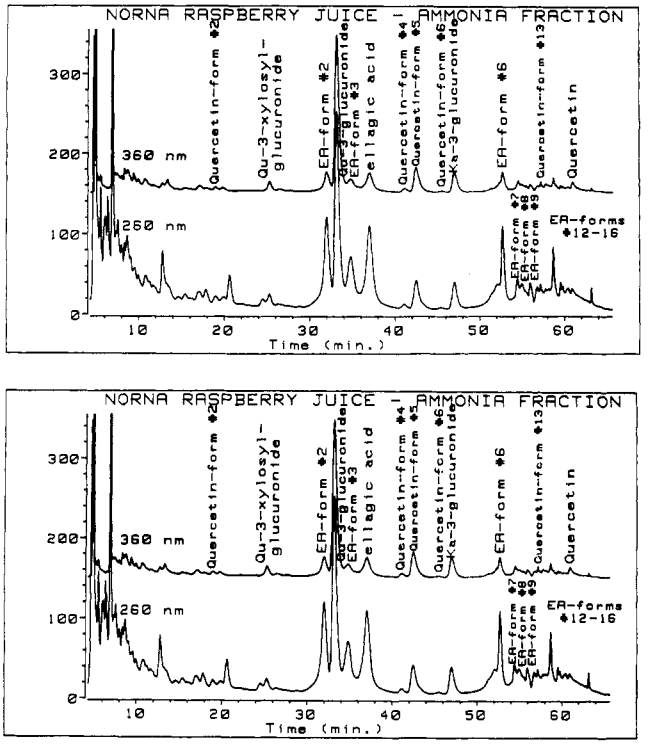

Figure 3. HPLC chromatogram of the ammonia/methanol fraction (separated on Polyamide 6) of red raspberry juice made from (a, top) ripe Willamette cultivar and (b, bottom) ripe Norna cultivar. Ellagic acid and ellagic acid derivatives were detected at 260 nm; flavonol glucuronides, acylated flavonol glycosides, and flavonol aglycons were detected at 360 nm.

and total ellagic acid forms than the other commerical samples (Rommel and Wrolstad, 1993). We believe that these facts clearly indicate that samples COM-G-65 and COM-H-66 were not authentic red raspberry juice con-

Table III. Quercetin and Kaempferol Forms Present in Trace Amounts[a] in Raspberry Juices in Both Fractions

quercetin glycoside 3 (M)[b]	5 experimental samples, 1 commercial sample
quercetin glycoside 7 (M)	2 commercial samples
quercetin form 1 (A)[c]	1 Willamette (overripe) sample
quercetin form 3 (A)	4 Willamette, 2 (of 3) Malling Seedling samples
quercetin form 6 (A)	Golden (4.4 ppm); centrif (1.7 ppm); 24 experimental, 6 commercial samples
quercetin form 7 (A)	all Heritage, Golden, 3 Willamette, 1 commercial sample
quercetin form 8 (A)	Golden, 2 commercial
quercetin form 9 (A)	16 experimental, 3 commercial samples
quercetin form 10 (A)	Golden
quercetin form 11 (A)	Golden
quercetin form 12 (A)	Golden
quercetin form 13 (A)	32 experimental, 4 commercial samples
quercetin form 14 (A)	Golden, 1 Willamette, 1 Heritage (overripe) sample
kaempferol 3-glucoside[d] (M)	4 Meeker, all Norna, all Veten, Skeena, 1 osmosis sample
kaempferol glycoside 1 (M)	1 commercial sample (COM-H-66)
kaempferol glycoside 2 (M)	22 experimental, 2 commercial samples
kaempferol glycoside 3 (M)	6 commercial samples
kaempferol glycoside 4 (M)	6 (of 7) Meeker, 1 commercial sample
kaempferol glycoside 5 (M)	1 Norna sample
kaempferol glycoside 6 (M)	Golden, 3 Malling, 1 Heritage (OR), 1 Willamette, 1 commercial sample
kaempferol form 1 (A)	all centrif, 1 Meeker (OR), 2 Willamette samples, 1 commercial sample
kaempferol form 2 (A)	all centrif, all Heritage samples
kaempferol form 3 (A)	centrif + SP-enzyme (1.4 ppm); centrif + BE-enzyme
kaempferol form 4 (A)	1 Veten sample (1.5 ppm), Chilcotin
kaempferol form 5 (A)	1 Meeker, 1 Willamette, 1 Malling Seedling sample
kaempferol form 6 (A)	Golden, 1 Norna, 1 Veten sample
flavonol glycoside 1 (M)	2 commercial samples
flavonol glycoside 2 (M)	1 commercial sample
flavonol glycoside 3 (M)	29 experimental, 6 commercial samples
flavonol glycoside 4 (M)	29 experimental, all commercial samples (COM-H-66: 44.4 ppm)
flavonol glycoside 5 (M)	24 experimental, 6 commercial samples
flavonol glycoside 6 (M)	1 osmosis sample; diffusion extraction; 1 commercial sample
flavonol glycoside 7 (M)	all Heritage samples
flavonol glycoside 8 (M)	26 experimental, 8 commercial samples
flavonol glycoside 9 (M)	22 experimental, 4 commercial samples
flavonol glycoside 10 (M)	all Heritage, 2 Malling samples, 1 commercial sample

