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Recent Advances in Strawberry Metabolomics

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ABSTRACT

The recent developments in metabolomics technologies have facilitated a comprehensive examination of the rich chemical composition of plants. Both gas and liquid chromatography based separation combined to high mass accuracy mass spectrometry as well as structural elucidation utilizing 2D NMR are currently frequently applied in metabolite profiling approaches for various plant species, including strawberry. With these technologies, the knowledge of the metabolite composition of strawberry has been expanded to include numerous different derivatives of well-known metabolites. Furthermore, metabolite classes previously unknown for this species have been identified. As in other plants, the array of natural products generated in different organs and cell layers of strawberry forms the basis for the chemical defense and interaction with the environment. The same compounds, when consumed in the diet are responsible for the bioactivity mediating beneficial health effects in humans. Strawberry produces large amounts of commonly occurring phenolic compounds such as phenolic acids, flavonols and anthocyanidins. The early developmental stages of strawberry fruit are characterized by abundant accumulation of proanthocyanidin polymers that protect the developing fruit against pests, and give an astringent taste rendering it unappealing for consumption. One of the most abundant metabolite classes of strawberry fruit is ellagitannins, group of compounds restricted to a small number of plant species. Ellagitannins are likely to contribute to the beneficial health effects claimed for strawberry, as these compounds show e.g. anticarcinogenic activity *in vitro*. In this review we discuss the phytochemicals produced in the vegetative and reproductive organs of strawberry, both in terms of the plant's physiology and as a constituent of the human diet. The metabolome of strawberry is described in light of recent developments and application of cutting-edge analytical chemistry-based approaches for metabolomics analysis of complex plant matrices.

Keywords: strawberry, *Fragaria* × *ananassa*, phytochemical, metabolite profiling, metabolomics, phenolic compounds

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INTRODUCTION

Strawberry, along with other fruits of the *Rosaceae* family including apples, pears, plums, peaches and raspberries, has a particularly rich secondary metabolite composition. The chemical profiles include hundreds of non-volatile and volatile compounds, the latter ones being responsible for the typical fruit aroma bouquet. These metabolites have been the subject of intensive investigations for decades. The focus has been either on a wide-range non-targeted metabolite profiling, quantification of specific metabolite classes, or structural characterization of single phytochemicals. The metabolites most frequently analyzed from strawberry were phenolic compounds such as phenolic acids, flavonols (kaempferol and quercetin derivatives), anthocyanins (cyanidin and pelargonidin derivatives), proanthocyanidins, galloylglucoses and ellagitannins. Additionally, compounds of the terpenoid class, some nitrogen-containing metabolites, as well as various volatile metabolites have been identified in strawberry. Metabolites classified as micronutrients such as vitamin C and folate have been analysed to determine the nutritional quality of strawberry. These phytochemical analyses have served to develop a database for the nutritional composition and health considerations but have also increased the knowledge about strawberry physiology. Both aspects will be reviewed here.

METHODS USED FOR STRAWBERRY METABOLITE ANALYSIS

By far the most frequently applied method in the analysis of strawberry metabolite composition is Liquid Chromatography Mass Spectrometry (LC-MS) coupled with UV detection (Määttä-Riihinen *et al*. 2004; Seeram *et al*. 2006b; Aaby *et al*. 2007a; Hukkanen *et al*. 2007). The most recent applications, which combine efficient separation by Ultra Performance LC (UPLC) and accurate mass measurement with high-resolution mass spectrometers, allow qualitative

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Invited Review

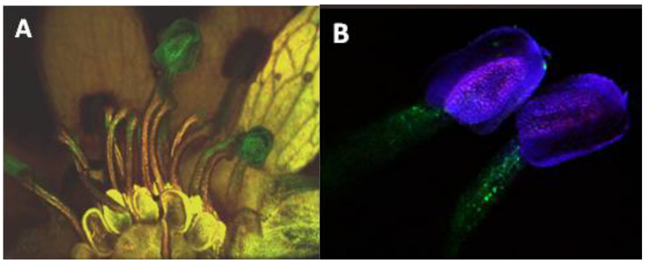

Fig. 1 Confocal microscopic examination of A. Whole strawberry flower, B. Mature stamen. The fluorescent images were obtained with an Ultraview® confocal scanner (Perkin Elmer Life Sciences, Wallac-LSR, Oxford, UK), on a Nikon Eclipse TE300 microscope (Nikon, Tokyo, Japan). The wavelengths were: Green: excitation 488 nm, emission 525 nm; red: excitation 568 nm, emission 607 nm; blue: excitation 647 nm, emission 700 nm. In A wavelengths for only green and red were used.

analysis of over hundred compounds in a single run (Fait *et al*. 2008; Hanhineva *et al*. 2008). Gas Chromatography-MS (GC-MS) is widely used in the analysis of polar metabolites in derivatized extracts (often primary/central metabolites; Fait *et al*. 2008) and aroma (volatile) compounds (Zabetakis and Holden 1997; Aubert *et al*. 2005). Nuclear Magnetic Resonance (NMR) spectroscopy has mainly been employed for unambiguous structure elucidation of strawberry secondary metabolites, often in combination with LC-MS analysis (Ishimaru *et al*. 1995; Hirai *et al*. 2000; Hilt *et al*. 2003; Hanhineva *et al*. 2009b).

Several less common metabolite analysis techniques such as Fourier Transform Ion Cyclotron-MS (FTICR-MS; Aharoni *et al*. 2002) and Colloidal Graphite-Assisted Laser Desorption/Ionization MS (GALDI, Zhang *et al*. 2007) have also been applied for strawberry. Direct Infusion MS (DIMS) analysis has been demonstrated to be useful particularly in a quick comparison of the rich tannin signals in a large set of samples (McDougall *et al*. 2008). Laser-Induced Fluorescence Spectroscopy (LIFS) has been tested as a non-destructive method to characterize phenolic compounds in the surface of strawberry fruit (Wulf *et al*. 2008). Finally, the autofluorescence of phenolic compounds can be used for simple visualization of differences in the composition of this metabolite class (**Fig. 1**).

STRAWBERRY METABOLITE COMPOSITION

The array of phenolic secondary metabolites found in strawberry is listed in **Table 1**. Several large-scale, non-targeted metabolite profiling studies have been carried out on strawberry fruit (Aharoni *et al*. 2002; Määttä-Riihinen *et al*. 2004; Seeram *et al*. 2006b; Aaby *et al*. 2007a; Fait *et al*. 2008), flowers (Hanhineva *et al*. 2008) and leaves (Hukkanen *et al*. 2007; Hanhineva *et al*. 2009a) by using LC-MS. A number of studies have focused on specific metabolite classes, e.g. phenolic acids (Mattila and Kumpulainen 2002; Mattila *et al*. 2006), ellagitannins (Okuda *et al*. 1992; Cerda *et al*. 2005), anthocyanins (Nyman and Kumpulainen 2001; Lopes da Silva *et al*. 2002; Koponen *et al*. 2007), proanthocyanidins (Gu *et al*. 2003; Buendía *et al*. 2009; Hellström *et al*. 2009) and flavonols (Häkkinen and Auriola 1998). Several strawberry secondary metabolites have been identified based on structural elucidation with 2D NMR. These include, the characterization of phenylpropanoid derivatives (Hanhineva *et al*. 2009b); phloridzin (Hilt *et al*. 2003); 1-*O*-trans-cinnamoyl-β-D-glucopyranose (Latza *et al*. 1996); E-cinnamic acid derivatives in the progenitor of the garden strawberry (*Fragaria chiloensis*) (Cheel *et al*. 2005); ellagic acid derivatives (Heur *et al*. 1992); valerophenone derivative (Tsukamoto *et al*. 2004); 5-carboxypyranopelargonidin (Andersen *et al*. 2004); anthocyanin-flavan-3-ol metabolites (Fossen *et al*. 2004); taxifolin 3-arabinoside in strawberry roots (Ishimaru *et al*. 1995); and triterpenes (Hirai *et al*. 2000).

