

Towards new properties of strawberry: Chemical composition and sensory properties of species-reconstructed garden strawberry progenies

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ABSTRACT

Garden strawberry (*Fragaria* × *ananassa*) is a hybrid species with a narrowed allelic diversity due to intensive breeding. We studied the chemical composition and sensory properties of 13 progenies (reconstructed garden strawberry from diverse *F. chiloensis* and *F. virginiana* germplasms) and compared to cultivars ‘Honeoye’, ‘Korona’ and ‘Polka’. Polar metabolite profiles (¹H NMR) were associated with ripeness and origin of the wild parent. Volatile compounds not commonly present in commercial cultivars were observed, and the total content of volatiles and specifically esters were present in higher concentrations in the hybrids (HS-SPME-GC-MS). Descriptive sensory analysis indicated the hybrids were less red, and the anthocyanin contents (UHPLC-DAD), especially pelargonidin glycosides, were lower in the hybrid strawberries. Hybrids were also smaller, and had higher overall odour and overall flavour intensities. Hence, the reconstruction from wild sources enabled the introduction of new chemical, colour and flavour properties of high interest into the garden strawberry.

1. Introduction

Strawberries are among the most consumed berries with global production increasing annually, reaching 9.6 million ton in 2022 (FAOSTAT, 2022). Given its popularity, the chemical composition of strawberries is widely studied (Alvarez-Suarez et al., 2014; Giampieri et al., 2012; Kårlund et al., 2015; Prat et al., 2014; Scott et al., 2021; Song et al., 2016; Ulrich & Olbricht, 2016; Yan et al., 2018). Strawberry cultivar selection, pre-harvest and post-harvest factors are well-known to affect the comprehensive quality of strawberries (Alvarez-Suarez et al., 2014).

Garden strawberry (*Fragaria* × *ananassa*) first originated in Europe from chance hybridisations between a few introduced accessions of South-American *F. chiloensis* and North-American *F. virginiana*, two sister species with highly polyploid (octoploid) genome compositions (Darrow, 1966). Subsequently, the high allelic diversity of the early hybrids declined as inbreeding in strawberry breeding programmes eradicated in particular the *F. chiloensis* -derived allelic forms (Fan & Whitaker, 2023; Hardigan et al., 2021). However, the genetic and phenotypic variation from wild and landrace germplasms are accessible

because there are no reproductive barriers between the cultivated strawberry and its progenitor species. To truly re-introduce the wide genetic diversity into breeding gene pools, a complete species reconstruction approach has been suggested and demonstrated (Hancock et al., 2010; Hancock & Luby, 1993). In this approach, improved *F. chiloensis* and *F. virginiana* elite individuals were created by within-species crosses between accessions from different geographic areas. The two species provide highly diverse novel parent materials for breeders to use in subsequent between-species reconstruction crosses (Hancock et al., 2010; Hancock & Luby, 1993). These elite germplasms support the possibility of newly reconstructing the garden strawberry from its progenitor species and the introduction of multiple traits for adaptation to climate change, as well as for improvement of its sensory and chemical characteristics.

F. chiloensis, the Chilean strawberry, has four recognised subspecies, namely ssp. *chiloensis*, ssp. *pacifica*, ssp. *lucida*, and ssp. *sandwicensis*, with different morphologies (Hokanson et al., 2006). *F. chiloensis* ssp. *chiloensis* originates from Chile, and ssp. *pacifica* and ssp. *lucida* are found from Northern hemisphere as far as Alaska. In fully ripe stage, the fruits of cultivated *F. chiloensis* ssp. *chiloensis* landraces are large and

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their colour is dull white-pink, whereas fruits from wild *F. chiloensis* ssp. *chiloensis* f. *patagonica*, ssp. *pacifica*, and ssp. *lucida* are light or dull red (Hancock et al., 2003; Morales-Quintana & Ramos, 2019). Chilean strawberry has a cultivation history of at least 1000 years in South America (Hancock et al., 1999). The Virginia strawberry, *F. virginiana*, originates from Northern America. It has four recognised subspecies, namely ssp. *glauca*, ssp. *platypetala*, ssp. *grayana*, and ssp. *virginiana* Mill (Hokanson et al., 2006); these have scarlet or crimson red fruits with mostly white interior (Hancock, 2020).

Sensory properties are one of the most important cultivar characteristics, which dictate how well new cultivars will gain consumer acceptance and succeed in the markets. Especially the sweetness and flavour are among the most important factors affecting consumer acceptance of strawberries (Fan et al., 2021). However, sweetness of strawberries is not only ascribed to sugars and acids, but certain volatile compounds, such as γ -dodecalactone, can enhance the perceived sweetness independently of sugars and are therefore important factors to consider in breeding processes (Fan et al., 2021). Some volatile compounds, such as furanones, and branched esters, may negatively affect consumer preferences (Ulrich & Olbricht, 2016). On the other hand, certain volatile compounds, such as methyl anthranilate and γ -decalactone, have antifungal activities and thus, reduce fruit spoilage and improve harvest quality (Chambers et al., 2013). Anthocyanins are responsible for red colour of strawberries (Aaby et al., 2012).

Anthocyanins found in strawberries are mainly pelargonidin and cyanidin glycosides. In *F. × ananassa*, the main anthocyanin is pelargonidin-3-O-glucoside, but in white berried *F. chiloensis* ssp. *chiloensis* (form *chiloensis*) the main anthocyanin is cyanidin-3-O-glucoside (Salvatierra et al., 2010). Flavonoids and flavan-3-ols are positively correlated to the intensities of bitterness and astringency, flavonol glycosides to astringency intensities, and ellagic acid to the intensity of the bitter flavour in the strawberries (Kårlund et al., 2015).

While the potentially aroma active compounds are numerous and their biochemical pathways have complex interrelations and low heritability values, family-based selection scheme has been suggested: future parents are selected based on the performance of their progenies (Carrasco et al., 2005). Thus, the aim of this study was to characterise the fruit chemical composition of strawberry progenies, created by crosses between various improved selections of *F. chiloensis* (three subspecies) and *F. virginiana* ssp. *virginiana*, and the relationship of their chemical compositions to sensory properties. The compositional and sensory profiles of strawberry hybrid progenies were expected to differ from the commercial strawberry cultivars, as new properties from the wide taxonomic and geographic range of their parents have been inherited.

Table 1

Reconstructed strawberry progenies and fruit sampling.

Progeny/ Cultivar	Fragaria chiloensis founders				Sample code	Average fruit weight (g/ berry) ^e	Harvest earliness ^f	Included in analysis			
	Countries of origin ^a	Continent of origin ^b	Admixture with cultivated strawberry ^c	Sub- species ^d				Volatiles	Phenolics	Sensory	NMR
Reconstruction progenies						2.76	2.2				
HS01	US, CL	N, S	yes	l × c	HS01.1	1.93	1.1	x			x
HS02	CA, US	N	yes	p × l	HS01.2	2.25	1.7	x	x		x
					HS02.1	2.65	3.1	x	x		x
					HS02.2	1.91	3.3	x	x	x	x
					HS02.3	2.57	3.1	x			x
HS03	US	N	no	p × l	HS03.1	3.07	2.3	x	x	x	x
					HS03.2	3.35	2.1	x			x
					HS04.1	3.95	2.5	x	x	x	x
HS04	US	N	no	l	HS04.2	3.08	2.2	x			x
					HS05.1	2.95	1.0	x	x		x
HS05	EC, PE	S	yes	c	HS06.1	3.08	2.2	x			x
HS06	EC, PE	S	yes	c	HS06.2	2.25	1.6	x	x		x
					HS06.3	3.82	1.4	x			x
					HS07.1	1.44	3.1	x	x	x	x
HS07	EC, CL	S	no	c	HS07.2	2.19	3.3	x			x
					HS07.3	1.62	3.3	x			x
					HS08.1	1.80	2.3	x	x		x
					HS08.2	2.65	2.2	x			x
HS08	EC, CL	S	no	c	HS08.3	n.d.	2.3				x
					HS09.1	2.69	1.7	x	x		x
					HS09.2	3.19	2.5	x			x
HS09	CL	S	yes	c	HS10.1	2.98	1.7	x	x	x	x
					HS10.2	2.72	2.2	x			x
HS10	US, CL	N, S	no	p × c	HS11.1	2.63	1.8	x	x		x
					HS11.2	n.d.	1.3				x
HS11	US, CL	N, S	no	p × c	HS12.1	5.01	1.8	x	x		x
HS12	US, CL	N, S	no	p × c	HS13.1	2.93	1.9	x			x
HS13	US	N	no	p × l	HS13.2	3.05	2.2	x	x		x
Cultivars						7.35	2.6				
‘Korona’						5.77	2.1	x	x		
‘Polka’						7.13	1.8	x	x		
‘Honeoye’						9.14	3.8	x	x	x	

^a US, the United States of America; CL, Chile; CA, Canada; EC, Ecuador; PE, Peru.

