Ellagic Acid Content of Red Raspberry Juice As Influenced by Cultivar, Processing, and Environmental Factors†

Angelika Rommel and Ronald E. Wrolstad*

Department of Food Science and Technology, Wiegand Hall, Oregon State University, Corvallis, Oregon 97331-6602

Ellagic acid and its derivatives present in pilot-plant-processed raspberry juices and commercial juice concentrates were characterized and concentrations estimated using HPLC/diode array spectral techniques. Experimental juice samples (n = 45) contained a mean concentration of 10 ppm of ellagic acid and ≤16 derivatives of ellagic acid with individual mean concentrations of up to 3 ppm. Commercial juices (n = 7) contained more ellagic acid and derivatives than experimental juices, with a mean concentration of 30 ppm of ellagic acid and up to 6.7 ppm for individual ellagic acid derivatives. The mean total concentration of ellagic acid and its derivatives in experimental juices was 28 ppm and in commercial juices 52 ppm. Qualitatively, the chromatographic profiles were very similar for the juices studied, but quantitatively, there were great differences due to cultivar (n = 10) and processing method. Williamette and Meeker cultivars contained the most ellagic acid and its forms. Juices made by diffusion extraction and a standard process had by far the highest concentrations of ellagic acid and its forms. High-speed centrifugation reduced total ellagic acid forms by half compared to diffusion extraction; depectinization and concentration decreased total forms even further.

INTRODUCTION

Our investigation of the phenolic composition of red raspberry juice was stimulated by the need for an expanded compositional database for determining juice authenticity. The phenolic composition is also of great interest because of the posssible positive effects on human health (Daniel et al., 1989; Maas et al., 1991a). Bate-Smith (1959) first reported the presence of ellagic acid in members of the plant family Rosaceae and specifically in strawberries, raspberries, and blackberries; only certain subfamilies and genera of the Rosaceae produce ellagic acid (Bate-Smith, 1961a,b). Of the foods normally consumed by humans, raspberries, blackberries, and strawberries contain by far the most ellagic acid; they have about 3 times as much as walnuts and pecans and at least 15 times as much as other fruits and nuts (Daniel et al., 1989; Maas et al., 1991b; Wang et al., 1990). Daniel et al. (1989) report the presence of ellagic acid in raspberry pulp at 9.1 ± 0.1 ppm (wet weight), in seeds as 275 ± 4 ppm (wet weight), and in blended freeze-dried raspberries at 90 ± 70 and 400 ± 100 ppm (dry weight) for extraction with acetone and methanol, respectively. After hydrolysis of ellagic acid glucosides with trifluoroacetic acid, the freeze-dried raspberry samples contained ellagic acid at 1500 ± 100 and 1900 ± 300 ppm (dry weight) for extraction with methanol and acetone, respectively. Raspberry juice (hand-squeezed) did not contain measurable amounts of ellagic acid. Daniel et al. (1989) investigated raspberries from three local markets, purchased within 2 months. There is no information on the influence of cultivar, processing, or environmental factors on the contents of ellagic acid in raspberries or raspberry juice. For strawberries, large differences in ellagic acid contents have been found among cultivars, and green (unripe) fruit pulp has been found to contain about twice as much ellagic acid as red fruit pulp (Maas et al., 1991b).

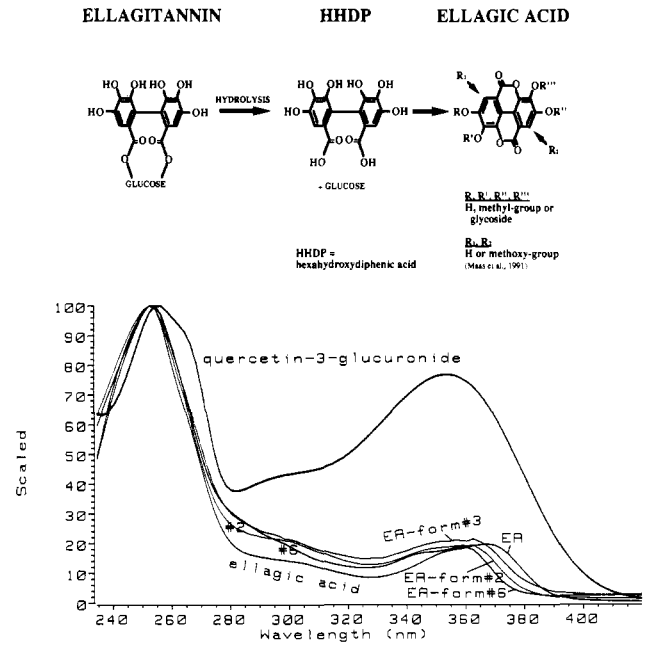

Figure 1. (a, top) Structures of ellagic acid and derivatives and their precursors in plant cell walls. (b, bottom) Absorbance spectra of ellagic acid (EA), three ellagic acid derivatives, and quercetin 3-glucuronide present in ammonia/methanol fractions of red raspberry juice.

Ellagic acid (Figure 1), a dimeric derivative of gallic acid, exists in plants combined with its precursor hexahydroxydiphenic acid (HHDP) or bound as ellagitannins, esterified with glucose; the glucose molecule is often further esterified with gallic acid (Bate-Smith, 1972; Haddock et al., 1982; Maas et al., 1991a). Ellagic acid is released from plant cell walls through hydrolysis of ellagitannins to glucose and hexahydroxydiphenic acid, which forms an inner dilactone spontaneously, called ellagic acid (Bate-Smith, 1959, 1972; Wilson and Hagerman, 1990; Figure 1b). The major ellagitannins in raspberries are α- and β-1-*O*-galloyl-2,3:4,6-bis(hexahydroxydiphenoyl)-D-glucose and a dimer of these two forms (MW 1870); also

* Author to whom correspondence should be addressed [phone (503) 737-3591; fax (503) 737-1877].

† Technical Paper 9959 from the Oregon State University Agricultural Experiment Station.

0021-8561/93/1441-1951$04.00/0

present are the β-1,2,3-tri-O-galloyl-4,6-, 2,3:4,6-bis-, and 2,3-di-O-galloyl-4,6- derivatives (Haddock et al., 1982; Haslam and Lilley, 1985). When fruits and nuts are consumed by animals and humans, the glucose moieties of ellagitannins are probably removed by enzymatic activity in the bowel, thus "freeing up" ellagic acid for absorption (Stoner, 1989). Numerous derivatives of ellagic acid exist in plants, formed through methylation, glycosylation, and methoxylation of its hydroxyl groups (Maas et al., 1991a), which differ in solubility, mobility, and reactivity in plant as well as in animal systems. Ellagic acid has also been reported to complex readily with metallic cations, e.g., Mg^{2+} and Ca^{2+} (Press and Hardcastle, 1969).

There is particular interest in the amounts of ellagic acid in fruits because of the increasing evidence for its anticarcinogenic effects (Rommel et al., 1992). Daniel et al. (1989) and Maas et al. (1991a) have summarized ellagic acid's effect against a wide range of carcinogens in several tissues. Significant inhibition of colon, esophageal, liver, lung, tongue, and skin neoplasms has been shown in rats and mice (Chang et al., 1985; Daniel et al., 1989; Das et al., 1985; Del Tito et al., 1983; Lesca, 1983; Maas et al., 1991a; Mandal and Stoner, 1990; Mukhtar et al., 1984; Tanaka et al., 1988, 1992). In addition, ellagic acid has been found to have antimutagenic, antiviral, and antioxidative properties, to control hemorrhage, and possibly to have hypotensive and sedative effects (Damas and Remacle-Volon, 1987; Maas et al., 1991a).