The structurally simplest phenolics are phenolic acids, *i.e.* the hydroxylated derivatives of benzoic and cinnamic acids, which are frequently conjugated with sugars. They serve as precursors for a wide array of secondary metabolites including benzoates, salicylates, coumarins, lignans, lignin and flavonoids. In strawberry fruit, the predominant phenolic acid is coumaric acid present as glycosides (Mattila *et al*. 2006). It is also found in other organs including leaves (Hukkanen *et al*. 2007; Hanhineva *et al*. 2009a) and flowers (Hanhineva *et al*. 2008). It may also be present as a substituent in other compounds such as flavonols and spermidines (Hanhineva *et al*. 2008). Other phenolic acids frequently detected in strawberry, especially in the fruit, are glucose derivatives of cinnamic, caffeic, ferulic and sinapic acids (**Table 1**).

All strawberry flavonoids contain a flavonoid backbone hydroxylated in positions 3' and/or 4'of the B-ring (**Fig. 2**). Unlike in many other flavonoid-rich plants, enzymatic activity for the hydroxylation of the B-ring 5' position has not been reported in strawberry, and thus the main flavonoid metabolites are derivatives of the flavonols kaempferol and quercetin, the anthocyanidins, cyanidin and pelargonidin, and the flavan 3-ols (epi)catechin and (epi)epiafzalechin. *In planta*, flavonoids do not normally occur as free aglycones but are decorated e.g. with sugars and phenolic acids (**Table 1**).

The presence of two metabolite groups with large macromolecular structures, i.e. the proanthocyanidins (condensed tannins) and ellagitannins (hydrolyzable tannins) are typical to strawberry. The proanthocyanidins occur as linear molecules of the flavan 3-ol units (epi)catechin and (epi)afzalechin linked via a C4→C8 bond (B-type interlinkage). They are typically present as oligomers (Gu *et al*. 2003), but also polymers as large as decamers have been reported, especially at the early developmental stages of strawberry fruit (Fait *et al*. 2008). A recent analysis of proanthocyanidins showed variation both in the degree of polymerization and the quantity among fifteen strawberry cultivars (Buendía *et al*. 2010).

Ellagitannins occur in plants much less frequently than do proanthocyanidins but they are often produced by species in the *Rosaceae* family (Okuda *et al*. 1992). Unlike the majority of phenolic compounds generated via the phenylpropanoid pathway, ellagitannins are synthesized from gallic acid units that are intermediates in the shikimate pathway (Gross 1994). Ellagitannins occur as a myriad of different combinations of sugar core units with several conjugated gallic acid moieties, which can be further interlinked to form hexahydroxydiphenyl (HHDP) units (**Fig. 2**). Qualitative analysis of strawberry ellagitannins is in its early stages but recent reports indicate that several parts of the strawberry plant are rich in ellagitannins (Fait *et al*. 2008; Hanhineva *et al*. 2008). The most abundant macromolecular ellagitannins identified in strawberry fruit include lambertianin C, sanguiin H-6 and galloyl-bis-HHDP-glucose (Seeram *et al*. 2006b; Aaby *et al*. 2007a; Buendía *et al*. 2010). The HHDP units are easily released from ellagitannins, leading to the formation of ellagic acid, which is found in strawberry fruit together with various precursors of ellagitannins, i.e. galloyl glucoses (**Table 1**).

In addition to the commonly occurring phenolic compounds, strawberry contains some metabolites that have received little attention, such as the phenylethyl derivatives of phenylpropanoids (Hanhineva *et al*. 2009a, 2009b). One of the most intensively studied natural products, resveratrol, has been rarely reported in strawberry fruit and achenes (Ehala *et al*. 2005; Wang *et al*. 2007). Resveratrol has never been found in strawberry in profiling studies, as it is most likely present in detectable quantities only after induction or after specific purification. The lignans secoisolariciresinol and matairesinol were found in strawberry some years ago when their metabolism to enterolactone and enterodiol (lignan derivatives formed in mammals from plant lignans by intestinal bacteria) was studied by GC-MS (Mazur *et al*. 2000). An interesting flavonoid known to occur in strawberries but rarely reported in metabolomics studies is fisetin,

Table 1 Aromatic and phenolic metabolites reported in strawberry plants.