^b N, North America; S, South America.

^c Admixture in one of the founders with cultivated strawberry (*F. × ananassa*): admixed contribution 1/8 or less.

^d *F. chiloensis* subspecies: l, *lucida*; p, *pacifica*; c, *chiloensis*.

^e Approximately 50 g of fruits was weighed and divided by the number of fruits.

^f Harvest earliness was assessed at scale 1–4 where values <1 = late, 1–2 = mid-season and > 2–4 = early or very early, calculated on the mean values of the number of plants with ripe fruits at three time points across plots.

2. Materials and methods

2.1. Reconstructed hybrid progenies and fruit samples

The creation of a multi-parental population of *F. chiloensis* and *F. virginiana* interspecific hybrid strawberries (HS) was described in Haikonen et al. (2021) and Rehman et al. (2024). Briefly, improved clones of garden strawberry progenitor species *F. chiloensis* and *F. virginiana* initially created through crosses between wild-collected accessions from multiple geographic areas (Hancock et al., 2010) were used as parents to produce 13 reconstructed progenies (Table 1). All *F. virginiana* parents were originally derived from four accessions of subspecies *virginiana*. In contrast, the *F. chiloensis* parents were based on taxonomically and geographically diverse founder accessions, thus, the HS progenies were classified into those with only Northern American (N), those with only Southern American (S), and those with both Northern and Southern American (NS) *F. chiloensis* founders in their pedigrees. Admixture in the *F. chiloensis* founders with old cultivars of *F. × ananassa* is present in five of the progenies, indicating reduced contribution of *F. chiloensis* in those HS progenies (HS01, HS02, HS05, HS06 and HS09) (Table 1).

The seeds of HS progenies were germinated at the Natural Resources Institute Finland (Luke) in Piikkiö, Finland. The progeny individuals (in total, 312 F1-seedlings) were randomly assigned into 2–3 subgroups per progeny (Fig. S1) and clonally propagated for planting in plots of four clonal plants in the field trial (Rehman et al., 2024). Harvest earliness was indexed from bi-weekly observations of ripening onset per plot, and harvest earliness per HS progeny sub-group were calculated as a mean over plots. Fruit samples ($n = 28$) were harvested per progeny subgroup, with calyxes; each sample contained fruits from 6 to 10 F1 individuals and each progeny (HS01–HS13) was represented by 1–3 sub-groups (Table 1). Samples from three commercial *F. × ananassa* cultivars ('Honeye', 'Polka' and 'Korona') were harvested from the same experimental set-up. Harvest was done during a single day on 5th of July 2021. The calyxes were removed and the samples were cooled immediately after harvest, frozen and stored in plastic containers at $-80\text{ }^{\circ}\text{C}$. For phenolic compound and NMR analysis, the berries were freeze-dried (48 h; Lyovapor L-200, BÜCHI Labortechnik AG, Flawil, Switzerland) and grinded with mortar and pestle. The strawberry powder was sealed in a vacuum bag and stored at $-20\text{ }^{\circ}\text{C}$.

2.2. Chemicals

n-Butanol was purchased from Riedel-de Haën (Morris Plains, NJ). Ethanol ($\geq 99.5\%$) was purchased from ALTIA Oy (Rajamäki, Finland). Methanol was purchased from Riedel-de Haën (Morris Plains, NJ), acetone was purchased from Sigma–Aldrich (St. Louis, MO), and acetic acid and formic acid were purchased from Avantor (Radnor, PA).

2.3. Standard compounds

2-Methyl-1-propyl acetate, 3-methyl-1-butanol acetate, benzaldehyde, ethyl decanoate, hexanal, hexanoic acid, 3,7-dimethylocta-1,6-dien-3-ol (linalool), methyl butanoate, methyl hexanoate, nonanal, octanal, octanoic acid, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl pentanoate, 3-methylbutyl hexanoate, hexyl acetate, 2-propyl butanoate, methyl thiobutanoate, butyl butanoate, 3-methylbutyl butanoate, butyl hexanoate, hexyl butanoate, methyl decanoate, hexyl hexoate, methyl dodecanoate, ethyl dodecanoate, ethyl (*E*)-cinnamate, methyl anthranilate, 2-propyl acetate, (*Z*)-3-hexenyl acetate, octyl acetate, heptanoic acid, nonanoic acid, and δ -decalactone were purchased from Sigma–Aldrich (St. Louis, MO). Methyl 3-methylbutanoate, butanoic acid, ethyl hexanoate, methyl 2-methylbutanoate, methyl 3-methylbutanoate, methyl octanoate, butyl acetate, and 2-heptanone were purchased from Fluka Chemicals (Neu Ulm, Switzerland). 2-Methyl-propyl butanoate was purchased from Haarmann&Reimer (Teterboro,

NJ). Standards of pelargonidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, quercetin-3-*O*-glucoside, and kaempferol-3-*O*-glucoside were purchased from Extrasynthese (Genay, France). All standards used in GC–MS analysis had purity of $\geq 95\%$, and standards for UHPLC had purity of $\geq 99.8\%$.

2.4. Determination of average fruit weight

Approximately 50 g of each commercial and HS fruit samples were weighed, the number of fruits was counted, and the average fruit weight was calculated by dividing the weight by the number of fruits.

2.5. Determination of volatile compounds with GC–MS

Approximately 50 g of frozen strawberries were weighed in a beaker and the same amount of 20% NaCl solution was added to increase volatility of volatile compounds, to prevent enzymatic activity, and degradation of certain volatile compounds. Strawberries were crushed and mixed with NaCl solution with a blender (Bamix, Switzerland) while they were still frozen. Two grams of the strawberry mash was moved to a 20 mL glass vial with 10 μL of internal standard mixture (4-methyl-2-pentanol 80.2 $\mu\text{g/mL}$ and neryl acetate 90 $\mu\text{g/mL}$ in methanol). Three analytical replicates of each sample were prepared. Samples were analysed the same day as prepared.

The samples were analysed using headspace solid phase micro-extraction coupled with gas chromatography mass spectrometry (HS-SPME-GC–MS). The volatile compounds were extracted from the headspace with a 2 cm DVB/CAR/PDMS fibre (50/30 μm , Supelco, Bellefonte, PA) at $45\text{ }^{\circ}\text{C}$ for 30 min after 10 min of incubation. The fibre was conditioned at $230\text{ }^{\circ}\text{C}$ prior to sample extraction. After the extraction, the SPME fibre was immediately transferred to the injection port of a Trace 1310 gas chromatograph equipped with a TSQ 7000 EVO mass spectrometer (Thermo Fisher Scientific, Waltham, MA) to be thermally desorbed in the splitless mode at $220\text{ }^{\circ}\text{C}$ for 3 min. A DB-WAX polar capillary column (60 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm film thickness (J&W Scientific, Folsom, CA) was used to separate the volatile compounds of the samples. Helium was used as the carrier gas at a flow rate of 1.6 mL/min. The initial column temperature was set at $40\text{ }^{\circ}\text{C}$ and held for 3 min. Afterwards, the temperature was increased to $220\text{ }^{\circ}\text{C}$ at a rate of $5\text{ }^{\circ}\text{C}/\text{min}$ and held at $220\text{ }^{\circ}\text{C}$ for 10 min. Mass spectra were detected in electron impact (EI) mode at 70 eV with a scan range from m/z 33 to m/z 300. The temperatures of the MS transfer line and the ionisation source were 220 and $240\text{ }^{\circ}\text{C}$, respectively.

