

# Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectrometry

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## Abstract

Strawberry (*Fragaria x ananassa* Duch.) fruits contain phenolic compounds that have antioxidant, anticancer, antiatherosclerotic and anti-neurodegenerative properties. Identification of food phenolics is necessary since their nature, size, solubility, degree and position of glycosylation and conjugation influence their absorption, distribution, metabolism and excretion in humans. Freeze-dried whole strawberry fruit powder and strawberry fruit extracts were analyzed by liquid chromatography electrospray ionization mass spectrometry (LC–ESI–MS) methods. Phenolics were identified as ellagic acid (EA), EA-glycosides, ellagitannins, gallotannins, anthocyanins, flavonols, flavanols and coumaroyl glycosides. The anthocyanidins were pelargonidin and cyanidin, found predominantly as their glucosides and rutosides. The major flavonol aglycons were quercetin and kaempferol found as their glucuronides and glucosides. LC–ESI–MS/MS methods differentiated EA from quercetin conjugates since both aglycons have identical molecular weights (302 g/mol). The identification of strawberry phenolics is necessary to generate standardized materials for in vitro and in vivo studies and for the authentication of strawberry-based food products.

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**Keywords:** Strawberries; Ellagic acid; Ellagitannins; Anthocyanins; Flavonols; LCMS

## 1. Introduction

It is now well established that a diet high in fruits and vegetables is associated with a reduced risk of oxidative stress mediated diseases such as cancer, cardiovascular and neurodegenerative diseases (Halliwell, 1994). The health beneficial effects of fruits and vegetables are attributed to their high levels of a wide variety of phytochemicals, of which phenolics constitute the greatest proportion. Phenolic compounds contain aromatic ring(s) bearing hydroxyl group(s) and can range from simple molecules to very large oligomers (Fig. 1). They frequently occur naturally in glycosylated forms, which make them more water-soluble although the higher molecular weight oligomers are more insoluble (Bravo,

1998). Phenolic compounds are abundant in highly colored berry fruits, and due to their popularity and high consumption, these berries serve as one of our most important dietary sources of phenolics (Kahkonen, Hoppa, & Heinonen, 2001; Williner, Pirovani, & Guemes, 2003). Berry fruits are reported to contain a wide variety of phenolics including hydroxybenzoic and hydroxycinnamic acid derivatives, anthocyanins, flavonols, flavanols, condensed tannins (proanthocyanidins) and hydrolyzable tannins (Machiex, Fleuriet, & Billot, 1990).

Strawberry (*Fragaria x ananassa* Duch.) fruits are very popular among berries and are widely consumed in fresh forms and as food-products such as preserves, jams, yogurts and ice creams. Strawberry fruits are reported to have antioxidant, anticancer, anti-inflammatory and anti-neurodegenerative biological properties (reviewed in Hannum, 2004). Because of the reported

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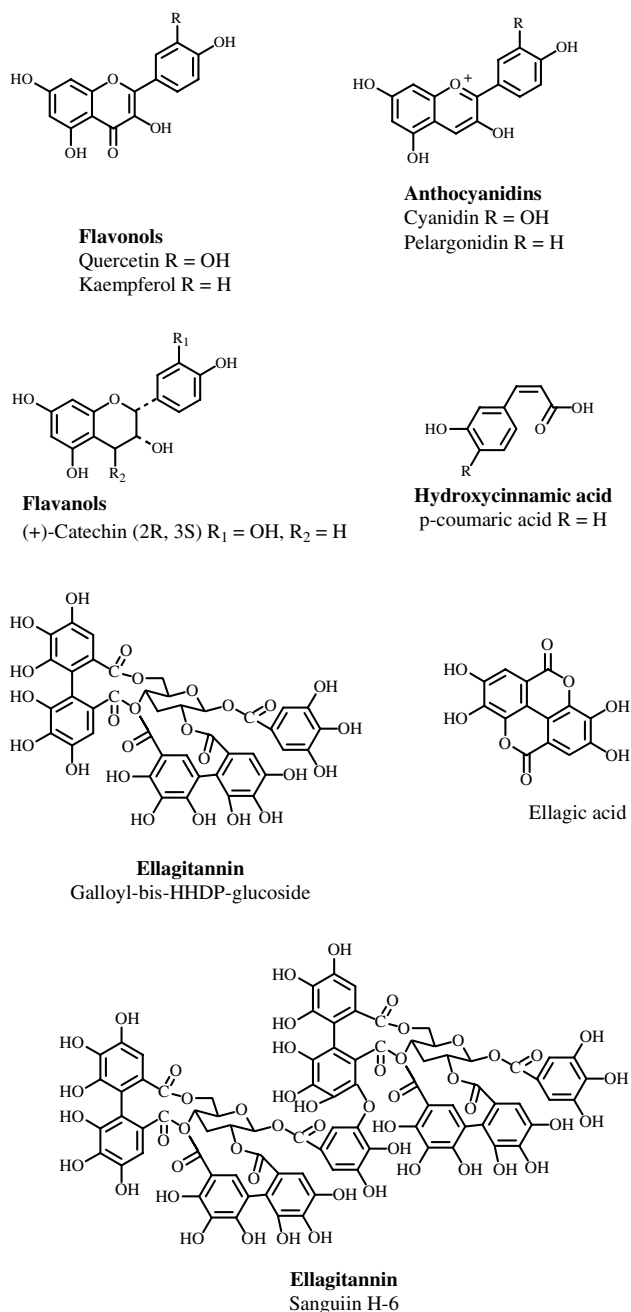


Fig. 1. Examples of phenolic compounds found in strawberry fruits.

biological properties associated with strawberry fruits, the identification of their phytochemicals is necessary for the evaluation of strawberry consumption on human health. The nature, size, structure, solubility, degree and position of glycosylation, and conjugation of phenolics with other compounds can influence their bioavailability, absorption, distribution, metabolism and excretion in humans (Aherne & O'Brien, 2002; Hollman, 2001). However, it is also necessary to identify strawberry phenolics in the different forms in which the fruit has been studied, namely, as freeze-dried whole fruits (Cao, Russell, Lischner, & Prior, 1998; Carlton et al.,

2001; Chung, Lee, & Sung, 2002; Joseph et al., 1998; Stoner, Kresty, Carlton, Siglin, & Morse, 1999), and extract forms (Meyers, Watkins, Pritts, & Liu, 2003; Seeram, Momin, Bourquin, & Nair, 2001; Xue et al., 2001). Due to the lack of standard methods for sample preparation, extraction and analyses, there is no general consensus on a standard protocol for quantitation of phenolic compounds in fruits and other foods (Naczek & Shahidi, 2004). Quantitation of fruit phenolics is also complicated by factors such as differences in fruit cultivars, growing conditions, degree of ripeness, handling after harvest, etc. (Bravo, 1998). Our study is focused on the identification and not quantitation of phenolic compounds in strawberry fruits as freeze-dried whole strawberry fruit powder (SFP) and strawberry fruit extracts (SFE).