[a] Trace ≤ 1 ppm. [b] M, methanol fraction. [c] A, ammonia/methanol fraction. [d] Reported previously for fresh raspberries by Henning (1981).

centrates but that they had been adulterated by other fruit juices and/or colorants.

These two samples were therefore excluded before the mean flavonol concentrations were calculated for the commercial samples (n = 7): total flavonol forms, 122 ppm; total quercetin forms, 121 ppm; total kaempferol forms, 3.36 ppm; quercetin 3-glucuronide, 51.3 ppm; quercetin 3-sophoroside, 33.0 ppm; quercetin, 6.83 ppm; quercetin glycoside 4 (methanol fraction), 5.60 ppm; quercetin 3-glucoside, 5.07 ppm; quercetin form 2 (ammonia/methanol), 4.06 ppm; quercetin glycoside 8 (methanol), 2.97 ppm; quercetin glycoside 1 (MeOH), 2.86 ppm; quercetin 3-galactoside, 2.69 ppm; quercetin 3-xylosylglucuronide, 2.33 ppm; kaempferol 3-glucuronide, 1.93 ppm; quercetin form 5 (ammonia/methanol), 1.94 ppm; kaempferol, 1.29 ppm; quercetin glycosides 5 and 6 (methanol), traces; kaempferol 3-glucoside, not detected.

Mean concentrations and ranges of quercetin and kaempferol glycosides, glucuronides, and other forms measured in both juice fractions of experimental (except the moldy sample) and commercial juices, respectively, as well as standard errors and percentages of variances for the experimental samples are summarized in Table I. Where the percentage of total variance attributable to differences between replicate sample preparations is high, accuracy of the reported flavonol concentrations will be low. Quercetin, present almost entirely in glycosylated form, was the dominant flavonol aglycon in all raspberry juices. The mean total concentration of flavonols in the 45 experimental samples was 122 ppm, that of quercetin forms was 118 ± 8.5 ppm, and that of kaempferol forms was 3.6 ± 0.6 ppm. Quercetin 3-glucuronide, kaempferol 3-glucuronide, quercetin 3-glucoside, and quercetin were measured in all experimental samples. Quercetin 3-glucoside was present with the highest mean concentration (54 ± 4.5 ppm) in experimental samples; it ranged from 25 to 89 ppm (mean of 51 ppm exluding the two adulterated samples) in commercial samples. Quercetin glycoside 2 (quercetin 3-sophoroside?), quercetin 3-galactoside, quercetin 3-xylosylglucuronide, kaempferol, two quercetin glycosides, and two other quercetin forms were present in the majority of experimental samples. Quercetin 3-sophoroside was present in the second highest concentration in experimental samples (mean of 29 ± 4.9 ppm); it ranged from 1.8 to 68 ppm (mean of 33 ppm excluding the two adulterated samples) in commercial samples.

Table III summarizes 36 additional quercetin and kaempferol forms which were measured in trace amounts (i.e., <1 ppm) and were found to varying degrees in both fractions of the 55 juices investigated. Only certain cultivars seemed to contain some of these forms, and certain processes appeared to enhance their concentrations. These flavonol forms were not identified conclusively; however, we speculate that those present in the methanol fraction were quercetin or kaempferol glycosides, while those present in the ammonia/methanol fraction may have been flavonol glucuronides or acylated flavonol glycosides, following Wald and Galensa (1989). Alternatively, juice processing may have produced new flavonols, not present in fresh berries, by the influences of enzymes, high temperature, or oxidation.