The retention indices (RI) of the volatiles were calculated via co-injection with an alkane mixture (C7–C30; Sigma–Aldrich, St. Louis, MO). Volatiles were identified by matching the obtained mass spectra with the standard NIST14 library and by comparing the RIs to those of the compounds reported in the literature and the NIST Webbook (<https://webbook.nist.gov/chemistry/>). Moreover, the identification of a selected number of volatile compounds was confirmed by comparing the RIs and mass spectra with those of the authentic reference compounds.

The semi-quantification of the volatile compounds was performed by dividing each of the individual compound area with the area of internal standard and multiplied with 1000 to make all data more legible.

2.6. Determination of phenolic compounds with UHPLC-DAD

For the phenolic compounds, the extraction method and solvent were a modified version of Kajdžanoska et al. (2011). The extraction solvent was a mixture of acetone, water and acetic acid (70:29:1, v/v/v). Due to the limitations in the sample amount, all samples were freeze-dried for both phenolic and polar metabolite analyses. Preliminary tests showed the absence of water in the samples hindered the extraction of water-soluble phenolics, especially anthocyanins. Therefore, water was added to the solvent. Freeze-dried strawberry powder (1 g) was mixed with 10 mL of the extraction solvent, vortexed for 30 s, sonicated

for 20 min and centrifuged at $3197 \times g$ for 20 min at 20 °C. The extraction procedure was performed three times, and the supernatants were combined and transferred to a boiling flask. The combined extract was concentrated using a rotary evaporator (Heidolph, Schwabach, Germany) at low temperatures (maximum 35 °C). After removal of the solvents, the residue was recollected in 4 mL of methanol, filtered through a 0.45 µm polytetrafluoroethylene (PTFE) syringe filter (Thermo Fisher Scientific, Waltham, MA), and stored at −80 °C until analysis.

Phenolic compounds were analysed using an Ultra-High-Performance Liquid Chromatography (UHPLC) equipment (Agilent 1290 Infinity II, Santa Clara, CA) coupled with an autosampler, and a G7117B 1290 diode array detector (DAD). A BioZen Peptide XB-C18 column (150 × 2.1 mm, 1.7 µm; Phenomenex, Torrance, CA) was used to separate the compounds with an oven temperature of 35 °C. The mobile phases were water (A) and methanol (B), both containing 1% formic acid (v/v), with a gradient of mobile phase B: 0–2.4 min, 5%; 2.4–21.3 min, 5–50%; 21.3–23.6 min, 50–100%; 23.6–28.3 min, 100%; 28.3–29 min, 100–5%; and 29–30 min, 5%. The flow rate was 0.3 mL/min, and the injection volume was 4 µL. Anthocyanins were recorded at 520 nm and flavonols at 360 nm. Other compounds were detected at the 280 nm chromatograms. Identification of phenolic compounds was done using OpenLAB CDS software (Agilent, Santa Clara, CA) and achieved by comparison to reference elution orders and UV-spectra to literature (Table 2). Reference compounds pelargonidin-3-O-glucoside and cyanidin-3-O-glucoside were used for quantifying anthocyanins. Reference

compounds quercetin-3-O-glucoside and kaempferol-3-O-glucoside were used for quantifying flavonols. Five-point calibration curves had a concentration range of 5–100 µg/mL.

2.7. Sensory evaluation

Ethical review complying with the guidelines presented by the Finnish National Board on Research Integrity (Kohonen et al., 2019) was approved before the sensory evaluation was conducted. The research methods were evaluated based on the ethical principles regarding the human involvement in studies performed in Finland, including data management, sensitive information collected, exposition to strong stimuli, and consent acquisition. Panellists gave their consent to participate in the evaluation prior to start of training sessions, and were informed on the characteristics of the study, including sensitive data collection and management.

The panel for the descriptive sensory analysis had nine volunteer panellists, familiar with strawberries, both students and staff of the University of Turku with prior experiences in sensory tests. Four training sessions and three evaluation sessions were carried out in the sensory laboratory of the University of Turku, which follows the general guidance for sensory evaluation rooms (ISO 8589 2007). The procedures followed a modified version of the ones described by Kårlund et al. (2015). Four training sessions of 1 h each were performed in accordance with ISO guidelines (ISO 8586 1993). The first session defined the most relevant attributes from a commercial cultivar sample and evaluated the concentration of standard solutions. The following sessions defined the evaluation attributes (3 appearance, 5 odour, and 7 flavour attributes) and reference products representing each of these attributes, as well as its intensity values (Table S1). The last two training sessions focused on guiding panellists on the usage of the scale. Standard solutions were prepared using carbon-filtered water. Strawberry juice and standard solutions were placed in 50 mL beakers, while others were placed in small glass containers. All standards had small glass lids to allow the stabilisation of odours in the headspace.

Samples representing five progeny subgroups (HS02.2, HS03.1, HS04.1, HS07.1, HS10.1) and one commercial cultivar ('Honeoye') were chosen for the evaluation based on their volatile compound profiles. Priority was given to strawberry hybrid progenies that had different volatile composition from each other and from the commercial strawberries. The samples were thawed using a low-settings microwave oven for 20 s, blended and homogenised. Each sample had 4–5 g of strawberry puree, and were kept at room temperature in covered containers to stabilise the headspace and serving temperature. Three-digit code and Williams Design randomisation created by Compusense 23.0.19 (Guelph, ON) were used to identify and present each sample. Evaluations were conducted in controlled laboratory conditions, which include room temperatures of approximately +22–24 °C, relative humidity of 45–55%, and atmosphere free of distractions, odours, and noise. In addition, carbon-filtered water and unsalted crackers were provided to cleanse the palate between the tastings. Samples were served monadically one-by-one. Line scale was used in the intensity evaluation of attributes, ranging from 0 (absent) to 10 (very intense), except for the 'number of seeds' (achenes) which ranged from 1 (no seeds) to 5 (a lot of seeds).

2.8. Determination of polar metabolites with targeted ¹H NMR metabolomics

For the NMR samples, 60 mg of freeze-dried strawberry powder was weighed and combined with 1.2 mL sodium phosphate buffer (0.2 M, pH 7), vortexed for 2 min and centrifuged at $14,000 \times g$ for 10 min (+4 °C). The supernatant was filtered with a 0.2 µm RC syringe filter and an aliquot of the filtrate (630 µL) was mixed with 70 µL of internal standard solution containing 4.67 mM deuterated DSS (DSS-*d*₆) and 0.03% w/v sodium azide (NaN₃) in D₂O (Chenomx Inc., Edmonton, AB).

Table 2

Tentative identification of phenolic compounds in strawberries showing all quantified anthocyanins (*n* = 5) and flavonols (*n* = 3) and each compound as a peak from UHPLC-DAD chromatograms and respective retention times, absorption maxima, compound names and references.