COMPOUND	MW	λ_{max} (nm)	Plant part	Analytics	Reference
Benzoic acid derivatives					
benzoic acid	122		fruit	FTMS	6
hydroxybenzoylhexose	300	262	fruit	LC-MS	2, 21
hydroxybenzoic acid	138		fruit	FTMS	6
vanillic acid	168		fruit	FTMS	6, 21
di-hydroxybenzoquinone	140		fruit	FTMS	6
di-hydroxy benzoic acid hexose	316		fruit	LC-MS	21
Cinnamic acid derivatives					
p-coumaric acid glucoside	326	264, 293	fruit, flower, leaf	LC-MS	1, 19, 20, 22
p-coumaroyl hexose	326	236, 300sh,310	fruit	LC-MS	1, 2, 3, 5
p-coumaroylhexose-4-*O*-hexoside	488	312	fruit	LC-MS	2
p-coumaroyl-ester	356	235, 330	fruit	LC-MS, NMR	3, 18
di-coumaroyl hexose	472		flower, leaf	LC-MS	19, 20
caffeoylglucose, caffeic acid hexose	342	264, 300sh, 330	fruit, flower	LC-MS	1, 19, 22
caffeate	180		fruit	FTMS	6
ethyl cinnamate	176		fruit	FTMS	6
methyl cinnamate	162		fruit	FTMS	6
hydroxyferulate	210		fruit	FTMS	6
4-coumarate	164		fruit	FTMS	6
sinapyl alcohol	210		fruit	FTMS	6
cinnamate glucose	310		fruit	FTMS	6
cinnamoyl-xylopyranoside	280	284	fruit	NMR	10
cinnamoyl-rhamnopyranoside	294	284	fruit	NMR	10
cinnamoyl-xylofuranosyl-glucopyranose	442	284	fruit	NMR	10
cinnamoyl-glucopyranoside		287	fruit	NMR	14
chlorogenic acid	354	sh-323	flower, leaf, fruit	LC-MS	19, 20, 22
ferulic acid hexose	356	sh-328	Flower, fruit	LC-MS	19, 22
galloyl caffeoyl hexose	494	252, 367	flower	LC-MS	19
galloyl coumaroyl hexose	478		flower, leaf	LC-MS	19, 20
coumaroyl quinic acid	338		flower	LC-MS	19
Phenylethyl derivatives of phenylpropanoid glucosides					
hydroxyphenylethyl coumaroyl glucopyranoside (Eutigoside A)	446	311	fruit, leaf	LC-MS	21, 22
hydroxyphenylethyl feruoyl glucopyranoside (Grayanoside A)	476	320	fruit, leaf	LC-MS	21, 22
hydroxyphenylethyl caffeoyl glucopyranoside	462		leaf	LC-MS	21
Gallic acid and ellagic acid derivatives					
ellagic acid 4-pentoside	435	252, 362	fruit	LC-MS	1
ellagic acid pentoside	434	254, 360	fruit, leaf, flower	LC-MS	2, 5, 19
ellagic acid	302	252, 368	fruit, leaf, flower	FTMS, LC-MS	1, 2, 3, 5, 6, 12, 19, 22
ellagic acid acetylpentoside	476	254, 358	fruit, leaf	LC-MS	1
ellagic acid deoxyhexoside	448	254, 362	fruit, leaf, flower	LC-MS	2, 5, 19, 22
methyl-ellagic acid pentose	448	250, 370	fruit	LC-MS	3
ellagic acid hexose	464		flower, leaf	LC-MS	5, 19
glucogallin, galloylglucose	332	276	fruit, leaf, flower	FTMS, LC-MS	6, 19, 20, 22
galloylquinic acid	344	270	fruit, flower, leaf	LC-MS	19, 20, 22
di-galloylquinic acid	496		fruit, flower	LC-MS	19, 22
di-galloylglucose	484	276	flower	LC-MS	19
tri-galloylglucose	636	272	fruit, flower, leaf	LC-MS	19, 20, 22
tetra-galloylglucose	788	278	fruit, flower	LC-MS	19, 22
penta-galloylglucose	940	277	fruit, flower, leaf	LC-MS	19, 20, 22
Ellagitannins					
HHDP-glucose	482	slope	fruit, flower	LC-MS	19, 22
bis-HHDP-glucose	784	232, slope	fruit, leaf, flower	LC-MS	2, 19, 20, 22
galloyl-HHDP-glucose	634	232, slope	fruit, leaf, flower	LC-MS	2, 21, 20, 22
HHDP-galloyl-glucose	634	300sh, 284	fruit	LC-MS	2
galloyl-bis-HHDP-glucose	936	234	fruit, leaf	LC-MS	2, 20, 22
di-galloyl HHDP glucose	786	270	fruit, flower	LC-MS	19, 22
sanguiin H6	1870	260, 345	fruit	LC-MS	3
sanguiin H10, (bis HHDP glucose)-dimer	1568	230, 280sh	fruit, leaf	LC-MS	5, 22
tri-galloyl-HHDP glucose	938		fruit, flower, leaf	LC-MS	19, 20, 22
di(HHDP-galloylglucose)-pentose	1416	225	leaf	LC-MS	5
casuarictin	936	225, 280sh	fruit, flower, leaf	LC-MS	5, 19, 22
trigalloyl-triHHDP-diglucose	1718	230, 280sh	leaf	LC-MS	5
potentillin	936	230, 260sh, 280sh	fruit, flower, leaf	LC-MS	5, 19, 20, 22
agrimoniin	1870	230, 260sh, 280sh	fruit, flower, leaf	LC-MS	5, 19, 20, 22
lambertiain C	2804		fruit	LC-MS	22
pedungulagin			root	NMR	7
Chalcones					
phloretin	274		fruit	LC-MS, NMR	15
phloridzin	436		fruit	LC-MS, NMR	15
naringenin/naringenin chalcone hexose	434		fruit	LC-MS	22

Table 1 (Cont.)

COMPOUND	MW	λ_{max} (nm)	Plant part	Analytics	Reference
Flavanones					
dihydrokaempferol (aromadendrin)	288		fruit	FTMS	6
dihydroquercetin (taxifolin)	304		fruit	FTMS	6
taxifolin 3-arabinofuranoside	436		root	NMR	7
eriodictyol hexose	450		fruit	LC-MS	22
Flavones					
apigenin	270		fruit	GALDI-MS	16
apigenin glucoside	432		fruit	GALDI-MS	16
Flavan-3-ols, proanthocyanidin					
(+)-catechin	290	278/280	fruit, flower, leaf, root	FTMS, LC-MS, NMR	1, 2, 3, 6, 7, 19, 20, 22
(-)-epicatechin	290	278	fruit	LC-MS	1, 22
(+)-afzelechin-catechin			root	NMR	7
dimer B2	578	278	fruit, leaf, flower	LC-MS	1, 5, 19, 20, 22
proanthocyanidin B1	578	310,286	fruit	LC-MS	2
proanthocyanidin B3	578	312sh, 284	fruit, root	LC-MS, NMR	2, 7
procyanidin tetramer	1154	277	fruit, flower	LC-MS	19, 22
procyanidin pentamer	1442	277	fruit, flower	LC-MS	19, 22
proanthocyanidin trimer (EC-4,8-EC-4,8-C)	866	284	fruit, leaf, flower	LC-MS	2, 19, 20, 22
procyanidin B6			root	NMR	7
propelargonidin dimer (afz-cat)	562	277	fruit, flower	LC-MS	17, 19, 22
propelargonidin trimer (afz-cat-cat)	850	276	fruit, flower	LC-MS	17, 19, 22
propelargonidin tetramer (afz-cat-cat-cat)	1138		fruit	LC-MS	22
propelargonidin trimer (afz-afz-cat)	834		fruit	LC-MS	22
Flavonols					
quercetin 3-glucoside (quercetin hexose)	464	354, 285	fruit, flower	LC-MS, FTMS	1, 3, 6, 19
quercetin di-hexose	626		flower	LC-MS	19
quercetin hexose glucuronide	640	260, 353	flower	LC-MS	19
quercetin pentose glucuronide	610	255, 353	flower, leaf	LC-MS	19, 20
quercetin 3-glucuronide	478	354, 258	fruit, leaf, flower	LC-MS	1, 2, 3, 5, 19, 20, 22
quercetin 3-glucurone-deoxyhexoside	624	254, 300sh, 354	fruit	LC-MS	1
quercetin-3-malonylglucoside, (quercetin malonylhexose)	550	256, 354	fruit, flower	LC-MS	2, 19
quercetin-rutinoside (rutin)	610	255, 355	fruit	LC-MS	3, 22
quercetin-deoxyhexose-hexose (not rutin)	610	255, 295sh, 350	leaf	LC-MS	5
kaempferol 3-glucuronide	462	348, 265	fruit, leaf, flower	LC-MS	1, 3, 5, 19, 20, 22
kaempferol-3-glucoside, kaempferol hexose	448	266, 348	fruit, flower	LC-MS	2, 19, 22
kaempferol 3-malonylglucoside, kaempferol malonylhexose	534	266, 346	fruit, flower	LC-MS	2, 5, 19, 22
kaempferol 3-coumaroylglucoside (tiliroside)	594	268, 314/250	fruit, leaf, flower	LC-MS, NMR	2, 3, 11, 18, 19, 20, 22
kaempferol acetylhexose	490		fruit	LC-MS	22
kaempferol di-hexose glucuronide	786	264, 345	flower	LC-MS	19
kaempferol di-pentose hexose glucuronide	888	265, 345	flower	LC-MS	19
kaempferol di-hexose	610		flower	LC-MS	19
kaempferol hexose glucuronide	624	264, 344	flower, leaf	LC-MS	19, 20
kaempferol pentose glucuronide	594	265, 345	flower, leaf	LC-MS	19, 20
isorhamnetin hexose	478		fruit	LC-MS	22
isorhamnetin 3-glucuronide	492	254, 300sh, 354	fruit, flower	LC-MS	19, 22
isorhamnetin sophorose hexose	802		flower	LC-MS	19
isorhamnetin di-hexose	640	253, 362	flower	LC-MS	19
isorhamnetin rutinose	624		flower	LC-MS	19
isorhamnetin hexose malonylhexose	726	253, 360	flower	LC-MS	19
leucocyanidin	306		fruit	FTMS	6
Anthocyanins					
cyanidin 3-glucoside, (cyanidin hexose)	449	280, 516	fruit	LC-MS, NMR, FTMS	1, 2, 3, 4, 6, 18, 22
cyanidin hexose- deoxyhexoside	595	280, 516	fruit	LC-MS	1
cyanidin 3-sophoroside	611	280, 516	fruit	LC-MS	1
cyanidin 3-(2^G-glucosylrutinoside)	757	280, 516	fruit	LC-MS	1
cyanidin 3-rutinoside	595	280, 516	fruit	LC-MS	1, 4
cyanidin 3-malonylglucose-5-glucose	697	524	fruit	LC-MS	4
pelargonidin 3-glucoside	433	276, 504, 428sh	fruit	LC-MS, NMR, FTMS	1, 2, 3, 4, 5, 6, 8, 9, 18, 22
pelargonidin 3-rutinoside	579	276, 504	fruit	LC-MS, NMR	1, 3, 4, 18, 22
pelargonidin 3-malonylglucoside	519	276, 504, 430sh	fruit	LC-MS	1, 2, 5, 22
pelargonidin 3-succinylglucoside	533	276, 504	fruit	LC-MS	1
5-pyranopelargonidin-3-glucoside	501	492, 358, 262sh	fruit	LC-MS	2
pelargonidin 3-malonylrhamnoside or 3-succinylarabinoside	503	280, 430sh, 506	fruit	LC-MS	2
pelargonidin diglucoside	594/ 595	275, 520/500	fruit	LC-MS	3, 4
pelargonidin 3-malylglucoside	549	503	fruit	LC-MS	4
pelargonidin hexose pentose acylated with acetic acid	607	503	fruit	LC-MS	4
pelargonidin 3-acetylglucoside	475	504	fruit	LC-MS	4
catechin-4,8-pelargonidin-3-glucoside	721	518, 438	fruit	NMR	8
epicatechin-4,8-pelargonidin-3-glucoside	721	518, 433	fruit	NMR	8