Influence of Cultivar. The total mean concentration of quercetin forms (Figure 4a) ranged from ca. 10 to 160 ppm among experimental cultivars, with Heritage, Willamette, and Norna containing the highest concentrations. The concentration pattern for quercetin 3-glucuronide (Figure 4b) was similar, Heritage, Golden, Malling Promise, and Norna cultivars having the highest mean concentrations (between ca. 75 and 125 ppm). Quercetin 3-sophoroside (Figure 4c) was present in much greater amounts in Willamette than in nay other cultivar (between ca. 30 and 60 ppm, depending on ripeness) and was not detected in the cultivar Golden. Quercetin 3-glucoside (Figure 4d) was measured in all cultivars at 5 ppm or less, Heritage, Willamette, and Malling Promise having the highest concentrations. Quercetin 3-galactoside (Figure 4e) was present in Norna and Veten cultivars at significantly higher concentrations (between ca. 7 and 8 ppm) than in all other cultivars and was not detected in Heritage.

Meeker, Norna, and Veten cultivars contained much

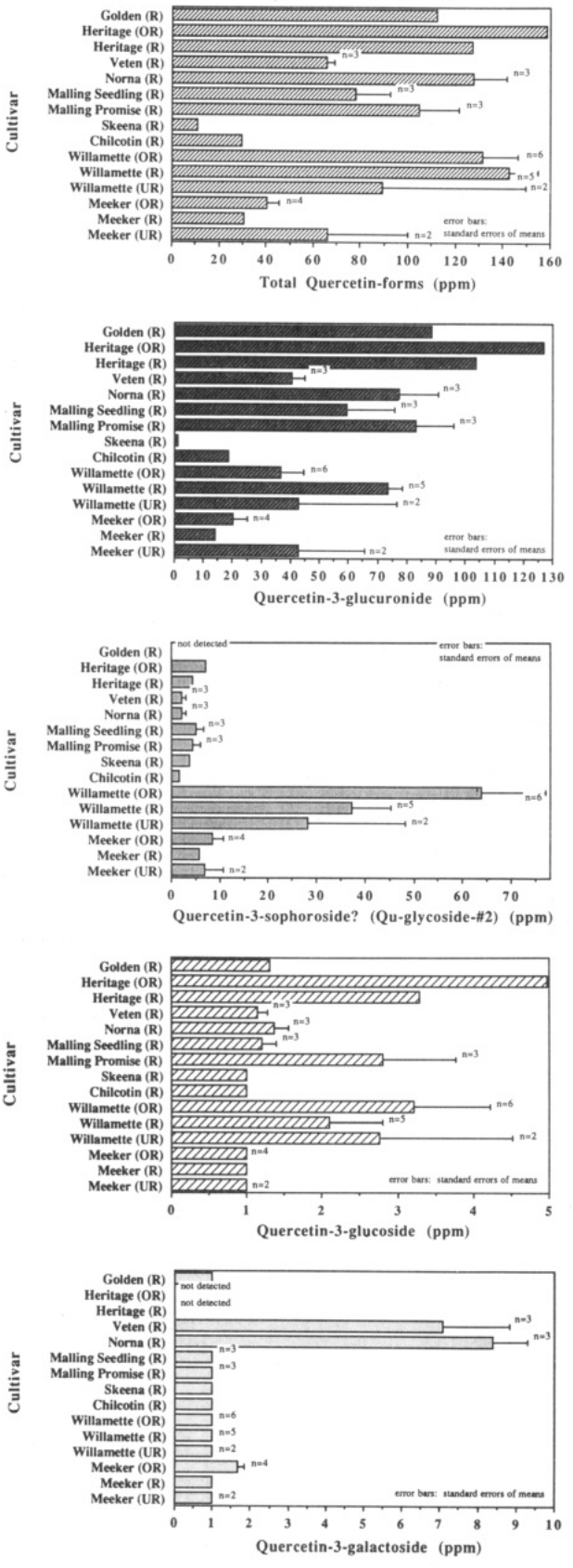

Figure 4. (a–e, top to bottom) (a) Total concentrations of quercetin forms in juices made from different raspberry cultivars. (b) Concentrations of quercetin 3-glucuronide in juices made from different raspberry cultivars. (c) Concentrations of quercetin glycoside 2 in juices made from different raspberry cultivars. (d) Concentrations of quercetin 3-glucoside in juices made from different raspberry cultivars. (e) Concentrations of quercetin 3-galactoside in juices made from different raspberry cultivars. UR; underripe; R, ripe; OR, overripe.