Peak	t _R (min)	λ _{max} (nm)	Compound	Reference ^a
1	1.21	244	Ascorbic acid	
2	6.72	280	Flavan-3-ol	1, 5
3	8.65	278	Flavan-3-ol	1, 5
4	9.66	278	(+)-Catechin	1, 4
5	10.44	314	<i>p</i> -Coumaroylhexose	1, 4, 5
6	11.11	284, 310	Unknown phenolic acid	
7	11.52	278	Flavan-3-ol	1, 5
8 ^b	12.10	278, 514	Cyanidin-3-O-glucoside	std
9 ^b	12.89	276, 428, 502	Pelargonidin-3-O-glucoside	std
10a ^b	13.99	276, 428, 502	Pelargonidin-3-O-rutinoside	1, 3, 5
10b	14.23	278	Unknown	
11a ^b	15.86	284, 510	Cyanidin-3-O-malonylglucoside	1, 2, 3, 5
11b	15.86	284	Unknown	
12 ^b	16.61	284, 430, 504	Pelargonidin-3-O-malonylglucoside	1, 3, 4, 5
13 ^b	18.63	256, 294, 354	Quercetin-3-O-glucoside	std
14	18.87	364	Ellagic acid derivative	6
15	19.25	254, 366	Ellagic acid derivative	1, 6
16	19.49	356	Ellagic acid derivative	6
17 ^b	20.41	264, 296, 348	Kaempferol-3-O-glucoside	std
18	21.15	376	Unknown	
19 ^b	21.39	254, 348	Kaempferol-3-O-malonylglucoside	
20a	23.18	264, 348	Kaempferol-3-O-coumaroylglucoside	1, 2, 5
20b	23.18	284	Unknown	
21	23.43	268	Unknown	
22	23.74	364	Ellagic acid derivative	6
23	23.96	280	Unknown	

^a std.: external standard used in identification. 1: Aaby et al. (2012), 2: Kajdžanoska et al. (2011), 3: Lopes da Silva et al. (2007), 4: Seeram et al. (2006), 5: Simirgiotis et al. (2009), 6: Määttä-Riikinen et al. (2004).

^b Quantified using standards and used as a data source for other Figures.

A volume of 650 μL was transferred to a 5-mm NMR tube. The prepared samples were stored at +4–6 $^{\circ}\text{C}$ until analysis.

The ^1H NMR spectra were recorded using 600 MHz AVANCE-III NMR-system (Bruker Biospin, Rheinstetten, Germany) equipped with Prodigy TCI cryoprobe and automated SampleJet sample changer. Proton spectra were acquired at 298 K with 1D NOESY pulse program with presaturation (*noesygppr1d*) with following parameters: spectral width, 12 ppm; number of scans, 128; number of dummy scans, 16; acquisition time, 2.5 s; relaxation delay d1, 2.5 s; mixing time d8, 0.10 s. The spectra were zero-filled to 128k, and phase, baseline and shim corrected with Chenomx NMR Suite v9.0. Line-broadening of 0.5 Hz was also applied. The metabolites were quantified with the Chenomx Profiler.

2.9. Statistical analysis

Heatmaps were constructed with all volatile compounds ($n = 78$) and with a subset of selected volatile compounds that have been reported to be important for strawberry aroma ($n = 41$) using online data analysis platform MetaboAnalyst 6.0 (Xia et al., 2009). Volatile data were normalised as mean-centred and divided by the standard deviation before construction.

Kolmogorov–Smirnov test was used to confirm the normality of the data, one-way analysis of variance (ANOVA) with Tukey's *post hoc* test was performed to analyse the phenolic compounds samples ($n = 48$) and sensory properties samples ($n = 6$) separately (SPSS 28.0.0.0; IBM SPSS Statistics, Inc., Chicago, IL). Principal component analysis (PCA) performed by Unscrambler 11 (CAMO, Inc., Oslo, Norway) was used to analyse the relationships between chemical and sensory properties. The performance of the sensory panel was assessed with three-way ANOVA using Panel Check v1.4.2 (Nofima, Tromsø, Norway).

3. Results and discussion

3.1. Volatile compounds

A total of 78 volatile compounds were identified in the strawberry fruit samples, of which 42 were ethyl or methyl esters, 12 acetates, 8 volatile acids, 4 ketones, 6 aldehydes, 3 lactones, 2 terpenes, and ethanol (Table 3). Esters of butanoic acid ($n = 18$; butanoates) contained the highest number of individual esters, followed by acetates (12), hexanoates (10), and octanoates (4). Concentrations of volatile compounds were semi-quantified by peak area normalisation with internal standard (Table S2). The highest total volatile and ester semi-quantified contents were detected in the HS06 progeny. In general, the HS progenies had twice the total contents of esters, acetates, and volatile acids than the commercial cultivar samples and 24% higher contents of total aldehydes, when the average values were calculated from all the HS progenies and compared to the total contents in the commercial strawberries (data not shown). In particular, there were a number of ester compounds identified in the HS progenies that were not detectable in cultivar samples. Similar results regarding the markedly higher diversity and abundance of ester compounds in *F. chiloensis*-derived introgression lines as compared to commercial cultivars have also been reported by Ulrich and Olbricht (2016) and Gonz  lez et al. (2009). The lowest total volatile and total ester contents of all samples was in the commercial 'Honeoye' cultivar. These results were consistent with the results reported by Hakala et al. (2002) and Yang et al. (2021). Hakala et al. (2002) observed 'Honeoye' to have the lowest ester content compared to four commercial varieties, including 'Polka' and 'Korona'. Yang et al. (2021) reported almost three times higher ester content in 'Polka' compared to 'Honeoye', while we observed 3.7 times higher ester content in 'Polka' compared to 'Honeoye'. Furthermore, the highest relative ester content was observed in the HS12 (82% of a total volatile content; data not shown). The lowest relative ester content was again observed in 'Honeoye' (40%). Out of all fruit samples, only in the 'Honeoye' samples the relative ester contents were less than 50% of a total volatile content.

Further considering differences among the cultivars as well as between the cultivars and the HSs, 'Honeoye' had also the lowest acetate contents, 69% less than the levels in HS progenies. On the other hand, 'Korona' had the highest contents of total esters and acetates compared to other two commercial cultivars, although the contents of these compounds were 22% and 30% less than the levels in HSs, respectively. All commercial strawberry samples had higher content of total ketones (15–44% more) and lower content of total volatile acids (33–87% less) compared to HSs. 'Honeoye' and 'Korona' had higher lactone contents (73% and 44% more, respectively) compared to HSs, except for HS02 that had higher content of lactones compared to the commercial strawberries. 'Korona' had also higher content (22% more) of the total aldehydes than HSs. Finally, 'Honeoye' had 68% higher total terpene content compared to the HSs.

HS06 had the highest total volatile acid content, which was 1.2 times higher than in HS05, which contained the second highest amount. The lowest volatile acid content was observed in the commercial 'Polka', which had 16.3 times lower total amount of volatile acids compared to HS06. The high volatile acid content in HS06 can indicate riper fruit (M  nager et al., 2004). All strawberries were harvested at the same time, possibly causing some differences in the stage of ripeness. M  nager et al. (2004) reported increased relative volatile acid contents in dark red coloured, fully matured strawberry fruits. They also reported slight decrease in the relative ester content in the dark red coloured fruits, however in the HS05 and HS06 samples the total ester contents remained high and the average ripening time in these progenies was among the latest in the whole HS population (Table S2).