This paper reports the separation and measurement of ellagic acid and its derivatives in raspberry juice. The information should be useful for investigating the authenticity of red raspberry juice and for evaluating the effects of raspberry juice on health. The flavonol (Rommel and Wrolstad, 1993) and anthocyanin (Boyles and Wrolstad, 1993) compositions of these same samples are reported in other papers.

MATERIALS AND METHODS

Samples. Fifty-five red raspberry juice samples were analyzed (Table I). These included experimental juice samples processed in our pilot plant (n = 21) or laboratory (n = 17; Table IA), samples available from investigations of alternative processing methodologies (n = 8; Table IB), and commercial red raspberry juice concentrates (n = 9; Table IC).

Experimental Samples. The 46 experimental samples were produced from berries grown in 1988–1990. In the United States most raspberries are grown in Oregon and Washington, and most Canadian raspberries are produced in British Columbia. Many of the raspberries imported to the United States are of eastern European origin. Experimental juices were made from 10 different cultivars representing the principal varieties grown commercially in Oregon and Washington, British Columbia, and Poland. Two lesser known cultivars, Heritage (which ripens in September) and Golden (a yellow-colored raspberry), were included in this study for comparison to the more common ones. In addition, several environmental factors were evaluated: geographic origin (United States, Canada, Poland), maturity (underripe, ripe, overripe), harvesting technique (machine- vs hand-picking), and mold contamination. One batch of very moldy ripe berries (Meeker cultivar), which was partially fermented (as evident by a low content of soluble solids of the raw juice), was included.

Parts A and B of Table I summarize the characteristics [cultivar, geographic origin, degree of ripeness, harvesting method, processing method, microbiological quality, soluble solids content (°Brix), fruit source, picking date] of the different experimental berry samples. The standard juice-processing method was simulated in the laboratory when not enough berries were available for juice processing in the pilot plant. All fruit samples from Oregon were obtained with the assistance of the Oregon Caneberry Commission. Some of these samples were grown at the North Willamette Research and Extension Center and others at a private farm (Lucy Wisniewski, Salem, OR). Several berry samples were obtained from commercial fruit-processing companies (Clermont Inc., J. M. Smucker Co., Kerr Concentrates, Inc.) and two from the East Chilliwack Co-op in Chilliwack, BC. Both machine-harvested and hand-picked berries were obtained. We also requested varying berry maturities, and a number of the samples received were designated by suppliers as being underripe, ripe, or overripe. Fruit samples were frozen at ca. −30 °C by suppliers and stored at −23 °C on receipt in the Department of Food Science and Technology at Oregon State University.

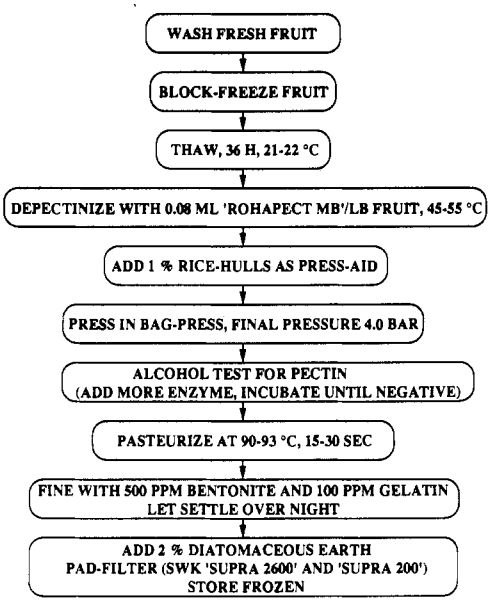

Figure 2. Flow diagram for juice processing by a standard technique.

Twelve samples of freeze-dried red raspberries were obtained from Dr. Witold Plocharski of the Research Institute of Pomology and Floriculture, Skierniewice, Poland. There were three subsamples (each representing a single picking) for each of four different cultivars. These berries were grown at the Polish Research Station in Prusy. Two-kilogram lots of fruit were freeze-dried in a Heraeus-Leybold laboratory freeze-dryer (temperature ≤30 °C). After receipt, the freeze-dried samples were stored at −28 °C until processing. These samples were rehydrated to their original weight of 2 kg with distilled water 1 day before being made into juice.

The experimental samples were coded as follows. Cultivar was designated by two letters: CH, Chilcotin; GD, Golden; HR, Heritage; ME, Meeker, MP: Malling Promise; MS, Malling Seedling; NR, Norna; SK, Skeena; VT, Veten; WI, Willamette. Geographic origin was designated by the next two-letter set: B. C. British Columbia; OR, Oregon; PO, Poland. Ripeness was designated by the next pair as UR, underripe; R, ripe; or OR, overripe. Harvesting method was designated MH, machine-harvested, or HP, hand-picked. Processing procedures were designated PP for pilot-plant-processed juices and LP for laboratory-processed juices. The one very moldy sample was designated ME-OR-R-PP-HP-MDY.

Commercial Samples. Commercial red raspberry juice concentrates (Table IC) were obtained from the following firms: Clermont Inc., Hillsboro, OR; Endurance Fruit Processing Inc., Wapato, WA; Kerr Concentrates Inc., Salem, OR; Milne Fruit Products, Inc., Prosser, WA; Rudolf Wild GmbH & Co. KG, Heidelberg, Germany; Sanofi Bio-Industries, Wapato, WA; and the J. M. Smucker Co., Woodburn, OR. The concentrates varied as to degree of concentration (some were 45 °Brix, others 65 °Brix). When samples were solicited, companies were assured that individual sample identities would be kept confidential. The commercial samples were coded COM-A to COM-I with 45, 65, or 66 as a designator for °Brix.

Juice-Processing Methods. *Standard Pilot Plant Juice Processing Method.* A "standard" process (Figure 2), typical of commercial practice, was used to produce juices in the pilot plant of the Department of Food Science, Oregon State University, from batches of berries ranging in weight from 4 to 15 kg. Juices were depectinized with Rohapect MB liquid pectinase (Rohm

Tech Inc., Malden, MA). Pressing, the alcohol test for pectin, and fining were done as described by Rommel et al. (1990). Juices were pasteurized using an APV-Crepaco high-temperature short-time (HTST) unit (size R1R, APV Crepaco, Inc., Chicago, IL) and filtered with a multiple-pad filtration unit (Herrmann Strassburger KG Filter- und Filterschichtenfabrik, Westhofen bei Worms, Germany) using SWK-Supra 80 filters (SWK Filtration, Inc., Petaluma, CA). Single-strength (i.e., unconcentrated) juices produced by the standard process were stored frozen at −23 °C.

Standard Laboratory Juice Processing Method. For the Polish, Heritage, and Golden raspberries (batch sizes ≤ 4 kg), a standard juice-making process was simulated in the laboratory. Two batches (each 4 kg) of Willamette berries (Oregon), processed in larger batches in the pilot plant, were also processed according to the laboratory procedure for comparison. For this simulated process, a basket centrifuge, type Supreme Juicerator, Model 6001 (Acme Juicer Manufacturing Co., Sierra Madre, CA) was used to separate juice from solids and for filtration; the inner wall of the basket was covered with glass-fiber paper, grade GF/B (Whatman International Ltd., Maidstone, U.K.) during filtration. The recovered juice was pasteurized in 1-L glass bottles in a steam kettle for 2 min at 88 °C. All other steps of processing were the same as in the pilot plant process described in Figure 2.