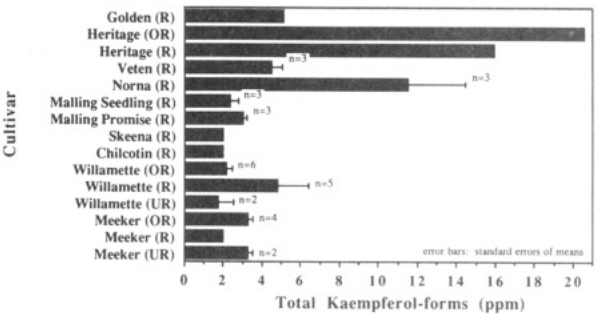

Figure 5. Total concentrations of kaempferol forms in juices made from different raspberry cultivars. UR, underripe; R, ripe; OR, overripe.

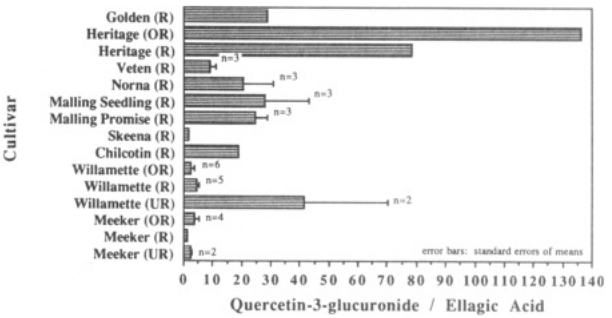

Figure 6. Ratios of quercetin 3-glucuronide to ellagic acid for juices made from different raspberry cultivars. UR, underripe; R, ripe; OR, overripe.

greater quantities of quercetin glycoside 1 (means of 3.2, 5.8, and 4.0 ppm, respectively) than all other cultivars (Table I). Cultivars that contained quercetin glycoside 1 appeared to contain this flavonol in preference to high concentrations of quercetin glycoside 2 (quercetin 3-sophoroside?), e.g., Meeker. Quercetin glycoside 4 was present in significant quantities only in the cultivar Willamette (Table II). Quercetin glycosides 5 and 6 (methanol fraction) were measured above trace amounts only in Norna and Veten cultivars and in one overripe Willamette sample (for glycoside 6; Table II). Norna contained considerably more quercetin form 5 (ammonia/methanol fraction) than other cultivars (Table II). Quercetin forms 10–12 (ammonia/methanol fraction) were detected only in the cultivar Golden (Table III).

There were large differences in the concentrations of total kaempferol forms among cultivars (Figure 5). Heritage and Norna cultivars contained between ca. 11 and 20 ppm, while all others contained trace amounts or a few parts per million. Heritage contained much more kaempferol 3-glucuronide (13–16 ppm) than all other cultivars (except one Norna sample; Table II). Kaempferol 3-glucoside was detected only in Meeker, Skeena, Norna, and Veten cultivars.

The ratio of quercetin 3-glucuronide to ellagic acid was calculated to determine if it could be used as an index to detect adulteration. However, this ratio differed greatly among cultivars (Figure 6), making it unsuitable as an index.

Influences of Environmental Factors. *Geographic Origin.* Juices made by the standard process from cultivars grown in the same region (British Columbia, Poland, Oregon) contained very different amounts of total flavonols (Figures 4a and 5) and individual quercetin glycosides (Figure 4b–e). It was not possible therefore to distinguish if differences in concentrations resulted from differences due to cultivar alone or if growing region also had an influence.

Maturity. Juice made from overripe Heritage rasp-

berries contained higher concentrations of total quercetin and kaempferol forms, quercetin 3-glucuronide, quercetin glycoside 2 (quercetin 3-sophoroside?), quercetin 3-glucoside, kaempferol 3-glucuronide, and other flavonols than juice made from ripe Heritage berries (Figures 4a–d and 5; Table II). However, there was no obvious correlation between ripeness and the concentrations of total quercetin forms (Figure 4a), total kaempferol forms (Figure 5), and quercetin 3-glucuronide (Figure 4b) in juices made from the cultivars Meeker and Willamette; e.g., ripe Meeker juice contained the least total quercetin forms, total kaempferol forms, and quercetin 3-glucuronide, while ripe Willamette juice contained the most of these flavonols. Quercetin glycoside 3-sophoroside (Figure 4c) increased in Willamette juice with increasing ripeness, while there was no such trend for quercetin 3-glucoside (Figure 4d) in the same cultivar.