A heatmap was constructed to visualize clustering of the progenies and cultivars by 41 important volatile compounds relevant for strawberry flavour, selected according to Carrasco et al. (2005), Prat et al. (2014), Schieberle and Hofmann (1997) and Yan et al. (2018). In addition to individual flavour-related compounds, the heatmap also includes the total contents of all detected acetates, esters, aldehydes, ketones, volatile acids, lactones, and terpenes (Fig. 1). The heatmap showed two main clusters. The first main cluster from the right included the samples HS02, HS04, HS05, and HS06. In this main cluster, HS02 formed own sub-cluster and other three HSs were in another sub-cluster. HS05 and HS06 had a similar *F. chiloensis* parent, which originates from subspecies *chiloensis* from South America admixed with *F. × ananassa* (Table 1), whereas HS04 originates from a cross between non-admixed accessions of North-American subspecies *lucida* and HS02 from across between accessions of *lucida* and *patagonica*. In addition, these samples are clustered with HS10 (non-admixed founder *patagonica* × *lucida*) in the first main cluster in the heatmap constructed with all the volatile compounds (Fig. S2). The mixed backgrounds of the progenies observed in the major clusters was consistent with the suggestion that inherited volatile profiles are not taxonomically restricted, but taxa contain inherent genetic variation (Carrasco et al., 2005; Ulrich & Olbricht, 2016). The samples in the first main cluster contained high amounts of straight ethyl esters, such as ethyl butanoate, ethyl hexanoate, ethyl decanoate, ethyl dodecanoate, methyl thiobutanoate, and butyl butanoate, which are some of the volatile compounds characteristic to strawberry (Carrasco et al., 2005; Yan et al., 2018). Not surprisingly, the total content of esters was also high in this cluster. Progenies of the first cluster also contained high amounts of methyl anthranilate and methyl nicotinate, which are mainly found from the wild small-fruited woodland strawberry, *F. vesca* (Dong et al., 2013; Pyysalo et al., 1979), and detected in some accessions of *F. chiloensis* ssp. *lucida*, but not in *F. virginiana* (Olbricht et al., 2008).

The second main cluster included all the other samples (Fig. 1). It was divided further into two sub-clusters. The first sub-cluster included progenies HS09, HS08, HS11 (first group), HS13, HS10, and HS03 (second group). The second sub-cluster contained commercial samples 'Honeoye' and 'Korona', and progenies HS07 and HS12. The group including HS09, HS08, and HS11 represented high contents of aldehydes, such as nonanal, hexanal, 2-hexenal and a total content of

Table 3
Identification of volatile compounds.

Number	Peak Name	Abbreviation ^a	Calculated RI ^b	Literature RI ^c	Identification ^d
<i>Esters</i>					
1	Methyl butanoate		992	983	MS, RI, STD
2	Methyl 2-methylbutanoate	Met2MetButanoate	1016	1010	MS, RI, STD
3	Methyl 3-methylbutanoate	Met3MetButanoate	1025	1019	MS, RI, STD
4	Ethyl butanoate		1045	1036	MS, RI
5	2-Propyl butanoate		1048	1039	MS, RI, STD
6	Ethyl 2-methylbutanoate	Et2MetButanoate	1059	1049	MS, RI, STD
7	Ethyl 3-methylbutanoate	Et3MetButanoate	1073	1072	MS, RI, STD
8	Ethyl pentanoate		1139	1140	MS, RI, STD
9	2-Methylpropyl butanoate	2MetProButanoate	1164	1161	MS, RI, STD
10	Ethyl (<i>E</i>)-2-butenate	Et2Butenoate	1170	1166	MS, RI
11	Methyl hexanoate		1193	1192	MS, RI, STD
12	Methyl thiobutanoate	MetThioButanoate	1203	1215	MS, RI, STD
13	Butyl butanoate		1225	1219	MS, RI, STD
14	Ethyl hexanoate		1242	1248	MS, RI, STD
15	3-Methylbutyl butanoate	3MetButButanoate	1271	1266	MS, RI, STD
16	Propyl hexanoate		1321	1321	MS, RI
17	Ethyl 2-hexenoate		1351	1345	MS, RI
18	2-Methylpropyl hexanoate	2MetProHexanoate	1357	1353	MS, RI
19	Methyl octanoate		1393	1387	MS, RI, STD
20	2-Hexenyl propanoate	2HexPropanoate	1401	1392	MS, RI
21	Butyl hexanoate		1417	1414	MS, RI, STD
22	Hexyl butanoate		1420	1419	MS, RI, STD
23	2-Propyl octanoate		1435		MS
24	Ethyl octanoate		1440	1446	MS, RI
25	Hexyl 3-methylbutyrate	Hex3MetButanoate	1448	1447	MS, RI
26	3-Methylbutyl hexanoate	3MetButHexanoate	1464	1458	MS, RI, STD
27	Methyl 3-hydroxybutanoate	Met3HydButanoate	1493	1466	MS, RI
28	Methyl nonanoate		1496	1500	MS, RI
29	(<i>E</i>)-Hex-2-en-1-yl 3-methylbutanoate	2Hexe3MetBut	1504		MS
30	Methyl decanoate		1598	1599	MS, RI, STD
31	Hexyl hexoate		1616	1612	MS, RI, STD
32	Butyl octanoate		1620	1613	MS, RI
33	Octyl butanoate		1624	1624	MS, RI
34	Ethyl decanoate		1645	1644	MS, RI, STD
35	(<i>E</i>)-2-Hexenyl hexanoate	2HexeHexanoate	1676	1668	MS, RI
36	Methyl nicotinate		1800	1793	MS, RI
37	Methyl dodecanoate		1810	1814	MS, RI, STD
38	Octyl hexanoate		1821	1815	MS, RI
39	Decyl butanoate		1831	1822	MS, RI
40	Ethyl dodecanoate		1854	1843	MS, RI, STD
41	Ethyl (<i>E</i>)-cinnamate		2149	2130	MS, RI, STD
42	Methyl anthranilate	MetAnthranilate	2245	2248	MS, RI, STD
<i>Acetates</i>					
43	Methyl acetate		827	827	MS, RI
44	Ethyl acetate		882	887	MS, RI
45	2-Propyl acetate		895	901	MS, RI, STD
46	2-Methylpropanol acetate	2MetPropyl acetate	1020	1014	MS, RI, STD
47	Butyl acetate		1077	1072	MS, RI, STD
48	3-Methyl-1-butanol acetate	3Met1ButAcetate	1126	1126	MS, RI, STD
49	Hexyl acetate		1278	1276	MS, RI, STD
50	(<i>Z</i>)-3-Hexenyl acetate		1311	1319	MS, RI, STD
51	2-Hexenyl acetate		1338	1333	MS, RI
52	Octyl acetate		1480	1476	MS, RI, STD
53	2,3-Butanediyl diacetate	2,3-ButDiacetate	1491	1484	MS, RI
54	Benzyl acetate		1743	1743	MS, RI
<i>Alcohols</i>					
55	Ethanol		942	930	MS, RI
<i>Volatile acids</i>					
56	Acetic acid		1457	1453	MS, RI
57	2-Methylpropanoic acid	2MetPropanoic acid	1578	1579	MS, RI
58	Butanoic acid		1636	1630	MS, RI
59	2-methyl butanoic acid	2MetButanoic acid	1681	1674	MS, RI
60	Hexanoic acid		1857	1860	MS, RI, STD
61	Heptanoic acid		1969	1963	MS, RI, STD
62	Octanoic acid		2079	2083	MS, RI
63	Nonanoic acid		2176	2173	MS, RI, STD
<i>Ketones</i>					
64	3-Penten-2-one		1134	1137	MS, RI
65	2-Heptanone		1189	1185	MS, RI, STD
66	Mesifuran		1606	1580	MS, RI
67	Furaneol		2055	2031	MS, RI
<i>Aldehydes</i>					
68	3-Methyl butanal		915	913	MS, RI

(continued on next page)

Table 3 (continued)

Number	Peak Name	Abbreviation ^a	Calculated RI ^b	Literature RI ^c	Identification ^d
69	Hexanal		1085	1083	MS, RI, STD
70	2-Hexenal		1227	1226	MS, RI
71	Octanal		1293	1297	MS, RI, STD
72	Nonanal		1397	1396	MS, RI, STD
73	Benzaldehyde		1536	1529	MS, RI, STD
	<i>Lactones</i>				
74	γ -Decalactone		2166	2177	MS, RI
75	δ -Decalactone		2213	2220	MS, RI, STD
76	γ -Dodecalactone		2362	2365	MS, RI
	<i>Terpenes</i>				
77	Linalool		1553	1549	MS, RI, STD
78	Unknown terpinoid				
79	(E)-Nerolidol		2050	2042	MS, RI
	<i>Internal standards</i>				
	4-methyl-2-pentanol		1179	1168	
	Neryl acetate		1735	1730	

^a Abbreviations used in the heatmap (Fig. 1 and Fig. S2).