Alternative Juice Processing Methods. Another investigation (Wrolstad et al., 1993) compared the sensory quality and composition of red raspberry juice concentrated by a direct osmotic process ($n = 3$) and conventional evaporative technology ($n = 1$) to single-strength juice ($n = 1$). In that study, single-strength red raspberry juice was produced from one 150-kg batch of individually quick frozen (IQF) raspberries (Willamette cultivar, from Oregon) using the above-described standard pilot plant process (Figure 2); one-fifth of the juice produced was retained as a control (code: WI-OR-R-PP). Another fifth of the standard processed juice was concentrated to 44 °Brix using a Centri-Therm centrifugal evaporator (Model CT-1B (Alfa-Laval, Inc., Newburyport, MA) at a maximum temperature of 86 °C and a vacuum of −0.88 kg/cm^3. Osmotic concentration was performed on the remaining three-fifths of the juice at Osmotek, Inc., Corvallis, OR, using a module and process as described by Beaudry and Lampi (1990a,b). In this process, juice was separated from high-fructose corn syrup, as the osmotic agent, by a membrane and concentrated by direct osmosis to a desired level (up to 45–50 °Brix). Two lots of juice were concentrated using membrane A (25–100-μm thickness; molecular weight cutoff of ca. 100), one at chilled conditions (8 °C) with a total process time of 10.3 h and the other at room temperature (26 °C) with a total processing time of 5.8 h. Another lot was concentrated using an experimental membrane (B) at room temperature (26 °C) with a total processing time of 5.8 h. The chilled sample was subjected to more pumping than the other two samples. For more details about processing procedures and additional compositional and sensory data, refer to Wrolstad et al. (1993). These samples coded WI-OR-R were further classified as VC for vacuum concentration and OS8A, OS26A, and OS26B for osmotic concentration at 8 and 26 °C using membrane A or B.

Additional red raspberry juices were available from experimental processing trials conducted in cooperation with the Agriculture Canada Research Station in Summerland, BC. These processing trials included high-speed centrifugation, by itself or in combination with two different commercial pectolytic enzyme preparations ($n = 3$), as well as diffusion extraction ($n = 1$). These trials were conducted on one batch of block frozen fruit (Willamette cultivar) grown in Chilliwack, BC. These samples coded WI-BC-R were further designated DE for diffusion extraction, CT for centrifugation, or ESP and EBE for Pectinex Ultra SP enzyme and Pectinex BE enzyme (Novo Laboratories, Danbury CT), respectively.

(*a*) *High-Speed Centrifugation Juice Processing Method.* This technique used centrifugation (Figure 3) rather than pressing to extract juice. Beveridge et al. (1988) provide a comprehensive review of this technique and of the equipment required. Juice was extracted from 40-kg batches of berries by centrifugation and by centrifugation in combination with two pectolytic enzymes, respectively. The enzymes Pectinex BE and Pectinex Ultra SP were included to study their effects on juice composition.

(*b*) *Diffusion Extraction Process.* Diffusion extraction (Figure 4) has been used successfully in the production of apple and pear juices (Schobinger et al., (1978), not however, for juices made from berries. Diffusion extraction and equipment used are described in detail by Luethi and Glunk (1974) and Schobinger et al. (1978). Water (63 °C) was used to leach components from 200 kg of berries. Raw juice was concentrated following depectinization (with Irgazyme 100, Ciba-Geigy Corp., Ardsley, NY) and pasteurization. Single-strength juices were stored frozen at −23 °C.

Sample Preparation, HPLC Analysis, and Peak Characterization. Juice samples were separated into two fractions, a methanol fraction and a 0.5% ammonia in methanol fraction, by use of Polyamide 6. Methodology for sample preparation, HPLC separation, and peak characterization are reported in another paper (Rommel and Wrolsted, 1993). Ellagic acid (Sigma Chemical Co., St. Louis, MO) was used as a standard to characterize ellagic acid and its derivatives in juice. Absorbance spectra of ellagic acid and ellagic acid derivatives were very similar and very different from those of flavonols (e.g., quercetin 3-glucuronide); examples of spectra are shown in Figure 1b.

Quantification Method. Ellagic acid and its forms were measured using both internal and external standards (Rommel and Wrolstad, 1993). 4-Methylumbelliferyl β-D-glucuronide (Sigma) was used as the internal standard and ellagic acid (Sigma) as the external standard for the ammonia/methanol fraction, which contained ellagic acid and its derivatives. The quantification of ellagic acid and its derivatives was limited by decreased peak sharpness of the external standard (ellagic acid) due to its low solubility under neutral or acidic conditions. High concentrations of ethanol (80% or greater) are required for solubilization of the pure standard. While ellagic acid is sufficiently soluble in alkaline solutions (Press and Hardcastle, 1969), such solutions cannot be used as the C_{18} columns will degrade and ellagic acid will decompose with time. The low solubility most likely contributes to the considerable variation in ellagic acid measurement with replicate analyses (Table III).

Statistical Analysis. To evaluate the reproducibility of the data we determined (1) the standard errors of the means of concentrations of ellagic acid and its derivatives and (2) the percentages of variances due to differences between samples and replicate sample preparations with analysis of variance (ANOVA; level of significance, $\alpha = 0.05$). Standard errors of means are listed in preference to standard deviations because of high variability of preparation replicates. The values reported should therefore be regarded as estimates. 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 an overall perspective of the amounts of ellagic acid derivatives present in red raspberry juices.

RESULTS AND DISCUSSION

Ammonia/methanol juice fractions contained ellagic acid, ellagic acid derivatives, flavonol glucuronides, acylated flavonol glycosides, and flavonol aglycons. The results for the flavonols are reported in another paper (Rommel and Wrolstad, 1993). Examples of HPLC chromatograms of ammonia/methanol fractions of juices made from Willamette and Norna cultivars are shown in Figure 5. The differences among chromatographic profiles of ammonia/methanol fractions were primarily quantitative. For instance, the ratio of ellagic acid to other ellagic acid forms is much greater in Willamette as compared to the Norna cultivar (Figure 5).

The concentrations of ellagic acid and 16 ellagic acid forms in experimental and commercial samples are given in Table II. These ellagic acid forms could be easily distinguished from other phenolics from their UV spectra (Figure 1b). These compounds were not further characterized, but they may be ellagic acid derivatives with varying methylation, glycosylation, or methoxylation patterns (Maas et al., 1991a) or monomeric and dimeric ellagitannins (Haddock et al., 1982; Haslam and Lilley, 1985). Two of the commercial samples (COM-G-65, COM-

Table I. Experimental Raspberry Juice Samples Produced by Standard and Alternative Processes[a]