Four cultivars grown in Poland (Malling Promise, Malling Seedling, Norna, Veten) were picked within 5 days (on July 6, 8, 11, 1988). The mean concentrations of total quercetin forms decreased in both Malling Seedling (from 95.3 to 49.0 ppm; Table II) and Veten (from 72.1 to 60.6 ppm) juices over this time period; there was no such trend for the other two cultivars. The mean concentrations of quercetin 3-glucuronide decreased in Malling Seedling (from 79.4 to 27.1 ppm), Veten (from 49.3 to 33.2), and Norna (from 104 to 61.5 ppm) juices, while there was no such trend for juice made from the cultivar Malling Promise. In contrast, total kaempferol forms increased in both Malling Seedling (from 1.5 to 3.0 ppm) and Veten (from 3.5 to 5.1 ppm) juices, while they decreased in both Malling Promise (from 3.4 to 2.6 ppm) and Norna (from 17.3 to 7.3 ppm) juices.

With the exception of the cultivar Heritage, there was no apparent correlation between flavonol concentrations and ripeness of raspberries for the juices investigated in our study. Such a correlation as reported for other fruits could not be confirmed; e.g., in black currants the amount of quercetin glycosides, particularly myricetin glycosides, increases markedly during ripening of the berries (Herrmann, 1976), and darker (i.e., riper) cranberries contain significantly more quercetin and myricetin than medium-colored berries (Bilyk and Sapers, 1986).

Harvesting Method. Willamette and Meeker cultivars at varying degrees of ripeness, hand-picked or machine-harvested, were available for juice-making by a standard process. The concentrations of total quercetin forms in these samples showed considerable variation. The degree of ripeness could also contribute to the variation within cultivar; hence, it was not possible to conclude whether or not harvesting method influenced the concentrations of flavonols in juices.

Mold Contamination. Mold decreased the contents of quercetin glycosides and glucuronides considerably in the cultivar Meeker, illustrated by the total concentration of quercetin forms (Table II, sample 17). Mold did not, however, appear to have an effect on ellagic acid and its derivatives (Rommel and Wrolstad, 1993). We speculate that enzymes produced by mold cleaved the glycosidic bond of flavonol glycosides but left the ester bonds of ellagic acid derivatives untouched.

Influences of Processing. Single-strength juices were made by a standard pilot plant process, with or without concentration (by vacuum or osmosis), from equal aliquots of the same batch of ripe Willamette raspberries, grown in Oregon. Juices processed by two alternative methodologies, diffusion extraction and high-speed centrifugation, were made from aliquots of another batch of ripe Willamette raspberries, grown in British Columbia. For comparison, juices were made by a standard process in the laboratory from small batches of underripe, ripe, and overripe Willamette berries, grown in Oregon.

Alternative Processes. The influences of these juice-making techniques on the concentrations of total quercetin forms are presented in Table II. Juice produced by high-speed centrifugation contained the most total quercetin forms (ca. 280 ppm), far more than did juices made by the other techniques. The use of pectinases combined with centrifugation decreased the concentrations of total quercetin forms considerably (to ca. 190 ppm) compared to centrifugation alone. The two pectinases used in this study (Pectinex BE and Pectinex Ultra SP, Novo Laboratories Inc., Danbury, CT) had a similar effect on total quercetin concentrations. It is likely that these pectinases deglycosylated flavonol glycosides to less stable aglycons. Diffusion-extracted juice contained even less total quercetin forms (ca. 130 ppm), which might have resulted from accelerated breakdown of flavonol glycosides by the combined effects of depectinization and exposure to high temperature (63 °C) for several hours. However, the same diffusion-extracted juice contained much higher concentrations of total ellagic acid forms than other juices (Rommel and Wrolstad, 1993); this probably resulted from the release of ellagic acid from cell walls during this slow, high-temperature, extraction process.

Standard Pilot Plant Process and Concentration Techniques. There were fewer differences in the concentrations of total quercetin forms among juices produced by the standard process (with or without concentration; Table II). Osmotic concentration by membrane A decreased total quercetin more than by membrane B, and osmotic concentration at room temperature (using membrane A) had a greater decreasing effect than concentration at chilled temperature (using membrane A). Membrane A evidently retained more flavonols than membrane B, and osmotic concentration at higher temperature may have led to breakdown of more glycosides than concentration at chilled conditions. Vacuum concentration decreased the contents of total quercetin forms compared to standard pilot plant processing without concentration (control); however, standard single-strength juice produced from a smaller batch of ripe raspberries contained less total quercetin forms than vacuum concentrated juice.