^b Retention index (RI) calculated according to Kovat's equation in a DB-WAX column.

^c RI from NIST Webbook (<https://webbook.nist.gov/chemistry/>).

^d STD identification by comparison of GC and mass spectra with those of reference compounds, MS tentatively identified by library mass spectral match, RI identification by comparison of calculated and reference literature RI.

aldehydes, and low amounts of other volatile compounds. High levels of aldehydes and low levels of esters has been previously reported to indicate lower stage of ripeness in strawberry by Azodanlou et al. (2004). However, the ripening times of HS09 and HS08 progenies were early season and that of HS11 mid-season (Table 1) suggesting they were not unripe during the harvest. The group including progenies HS13, HS10, HS01, and HS03 represented high levels of different acetates and, thus, also total level of acetates. Dong et al. (2013) discussed in their study that a higher proportion of acetates compared to other esters in woodland strawberry *F. vesca* gives its pronounced floral aroma compared to cultivated *F. × ananassa* strawberries. Finally, the second sub-cluster including samples 'Honeoye' and 'Korona', HS07 and HS12 represented many branched esters, such as ethyl-3-methylbutanoate, ethyl-2-methylbutanoate, and methyl-2-methylbutanoate, γ -decalactone and γ -dodecalactone, and some volatile acids. γ -Dodecalactone and γ -decalactone, among other compounds, have been reported to highly correlate with sweetness perception of strawberries and γ -dodecalactone to affect consumers liking of strawberries independently from sugars (Fan et al., 2021), indicating these samples to possible be liked by consumers.

Ulrich et al. (1997) divided strawberries into two distinct aroma types: a methyl anthranilate-containing and methyl anthranilate-free types. Methyl anthranilate has a unique 'wood strawberry' and 'grape-like' odour, which are preferred in strawberry flavour. They further divided the methyl anthranilate-free type to the ester-type with a higher total ester content and pleasant sensory properties and to the DHF-type (2,5-dimethyl-4-hydroxy-3(2H)-furanone; furaneol) with less esters and higher hexanoic acid, butanoic acid, 2-methyl butanoic acid, and γ -decalactone contents and medium or poor flavour properties. Progenies HS02 and HS12 and cultivar samples 'Korona' and 'Polka' did not contain any methyl anthranilate (Table S2). However, methyl anthranilate was detected in all other HS samples and in a minor quantity in cultivar 'Honeoye'. The strawberry samples HS05, HS03, HS04, HS06, and HS11 had the highest methyl anthranilate contents, making them mostly likely to belong to the methyl anthranilate-containing aroma type. Among the methyl anthranilate free samples, the progeny HS12 was the best candidate for ester type, while HS2 for the DHF type.

3.2. Phenolic compounds

A total of 20 phenolic compounds were tentatively identified (Table 2 and Figure S3A–C), of which 5 were anthocyanins and 3 flavonols. The remaining compounds were mainly ellagic acid derivatives, phenolic acids and flavanols. The most abundant group was flavonoids,

mainly composed of anthocyanins and flavonols. Anthocyanins were the most abundant subgroup in all commercial and hybrid strawberries (HSs), similar as previously observed in strawberry (Aaby et al., 2012; Kajdžanoska et al., 2011; Määttä-Riihinen et al., 2004). Anthocyanins form one of the most important group of phenolic compounds in strawberries since they are the main pigments contributing to the red colour in strawberries (Aaby et al., 2012). Tannins are reported to be the second most abundant group of phenolic compounds in strawberries, with ellagitannins being the second most abundant subgroup (Giampieri et al., 2012). Ellagitannins contribute to the antioxidative and other bioactive properties of strawberries. In addition, they may also contribute to the astringent mouthfeel, similarly to other tannins. However, precipitation during the phenolic compound extraction was observed, resulting in no confirmation of tannins in the chromatograms. This is consistent with tannins being the main compounds precipitating in acidic conditions (Li et al., 2014). Precipitation was observed in both the hybrid and the commercial strawberries. Additional extraction steps and analyses focused on the identification and quantification of certain phenolic groups are typically necessary for further characterisation. Structural information obtained from other analytical methods, such as MS, coupled with the UV spectra allow for the confirmation of the identity of more complex compounds, i.e. ellagitannins. Due to all of this, the quantitative characterisation was done to anthocyanins and flavonols.

In the anthocyanin subgroup, pelargonidin glycosides had a maximum UV spectra absorption of 504 nm, while for cyanidin glycosides it was 514 nm. Peak 9 (Table 2; Fig. S3A) was identified as pelargonidin-3-O-glucoside, the most abundant anthocyanin in all samples, which is consistent with the previously reported results (Aaby et al., 2012; Kajdžanoska et al., 2011; Määttä-Riihinen et al., 2004), and ranged from 14.84 to 59.15 mg/100 g of strawberry. Commercial strawberry cultivars had high concentrations of pelargonidin-3-O-glucoside and except for HS13, significantly higher than the HS samples ($p < 0.05$) (Table S3). For pelargonidin-3-O-rutinoside (peak 10a), almost all HSs had lower concentrations when compared to the commercial cultivars, except for HS02 and HS12. However, at 280 nm there is an unknown compound overlapping with pelargonidin-3-O-rutinoside that was not identified in this study (peak 10b) (Figure S3C). Pelargonidin-3-O-malonylglucoside (peak 12) was also observed in lower concentrations in HSs than in the commercial cultivars ($p < 0.05$). Pelargonidin-3-O-glucoside is the most abundant anthocyanin compound in red-fruited strawberry independent of growing conditions or cultivar (Giampieri et al., 2012). Aaby et al. (2012) found similar proportions of anthocyanins in 'Honeoye', 'Polka' and 'Korona' cultivated