Many previous methods to identify strawberry fruit phenolics were optimized for particular groups of compounds such as anthocyanins (Lopes-da-Silva, Pascual-Teresa, Rivas-Gonzalo, & Santos-Buelga, 2002) or ellagitannin-based compounds (Hakkinen, Karenlampi, Mykkanen, Heinonen, & Torronen, 2000; Maatta-Riihinen, Kamal-Elin, & Torronen, 2003; Williner et al., 2003). In addition, many previous methods to identify strawberry fruit phenolics were based on spectrophotometry (Meyers et al., 2003) or high performance liquid chromatography with UV detection (HPLC–UV) [also commonly referred to as photodiode array detection (HPLC–PDA) or diode array detection (HPLC–DAD)] (Gil, Holcroft, & Kader, 1997; Kosar, Kafkas, Paydas, & Baser, 2004; Wang and Zheng, 2001). However, HPLC–UV has its limitations since it relies on the comparison of characteristic absorbance wavelength spectra, wavelength maxima ( $\lambda_{\max}$ ), and chromatographic retention times ( $t_R$ ) with authentic standards. On the other hand, the use of HPLC with mass spectrometry (HPLC–MS) detection provides useful structural information and allows for tentative compound identification when standard reference compounds are unavailable and when peaks have similar  $t_R$  and similar UV-absorption spectra (Hakkinen, Karenlampi, Heinonen, Mykkanen, & Torronen, 1999; Lopes-da-Silva et al., 2002; Maatta-Riihinen, Kamal-Elin, & Torronen, 2004). In addition, tandem mass spectrometric ( $MS^n$ ) techniques are useful for distinguishing compounds with identical molecular weights (Mullen, Yokota, Lean, & Crozier, 2003).

The objective of our study was to identify the phenolic compounds and generate characteristic chromatographic 'fingerprints' of SFP and SFE by liquid chromatography electrospray ionization mass spectrometry (LC–ESI–MS) methods. We also report on the use of LC–MS/MS methods to differentiate between the sugar conjugates of quercetin and ellagic acid (EA) present in strawberries since both aglycons have identical molecular weights (MW 302 g/mol). Our study provides useful

information required for the generation of standardized strawberry materials for in vitro and in vivo studies and for the authentication of strawberry-based food products.

## 2. Experimental

### 2.1. Reagents

All solvents were HPLC grade and purchased from Fisher Scientific Co. (Tustin, CA). EA, catechin, epicatechin and quercetin standards were purchased from Sigma Aldrich Co. (St. Louis, MO). Pelargonidin and cyanidin aglycons and their respective 3-glucosides were purchased from Chromadex Inc. (Santa Ana, CA).

### 2.2. Strawberry fruit powder (SFP) and strawberry fruit extract (SFE)

Fresh strawberry fruits and freeze-dried whole strawberry fruit powder (SFP) were provided by the California Strawberry Commission (Watsonville, CA). Strawberry fruits (975 g) were separately blended (Waring Blender, New Hartford, Conn., USA) with either methanol (0.1% HCl) or acetone: water (7:3, v/v) and then centrifuged. The supernatant liquids were concentrated in vacuo (Buchi Rotavap) at low temperature (37 °C) to yield methanol and aqueous acetone strawberry fruit extracts (SFE), respectively. The SFE or SFP (~1 mg/ml) were dissolved by sonication in water: acidic methanol (1:1, v/v), and injected directly for HPLC–MS analyses.

### 2.3. Liquid chromatography (LC) diode array detection (DAD) and electrospray ionization mass spectrometry (ESI–MS) methods

Samples were analyzed on a Surveyor HPLC system equipped with a diode array absorbance detector (DAD), scanning from 250 to 600 nm, and an autosampler cooled to 4 °C (Thermo Finnigan, San Jose, USA). A Symmetry C-18 column, 250 × 4.6 mm, i.d. 5 μm (Waters, MA, USA), was used and solvent elution consisted of a gradient system over 70 min of acetonitrile (ACN) and H<sub>2</sub>O (1% formic acid) at a flow rate of 1 ml/min. The linear gradient system consisted of 10% ACN in H<sub>2</sub>O (1% formic acid) for 10 min to eventually 20% ACN in H<sub>2</sub>O (1% formic acid) at 70 min. The column was maintained at 25 °C. After passing through the flow cell of the DAD, the column eluate was split and 0.2 ml/min was directed to a LCQ Advantage ion trap mass spectrometer fitted with an electrospray (ESI) interface. Analyses utilized the positive ion mode ( $m/z$  M + H<sup>+</sup>) for detection of anthocyanins and negative ion mode ( $m/z$  M – H<sup>+</sup>) for all other compounds. Preli-

minary analyses were carried out using full scan, data dependent MS/MS scanning from  $m/z$  250–2000. The capillary temperature was 275 °C, sheath gas and auxiliary gas were 45 and 0 units/min respectively, and source voltage was 4 kV. MS/MS fragmentation was carried out with 50% energy. Zoom scan analyses were carried out to determine the charge state of some of the ellagitannin-based compounds. Identities of the compounds were obtained by matching their molecular ions ( $m/z$ ) obtained by LC–ESI–MS and LC–ESI–MS/MS with literature data (Lopes-da-Silva et al., 2002; Maatta-Riihinen et al., 2004; Mullen et al., 2003).

## 3. Results and discussion

### 3.1. General

HPLC–DAD–ESI–MS methods were used to analyze SFP and SFE. Common solvents used for extraction of phenolic compounds from foods include water, methanol, aqueous acetone, ethanol and ethyl acetate (Naczk & Shahidi, 2004). Our preliminary extractions with different solvents showed that the methanol (0.1% HCl) and aqueous acetone extracts were similar and yielded the most peaks in the HPLC–DAD chromatogram when monitored at 280, 360 and 520 nm, absorbance wavelengths typical of phenolics including hydroxycinnamic acids, flavonols, flavanols and anthocyanins (Fig. 1). However, because acidic methanol is also the widely accepted solvent of choice for the extraction of anthocyanins (Naczk & Shahidi, 2004), the SFE discussed in our studies was generated using this solvent.

Fig. 2A–C show the HPLC–DAD profiles of SFP at 280, 360 and 520 nm, respectively. Fig. 3A–C show the HPLC–DAD profiles of SFE at 280, 360 and 520 nm, respectively. Table 1 shows the identification of the phenolic compounds present in strawberry fruits labeled as peaks 1–22 following the elution orders in the HPLC–DAD chromatograms. Whenever available, reference standards of phenolics (see Section 2) were used to substantiate the identification of peaks in the SFP and SFE. Tentative identities of phenolics that were not available as standard reference materials were obtained by matching their molecular ions (M + H<sup>+</sup>, for anthocyanins) or (M – H<sup>+</sup>, for other phenolics) obtained by LC–ESI–MS and LC–MS/MS methods with the theoretical molecular weights from literature data.