Table 1 (Cont.)

COMPOUND	MW	λ_{max} (nm)	Plant part	Analytics	Reference
Anthocyanins (Cont.)					
afzelechin-4,8-pelargonidin-3-glucoside		516, 434	fruit	NMR	8
epiafzelechin-4,8-pelargonidin-3-glucoside	705	520, 432	fruit	NMR, LC-MS	8, 22
5-carboxypyranopelargonidin-3-glucoside	501	360, 496	fruit	LC-MS, NMR	9
5-carboxpyranocyanidin-3-glucoside	517	278, 351, 505	fruit	LC-MS, NMR	9
Phenolic polyamine derivatives					
di-caffeoyl coumaroyl spermidine	615	218, 292	Flower	LC-MS	19
caffeoyl di-coumaroyl spermidine	599	218, 292	Flower	LC-MS	19
caffeoyl coumaroyl feruoyl spermidine	629	225, 301	Flower	LC-MS	19
tri-coumaroyl spermidine	583	290	Flower	LC-MS	19
di-coumaroyl feruoyl spermidine	613	292	Flower	LC-MS	19
coumaroyl di-feruoyl spermidine	643	292	Flower	LC-MS	19
Others					
L-(+)-ascorbic acid	176	244	fruit	FTMS, LC-MS	6, 2
quinic acid	192	225, 270	fruit, leaf	FTMS, LC-MS	5, 6
gentisic/protocatechuic acid	154		fruit	FTMS	6
N-propyl carbazole	209		fruit	FTMS	6
3-methylcatechol	124		fruit	FTMS	6
1,4-benzoquinone	1008		fruit	FTMS	6
2-glucopyranosyloxy-4,6,-dihydroxyisovalerophone	372	225, 286	fruit	NMR	11
trans-resveratrol	228	320	fruit	LC-MS	13
cis-resveratrol	228	288	fruit	LC-MS	13
3,4,5-trihydroxyphenyl acrylic acid			fruit	LC-MS	18

MW, molecular weight; HHDP, hexa-hydroxyl di-phenyl; afz, afzelechin; cat, catechin

References: 1: Määttä-Riihinen *et al.* 2004; 2: Aaby *et al.* 2007a; 3: Seeram *et al.* 2006b; 4: Lopes da Silva *et al.* 2002; 5: Hukkanen *et al.* 2007; 6: Aharoni *et al.* 2002; 7: Ishimaru *et al.* 1995; 8: Fossen *et al.* 2004; 9: Andersen *et al.* 2004; 10: Cheel *et al.* 2005; 11: Tsukamoto *et al.* 2004; 12: Heur *et al.* 1992; 13: Wang *et al.* 2007; 14: Latza *et al.* 1996; 15: Hilt *et al.* 2003; 16: Zhang *et al.* 2007; 17: Gu *et al.* 2003; 18: Zhang *et al.* 2008; 19: Hanhineva *et al.* 2008; 20: Hanhineva *et al.* 2009a; 21: Hanhineva *et al.* 2009b; 22: Fait *et al.* 2008.