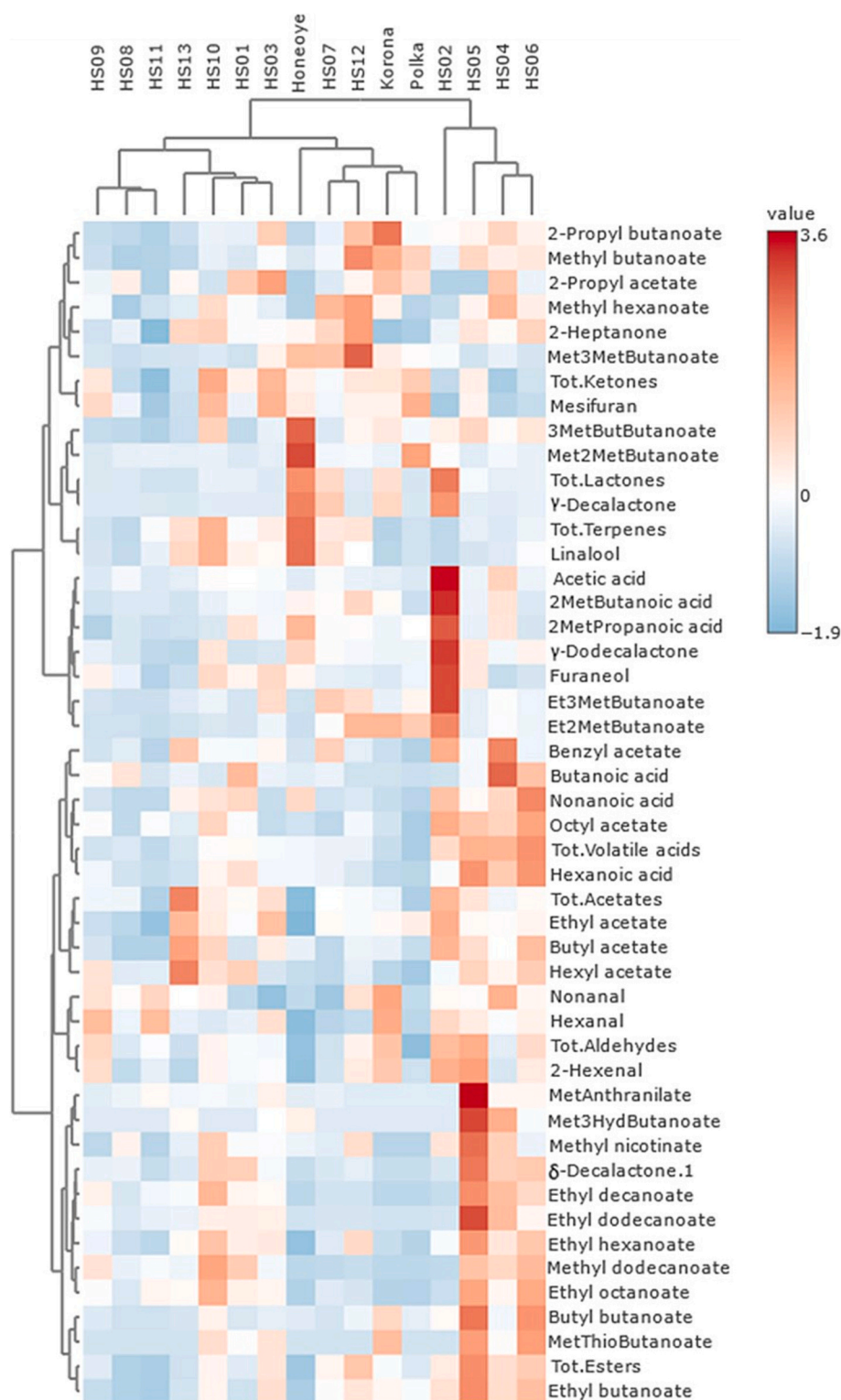


Fig. 1. Heatmap visualisation of hierarchical clustering of key identified volatile compounds ($n = 41$) and their total amounts. Compounds were selected for their reported importance for strawberry aroma according to Carrasco et al. (2005), Prat et al. (2014), Schieberle and Hofmann (1997) and Yan et al. (2018). Abbreviations refer to Table 3.

in Norway, a country with relatively similar growing season temperatures and day lengths to Finland. Aaby et al. (2012) also found that the anthocyanin content is closely related to the cultivar, and not as dependent of the growing conditions.

Cyanidin-3-*O*-glucoside is present in lower concentrations in strawberries than pelargonidin-3-*O*-glucoside, but it is the second most

abundant anthocyanin in red-fruited cultivars (Giampieri et al., 2012) and the main anthocyanin in white-fruited forms of the Chilean strawberry (Cheel et al., 2005, Cheel et al., 2007). Its concentrations ranged from 2.00 to 18.27 mg/100 g of fruit. Both cyanidin-3-*O*-glucoside and cyanidin-3-*O*-malonylglucoside, shown as peak 8 and 11a (Table 2), respectively, did not show significant differences between the HS and

commercial cultivars. However, HS08 and HS13 had higher concentrations of these anthocyanins than any other sample. An unknown compound eluted at the same time as peak 11a, and was marked as peak 11b. Its identification was not possible due to the reasons mentioned before.

Unknown compounds with UV-spectra resembling anthocyanins remained unidentified. Two small peaks between compounds 12 and 13 (Fig. S3B) had maximum wavelengths of 500 and 506 nm, respectively. The first two unmarked peaks (Fig. S3C) between peaks 10 and 11 had

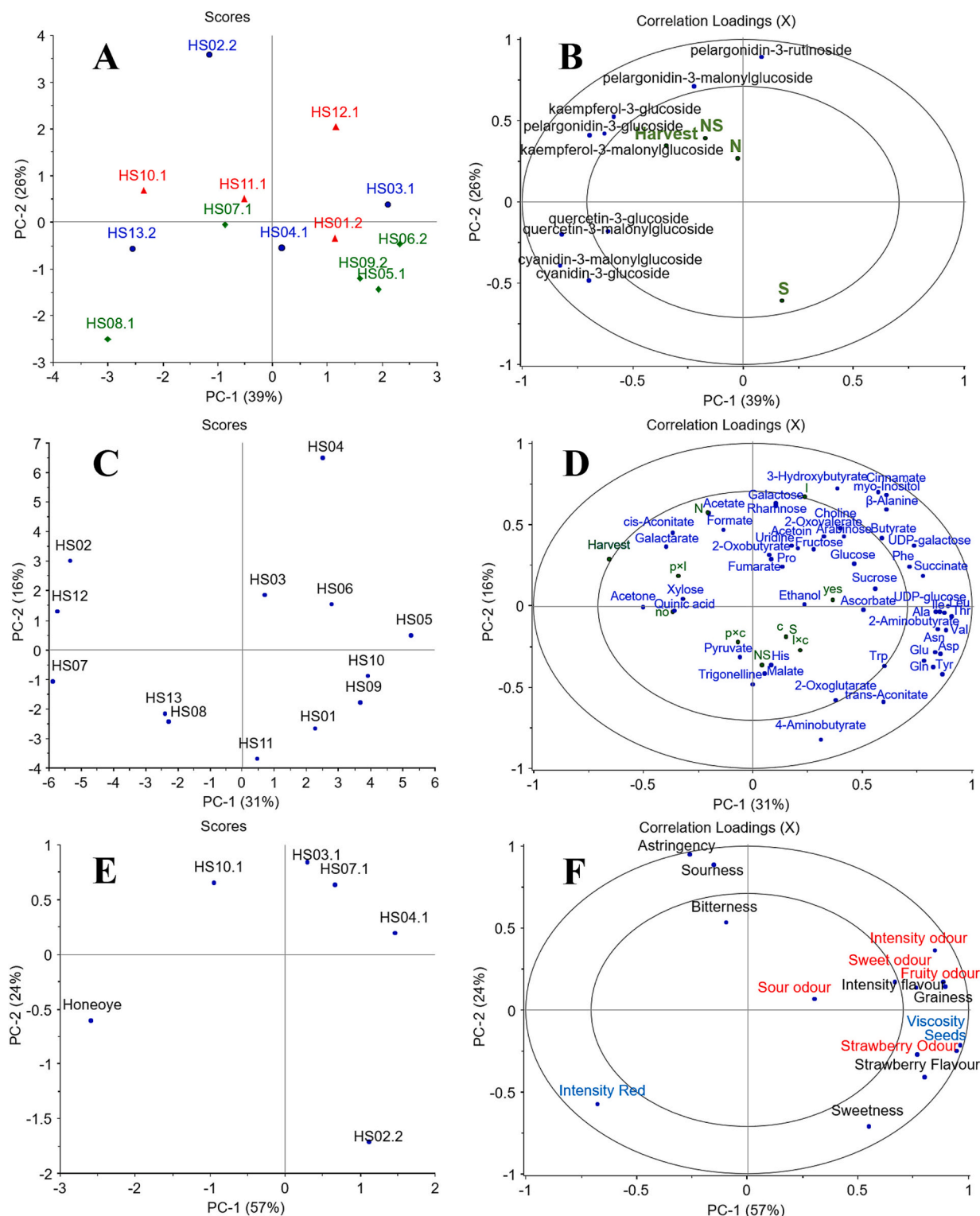


Fig. 2. Principal component analysis of strawberries as samples and (A) phenolic compounds ($n = 9$) including 'harvest' and 'origin' as dummy variables, (B) polar metabolites ($n = 46$) including 'harvest', 'origin' (N, North; S, South), 'admixture' (yes/no) and 'sub-species' (c, l, l × c, p × c, p × l) as dummy variables, and (C) sensory attributes ($n = 15$) as variables. (A) blue circles, North America (N); green diamonds, South America (S); red triangles, mix of North and South America (NS); (F) Red text, odour descriptions; black text, taste descriptions; blue text, descriptions by looking. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

UV spectra profile resembling anthocyanins with maximum absorption of 502 nm.