LC–MS/MS methods were also used to distinguish between conjugates of quercetin and EA since their aglycons produce identical molecular ions on fragmentation (M – H<sup>+</sup>,  $m/z$  301) (Mullen et al., 2003). On MS/MS analyses, the quercetin  $m/z$  301 ion further fragments to form characteristic  $m/z$  179 and 151 ions (Fig. 4A) whereas the equivalent EA  $m/z$  301 ion yields ions at  $m/z$  257 and 229 (Fig. 4B). The use of LC–MS/MS methods

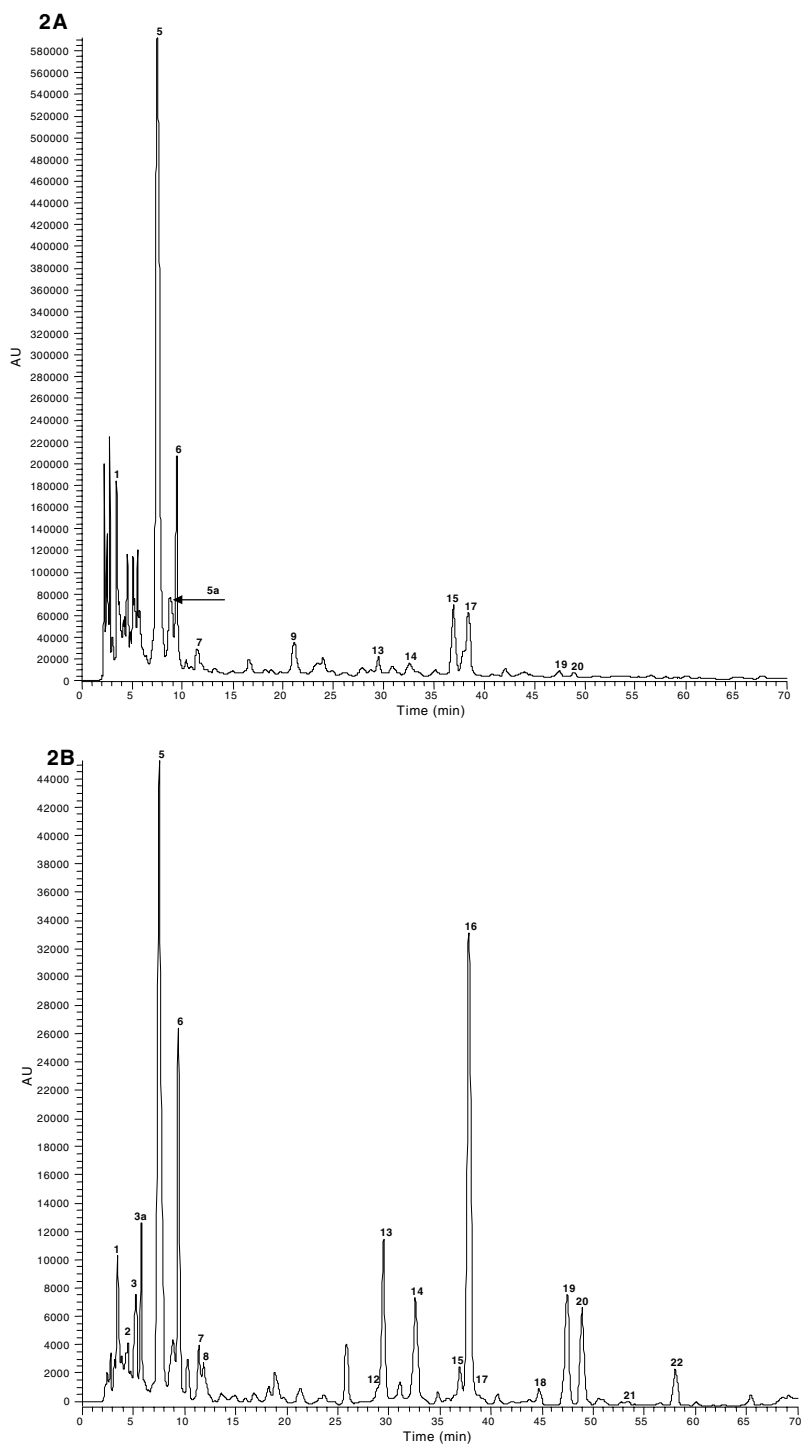


Fig. 2. HPLC–DAD profiles of freeze-dried whole strawberry fruit powder (SFP). A gradient solvent elution system was used over 70 min with acetonitrile and 1% aqueous formic acid at a flow rate of 1 ml/min. Detection is shown at (A) 280 nm, (B) 360 nm and (C) 520 nm. Peaks were identified by comparison with reference standards when available or by the LC–ESI–MS and LC–ESI–MS/MS data. Numbering of peaks refers to their identification as shown in Table 1.

is therefore useful to differentiate between EA and quercetin aglycons that are ubiquitous to berry fruits.

The basis for identification of the phenolic compounds grouped as hydrolyzable tannins [ellagitannins

(ETs), gallotannins (GTs), and EA], anthocyanins, flavonols, hydroxycinnamic acid derivatives and their esters, and flavanols (including catechin) is described below.

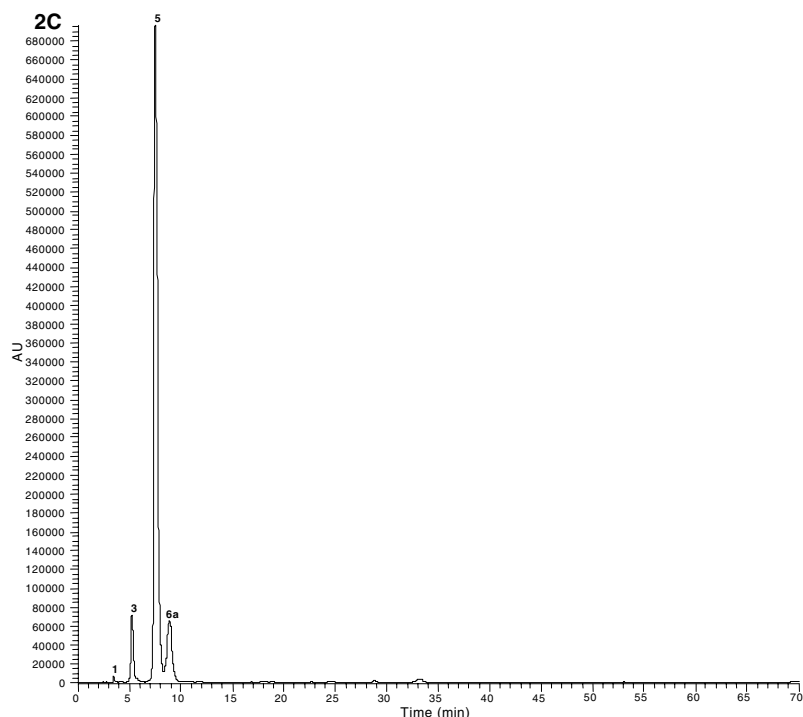


Fig. 2 (continued)

