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Title: Phenolic Composition of Red Raspberry Juice: Influences of Cultivar, Processing and Environmental Factors

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Flavonols and ellagic acid compounds were characterized and measured in 55 experimental and commercial red raspberry juices by high performance liquid chromatography (HPLC) and diode array spectral techniques. Samples were prepared using Polyamide-6; a fraction eluted with methanol contained ≤ 8 quercetin-glycosides, quercetin and kaempferol. Another fraction, eluted subsequently with 0.5% ammonia in methanol, contained 3 flavonol-glucuronides, 2 flavonol-forms, ellagic acid and ≤ 16 ellagic acid derivatives. In addition, 36 flavonol-forms were measured in trace amounts. Experimental (n=45) and commercial (n=7) juices contained mean concentrations of 10 and 30 ppm ellagic acid and ≤ 3 and 6.7 ppm individual derivatives, respectively. Quercetin-3-glucuronide was the major flavonol in all juices (mean: 51-54 ppm) and a flavonol presumed to be quercetin-3-sophoroside the second major flavonol (29-33 ppm). Mean total concentrations in experimental and commercial juices were, respectively: ellagic acid forms, 28 and 52 ppm; quercetin-forms, 118 and 121 ppm; kaempferol-forms, 3.6 and 3.4 ppm; flavonols, 122 and 125 ppm.

Qualitatively, the chromatographic profiles of juices were similar but quantitatively, there were great differences due to cultivar (n=10) and processing method. The cultivars Willamette and Meeker contained the most ellagic acid forms; Heritage, Willamette and

Norna the most total quercetin-forms and Heritage and Norna the most total kaempferol-forms. Juices made by diffusion extraction or a standard process had the highest concentrations of ellagic acid forms, while juice produced by high-speed centrifugation contained the most total quercetin-forms. Centrifugation reduced total ellagic acid by half compared to diffusion extraction; depectinization and concentration (by vacuum or direct osmosis) decreased total ellagic acid and quercetin-forms further. Mold reduced quercetin-glycosides and glucuronides considerably, while it appeared not to effect ellagic acid.

Two commercial juice concentrates were found to be adulterated based on their anthocyanin, flavonol and ellagic acid compositions. Flavonol and ellagic acid HPLC profiles have great potential as an auxiliary technique for detecting adulteration.

Hydrolysis simplified the chromatographic profile of red raspberry juice phenolics dramatically. In acid-hydrolyzed juices, ellagic acid, 2 ellagic acid compounds, 3 hydroxybenzoic acids, 3 hydroxycinnamic acids, 2 flavan-3-ols, quercetin and kaempferol were identified. In base-hydrolyzed samples one ellagic acid compound was absent and the other was present in smaller quantity. Hydrolysis could become a very useful tool for rapidly screening juices for adulteration and measuring total quantities of phenolic aglycons.

Ellagic acid and quercetin (in glycosylated form) were present in the juices in concentrations which have been shown to have anticarcinogenic effects. Medical evaluations of the positive effects of red raspberry juice on human health could be based on our initial phenolic compositional data.

Phenolic Composition of Red Raspberry Juice: Influences of Cultivar, Processing and Environmental Factors

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TABLE OF CONTENTS

(TABLE OF CONTENTS continued)

LIST OF FIGURES

(LIST OF FIGURES continued)

CHAPTER III

(LIST OF FIGURES continued)

CHAPTER IV

(LIST OF FIGURES continued)

LIST OF TABLES

(LIST OF TABLES continued)

CHAPTER IV

PHENOLIC COMPOSITION OF RED RASPBERRY JUICE: INFLUENCES OF CULTIVAR, PROCESSING AND ENVIRONMENTAL FACTORS

I. Introduction

The chemical composition of red raspberry juice is of much interest to the food industry and regulatory agencies in determining juice authenticity and protecting the consumer from fraudulent products. Raspberry juices and concentrates are targets for adulteration because of their high commercial value. In recent years, the composition of raspberry juice has become of ever increasing interest because of positive health effects associated with its high phenolic content, specifically the anticarcinogenic potencies of quercetin and ellagic acid.

This thesis is a contribution to an extensive study by Dr. Wrolstad and his group which will create an expanded database for the phenolic, sugar and acid composition of red raspberry juice. It is restricted to the separation, characterization and quantification of flavonols and ellagic acid in raspberry juices, as well as the separation of the phenolics present in selected hydrolyzed red raspberry juices. The anthocyanin composition of the red raspberry juices analyzed in this study is discussed in Boyles (1992); the compositions of acids and sugars in these samples will be reported elsewhere.

The fundamental questions addressed in this thesis are: 1) to establish whether or not non-anthocyanin flavonoids and other phenolics can be used as reliable supplementary indicators together with anthocyanins, sugars and organic acids to determine red raspberry juice authenticity; 2) to determine if the phenolic compounds, which have been reported as having potentially positive effects on human health, are present in raspberry juices.

The specific objectives of this study were: 1) to identify and quantify flavonols and ellagic acid in juices, made from different red raspberry cultivars by different processing techniques, and subjected to various environmental factors so that they can be used as

a) supplementary authenticity indicators together with anthocyanins, sugars and acids and

b) a data-base for evaluating the effects of raspberry juice on health; 2) to separate and characterize the phenolics present in selected hydrolyzed red raspberry juices.

Food companies and regulatory agencies need HPLC profiles of the flavonols, ellagic acid and other phenolics present in red raspberry juice for detecting adulteration, e.g. in cases where non-red juices (apple, pear) have been used as adulterants. The HPLC profile of hydrolyzed raspberry juice phenolics will allow to screen raspberry juices rapidly for adulteration. In addition, a hydrolysis profile could become a useful tool for measuring the total quantities of aglycons present, e.g. for the evaluation of health effects of raspberry juices. This thesis provides the concentrations of the flavonols and ellagic acid compounds in raspberry juices, including those of compounds with anticarcinogenic properties. These data could be used in medical evaluations of the beneficial effects of consuming red raspberry products, which in turn could lead to dietary recommendations.

LITERATURE REVIEW

Phenolic compounds seem to be universally distributed in plants (Herrmann, 1989). Plants produce thousands of compounds which contain one or more phenolic residues (Goodwin and Mercer, 1983). Commonly, a plant species contains 10 or more phenolics in very different concentrations, but it is possible that 20 to 30 or more phenolics are present in a single plant (Herrmann, 1989). Phenolics are classified according to the number of carbon atoms in their skeletons (Goodwin and Mercer, 1983).

FLAVONOIDS (C_6-C_3-C_6 compounds)

Flavonoids, which are glycosides, constitute one of the most characteristic classes of phenolic compounds in higher plants (Hahlbrock, 1981; Herrmann, 1976). Their aglycons (C_6-C_3-C_6 compounds or diphenylpropanes) consist of two aromatic rings joined in a chromane structure by a 3-carbon heterocyclic (pyran or C) ring; this ring is usually cyclized with oxygen (Goodwin and Mercer, 1983; Hahlbrock, 1981; Herrmann, 1976). One aromatic ring (A) has the 3-carbon heterocyclic ring attached which bears the second aromatic ring (B) in position 2, 3 or 4 (Hahlbrock, 1981; Herrmann, 1976). Individual carbon atoms are referred to by a numbering system which utilizes ordinary numerals for the A and C rings and 'primed' numerals for the B-ring (with the exception of chalcones) (Markham, 1982).

Flavonoids differ in the number and distribution of substituent hydroxyl groups and their methylation, degree of unsaturation, and degree of oxidation of the heterocyclic ring (Herrmann, 1974). Flavonoid aglycons are classified according to the oxidation state of the C3 residue of their heterocyclic ring (Goodwin and Mercer, 1983). The major flavonoid classes are: anthocyanidins, chalcones, flavanones, flavan-3-ols (or 'catechins'), flavan-3,4-diols (proanthocyanidins or leucoanthocyanidins), flavones, flavonols (or 3-hydroxyflavones; Figure I.1), and the polymeric proanthocyanidins (procyanidins or condensed tannins) (Goodwin and Mercer, 1983; Hahlbrock, 1981; Herrmann, 1976;

Spanos, 1988). Anthocyanidins, flavanones, flavones, and flavonols have ring B in position 2 of the heterocyclic ring (Hahlbrock, 1981). Minor groups are aurones, biflavonoids, dihydroflavonols, isoflavones, dihydrochalcones, neoflavones (or 4-phenyl-coumarins), and oligomeric flavonoids (Goodwin and Mercer, 1983; Hahlbrock, 1981; Herrmann 1976). Aurones are not strictly flavonoids, but are closely related and therefore included in this group (Goodwin and Mercer, 1983). Flavonoid classes of importance in our study are anthocyanins, flavones, flavonols, and flavan-3-ols.

Flavonoid substitution. a) Glycosylation. There are many hundreds of differently substituted flavonoid aglycons (Hahlbrock, 1981). They usually occur as flavonoid-O-glycosides in which one or more of the flavonoid hydroxyl groups is bound to a sugar or sugars by an acid-labile hemiacetal bond (Hahlbrock, 1981; Markham, 1982). The purpose of glycosylation is to render the flavonoid less reactive and more water (i.e. sap) soluble, the latter property permitting storage of flavonoids in the cell vacuole (Markham, 1982). Hydroxyls in certain sites have a much higher probability of being glycosylated than others, e.g. the 3- (and 5-) OH in anthocyanidins; the 7-OH in flavones, isoflavones, and dihydroflavones; and the 3- (and 7-) OH in flavonols (Figure I.1) and dihydroflavonols (Markham, 1982).

Glucose is the sugar most commonly involved in glycosylation; also common are galactose, rhamnose, xylose, and arabinose (Markham, 1982). The glycosidic linkage is usually beta except in the case of the L-rhamnosides and L-arabinosides where it is alpha (Goodwin and Mercer, 1983; Herrmann, 1976). Allose, mannose, fructose, apiose, and glucuronic and galacturonic acids are less frequently found (Markham, 1982). Disaccharides such as sophorose (2-O-beta-D-glucosyl-D-glucose), gentiobiose (6-O-beta-D-glucosyl-D-glucose), rutinose (6-O-alpha-L-rhamnosyl-D-glucose), and neohesperidose (2-O-alpha-L-rhamnosyl-D-glucose) are often associated with flavonoids, and occasionally tri and even tetrasaccharides (Markham, 1982).

b) Acylation. The sugar hydroxyls of flavonoids are often further substituted by one or

more acyl residues (e.g. malonate, 4-coumarate, caffeate, ferulate); the bond in this case is an ester bond, the acid effectively being esterified by the sugar (Hahlbrock, 1981; Markham, 1982). Generally, there are two types of acylation: 1) with either an aromatic acid (e.g. caffeic, p-coumaric) or the simple aliphatic acid acetic acid, and 2) with aliphatic dicarboxylic acids (e.g. malonic, malic, succinic acids) (Harborne, 1986). Based on rather limited sampling, Harborne (1986) found that aliphatic dicarboxylic acids appear to be absent in all Rosaceae species, which include raspberries, blackberries, strawberries. A survey of a large number of families and species has shown that presence of acylated flavonoids in a certain family makes presence in related species very likely (Harborne, 1986). Multiple acylation of flavonoids is possible, considering the number of free sugar hydroxyl groups present in flavonoid glycosides; in addition, both types of acylation can be present in the same flavonoid (Harborne, 1986). However, the great majority of acylated flavonoids have only one acid substitution and the position of this substitution is usually at the 6-hydroxyl of glucose (Harborne, 1986).

Anthocyanins

Anthocyanins, the glycosides of anthocyanidins, are the red pigments of many flowers and fruits (Markakis, 1982); they occur in living cells almost exclusively as O-glycosides (Spanos, 1988). Among the flavonoids, anthocyanins are unique as they possess ionic character (Goodwin and Mercer, 1983). Because of this charge, both the intensity and shade of color of anthocyanins vary with changes of pH, ranging from red over colorless to blue (Goodwin and Mercer, 1983). Anthocyanidins differ in the number of hydroxyl-groups in ring B; the most common ones are pelargonidin, delphinidin and cyanidin (Goodwin and Mercer, 1983). Harborne et al. (1975) and Markakis (1982) give a comprehensive review of anthocyanins.

Presence in red raspberries. Spanos and Wrolstad (1987) analyzed a number of red raspberries juices for their anthocyanin composition and give a comprehensive review of

work done previously. The major red raspberry anthocyanins are cyanidin-3-sophoroside (ca. 70%), cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside, pelargonidin-3-sophoroside, cyanidin-3-rutinoside, and pelargonidin-3-glucosylrutinoside; however, significant varietal differences exist (Rommel et al., 1990; Spanos and Wrolstad, 1987). Boyles (1992) has analyzed the same red raspberry juice samples used for this investigation to determine their anthocyanin concentrations.

Flavones and Flavonols

Flavones (e.g. apigenin, chrysoeriol, luteolin, tricin) and flavonols (Figure I.1) (e.g. kaempferol, myricetin, quercetin, isorhamnetin = 3'-methyl-quercetin) are found in almost all plants, particularly in leaves and petals; flavonols occur more frequently than flavones (Herrmann, 1976). The concentrations of flavones and flavonols are particularly high in leaves, while they are often low in fruits (Herrmann, 1974). Flavones and flavonols only contribute markedly to the colorization (ivory to light yellow) of plants where they occur in very high concentration or when they are complexed with metals (Goodwin and Mercer, 1983; Herrmann, 1976). Flavonols which make an essential contribution to the yellow color of flowers differ from the usual hydroxylation pattern by additional hydroxyl groups attached to the nucleus at the 6 and 8-positions (Goodwin and Mercer, 1983; Herrmann, 1976).

Flavones and flavonols are often similar in their substitution patterns, occurring in the same plant tissues (Hahlbrock, 1981; Herrmann, 1976). A major difference is frequently related to the substitution pattern of the 3-hydroxyl group of flavonols (i.e. glycosylation, alkylation, acylation). In common with other flavonoids, the flavones and flavonols most frequently found are those with B-ring hydroxylation in the 3' and 4' positions (e.g. quercetin; Figure I.1); second most frequently found are those with hydroxylation only in the 4' position (e.g. kaempferol) (Herrmann, 1976). Some very highly methoxylated flavones have been found in orange peel, which so far have not been found in other plants

(Herrmann, 1976).

Flavones and flavonols occur in living cells almost exclusively as O-glycosides (Spanos, 1988). In flavonols the preferred bonding site for sugar radicals is the 3-position, much less frequently the 7-position, and only rarely the 4', 3' and 5-positions; in the case of diglycosides, 3-O-biosides and 3,7-di-O-glycosides occur most frequently. Flavones occur mainly as 7-O-glycosides. The sugar attached most frequently is D-glucose; also found are D-galactose, L-rhamnose, L-arabinose, D-xylose, D-apiose, D-glucuronic acid and rarely D-galacturonic acid (Spanos, 1988). In addition, the sugars of flavone and flavonol-glycosides have been found acylated (i.e. esterified) with p-coumaric, ferulic, caffeic, p-hydroxybenzoic, benzoic, gallic, acetic, malonic and 3-hydroxy-3-methylglutaric acids (Harborne and Williams, 1975; Herrmann, 1976; Wald et al., 1986).

Presence in fruits. The mean concentrations, ranges of means, or occurrences of flavonol-glycosides and glucuronides in various fruits as reported in the literature are summarized in Table I.1. Because the formation of flavonol-glycosides depends on light, they are found mainly in the skins of fruits (Herrmann, 1976).

The non-anthocyanin flavonoids present in red raspberries are mainly flavonol-glycosides (Table I.2; Figure I.1), catechins (Table I.3) and trace amounts of flavones (Henning, 1981; Herrmann, 1974 and 1976; Mosel and Herrmann, 1974; Ryan & Coffin, 1971). The very similar glycoside patterns of raspberry, strawberry and blackberry are distinctly different from those of pomaceous and stone-fruits (Henning, 1981); flavonols are apparently present in these berries as 3-glycosides of the flavonols quercetin and kaempferol (Henning, 1981; Herrmann, 1974 and 1976; Ryan & Coffin, 1971; Table I.2). In addition, these berries contain several 3,7-glycosides as minor glycosides (Henning, 1981). However, 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.

Flavan-3-ols and Procyanins

In terms of volume, flavan-3-ols (or 'catechins': (+)-catechin, (-)-epicatechin, (+)-gallocatechin and (-)-epigallocatechin) and polymeric proanthocyanins (procyanins or condensed tannins) are the most important phenolic compounds besides hydroxycinnamic acid esters in fruits found in cool to moderate climates; they are not found in citrus fruits and in vegetables (Herrmann, 1974). Both monomeric 'catechins' and dimeric procyanidins are colorless; they are usually not glycosylated or esterified in plants (Herrmann, 1974). 'Catechins' possess asymmetric carbon atoms and so exhibit optical activity, i.e. they rotate plane polarized light (the trans-configuration is symbolized by (+) and the cis-configuration by (-) (Markham, 1982). (-)-Epicatechin is the most important 'catechin' in most fruits; however, (+)-gallocatechin is the primary 'catechin' in peaches, and some plums, strawberries, currants and gooseberries (Herrmann, 1974). If at all present, (+)-gallocatechin and (-)-epigallocatechin do not occur until fruit ripeness.

Dimeric procyanidins (flavan-3-ol and flavan-3,4-ol dimers) are compounds yielding anthocyanidins and 'catechins' during heating with acid (e.g. apple, pear: cyanidin and (-)-epicatechin; peach, cranberry: cyanidin and (+)-catechin) (Hahlbrock, 1981; Herrmann, 1974; Spanos, 1988). The 'Group B' procyanidin dimers, B1 to B4, consist of (-)-epicatechin and (+)-catechin combined with themselves or each other through a C4-C8 covalent bond (Spanos, 1988).

Presence in red raspberries. Blackberries, raspberries and strawberries contain mainly (-)-epicatechin and (+)-catechin; occasionally (+)-gallocatechins are found (Mosel and Herrmann, 1974; Table I.3). Concentrations of these catechins peak during ripeness of these berries (Mosel and Herrmann, 1974).

HYDROXYCINNAMIC ACIDS (C_6-C_3 compounds)

C_6-C_3 compounds include hydroxycinnamic acids, phenylpropenes, coumarins, isocoumarins and chromones (Goodwin and Mercer, 1983). Hydroxycinnamic acid

compounds (Figure I.2) are almost exclusively derived from the widespread p-coumaric, caffeic, and ferulic acids; sinapic acid is relatively rare (Herrmann, 1989; Spanos, 1988). Natural forms of cinnamic acids apparently have trans-configuration but tend to isomerize to cis with the exposure to UV-light during extraction from plants; oxidation in the O-position also occurs as an artifact (Herrmann, 1989; Spanos, 1988). Hydroxycinnamic acids may be bound to cell wall polymers (Herrmann, 1989). However, they occur in plants (fruit species) most frequently as simple esters with quinic acid (cyclitol) or glucose but also with carboxylic acids (e.g. malic, tartaric, galactaric); amides also occur (Herrmann, 1989; Schuster and Herrmann, 1985). The range of esters of the hydroxycinnamic acids is far greater than that of any other plant phenols (Goodwin and Mercer, 1983). The most common hydroxycinnamoylquinic acids are chlorogenic (5-caffeoylquinic) and neochlorogenic (3-caffeoylquinic) acids; less common are cryptochlorogenic (4-caffeoylquinic), p-coumaroylquinic, feruloylquinic and di-hydroxycinnamoylquinic acids (Herrmann, 1989). Frequently found glucose esters of hydroxycinnamic acids are p-coumaroylglucose, caffeoylglucose, and feruloylglucose (Herrmann, 1989).

Presence in red raspberries. In raspberries and blackberries the ß-D-glucoside of p-coumaric acid occurs regularly (Schuster and Herrmann, 1985). The following cinnamic acid glucosides (in the cultivars Glen Cova, Malling Exploit, Malling Promise) and esters (in Glen Cova and Malling Exploit) have been quantified in red raspberries (Herrmann, 1989; Schuster and Herrmann, 1985):

p-coumaric acid-ß-D-glucoside (4 - 10 ppm, i.e. mg/kg fresh weight):

ferulic acid-ß-D-glucoside (traces - 2 ppm),

1-O-coumaroyl-ß-D-glucopyranose (6 - 14 ppm),

1-O-feruloyl-ß-D-glucopyranose (4 - 7 ppm),

1-O-caffeoyl-ß-D-glucopyranose (3 - 7 ppm),

5-p-coumaroylquinic acid (1 - 2 ppm),

5-caffeoylquinic acid (= chlorogenic acid, traces - 1 ppm),

5-feruloylquinic acid (traces).

Hydroxycinnamic acid amides have been identified in the reproductive organs of food plants including raspberry, but are absent from the green parts, petals and sepals (Herrmann, 1989).

BENZOIC ACIDS (C_6-C_1 compounds)

Benzoic acids are a widespread group of phenolic acids in plants (Goodwin and Mercer, 1983; Herrmann, 1989; Spanos, 1988). The most common compounds are derivatives of the following hydroxybenzoic acids (Figure I.3): p-hydroxybenzoic, protocatechuic, vanillic, and gallic acids; salicylic, syringic and gentisic acids are less common (Herrmann, 1989; Spanos, 1988). Hydroxybenzoic acids are mainly present in the form of glucosides; esters with glucose are apparently found only occasionally (Herrmann, 1989). However, gallic acid is mainly esterified to quinic acid or catechins and usually present in polymeric forms as soluble tannins (condensation products) (Goodwin and Mercer, 1983; Herrmann, 1989). Such tannins are discussed below in the chapter on ellagic acid. Non-hydroxylated benzoic acid compounds, such as esters with glucose, also occur generally in fruits in trace amounts, e.g. 6-benzoylglucose in cranberries (Herrmann, 1989). Presence of free hydroxybenzoic acids can result from hydrolysis of flavonoids (e.g. anthocyanins) and of hydroxybenzyl-glucosinolates using alkali or non-specific enzymes (Herrmann, 1989) during extraction from plants or juice processing.

Presence in red raspberries. In raspberries and blackberries the ß-D-glucosides of p-hydroxybenzoic and protocatechuic acids occur regularly; in raspberries the content of p-hydroxybenzoic acid-ß-D-glucoside is distinctly higher than in other rosaceaous fruits (Schuster and Herrmann, 1985). The following hydroxybenzoic acid glucosides (in the cultivars Glen Cova, Malling Exploit, Malling Promise) and esters (in Glen Cova and Malling Exploit) have been quantified in red raspberries (Herrmann, 1989; Schuster and

Herrmann, 1985):

p-hydroxybenzoic acid-ß-D-glucoside (32 - 59 ppm, i.e. mg/kg fresh fruit),

protocatechuic acid-4-ß-D-glucoside (traces),

gallic acid-4-ß-D-glucoside (traces),

5-galloylquinic acid (traces),

1-O-galloyl-ß-D-glucopyranose (traces).

Swain et al. (1985) report presence of salicylic acid in raspberries ranging in concentration from 38.8 to 51.4 ppm (mg/kg edible fruit portion). To Herrmann (1989) these values seem to be too high; his group determined concentrations ranging from traces to 1 ppm (Mosel and Herrmann, 1974).

ELLAGIC ACID, ELLAGIC ACID DERIVATIVES AND PRECURSORS

Ellagic acid (MW 302.19; Figure I.4), a dimeric derivative of gallic acid (i.e. a C_6-C_1 dimer), exists in nature mainly in the form of 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). The major ellagitannins in raspberries are alpha- and beta-1-O-galloyl-2,3:4,6-bis-hexahydroxydiphenoyl-D-glucose and a dimer of these two forms (MW 1870); also present are the beta-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). 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 I.4). Numerous derivatives of ellagic acid exist in plants, formed through methylation, glycosylation and methoxylation of its hydroxyl-groups and differ in solubility, mobility, and reactivity in plant as well as in animal systems (Maas et al., 1991a). Ellagic acid has also been reported to complex readily with metallic cations, e.g. Mg^{2+} and Ca^{2+} (Press and Hardcastle, 1969).

Ellagic acid in fruits. The analysis of ellagic acid in fruits has been rather limited. 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 three 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) detected ellagic acid in raspberry pulp at 9.1 ± 0.1 ppm (wet-weight), in seeds at 275 ± 4 ppm (wet-weight), and in blended freeze-dried raspberries at 90 ± 70 ppm (dry-weight) 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 ppm (dry-weight) 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 two 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, which would be of great value to the consumer. For strawberries, large differences in ellagic acid contents were found among cultivars, and green (unripe) fruit pulp was found to contain about twice as much ellagic acid as red fruit pulp (Maas et al., 1991b).

RED RASPBERRY JUICE AUTHENTICITY

Of the constituents of raspberries, flavonoids and other phenolics (i.e. secondary plant metabolites) are particularly reliable indicators of authenticity as they are present within a finite range of concentrations, varying according to ripeness, cultivar, berry size, and growing conditions (Herrmann, 1976). Previous research has focussed on the use of

anthocyanins as juice authenticity indicators (Rommel et al., 1990; Spanos and Wrolstad, 1987). Anthocyanins are, however, sometimes inconclusive in detecting adulteration, which makes consideration of additional phenolic authenticity indicators, such as non-anthocyanin flavonoids (e.g. flavonols, catechins), benzoic and cinnamic acids, essential. For example, non-anthocyanin flavonoids would be particularly suitable in detecting adulteration by potential adulterants such as relatively inexpensive, non-red fruit juices (e.g. apple, pear). Most non-anthocyanin flavonoids are not commercially available and so cannot be added to hide adulteration.

HEALTH EFFECTS OF FLAVONOLS AND ELLAGIC ACID

Flavonols

The composition of raspberry juice is of increasing interest because of the positive health effects associated with its high phenolic content. The flavonol shown to have anticarcinogenic effects in mammals is quercetin but also kaempferol and the quercetin-glycoside rutin (e.g. Deschner, 1991; Leighton et al., 1991; Verma, 1991; Weisburger, 1991; Yasukawa et al., 1988). Quercetin also exhibits antiviral effects *in vitro* (Vlietinck et al., 1988) and *in vivo* (Anton, 1988); quercetin and kaempferol have anti-inflammatory potencies (Anton, 1988); and kaempferol shows antifertility activities (Anton, 1988).

Although quercetin has been demonstrated to be mutagenic in bacterial assay systems, it has been found not to be carcinogenic or teratogenic *in vitro* and *in vivo* (rodents) by many research groups (Deschner, 1991). Rather, quercetin has been found to be a potent anticarcinogen against skin, colon and mammary cancers in rodents (e.g. Deschner, 1991; Leighton et al., 1991; Verma, 1991; Weisburger, 1991; Yasukawa et al., 1988). For example, quercetin has been shown to have a very pronounced inhibitory effect on the carcinogen benzo[a]pyrene (BaP) in an *in vitro* mutagenicity assay (Stavric et al., 1991). *In vivo*, quercetin has also been shown to reduce the uptake of BaP (by 21%) in rats fed quercetin at 2% in their diet and to reduce mutagenicity of BaP by up to 72% (Stavric et al.,

1991). Quercetin (30 μmol applied topically) inhibits mouse skin tumor formation in a dose-dependent manner, both when initiated with 7,12-dimethylbenz[a]anthracene (DMBA; 43% fewer papillomas/mouse) and when promoted with 12-O-tetradecanoylphorbol-13-acetate (TPA; 60-75% fewer papillomas/mouse; Verma, 1991). Quercetin, rutin and kaempferol, applied to mouse skin (at 5 μmol) before and with TPA-tumor promoter inhibit the number of papillomas per mouse by 58%, 45% and 56%, respectively (Yasukawa et al., 1988).

Quercetin and rutin fed to mice at 2% and 4% in their diet, respectively, reduce azoxymethanol (AOM)-induced hyperproliferation of colonic epithelial cells significantly and suppress colon tumor multiplicity; 2% quercetin in mouse diet for 46 weeks significantly depresses colon tumor incidence (25% vs. 5.9% in controls; Deschner, 1991). Quercetin fed at 5% in rat diet inhibits DMBA-induced mammary tumors by > 50%; with induction by NMU the effect is not quite as dramatic but still very significant (Verma, 1991). Kaempferol-3-O-ß-D-glucopyranoside has also been found to be a potent anticarcinogen *in vivo* (Lee, 1991).

Quercetin may also inhibit the induction of human cancers (Verma, 1991); it markedly inhibits the growth of human gastric cancer cells and blocks cell progression *in vitro* (Yoshida et al., 1990). In *in vitro* transfection assays with the human H-*ras* oncogene, quercetin blocks the appearance of transformed loci and selectively inhibits the proliferation of tumor cells transformed with a panel of diverse oncogenes (65-90% vs. 30-40% for normal cells; Leighton et al., 1991). Epidemiological evidence supports the theory that flavonols have anticarcinogenic effects in humans, e.g. there is an inverse correlation between individuals who consume a diet rich in fruits and vegetables and their risk of developing cancer (Committee on Diet, Nutrition, and Cancer, 1982; Stich and Rosin, 1984; Weisburger, 1991).

Quercetin is the most abundant flavonoid in the human diet (Stavric, 1991). The daily intake by humans of glycosides of the flavonols kaempferol and quercetin is approximately

50-100 mg (MacGregor, 1986; Mazaki et al., 1982). Quercetin is formed in the human mouth and gastrointestinal tract via bacterial hydrolysis of quercetin-glycosides and glucuronides (Bokkenheuser and Winter, 1988; Deschner, 1991; Leighton et al., 1991; Shillitoe et al., 1984; Verma, 1991). However, quercetin appears to be poorly absorbed in the gastrointestinal tract (probably less than 1% of ingested quercetin; Deschner, 1991; Stavric et al., 1991; Verma, 1991). Absorption seems to be increased at some optimum fat content of diet; e.g. the anticarcinogenic effects of quercetin and rutin have been shown to be best in a mouse diet that contains 5% corn oil (Deschner, 1991).

Ellagic Acid

Ellagic acid has been shown to have anticarcinogenic properties (e.g. Daniel et al., 1989 and Maas et al., 1991a). It has also been found to have antimutagenic, antiviral and antioxidative properties, to control hemorrhage in animals and humans, and possibly to have hypotensive and sedative effects (Anonymous, 1986; Damas and Remacle-Volon, 1987; Maas et al., 1991a).

The increasing evidence for the anticarcinogenic effect of ellagic acid against a wide range of carcinogens in several tissues is summarized by Daniel et al. (1989) and Maas et al. (1991a). Significant inhibition of colon, esophageal, liver, lung, tongue, and skin neoplasms has been shown in rats and mice (e.g. 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 and 1991). *In vitro*, ellagic acid has an inhibitory effect on the mutagenicity of several carcinogens (e.g. benzo[a]pyrene, benzo[a]pyrene-diol-epoxides, N-methyl-N-nitrosurea) and greatly inhibits the carcinogenicity of benzo[a]pyrene (Del Tito et al., 1983; Dixit and Gold, 1987), while it enhances the effects of other mutagens (Dixit and Gold, 1987; Wood et al., 1982). *In vivo* however, ellagic acid reduces the mutagenicity of benzo[a]pyrene by up to 72%, and oral dosing of ellagic acid (at 2% in rat chow) reduces the uptake of the benzo[a]pyrene by

about 60% (Stavric et al., 1991). Ellagic acid, i.p. administered or added to diet, inhibits benzo[a]pyrene-induced lung-tumor formation in mice by over 50% (Lesca, 1983). Dietary ellagic acid (at 0.4 and 4 g/kg diet) significantly inhibits (21-55%) esophageal tumorigenesis in rats, induced by N-nitrosobenzylmethylamine, after 20 and 27 days (Mandal and Stoner, 1990). Exposure of mice to only 3 ppm ellagic acid in drinking water reduces the risk of developing skin tumors induced by 3-methylcholanthrene (Mukhtar et al., 1986). Ellagic acid, applied topically to mouse skin, is a potent inhibitor of skin cancer induced by benzo[a]pyrene, 3-methylcholanthrene and 7,12-dimethylbenz[a]anthrene (Chang et al., 1985; Das et al., 1985; Del Tito et al., 1983; Lesca, 1983).