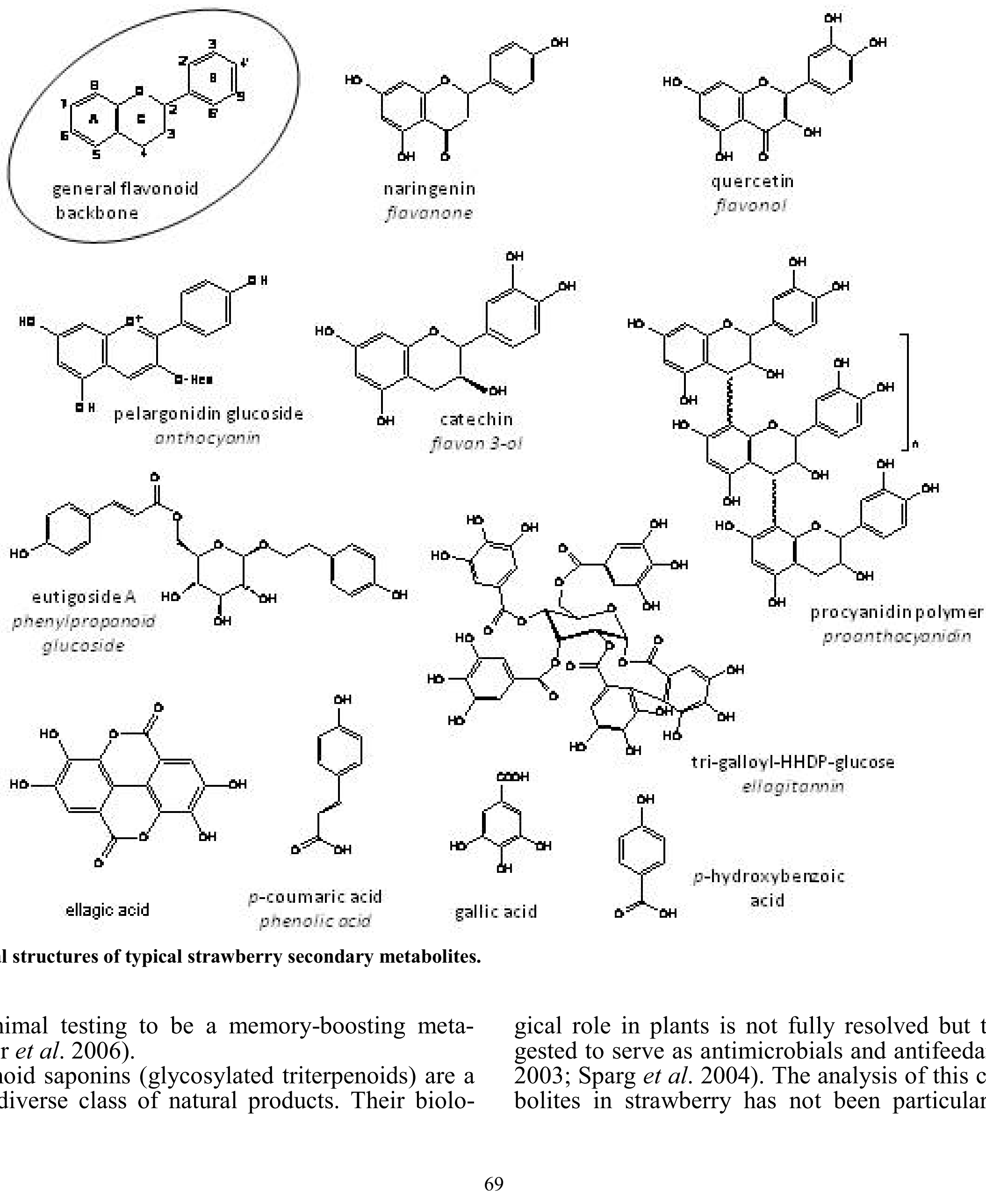

Fig. 2 Chemical structures of typical strawberry secondary metabolites.

shown in animal testing to be a memory-boosting metabolite (Maher *et al.* 2006).

Triterpenoid saponins (glycosylated triterpenoids) are a structurally diverse class of natural products. Their biological role in plants is not fully resolved but they are suggested to serve as antimicrobials and antifeedants (Osbourn 2003; Sparg *et al.* 2004). The analysis of this class of metabolites in strawberry has not been particularly intensive,

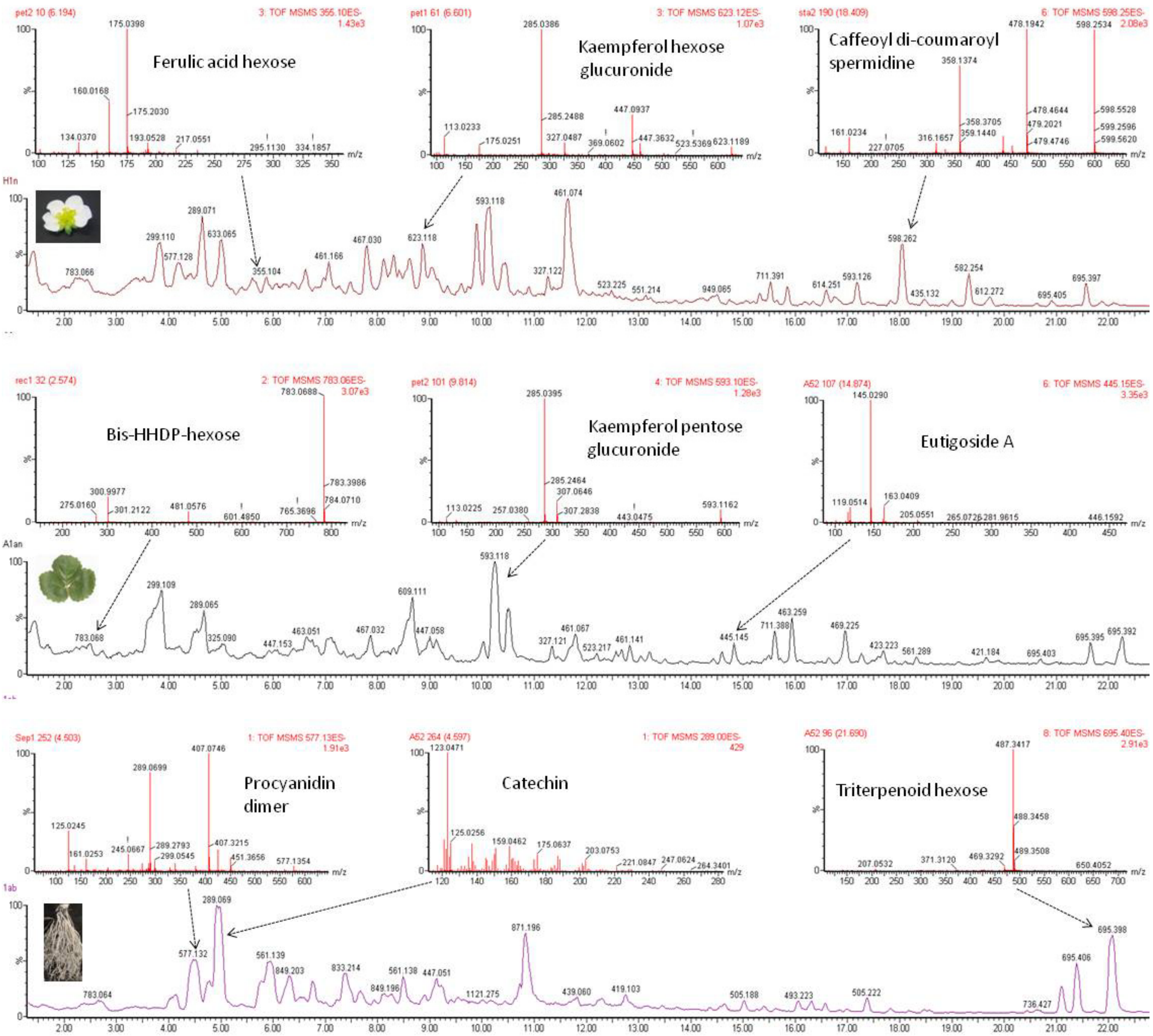

Fig. 3 Total ion chromatograms of strawberry flower (upper panel), mature green leaf (middle panel) and root (lower panel) obtained by UPLC-qTOF-MS analysis with ES(-) ionization. Examples of metabolites are indicated with MS/MS spectrum in ES(-).

although a few reports showed that at least the triterpenoids and their glucose derivatives are found in several strawberry organs (Hirai *et al*. 2000; Fait *et al*. 2008; Hanhineva *et al*. 2008). Interestingly, visual comparison of total ion chromatograms of different strawberry organs indicates that the last few minutes in the chromatogram, which is the region in which saponins (triterpenoid glucosides) are typically eluted, is particularly rich in the roots (**Fig. 3**).

Polyamines are nitrogenous compounds which can be conjugated with small molecules such as phenolic acids. These conjugates have been studied particularly in the flowers (Martin-Tanguy 1997), and were shown to be present as different sets of phenolic acid conjugates (Hanhineva *et al*. 2008).