### 3.2. Hydrolyzable tannins (ETs, GTs and EA)

ETs are esters of hexahydroxydiphenic acid (HHDP: 6,6'-dicarbonyl-2,2',3,3',4,4'-hexahydroxybiphenyl moiety) and a polyol usually glucose or quinic acid (Quideau & Feldman, 1996). A key feature of ETs is their ability to release the bislactone, EA, which is formed from the hydrolytic release of HHDP esters groups, which undergo rapid, facile and unavoidable lactonization. GTs can also be transformed to ETs by oxidative C–C coupling between spatially adjacent galloyl groups to form HHDP groups. ETs are known to be abundant in berry fruits and after hydrolytic conversion, are commonly detected and quantified in the form of EA (Amakura, Okada, Tsuji, & Tonogai, 2000). Hence EA may be considered as a chemical marker compound for hydrolyzable tannins. We have also recently reported that EA may be considered as a biomarker of ETs in human bioavailability studies (Seeram, Lee, & Heber, 2004).

Peak 9 was identified as a galloyl-bis-HHDP-glucose molecule (MS  $m/z$  = 935.0; MS/MS  $m/z$  = 898, 633, 463, 301, 257, 229) due to its key fragments in MS/MS analyses at  $m/z$  633 and 301 (Maatta-Riihinen et al., 2004). In addition, the  $m/z$  257 and 229 ions in the MS/MS analysis showed that an EA moiety, and not quercetin, was associated with this molecule (Fig. 4B). Galloyl-bis-HHDP-glucose (Fig. 1) is a basic unit of many ETs, for example, sanguin H-6 and lambertianin C con-

tain 2 and 3 units, respectively (Maatta-Riihinen et al., 2004).

Similarly, peaks 2, 3a, 4, 13, 14, 17 and 19 were also ascertained as being EA-based by MS/MS analyses. Peak 14 was identified as free EA from its HPLC–DAD and LC–MS data and by co-elution and comparison of its  $t_R$  with a reference standard of EA. Peak 17 was identified as sanguin H-6 (MS  $m/z$  = 1869; MS/MS  $m/z$  = 1567, 1265, 935, 633, 301, 257, 229) (Fig. 1) by comparison of its LC–MS/MS data with literature reports (Maatta-Riihinen et al., 2004; Mullen et al., 2003).

A key tool used to aid in the identification of the ET-based compounds was the use of high-resolution 'zoom scan' analyses. This technique improves resolution of the  $^{12}\text{C}/^{13}\text{C}$  isotopes of compounds, allowing their charge state to be determined and hence facilitating the correct determination of their molecular weights (MW) (Mullen et al., 2003). Therefore, peak 17 had a  $(\text{M} - \text{H}^+)$  ion at  $m/z$  934, which was shown to be doubly charged by zoom-scan analysis (Fig. 5A), giving MW 1870 for this compound. However, zoom scan analysis of peak 9 showed a singly charged ion at  $m/z$  935, giving MW 936, and confirming the presence of a free molecule of galloyl-bis-HHDP-glucose (Fig. 5B). Similarly, peaks 2 and 3a (both MS  $m/z$  = 783; MS/MS  $m/z$  = 481, 301, 257, 229) were shown to be a singly charged ions by zoom-scan analysis, giving a MW of 784 for these compounds. Peaks 2 and 3a were tentatively identified as isomeric forms of an EA-based compound.