Tanaka et al.'s (1991) data suggest that ellagic acid is a possible chemopreventative agent in human carcinogenesis. In human bronchial epithelial cells, ellagic acid (1.5 and 3.0 μg/ml) inhibits binding of benzo[a]pyrene metabolites to DNA (Teel et al., 1986); ellagic acid also has marginal cytotoxicity to human cancer cells (ED_{50} = 4.6 μg/ml; Lee, 1991). There is an inverse correlation between individuals who consume a diet rich in fruits and vegetables and their risk of developing cancer (Committee on Diet, Nutrition, and Cancer, 1982; Stoner, 1989; Weisburger, 1991), with meat eaters having a higher risk of developing breast and colon cancers (Weisburger, 1991).

Nonetheless, there is still some conflicting evidence as to the anticarcinogenic effectiveness of ellagic acid (Maas et al., 1991a). Furthermore, it has not been resolved how much ellagic acid is absorbed into the body from dietary sources (Maas et al., 1991a).

Table I.1. Mean Concentrations, Ranges of Means or Occurrences of Flavonol-glycosides and Glucuronides in Various Fruits as Reported in the Literature

Flavonol-glycoside (ppm, i.e. mg/kg fresh fruit)	Raspberry	Blackberry	Strawberry	Cranberry	Blueberry	Black Currant	Red Currant	Sour Cherry	Sweet Cherry	Apple	Pear
References Number of varieties	1, 2, 5, 8, 12 5 ?	1,3,4,5,8,12 5	1, 5, 12 20	3, 5, 8, 12	3, 8, 5, 8, 12	5, 6, 7, 8, 12	5, 7, 8, 12	5, 8, 9	5, 8, 9	5, 8, 10 16	5, 8, 10, 11 12
Qu-3-glucuronide + Ka-3-glucoside	17.2 (15-21)	41.6 (22-96)	X (see below)								
Qu-3-xylosylglucuronide	6.2 (traces-10)	22.6 (18-30)	4 (1-16)								
Qu-3-glucoside	12.2 (9-14)	44.6 (36-55)	5 (2-15)			XX (16-140)	XX (3-23)	X (2-3)	1	X (3-16)	X (1-20)
Qu-3-glucosyl-7-diglucoside								X ?			
Qu-3-diglucoside										X ?	X ?
Qu-3-O-(6"-O-malonyl)-ß-glucoside											n.d.-60
Qu-3-galactoside	11.6 (4-20)	42.4 (22-80)	5 (traces-15)	XX				traces	n.d.-traces	XX (5-105)	X (1-9)
Qu-3-galactosyl-7-diglucoside								X ?			
Qu-3-[6"-(3-HO-3-methyl-glutaryl)-ß-galactoside]		X									
Qu-3-rutinoside (Rutin)		21.2 (12-25)				XX (13-46)	X (8-29)	XX (9-18)	X (3-11)	X	X
Qu-3-(2G-rhamnosyl-rutinoside)							XX (2-18)				
Qu-3-rutinosyl-4'-diglucoside								XX	XX		
Qu-3-rhamnoside				X					X	X	
Qu-3-xyloside										X	X
Qu-3-arabinoside				X						X	
Qu-4'-glucoside								X ?			
Qu-7-xyloside											X
Ka-3-glucuronide	12 (7-16)	10.2 (6-19)	22 (8-42)								
Ka-3-xylosylglucuronide	8 (2-14)	40.8 (14-90)	5 (2-10)								
Ka-3-glucoside	X (see above)	X (see above)	22 (8-55)			X (4-19)		1	traces-1		
Ka-3-glucosyl-7-diglucoside									X ?		

Table I.1. continued

Flavonol-glycoside (ppm, i.e. mg/kg fresh fruit)	Raspberry	Blackberry	Strawberry	Cranberry	Blueberry	Black Currant	Red Currant	Sour Cherry	Sweet Cherry	Apple	Pear
References	1, 2, 5, 8, 12	1,3,4,5,8,12	1, 5, 12	3, 5, 8, 12	3, 8, 5, 8, 12	5, 6, 7, 8, 12	5, 7, 8, 12	5, 8, 9	5, 8, 9	5, 8, 10	5, 8, 10, 11
Ka-3-O-(6"-O-malonyl)-ß-glucoside											n.d.-14
Ka-3-galactoside	16.8 (6-21)	22 (12-40)	6 (traces-16)					traces	traces		
Ka-3-galactosyl-7-diglucoside								XX	X ?		
Ka-3-xylosylglucoside	0.8 (n.d.-2)		traces (n.d.-5)								
Ka-3-rutinoside						X (3-14)	X (n.d.-4)	XX (12-19)	XX (1-6)		
Ka-3-rutinosyl-4'-diglucoside								X	X		
Ka-3-rhamnosylglucoside								X			
Ka-3-rhamnosyl-4'-galactoside								X			
Ka-7-glucoside			X ?								
My-3-glucoside						XX (13-38)	X (n.d.-4)				
My-3-arabinoside				X							
My-3-rutinoside						XX	traces				
My-3-digalactoside				X							
IsoRh-3-O-(6"-O-malonyl)-ß-glucoside						(15-108)					1-102
Total quercetin	47 (28-65)	172 (110-286)	14 (3-46)	34-250	24-32	33	27	80	6	1-2 (flesh) 58-263 (skin)	< 1 (flesh) 28 (skin)
Total kaempferol	38 (15-53)	73 (32-149)	55 (18-128)	< 1-27		< 0.1	2	17	6	0-0.1 (flesh) 1-7 (skin)	0 (flesh) 12 (skin)
Total myricetin				nd-3		55	< 0.1				
Total isorhamnetin		(4?)									
Total flavonols	85 (43-118)	245 (142-435)	69 (21-174)	30-279	30-40	88-90	29-40	97	12	1-2 (flesh) 59-270 (skin)	< 1 40 (skin)

References: 1) Henning (1981); 2) Ryan and Coffin (1971); 3) Bilyk and Sapers (1986); 4) Wald et al. (1986); 5) Macheix et al. (1990); 6) Koeppen and Herrmann (1977); 7) Siewek et al. (1984); 8) Wildanger and Herrmann (1973); 9) Henning and Herrmann (1980); 10) Herrmann (1976); 11) Wald et al. (1989); 12) Herrmann (1974)
Abbreviations: Qu-quercetin; Ka-kaempferol; My-myricetin; IsoRh-isorhamnetin; XX-present as a primary flavonol; X-present in smaller quantities.

Table I.2. Flavonol-glycosides and Glucuronides Present in Red Raspberries, Strawberries and Blackberries as Reported in the Literature

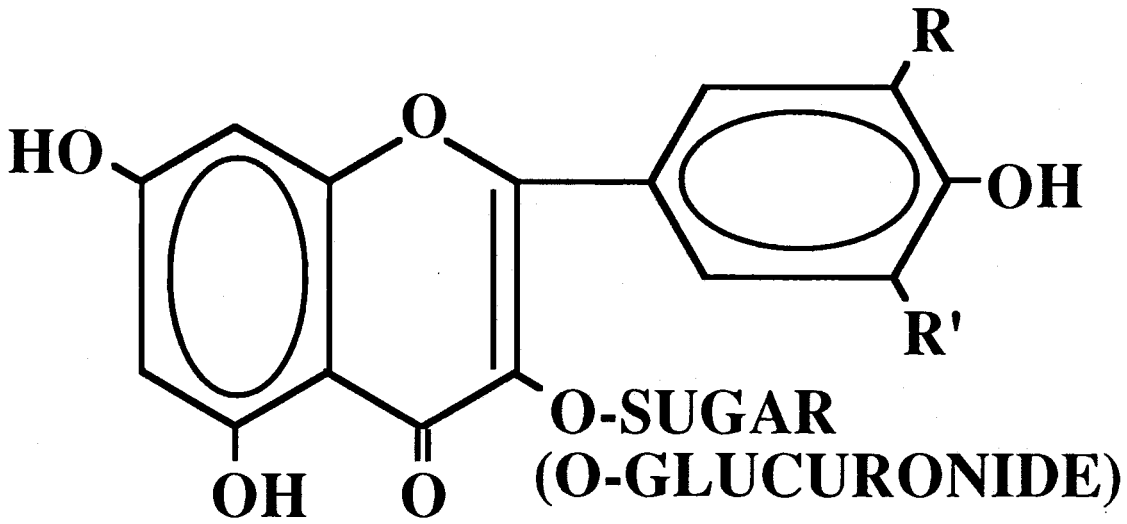
Flavonol-glycoside (ppm, i.e. mg/kg fresh fruit)	Total flavonols	Total Qu	Total Ka	Qu3gluc-uronide	Ka3gluc-uronide	Qu-3-glucoside	Qu-3-galactoside	Ka-3-galactoside	Qu-3-xylosyl-glucuron.	Ka-3-xylosyl-glucuron.	Ka-3-xylosyl-glucoside	Qu3rutin-oside (Rutin)	
RASPBERRIES													
Varieties													Reference
unknown				X	X	?	?						Herrmann, 1976
unknown				XX	X	?	?						Ryan & Coffin, 1971
Glen Isla	76	42	34	15*	16	9	8	6	10	10	2		Henning, 1981
Schoenemann	76	47	29	21*	7	14	12	20	trace	2	nd		"
Veten	98	55	43	16*	14	13	20	21	6	6	2		"
Zeva I	72	40	32	17*	7	12	4	17	7	8	nd		"
Zeva II	102	52	50	17*	16	13	14	20	8	14	nd		"
mean	85	47	37	17*	12	12	12	17	6	8	1		"
STRAWBERRIES													
(20 varieties, mean)	69	36	33	22*	22	5	5	6	4	5	trace		"
var. Senga Sengana	≤ 1000												Wildanger & Herrmann, 1973
BLACKBERRIES													
(5 varieties, mean)	246	173	73	42*	10	45	42	22	23	41	nd	21	Henning, 1981

Abbreviations: Qu-quercetin; Ka-kaempferol; XX-present as a major flavonol; X-present in smaller quantities; *coelution with kaempferol-3-glucoside.

Table I.3. Flavan-3-ols Present in Fresh Raspberries, Blackberries and Strawberries as Reported in the Literature

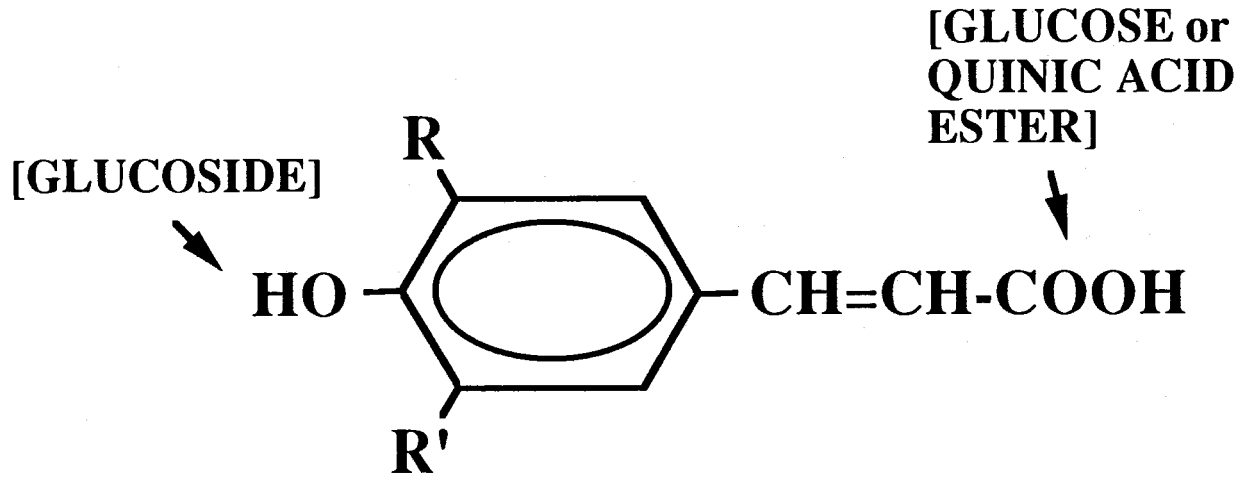
Flavan-3-ol (ppm, i.e. mg/kg fresh fruit)	**Total flavan-3-ols**	**(+)-catechin**	**(-)-epicatechin**	**(+)-gallocatechin**	
Raspberries					**Reference**
Varieties					
Unknown	133	22	111		Mosel & Herrmann, 1974
Gevalo	46	1.1	45		"
Malling Jewel	48	6.2	42		"
Golden Queen	32	12	20		"
Promiloy	49	14	35		"
mean	62	11	51	traces*	"
Blackberries (6 varieties, mean)	131	19	112	traces*	"
Strawberries (16 varieties, mean)	47	33	6.9	7.4	Stoehr & Herrmann, 1975

*in some samples



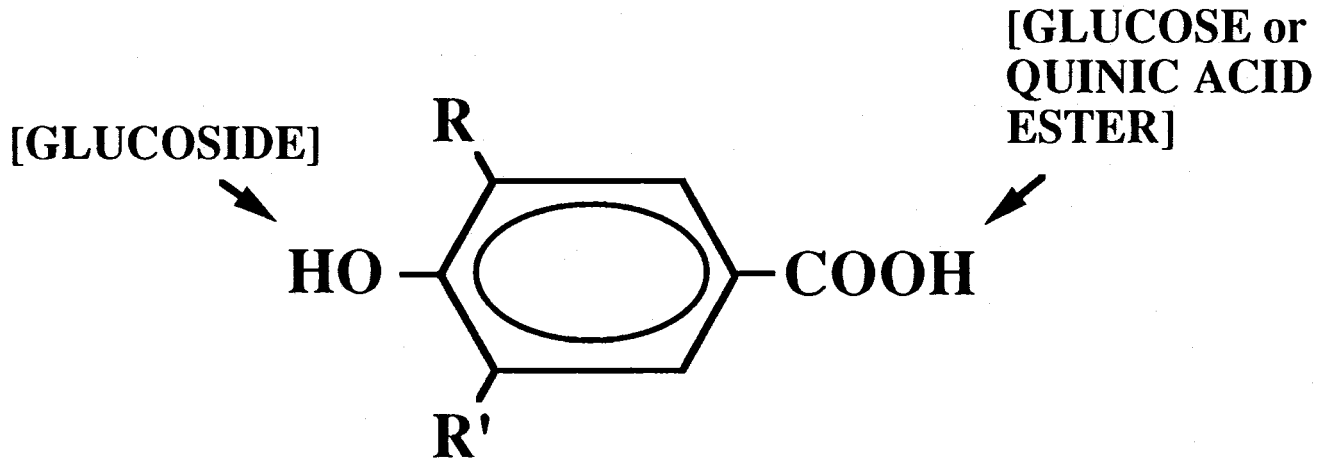
R = R' = H	KAEMPFEROL
R = OH R' = H	QUERCETIN
R = R' = OH	MYRICETIN
R = OMe R' = H	ISORHAMNETIN
R = R' = OMe	SYRINGETIN

Figure I.1 Structures of flavonol-glycosides and glucuronides



R = R' = H	p-COUMARIC ACID
R = OH R' = H	CAFFEIC ACID
R = OMe R' = H	FERULIC ACID
R = R' = OMe	SINAPIC ACID

Figure I.2. Structures of hydroxycinnamic acids and derivatives



R = R' = H	p-HYDROXYBENZOIC ACID
R = OH R' = H	PROTOCATECHUIC ACID
R = R' = OH	GALLIC ACID
R = OMe R' = H	VANILLIC ACID
R = R' = OMe	SYRINGIC ACID

Figure I.3. Structures of hydroxybenzoic acids and derivatives

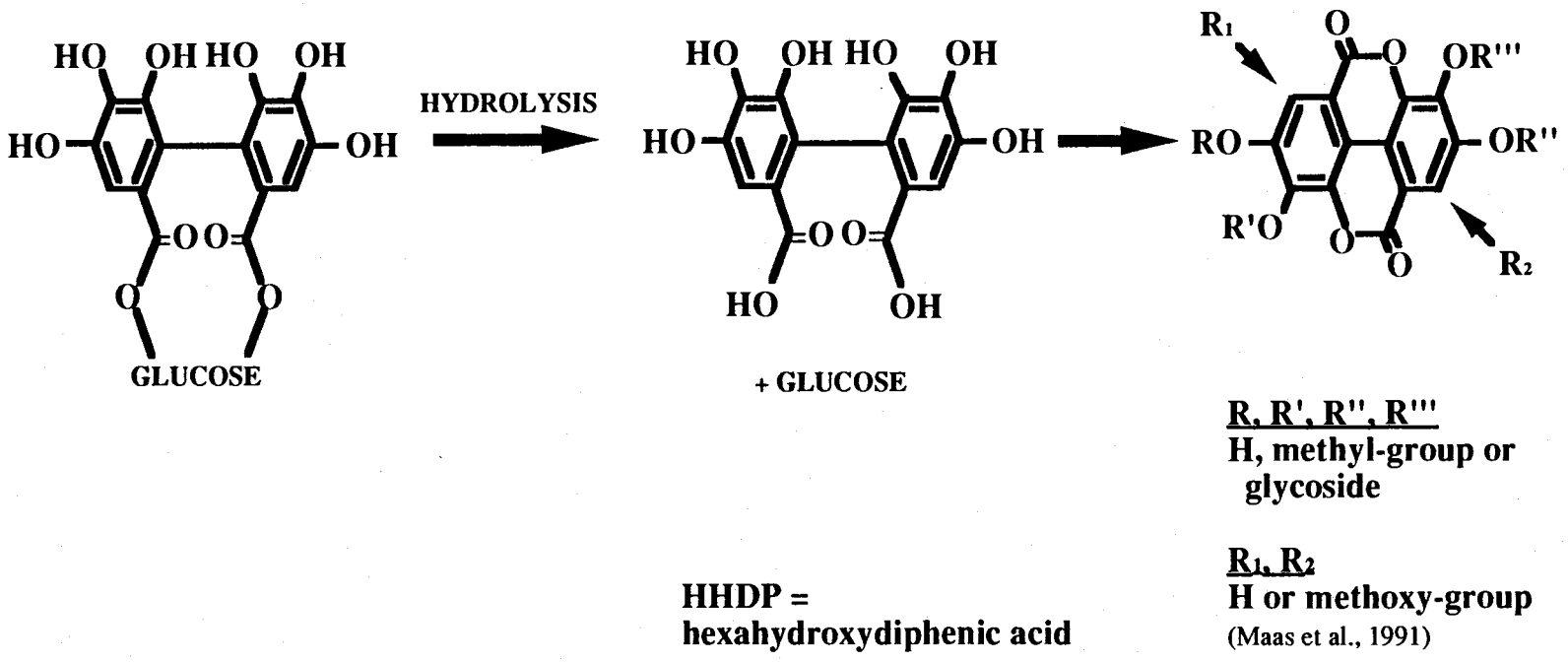


Figure I.4. Structures of ellagic acid and derivatives and their precursors in plant cell walls

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II. Composition of Ellagic Acid and Derivatives in Red Raspberry Juice as Influenced by Cultivar, Processing and Environmental Factors

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ABSTRACT

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 ellagic acid and ≤ 16 derivatives of ellagic acid with individual mean concentrations of up to 3 ppm. Commercial juices (n=7) contained much more ellagic acid and derivatives than experimental juices, with a mean concentration of 30 ppm 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. Willamette 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

Determining the authenticity of red raspberry juice by its phenolic composition is of much importance to the food industry and regulatory agencies as red raspberries are very expensive and so are a prime target for adulteration. The phenolic constituents of raspberry

juice are also of great interest because of their positive effects on human health, specifically ellagic acid (e.g. Daniel et al., 1989 and Maas et al., 1991a) and quercetin, which have been shown to have anticarcinogenic properties; the anticarcinogenic effect of quercetin is reviewed in Rommel and Wrolstad (manuscript in prep.). In addition, ellagic acid has been found to have antimutagenic, antiviral and antioxidative properties, to control hemorrhage in animals and humans, and possibly to have hypotensive and sedative effects (Anonymous, 1986; Damas and Remacle-Volon, 1987; Maas et al., 1991a).

Anticarcinogenicity of ellagic acid. The increasing evidence for the anticarcinogenic effect of ellagic acid against a wide range of carcinogens in several tissues is summarized by Daniel et al. (1989) and Maas et al. (1991a). Significant inhibition of colon, esophageal, liver, lung, tongue, and skin neoplasms has been shown in rats and mice (e.g. 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 and 1991). *In vitro*, ellagic acid has an inhibitory effect on the mutagenicity of several carcinogens (e.g. benzo[a]pyrene, benzo[a]pyrene-diol-epoxides, N-methyl-N-nitrosurea) and greatly inhibits the carcinogenicity of benzo[a]pyrene (Del Tito et al., 1983; Dixit and Gold, 1987), while it enhances the effects of other mutagens (Dixit and Gold, 1987; Wood et al., 1982). *In vivo*, however, ellagic acid reduces the mutagenicity of benzo[a]pyrene by up to 72%, and oral dosing of ellagic acid (at 2% in rat chow) reduces the uptake of benzo[a]pyrene by about 60% (Stavric et al., 1991). Ellagic acid, i.p. administered or added to diet, inhibits benzo[a]pyrene-induced lung-tumor formation in mice by over 50% (Lesca, 1983). Dietary ellagic acid (at 0.4 and 4 g/kg diet) significantly inhibits (21-55%) esophageal tumorigenesis in rats, induced by N-nitrosobenzyl-methylamine, after 20 and 27 days (Mandal and Stoner, 1990). Exposure of mice to only 3 ppm ellagic acid in drinking water reduces the risk of developing skin tumors induced by 3-methylcholanthrene (Mukhtar et al., 1986). Ellagic acid, applied topically to mouse skin, is a potent inhibitor of skin cancer induced by benzo[a]pyrene, 3-methylcholanthrene and

7,12-dimethylbenz[a]anthrene (Chang et al., 1985; Das et al., 1985; Del Tito et al., 1983; Lesca, 1983).

Tanaka et al.'s (1991) data suggest that ellagic acid is a possible chemopreventative agent in human carcinogenesis. In human bronchial epithelial cells, ellagic acid (1.5 and 3.0 μg/ml) inhibits binding of benzo[a]pyrene metabolites to DNA (Teel et al., 1986); ellagic acid also has marginal cytotoxicity to human cancer cells (ED_{50} = 4.6 μg/ml; Lee, 1991). There is an inverse correlation between individuals who consume a diet rich in fruits and vegetables and their risk of developing cancer (Committee on Diet, Nutrition, and Cancer, 1982; Stoner, 1989; Weisburger, 1991), with meat eaters having a higher risk of developing breast and colon cancers (Weisburger, 1991).

Nonetheless, there is still some conflicting evidence as to the anticarcinogenic effectiveness of ellagic acid (Maas et al., 1991a). Furthermore, it has not been resolved how much ellagic acid is absorbed into the body from dietary sources (Maas et al., 1991a).

Ellagic acid in fruits. The analysis of ellagic acid in fruits has been rather limited. 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 three 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 at 275 ± 4 ppm (wet-weight), and in blended freeze-dried raspberries at 90 ± 70 ppm (dry-weight) 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 ppm (dry-weight) 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 two 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, which would be of great value to the consumer. 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).

Chemistry of ellagic acid. Ellagic acid (MW 302.19), 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 II.1a). The major ellagitannins in raspberries are alpha- and beta-1-O-galloyl-2,3:4,6-bis-hexahydroxydiphenoyl-D-glucose and a dimer of these two forms (MW 1870); also present are the beta-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).

This discussion is restricted to the separation and quantification of ellagic acid and its derivatives in raspberry juices so that they can be used as a data-base for determining the

authenticity of red raspberry juice and evaluating the effects of raspberry juice on health. The composition of non-anthocyanin flavonoids (flavonols) in the same samples will be reported in Rommel and Wrolstad (manuscript in prep.); that of ellagic, benzoic and cinnamic acids as well as catechins in selected hydrolyzed samples will be reported in Rommel et al. (1992).

MATERIALS AND METHODS

Samples

Fifty-five red raspberry juice samples were analyzed (Table II.1). These included experimental juice samples processed in our pilot plant (n=21) or laboratory (n=17) by a standardized process (Table II.1a) and available from investigations of alternative processing methodologies (n=8; Table II.1b), as well as commercial red raspberry juice concentrates (n=9; Table II.1c).

Experimental Samples. The 46 experimental samples were produced from berries grown in 1988-1990, inclusive. 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 therefore 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.

Tables II.1a and b 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 where 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, Oregon). 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, British Columbia (Canada). 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.

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 3 sub-samples (each representing a single packing) for each of 4 different cultivars. These berries were grown at the Polish Research Station in Prusy. Two kg lots of fruit were freeze dried in a Heraeus-Leybold laboratory freeze dryer (temperature $\leq$ 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 one 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: BC, British Columbia; OR, Oregon; PO, Poland. Ripeness was designated by the next pair as UR, underripe; R, ripe; OR,

overripe. Harvesting method was designated as MH, machine-harvested; and HP, hand-picked. Processing procedures were designated as PP for pilot plant processed juices and LP for laboratory processed juices. The one very moldy sample was designated as ME-OR-R-PP-HP-MDY.

Commercial Samples. Commercial red raspberry juice concentrates (Table II.1c) 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 Company, Woodburn, OR. The concentrates varied as to degree of concentration (some were 45°Brix, others 65°Brix). In soliciting the samples, 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 attached for °Brix.

Juice Processing Methods

Standard pilot plant juice processing method. A 'standard' process (Figure II.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 02148). 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-filters (SWK Seitz-Filter-Werke GmbH & Co., Germany). 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 by the laboratory procedure for comparison. For this simulated process, a basket centrifuge, type 'Supreme Juicerator', model 6001 (Acme Juicer Mfg. 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, United Kingdom) 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 II.2.

Alternative juice processing methods. Another investigation (Wrolstad et al., manuscript in prep.) 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 an unconcentrated control 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 II.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 01950) at a maximum temperature of 86°C and a vacuum of -0.88 kg/cm^3. Osmotic concentration was performed on the remaining three fifth of the juice at Osmotek, Inc., Corvallis, Oregon, 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 cut-off of ca. 100), one at chilled conditions (8°C) with a total process time of 10.3 hours, the other at room temperature (26°C) with a total processing

time of 5.8 hours. Another lot was concentrated using an experimental membrane (B) at room temperature (26°C) with a total processing time of 5.8 hours. 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. (manuscript in prep.). 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 membranes A or B.

Additional red raspberry juices were available from experimental processing trials conducted in cooperation with the Agriculture Canada Research Station in Summerland, British Columbia (Canada). 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, British Columbia. These samples coded WI-BC-R were further designated DE for diffusion extraction, CT for centrifugation, ESP and EBE for Pectinex Ultra SP enzyme and Pectinex BE enzyme (Novo Laboratories Inc., Danbury, CT 06810-5101), respectively.

High-speed centrifugation juice processing method. This technique used centrifugation (Figure II.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 in order to study their effects on juice composition.

Diffusion extraction process. Diffusion extraction (Figure II.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 out fruit components from a batch of berries of about 200 kg. Raw-juice

was concentrated following depectinization (with Irgazyme 100, Ciba-Geigy Corp., Ardsley, NY 10502) and pasteurization. Single-strength juices were stored frozen at -23°C.

Juice Sample Preparation, HPLC Analysis and Peak Characterization

Samples were separated into fractions by use of Polyamide-6; two fractions (a methanol fraction and a 0.5% ammonia in methanol fraction) were recovered, analyzed and peaks characterized as described by Rommel and Wrolstad (manuscript in prep.). Ellagic acid (Sigma Chemical Co., St. Louis, MO 63178) 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 II.1b.

Quantification Method

Ellagic acid and its forms were quantified on the basis of internal and external standards as described by Rommel and Wrolstad (manuscript in prep.). Four-methylumbelliferyl-ß-D-glucuronide (Sigma Chemical Co.) was used as the internal standard and ellagic acid (Sigma Chemical Co.) 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 hindered by uncertainty as to the reproducibility of sample-preparation and by decreased peak sharpness of the external standard (ellagic acid); the latter resulting from injection of the standard mix into the HPLC in ≥ 80% ethanol. In neutral or acid conditions ellagic acid is only soluble in high concentrations of ethanol (Anonymous, 1986). While ellagic acid is sufficiently soluble in alkaline solutions (Press and Hardcastle, 1969), such solutions cannot be used as they degrade C_{18}-columns and because ellagic acid decomposes at high pH over time.

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 preparation-replicates varied greatly among juices. Nonetheless, we think that reporting the calculated concentrations is still of value. While this variation limited the conclusions that can be drawn from treatment effects, the quantitative estimates are still useful for evaluating trends and providing an overall perspective.

RESULTS AND DISCUSSION

Ammonia/methanol juice fractions contained ellagic acid, ellagic acid derivatives, flavonol-glucuronides, acylated flavonol-glycosides and flavonol-aglycons. Flavonols are discussed in Rommel and Wrolstad (manuscript in prep.). Examples of HPLC-chromatograms of ammonia/methanol fractions of juices made from Willamette and Norna cultivars are shown in Figures II.5a and II.5b. The differences among chromatographic profiles of ammonia/methanol fractions were primarily quantitative. For instance, in the cases shown in Figure II.5, the ratio of ellagic acid to other ellagic acid forms was much greater in Willamette than Norna cultivar.

The concentrations of ellagic acid and 16 ellagic acid forms in experimental and commercial samples are tabulated in Table II.2. These ellagic acid forms, which were detected in most experimental and commercial samples, had UV-spectra very similar to that of ellagic acid (Figure II.1b). These compounds may be ellagic acid derivatives formed through methylation, glycosylation or methoxylation, as described by Maas et al. (1991a), or monomeric and dimeric ellagitannins as reported for raspberries by Haddock et al. (1982) and Haslam and Lilley (1985). Two of the commercial samples (COM-G-65, COM-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 commercial samples were found to be adulterated based on their flavonol (Rommel and Wrolstad, manuscript in prep.) and anthocyanin compositions (Boyles, 1992) and were therefore excluded before calculating mean concentrations of ellagic acid and its forms. The mean concentrations of ellagic acid compounds in commercial samples (n=7) were: 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, respectively; form #7, 0.8 ppm; forms #5, #10, #11, #13, #14, #15, and #16, trace amounts, respectively.

Table II.3 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 9 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 and between traces and 24 ppm for commercial samples. The total concentration of ellagic acid and its derivatives in experimental juices was 28 ppm ± 2.3 ppm (mean) and in 9 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 II.6a); different maturities were available for three cultivars. The concentrations of

ellagic acid for the same juices were also compared (Figure II.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 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 II.6a&b). Juices made from two of the cultivars grown in Canada (Chilcotin, Skeena) by 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 cultivar Meeker (Figure II.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 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 the 6th, 8th and 11th July 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 summed forms; from 2.6 to 9.8 ppm ellagic acid), while they decreased in juice made

from Veten (from 29.5 to 11.3 ppm summed forms; from 7.5 to 2.5 ppm 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 II.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 than 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 effect the concentrations of ellagic acid and ellagic acid derivatives in juices made from the cultivar Meeker (Figure II.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. Mold decreased, however, the contents of quercetin-glycosides and glucuronides considerably in these samples (Rommel and Wrolstad, manuscript in prep.).