Compounds that contribute to the flavor and aroma of ripe strawberry fruit, and several enzymes involved in their biosynthesis are well characterized (Aharoni *et al*. 2000; Lunkenbein *et al*. 2006a). Terpenoids are among the most important contributors to the aroma, and in cultivated strawberry the monoterpene linalool and the sesquiterpene nerolidol are the most characteristic compounds (Aharoni *et al*. 2004). Additionally, many other methylated volatile derivatives of phenolic acid precursors synthesized in a branch of phenylpropanoid pathway contribute to the aroma (Zabetakis and Holden 1997). The primary metabolite content and the volatile aroma compounds of strawberry, including acids, alcohols, aldehydes, ketones, esters, lactones, acetals, furans, sulphur containing compounds and terpenes have been reviewed by Zabetakis and Holden (1997). A more recent report deals with common aroma volatiles during ripening of wild strawberry fruit (Gonzalez *et al*. 2009).

DEVELOPMENTAL EFFECTS ON STRAWBERRY METABOLITES

The phytochemical composition of plants is highly responsive to internal and external stimuli. Thus, metabolite composition varies with the developmental stage, in different organs as well as in response to environmental perturbations such as UV radiation and disease. A clear difference in the metabolite profile of various parts of the strawberry plant is shown in **Figs. 3** and **4**. Interestingly, strawberry root appears to contain a wide array of secondary metabolites but this organ has not been extensively studied, as only few papers mention the analysis of secondary metabolites in strawberry root (Ishimaru *et al.* 1995; Aharoni *et al*. 2004). The roots clearly represent an almost unexplored resource for phytochemical research of strawberry, as the total ion chromatograms indicate that they are rich in semi-polar compounds (**Figs. 3** and **4**). In contrast to the roots, the phy-

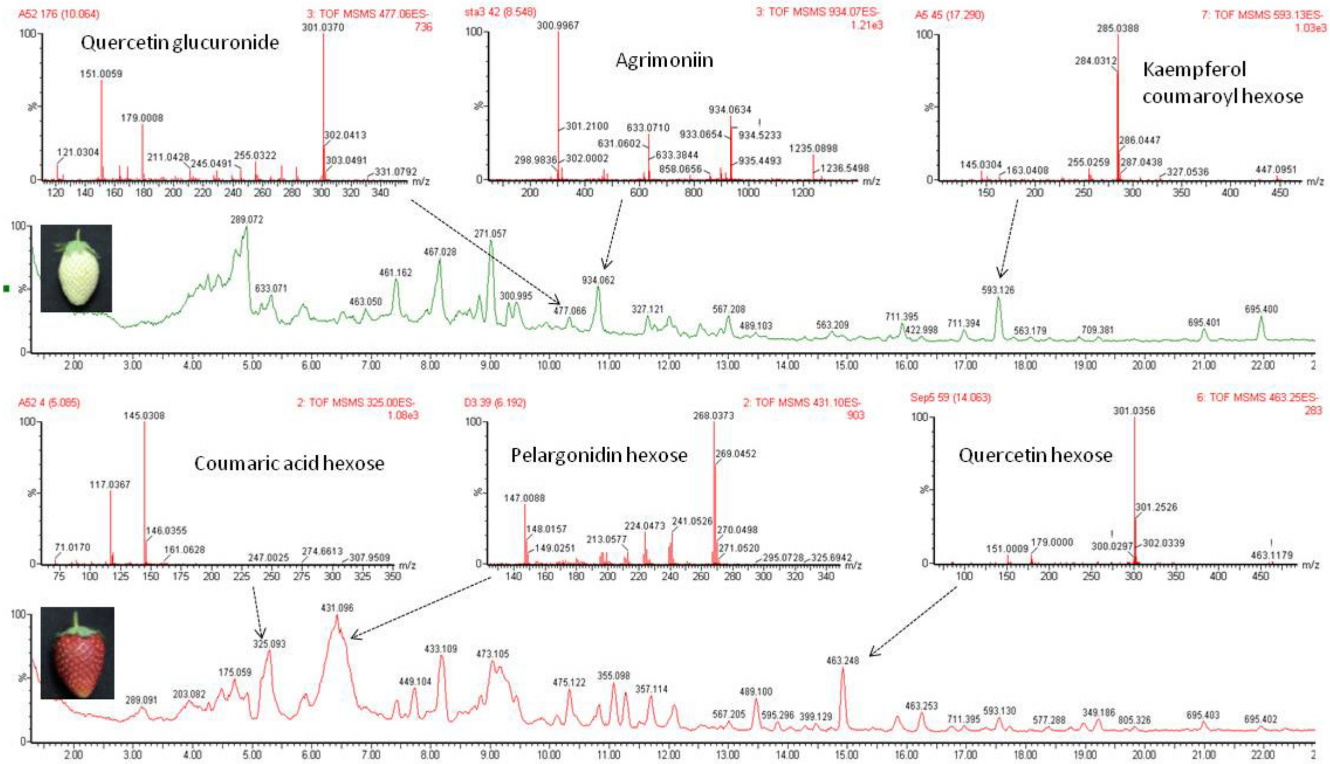

Fig. 4 Total ion chromatograms of immature white strawberry fruit (upper panel) and mature red fruit (lower panel) obtained by UPLC-qTOF-MS analysis with ES(-) ionization. Examples of metabolites are indicated with MS/MS spectrum in ES(-).

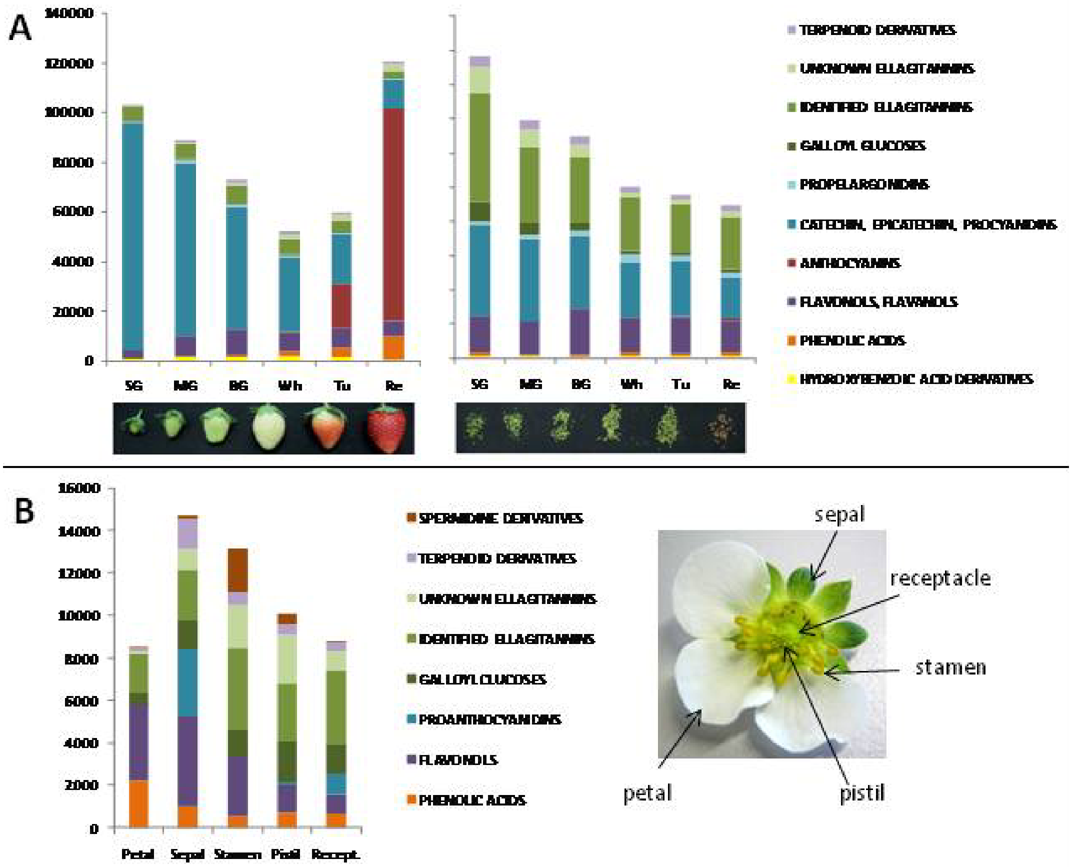

Fig. 5 Distribution of metabolites belonging to different metabolite classes in A. Strawberry fruit tissues (receptacle and achenes) and B. Floral organs. The blocks in the columns represent the sum of the average peak areas (Y-axis) of all identified metabolites in non-targeted metabolite profiling analysis.

tochemicals present in strawberry fruit have been described extensively. More than a hundred metabolites have been reported in the literature, showing both qualitative and quantitative differences among different cultivars and organs (**Table 1** and references therein).