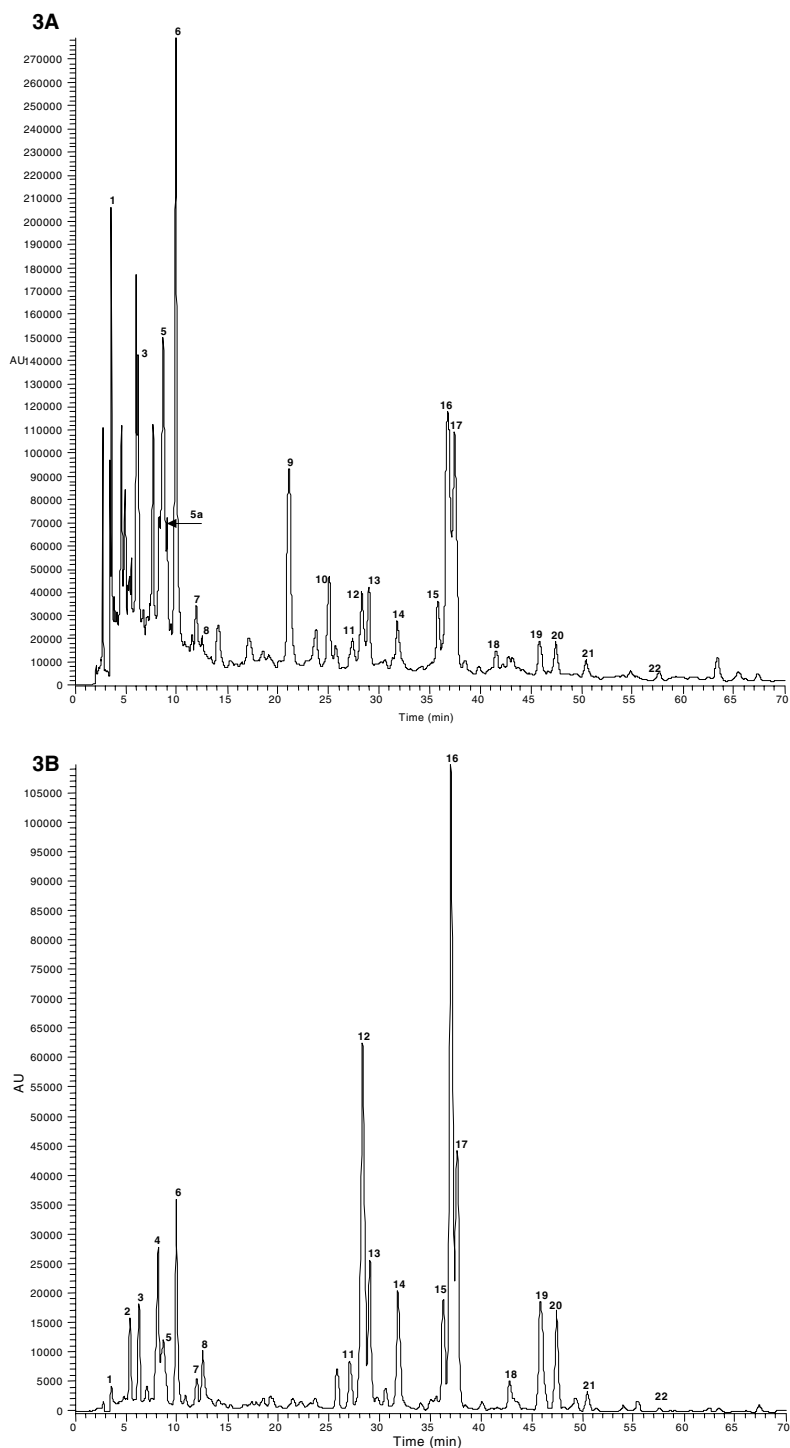


Fig. 3. HPLC–DAD profiles of strawberry fruit extract (SFE). A gradient solvent elution system was used over 70 min with acetonitrile and 1% aqueous formic acid at a flow rate of 1 ml/min. Detection is shown at (A) 280 nm, (B) 360 nm and (C) 520 nm. Peaks were identified by comparison with reference standards when available or by the LC–ESI–MS and LC–ESI–MS/MS data. Numbering of peaks refers to their identification as shown in Table 1.

Peak 4 (MS  $m/z$  = 639; MS/MS  $m/z$  = 463, 301, 257, 229), was also tentatively assigned as an EA-based compound based on its LC–MS/MS data. Peaks 13 and 19 were identified as methyl–EA–pentose conjugates (MS  $m/z$  = 447; MS/MS  $m/z$  = 315), as previously reported

(Mullen et al., 2003). The significantly different  $t_R$  of these compounds in the HPLC–DAD trace may possibly be attributed to isomeric forms due to differences in the point of attachment of the methyl and pentose substituents.



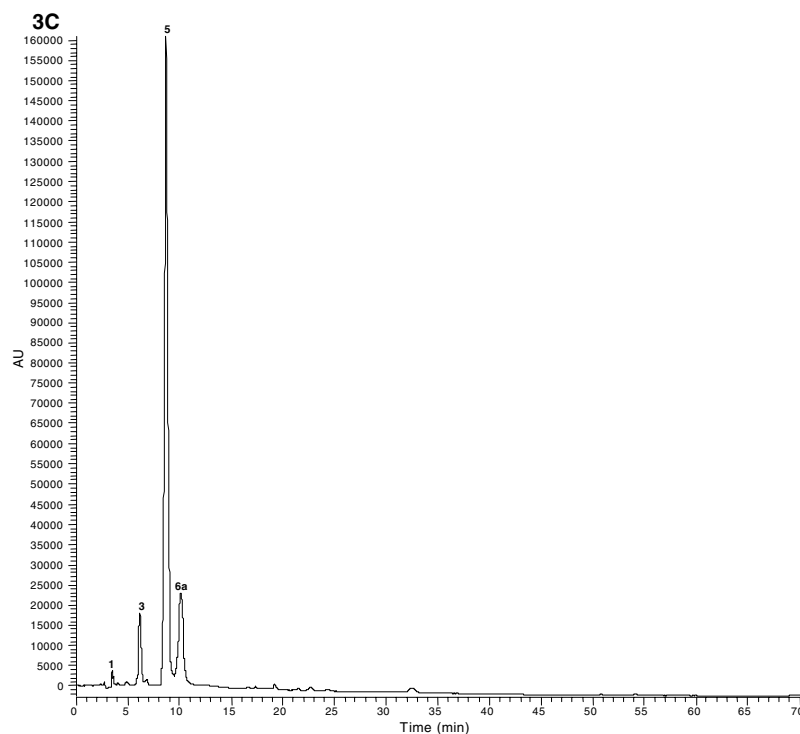


Fig. 3 (continued)

Table 1

Identification of phenolic compounds in strawberry fruits by using their HPLC–DAD, LC–MS and LC–MS/MS data<sup>a</sup>

Peak #	$t_R$ (min)	$\lambda_{max}$ (nm)	MW	MS	Ion (+/–)	MS/MS	Tentative ID
1	3.20	275, 520	594	595	+	433, 271	Plg-diglucoside
2	5.37	235, 350	784	783	–	481, 301, 257, 229	GT/ET
3	6.16	275, 520	448	449	+	287	Cy-glucoside
3a	6.20	235, 350	784	783	–	481, 301, 257, 229	GT/ET
4	8.13	235, 350	640	639	–	463, 301, 257, 229	GT/ET
5	9.12	275, 520	432	433	+	271	Plg-glucoside
5a	9.20	280	290	289	–	245	Catechin
6	9.95	235, 310	326	325	–	265, 187, 163, 145	<i>p</i> -Coumaroyl-glucoside
6a	10.13	310, 505	578	579	+	433, 271	Plg-rutinoside
7	11.95	235, 310	326	325	–	265, 187, 163, 145	<i>p</i> -Coumaroyl-glucoside
8	12.55	235, 330	356	355	–	295, 217, 193, 175, 134	<i>p</i> -Coumaroyl-ester
9	21.3	235, 250	936	935	–	898, 633, 463, 301, 257, 229	Galloyl-HHDP-glucoside
10	25.10	240, 325	326	325	–	265, 187, 163, 145	<i>p</i> -Coumaroyl-glucoside
11	27.07	265, 350	596	595	–	300, 271, 179	Unknown
12	28.32	255, 355	610	609	–	301, 179, 151	Q-rutinoside
13	29.02	250, 370	448	447	–	315	Methyl-EA-pentose
14	31.80	250, 370	302	301	–	257, 229	EA
15	36.27	285	464	463	–	301, 179, 151	Q-glucoside
16	37.03	265, 355	478	477	–	301, 179, 151	Q-glucuronide
17	37.63	260, 345	1870	1869	–	1567, 1265, 935, 633, 301, 257, 229	Sanguin-H6
18	42.80	250	594	593	–	285	K-coumaroyl-glucoside
19	45.85	260, 345	448	447	–	315	Methyl-EA-pentose
20	47.43	265, 345	462	461	–	285	K-glucuronide
21	50.47	325	462	461	–	285, 179, 161	K-glucuronide
22	55.43	265, 345	490	489	–	285	Unknown

Identification were aided by comparison with reference standards where available and by correlation with previous literature reports.

<sup>a</sup> Peak numbers and retention times ( $t_R$ ) refer to HPLC chromatograms in Figs. 2 and 3. Peaks 3/3a; 5/5a and 6/6a co-elute. GT, gallotannin; ET, ellagitannin; Q, quercetin; K, kaempferol; Plg, pelargonidin; Cy, cyanidin; EA, ellagic acid; HHDP, hexahydroxydiphenyl.