Influences of Processing

Standard pilot plant process and alternative processes. The influences of the different processing techniques on total concentrations of ellagic acid forms in raspberry juices made from the cultivar Willamette are shown in Figure II.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); however, these juices were made from berries of different origins and so 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 II.1; Bate-Smith, 1959, 1972; Wilson and Hagerman, 1990). Depectinization combined with centrifugation decreased total ellagic acid forms considerably compared 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 II.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 II.7 and II.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 and WI-OR-R-OS26B), however, reduced total ellagic acid concentrations less than vacuum evaporation and the third 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. (manuscript in prep.) found no significant differences in total anthocyanin contents and HPLC-anthocyanin profiles, respectively, among the same single-strength, vacuum and osmosis concentrated samples (using membrane A at both temperatures).

Comparison of Experimental and Commercial Samples

Commercial samples contained much higher concentrations of total ellagic acid forms than experimental juices (mean of 52 ppm for commercial samples (n=7) compared to a mean of 18 ppm for experimental samples (n=20); Figure II.10). However, 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. The pattern of concentration of ellagic acid for different cultivars was similar to that of summed ellagic acid forms, Meeker and Willamette containing most. Commercial samples contained much more ellagic acid and its forms than experimental juices. Diffusion extraction and the standard process produced juices with by far the highest total concentrations of ellagic acid forms. High-speed centrifuged juice contained about one half of total ellagic acid compared to diffusion extraction. Juice depectinization and concentration decreased total ellagic acid forms even further. Mold appeared not to 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.

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 ellagic acid in 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, it remains 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.

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Table II.1a. Experimental Raspberry Juice Samples Produced by a Standard Process

Sample No.	Sample Code	Cultivar	Geographic Origin	Ripeness UR	R	OR	MH	HP	Processing Method STD PP	STD LP*	STD+ VC	STD+ OS	CT	CT+ Enz.	DE	MDY	Juice °Brix	Juice Conc. °Brix	Source	Picking Date
8	ME-OR-UR-HP-PP	Meeker	USA (OR)	X				X	X								8.5		J.M. Smucker	11-Jul-88
16	ME-OR-UR-MH-PP	Meeker	USA (OR)	X			X		X								9.8		J.M. Smucker	21-Jul-88
11	ME-OR-R-MH-PP	Meeker	USA (OR)		X		X		X								7.5		J.M. Smucker	12-Jul-88
10	ME-OR-OR-HP-PP	Meeker	USA (OR)			X		X	X								13.0		Clermont	23-Jul-88
15	ME-OR-OR-HP-PP	Meeker	USA (OR)			X		X	X								10.0		J.M. Smucker	11-Jul-88
3	ME-OR-OR-MH-PP	Meeker	USA (OR)			X	X		X								13.0		Clermont	23-Jul-88
5	ME-OR-OR-MH-PP	Meeker	USA (OR)			X	X		X								10.2		Clermont	8-Jul-88
17	ME-OR-R-PP-HP-MDY	Meeker	USA (OR)		X			X	X							X	2.5		J.M. Smucker	12-Jul-88
18	WI-OR-UR-HP-PP	Willamette	USA (OR)	X				X	X								8.4		NWResExtCen	22-Jul-88
20	WI-OR-UR-HP-PP	Willamette	USA (OR)	X				X	X								9.0		NWResExtCen	1-Jul-88
34 A	WI-OR-R-MH-PP	Willamette	USA (OR)		X		X		X								9.1		Kerr Concentra.	19-Jun-90
34 B	WI-OR-R-MH-PP	Willamette	USA (OR)		X		X		X								8.0		Kerr Concentra.	19-Jun-90
34 C	WI-OR-R-MH-LP	Willamette	USA (OR)		X		X			X							9.2		Kerr Concentra.	19-Jun-90
34 D	WI-OR-R-MH-LP	Willamette	USA (OR)		X		X			X							10.0		Kerr Concentra.	19-Jun-90
6	WI-OR-OR-HP-PP	Willamette	USA (OR)			X		X	X								10.5		J.M. Smucker	11-Jul-88
14	WI-OR-OR-HP-PP	Willamette	USA (OR)			X		X	X								9.2		NWResExtCen	22-Jul-88
19	WI-OR-OR-HP-PP	Willamette	USA (OR)			X		X	X								8.0		NWResExtCen	1-Jul-88
4	WI-OR-OR-MH-PP	Willamette	USA (OR)			X	X		X								9.8		Clermont	8-Jul-88
7	WI-OR-OR-MH-PP	Willamette	USA (OR)			X	X		X								13.4		J.M. Smucker	21-Jul-88
9	WI-OR-OR-MH-PP	Willamette	USA (OR)			X	X		X								13.0		J.M. Smucker	21-Jul-88
1	CH-BC-R-PP	Chilcotin	Canada (BC)		X				X								9.4		EChilliwackCP	20-Jul-88
2	SK-BC-R-PP	Skeena	Canada (BC)		X				X								9.0		EChilliwackCP	20-Jul-88
32	HR-OR-R-HP-LP	Heritage	USA (OR)		X			X		X							13.2		private farm	23-Sep-89
31	HR-OR-OR-HP-LP	Heritage	USA (OR)			X		X		X							15.0		private farm	23-Sep-89
33	GD-OR-R-HP-LP	Golden	USA (OR)		X			X		X							11.3		private farm	Jul-89
30	MP-PO-R-HP-LP	Malling Promise	Poland		X			X		X							9.4		ResInstPo&Flo	6-Jul-88
29	MP-PO-R-HP-LP	Malling Promise	Poland		X			X		X							8.0		ResInstPo&Flo	8-Jul-88
24	MP-PO-R-HP-LP	Malling Promise	Poland		X			X		X							11.0		ResInstPo&Flo	11-Jul-88
13	MS-PO-R-HP-LP	Malling Seedling	Poland		X			X		X							8.5		ResInstPo&Flo	6-Jul-88
28	MS-PO-R-HP-LP	Malling Seedling	Poland		X			X		X							7.0		ResInstPo&Flo	8-Jul-88
27	MS-PO-R-HP-LP	Malling Seedling	Poland		X			X		X							8.5		ResInstPo&Flo	11-Jul-88
26	NR-PO-R-HP-LP	Norna	Poland		X			X		X							9.0		ResInstPo&Flo	6-Jul-88
21	NR-PO-R-HP-LP	Norna	Poland		X			X		X							7.9		ResInstPo&Flo	11-Jul-88
22	NR-PO-R-HP-LP	Norna	Poland		X			X		X							9.0		ResInstPo&Flo	8-Jul-88
12	VT-PO-R-HP-LP	Veten	Poland		X			X		X							8.5		ResInstPo&Flo	6-Jul-88
23	VT-PO-R-HP-LP	Veten	Poland		X			X		X							8.8		ResInstPo&Flo	8-Jul-88
25	VT-PO-R-HP-LP	Veten	Poland		X			X		X							9.8		ResInstPo&Flo	11-Jul-88

Table II.1b. Experimental Raspberry Juice Samples Produced by Alternative Processes

Sample No.	Sample Code	Cultivar	Geographic Origin	Ripeness UR	R	OR	MH	HP	Processing Method STD PP	STD LP*	STD+ VC	STD+ OS	CT	CT+ Enz.	DE	MDY	Juice °Brix	Juice Conc. °Brix	Source	Picking Date
37 D	WI-OR-R-PP	Willamette	USA (OR)		X				X								10.8		Kerr Concentra.	19-Jun-90
37 E	WI-OR-R-VC	Willamette	USA (OR)		X						X						10.0	43.5	Kerr Concentra.	19-Jun-90
37 A	WI-OR-R-OS8A	Willamette	USA (OR)		X							X 8A					10.0	44.8	Kerr Concentra.	19-Jun-90
37 B	WI-OR-R-OS26A	Willamette	USA (OR)		X							X 26A					10.0	43.5	Kerr Concentra.	19-Jun-90
37 C	WI-OR-R-OS26B	Willamette	USA (OR)		X							X 26B					10.0	45.5	Kerr Concentra.	19-Jun-90
35 A	WI-BC-R-MH-CT	Willamette	Canada (BC)		X		X						X				11.9		EChilliwackCP	20-Jul-88
35 B	WI-BC-R-MH-CTESP	Willamette	Canada (BC)		X		X							X ESP			12.4		EChilliwackCP	20-Jul-88
35 C	WI-BC-R-MH-CTEBE	Willamette	Canada (BC)		X		X							X EBE			12.2		EChilliwackCP	20-Jul-88
36	WI-BC-R-MH-DE	Willamette	Canada (BC)		X		X								X		7.6		EChilliwackCP	20-Jul-88

Table II.1c. Commercial Red Raspberry Juice Concentrates

Sa. No.	Sample Code	Company Code	ESR	°Brix
38	COM-A-45	A	yes	45
39	COM-B-65	B		65
40	COM-C-45	C	yes	45
41	COM-D-45	D	no	45
42	COM-E-45	E	yes	45
43	COM-F-45	F	no	45
44	COM-G-65	G		65
45	COM-H-66	H		66
46	COM-I-65	I		65

MH-machine harvested; HP-hand-picked; MDY-moldy; UR-underriped; 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.-enzyme (pectinase); DE-diffusion extraction; ME-Meeker; 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 concentration at 8°C 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; North Willamette Research and Extension Center, Aurora, Oregon; East Chilliwack Co-op, Chilliwack, BC (Canada); Research Institute of Pomology and Floriculture, Skierniewice, Poland.

Table II.2. Concentrations of Ellagic Acid and its Forms in Experimental and Commercial Juices

Cultivar (Sample No.)	EA	Ellagic Acid Forms (in ppm)																
		#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
Willam. 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
Willam. 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
Mall. Prom. 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
Mall. Prom. 2 (29)	2.1	0.9	0.8	tr	tr	tr	tr	nd	nd	nd	nd	nd	nd	nd	nd	tr	tr	7.3
Mall. Prom. 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
Mall. Seedl. 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
Mall. Seedl. 2 (28)	1.2	0.8	tr	1.5	nd	tr	tr	nd	nd	tr	nd	nd	nd	nd	nd	tr	tr	7.1
Mall. Seedl. 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
Willam. 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
Willam. 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
Willam. 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
Willam. 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
Willam. 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
Willam. 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
Willam. 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
Willam. 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

UR-underripe; R-ripe; OR-overripe; LP-laboratory process; Mall.-Malling; Prom.-Promise; Seedl.-Seedling; STD-standard process; VC-vacuum concentration; OS8A, OS26A-osmotic concentration at 8°C 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; EA-ellagic acid; tr-trace (≤ 0.6 ppm); nd-not detected.

Table II.3. Mean Concentrations of Ellagic Acid and its Forms in Experimental Raspberry Juices and Concentration-Ranges in Commercial Samples

Ellagic Acid Compound	**Concentration in Experimental Juices (ppm)**				**Concentration-Range in Commercial Juices (ppm; n=9)**
	n	Mean ± Std. Error	% Variance due to Difference Among Juices	Between Repl.	
Ellagic Acid (EA)*	45	10.12 ± 1.53	71.7	28.3	5.48 - 52.14
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	trace - 1.88
EA-form #4	13	1.65 ± 0.25	22.0	78.0	n.d. - 2.40
EA-form #1	20	1.52 ± 0.44	13.1	86.9	2.06 - 23.51
EA-form #12	44	1.12 ± 0.10	83.3	16.7	trace - 1.48
EA-form #8	32	0.77 ± 0.08	38.4	61.6	trace - 2.44
EA-form #9	32	0.74 ± 0.05	1.6	98.4	n.d. - 2.46
EA-form #7	37	0.71 ± 0.04	23.5	76.5	n.d. - 1.49
EA-form #10	26	0.69 ± 0.04	12.7	87.3	n.d. - 0.85
EA-form #11	31	0.62 ± 0.01	32.9	67.1	trace
EA-form #15	32	0.62 ± 0.01	30.4	69.6	n.d. - 0.99
EA-form #13	38	0.61 ± 0.01	31.8	68.2	trace
EA-form #14	35	0.61 ± 0.00	32.6	64.4	n.d. - 0.71
EA-form #16	44	0.61 ± 0.01	24.3	75.7	trace - 0.77
EA-form #5	34	trace• ± 0.00	33.3	66.7	n.d. or trace
EA + 16 EA-forms	45	28.17 ± 2.34	69.9	30.0	22.36 - 80.36

*reported previously for fresh raspberries by Bate-Smith (1959) and Daniel et al. (1989); •trace≤ 0.6 ppm.

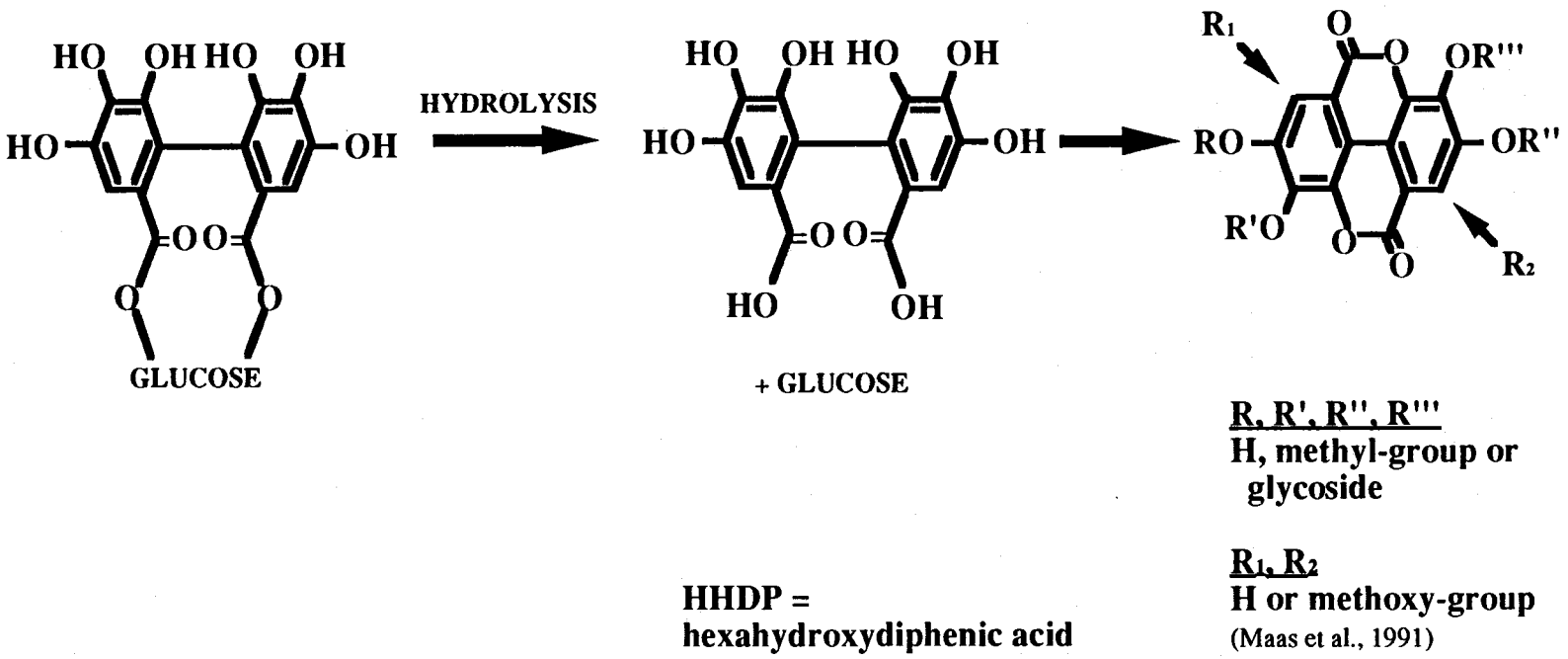

Figure II.1a. Structures of ellagic acid and derivatives and their precursors in plant cell walls

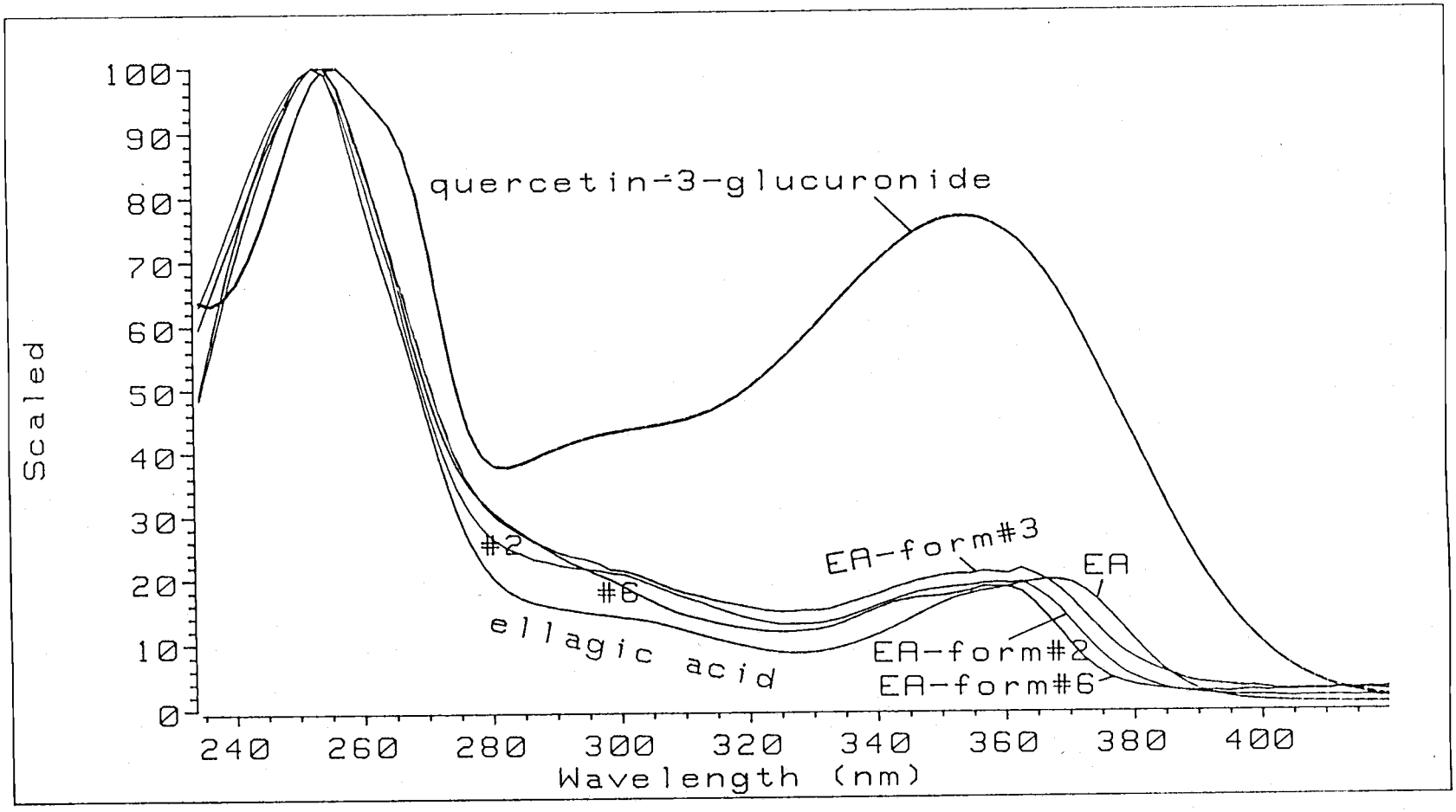

Figure II.1b. Absorbance spectra of ellagic acid (EA), 3 ellagic acid derivatives and quercetin-3-glucuronide present in ammonia/ methanol fractions of red raspberry juices.

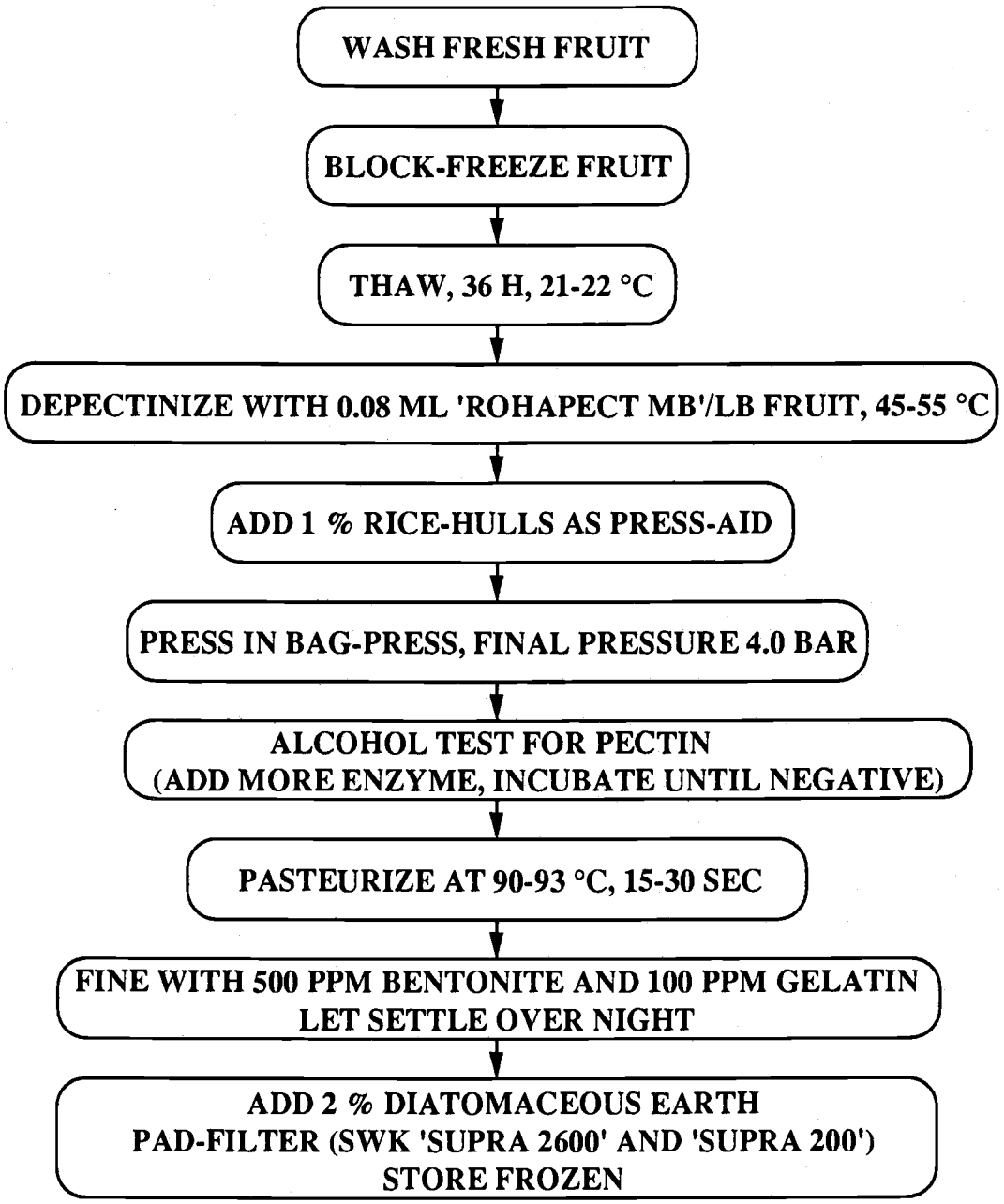

Figure II.2. Flow diagram for juice processing by a standard technique.

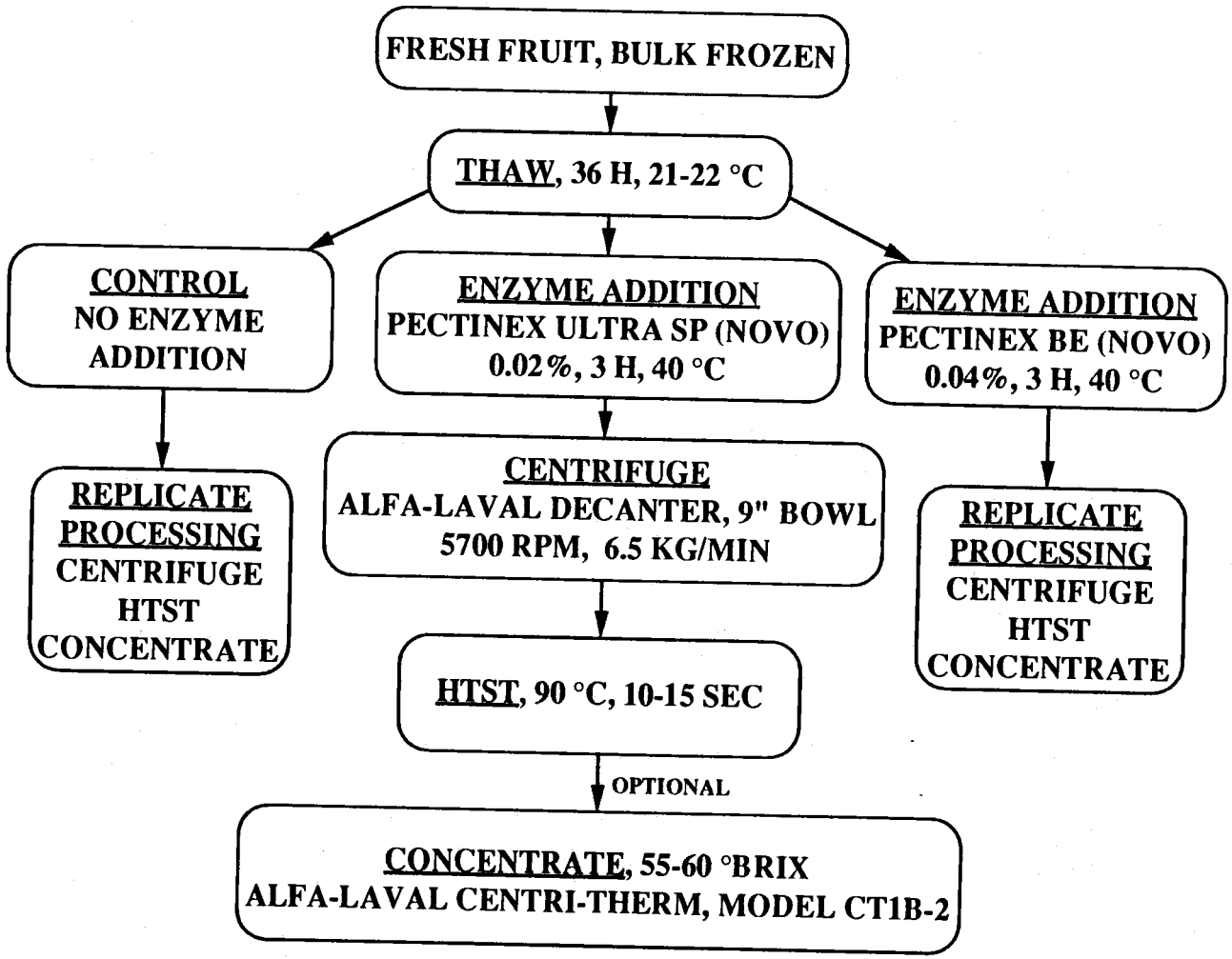

Figure II.3. Flow diagram for juice processing by centrifugation, with and without depectinization.

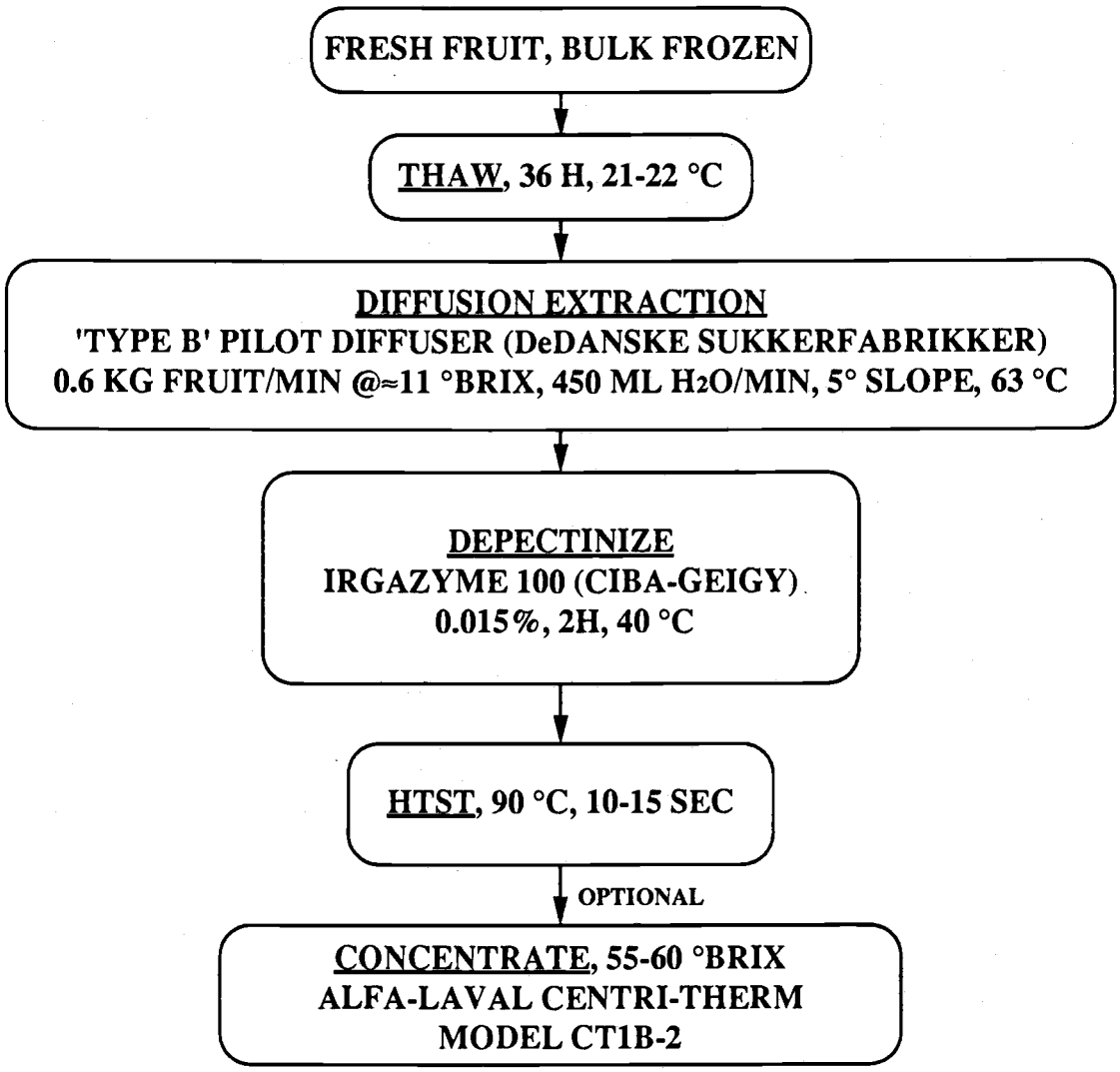

Figure II.4. Flow diagram for juice processing by diffusion extraction.