sample	sample code	cultivar	geographic origin	ripeness					processing method									juice °Brix	juice concentrate °Brix	source	picking date
				UR	R	OR	MH	HP	STD PP	STD LP*	STD + VC	STD + OS	CT	CT + enz	DE	MDY					
									A. Standard Process												
8	ME-OR-UR-HP-PP	Meeker	U.S.A. (OR)	X				X	X									8.5		Smucker	7/11/88
16	ME-OR-UR-MH-PP	Meeker	U.S.A. (OR)	X			X		X									9.8		Smucker	07/21/88
11	Me-OR-R-MH-PP	Meeker	U.S.A. (OR)		X		X		X									7.5		Smucker	07/12/88
10	ME-OR-OR-HP-PP	Meeker	U.S.A. (OR)			X		X	X									13.0		Clermont	07/23/88
15	ME-OR-OR-HP-PP	Meeker	U.S.A. (OR)			X		X	X									10.0		Smucker	07/11/88
3	ME-OR-OR-MH-PP	Meeker	U.S.A. (OR)			X	X		X									13.0		Clermont	07/23/88
5	ME-OR-OR-MH-PP	Meeker	U.S.A. (OR)			X	X		X									10.2		Clermont	07/21/88
17	ME-OR-R-PP-HP-MDY	Meeker	U.S.A. (OR)		X			X	X								X	2.5		Smucker	07/12/88
18	WI-OR-UR-HP-PP	Willamette	U.S.A. (OR)	X				X	X									8.4		NWREC	07/22/88
20	WI-OR-UR-HP-PP	Willamette	U.S.A. (OR)	X				X	X									9.0		NWREC	07/01/88
34 A	WI-OR-R-MH-PP	Willamette	U.S.A. (OR)		X		X		X									9.1		Kerr	06/19/90
34 B	WI-OR-R-MH-PP	Willamette	U.S.A. (OR)		X		X		X									8.0		Kerr	06/19/90
34 C	WI-OR-R-MH-LP	Willamette	U.S.A. (OR)		X		X			X								9.2		Kerr	06/19/90
34 D	WI-OR-R-MH-LP	Willamette	U.S.A. (OR)		X		X			X								10.0		Kerr	06/19/90
6	WI-OR-OR-HP-PP	Willamette	U.S.A. (OR)			X		X	X									10.5		Smucker	07/11/88
14	WI-OR-OR-HP-PP	Willamette	U.S.A. (OR)			X		X	X									9.2		NWREC	07/22/88
19	WI-OR-OR-HP-PP	Willamette	U.S.A. (OR)			X		X	X									8.0		NWREC	07/01/88
4	WI-OR-OR-MH-PP	Willamette	U.S.A. (OR)			X	X		X									9.8		Clermont	07/08/88
7	WI-OR-OR-MH-PP	Willamette	U.S.A. (OR)			X	X		X									13.4		Smucker	07/21/88
9	WI-OR-OR-MH-PP	Willamette	U.S.A. (OR)			X	X		X									13.0		Smucker	07/21/88
1	CH-BC-R-PP	Chilcotin	Canada (BC)		X				X									9.4		E Chilliwack	07/20/88
2	SK-BC-R-PP	Skeena	Canada (BC)		X				X									9.0		E Chilliwack	07/20/88
32	HR-OR-R-HP-LP	Heritage	U.S.A. (OR)		X				X	X								13.2		private farm	09/23/89
31	HR-OR-OR-HP-LP	Heritage	U.S.A. (OR)			X		X		X								15.0		private farm	09/23/89
33	GD-OR-R-HP-LP	Golden	U.S.A. (OR)		X			X		X								11.3		private farm	07/00/89
30	MP-PO-R-HP-LP	Malling Promise	Poland		X			X		X								9.4		RIPF	07/06/88
29	MP-PO-R-HP-LP	Malling Promise	Poland		X			X		X								8.0		RIPF	07/08/88
24	MP-PO-R-HP-LP	Malling Promise	Poland		X			X		X								11.0		RIPF	07/11/88
13	MS-PO-R-HP-LP	Malling Seedling	Poland		X			X		X								8.5		RIPF	07/06/88
28	MS-PO-R-HP-LP	Malling Seedling	Poland		X			X		X								7.0		RIPF	07/08/88
27	MS-PO-R-HP-LP	Malling Seedling	Poland		X			X		X								8.5		RIPF	07/11/88
26	NR-PO-R-HP-LP	Norna	Poland		X			X		X								9.0		RIPF	07/06/88
21	NR-PO-R-HP-LP	Norna	Poland		X			X		X								7.9		RIPF	07/11/88
22	NR-PO-R-HP-LP	Norna	Poland		X			X		X								9.0		RIPF	07/08/88
12	VT-PO-R-HP-LP	Veten	Poland		X			X		X								8.5		RIPF	07/06/88
23	VT-PO-R-HP-LP	Veten	Poland		X			X		X								8.8		RIPF	07/08/88
25	VT-PO-R-HP-LP	Veten	Poland		X			X		X								9.8		RIPF	07/11/88
									B. Alternative Processes												
37 D	WI-OR-R-PP	Willamette	U.S.A. (OR)		X				X									10.8		Kerr	06/19/90
37E	WI-OR-R-VC	Willamette	U.S.A. (OR)		X						X							10.0	43.5	Kerr	06/19/90
37 A	WI-OR-R-OS8A	Willamette	U.S.A. (OR)		X							X 8A						10.0	44.8	Kerr	06/19/90
37 B	WI-OR-R-OS26A	Willamette	U.S.A. (OR)		X							X 26A						10.0	43.5	Kerr	06/19/90
37C	WI-OR-R-OS26B	Willamette	U.S.A. (OR)		X							X 26B						10.0	45.5	Kerr	06/19/90
35 A	WI-BC-R-MH-CT	Willamette	Canada (BC)		X		X						X					11.9		E Chilliwack	07/20/88
35 B	WI-BC-R-MH-CTESP	Willamette	Canada (BC)		X		X							X ESP				12.4		E Chilliwack	07/20/88
35 C	WI-BC-R-MH-CTEBE	Willamette	Canada (BC)		X		X							X EBE				12.2		E Chilliwack	07/20/88
36	WI-BC-R-MH-DE	Willamette	Canada (BC)		X		X								X			7.6		E Chilliwack	07/20/88

Table I (Continued)

						C. Commercial Red Raspberry Juice Concentrates						
sample	sample code	company code	ESR	°Brix	sample	sample code	company code	ESR	°Brix	sample	sample code	company code	ESR	°Brix
38	COM-A-45	A	yes	45	41	COM-D-45	D	no	45	44	COM-G-65	G		65
39	COM-B-65	B		65	42	COM-E-45	E	yes	45	45	COM-H-66	H		66
40	COM-C-45	C	yes	45	43	COM-F-45	F	no	45	46	COM-I-65	I		65

[a] MH, machine harvested; HP, hand-picked; MDY, moldy; UR, underripe; R, ripe; OR, overripe; STD, standard; PP, pilot plant process; LP, laboratory process; *, simulation of a standard juice-making process in the laboratory; VC, vacuum concentration with a Centritherm centrifugal evaporator; OS, direct osmotic concentration; CT, high-speed centrifugation; enz, enzym (pectinase); DE, diffusion extraction; ME, Mesker, WI; Willamette; CH, Chilcotin; SK, Skeena; HR, Heritage; GD, Golden; MP, Malling Promise; MS, Malling Seedling; NR, Norna; VT, Veten; OR, Oregon; BC, British Columbia; PO, Poland; OS8A, OS26A, osmotic concentrations at 8 and 26 °C, respectively, using membrane A; OS26B, osmotic concentration at 26 °C using membrane B; ESP, Pectinex Ultra SP enzyme; EBE, Pectinex BE enzyme; ESR, essence returned; COM, commercial; NWREC, North Willamette Research and Extension Center, Aurora, OR; E Chilliwack, East Chilliwack Co-op, Chilliwack, BC, Canada; RIPF, Research Institute of Pomology and Floriculture, Skierniewice, Poland.

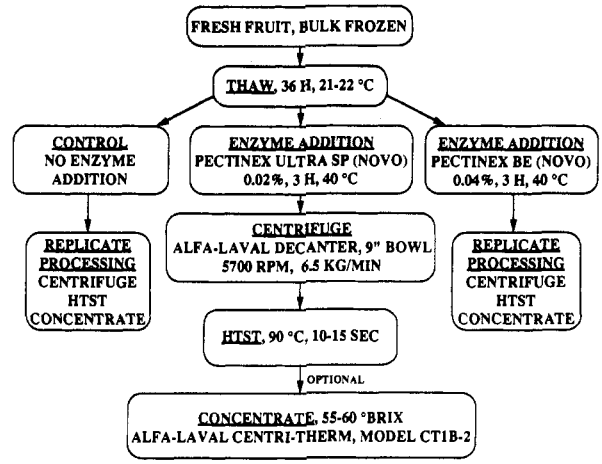

Figure 3. Flow diagram for juice processing by centrifugation, with and without depectinization.