Comparison of Experimental and Commercial Samples. There was considerable variation among commercial juice samples with respect to concentration of total quercetin forms (Table II). The two samples with the lowest quercetin concentrations were found to be adulterated. Variation among the remaining seven concentrates could be due to either different cultivars or processing methods. Three commercial samples had very high contents of total quercetin forms (ca. 150–210 ppm).

SUMMARY AND CONCLUSIONS

The chromatographic profiles of flavonols were qualitatively quite similar for all cultivars investigated; however, there were great quantitative differences due to differences in cultivar, processing technique, and environmental factors. Quercetin was the primary flavonol aglycon in all raspberry juices, present almost entirely in glycosylated form. Quercetin 3-glucuronide (the primary flavonol), kaempferol 3-glucuronide, quercetin 3-glucoside, and quercetin were measured in all experimental samples. Also present in the majority of experimental samples were quercetin glycoside 2 (presumably quercetin 3-sophoroside and the secondary primary flavonol), quercetin 3-galac-

toside, quercetin 3-xylosylglucuronide, kaempferol, two quercetin glycosides, and two other unidentified quercetin forms. Thirty-six additional flavonol forms were detected in trace amounts.

The cultivars Heritage, Willamette, and Norna contained the largest amounts of total quercetin forms; Heritage and Norna contained the most total kaempferol forms. Heritage, Golden, Malling Promise, and Norna cultivars had the highest concentrations of quercetin 3-glucuronide. Quercetin glycoside 2 (quercetin-3-sophoroside?) was present in Willamette in much greater quantity than in other cultivars. Cultivars grown in the same region showed great variation in flavonol contents, making it impossible to determine if harvesting method had influenced the concentrations of flavonols present in these juices. No apparent correlation between flavonol concentrations and ripeness of raspberries could be determined. Mold decreased the contents of quercetin glycosides and glucuronides considerably in juices. Juice produced by high-speed centrifugation contained the most total quercetin forms and much more than the juices made by other techniques. The use of pectinases combined with centrifugation decreased the concentrations of total quercetin forms considerably. Diffusion-extracted juice contained even less total quercetin forms. Commercial samples had greatly varying flavonol concentrations. The two commercial red raspberry juice concentrates with the lowest content of quercetin glycosides were judged to be adulterated because of their anthocyanin, flavonol, and ellagic acid composition. One of the samples contained a qualitatively different flavonol glycoside in substantial concentrations. This illustrates the usefulness of flavonol HPLC profiles as an auxiliary technique for detecting adulteration.

Quercetin was present (in glycosylated form) in our red raspberry juices in substantial quantities, definitely within the concentration range that has been shown to have anticarcinogenic effects in rodents. Its presence as glycosides and glucuronides is no hindrance as glycosidic bonds are broken down readily by gastrointestinal bacteria. However, the rate of deglycosylation in the gut and the degree of quercetin absorption into the body need to be studied further. Poor absorption of quercetin may require consumption of great amounts of this flavonol to generate anticarcinogenic effects. These compositional data for flavonol concentrations in red raspberry juices should be useful in evaluating the potential positive effects of raspberry juice on human health.

ACKNOWLEDGMENT

We thank David Thomas, Department of Statistics, Oregon State University, for his advice on statistical analyses and Bob Durst for advice on analytical methodology. We also thank Prof. Dr. Herrmann, Institute of Food Chemistry, University of Hannover, Germany, for supplying the flavonol standards. We appreciate the cooperation of T. H. J. Beveridge and D. B. Cumming, Agriculture Canada Research Station, Summerland, BC, in providing juice samples. The following organizations and individuals provided fruit samples: Oregon Caneberry Commission, Salem, OR; North Willamette Research and Extension Center, Aurora, OR; Witold Plocharski, Research Institute of Pomology and Floriculture, Skierniewice, Poland; and Lucy Wisniewski, Salem, OR. We thank the following firms for their research support through contributions to the Oregon State University Agricultural Research Foundation: Beech-Nut Nutrition Corp., Certified Pure Ingredients Inc., Clermont Inc.; Everfresh Juice Co.; Genencor International Inc.; General Foods Corp., Gerber Products Co., Jugos Del Sur S. A., Kerr Concentrates Inc., Minot Food Packers Inc., Ocean Spray Cranberries Inc., Rudolf Wild GmbH & Co. KG, Sanofi Bio-Industries, The J. M. Smucker Co., Tree Top Inc., and Welch Foods Inc.

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Received for review July 23, 1993. Accepted July 28, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, October 1, 1993.