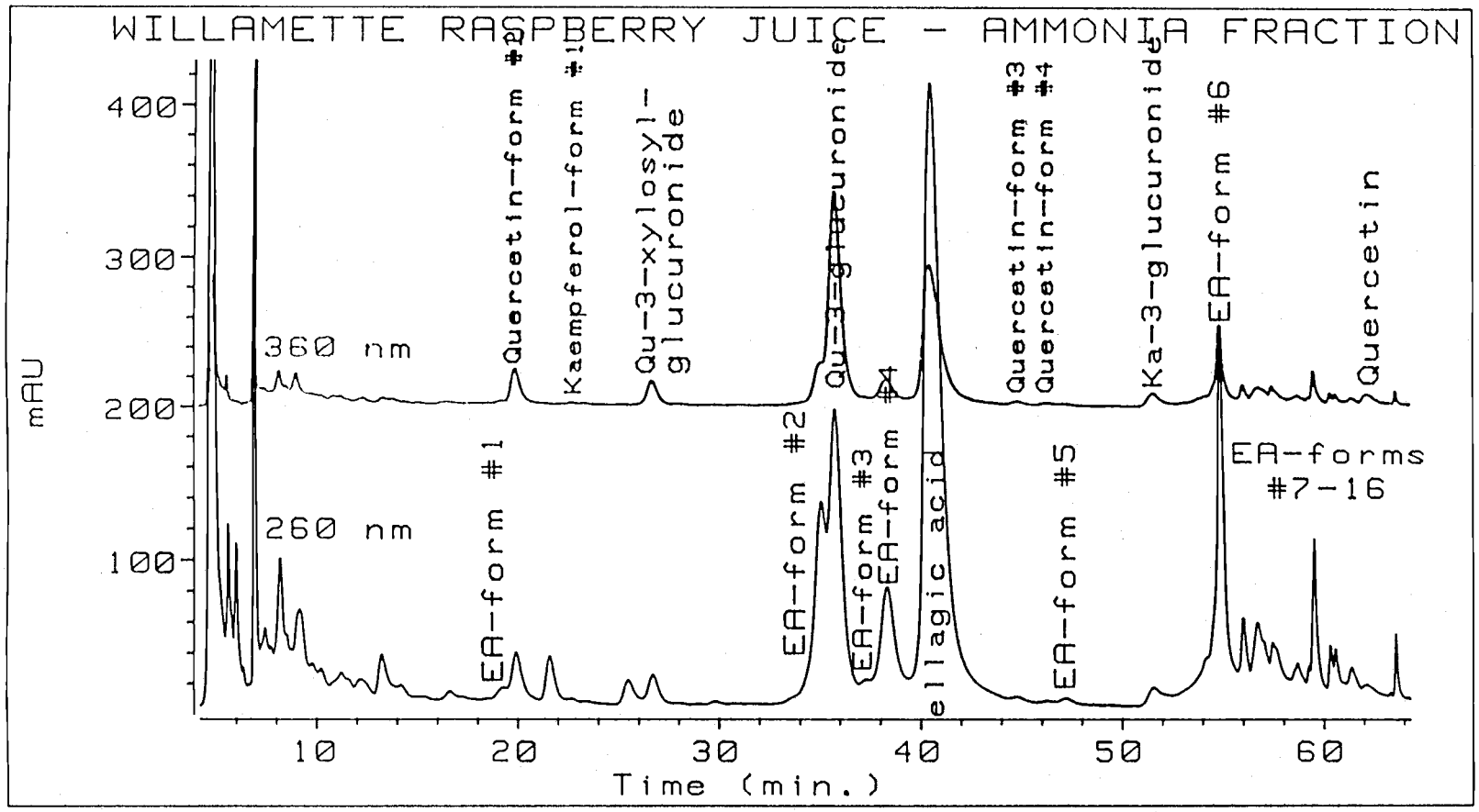

Figure II.5a. HPLC chromatogram of the ammonia/methanol fraction (separated on Polyamide-6) of red raspberry juice made from ripe Willamette cultivar. Ellagic acid and ellagic acid derivatives were detected at 260 nm; flavonol-glucuronides, acylated flavonol-glycosides, and flavonol aglycons were detected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

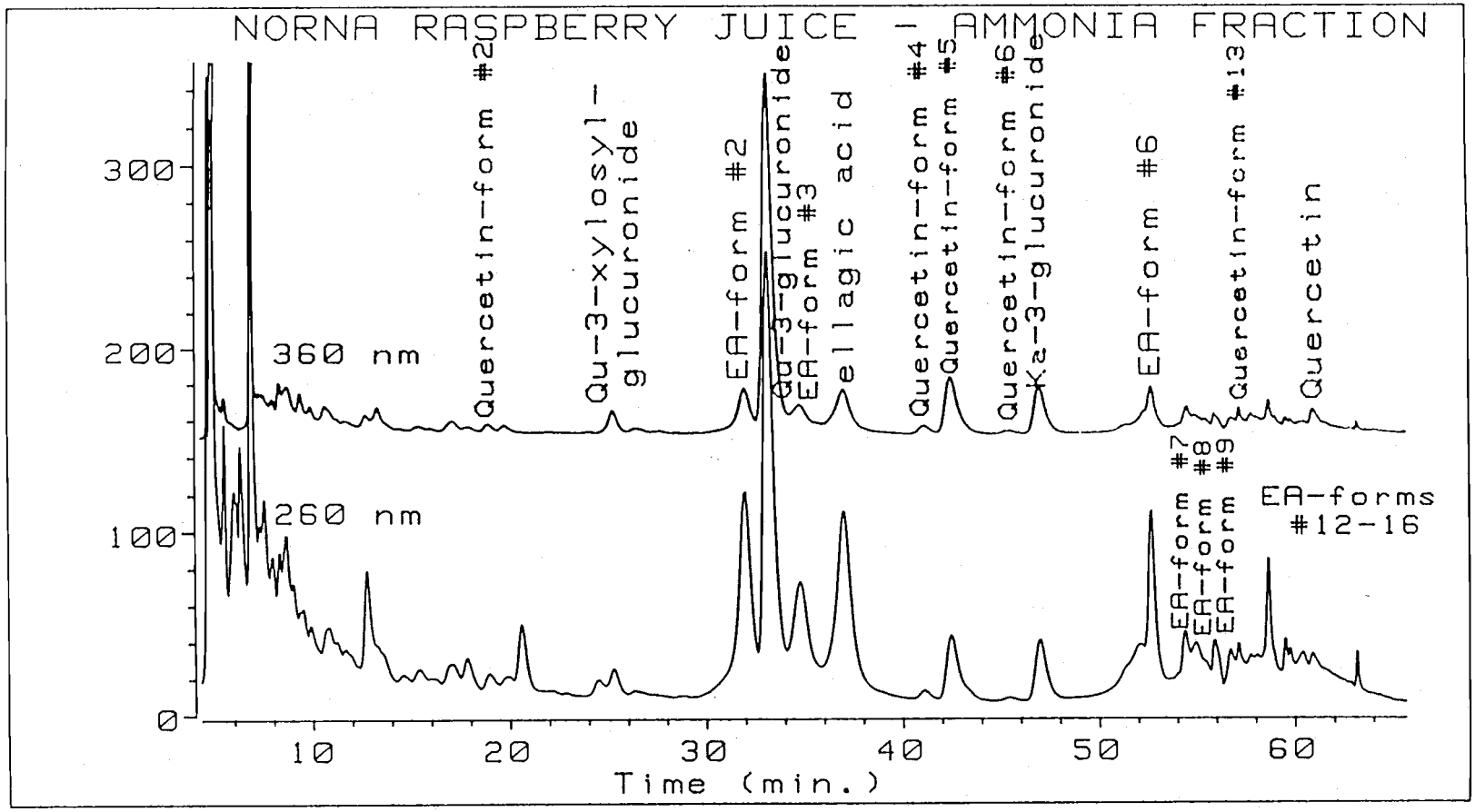

Figure II.5b. HPLC chromatogram of the ammonia/methanol fraction (separated on Polyamide-6) of red raspberry juice made from ripe Norna cultivar. Ellagic acid and ellagic acid derivatives were detected at 260 nm; flavonol-glucuronides, acylated flavonol-glycosides, and flavonol aglycons were detected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

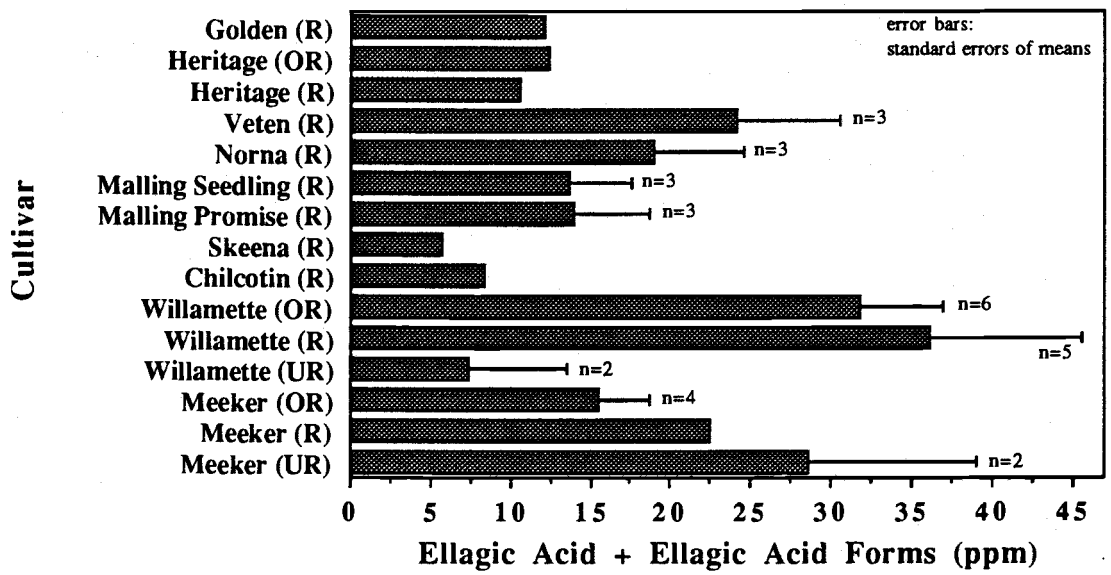

Figure II.6a. Total concentrations of ellagic acid forms in juices made from different raspberry cultivars (UR-underripe, R-ripe, OR-overripe).

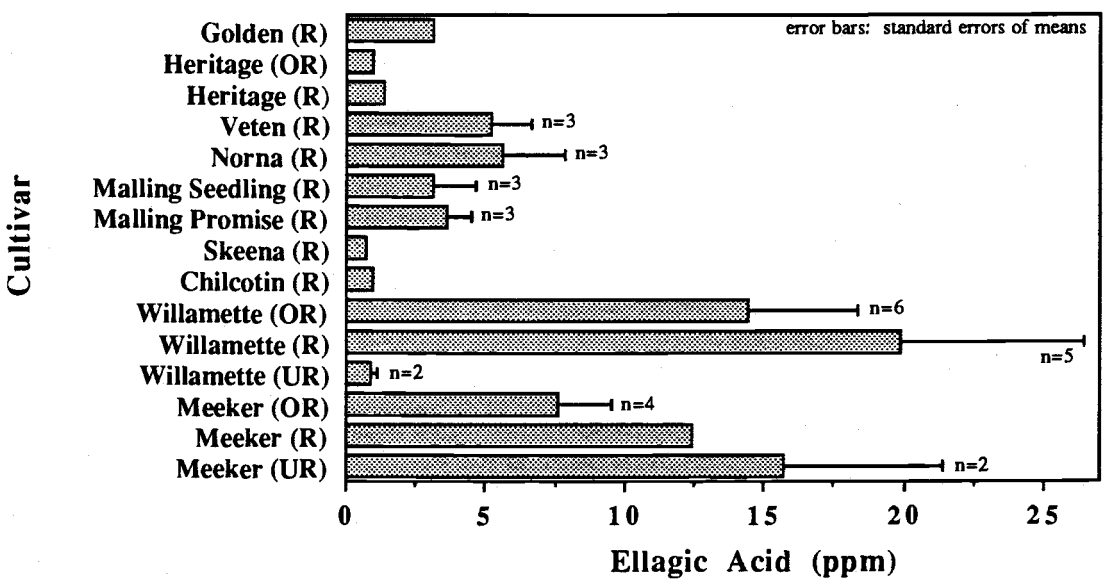

Figure II.6b. Concentrations of ellagic acid in juices made from different raspberry cultivars (UR-underripe, R-ripe, OR-overripe).

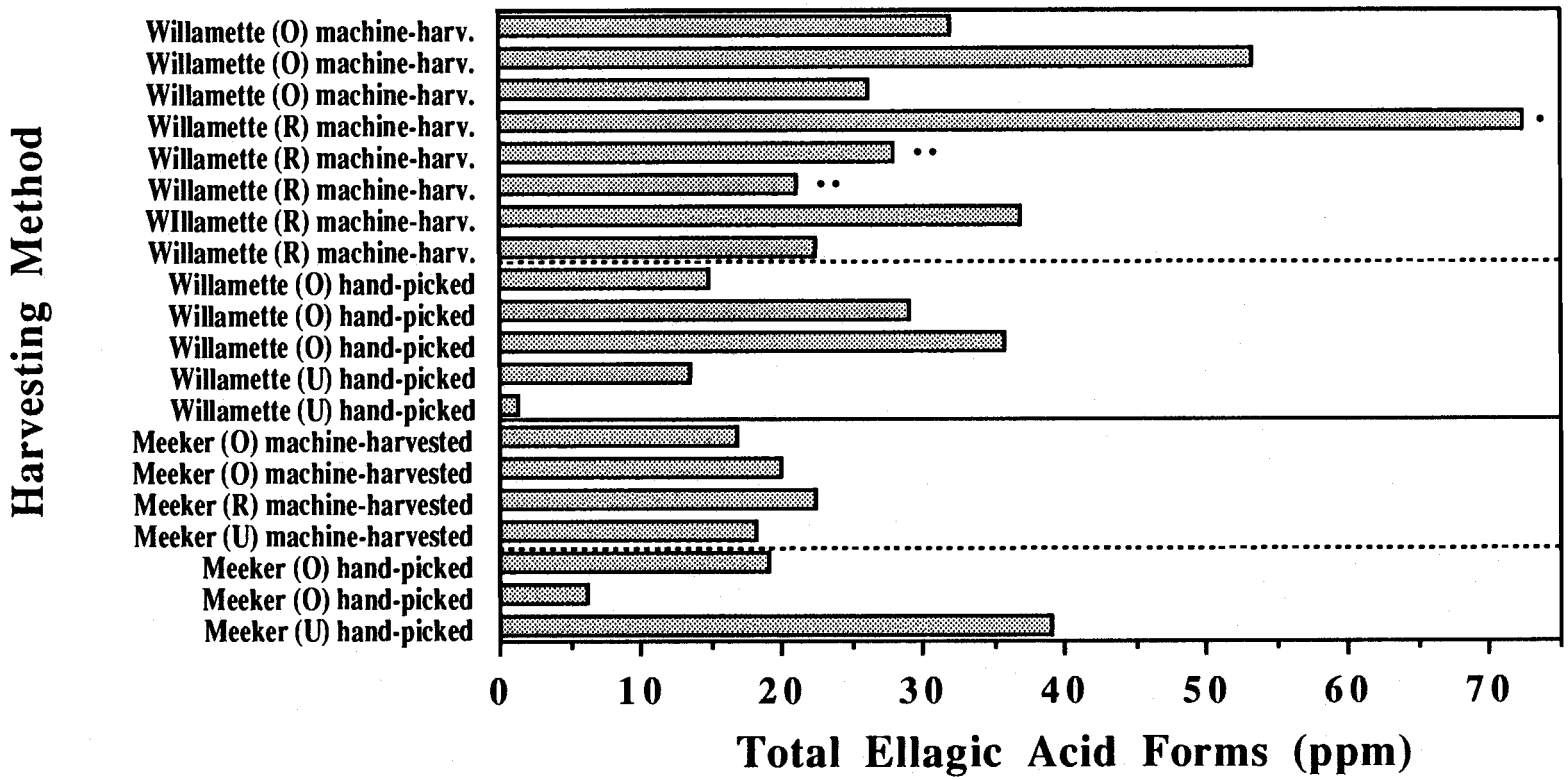

Figure II.7. Total concentrations of ellagic acid forms in juices made from raspberries picked by hand or harvested by machines (U-underripe, R-ripe, O-overripe, •large batch, ••standard process simulated in the laboratory).

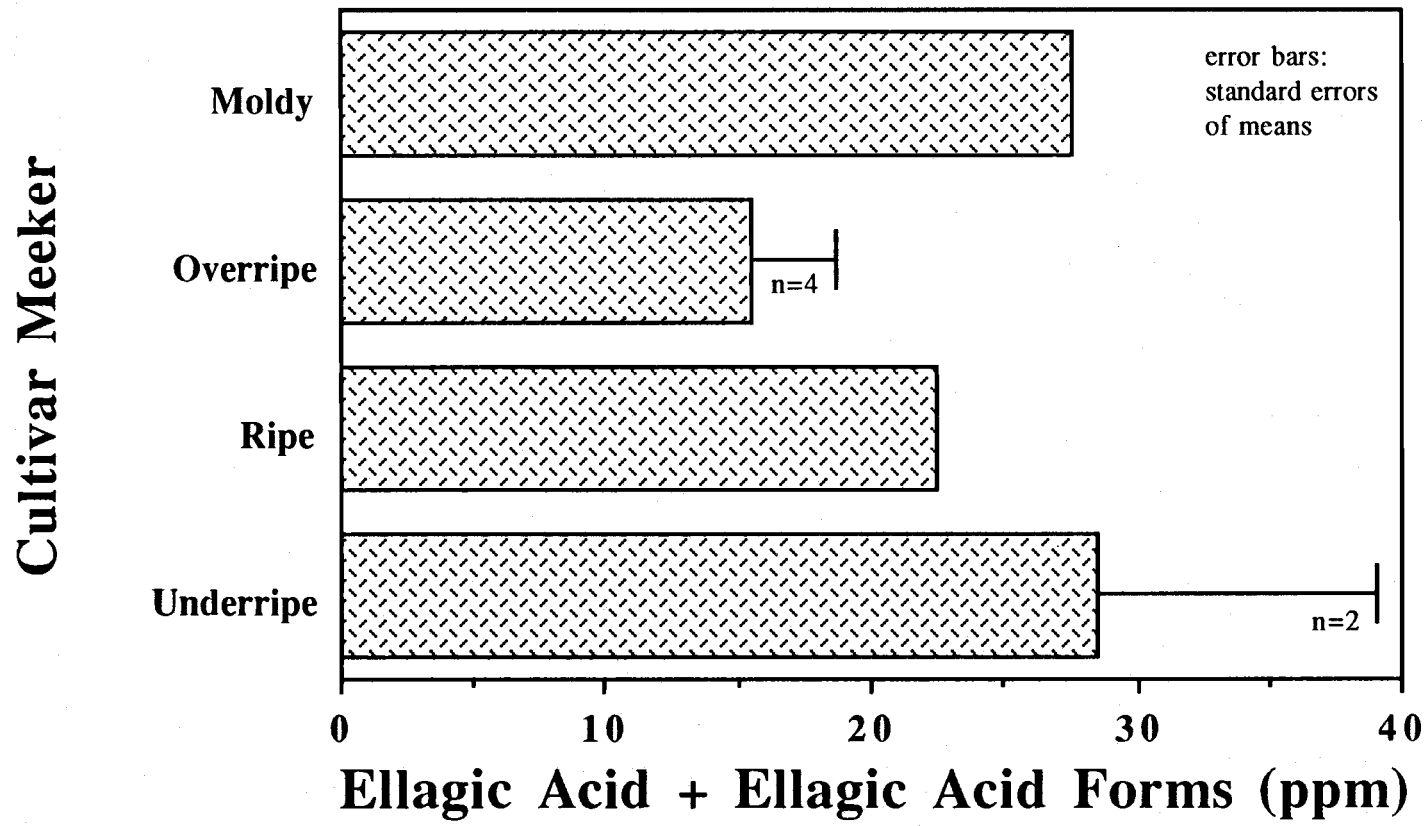

Figure II.8. Effect of mold contamination on total concentration of ellagic acid forms in raspberry juices made from the cultivar Meeker.

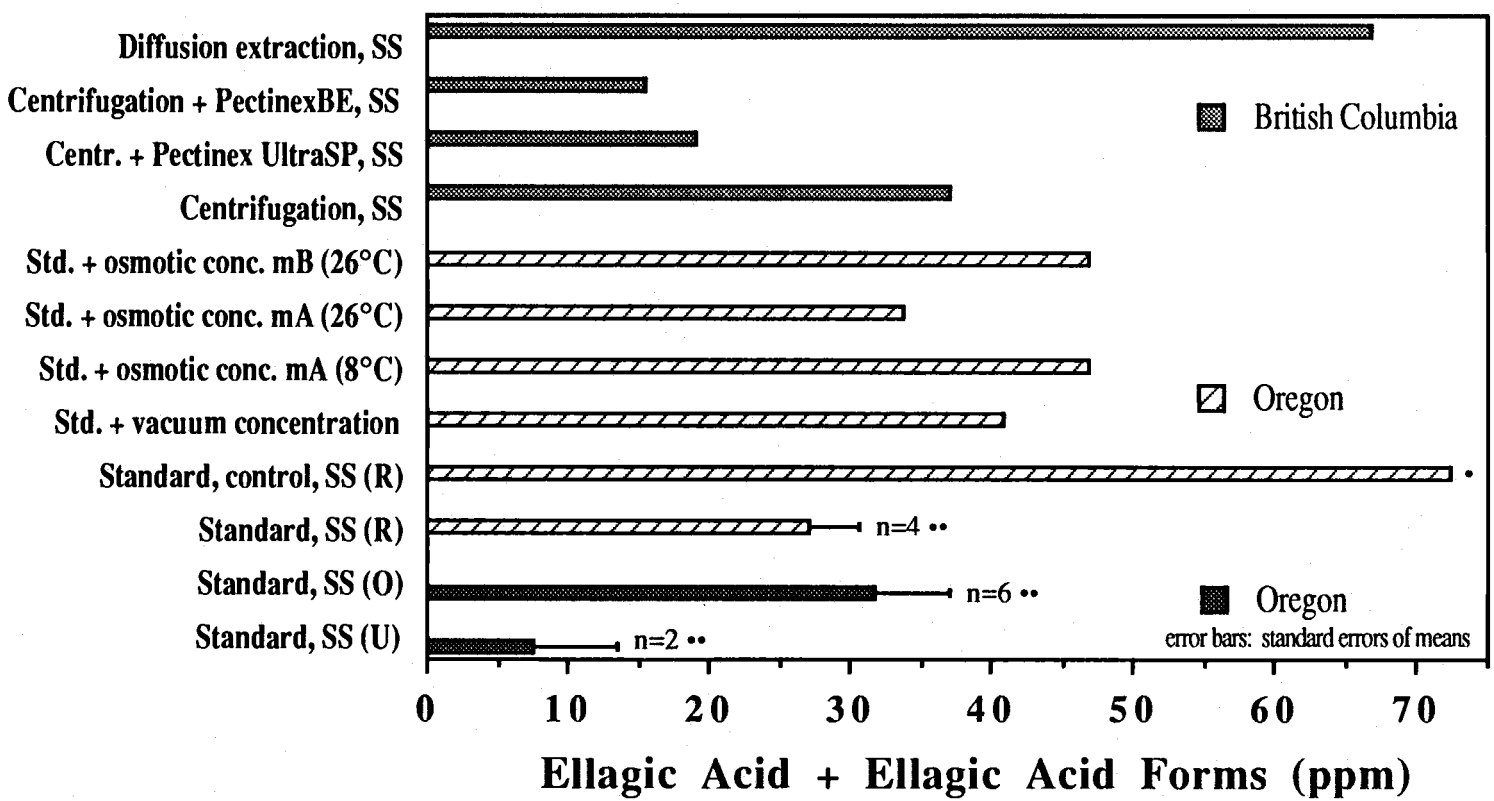

Figure II.9. Effects of different processing techniques on total concentration of ellagic acid forms in juices made from the cultivar Willamette (m-membrane, SS-single strength, UR-underripe, R-ripe, OR-overripe, •large batch, ••small batch).

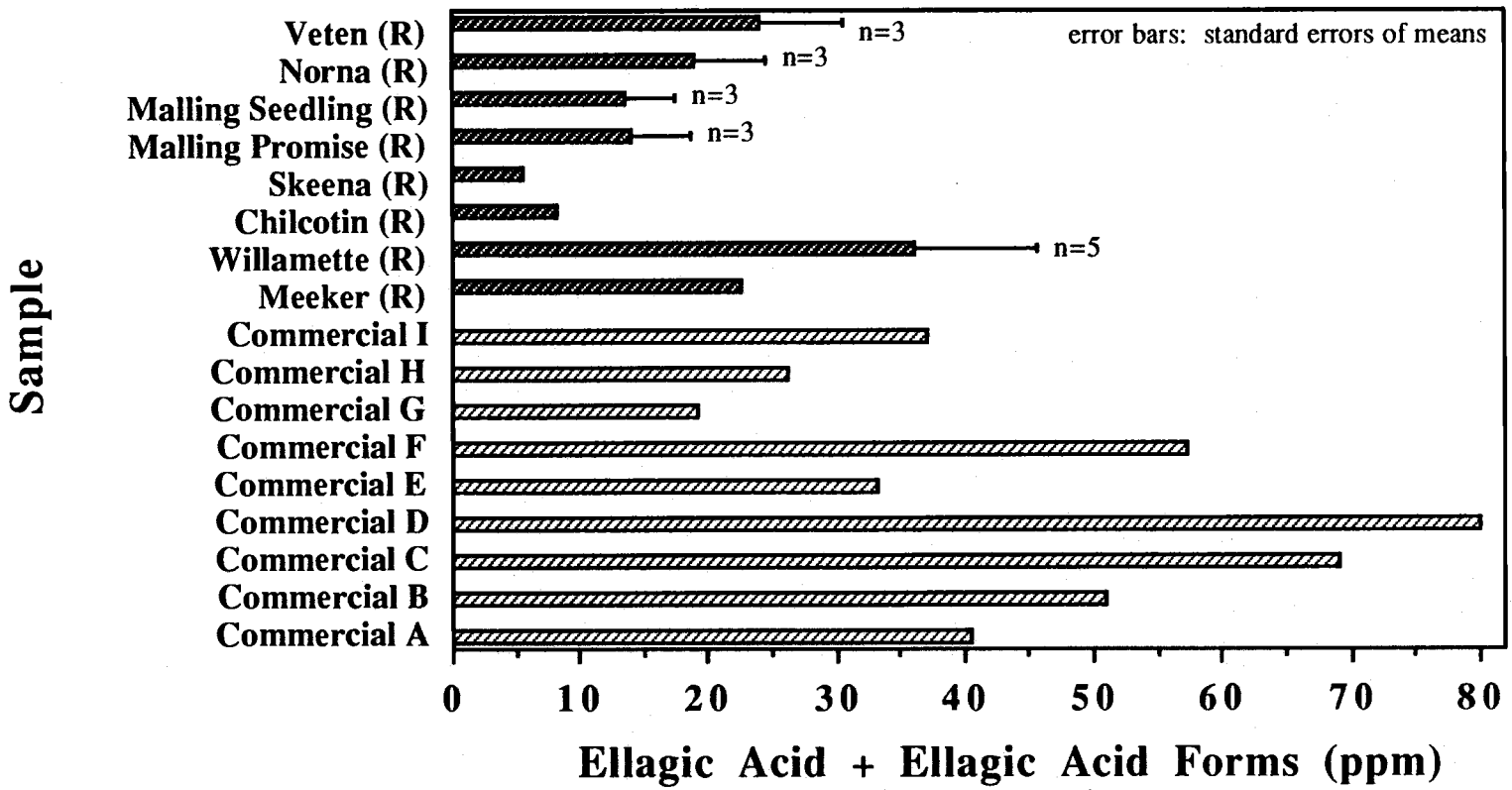

Figure II.10. Total concentrations of ellagic acid in commercial and experimental raspberry juices. (Commercial juices were made by diluting concentrates to soluble solids contents of 10°Brix.)

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III. Composition of Flavonols in Red Raspberry Juice as Influenced by Cultivar, Processing and Environmental Factors

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ABSTRACT

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 mini-columns, packed with Polyamide-6. A fraction eluted with methanol contained ≤ 8 quercetin-glycosides, quercetin and kaempferol. A second fraction eluted subsequently with 0.5% ammonia in methanol contained 3 flavonol-glucuronides, 2 flavonol-forms, aglycons, ellagic acid and its derivatives. Quercetin-3-glucuronide was the major flavonol in experimental and commercial juices, respectively (means 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 ppm and 3.6 ppm and in commercial juices (n=7) were 121 ppm and 3.4 ppm. The mean total flavonol concentrations in experimental and commercial juices were 122 ppm and 125 ppm. 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 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 focussed on the use of anthocyanins, the red pigments and major flavonoids of raspberries, as juice authenticity indicators (Spanos and Wrolstad, 1987). Anthocyanins are sometimes, however, inconclusive in detecting adulteration, which makes additional phenolic authenticity indicators, such as non-anthocyanin flavonoids (e.g. flavonols, catechins), benzoic and cinnamic acids, essential. For example, non-anthocyanin flavonoids would be particularly suitable in detecting adulteration by potential adulterants such as relatively inexpensive, non-red fruit juices (e.g. apple, 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 III.1), catechins and trace amounts of flavones (Henning, 1981; Herrmann, 1974 and 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 and 1976; Ryan & 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.

Health effects. The composition of raspberry juice is of increasing interest because of the positive health effects associated with its high phenolic content. The phenolics present in raspberries, which have been shown to have anticarcinogenic effects in mammals, are quercetin, ellagic acid and also kaempferol and other phenolics. Quercetin also exhibits

antiviral effects *in vitro* (Vlietinck et al., 1988) and *in vivo* (Anton, 1988); quercetin and kaempferol have anti-inflammatory potencies (Anton, 1988); and kaempferol shows antifertility acitivities (Anton, 1988). A review of the anticarcinogenic effects of ellagic acid is given in Daniel et al. (1989) and Maas et al. (1991).

Although quercetin has been demonstrated to be mutagenic in bacterial assay systems, it has been found not to be carcinogenic or teratogenic *in vitro* and *in vivo* (in rodents) by many research groups (Deschner, 1991). Rather, quercetin has been found to be a potent anticarcinogen against skin, colon and mammary cancers in rodents (e.g. Deschner, 1991; Leighton et al., 1991; Verma, 1991; Weisburger, 1991; Yasukawa et al., 1988). For example, quercetin has been shown to have a very pronounced inhibitory effect on the carcinogen benzo[a]pyrene (BaP) in an *in vitro* mutagenicity assay (Stavric et al., 1991). *In vivo*, quercetin has also been shown to reduce the uptake of BaP (by 21%) in rats fed quercetin at 2% in their diet and to reduce mutagenicity of BaP by up to 72% (Stavric et al., 1991). Quercetin (30 µmol applied topically) inhibits mouse skin tumor formation in a dose-dependent manner, both when initiated with 7,12-dimethylbenz[a]anthracene (DMBA; 43% fewer papillomas/mouse) and when promoted with 12-O-tetradecanoylphorbol-13-acetate (TPA; 60-75% fewer papillomas/mouse; Verma, 1991). Quercetin, rutin and kaempferol, applied to mouse skin (at 5 µmol) before and with TPA-tumor promoter inhibit the number of papillomas per mouse by 58%, 45% and 56%, respectively (Yasukawa et al., 1988). Quercetin and rutin fed to mice at 2% and 4% in their diet, respectively, reduce azoxymethanol (AOM)-induced hyperproliferation of colonic epithelial cells significantly and suppress colon tumor multiplicity; 2% quercetin in mouse diet for 46 weeks significantly depresses colon tumor incidence (25% vs. 5.9% in controls; Deschner, 1991). Quercetin fed at 5% in rat diet inhibits DMBA-induced mammary tumors by over 50%; with induction by NMU the effect is not quite as dramatic but still very significant (Verma, 1991). Kaempferol-3-O-ß-D-glucopyranoside has also been found to be a potent anticarcinogen *in vivo* (Lee, 1991).