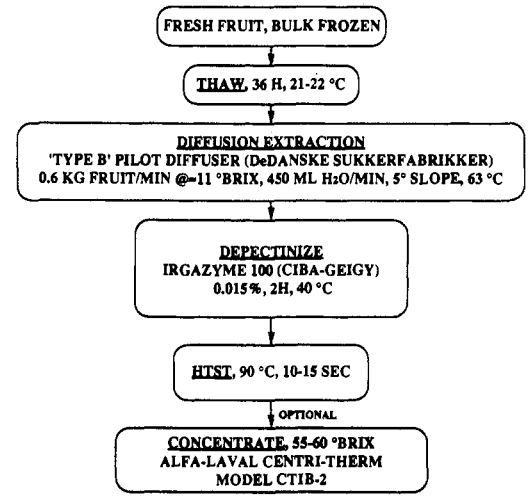

Figure 4. Flow diagram for juice processing by diffusion extraction.

H-66) had considerably lower concentrations of ellagic acid (5.5, 14 ppm), ellagic acid form 6 (0.9, 0.8 ppm), and total ellagic acid forms (22, 26 ppm) than the other commercial samples. Sample COM-H-66 also contained less ellagic acid form 2 (0.9 ppm), while sample COM-G-65 contained much more form 2 (7.2 ppm), more than all other commercial samples. These two commercial samples were found to be adulterated on the basis of their flavonol (Rommel and Wrolstad, 1993) and anthocyanin (Boyles and Wrolstad, 1993) compositions; therefore, they were excluded from the calculated mean concentrations of ellagic acid and its forms for the commercial samples. The mean concentrations of ellagic acid compounds in commercial samples ($n = 7$) were as follows: total ellagic acid forms, 52.4 ppm; ellagic acid, 29.8 ppm; form 1, 6.7 ppm; form 2, 3.3 ppm; form 6, 2.3 ppm; form 9, 1.3 ppm; forms 3, 4, 8, and 12, 1 ppm of each; form 7, 0.8 ppm; forms 5, 10, 11, and 13–16, trace amounts of each.

Table III summarizes mean concentrations and ranges of ellagic acid and derivatives measured in experimental (except the moldy sample) and commercial juices, respectively, as well as standard errors and percentages of variances for the experimental samples. Ellagic acid was the major ellagic acid compound in all juice samples analyzed, with a mean concentration of 10 ± 1.5 ppm for 45 experimental samples. In the nine commercial samples ellagic acid ranged from 5.5 to 52 ppm; the mean concentration ($n = 7$) was 30 ppm. Individual concentrations of ellagic acid derivatives ranged from trace amounts to 3 ± 0.3 ppm (means) in experimental juices

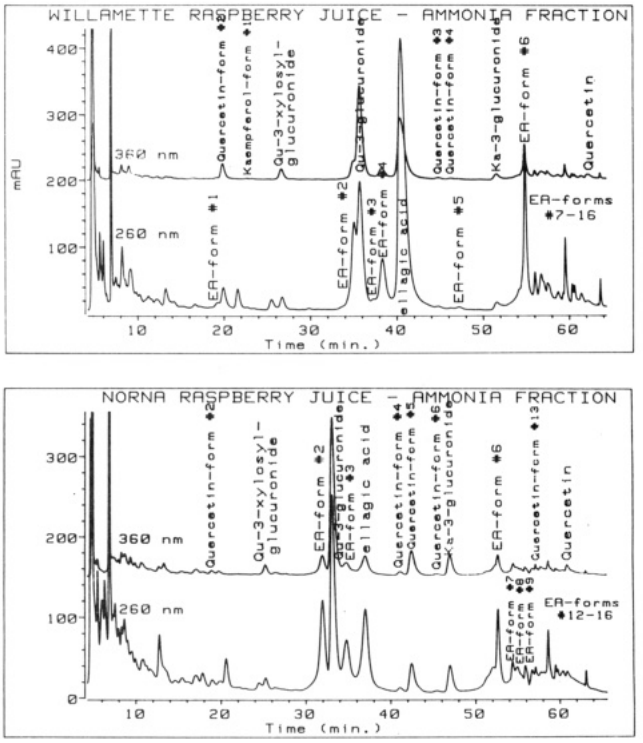

Figure 5. HPLC chromatogram of the ammonia/methanol fraction (separated on Polyamide 6) of red raspberry juice made from (a, top) ripe Willamette cultivar (b, bottom) ripe Norna cultivar. Ellagic acid and ellagic acid derivatives were detected at 260 nm flavonol glucuronides, acylated flavonol glycosides, and flavonol aglycons were detected at 360 nm.

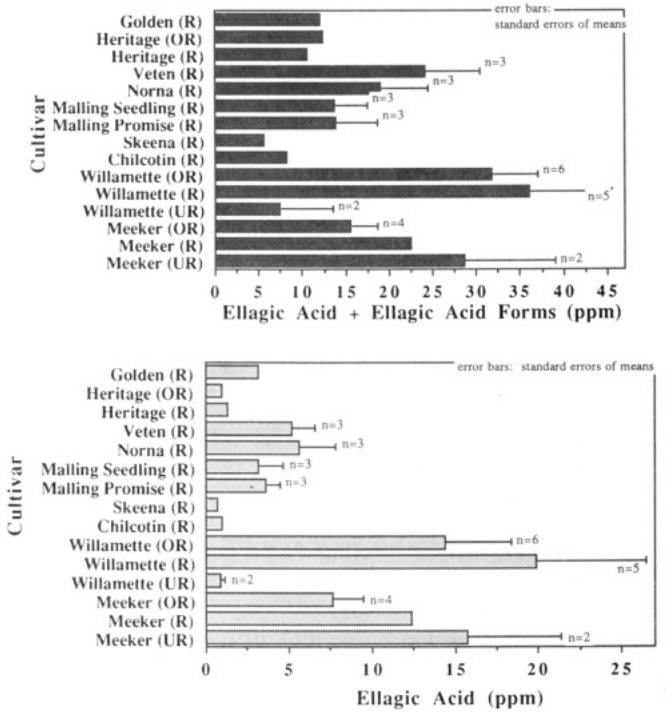

Figure 6. (a, top) Total concentrations of ellagic acid forms in juices made from different raspberry cultivars. (b, bottom) Concentrations of ellagic acid in juices made from different raspberry cultivars. UR, underripe; R, ripe; OR, overripe.

and between traces and 24 ppm for commercial samples. The total concentration of ellagic acid and its derivatives in experimental juices was 28 ± 2.3 ppm (mean) and in nine commercial juices ranged from 22 to 80 ppm (mean of 52 ppm).

Influence of Cultivar. Total concentrations of ellagic acid forms in juices made from 10 cultivars were compared (Figure 6a); different maturities were available for three cultivars. The concentrations of ellagic acid for the same juices were also compared (Figure 6b). The distribution of ellagic acid concentrations among cultivars largely reflected that of total ellagic acid forms. However, cultivars differed considerably in their contents of ellagic acid and summed forms. Willamette and Meeker cultivar contained most summed ellagic acid forms (between ca. 7 and 36 ppm) and ellagic acid (up to ca. 20 ppm); Veten and Norna cultivars contained medium amounts of ellagic acid and total forms.

Influences of Environmental Factors. *Geographic Origin.* From our set of samples it was difficult to determine whether the differences in ellagic acid concentrations resulted solely from differences among cultivars or also from differences in growing region. For example, of the juices grown in Poland and processed by the standard technique, two of the four cultivars (Veten, Norna) showed quite differing ellagic acid and total ellagic acid forms contents, while the two Malling varieties were very similar (Figure 6a,b). Juices made from two of the cultivars grown in Canada (Chilcotin, Skeena) according to the standard process had very low contents of ellagic acid and total forms compared to other juices. The remaining juices, which were made from berries grown in Oregon, showed greatly differing ellagic acid concentrations.