One of the most extensively studied topics is the differential production of metabolites during fruit development. Alterations in fruit secondary metabolism are well documented and demonstrate a shift from the accumulation of the astringent proanthocyanidin polymers to coloured anthocyanins during maturation, as well as several other distinct compositional changes (Aharoni *et al.* 2002; Kosar *et al.* 2004; Halbwirth *et al.* 2006; Fait *et al.* 2008). The amount of phenylalanine is very high at the early stages of development as it serves as a precursor for proanthocyanidins, and its amount rises again at the very last stage of maturation enabling the development of anthocyanin colouration (Aharoni *et al.* 2002; Halbwirth *et al.* 2006). Changes also occur in the content of primary metabolites (Fait *et al.* 2008) as well as in volatile aroma and flavour components in garden strawberry (Menager *et al.* 2004) and its progenitor *F. chiloensis* (Gonzalez *et al.* 2009). Maximal volatile (e.g. furanones) production (Menager *et al.* 2004) as well as increase in esters (Gonzalez *et al.* 2009) take place in fully ripe red fruit. The phenolic composition of strawberry achenes has been analysed separately from the fruit flesh (receptacle). It appears that the achenes are extremely rich in phenolics both qualitatively and quantitatively (Aaby *et al.* 2005, 2007b; Fait *et al.* 2008).

Hanhineva *et al* (2008) recently published a detailed characterization of the metabolites in the floral organs of strawberry, which demonstrates the spatial distribution of different phytochemical classes and diverse derivatives of each metabolite class in the different parts of the flower (**Fig. 5**; Hanhineva *et al.* 2008). An overall difference in the phenolic composition of strawberry floral organs is illustrated in **Fig. 1**; the autofluorescence of phenolics is clearly visible under fluorescence confocal microscopy, pointing out the different distribution among the organs and even organ compartments (as shown by the different colours in the anther and filament of the stamen in **Fig. 1b**). Various flavonols as well as their sugar conjugates showed both qualitative and quantitative differences (Hanhineva *et al.* 2008), suggesting that they play distinct roles in the flower. Flavonols are known to play a central role in plant fertility, being essential for pollen tube germination (Mo *et al.* 1992; Ylstra *et al.* 1994).

INFLUENCE OF ENVIRONMENTAL FACTORS AND GENETIC MODIFICATION ON STRAWBERRY METABOLITE PRODUCTION

Phytochemicals are important for the adaptation of sessile plants to their changing environmental conditions. One of the most important roles of phenolic compounds *in planta* is the protection against fungal and bacterial infections and other harmful environmental conditions such as UV radiation. This has been demonstrated by the increase in phenolic compounds in the leaves after treatment of strawberry plants with benzothiadiazole, which induces natural plant defence (Hukkanen *et al.* 2007). On the other hand, when flavonoid synthesis was down regulated by genetic modification (suppression of *CHALCONE SYNTHASE*), the strawberries had increased susceptibility to fungal infection, which was suggested to be due to the depletion of flavonols and other flavonoids (Hanhineva *et al.* 2009a). Antifungal metabolites have been found among the volatile compounds (Arroyo *et al.* 2007), triterpenes (Hirai *et al.* 2000; Terry *et al.* 2004) and phenolics (Terry *et al.* 2004) of strawberry fruit. Preharvest conditions and treatments clearly affect the levels of phenolic metabolites in strawberry (Anttonen *et al.* 2006; Hukkanen *et al.* 2007; Wang *et al.* 2007).

Metabolomics has proven indispensable in the characterisation of strawberries with genetically modified phenylpropanoid pathway (Lunkenbein *et al.* 2006b; Hanhineva *et al.* 2009). A non-targeted profiling method was used to identify the changes that occurred in secondary metabolites following the transfer of a stilbene synthase-encoding gene (Hanhineva *et al.* 2009a). Unexpectedly introduction of the gene did not result in the production of resveratrol, but rather the accumulation of several phenolic acid derivatives in the central phenylpropanoid pathway was observed. Among the accumulating metabolites was also a group of compounds that could not be unambiguously identified by LC-MS. These compounds were subsequently subjected to 2D-NMR analysis that led to the discovery of a yet uncharacterized metabolite class in strawberry, i.e. the phenylethanol derivatives of phenylpropanoid glucosides (Hanhineva *et al.* 2009b). This metabolite class is not well-defined in plants, and clearly deserves more attention in the future.

STRAWBERRY METABOLITES AS BENEFICIAL COMPONENTS IN THE DIET

The importance of polyphenol-rich food in human health and prevention of diseases is well acknowledged, including anticarcinogenicity as well as lowering the risk of cardiovascular diseases and other aging-induced malfunctions. Especially ellagitannins have gained much attention because of their anticarcinogenicity (Kuo *et al.* 2007; Ross *et al.* 2007). Strawberries are among the most important polyphenol sources both as fresh fruit and processed products and could have vital effects on human health if consumed regularly as part of a healthy diet (Hannum 2004; Zafra-Stone *et al.* 2007; Tulipani *et al.* 2009). Estimations have been made about the phenolic content and intake of various food components in the diet, including the Finnish (Ovaskainen *et al.* 2008), French (Brat *et al.* 2006) and American diets (Chun *et al.* 2005). In the French diet, strawberries and apples are the main sources of polyphenols (Brat *et al.* 2006). Besides ellagitannins, the minor flavonoids in strawberry such as the flavonols kaempferol and quercetin, as well as their precursor phenolic acids are targets of intensive research in terms of assessing their bioactivity and bioavailability, and they most likely contribute to the heath-beneficial characteristics of strawberry.

The contents of several classes of polyphenol family and also of individual metabolites in strawberry consumables (i.e. food products) and fresh fruit are summarized in **Table 2**. Different studies include slightly different combinations of compounds, and the results may also vary depending on the analytical method. Most often the content of total phenolics has been estimated (Kähkönen *et al.* 2001; Ovaskainen *et al.* 2008; Vasco *et al.* 2009). Studies on the contents of different phenolic classes include ellagitannins (Koponen *et al.* 2007), anthocyanins (Nyman and Kumpulainen 2001; Koponen *et al.* 2007; Tulipani *et al.* 2008; Buendía *et al.* 2009), phenolic acids (Mattila and Kumpulainen 2002; Mattila *et al.* 2006) and proanthocyanidins (Buendía *et al.* 2009; Hellström *et al.* 2009). Although the studies were typically focused on fruit, strawberry leaves are also a rich source of phytochemicals that could have potential *e.g.* in the development of food supplements, and should thus not be overlooked (Mudnic *et al.* 2009).