Quercetin may also inhibit the induction of human cancers (Verma, 1991); it markedly inhibits the growth of human gastric cancer cells and blocks cell progression *in vitro* (Yoshida et al., 1990). In *in vitro* transfection assays with the human H-*ras* oncogene, quercetin has been found to block the appearance of transformed loci and also to selectively inhibit the proliferation of tumor cells transformed with a panel of diverse oncogenes (65-90% vs. 30-40% for normal cells; Leighton et al., 1991). Epidemiological evidence supports the theory that flavonols have anticarcinogenic effects in humans, e.g. there is an inverse correlation between individuals who consume a diet rich in fruits and vegetables and their risk of developing cancer (Committee on Diet, Nutrition, and Cancer, 1982; Stich and Rosin, 1984; Weisburger, 1991).

Quercetin is the most abundant flavonoid in the human diet (Stavric, 1991). The daily intake by humans of glycosides of the flavonols kaempferol and quercetin is approximately 50-100 mg (MacGregor, 1986; Mazaki et al., 1982). Quercetin is formed in the human mouth and gastrointestinal tract via bacterial hydrolysis of quercetin-glycosides and glucuronides (Bokkenheuser and Winter, 1988; Deschner, 1991; Leighton et al., 1991; Shillitoe et al., 1984; Verma, 1991). However, quercetin appears to be poorly absorbed in the gastrointestinal tract (probably less than 1% of ingested quercetin; Deschner, 1991; Stavric et al., 1991; Verma, 1991). Absorption seems to be increased at some optimum fat content of diet; e.g. the anticarcinogenic effects of quercetin and rutin have been shown to be best in a mouse diet that contains 5% corn oil (Deschner, 1991).

This manuscript is a contribution to an extensive study conducted by our group at the Department of Food Science and Technology to create an expanded database for the phenolic, sugar and acid composition of red raspberry juice composition. The specific objective of this study was to identify and quantify flavonols so that they can be used as a) supplementary authenticity indicators together with anthocyanins, sugars and organic acids and b) a data-base for evaluating the effects of raspberry juice on health. The composition of ellagic acid and derivatives and other phenolics will be reported in Rommel

and Wrolstad (manuscript in prep.) and Rommel et al. (1992). The anthocyanin composition of these same raspberry juices is reported in Boyles (1992); acid and sugar compositions of these samples will be reported elsewhere.

MATERIALS AND METHODS

Raspberry samples (n=55), juice processing methods, the environmental factors evaluated, and statistical methods are described in Rommel and Wrolstad (manuscript in prep.).

High Performance Liquid Chromatography (HPLC)

Preparation of samples. A clean separation of the compounds of interest into fractions 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 unexpectedly numerous flavonol forms and ellagic acid derivatives present in raspberries.

We obtained a clean separation, however, by adapting a fractionation procedure developed for large chromatography-columns by Wald and Galensa (1989), following Henning and Herrmann (1980), to minicolumns by using TLC-grade instead of SC-grade Polyamide-6 (Figure III.2). 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, aglycons, as well as ellagic acid and ellagic acid derivatives. The original technique apparently did not elute ellagic acid and/or derivatives in the ammonia/methanol fraction, probably because fresh fruit was used, whereas we used juices. Sample-preparation was replicated for each of the 55 juices analyzed.

Materials used: TLC-grade Polyamide-6, particle size < 100 µm (J.T. Baker Inc., Phillipsburg, NJ 08865); Bio-Rad minicolumns, 10 cm length (Bio-Rad Laboratories, Richmond, CA); glass beads, 212-300 µm size (Sigma Chemical Co., St. Louis, MO

63178); filters, 0.45 μm pore size, type HA (Millipore Corp., Bedford, MA 01730).

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. The columns usually used for separating flavonoids such as C_{18}-columns (with high C_{18}-carbon-load and end-capped) or polymer columns did not work in the case of 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', 5μ, 250 mm length, 4.6 mm ID (Alltech Associates, Inc., Deerfield, Il). A C_{18}-guard column, 5μ, 10 mm length (Supelco, Inc., Bellefonte, PA) was attached before the ODS-1 column.

HPLC separation conditions. Solvents: A--100% acetonitrile, 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. Compounds were detected at the following wavelengths (nm): ellagic acid and ellagic acid derivatives (260), flavonols (360).

Instrumentation. A Perkin-Elmer liquid chromatograph, series 400 (The 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) was used in the cases where standards were unavailable.

Standards for peak characterization. Flavonols: quercetin-3-glucoside, quercetin-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 Chemical Co., St. Louis, MO 63178). Ellagic acid (Sigma Chemical Co.)

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-methyl-umbelliferyl-ß-D-glucuronide (MUG in this discussion) for the ammonia/methanol fraction (both obtained from Sigma Chemical Co.). Half a ml of a naringin stock solution (500 ppm naringin in de-ionized water) and a MUG stock-solution (250 ppm in de-ionized 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 III.2. 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 used for

the ammonia/methanol fraction (both obtained from Sigma Chemical Co.). 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, 150 ppm (in de-ionized water); 100 ppm naringin and 50 ppm MUG were added to each standard. Set 2: ellagic acid at 0.6, 10, 20, 60 ppm (in 80% ethanol); 100 ppm 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 ppm). The concentration (C) of each individual flavonol or ellagic acid derivative was calculated from measured peak area (A) using the equation $C = I + S\,A$, 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. 1) At the data reduction stage of this project it became apparent that the internal standard naringin had degraded in the stock-solution, stored in the freezer over time. A cubic function was determined in order to estimate a corrected naringin peak area for the time when each juice sample was analyzed. 2) The ellagic acid used as an external standard is only soluble in ≥ 80% ethanol, resulting in decreased peak sharpness. 3) The influence of Polyamide-6 on reproducibility of sample preparation is presently unknown. The variable influences of these three factors probably explain a significant portion of the variance observed between replicate analyses.

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 III.3a) and Norna (Figure III.3b) cultivars. For flavonols present in low concentrations, however, there was also a qualitative difference between these two cultivars in that 3 kaempferol-glycosides (kaempferol-3-glucoside and 2 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 Figures III.4a and III.4b. The composition of ellagic acid and ellagic acid forms present in these fractions is described in Rommel and Wrolstad (manuscript in prep.).

The concentrations of quercetin and kaempferol-glycosides, glucuronides and other forms in experimental and commercial samples are tabulated in Table III.1. Eight of these flavonols were conclusively identified as quercetin-3-glucuronide, quercetin-3-glucoside, quercetin-3-xylosylglucuronide, kaempferol-3-glucuronide, quercetin-3-galactoside, kaempferol-3-glucoside, quercetin, and kaempferol. Presence of these 6 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 (Figures III.3a and

III.3b) 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, 1992) 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 III.1; Figure III.11). In addition, sample COM-H-66 contained 44.4 ppm of flavonol-glycoside #4 (methanol fraction), a glycoside which was either not detected in the other commercial and experimental juices or present only in trace amounts (Table III.3).

Kaempferol-glycoside # 1 was also only detected in sample COM-H-66 (in trace amounts; Table III.3). Boyles (1992), who analyzed the raspberry juices 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, 1992). These two samples also had much lower concentrations of ellagic acid and total ellagic acid forms than the other commercial samples (Rommel and Wrolstad, manuscript in prep.). We believe that these facts clearly indicate that samples COM-G-65 and COM-H-66 were not authentic red raspberry juice concentrates, but rather that they had been adulterated by other fruit juices and/or colorants.

These two samples were therefore excluded before calculating mean flavonol concentrations for the commercial samples (n=7): total flavonol-forms, 121.6 ppm; total quercetin-forms, 121.3; 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, respectively; 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 III.2. In cases where the percentage of total variance attributable to differences between replicate sample preparations was very high, we do not consider the reported flavonol concentrations to be very reliable.

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-glucuronide 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 excluding the 2 adulterated samples) in commercial samples. Quercetin-glycoside #2 (quercetin-3-sophoroside?), quercetin-3-galactoside, quercetin-3-xylosylglucuronide, kaempferol, 2 quercetin-glycosides, and 2 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 2 adulterated samples) in commercial samples. Table III.3 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 III.5a) 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 III.5b) was similar; Heritage, Golden, Malling Promise, and Norna cultivars having the highest mean concentrations (between ca. 75 and 125 ppm). Quercetin-3-sophoroside (Figure III.5c) was present in much greater amounts in Willamette than any other cultivar (between ca. 30 and 60 ppm, depending on ripeness) and was not detected in the cultivar Golden. Quercetin-3-glucoside (Figure III.5d) was measured in all cultivars at 5 ppm or less, Heritage, Willamette and Malling Promise having the highest concentrations. Quercetin-3-galactoside (Figure III.5e) was present in Norna and Veten 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 greater quantities of quercetin-glycoside #1 (means of 3.2, 5.8 and 4.0 ppm, respectively) than all other cultivars, in which this flavonol was not detected or present in trace amounts (Table III.1). Cultivars which contained quercetin-glycoside #1 appeared to contain this flavonol in favor of high concentrations of quercetin-glycoside #2 (quercetin-3-sophoroside?), e.g. Meeker; this could indicate that these two flavonols were closely related compounds. Quercetin-glycoside #4 was present in significant quantities only in the cultivar Willamette (Table III.1). Quercetin-glycosides #'s 5 and 6 (methanol fraction) were measured above trace amounts only in Norna and Veten cultivars and one overripe Willamette sample (for glycoside #6; Table III.1). Norna contained considerably more quercetin-form #5 (ammonia/methanol fraction) than other cultivars (Table III.1). Quercetin-forms #'s 10, 11 and 12 (ammonia/methanol fraction) were only detected in the cultivar Golden (Table III.3).

There were great differences in the concentrations of total kaempferol-forms among cultivars (Figure III.6). Heritage and Norna contained between ca. 11 and 20 ppm, while all others contained trace amounts or a few ppm. Heritage contained much more kaempferol-3-glucuronide (13-16 ppm) than all other cultivars (except one Norna sample;

Table III.1). Kaempferol-3-glucoside was detected only in Meeker, Skeena, Norna and Veten.

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 III.7), 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 (Canada-British Columbia, Poland, USA-Oregon) contained greatly differing amounts of total flavonol-forms (Figures III.5a and III.6) and individual quercetin-glycosides (Figures III.5b to III.5e). 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 raspberries 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 III.5a-d and III.6, Table III.1). However, there was no obvious correlation between ripeness and the concentrations of total quercetin-forms (Figure III.5a), total kaempferol-forms (Figure III.6) and quercetin-3-glucuronide (Figure III.5b) 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 III.5c) increased in Willamette juice with increasing ripeness, while there was no such trend for quercetin-3-glucoside (Figure III.5d) in the same cultivar.

Four cultivars grown in Poland (Malling Promise, Malling Seedling, Norna, Veten) were picked within 5 days (on the 6th, 8th and 11th July 1988). The mean concentrations of

total quercetin-forms decreased in both Malling Seedling (from 95.3 to 49.0 ppm; Table III.1) 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 are shown in Figure III.8. There was a lot of variation in total quercetin concentrations among samples of the same cultivar, degree of ripeness and harvested by the same method, making it impossible to determine 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 (Figure III.9). Mold did not, however, appear to have an effect on ellagic acid and its derivatives (Rommel and Wrolstad, manuscript in prep.). 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 (Canada). 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 illustrated in Figure III.10. 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 06810-5101) had a similar effect on total quercetin concentrations. It is likely that these pectinases de-glycosylated 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, manuscript in prep.); 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; Figure III.10). 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 (Figure III.11). The two samples with the lowest quercetin concentrations were found to be adulterated. Variation among the remaining 7 concentrates was probably due to differences among cultivars rather than processing techniques. 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 second primary flavonol), quercetin-3-galactoside, quercetin-3-xylosylglucuronide, kaempferol, 2 quercetin-glycosides, and 2 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 the 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 contents, however, were found to be adulterated based on their anthocyanin, flavonol and ellagic acid compositions. This demonstrated that flavonol HPLC profiles have great potential as an auxiliary technique for detecting adulteration, e.g. in cases where qualitative differences are found. However, their use could be enhanced by measuring flavonol concentrations more reproducibly.

Quercetin was present (in glycosylated form) in our red raspberry juices in very significant amounts, definitely within the concentration-range which 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 de-glycosylation 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 in order to generate anticarcinogenic effects. Further medical evaluations of the positive effects of raspberry juice on human health could be based on our initial compositional data of flavonol concentrations in red raspberry juices.

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Table III.1. Concentrations of Flavonol-glycosides, Glucuronides, and Other Forms in Experimental and Commercial Juices

Cultivar (Sample No.)	Flavonol Compound (in ppm)									
	Q3Guu	Q3So	QG4M	QF2A	QG6M	QG5M	Q3Glu	QG8M	QG1M	Q3XGuu
Meeker UR (8)	65.5	10.8	nd	4.7	nd	nd	tr	nd	8.2	3.1
Meeker UR (16)	19.6	2.8	nd	tr	nd	nd	tr	tr	1.1	tr
Meeker R (11)	14.0	5.7	nd	tr	nd	nd	tr	tr	2.9	tr
Meeker OR (5)	33.4	6.2	nd	1.5	nd	nd	tr	tr	3.4	tr
Meeker OR (3)	10.1	13.9	tr	tr	nd	nd	tr	nd	3.3	nd
Meeker OR (10)	17.9	4.4	nd	tr	nd	nd	tr	tr	tr	tr
Meeker OR (15)	19.8	9.7	tr	tr	nd	nd	tr	tr	2.8	tr
Meeker R moldy (17)	tr	tr	nd	tr	nd	nd	tr	tr	2.7	tr
Willamette UR (18)	9.1	8.2	tr	tr	nd	nd	tr	tr	nd	tr
Willamette UR (20)	76.5	48.3	5.5	1.1	nd	nd	4.5	3.8	tr	3.3
Willamette R (34A)	63.2	18.4	7.8	3.6	nd	nd	tr	2.0	nd	3.4
Willamette R (34B)	82.3	23.5	5.5	5.7	nd	nd	tr	1.5	tr	4.9
Willam. R LP (34C)	59.7	49.5	12.3	3.2	nd	nd	2.1	4.7	tr	2.5
Willam. R LP (34D)	80.8	34.8	10.5	5.4	nd	nd	1.5	4.4	tr	5.2
Willamette OR (7)	40.1	28.9	3.0	3.1	nd	nd	tr	tr	nd	1.8
Willamette OR (4)	53.4	87.1	15.4	13.2	nd	nd	2.7	3.1	nd	1.9
Willamette OR (6)	53.6	59.5	8.5	4.8	nd	nd	4.5	2.9	nd	2.6
Willamette OR (9)	46.3	30.1	7.4	4.5	nd	nd	1.5	1.3	nd	2.6
Willamette OR (14)	23.4	67.4	13.4	3.5	nd	nd	2.1	1.7	nd	tr
Willamette OR (19)	3.0	110.6	8.5	5.8	11.5	nd	7.6	5.2	tr	3.0
Chilcotin R (1)	18.8	1.6	nd	tr	nd	nd	tr	tr	tr	nd
Skeena R (2)	1.2	3.6	nd	nd	nd	nd	tr	nd	nd	nd
Heritage R (32)	103.7	4.4	nd	9.4	nd	nd	3.3	tr	nd	nd
Heritage OR (31)	126.8	7.0	nd	12.6	nd	nd	5.0	tr	nd	nd
Mall. Prom. 1 (30)	106.7	6.9	tr	1.3	nd	nd	3.0	4.3	nd	7.0
Mall. Prom. 2 (29)	62.6	1.4	tr	tr	nd	nd	tr	tr	nd	2.2
Mall. Prom. 3 (24)	80.2	4.8	tr	tr	nd	nd	2.7	3.2	nd	3.8
Mall. Seedl. 1 (13)	79.4	2.3	tr	tr	nd	nd	tr	1.5	nd	3.0
Mall. Seedl. 2 (28)	72.2	4.8	tr	tr	nd	nd	tr	1.5	nd	1.5
Mall. Seedl. 3 (27)	27.1	7.7	tr	tr	nd	nd	1.6	1.9	nd	1.2
Norna 1 (26)	103.9	1.8	nd	nd	3.5	9.7	1.3	3.0	4.5	1.4
Norna 2 (22)	66.7	tr	nd	tr	3.1	5.8	1.1	3.3	2.7	tr
Norna 3 (21)	61.5	3.7	nd	tr	3.9	6.8	1.7	3.7	10.3	tr
Veten 1 (12)	49.3	tr	nd	tr	tr	2.3	tr	tr	1.2	tr
Veten 2 (23)	38.3	1.5	nd	tr	1.9	1.9	1.4	tr	3.3	tr
Veten 3 (25)	33.2	3.8	nd	tr	1.3	2.8	tr	tr	6.8	tr
Golden R (33)	88.4	nd	tr	tr	tr	nd	1.3	tr	tr	nd
Willam. STD (37D)	81.4	60.6	9.4	8.2	nd	tr	4.8	4.1	tr	3.6
Willam. VC (37E)	61.9	58.4	9.1	4.3	nd	nd	4.1	2.3	tr	2.8
Willam. OS8A (37A)	54.7	54.7	8.1	4.9	nd	tr	3.8	2.0	tr	1.9
Willam. OS26A (37B)	48.7	48.1	6.7	5.3	nd	tr	3.1	1.2	tr	2.4
Willam. OS26B (37C)	78.2	55.1	7.9	6.9	nd	tr	3.9	1.7	tr	2.2
Willam. CT (35A)	78.5	123.3	30.9	9.9	nd	nd	10.7	9.5	tr	5.8
Willam. CT ESP (35B)	63.7	61.0	21.4	8.5	nd	nd	19.2	6.8	nd	5.6
Willam. CT EBE (35C)	50.9	82.2	20.1	5.6	nd	nd	14.8	9.8	nd	3.6
Willamette DE (36)	37.3	64.9	11.9	11.1	nd	nd	3.0	1.8	nd	tr
A (38)	40.9	28.2	1.7	1.2	nd	tr	8.4	tr	4.7	1.9
B (39)	28.3	12.8	nd	3.2	nd	nd	1.7	tr	6.2	tr
C (40)	88.5	64.3	14.8	9.0	tr	nd	6.1	6.5	2.0	5.7
D (41)	71.9	67.9	14.0	6.3	tr	nd	6.7	3.3	tr	2.1
E (42)	25.3	8.0	nd	1.1	nd	nd	tr	nd	5.1	tr
F (43)	57.6	51.2	7.7	5.2	nd	nd	8.9	3.7	nd	2.4
G (44)	9.7	1.4	4.1	tr	tr	nd	tr	tr	2.5	tr
H (45)	13.9	3.7	1.3	tr	nd	nd	6.3	2.0	tr	tr
I (46)	46.4	1.8	tr	2.4	tr	tr	2.7	5.3	tr	2.2

UR-underripe; R-ripe; OR-overripe; LP-laboratory process; Mall.-Malling; Prom.-Promise; Seedl.-Seedling; STD-standard process; VC-vacuum concentration; OS8A, OS26A-osmotic concentration at 8°C 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; 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 quercetin-glycoside#5

Table III.1 (continued)

Cultivar (Sample No.)	Flavonol Compound (in ppm)								
	K3Guu	Q3Gal	QF5A	K3Glu	QU	KA	QUsum	KAsum	FlavSum
Meeker UR (8)	tr	tr	tr	tr	tr	tr	100.1	3.5	103.6
Meeker UR (16)	tr	tr	nd	nd	tr	tr	32.3	3.0	35.3
Meeker R (11)	tr	tr	tr	nd	tr	nd	30.6	2.0	32.6
Meeker OR (5)	tr	1.6	1.1	tr	tr	tr	53.9	3.0	56.9
Meeker OR (3)	tr	2.0	nd	tr	tr	2.0	34.1	3.0	37.1
Meeker OR (10)	tr	1.2	2.2	tr	tr	tr	31.6	3.0	34.6
Meeker OR (15)	tr	1.9	nd	tr	tr	tr	42.6	4.0	46.6
Meeker R moldy (17)	tr	tr	tr	nd	tr	tr	13.7	2.0	15.7
Willamette UR (18)	tr	tr	tr	nd	tr	nd	29.3	tr	30.3
Willamette UR (20)	2.0	tr	tr	nd	tr	tr	149.5	2.5	152.0
Willamette R (34A)	tr	tr	nd	nd	tr	tr	106.9	3.5	110.4
Willamette R (34B)	tr	tr	nd	nd	tr	tr	131.3	3.0	134.3
Willam. R LP (34C)	tr	tr	tr	nd	1.6	8.6	141.6	11.1	152.7
Willam. R LP (34D)	tr	tr	nd	nd	4.6	tr	152.7	3.0	155.7
Willamette OR (7)	tr	tr	tr	nd	tr	tr	83.8	2.5	86.3
Willamette OR (4)	tr	tr	nd	nd	2.2	tr	180.5	2.5	183.0
Willamette OR (6)	tr	tr	tr	nd	1.6	tr	142.0	3.0	145.0
Willamette OR (9)	tr	tr	tr	nd	tr	tr	99.3	2.0	101.3
Willamette OR (14)	tr	tr	tr	nd	tr	tr	118.6	2.0	120.6
Willamette OR (19)	tr	tr	tr	nd	3.4	nd	163.6	tr	164.6
Chilcotin R (1)	tr	tr	tr	nd	tr	tr	29.4	2.0	31.4
Skeena R (2)	tr	tr	tr	tr	tr	nd	10.8	2.0	12.8
Heritage R (32)	13.0	nd	nd	nd	tr	tr	127.3	16.0	143.3
Heritage OR (31)	15.6	nd	nd	nd	tr	tr	158.4	20.6	179.0
Mall. Prom. 1 (30)	1.9	tr	tr	nd	tr	tr	135.2	3.4	138.6
Mall. Prom. 2 (29)	tr	tr	tr	nd	tr	tr	77.2	3.0	80.2
Mall. Prom. 3 (24)	1.1	tr	tr	nd	tr	tr	101.6	2.6	104.3
Mall. Seedl. 1 (13)	tr	tr	tr	nd	tr	nd	95.3	1.5	96.8
Mall. Seedl. 2 (28)	tr	tr	tr	nd	tr	tr	89.5	2.5	92.0
Mall. Seedl. 3 (27)	tr	tr	tr	nd	tr	tr	49.0	3.0	52.0
Norna 1 (26)	12.3	6.7	12.8	tr	2.6	tr	155.1	17.3	172.4
Norna 2 (22)	5.9	8.6	9.7	tr	tr	2.0	110.1	9.9	120.0
Norna 3 (21)	4.3	9.9	9.6	tr	tr	tr	117.7	7.3	125.0
Veten 1 (12)	2.5	8.8	tr	tr	tr	nd	72.1	3.5	75.6
Veten 2 (23)	1.3	8.8	tr	tr	tr	tr	64.7	5.0	69.7
Veten 3 (25)	1.6	3.6	tr	tr	tr	tr	60.6	5.1	65.7
Golden R (33)	1.1	tr	tr	nd	tr	tr	112.1	5.1	117.2
Willam. STD (37D)	1.5	tr	tr	nd	4.8	tr	181.5	3.5	185.0
Willam. VC (37E)	tr	tr	2.4	nd	2.2	tr	151.9	3.0	154.9
Willam. OS8A (37A)	tr	tr	tr	nd	2.1	tr	138.5	1.5	140.0
Willam. OS26A (37B)	tr	tr	tr	nd	tr	tr	121.9	1.5	123.4
Willam. OS26B (37C)	2.0	tr	1.5	nd	tr	tr	166.5	2.5	169.1
Willam. CT (35A)	3.0	1.9	tr	nd	3.9	tr	278.6	7.0	285.5
Willam. CT ESP (35B)	4.3	tr	tr	nd	7.8	tr	198.4	9.2	207.7
Willam. CT EBE (35C)	2.6	1.5	tr	nd	5.1	tr	197.6	7.1	204.6
Willamette DE (36)	tr	tr	tr	nd	4.6	tr	137.6	3.0	140.6
A (38)	1.7	tr	1.9	nd	12.6	2.0	105.9	4.2	110.1
B (39)	tr	3.2	tr	nd	5.2	tr	66.0	2.0	68.0
C (40)	4.5	4.8	2.4	nd	3.8	2.0	211.3	6.0	217.3
D (41)	2.4	1.2	tr	nd	11.5	tr	188.9	3.9	192.8
E (42)	tr	1.4	2.4	nd	1.9	tr	48.2	2.5	50.7
F (43)	1.9	6.2	1.8	nd	6.1	tr	152.6	2.9	155.5
G (44)	tr	tr	tr	nd	5.8	2.0	31.2	4.0	35.2
H (45)	tr	tr	nd	nd	1.9	tr	36.1	2.5	38.6
I (46)	tr	tr	tr	nd	6.7	tr	75.9	2.0	77.9

(MeOH fraction); Q3Glu quercetin-3-glucoside; QG8M 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.

Table III.2. Mean Concentrations of Quercetin and Kaempferol-Glycosides, Glucuronides, and Other Forms in Experimental Raspberry Juices and Concentration-Ranges in Commercial Samples

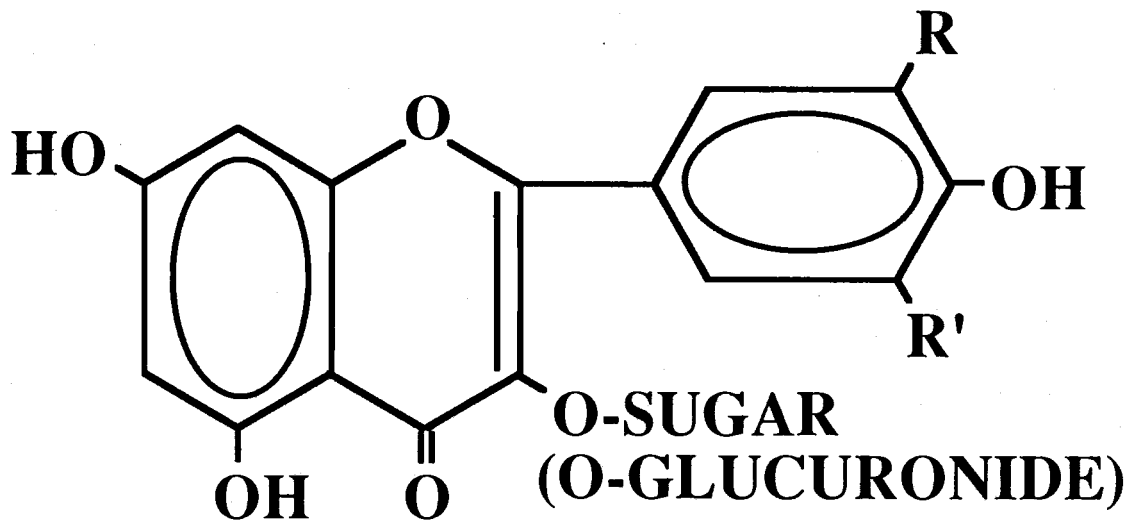
Flavonol Compound	**Concentration in Experimental Juices (ppm)**				**Concentration-Range in Commercial Juices (ppm; n=9)**
	n	Mean ± Std. Error	% Variance due to Difference Among Juices	Between Repl.	
Quercetin-3-glucuronide*	45	54.37 ± 4.48	64.2	35.8	9.72 - 88.51
Quercetin-glycoside #2 (3-sophoroside?)	44	29.21 ± 4.88	88.1	11.9	1.35 - 67.88
Quercetin-glycoside #4 (MeOH-fraction)	30	7.78 ± 1.32	75.7	24.3	n.d. - 14.76
Quercetin-form #2 (NH3-fraction)	43	3.86 ± 0.54	57.9	42.1	trace• - 8.96
Quercetin-glycoside #6 (MeOH-fraction)	8	3.41 ± 1.23	13.2	86.8	n.d. or trace
Quercetin-glycoside #5 (MeOH-fraction)	10	3.33 ± 0.97	64.0	36.0	n.d. or trace
Quercetin-3-glucoside*	45	2.99 ± 0.55	86.1	13.9	trace - 8.94
Quercetin-glycoside #8 (MeOH-fraction)	42	2.55 ± 0.33	58.3	41.7	trace - 6.46
Quercetin-glycoside #1 (MeOH-fraction)	26	2.48 ± 0.49	55.6	44.4	nd - 6.18
Quercetin-3-xylosylglucuronide*	39	2.43 ± 0.25	56.8	43.2	trace - 5.72
Kaempferol-3-glucuronide*	45	2.31 ± 0.48	96.4	3.6	trace - 4.50
Quercetin-3-galactoside*	43	2.03 ± 0.38	77.0	23.0	trace - 6.18
Quercetin-form #5 (NH3-fraction)	36	1.90 ± 0.46	93.9	6.1	n.d. - 2.37
Quercetin* (sum, both fractions)	45	1.75 ± 0.23	73.8	26.2	1.89 - 12.59
Kaempferol* (sum, both fractions)	39	1.25 ± 0.20	3.0	97.0	trace - 2.00
Sum, all Above Quercetin-forms	45	118.09 ± 8.47	77.1	22.9	31.15 - 211.31
Sum, all Above Kaempferol-forms	45	3.55 ± 0.64	79.2	20.8	2.00 - 6.00
Sum, all Above Flavonols	45	121.64			

*reported previously for fresh berries by Ryan and Coffin (1971) and/or Henning (1981); •trace ≤ 1ppm.

Table III.3. Quercetin and Kaempferol-forms Present in Trace Amounts in Raspberry Juices in Both Fractions•

Quercetin-glycoside #3 (M)	5 experimental samples, 1 commercial sample
Quercetin-glycoside #7 (M)	2 commercial samples
Quercetin-form #1 (A)	1 Willamette (overripe) sample
Quercetin-form #3 (A)	4 Willamette, 2 (of 3) Malling Seedling samples
Quercetin-form #6 (A)	Golden (4.4 ppm); centri. (1.7 ppm); 24 exp., 6 comm. spls.
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* (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 Will., 1 comm. sample
Kaempferol-form #1 (A)	all centri., 1 Meeker (OR), 2 Will. samples, 1 comm. sample
Kaempferol-form #2 (A)	all centrifugation, all Heritage samples
Kaempferol-form #3 (A)	centrifugation + SP-enzyme (1.4 ppm); centr.+ 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
Flavonolglycoside #1 (M)	2 commercial samples
Flavonolglycoside #2 (M)	1 commercial sample
Flavonolglycoside #3 (M)	29 experimental, 6 commercial samples
Flavonolglycoside #4 (M)	29 experimental, all comm. samples (COM-H-66: 44.4 ppm)
Flavonolglycoside #5 (M)	24 experimental, 6 commercial samples
Flavonolglycoside #6 (M)	1 osmosis sample; diffusion extraction; 1 comm. sample
Flavonolglycoside #7 (M)	all Heritage samples
Flavonolglycoside #8 (M)	26 experimental, 8 commercial samples
Flavonolglycoside #9 (M)	22 experimental, 4 commercial samples
Flavonolglycoside #10 (M)	all Heritage, 2 Malling samples, 1 commercial sample

*reported previously for fresh raspberries by Henning (1981); •trace ≤ 1 ppm; M, methanol fraction; A, ammonia/methanol fraction.