Maturity. The concentrations of ellagic acid and summed ellagic acid forms decreased with increasing ripeness in juices made from the cultivars Meeker (Figure 6a,b). In contrast, there was no such trend for Willamette juices; juice made from ripe berries contained the most ellagic acid and summed forms, while juice made from underripe fruit contained the least. Raspberry juice made from overripe Heritage cultivar contained slightly more total ellagic acid forms than that made from ripe berries; for ellagic acid the situation was reversed. The four cultivars grown in Poland were picked within 5 days (on July 6, 8, 11, 1988). The concentrations of total ellagic acid forms and of ellagic acid increased over this time period in juices made from the cultivar Norna (from 9.5 to 28.5 ppm of summed forms; from 2.6 to 9.8 ppm of ellagic acid), while they decreased in juice made from Veten (from 29.5 to 11.3 ppm of summed forms; from 7.5 to 2.5 ppm of ellagic acid). There was no trend for juices made from the two Malling cultivars. For the seven cultivars studied in this investigation there was no apparent correlation between ripeness and the concentrations of ellagic acid and its derivatives.

Harvesting Technique. Figure 7 shows concentrations of summed ellagic acid forms for juices made from Willamette and Meeker cultivars, either hand-picked or machine-harvested at three stages of maturity. Juices made from overripe Willamette berries harvested by machine appeared to have higher concentrations of total ellagic acid forms from juices made from hand-picked overripe Willamette berries. However, as there was much variation among juices made from berries of the same cultivar and maturity, it was not possible to determine conclusively if harvesting method influenced ellagic acid concentrations as well.

Mold Contamination. Mold did not seem to affect the concentrations of ellagic acid and ellagic acid derivatives in juices made form the cultivar Meeker (Figure 8). The concentration of total ellagic acid forms in the moldy sample was within the concentration range for juices made from berries at three stages of maturity. However, mold decreased the contents of quercetin glycosides and glucuronides considerably in these samples (Rommel and Wrolstad, 1993).

Influences of Processing. *Standard Pilot Plant*

Table II. Concentrations of Ellagic Acid and Its Forms in Experimental and Commercial Juices

		EA forms,[c] ppm																
cultivar[a] (sample no.)	EA[b]	2	6	3	4	1	12	8	9	7	10	11	15	13	14	16	5	total
Meeker (UR) (8)	21.4	3.5	3.2	1.2	2.4	tr	1.4	nd	tr	tr	tr	tr	tr	tr	tr	tr	tr	39.0
Meeker UR (16)	10.1	1.6	1.1	1.7	nd	tr	tr	tr	tr	tr	nd	nd	nd	nd	nd	tr	nd	18.1
Meeker R (11)	12.4	1.8	1.1	1.6	nd	0.9	tr	tr	nd	tr	tr	tr	nd	tr	nd	tr	tr	22.5
Meeker OR (5)	9.1	2.1	tr	1.5	nd	0.8	0.9	nd	tr	tr	nd	0.7	tr	tr	tr	tr	tr	20.0
Meeker OR (3)	8.0	1.2	1.0	1.9	nd	nd	0.7	nd	tr	tr	nd	tr	tr	tr	tr	tr	nd	16.9
Meeker OR (10)	2.2	0.7	0.7	0.9	nd	nd	tr	nd	nd	tr	nd	nd	nd	nd	nd	tr	nd	6.2
Meeker OR (15)	11.1	1.5	0.8	2.2	nd	nd	tr	nd	nd	tr	nd	tr	nd	tr	tr	tr	nd	19.1
Meeker R moldy (17)	22.2	nd	nd	nd	nd	5.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	27.5
Willamette UR (18)	0.7	nd	nd	nd	nd	nd	nd	nd	tr	nd	nd	nd	nd	nd	nd	nd	nd	1.3
Willamette UR (20)	1.1	3.4	1.5	2.0	nd	tr	0.9	tr	nd	tr	tr	nd	nd	tr	tr	tr	tr	13.6
Willamette R (34A)	10.3	2.7	1.6	1.5	nd	nd	0.9	nd	tr	tr	tr	tr	tr	tr	tr	tr	tr	22.5
Willamette R (34B)	18.6	4.5	3.0	2.4	nd	tr	1.0	1.2	0.8	tr	tr	tr	tr	tr	tr	tr	tr	36.8
Willamette R LP (34C)	10.5	1.4	1.3	1.2	nd	nd	0.7	tr	tr	tr	0.7	tr	tr	tr	tr	tr	tr	21.1
Willamette R LP (34D)	14.6	2.9	1.6	1.3	nd	tr	0.9	0.8	tr	tr	tr	tr	tr	tr	tr	tr	tr	28.1
Willamette OR (7)	10.0	3.6	2.6	1.3	1.3	tr	0.9	nd	tr	1.0	0.7	tr	tr	tr	tr	tr	tr	26.2
Willamette OR (4)	31.5	5.0	3.4	2.4	1.8	0.9	1.6	tr	1.3	tr	tr	tr	tr	tr	tr	tr	tr	53.3
Willamette OR (6)	17.9	4.4	3.0	3.1	nd	nd	1.1	tr	0.7	tr	tr	0.7	tr	tr	tr	tr	tr	35.8
Willamette OR (9)	13.8	4.5	2.8	3.3	nd	tr	1.4	tr	0.7	tr	tr	nd	tr	tr	tr	tr	tr	31.9
Willamette OR (14)	9.9	6.0	2.5	3.6	nd	nd	1.8	0.8	nd	0.8	nd	tr	tr	tr	tr	tr	tr	29.0
Willamette OR (19)	3.5	2.7	2.2	1.5	nd	nd	0.9	nd	tr	tr	nd	tr	nd	tr	tr	tr	tr	14.9
Chilcotin R (1)	1.0	1.5	1.0	3.1	nd	nd	tr	nd	nd	tr	nd	nd	nd	nd	nd	tr	nd	8.3
Skeena R (2)	0.7	0.8	tr	tr	tr	nd	tr	nd	nd	nd	nd	tr	nd	tr	nd	tr	nd	5.7
Heritage R (32)	1.3	1.5	1.1	1.3	nd	nd	tr	tr	tr	nd	nd	tr	tr	tr	tr	tr	tr	10.6
Heritage OR (31)	0.9	2.2	1.1	1.7	nd	nd	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	12.5
Malling Promise 1 (30)	3.8	1.2	0.6	1.6	nd	tr	tr	tr	nd	tr	nd	nd	nd	tr	nd	tr	tr	11.4
Malling Promise 2 (29)	2.1	0.9	0.8	tr	tr	tr	tr	nd	nd	nd	nd	nd	nd	nd	nd	tr	tr	7.3
Malling Promise 3 (24)	4.9	4.2	2.4	4.7	nd	nd	0.9	0.7	nd	tr	1.2	tr	tr	tr	tr	tr	tr	23.1
Malling seedling 1 (13)	6.1	2.6	1.6	3.8	nd	nd	1.3	0.8	tr	tr	nd	tr	tr	tr	tr	tr	nd	20.5
Malling seedling 2 (28)	1.2	0.8	tr	1.5	nd	tr	tr	nd	nd	tr	nd	nd	nd	nd	nd	tr	tr	7.1
Malling seedling 3 (27)	2.1	1.0	1.0	3.4	nd	nd	tr	tr	nd	tr	tr	tr	tr	tr	tr	tr	tr	13.5
Norna 1 (26)	2.6	2.2	1.0	1.8	nd	nd	tr	nd	tr	nd	nd	nd	nd	nd	nd	tr	nd	9.5
Norna 2 (22)	4.5	4.0	1.8	3.0	nd	nd	0.9	tr	tr	0.7	tr	nd	tr	tr	tr	tr	nd	19.1
Norna 3 (21)	9.8	5.2	2.8	3.9	nd	nd	1.2	tr	tr	0.7	1.3	nd	tr	tr	tr	tr	nd	28.5
Veten 1 (12)	7.5	7.3	2.5	5.1	nd	nd	1.8	1.1	tr	nd	nd	tr	tr	tr	tr	tr	tr	29.5
Veten 2 (23)	5.6	7.3	3.4	5.6	nd	nd	1.9	1.5	0.8	1.0	0.7	tr	0.9	tr	tr	tr	tr	31.5
Veten 3 (25)	2.5	2.1	0.9	1.0	nd	tr	tr	nd	nd	nd	tr	nd	tr	tr	tr	tr	tr	11.3
Golden R (33)	3.1	2.4	tr	tr	nd	nd	tr	nd	tr	nd	tr	tr	tr	tr	tr	tr	tr	12.2
Willamette STD (37D)	45.5	6.0	4.6	0.7	3.0	2.6	1.3	1.9	1.8	tr	tr	tr	0.8	tr	tr	tr	tr	72.3
Willamette VC (37E)	20.7	3.7	3.4	tr	2.4	2.3	1.0	1.0	0.7	0.8	tr	tr	tr	tr	tr	tr	tr	40.9
Willamette OS8A (37A)	26.8	3.2	3.4	2.0	1.0	2.2	1.3	1.1	0.7	0.7	tr	0.7	tr	tr	tr	tr	tr	46.8
Willamette OS26A (37B)	16.6	4.0	2.7	2.1	tr	tr	1.2	1.0	tr	0.6	nd	0.7	tr	tr	tr	tr	tr	33.7
Willamette OS26B (37C)	20.5	4.2	3.1	1.3	1.8	8.1	1.3	0.8	tr	1.0	tr	tr	tr	tr	tr	tr	tr	46.8
Willamette CT (35A)	8.3	1.8	6.3	5.6	2.4	nd	3.3	0.9	1.2	2.0	1.0	tr	tr	0.8	0.7	0.8	tr	37.0
Willamette CT ESP (35B)	tr	1.2	4.5	1.3	tr	nd	3.5	0.9	0.8	1.0	0.7	tr	tr	0.7	0.7	0.8	tr	19.1
Willamette CT EBE (35C)	1.2	1.1	3.8	1.5	nd	nd	2.8	tr	0.7	0.8	nd	nd	tr	tr	tr	tr	tr	15.5
Willamette DE (36)	38.5	4.2	4.3	tr	2.9	5.5	1.8	1.8	1.5	0.8	1.0	0.8	tr	tr	tr	tr	tr	66.7
A (38)	24.8	1.8	1.7	0.7	tr	5.0	1.5	1.0	1.9	0.8	0.8	tr	tr	tr	tr	tr	tr	44.1
B (39)	29.9	2.5	2.0	tr	1.4	5.0	tr	tr	tr	tr	0.7	tr	tr	tr	tr	tr	tr	48.2
C (40)	28.4	4.5	2.8	tr	2.4	23.5	1.1	0.9	tr	1.3	0.8	tr	tr	tr	tr	tr	tr	70.4
D (41)	52.1	5.1	3.8	0.8	1.1	5.8	1.0	2.4	2.5	0.7	0.9	tr	1.0	tr	0.7	0.8	tr	80.4
E (42)	20.6	1.9	1.7	0.8	tr	2.6	tr	tr	0.9	nd	nd	tr	nd	tr	tr	tr	tr	32.6
F (43)	36.1	4.1	2.4	1.6	tr	2.1	1.3	tr	2.2	1.5	nd	tr	tr	tr	tr	tr	tr	56.1
G (44)	5.5	7.2	0.9	1.2	tr	2.1	tr	tr	0.7	tr	nd	tr	nd	tr	nd	tr	tr	22.4
H (45)	14.0	0.9	0.8	0.8	nd	4.2	tr	tr	nd	tr	tr	tr	nd	tr	tr	tr	tr	26.0
I (46)	17.0	3.5	2.0	1.9	tr	3.0	1.2	tr	0.7	tr	tr	tr	tr	tr	tr	tr	tr	35.3