The phenolic content of strawberry fruit decreases during industrial (Hartmann *et al.* 2008) and domestic (Häkkinen *et al.* 2000a, 2000b) processing. Processing of juices and purees is a significant source of variation in the anthocyanin content as these compounds may undergo structural changes caused by pH, light and oxidating enzymes (Aaby *et al.* 2007; Hartmann *et al.* 2008). Processing also usually removes the achenes, which are a rich source of e.g. ellagitannins both quantitatively (Aaby *et al.* 2005) and qualitatively (Fait *et al.* 2008). Even though the achenes constitute only 1% of strawberry fresh weight, they account for 11% of total phenolics and 14% of antioxidative capacity (Aaby *et al.* 2005) which is an important point of consideration for strawberry processing. The achenes also help to preserve the phenolic content and antioxidative capacity of strawberry purees during storage (Aaby *et al.* 2007b).

While the exact mechanisms and contributing compounds have not been fully resolved (Crozier *et al.* 2009), the health-beneficial effects of polyphenol-rich fruits are most often ascribed to their antioxidative activity. However, in strawberry fruit, ascorbic acid is the most important single contributor to the antioxidative capacity while among

Table 2 Content of phenolic compounds in strawberry.

Compounds	Reported values	Reference
Polyphenols in total		
Finnish FCDB	178 mg/100 g	Ovaskainen *et al*. 2007
strawberry jam	59 mg/100 g	Ovaskainen *et al*. 2007
French consumption	268 mg of GAE/100 g FEP	Brat *et al*. 2006
Ecuadorian	531 mg/kg FW	Vasco *et al*. 2009
Finnish FCDB	1600-2410 mg/100 g DW	Kähkönen *et al*. 2001
Total anthocyanin		
fruit, nine cultivars	99-296 µg/g FW	Tulipani *et al*. 2008
comparison of several studies	15-75 mg/100 g edible portion	Rev. Lotito and Frei 2006
fruit	32-52 mg/100 g FW	Koponen *et al*. 2007
strawberry jam	3 mg/100 g FW	Koponen *et al*. 2007
flesh and achenes, freeze dried and puree	11-68 mg/100 g FW	Aaby *et al*. 2005
fruit, three cultivars	195-232 mg/100 g DW	Kähkönen *et al*. 2001
fruit, 15 cultivars	20-47 mg/100 g FW	Buendia *et al*. 2010
Ellagitannins		
fruit and achenes separately, freeze dried and puree	9-833 mg/100 g FW	Aaby *et al*. 2005
fruit	68-85 mg/100 g FW	Koponen *et al*. 2007
strawberry jam	25 mg/100 g FW	Koponen *et al*. 2007
fruit, three cultivars	81-184 mg/100 g DW	Kähkönen *et al*. 2001
fruit, 15 cultivars	10-23 mg/100g FW	Buendia *et al*. 2010
Proanthocyanidins		
fruit and achenes separately, freeze dried and puree	10-32 mg/100 g FW	Aaby *et al*. 2005
fruit, three cultivars	8-10 mg/100 g DW	Kähkönen *et al*. 2001
fruit, 15 cultivars	54-163 mg/100 g FW	Buendia *et al*. 2010
fruit	34-57 mg/100 g FW	Hellström *et al*. 2009
strawberry jam	12 mg/100 g FW	Hellström *et al*. 2009
Flavonols		
fruit, three cultivars	6-20 mg/100 g DW	Kähkönen *et al*. 2001
fruit, 15 cultivars	2-3 mg/100 g FW	Buendia *et al*. 2010
Phenolic acids		
fruit, three cultivars (hydroxycinnamic acid)	47-63 mg/100 g DW	Kähkönen *et al*. 2001
fruit, three cultivars (hydroxybenzoic acid)	11-55 mg/100 g DW	Kähkönen *et al*. 2001
fruit, 15 cultivars (p-coumaroyl glucose)	1-7 mg/100 g FW	Buendia *et al*. 2010
fruit	10-18 mg/100 g FW	Mattila *et al*. 2006
strawberry jam	12 mg/100 g FW	Mattila *et al*. 2006
Phytoestrogens		
Secoisolariciresinol	15046 µg/kg DW	Mazur *et al*. 2000
Matairesinol	781 µg/kg DW	Mazur *et al*. 2000
Other		
folate	13-96 µg/100 g FW	Rev. Tulipani *et al*. 2009

FW, fresh weight; DW, dry weight; FCDB, Finnish national food compositin database; GAE, gallic acid equivalent; FEP, fersh edible portion

the polyphenols, ellagitannins and anthocyanins exhibit the strongest antioxidative properties (Scalbert *et al*. 2000; Yu *et al*. 2005; Mertens-Talcott *et al*. 2006; Aaby *et al*. 2007a, 2007b). Although the conclusive proof for the mechanism of the proposed health-beneficial effects is lacking, new evidences are emerging from *in vitro* studies on cell lines showing antiproliferative effects on cancer cells (Olsson *et al*. 2006; Seeram *et al*. 2006a; Zhang *et al*. 2008) and anti-bacterial activity on intestinal pathogens (Puupponen-Pimiä *et al*. 2005; Nohynek *et al*. 2006) exposed to strawberry extracts.

Polyphenols have relatively low biavailability (Lotito and Frei 2006; Korkina 2007). Most studies focus on the soluble phenolic compounds that are readily absorbed from the fruit. Very few reports describe the importance of human colonic microbiota in the modification and absorption of the dietary polyphenols, including those from strawberry (del Rio *et al*. 2009). It is known, however, that a large proportion of the polyphenols is bound to the matrix (e.g. fiber) and is released only by the activity of colonic microbiota. The few studies on strawberry metabolites include an analysis of the formation of urolithin from ellagitannin precursors in strawberry-rich diet (Cerda *et al*. 2005), and the production of important bioactive molecules from strawberry lignans as a result of the activity of colonic microbiota, as well as their urinary excretion after consumption of strawberries (Mazur *et al*. 2000).

While the majority of studies focus on phenolic compounds, strawberry fruit contains other nutritionally important chemical constituents, such as vitamins and amino acids. Among the most promising nutrients is folate, the content of which in strawberry is 10 to 100 µg/100 g fresh weight (reviewed in Tulipani *et al*. 2009). There are thus several reasons to include strawberries in a healthy diet.

CONCLUSIONS

The recent year's outcome of metabolite analyses using metabolomics technologies provided a significant addition to the existing data generated through targeted, less comprehensive analyses. While in most cases, particularly with MS-based methods, the identification of various metabolites is not unambiguous, it still provides very valuable information for biological studies. However, minor constituents may have bioactivity and/or they can act synergistically with the more abundant metabolites and it is therefore essential to examine those compounds in detail as well. As described above, the repertoire of strawberry secondary metabolites is enormous, varying from organ to organ, in different tissues, and even from cell layer to layer. It is not clear at this stage if all of these chemicals are crucial for the plant life cycle. Therefore, a major future challenge will be to understand the role of this metabolite diversity in plant growth and fitness. The increasing knowledge about the composition of strawberry and other fruit species is also expected to inspire new discoveries regarding the value of strawberry and related species to human health and nutrition.

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