R = R' = H KAEMPFEROL
R = OH R' = H QUERCETIN

Figure III.1. Structures of kaempferol and quercetin-glycosides and glucuronides

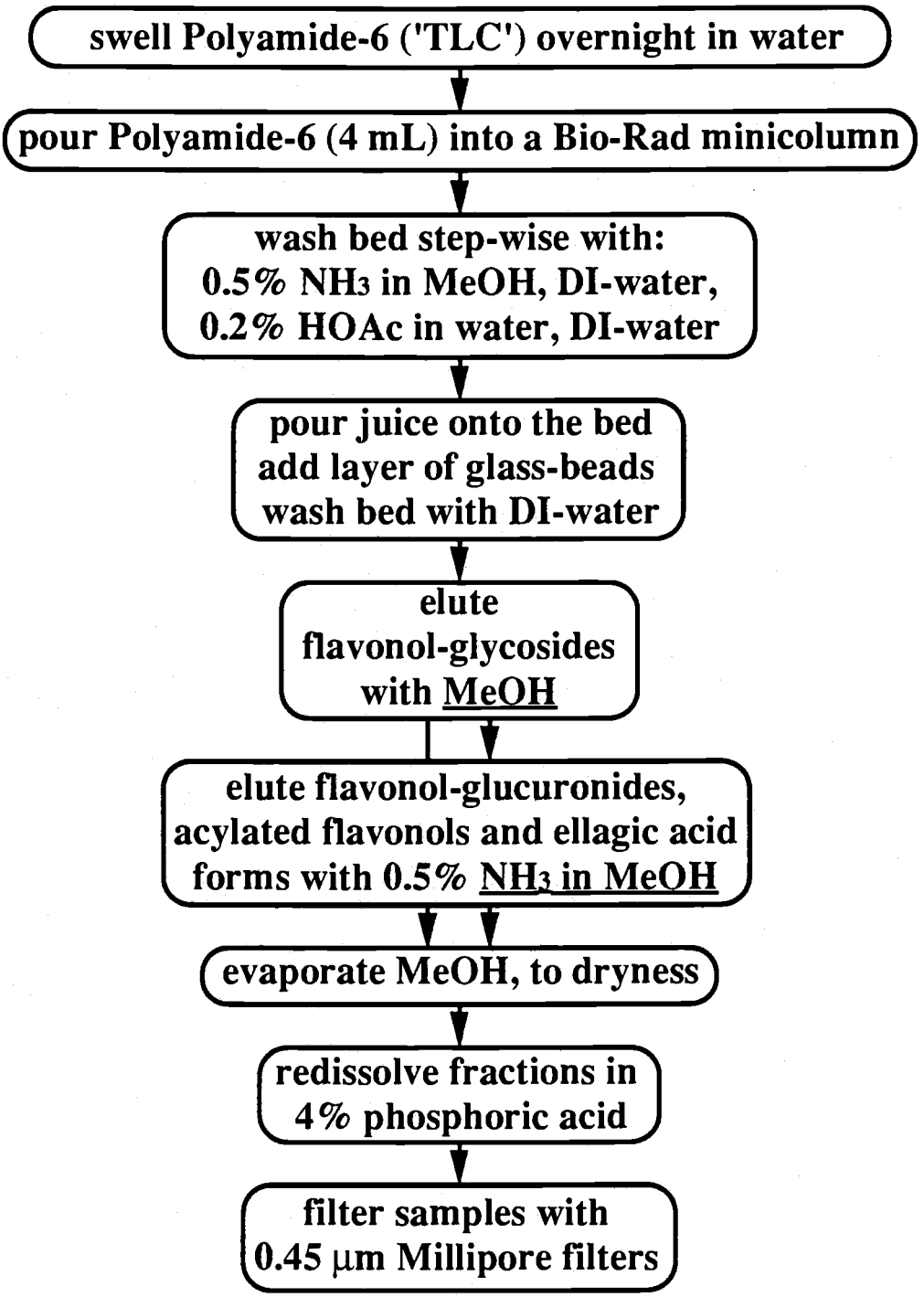


Figure III.2. Flow diagram of juice sample preparation for HPLC-analysis (procedure adapted from Wald and Galensa (1989) and Henning and Herrmann (1980); DI, de-ionized).

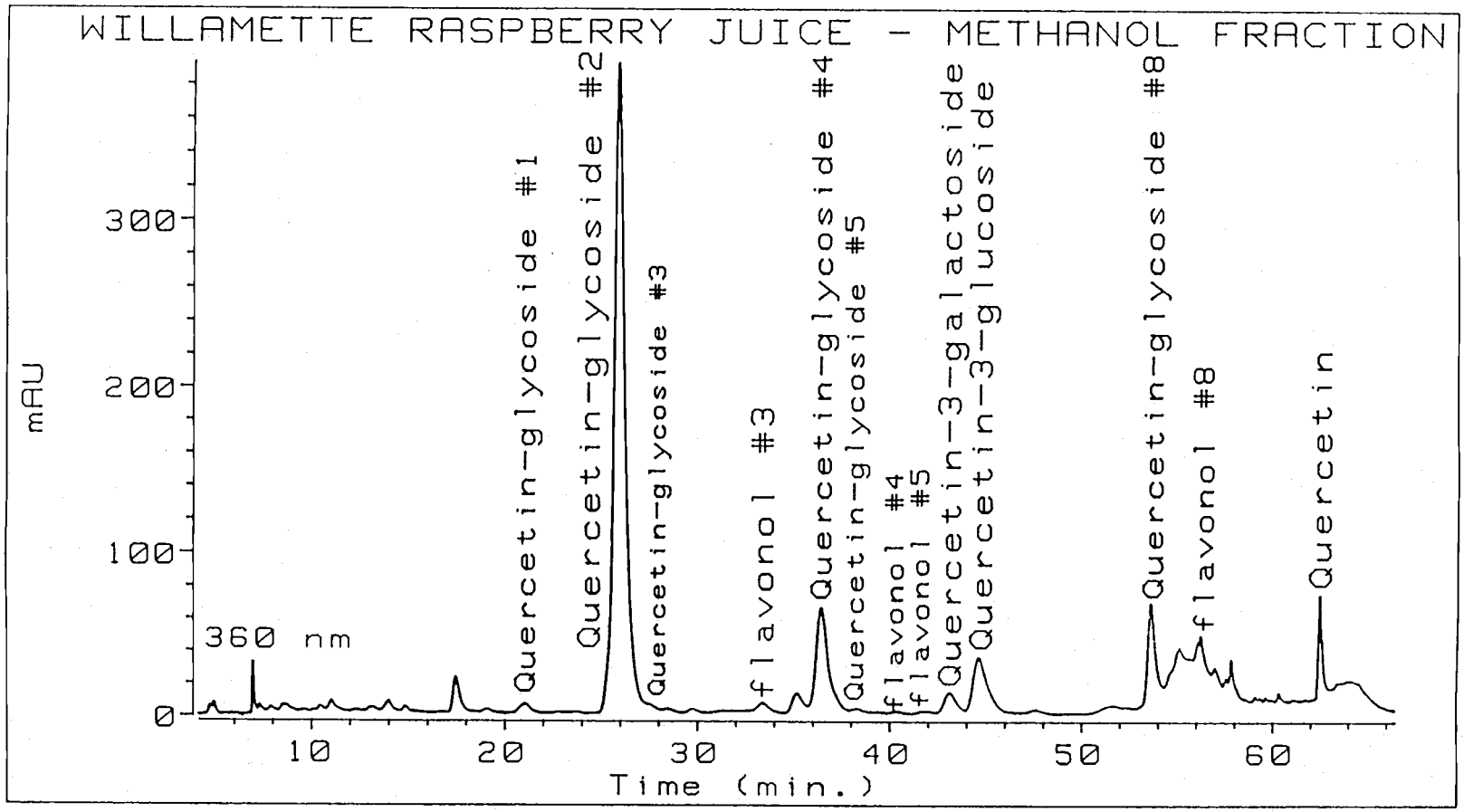


Figure III.3a. HPLC chromatogram of the methanol fraction (separated on Polyamide-6) of red raspberry juice made from ripe Willamette cultivar. Flavonol-glycosides and aglycons were detected at 360 nm.

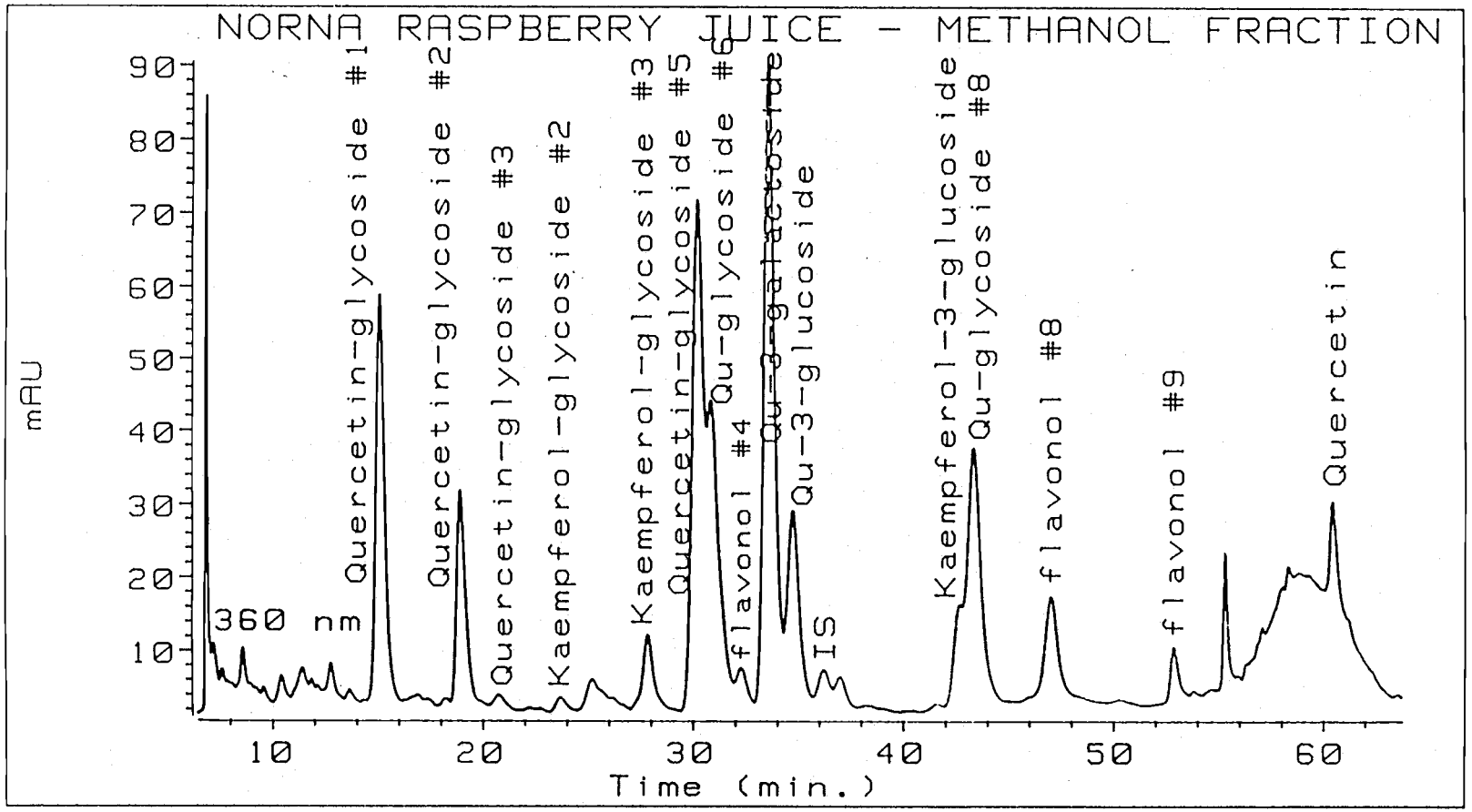


Figure III.3b. HPLC chromatogram of the methanol fraction (separated on Polyamide-6) of red raspberry juice made from ripe Norna cultivar. Flavonol-glycosides and aglycons were detected at 360 nm.

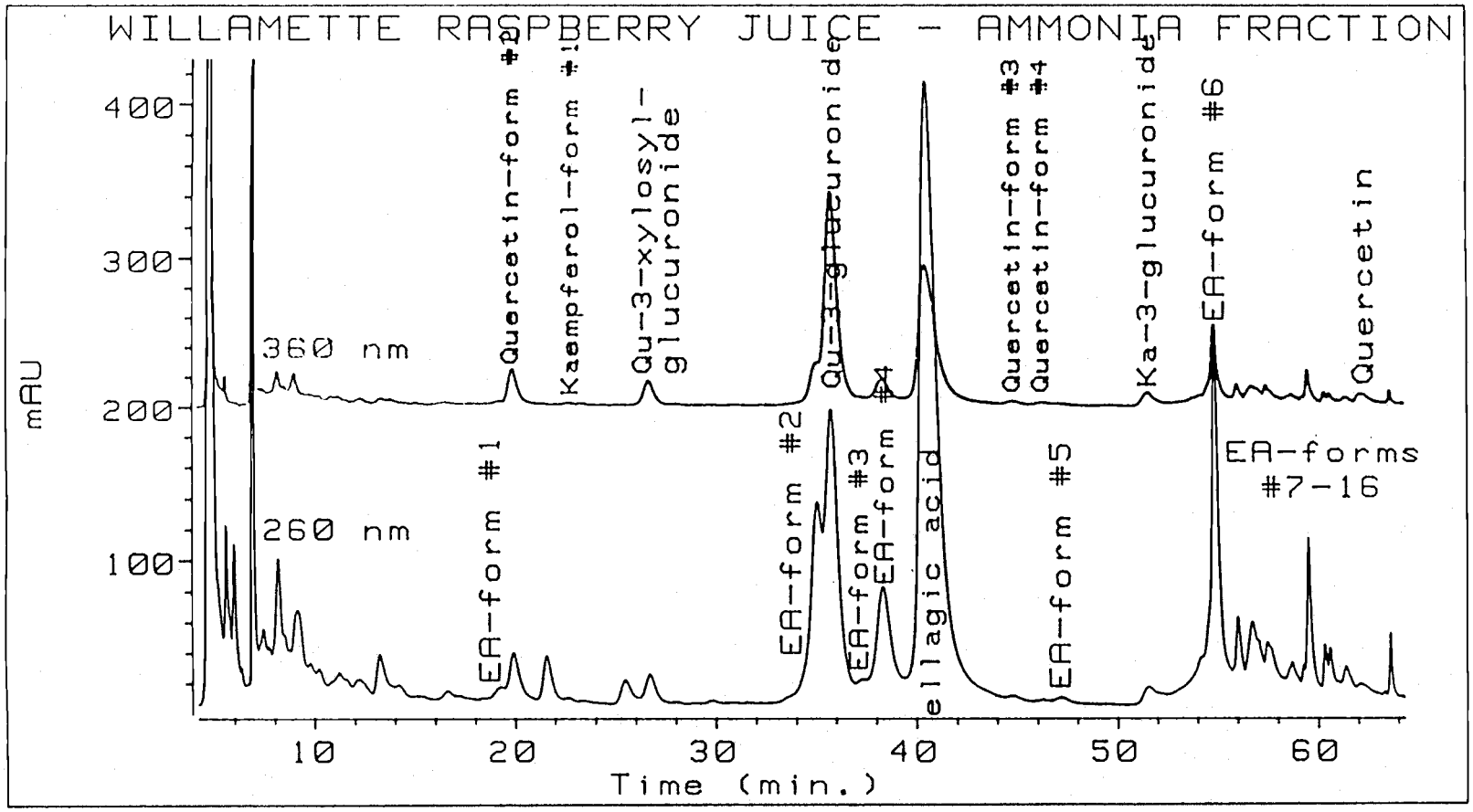


Figure III.4a. HPLC chromatogram of the ammonia/methanol fraction (separated on Polyamide-6) of red raspberry juice made from ripe Willamette cultivar. Ellagic acid and ellagic acid derivatives were detected at 260 nm; flavonol-glucuronides, acylated flavonol-glycosides, and flavonol-aglycons were detected at 360 nm.

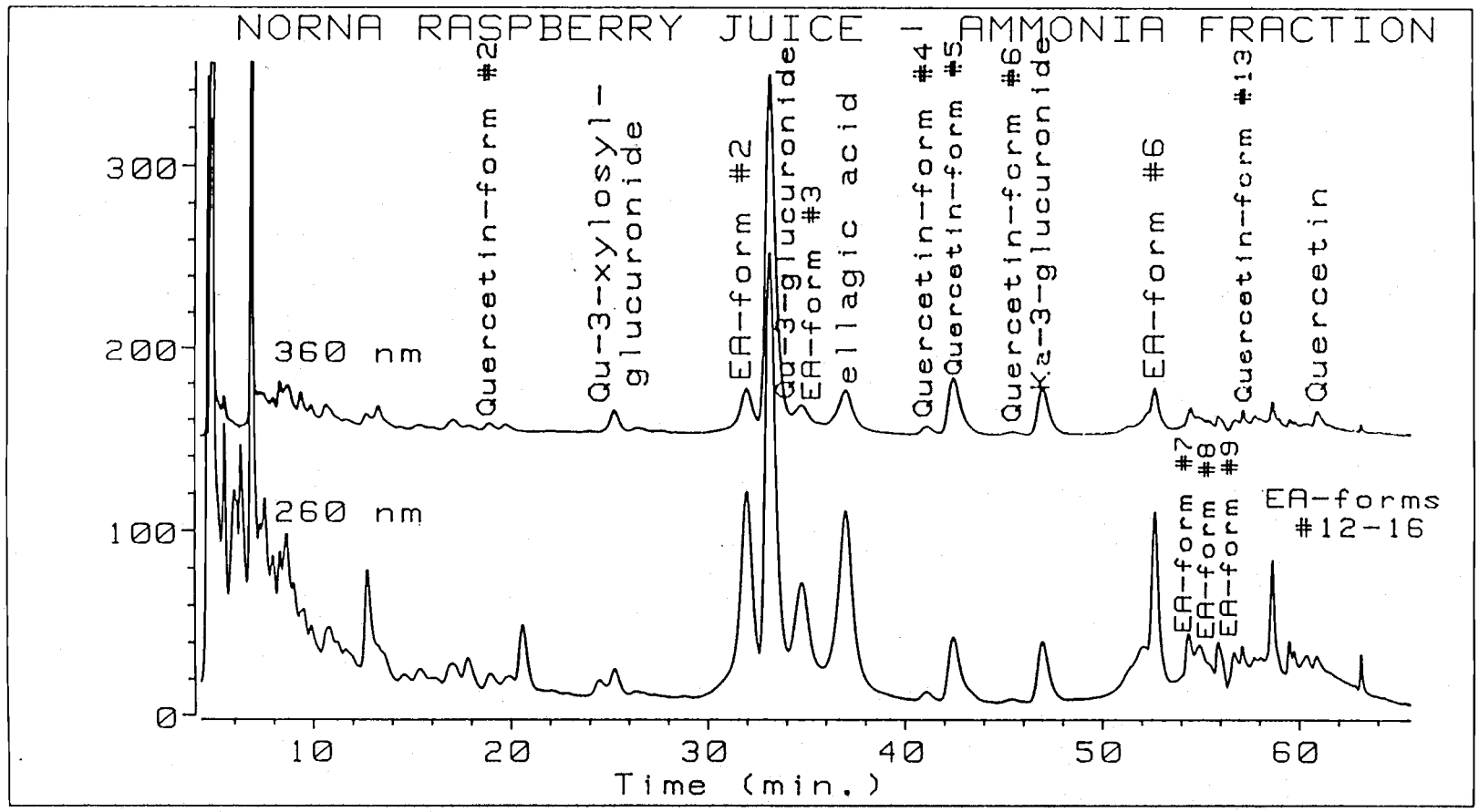


Figure III.4b. HPLC chromatogram of the ammonia/methanol fraction (separated on Polyamide-6) of red raspberry juice made from ripe Norna cultivar. Ellagic acid and ellagic acid derivatives were detected at 260 nm; flavonol-glucuronides, acylated flavonol-glycosides, and flavonol-aglycons were detected at 360 nm.

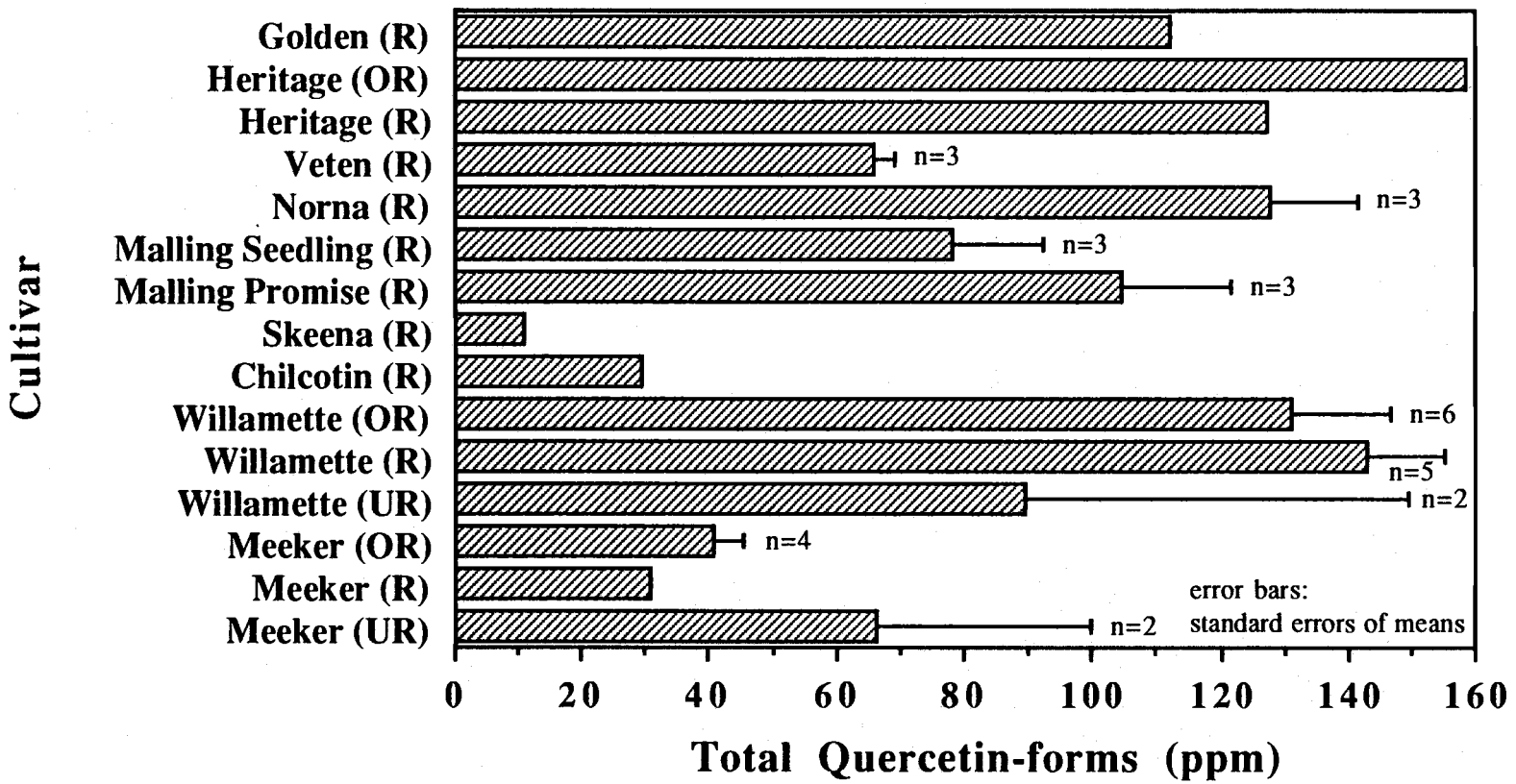


Figure III.5a. Total concentrations of quercetin-forms in juices made from different raspberry cultivars (UR-underripe, R-ripe, OR-overripe).

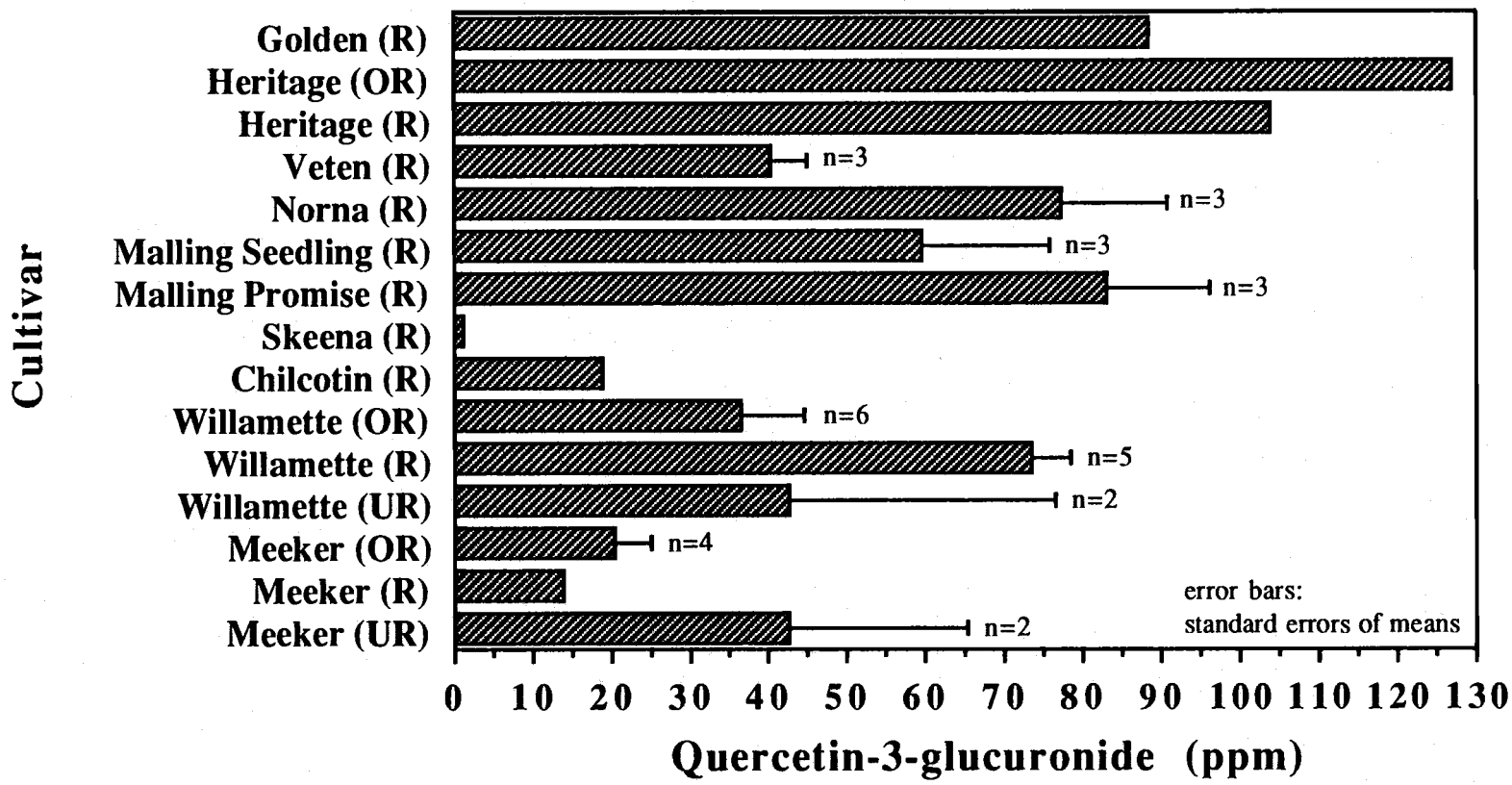


Figure III.5b. Concentrations of quercetin-3-glucuronide in juices made from different raspberry cultivars (UR-underripe, R-ripe, OR-overripe).

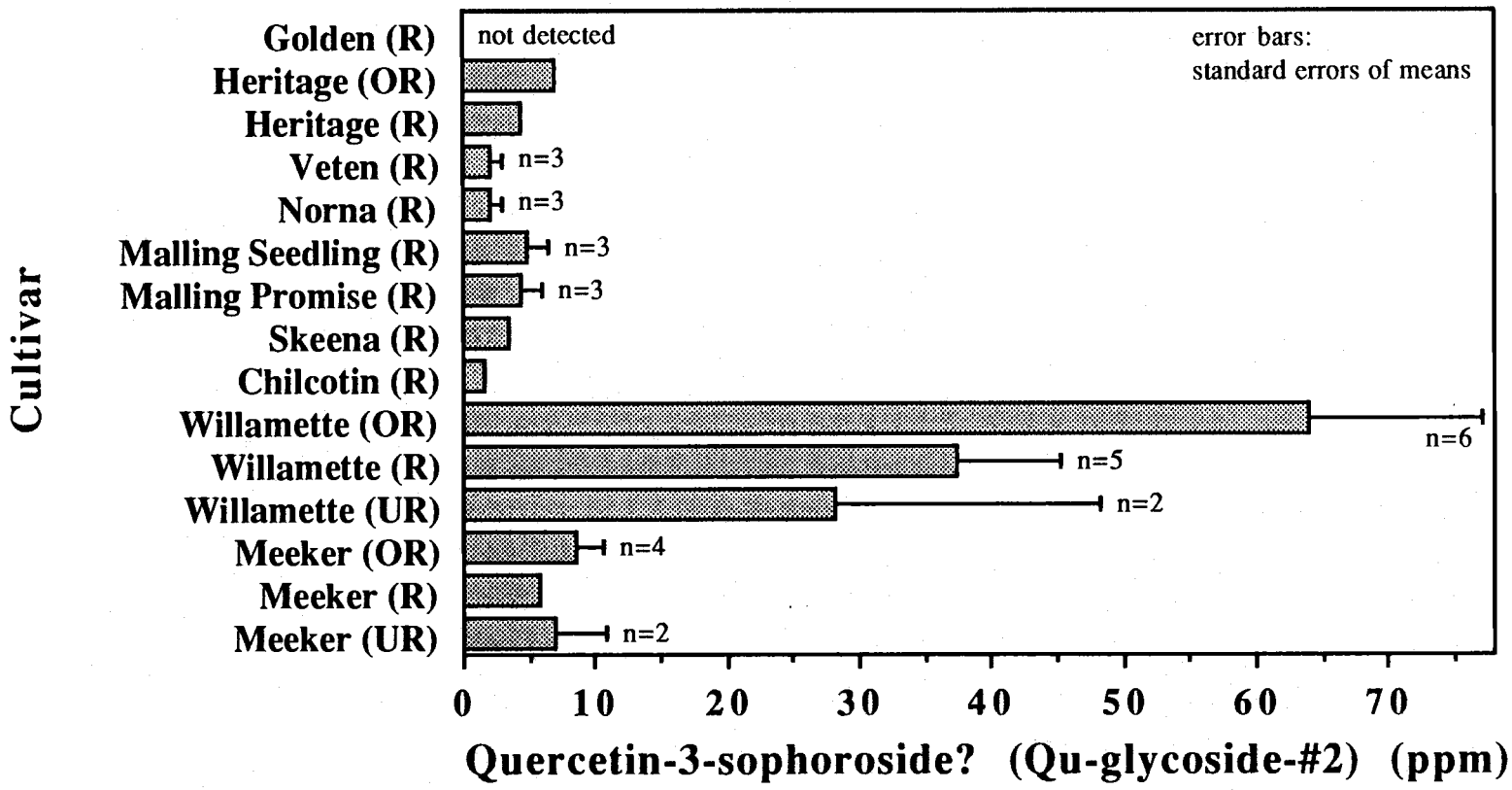


Figure III.5c. Concentrations of quercetin-glycoside #2 in juices made from different raspberry cultivars (UR-underripe, R-ripe, OR-overripe).