[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] EA, ellagic acid. [c] tr, trace (≤0.6 ppm); nd, not detected.

Process and Alternative Processes. The influences of the different processing technique on total concentrations of ellagic acid forms in raspberry juices made from the cultivar Willamette are shown in Figure 9. Diffusion extraction and the standard pilot plant process (single-strength, large batch) produced juices with by far the highest total concentrations of ellagic acid forms (ca. 70 ppm). All juices were not made from the same lot of fruit, so all samples are not directly comparable. Juice produced by diffusion extraction contained about twice as much summed ellagic acid forms as juice made by high-speed centrifugation from the same Canadian raspberries. Diffusion extraction exposed the berries to high temperature (63 °C) for several hours, which is likely to have increased the release of ellagic acid from cell walls through hydrolysis of ellagitannins (Figure 1; Bate-Smith, 1959, 1972; Wilson and Hagerman, 1990). Depectinization combined with centrifugation decreased total ellagic acid forms considerably compared

Table III. Mean Concentrations of Ellagic Acid and Its Forms in Experimental Raspberry Juices and Concentration Ranges in Commercial Samples

ellagic acid (EA) compd	concn in experimental juices, ppm		% variance due to difference		concn range in commercial juices, ppm (n = 9)
	n	mean ± SE	among juices	between repl	
ellagic acid[a]	45	10.1 ± 1.53	71.7	28.3	5.48–52.1
EA form 2	44	2.95 ± 0.27	70.6	29.4	0.87–7.19
EA form 6	44	2.13 ± 0.20	65.9	34.1	0.78–3.78
EA form 3	44	2.12 ± 0.20	25.5	74.5	tr[b]–1.88
EA form 4	13	1.65 ± 0.25	22.0	78.0	nd[c]–2.40
EA form 1	20	1.52 ± 0.44	13.1	86.9	2.06–23.5
EA form 12	44	1.12 ± 0.10	83.3	16.7	tr–1.48
EA form 8	32	0.77 ± 0.08	38.4	61.6	tr–2.44
EA form 9	32	0.74 ± 0.05	1.6	98.4	nd–2.46
EA form 7	37	0.71 ± 0.04	23.5	76.5	nd–1.49
EA form 10	26	0.69 ± 0.04	12.7	87.3	nd–0.85
EA form 11	31	0.62 ± 0.01	32.9	67.1	tr
EA form 15	32	0.62 ± 0.01	30.4	69.6	nd–0.99
EA form 13	38	0.61 ± 0.01	31.8	68.2	tr
EA form 14	35	0.61 ± 0.00	32.6	64.4	nd–0.71
EA form 16	44	0.61 ± 0.01	24.3	75.7	tr–0.77
EA form 5	34	trace- U 0.00	33.3	66.7	nd or tr
EA + 16 EA forms	45	28.2 ± 2.34	69.9	30.0	22.4–80.4

[a] Reported previously for fresh raspberries by Bate-Smith (1959) and Daniel et al. (1989). [b] tr, (trace), ≤ 0.6 ppm. [c] nd, not detected.