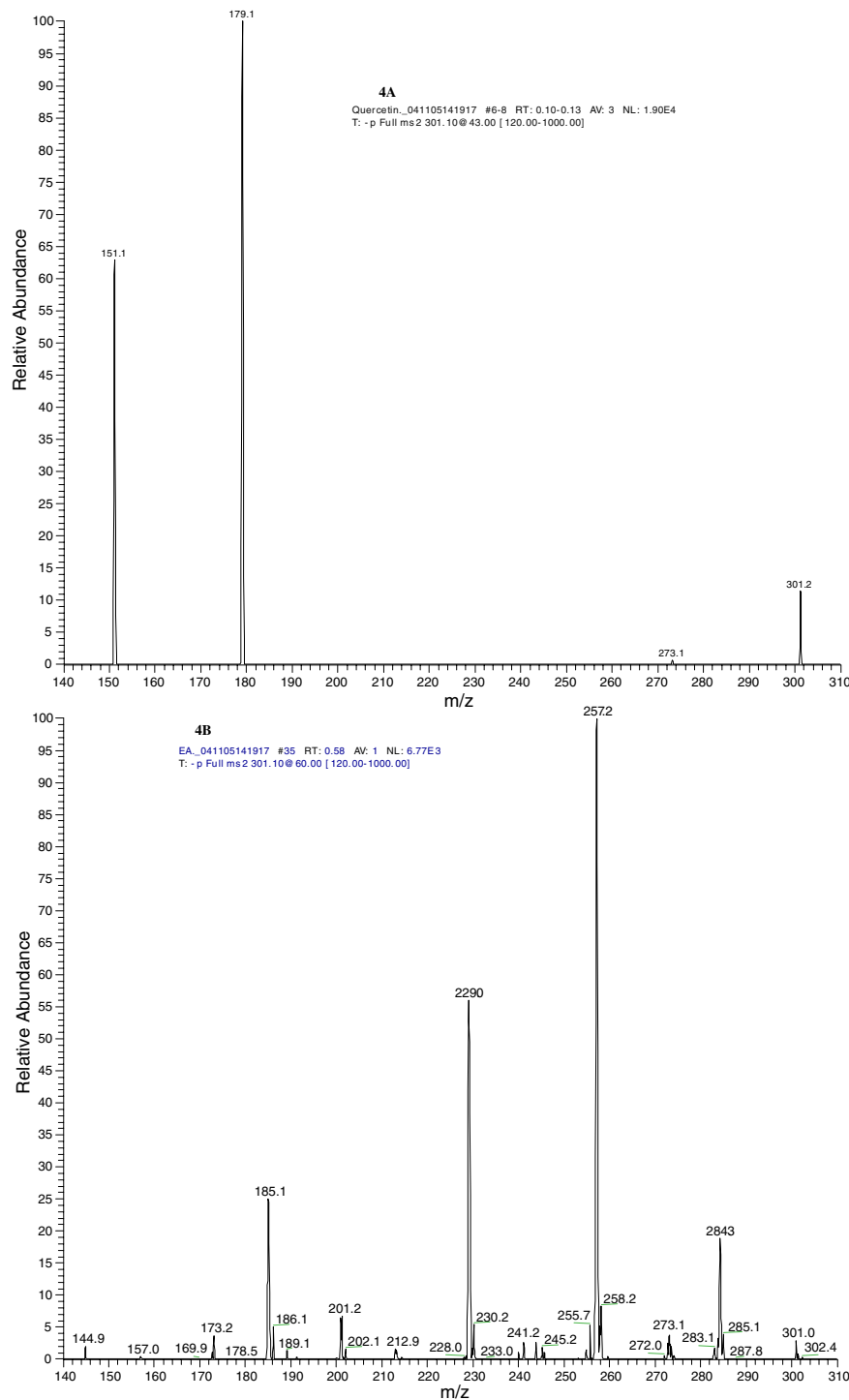


Fig. 4. LC-MS/MS trace by direct infusion of (A) quercetin ( $M - H$   $m/z$  301) showing its characteristic fragment ions at  $m/z$  179 and 151 and (B) ellagic acid (EA) ( $M - H$   $m/z$  301) showing its characteristic fragment ions at  $m/z$  257 and 229.

### 3.3. Anthocyanins

Anthocyanins are the glycosides of anthocyanidins responsible for the attractive colors of fruits and vegetables and have a characteristic absorption wavelength of approximately 500–530 nm. Anthocyanins are typically observed in LC-ESI-MS analyses in the positive mode

( $M + H^+$ ). Strawberry fruit anthocyanins are reported to be based on pelargonidin (Plg) and cyanidin (Cy) aglycons (Fig. 1) (Lopes-da-Silva et al., 2002). Strawberry anthocyanins were identified from their HPLC-DAD chromatograms at 520 nm (Fig. 2C for SFP and Fig. 3C for SFE), by comparison with reference standards when available, and from their LC-MS/MS data.