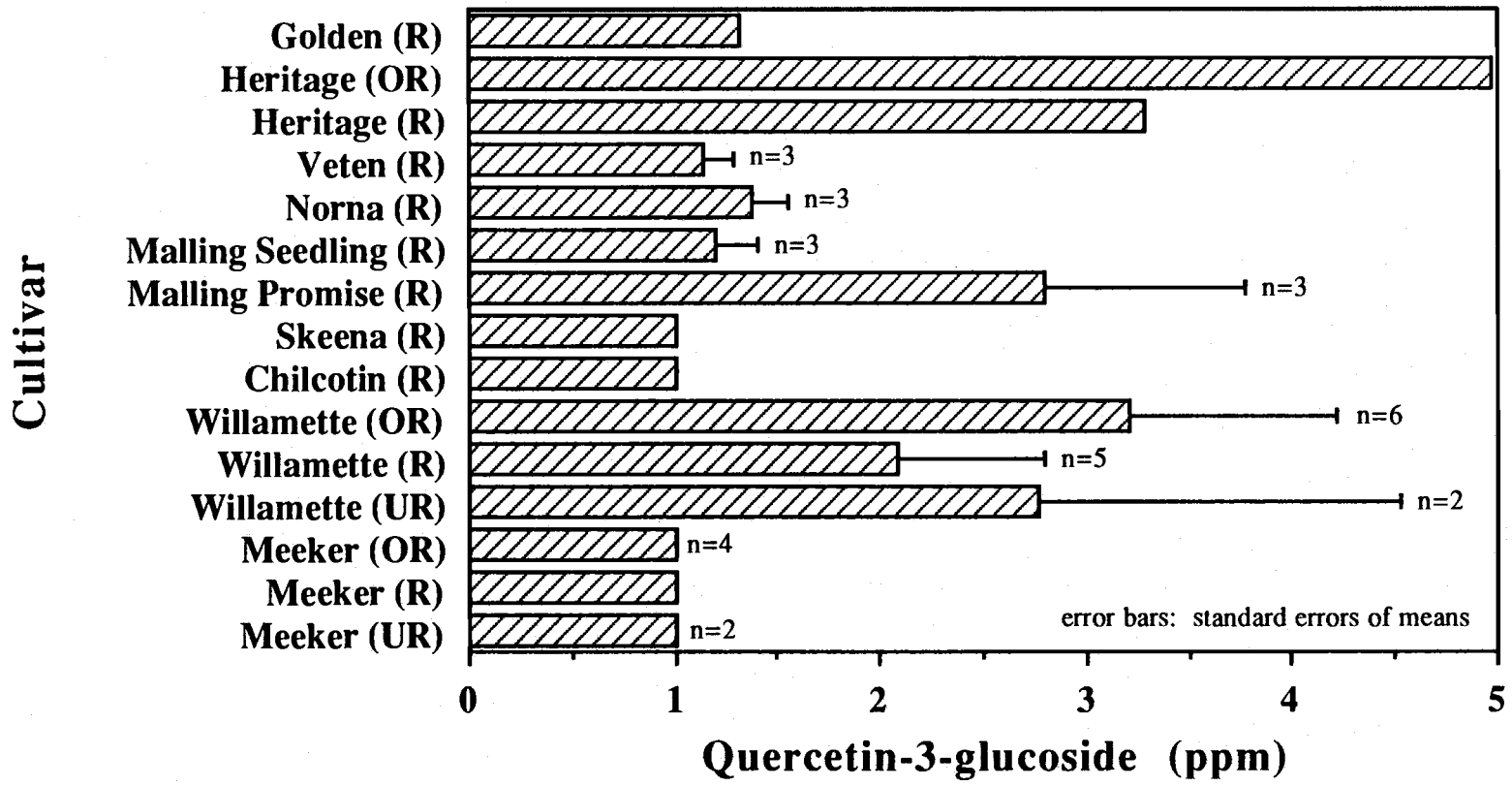


Figure III.5d. Concentrations of quercetin-3-glucoside in juices made from different raspberry cultivars (UR-underripe, R-ripe, OR-overripe).

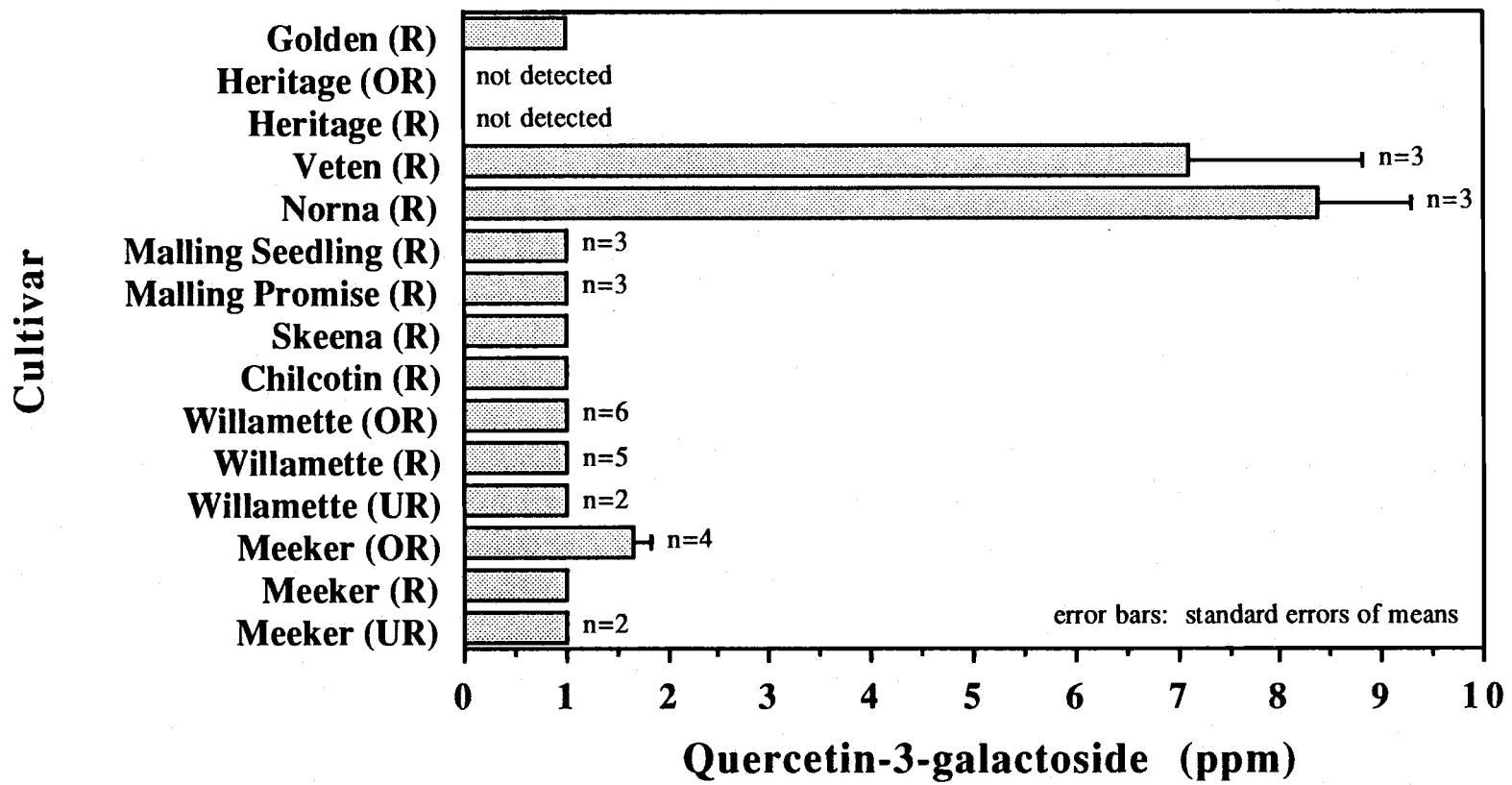


Figure III.5e. Concentrations of quercetin-3-galactoside in juices made from different raspberry cultivars (UR-underripe, R-ripe, OR-overripe).

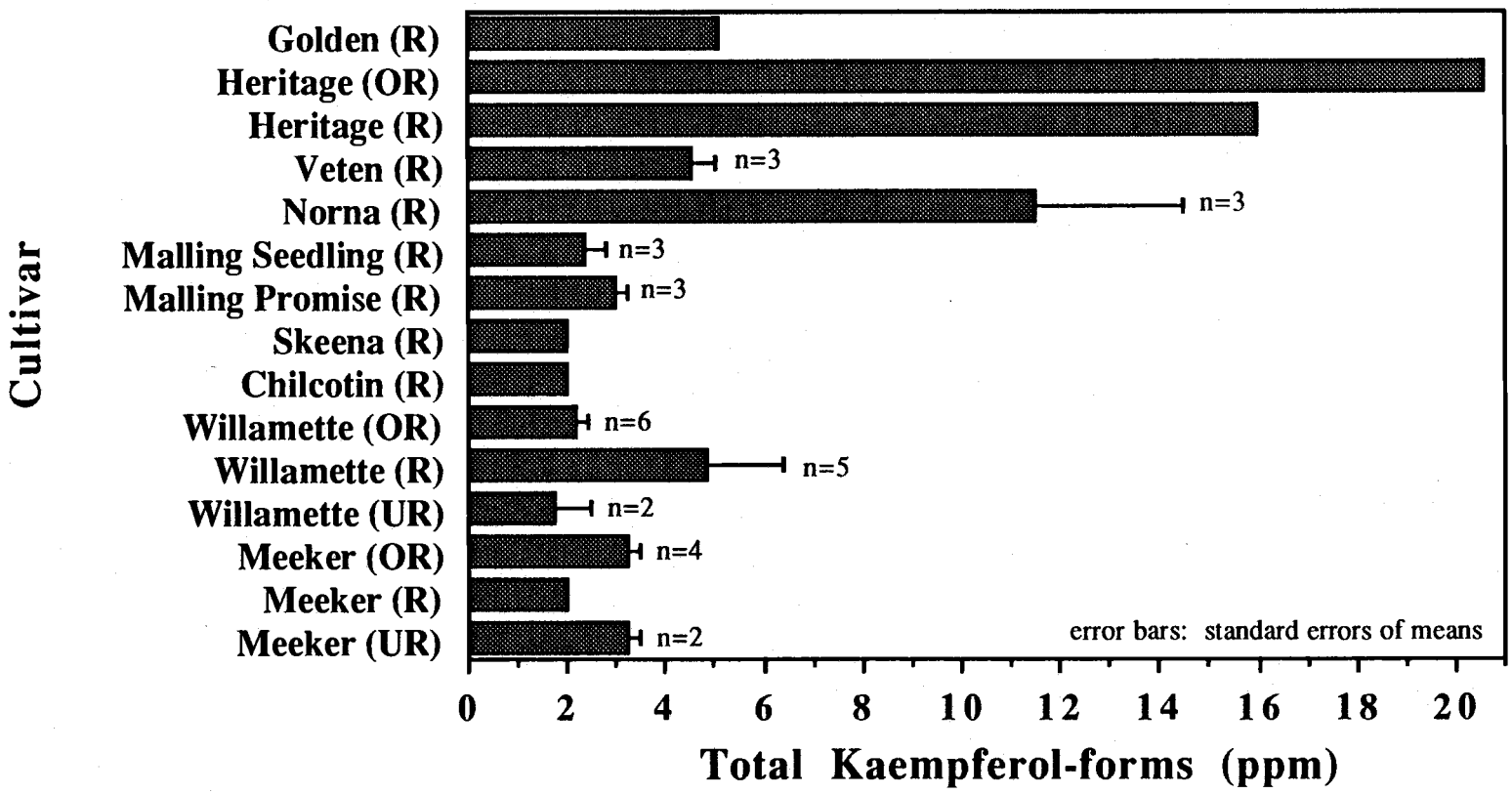


Figure III.6. Total concentrations of kaempferol-forms in juices made from different raspberry cultivars (UR-underripe, R-ripe, OR-overripe).

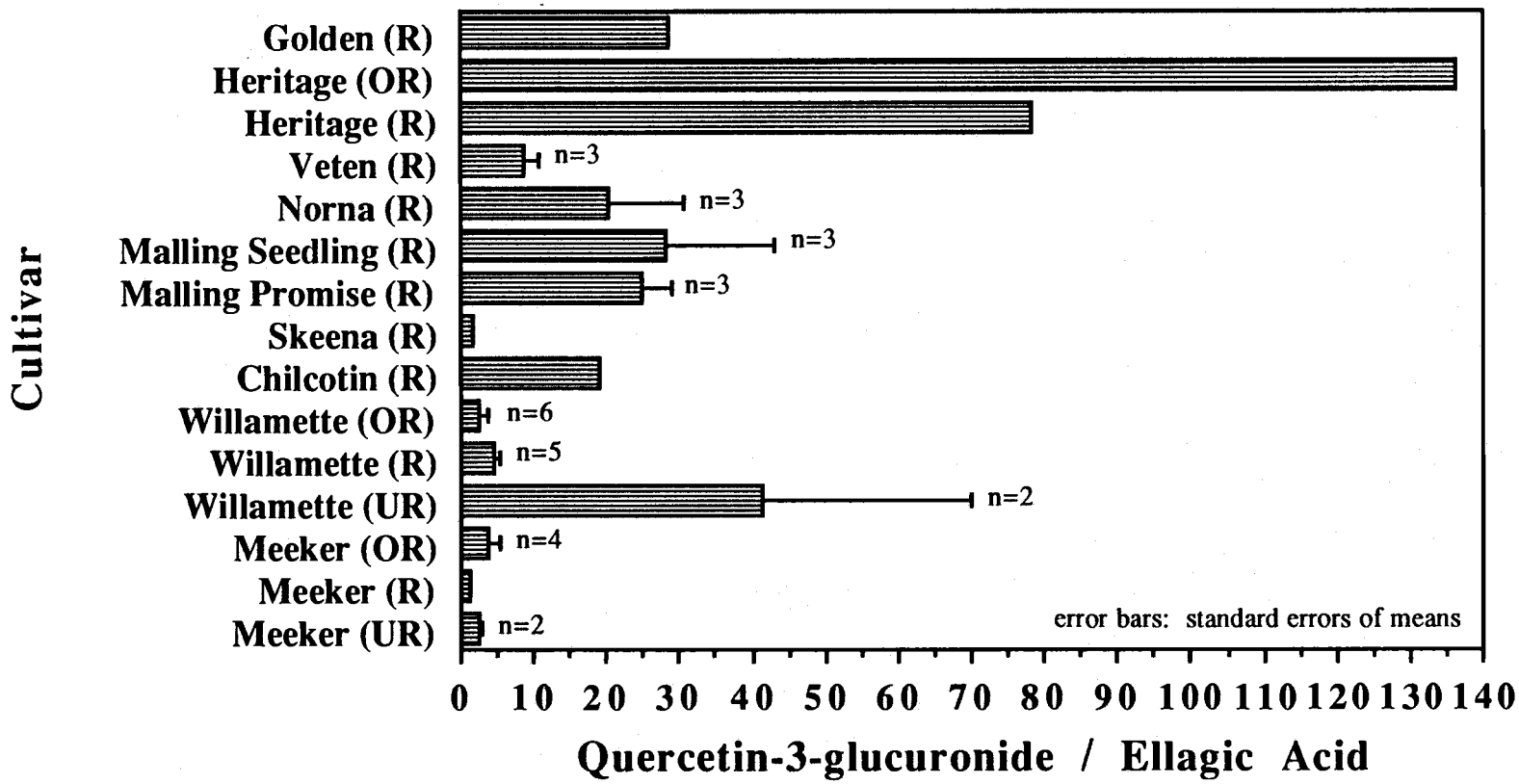


Figure III.7. Ratios of quercetin-3-glucuronide to ellagic acid for juices made from different raspberry cultivars (UR-underripe, R-ripe, OR-overripe).

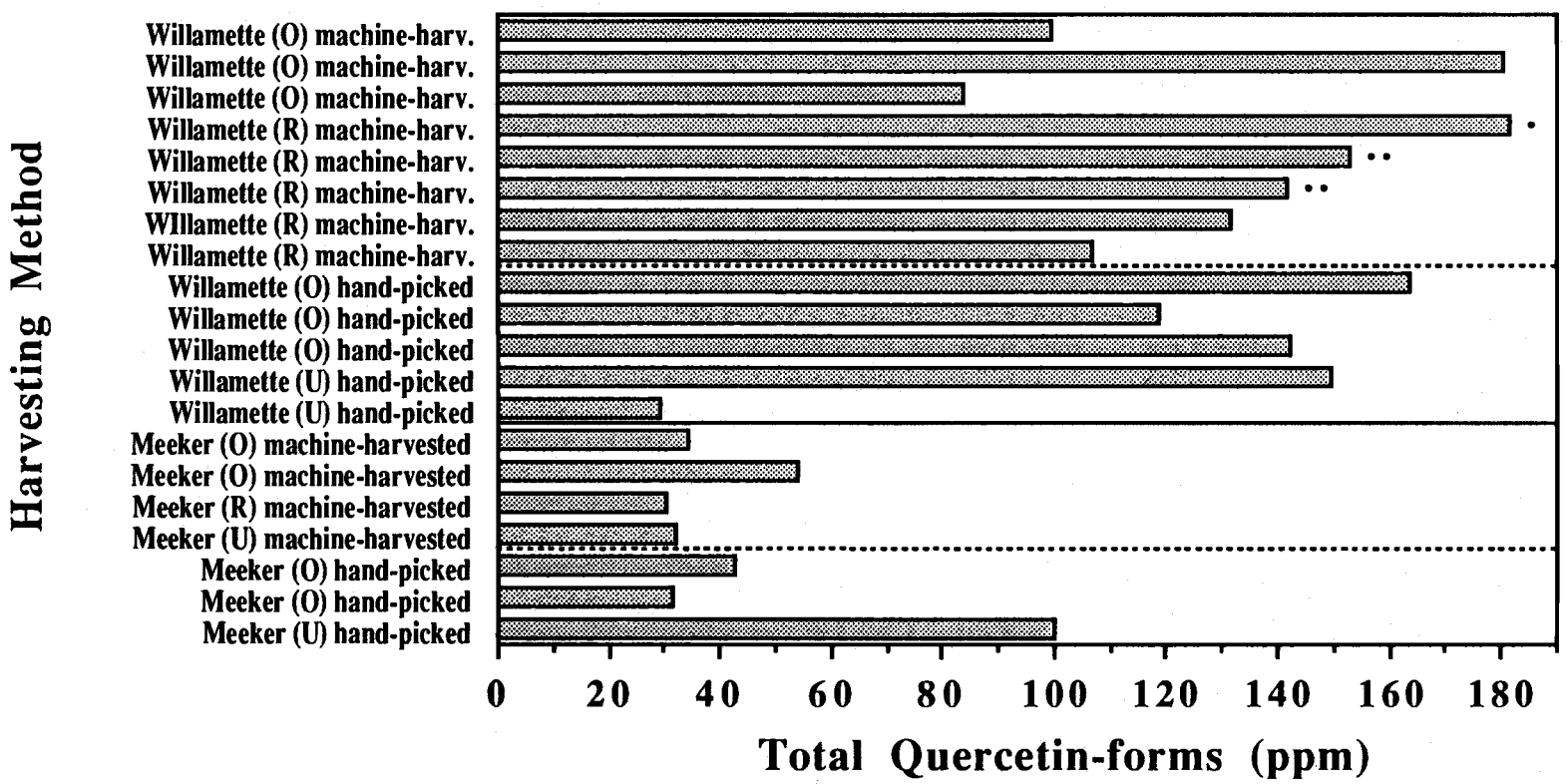


Figure III.8. Total concentrations of quercetin-forms in juices made from raspberries picked by hand or harvested by machines (U-underripe, R-ripe, O-overripe, •large batch, ••standard process simulated in the laboratory).

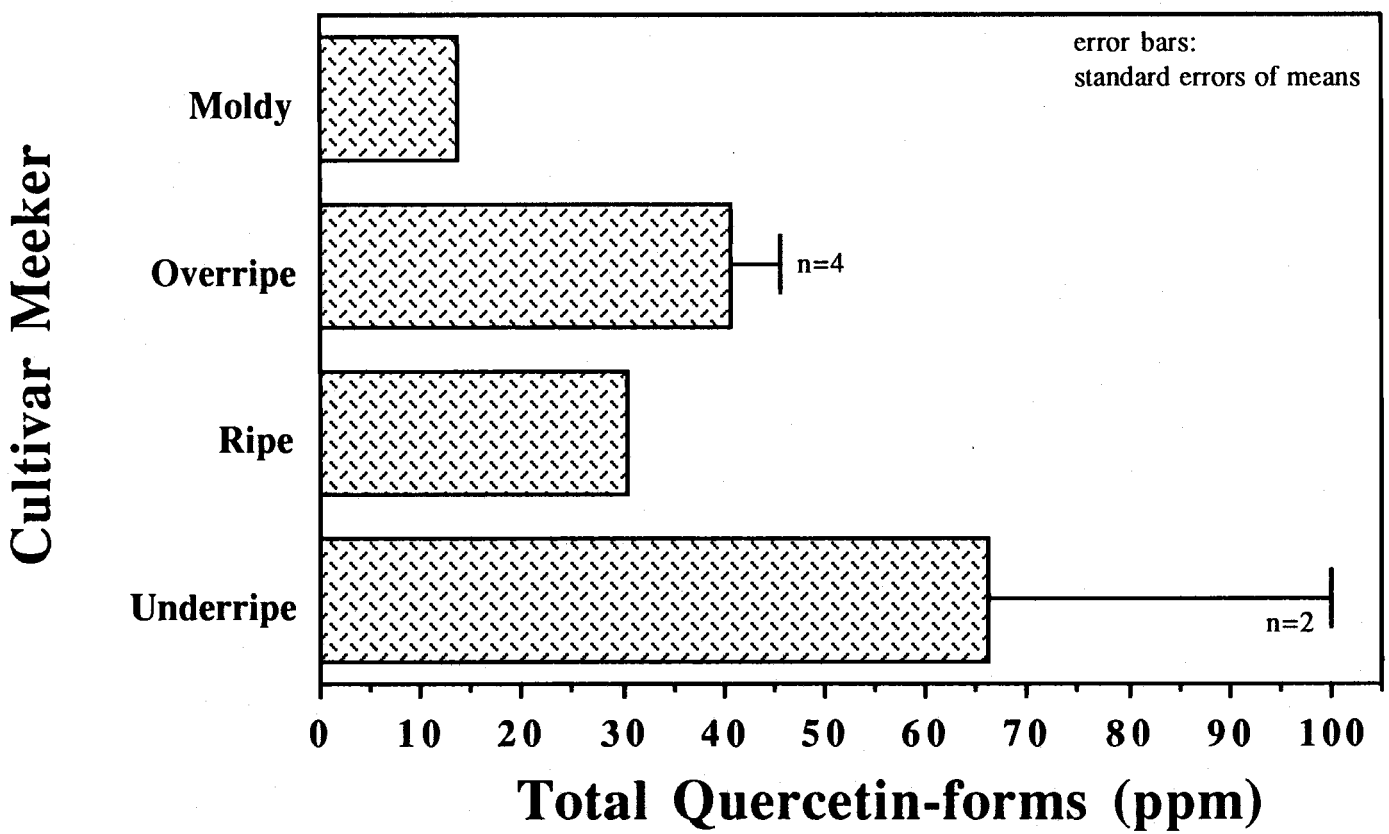


Figure III.9. Effect of mold-contamination on total concentration of quercetin-forms in raspberry juices made from the cultivar Meeker.

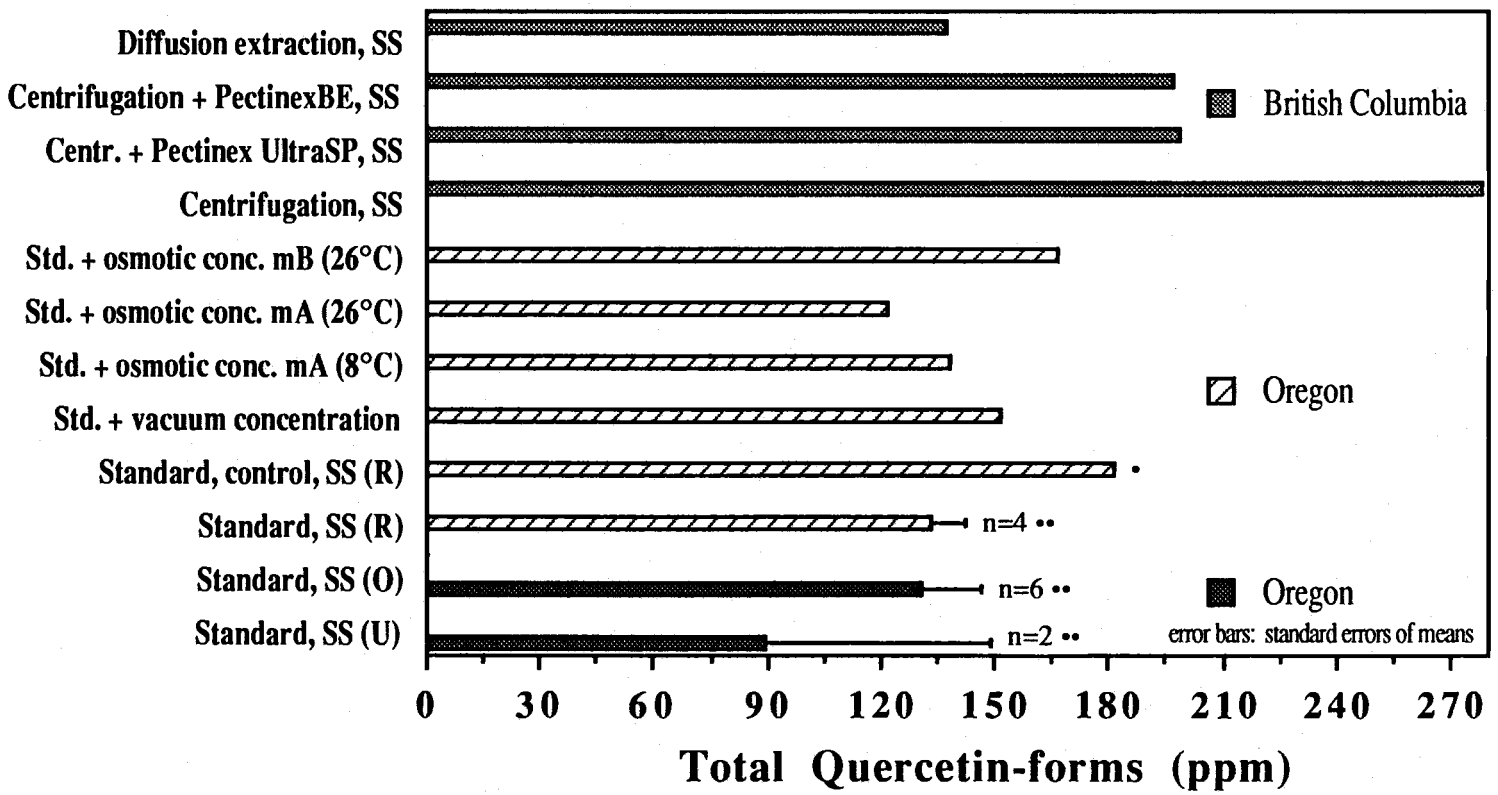


Figure III.10. Effects of different processing techniques on total concentration of quercetin-forms in raspberry juices made from the cultivar Willamette (m-membrane, SS-single strength, UR-underripe, R-ripe, OR-overripe, •large batch, ••small batch).

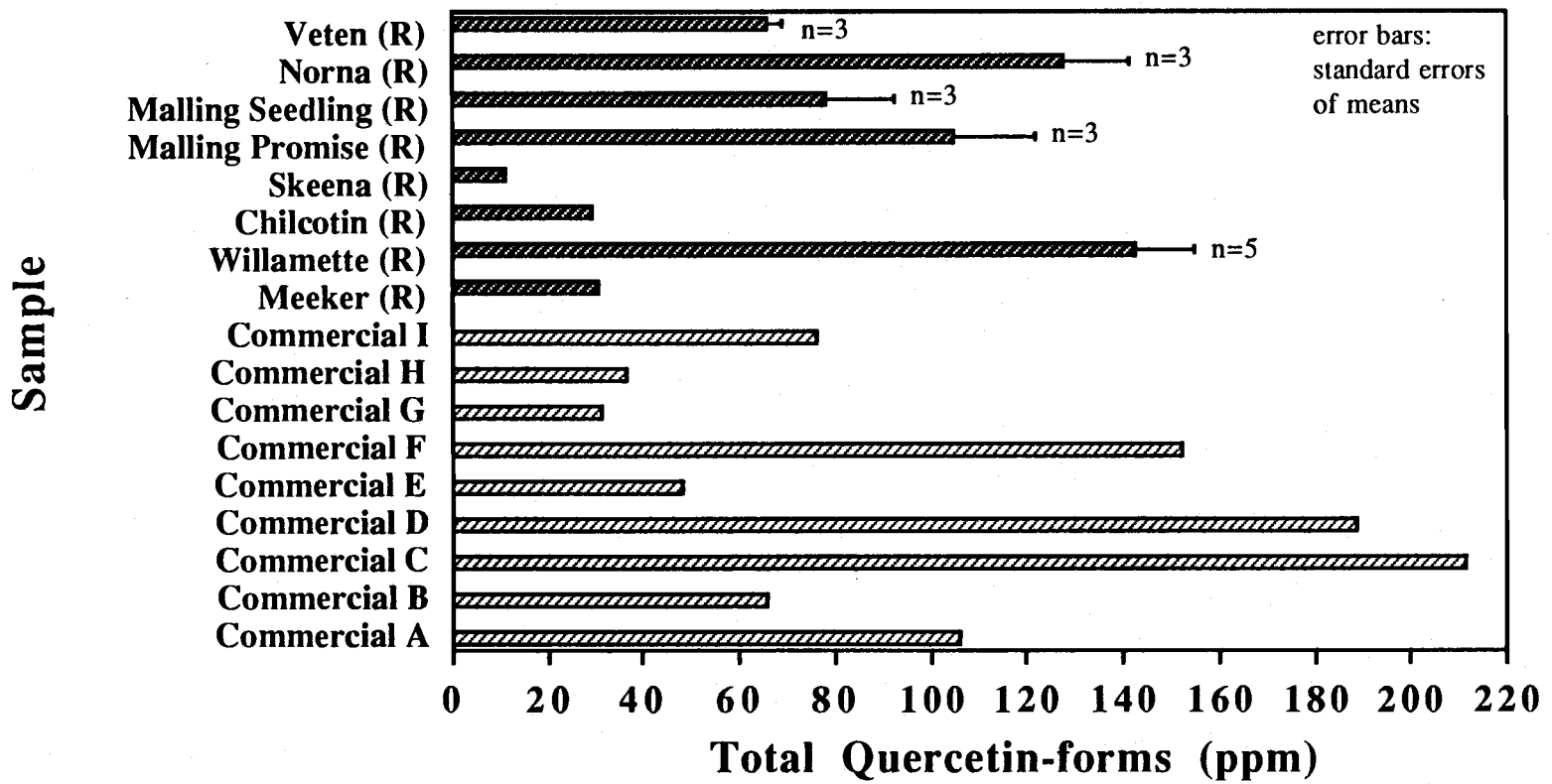


Figure III.11. Total concentrations of quercetin-forms in commercial and experimental raspberry juices. (Commercial juices were made by diluting concentrates to soluble solids contents of 10°Brix.)