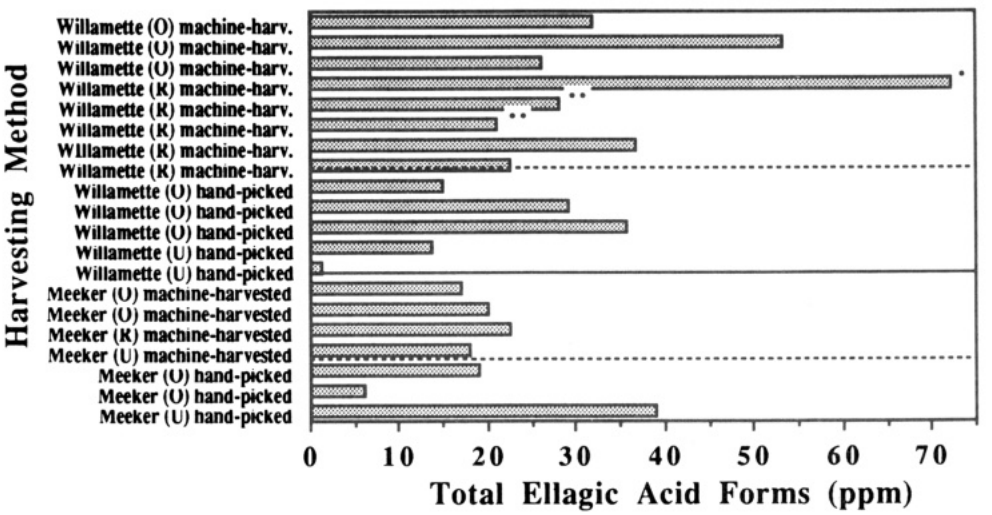

Figure 7. Total concentrations of ellagic acid forms in juices made from raspberries picked by hand or harvested by machines. U, underripe; R, ripe; O, overripe; large batch, standard process simulated in the laboratory).

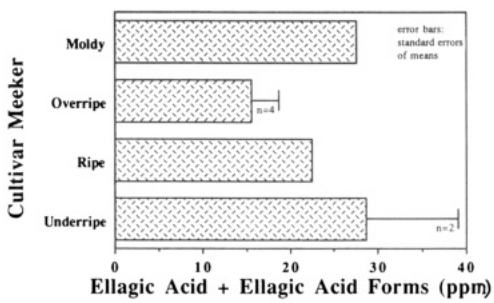

Figure 8. Effect of mold contamination on total concentration of ellagic acid forms in raspberry juices made from the cultivar Meeker.

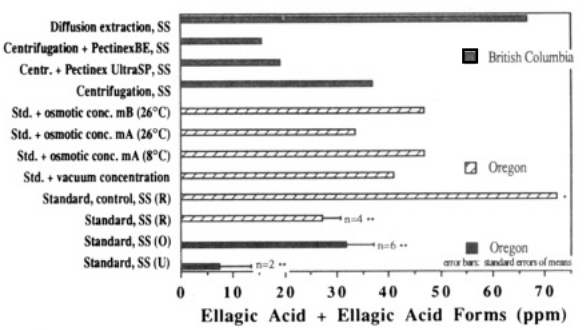

Figure 9. Effects of different processing techniques on total concentration of ellagic acid forms in juices made from the cultivar Willamette. M, membrane; SS, single strength; UR, underripe; R, ripe; OR, overripe; large batch, small batch.

to centrifugation alone; one enzyme (Pectinex BE) had a greater decreasing effect than the other (Pectinex Ultra SP).

Standard Laboratory Process. Juices made by this process contained about the same amounts of total ellagic acid forms as juices made by the standard pilot plant process from the same berries (Willamette cultivar) and the same small batch size (Figure 7). However, juice made by the standard pilot plant process from a much larger batch of the same Willamette berries contained over twice as much total ellagic acid forms as juice made from a small batch (Figures 7 and 9).

Concentration Methodologies. Both concentration techniques decreased total ellagic acid forms considerably compared to the control (standard pilot plant process). Two of the osmosis concentrated juices (WI-OR-R-OS8A, WI-OR-R-OS26B), however, reduced total ellagic acid concentrations less than vacuum evaporation and the third

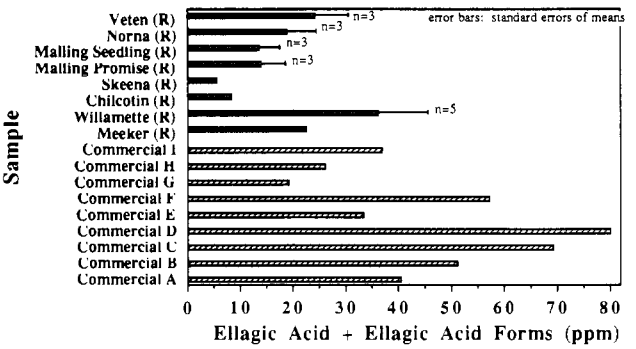

Figure 10. Total concentrations of ellagic acid in commercial and experimental raspberry juices. (Commercial juices were made by diluting concentrates to soluble solid contents of 10 °Brix.)

osmosis concentration process (membrane A, 26 °C). Osmotic concentration at chilled conditions took more time and subjected juice to more pumping, which might explain the higher concentration of total ellagic acid of this sample compared to the sample concentrated by the same membrane at higher temperature; ellagic acid is released from cell walls during juice processing through hydrolysis of ellagitannins. Wrolstad et al. (1993) found no significant differences in anthocyanin composition for these same samples.

Comparison of Experimental and Commercial Samples. Commercial samples contained much higher concentrations of total ellagic acid forms than experimental juices [Figure 10; mean of 52 ppm for commercial samples (n = 7) compared to a mean of 18 ppm for experimental samples (n = 20)]. The higher ellagic acid levels of commercial samples is likely to be related to the greater juice extraction efficiencies of commercial processes. The commercial samples showed considerable variation in their summed ellagic acid contents, ranging from ca. 33 to 80 ppm (excluding the two adulterated samples).

SUMMARY AND CONCLUSIONS

The chromatographic ellagic acid profiles were qualitatively very similar for all cultivars studied; however, quantitatively, there were great differences attributable to differences in cultivar and processing. Ellagic acid was present in all samples studied; 16 additional ellagic acid derivatives were detected. Cultivars differed considerably with respect to their amounts of ellagic acid and summed ellagic acid forms, with Meeker and Willamette containing the most. Commercial samples contained much more ellagic acid and its forms then experimental juices. Diffusion extraction and the standard process produced juices with the highest total concentrations of ellagic acid forms. High-speed centrifuged juice contained about half of the total ellagic acid compared to diffusion-extracted juice. Juice depectinization and concentration decreased total ellagic acid forms even further. Mold appeared to not have an effect on the contents of ellagic acid and its forms. Cultivars grown in the same region showed great variation in ellagic acid contents. There was no apparent correlation between ripeness or geographic origin of berries and the concentrations of ellagic acid and its derivatives, nor could the influence of harvesting method be determined with the samples available. Since ellagic acid does not have widespread distribution in fruits, its presence provides a useful marker in authenticity investigations; its high quantitative variability, however, limits its utility.

Ellagic acid was present in our red raspberry juices in concentrations within the range (or higher) that has been found to be anticarcinogenic in rodents; for example, 3 ppm of ellagic acid in the drinking water of mice reduces the risk of developing skin tumors induced by 3-methylcholanthrene. However, while many raspberry juices contained considerable amounts of ellagic acid and its derivatives, several things remain to be determined: whether different ellagic acid compounds are equally effective; the form in which they are absorbed; and what percentage of the ellagic acid and its derivatives consumed is absorbed into the human body.

ACKNOWLEDGMENT

We thank David Thomas, Department of Statistics, Oregon State University, for his advice on statistical analyses. Bob Durst advised on analytical methodology and helped with juice processing. We appreciate the cooperation of T. H. J. Beveridge and D. B. Cumming, Agriculture Canada Research Station, Summerland, BC, and Brian Yorgey, Oregon State University, in juice processing. 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 26, 1993. Accepted July 28, 1993.[⊛]

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