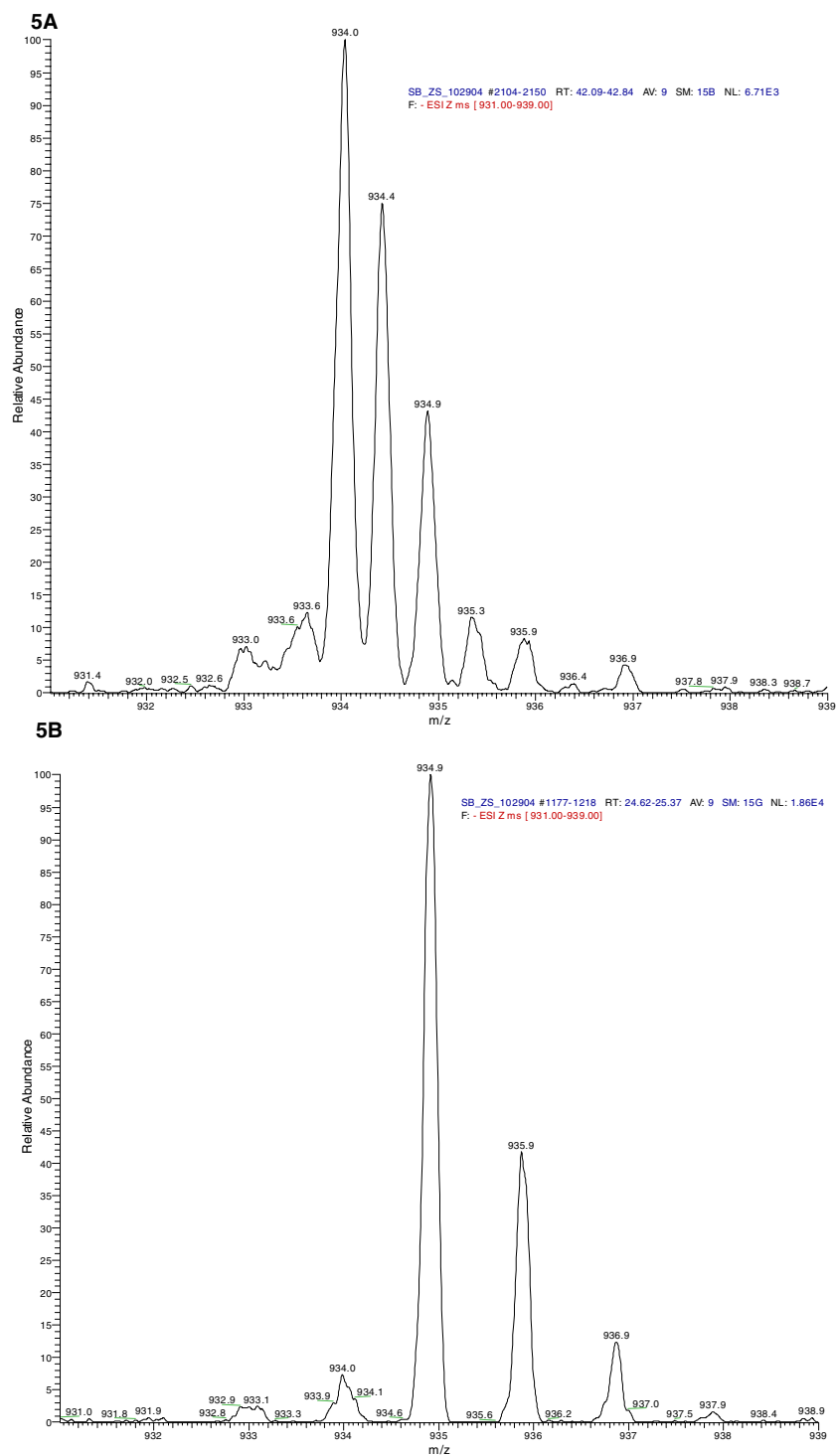


Fig. 5. Zoom scan mass spectra of (A) HPLC-DAD peak 17, showing the intervals between the  $m/z$  934 isotope peaks of 0.5 amu which demonstrates the doubly charged state of the ion corresponding to MW 1870; (B) HPLC-DAD peak 9, showing the intervals between the  $m/z$  935 isotope peaks of 1.0 amu which demonstrates the singly charged state of the ion corresponding to MW 936.

Peaks 1, 3, 5 and 6a were identified as Plg-diglucoside (MS  $m/z$  = 595; MS/MS  $m/z$  = 433, 271); Cy-glucoside (MS  $m/z$  = 449; MS/MS  $m/z$  = 287); Plg-glucoside (MS  $m/z$  = 433; MS/MS  $m/z$  = 271); and Plg-rutinoside (MS  $m/z$  = 579; MS/MS  $m/z$  = 433, 271), respectively. Peak

1 was confirmed as Plg-diglucoside and not Cy-rutinoside (both  $m/z$  595) by its MS/MS data which showed the fragment for the Plg aglycon at  $m/z$  271. The MS/MS analyses of anthocyanins containing the Cy aglycon would give a characteristic fragment at  $m/z$  287. The

identities of these anthocyanins in strawberries are in agreement with the previous literature report (Lopes-da-Silva et al., 2002).

### 3.4. Flavonol glycosides

Peaks were identified as flavonol glycosides based on their HPLC–DAD and LC–MS/MS data. Peaks 12, 15 and 16 were identified as quercetin-rutinoside (MS  $m/z$  = 609; MS/MS  $m/z$  = 301, 179, 151), quercetin-glucoside (MS  $m/z$  = 463; MS/MS  $m/z$  = 301, 179, 151), and quercetin-glucuronide (MS  $m/z$  = 477; MS/MS  $m/z$  = 301, 179, 151), respectively. The  $m/z$  179 and 151 ions in the MS/MS analysis showed that a quercetin aglycon, and not EA, was associated with these compounds (Fig. 4A).

Peak 18 was identified as kaempferol-coumaroyl-glucoside (MS  $m/z$  594; MS/MS  $m/z$  = 285) based on the previous report (Tsukamoto et al., 2004). Peaks 20 and 21 were identified as isomers of kaempferol-glucuronide (MS  $m/z$  = 461; MS/MS  $m/z$  = 285) and (MS  $m/z$  = 461; MS/MS  $m/z$  = 285, 179, 161), respectively (Maatta-Riihinen et al., 2004). The occurrence of the kaempferol aglycon in berry fruits is uncommon but reported to be present in strawberries, artichoke, and gooseberries (Hakkinen et al., 1999; Maatta-Riihinen et al., 2004).

There have also been other reports about the occurrence of flavonols as their glucuronides in berry fruits. Quercetin-glucuronide has been reported in raspberries and strawberries (Maatta-Riihinen et al., 2004; Ryan & Coffin, 1971), although Maatta-Riihinen et al. (2004), only detected kaempferol-glucuronide in strawberries. The pharmacokinetics of flavonols in humans is reported to differ significantly between food sources depending on the type of glycosides that they contain (Manach & Donovan, 2004). Hence, the occurrence of flavonols as their glucuronides in these berry fruits is interesting since many studies continue to probe the mechanisms of absorption, metabolism, biotransformation, and excretion of these dietary phenolics in humans. Flavonols are usually found in foods as their glycosides and are detected in biological fluids as their glucuronidated, as well as sulphated and methylated forms (Manach & Donovan, 2004). After ingestion, whether these molecules are transformed by gut bacteria enzymatic and/or physiological pH action from glucosides to glucuronides, or de-glycosylated then glucuronidated, or in the case of ingestion as naturally occurring glucuronide forms, whether they are absorbed intact as these glucuronides, is worth investigating.

### 3.5. Flavonols

Peak 5a was identified as catechin by comparison of its  $t_R$  (~9.2 min in the 280 nm chromatograms,

Figs. 2A and 3A) with an authentic reference standard and by its LC–MS data (MS  $m/z$  = 289; MS/MS  $m/z$  = 245). Although catechin has the same molecular weight as its isomer, epicatechin, we were able to differentiate between these two compounds due to their different  $t_R$  in HPLC–DAD. A reference standard of epicatechin showed that this isomer eluted at a later time than catechin using these HPLC conditions. Catechin has previously been reported to be present in strawberries (Hannum, 2004).

### 3.6. Hydroxycinnamic acid derivatives

Peaks 6, 7 and 10 (all MS  $m/z$  = 325; MS/MS  $m/z$  = 265, 187, 163, 145), were identified as isomeric forms of *p*-coumaroyl-glucoside (Maatta-Riihinen et al., 2004). Peak 8 (MS  $m/z$  = 355; MS/MS  $m/z$  = 295, 217, 193, 175, 134) was identified as *p*-coumaroyl sugar ester, as previously reported (Maatta-Riihinen et al., 2004). Free hydroxycinnamic acids (Fig. 1) are uncommon in fruits (Machiex et al., 1990) and are found more likely in their conjugated forms. It has also been reported that *p*-coumaric acid is the common hydroxycinnamic acid aglycon found in strawberries and raspberries (Maatta-Riihinen et al., 2004).