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IV. The Influence of Acid and Base Hydrolysis on the Phenolic Composition of Red Raspberry Juice

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ABSTRACT

Acid and base hydrolysis simplified the complex HPLC-chromatographic profile of red raspberry juice phenolics dramatically. In 3 acid-hydrolyzed juices, ellagic acid, two unidentified ellagic acid compounds, and gallic, p-hydroxybenzoic, protocatechuic, caffeic, p-coumaric and ferulic acids, as well as (+)-catechin, (-)-epicatechin, quercetin, and kaempferol were separated and identified using HPLC/diode array spectral techniques. The percentages of HPLC peak area for these phenolics were similar in the acid-hydrolyzed juices. In base-hydrolyzed samples the same phenolics, except the second ellagic acid compound, were present, and the first ellagic acid compound was present in smaller quantity. Sep-Pak C_{18}-cartridges were used for sample preparation. Hydrolysis could become a very useful tool for rapidly screening raspberry juices for qualitative deviations from authentic HPLC profiles, in order to determine adulteration by other fruit juices or phenolic mixes.

INTRODUCTION

It is of great importance to the food industry and regulatory agencies to guarantee the authenticity of red raspberry juices and concentrates, which are expensive and therefore targets for adulteration. Red raspberries and products made thereof are also of interest because of the anticarcinogenic effects of their phenolic constituents, particularly quercetin and ellagic acid. The health effects of these phenolics are reviewed in Rommel and Wrolstad (manuscripts in prep. a&b).

Secondary plant metabolites (e.g. flavonoids and other phenolics) are very suitable as authenticity indicators because they are present in plants within a finite range (Herrmann, 1976). Anthocyanins, the red pigments and major flavonoids of raspberries, have been used mainly to determine authenticity (Spanos and Wrolstad, 1987), however, they do not always provide sufficient evidence for adulteration. Additional phenolic authenticity indicators such as non-anthocyanin flavonoids (e.g. flavonols, catechins), benzoic and cinnamic acids are needed. However, the phenolic composition of red raspberries is very complex; flavonols (Henning, 1981; Rommel and Wrolstad, manuscript in prep. b), ellagic acid (Daniel et al., 1989; Maas et al., 1991; Rommel in Wrolstad, manuscript in prep. a; Wilson and Hagerman, 1990), cinnamic and benzoic acids (Herrmann, 1989; Schuster and Herrmann, 1985) are present in raspberries mainly as glycosides or esters. Acid and/or base hydrolysis transforms glycosylated and esterified phenolics into their aglycons (Hong and Wrolstad, 1986; Markham, 1982) and so reduces the number of raspberry juice phenolics significantly. A simplified HPLC phenolic profile of raspberry juice would facilitate more rapid screening of samples for adulteration and would also make it easier to determine total amounts of phenolic aglycons for the evaluation of health effects of raspberry juices.

The flavonol and ellagic acid compositions of red raspberries are reviewed in Rommel and Wrolstad (manuscripts in prep. a&b). The most common benzoic acid compounds are derivatives of the hydroxybenzoic acids p-hydroxybenzoic, protocatechuic, vanillic, and gallic acids (Figure IV.1); salicylic, syringic and gentisic acids are less common (Goodwin and Mercer, 1983; Herrmann, 1989; Spanos, 1988). Most hydroxybenzoic acids are present in the form of glucosides and some as esters with glucose; however, gallic acid is mainly esterified to quinic acid or catechins and usually present in polymeric forms as soluble tannins, i.e. condensation products (Goodwin and Mercer, 1983; Herrmann, 1989). The hydroxybenzoic acid glucosides and esters which have been quantified in red raspberries (Herrmann, 1989; Schuster and Herrmann, 1985) are summarized in Table

IV.1. In raspberries the content of p-hydroxybenzoic acid-ß-D-glucoside is distinctly higher than in other rosaceaous fruits (Schuster and Herrmann, 1985). Swain et al. (1985) report presence of high concentrations of salicylic acid in raspberries; however, Herrmann (1989) considers trace amounts more realistic. Non-hydroxylated benzoic acid compounds, such as esters with glucose (e.g. 6-benzoylglucose), also occur in fruits in trace amounts (Herrmann, 1989). Presence of free hydroxybenzoic acids can result from hydrolysis of flavonoids (e.g. anthocyanins) and of hydroxybenzyl-glucosinolates using alkali or non-specific enzymes (Herrmann, 1989), during extraction from plants or juice processing.

Hydroxycinnamic acid compounds (Figure IV.2) are almost exclusively derived from the widespread p-coumaric, caffeic, and ferulic acids (Herrmann, 1989; Spanos, 1988). These acids may be bound to cell wall polymers (Herrmann, 1989), however, they occur in plants most frequently as simple esters with quinic acid (cyclitol) or glucose and also with carboxylic acids (e.g. malic, tartaric, galactaric; Herrmann, 1989; Schuster and Herrmann, 1985). The range of esters of the hydroxycinnamic acids is far greater than that of any other plant phenols (Goodwin and Mercer, 1983). The hydroxycinnamic acid glucosides and esters which have been quantified in red raspberries (Herrmann, 1989; Schuster and Herrmann, 1985) are tabulated in Table IV.1. Natural forms of cinnamic acids apparently have the trans-configuration but tend to isomerize to cis with the exposure to UV-light during extraction from plants; oxidation in the O-position also occurs as an artifact (Herrmann, 1989; Spanos, 1988). Amides of hydroxycinnamic acids have been identified in the reproductive organs of raspberries, but are absent from the green parts, petals and sepals (Herrmann, 1989).

Flavan-3-ols (or 'catechins') and polymeric proanthocyanins (procyanins or condensed tannins) are the most abundant phenolic compounds besides hydroxycinnamic acid esters in fruits found in cool to moderate climates; they are not found in citrus fruits or in vegetables (Herrmann, 1974). Both monomeric 'catechins' and dimeric procyanidins (flavan-3-ol and

flavan-3,4-ol dimers) are usually not glycosylated or esterified in plants (Herrmann, 1974). (-)-Epicatechin is the most important 'catechin' in most fruits; however, (+)-gallocatechin is the primary 'catechin' in peaches, and some plums, strawberries, currants and gooseberries (Herrmann, 1974). If at all present, (+)-gallocatechin and (-)-epigallocatechin do not occur until fruit ripeness. Blackberries, raspberries and strawberries contain mainly (-)-epicatechin and (+)-catechin; occasionally (+)-gallocatechins are found (Mosel and Herrmann, 1974; Table IV.2). Concentrations of these catechins peak during ripening of these berries (Mosel and Herrmann, 1974). Dimeric procyanidins yield anthocyanidins and 'catechins' during heating with acid (Hahlbrock, 1981; Herrmann, 1974; Spanos, 1988). It was the objective of our study to create an expanded compositional database for red raspberry juice consisting of flavonoids and other phenolics, sugars, and organic acids. This report is restricted to ellagic acid, non-anthocyanin flavonoids and benzoic and cinnamic acids in hydrolyzed red raspberry juices. The objective was to separate and identify hydrolyzed phenolics in red raspberry juice so that they can be used a) as supplementary authenticity indicators together with anthocyanins, sugars and organic acids and b) as a database for evaluating the effects of raspberry juice on health.

MATERIALS AND METHODS

Several red raspberry juices made from cultivars commonly used in commercial processing (Willamette, Meeker) were investigated. Juices were processed in the pilot plant of the Department of Food Science and Technology at Oregon State University as described in Rommel and Wrolstad (manuscript in prep. a).

Juice Sample Preparation and Hydrolysis

The flavonoids and other phenolics of unconcentrated (single-strength) red raspberry juice (ca. 5 mL) were adsorbed onto activated Sep-Pak C_{18}-cartridges (Waters Associates, Milford, MA) using 'cleanup procedure I' of Hong and Wrolstad (1990);

the eluate containing sugars and organic acids was discarded. C_{18}-Cartridges were activated with methanol. Flavonoids and phenolics were eluted from cartridges with acidified methanol; this fraction was evaporated to dryness using a rotary evaporator and a 40°C water bath. The dried phenolics were redissolved in a few mL of 4% phosphoric acid before hydrolysis.

Acid and base hydrolysis. Juice samples were hydrolyzed a) in 2N HCl for 30 min in boiling water as described by Hong and Wrolstad (1986) and b) in 2N NaOH for 2 hours at room temperature in the dark in nitrogen atmosphere, as described by Markham (1982), followed by 30 min hydrolysis in 2N HCl in boiling water. Hydrolyzed samples were re-adsorbed onto C_{18}-cartridges and prepared as described above. Phenolics redissolved in 4% phosphoric acid were filtered with Millipore filters, type HA, 0.45 µm pore size (Millipore Corp., Bedford, MA 01730).

Analysis by High Performance Liquid Chromatography (HPLC)

HPLC separation conditions and instrumentation as well as a diode-array detection system were used as described in Rommel and Wrolstad (manuscript in prep. b). The following gradient elution program gave the best separation of the phenolics present in hydrolyzed juice samples: from 5 to 15% A in 35 min; to 25% A in 20 min; to 55% A in 18 min; to 100% A in 5 min; 5 min at 100% A; return to initial conditions in 7 min (total run time: 90 min). Ellagic and benzoic acids were detected at 260 nm, gallic acid and catechins at 280 nm, cinnamic acids at 320 nm and flavonols at 360 nm.

Peak Characterization

HPLC peaks were characterized by 1) UV-spectra, which are very characteristic of different classes of phenolics and sometimes of compounds within classes, e.g. kaempferol and quercetin, which have slightly different absorption maxima; 2) comparison to

standards: standards were separated by HPLC either by themselves or mixed with juices; and 3) relative retention-times of known and unknown peaks.

Standards used for peak characterization. Flavonols: kaempferol and quercetin (Sigma Chemical Co., St. Louis, MO 63178); ellagic acid (Sigma Chemical Co.); benzoic acids: gallic, protocatechuic, and p-hydroxybenzoic acids (Sigma Chemical Co.); cinnamic acids: caffeic, p-coumaric, and ferulic acids (Sigma Chemical Co.)

Quantification of phenolics was attempted, however, adsorption of benzoic acids (particularly gallic and protocatechuic acids) on C_{18}-cartridges was minimal (usually less than 10%) and very variable. Other cartridges and adsorption materials were tested, however, materials for reproducible sample preparation could not be obtained.

RESULTS AND DISCUSSION

The HPLC-chromatograms of the hydrolyzed red raspberry juice samples analyzed were very similar for both Meeker and Willamette cultivars. An example of Willamette juice, hydrolyzed in 2N HCl for 30 min, is shown in Figure IV.3. Table VI.3 summarizes percentages of HPLC peak area of the phenolics present in hydrolyzed red raspberry juices; peak areas were measured at the absorbance maximum of each compound (260, 280, 320 or 360 nm). The three acid-hydrolyzed juices had similar percentages of HPLC area for the phenolics as evident by the standard deviations for their means (Table VI.3).

The major peaks present were ellagic acid and an unidentified ellagic acid compound (#1), which eluted before ellagic acid (Figure IV.3). Another unidentified ellagic acid compound (#2) eluted about 12 min later than ellagic acid; both ellagic acid compounds had absorbance spectra very similar to that of ellagic acid. In unhydrolyzed red raspberry juices ellagic acid and up to 16 additional ellagic acid compounds were present (Rommel and Wrolstad, manuscript in prep. b). A reduction to only ellagic acid and two ellagic compounds during hydrolysis probably resulted from de-glycosylation, de-methylation and de-methoxylation of ellagic acid (Daniel et al., 1991; Maas et al., 1991) as well as from the

removal of glucose and gallic acid molecules from ellagitannins through cleavage of ester-bonds (Bate-Smith, 1959, 1972; Wilson and Hagerman, 1990). Hydrolysis may also have dissociated metal complexes of ellagic acid (Press and Hardcastle, 1969) and possibly open lactone-forms of ellagic acid.

Three hydroxybenzoic acids were identified: gallic, protocatechuic and p-hydroxybenzoic acids; protocatechuic acid had the greatest peak height. Salicylic acid was not detected conclusively. Also identified were the three hydroxycinnamic acids: caffeic, p-hydroxybenzoic and ferulic acids, with p-coumaric acid having the greatest peak height. Presence of the three hydroxybenzoic and the three hydroxycinnamic acids, reported by Herrmann (1989) and Schuster and Herrmann (1985) to be present as glucosides and esters, was confirmed. We confirmed further that salicylic acid is present, if at all, in raspberries in very small concentrations (Herrmann, 1989; Mosel and Herrmann, 1974) and not in great quantities (39-51 ppm) as reported by Swain et al. (1985).

The flavan-3-ols (-)-epicatechin and (+)-catechin were detected, however, as very small peaks; (+)-catechin was often present below the detection limit. Presence of these catechins confirmed the report of Mosel and Herrmann (1974). In addition, the flavonol-aglycons quercetin and kaempferol were identified; kaempferol was present below the detection limit in some samples. In unhydrolyzed red raspberry juices 13 quercetin and kaempferol-glycosides and up to 36 additional flavonols in trace amounts were measured (Rommel and Wrolstad, manuscript in prep. b); hydrolysis simplified the very complex flavonol profile of raspberry juice significantly. Several other peaks present in chromatograms could not be identified as they did not match any of the phenolic standards available in our laboratory.

A HPLC chromatogram of the same Willamette juice, hydrolyzed with 2N NaOH for 2 hours followed by 2N HCl for 30 min is shown in Figure IV.4. The phenolic pattern of this sample was very similar to that of the acid-hydrolyzed sample of the same juice, and the same compounds were present (Table IV.3). However, ellagic acid compound #2 was

absent and the ratio of the percentages of peak area of ellagic acid and ellagic acid compound #1 was greater (5.7) than that for the acid-hydrolyzed sample (3.0). We speculate that both of these ellagic acid compounds were esters of ellagic acid (i.e. ellagitannins), which were hydrolyzed in basic but not in acidic conditions.

SUMMARY AND CONCLUSIONS

The complex HPLC-chromatographic profile of red raspberry juice phenolics was simplified dramatically by both acid and base hydrolysis. In acid-hydrolyzed juices, ellagic acid and two unidentified ellagic acid compounds, and also gallic, p-hydroxybenzoic, protocatechuic, caffeic, p-coumaric and ferulic acids, as well as (+)-catechin, (-)-epicatechin, quercetin, and kaempferol were identified. In base-hydrolyzed samples the same phenolics, except the second ellagic acid compound, were present; the first ellagic acid compound was detected in smaller quantity.

Hydrolysis appeared to break down phenolic glycosides and esters into aglycons very well. The three acid-hydrolyzed juices had similar percentages of HPLC area for the phenolics present. Hydrolysis could become a very useful tool for rapidly screening raspberry juices for qualitative deviations from authentic HPLC profiles, in order to determine adulteration by other fruit juices or phenolic mixes. The development of a reproducible sample preparation procedure is still needed for determining whether hydrolysis is complete and if aglycons are degraded during hydrolysis, and for measuring total quantities of phenolic aglycons in hydrolyzed samples, e.g. for evaluating the health effects of raspberry juices.

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Table IV.1. Concentrations of Hydroxybenzoic and Hydroxycinnamic Acids Present in Fresh Red Raspberries*

- **Hydroxybenzoic acid glucosides** (cultivars: Glen Cova, Malling Exploit, Malling Promise):	
p-hydroxybenzoic acid-ß-D-glucoside	32 - 59 ppm (i.e. mg/kg fresh fruit)
protocatechuic acid-4-ß-D-glucoside	traces
gallic acid-4-ß-D-glucoside	traces
- **Hydroxybenzoic acid esters** (Glen Cova and Malling Exploit):	
5-galloylquinic acid	traces
1-O-galloyl-ß-D-glucopyranose	traces
- **Salicylic acid**	traces - 1 ppm (Mosel & Herrmann, 1974)
	39 - 51 ppm (Swain et al., 1985)
- **Hydroxycinnamic acid glucosides** (Glen Cova, Malling Exploit, Malling Promise):	
p-coumaric acid-ß-D-glucoside	4 - 10 ppm
ferulic acid-ß-D-glucoside	traces - 2 ppm
- **Hydroxycinnamic acid esters** (Glen Cova and Malling Exploit):	
1-O-coumaroyl-ß-D-glucopyranose	6 - 14 ppm
1-O-feruloyl-ß-D-glucopyranose	4 - 7 ppm
1-O-caffeoyl-ß-D-glucopyranose	3 - 7 ppm
5-p-coumaroylquinic acid	1 - 2 ppm
5-caffeoylquinic acid (=chlorogenic acid)	traces - 1 ppm
5-feruloylquinic acid	traces

*Herrmann (1989) and Schuster and Herrmann (1985)

Table IV.2. Flavan-3-ols Present in Fresh Raspberries, Blackberries and Strawberries as Reported in the Literature

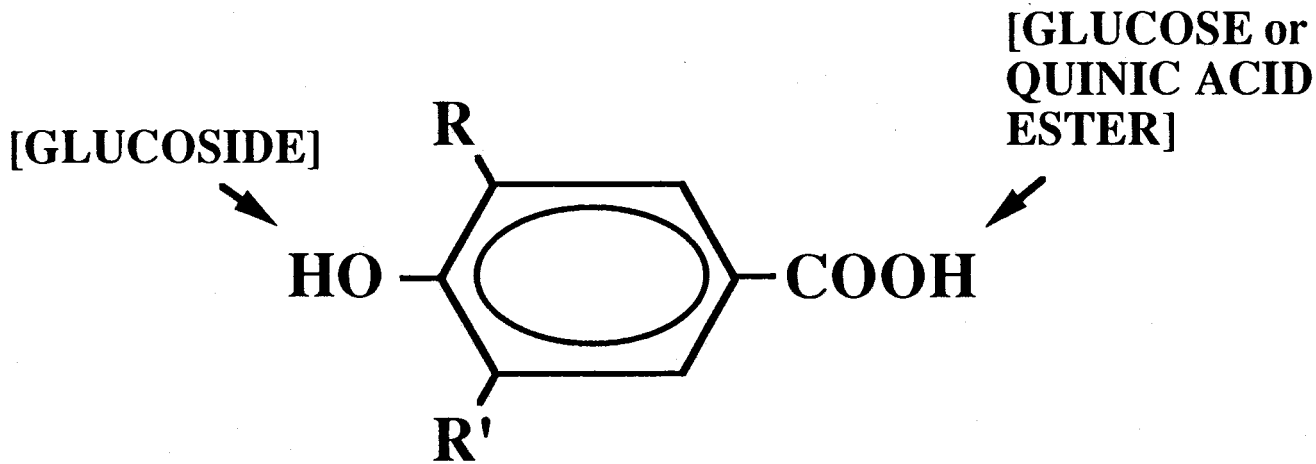
Flavan-3-ol (ppm, i.e. mg/kg fresh fruit)	**Total Flavan-3-ols**	**(+)-Catechin**	**(-)-Epicatechin**	**(+)-Gallocatechin**	
Raspberries					**Reference**
Varieties					
Unknown	133	22	111		Mosel & Herrmann, 1974
Gevalo	46	1.1	45		"
Malling Jewel	48	6.2	42		"
Golden Queen	32	12	20		"
Promiloy	49	14	35		"
mean	62	11	51	traces*	"
Blackberries (6 varieties, mean)	131	19	112	traces*	"
Strawberries (16 varieties, mean)	47	33	6.9	7.4	Stoehr & Herrmann, 1975

*in some samples

Table IV.3. Percentages of HPLC Peak Area of Phenolics Present in Acid and Base Hydrolyzed Red Raspberry Juices

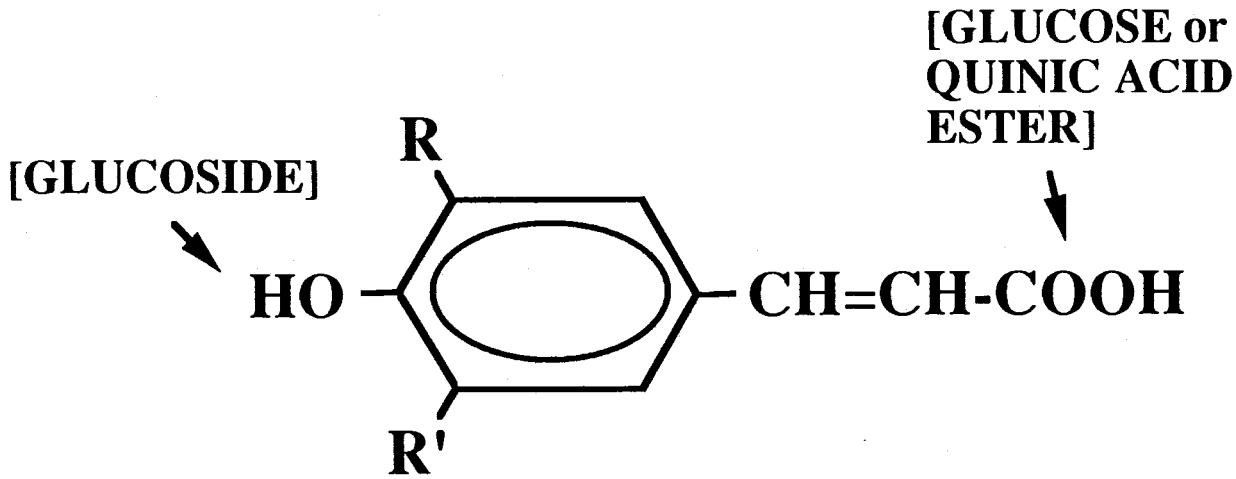
Hydrolysis Method	Base + Acid	Acid	Acid	Acid	Acid	Acid
Cultivar	Willamette	Willamette	Willamette	Meeker		
Sample Code	WI-OR-OR-HP-PP	WI-OR-OR-HP-PP	WI-BC-R-MH-DE	ME-OR-UR-HP-PP		
Ellagic Acid Compound					**Mean**	**± SD***
Ellagic Acid	68.34	67.05	60.24	68.29	65.19	4.33
Ellagic Acid-form #1	11.95	22.36	20.69	21.11	21.39	0.87
Ellagic Acid-form #2	not detected	2.40	6.27	1.35	3.34	2.59
Hydroxybenzoic Acids						
Gallic	0.31	0.32	0.16	0.08	0.19	0.12
Protocatechuic	4.11	1.39	0.75	0.44	0.86	0.48
p-Hydroxybenzoic	1.60	0.74	0.46	1.03	0.74	0.29
Hydroxycinnamic Acids						
Caffeic	0.74	0.49	0.38	0.74	0.54	0.18
p-Coumaric	2.72	1.52	2.01	1.91	1.81	0.26
Ferulic	4.58	1.71	1.85	1.14	1.57	0.38
Flavonol-aglycons						
Quercetin	3.47	1.07	4.06	3.38	2.84	1.57
Kaempferol	0.10	0.28	2.57	trace	1.43	1.62
Flavan-3-ols						
(+)-Catechin	trace	0.11	trace	0.18	0.15	0.05
(-)-Epicatechin	2.08	0.57	0.58	0.37	0.51	0.12

*standard deviation (alpha=0.05)



R = R' = H	p-HYDROXYBENZOIC ACID
R = OH R' = H	PROTOCATECHUIC ACID
R = R' = OH	GALLIC ACID

Figure IV.1. Structures of hydroxybenzoic acids and derivatives



R = R' = H	p-COUMARIC ACID
R = OH R' = H	CAFFEIC ACID
R = OMe R' = H	FERULIC ACID

Figure IV.2. Structures of hydroxycinnamic acids and derivatives

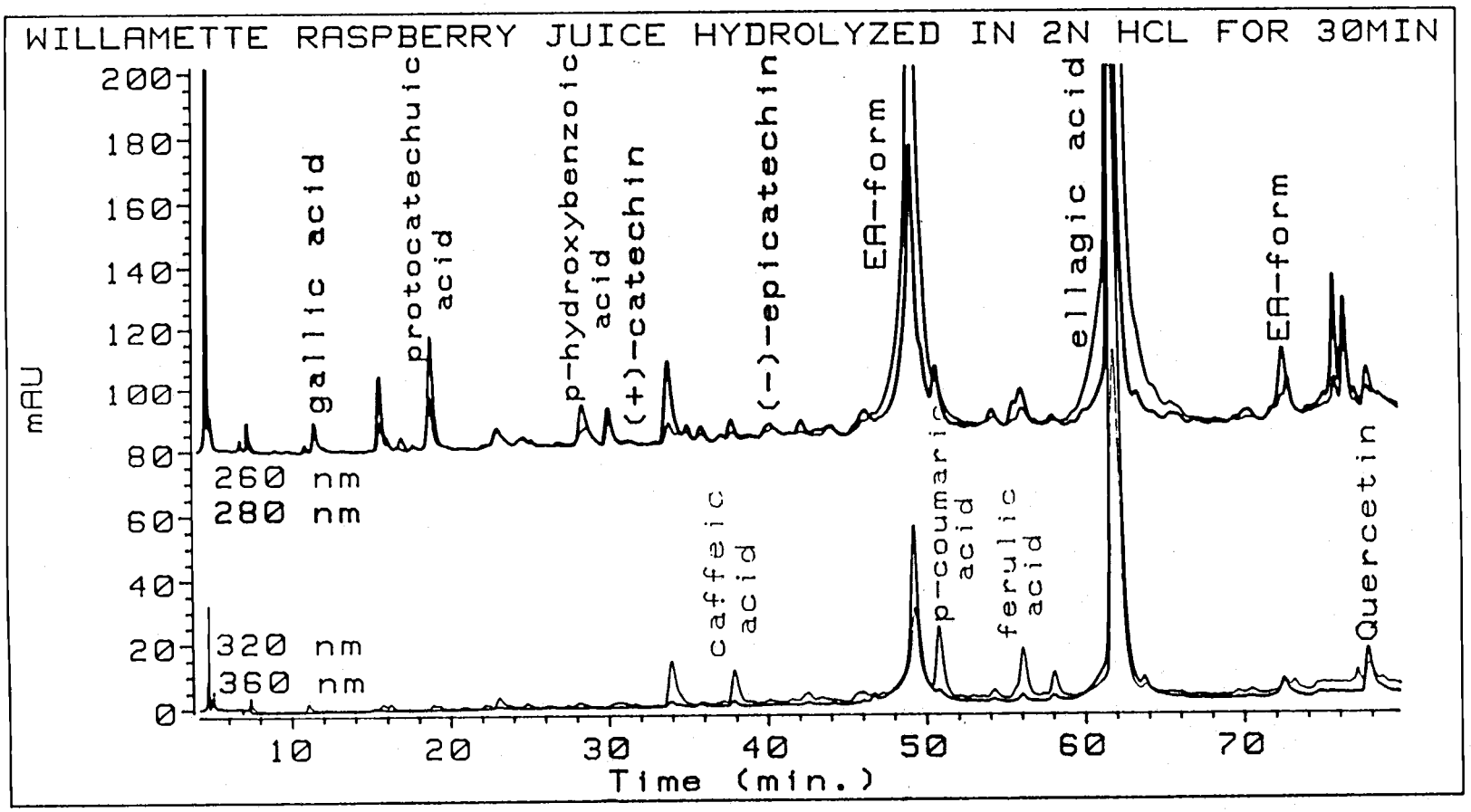


Figure IV.3. HPLC chromatogram of Willamette red raspberry juice hydrolyzed in 2N hydrochloric acid for 30 minutes. Phenolic compounds were detected at the following wavelengths (nm): ellagic acid, ellagic acid derivatives and benzoic acids (260); gallic acid and catechins (280); cinnamic acids (320); flavonols (360).

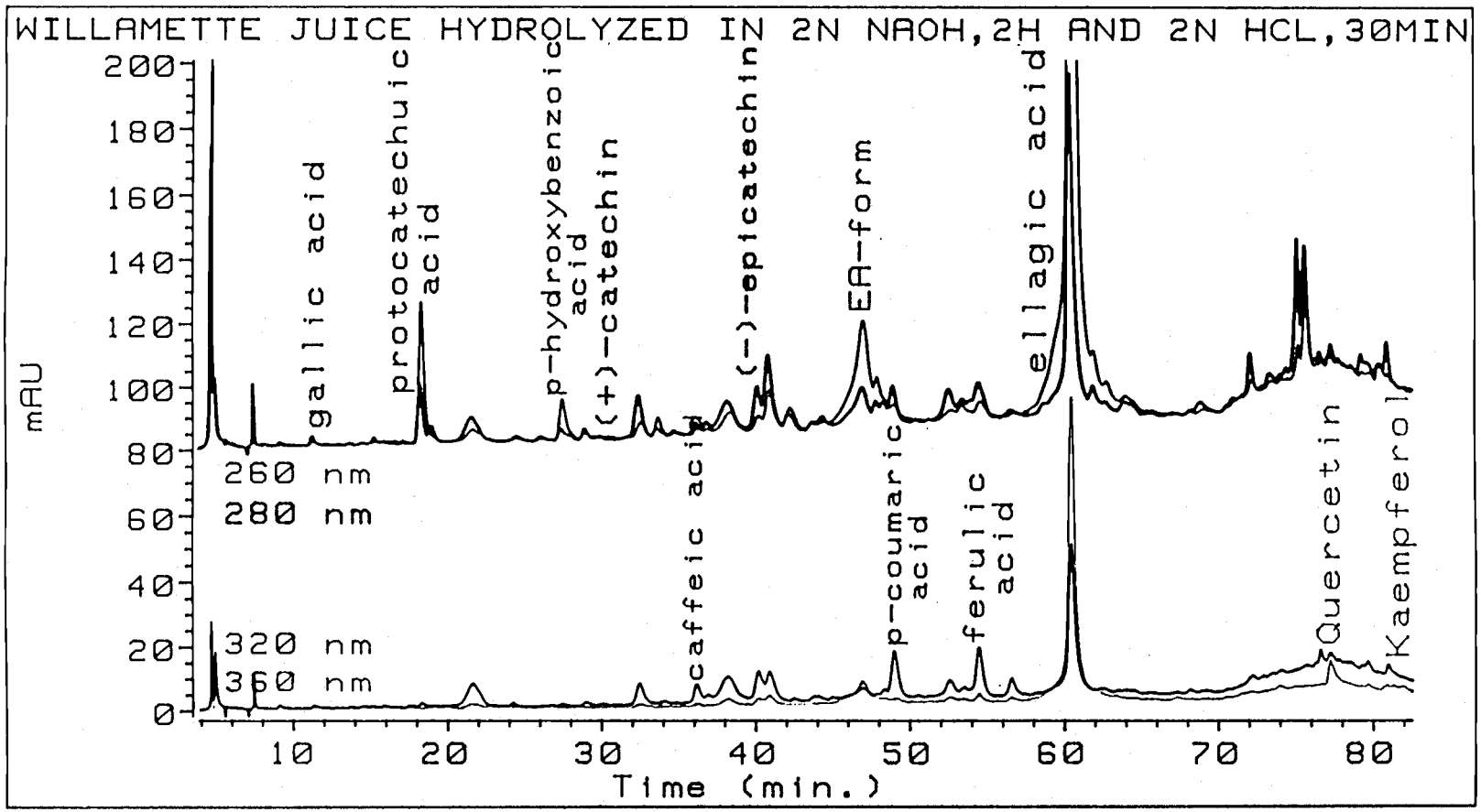


Figure IV.4. HPLC chromatogram of Willamette red raspberry juice hydrolyzed in 2N sodium hydroxide for 2 hours and in 2N hydrochloric acid for 30 minutes. Phenolic compounds were detected at the following wavelengths (nm): ellagic acid, ellagic acid derivatives and benzoic acids (260); gallic acid and catechins (280); cinnamic acids (320); flavonols (360).

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