### 3.7. Unidentified compounds

Peak 11 and the late-eluting peak 22, both for which LC–MS data were obtained, remain without further identification.

## 4. Conclusions

In conclusion, we have identified the major phenolic compounds present in strawberry fruits and established characteristic chromatographic profiles for whole freeze-dried strawberry fruit powder (SFP) and strawberry fruit extract (SFE). Because it may be more meaningful to evaluate the biological properties of strawberries as whole fruits rather than in extract forms, these methods can aid in the standardization of strawberry materials for in vitro and in vivo studies. The chromatographic fingerprinting of strawberries is also useful for the authentication of strawberry-based food products. The information provided by our study will aid in the evaluation of the importance of strawberry consumption on human health.

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## References

- Aherne, S. A., & O'Brien, N. M. (2002). Dietary flavanols: Chemistry, food content and metabolism. *Nutrition*, *18*, 75–81.
- Amakura, Y., Okada, M., Tsuji, S., & Tonogai, Y. (2000). High performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and processed fruits. *Journal of Chromatography A*, *896*, 87–93.
- Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. *Nutrition Reviews*, *56*, 317–333.
- Cao, G., Russell, R. M., Lischner, N., & Prior, R. L. (1998). Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine, or vitamin C in elderly women. *Journal of Nutrition*, *128*, 2383–2390.
- Carlton, P. S., Kresty, L. A., Siglin, J. C., Morse, M. A., Lu, J., Morgan, C., et al. (2001). Inhibition of *N*-nitrosomethylbenzylamine-induced tumorigenesis in the rat esophagus by dietary freeze-dried strawberries. *Carcinogenesis*, *22*, 441–446.
- Chung, M. J., Lee, S. H., & Sung, N. J. (2002). Inhibitory effect of whole strawberries, garlic juice or kale juice on endogenous formation of *N*-nitrosodimethylamine in humans. *Cancer Letters*, *182*, 1–10.
- Gil, M. I., Holcroft, D. M., & Kader, A. A. (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatment. *Journal of Agricultural and Food Chemistry*, *45*, 1662–1667.
- Hakkinen, S. H., Karenlampi, S. O., Heinonen, M., Mykkanen, H. M., & Torronen, A. R. (1999). Content of the flavanols quercetin, myricetin and kaempferol in 25 edible berries. *Journal of Agricultural and Food Chemistry*, *47*, 2274–2279.
- Hakkinen, S. H., Karenlampi, S. O., Mykkanen, H. M., Heinonen, M., & Torronen, A. R. (2000). Ellagic acid content in berries: Influence of domestic processing and storage. *European Food Research and Technology*, *212*, 75–80.
- Halliwell, B. (1994). Free radicals, antioxidants and human disease: Curiosity, cause or consequence. *The Lancet*, *344*, 721–724.
- Hannum, S. M. (2004). Potential impact of strawberries on human health. *Critical Reviews in Food Science and Nutrition*, *44*, 1–7.
- Hollman, P. C. (2001). Evidence for health benefits of plant phenols: Local or systemic effects? *Journal of the Science of Food and Agriculture*, *81*, 842–852.
- Joseph, J. A., Shukitt-Hale, B., Denisova, N. A., Prior, R. L., Cao, G., Martin, A., et al. (1998). Long-term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. *Journal of Neuroscience*, *18*, 8047–8055.
- Kahkonen, M. P., Hopia, A. I., & Heinonen, M. (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry*, *49*, 4076–4082.
- Kosar, M., Kafkas, E., Paydas, S., & Baser, K. H. C. B. (2004). Phenolic composition of strawberry genotypes at different maturation stages. *Journal of Agricultural and Food Chemistry*, *52*, 1586–1589.
- Lopes-da-Silva, F., Pascual-Teresa, S. D., Rivas-Gonzalo, J., & Santos-Buelga, C. (2002). Identification of anthocyanin pigments in strawberry (cv Camarosa) by LC using DAD and ESI-MS detection. *European Food Research and Technology*, *212*, 248–253.
- Maatta-Riihinen, K. R., Kamal-Elin, A., & Torronen, A. R. (2003). High-performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: Ribes species. *Journal of Agricultural and Food Chemistry*, *51*, 6736–6744.
- Maatta-Riihinen, K. R., Kamal-Elin, A., & Torronen, A. R. (2004). Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (Family Rosaceae). *Journal of Agricultural and Food Chemistry*, *52*, 6178–6187.
- Machiex, J. J., Fleuriet, A., & Billot, J. (1990). *Fruit phenolics*. Boca Raton, FL: CRC Press.
- Manach, C., & Donovan, J. L. (2004). Pharmacokinetics and metabolism of dietary flavanoids in humans. *Free Radical Research*, *38*, 771–785.
- Meyers, K. J., Watkins, C. B., Pritts, M. P., & Liu, R. H. (2003). Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry*, *51*, 6887–6892.
- Mullen, W., Yokota, T., Lean, M. E. J., & Crozier, A. (2003). Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC-MS/MS. *Phytochemistry*, *64*, 617–624.
- Nacz, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, *1054*, 95–111.
- Quideau, S., & Feldman, K. S. (1996). Ellagitannin chemistry. *Chemical Reviews*, *96*, 475–503.
- Ryan, J. J., & Coffin, D. E. (1971). Flavanol glucuronides from red raspberry, *Rubus idaeus* (Rosaceae). *Phytochemistry*, *10*, 1675–1677.
- Seeram, N. P., Lee, R., & Heber, D. (2004). Bioavailability of ellagic acid in human plasma after the consumption of ellagitannins from pomegranate (*Punica granatum* L.) juice. *Clinica Chimica Acta*, *348*, 63–68.
- Seeram, N. P., Momin, R. A., Bourquin, L. D., & Nair, M. G. (2001). Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine*, *8*, 362–369.
- Stoner, G. D., Kresty, L. A., Carlton, P. S., Siglin, J. C., & Morse, M. A. (1999). Isothiocyanates and freeze-dried strawberries as inhibitors of esophageal cancer. *Toxicological Sciences*, *52*(Supplement), 95–100.
- Tsukamoto, S., Tomise, K., Aburatani, M., Onuki, H., Hirota, H., Ishiharajima, E., et al. (2004). Isolation of cytochrome P450 inhibitors from strawberry fruit, *Fragaria ananassa*. *Journal of Natural Products*, *67*, 1839–1841.
- Williner, M. R., Pirovani, M. E., & Guemes, D. R. (2003). Ellagic acid content in strawberries of different cultivars and ripening stages. *Journal of the Science of Food and Agriculture*, *83*, 842–845.
- Xue, H., Aziz, R. M., Sun, N., Cassady, J. M., Kamendulis, L. M., Xu, Y., et al. (2001). Inhibition of cellular transformation by berry extracts. *Carcinogenesis*, *22*, 351–356.