In the flavonol subgroup, quercetin glycosides had a maximum UV spectra absorption of 354 nm, while for kaempferol glycosides it was 348 nm. Quercetin glycosides (1.05–4.21 mg/100 g of fruit) were also found in higher concentrations than kaempferol glycosides (0.28–1.49 mg/100 g of fruit) (Table S3), which was also found by other authors (Aaby et al., 2012; Kajdžanoska et al., 2011; Määttä-Riihinen et al., 2004). Aaby et al. (2012) reported similar concentrations for quercetin glycosides in ‘Honeoye’, ‘Polka’ and ‘Korona’ grown in Norway. In general, commercial strawberries had relatively high concentrations of quercetin-3-O-glucoside (peak 13; Table S3), but only progenies HS03 and HS06 had significantly lower concentrations than the cultivars and other HS progenies. Kaempferol-3-O-coumaroylglucoside (peak 17), was identified in only some of the samples and was consistently detected across replicates in three of the HS progenies, in concentrations ranging from 0.07 to 0.29 mg/100 g of fruit (data not shown). This compound eluted at the same time as unidentified compounds effecting on the detection. The same phenomenon was observed also with some quercetin glycosides. Anthocyanins and hydroxycinnamic acids have some absorption at 360 nm, which was also observed in this study (Fig. S3B). Peak 15 had a UV spectrum that resembled ellagic acid derivatives, and peaks 14, 16, and 22 had wavelength between 356 and 364 nm indicating it's possible to be ellagic acid derivatives. Finally, ascorbic acid was detected in the 280 nm chromatogram (Fig. S3C).

A PCA model was constructed with the HSs as samples ($n = 13$), identified anthocyanins and flavonols ($n = 9$) as variables, while the grandparental origin of the reconstructed strawberries and harvest earliness (Table 1) were included as dummy variables ($n = 4$; Fig. 2A and B). Commercial strawberry samples were excluded due to their high impact on the model and to better observe the differences between HS progenies. First two components explained 65% of the variance of the data (PC1 39% and PC2 26%). All phenolic compounds are present in the left side of the correlation loadings, except for pelargonidin-3-O-rutinoside. Roughly half of the HSs are positively correlated to the content of phenolic compounds (HS02, HS10, HS11.1, HS07, HS13, and HS08). Phenolic compounds also had higher loadings when compared to the dummy variables for harvest and parental origin. Some differences between the samples based on their parental origin are seen on PC2: All Southern origin samples are located on the bottom part of PCA, whereas most of the Northern $\times$ Southern samples are located on the upper part of PCA. Correlation loadings plot also shows clearly that Southern-dummy variable is negatively correlating with all chemical variables on both PCs. As expected, HS08 and HS07 were located on the opposite side of PC1 when compared to other Southern origin samples on the scores plot (HS05, HS06, and HS09) (Table 1). The *F. chiloensis* grandparents of HS08 and HS07 were not admixed with cultivated strawberry as were the others. In addition, they are positively correlating with cyanidin glycosides and negatively with pelargonidin glycosides, indicating less intense colour properties compared to Northern origin HSs. Furthermore, progeny subgroup HS02 is also derived from admixed parents, but contrary to other admixture-derived progenies it is derived from the cross of *ssp. pacifica* and *ssp. lucida*, whereas the others are derived from admixed *ssp. chiloensis*.

3.3. Polar metabolites

A total of 46 polar metabolites were quantitatively profiled from the 28 hybrid strawberry (HS) progeny samples: 2-aminobutyrate, 2-oxobutyrate, 2-oxoglutarate, 2-oxovalerate, 3-hydroxybutyrate, 4-aminobutyrate, β -alanine, acetate, acetoin, acetone, alanine, arabinose, ascorbate, asparagine, aspartate, butyrate, choline, cinnamate, ethanol, formate, fructose, fumarate, galactarate, galactose, glucose, glutamate, glutamine, isoleucine, leucine, malate, phenylalanine, proline, quinic acid, succinate, sucrose, threonine, trigonelline, tryptophan, tyrosine, UDP-galactose, UDP-glucose, uridine, valine, xylose, *myo*-inositol, and

trans-aconitate. Quantitation of citrate (the major organic acid in strawberries) was not successful due to line broadening. The data presented in a PCA model (Fig. 2C and D) represents the mean metabolite concentrations of the HS progenies, calculated as mean values from progeny subgroup samples (Table S4). The first two principal components explained 47% of the variance. The dummy variable ‘Harvest’ reflecting the level of ripeness had a strong influence on the first principal component (PC1), while the effect of ‘Origin’ was seen along the PC2.

Amino acids, succinate, sucrose, and UDP-glucose had a strong loading along the PC1. Samples HS02, HS07 and HS12 had a strong negative correlation to these, indicating a relatively low concentration of these metabolites. Amino acids, like alanine (Perez et al., 1992) and branched-chain amino acids are precursors for volatile esters. The relatively low free amino acid concentration is therefore in line with the high volatile esters content e.g. in HS12. UDP-glucose is involved in the glucosylation of furaneol (HDMF) during ripening to form a flavourless HDMF β -D-glucoside (Song et al., 2016). UDP-glucose also takes part in the formation of other glycosides and glucose esters (Lunkenbein et al., 2006).

4-Aminobutyrate (GABA) had a strong loading on the second quadrant of the PCA and it correlated with HS01 and HS11. 4-Aminobutyrate is derived from glutamate by deamination and is known to accumulate in plants in response to stress (Kinnersley & Turano, 2000). Increase of 4-aminobutyrate associated with low temperature and elevated CO₂ storage conditions can help maintain the postharvest quality of strawberries (Aghdam et al., 2022). Cinnamate had a strong loading in the second quadrant of the PCA and correlated best with HS04. In the phenylpropanoid pathway, cinnamate is derived from phenylalanine. It has a role in the biosynthesis of anthocyanins and volatile esters (Lunkenbein et al., 2006).

Ascorbate (vitamin C) is one of the main contributors to strawberries’ nutritional and postharvest quality and its content is associated with the growth and stage of ripeness (Morales-Quintana & Ramos, 2019). Ascorbate had a moderate loading along the first component (Fig. 2D), correlating positively e.g. with HS05, HS06 and HS10 and negatively with HS02, HS07 and HS12.

3.4. Sensory properties

The trained sensory panel performed well, and panellists were able to discriminate between samples for these attributes. The ‘product effect’ on the attributes, separately investigated based on the three-way ANOVA results (Table S5), was significant at low values for ‘intensity of the red colour’, ‘apparent viscosity’, and ‘number of seeds’ among the appearance attributes. The ‘product effect’ was also significant with a p -value of at least 0.01 for ‘total intensity of flavour’, ‘sweetness’, and ‘sourness’ among the flavour attributes, with ‘bitterness’ and ‘graininess’ being still significant with $p < 0.05$. The panellists were able to discriminate well between samples for these attributes. The significant results for ‘assessor effect’ and ‘assessor and product interaction’ showed that the panellists might have used the scale differently or had difficulties with the attributes.

One-way ANOVA with Tukey’s post hoc test was considered for further exploration of results and grouping of samples (Table 4). Hybrid strawberries (HSs) had lower values for the ‘intensity of red colour’, and higher intensity values for ‘viscosity by looking’ and the ‘number of seeds’ than ‘Honeoye’. ‘Graininess’ was more intense for HSs, which can also be related to the higher relative proportion of seeds in the samples, a consequence of their small fruit size. The ‘total intensity of flavour’ was higher for HSs, however, intensity of the ‘strawberry flavour’ was not significantly different between the samples. ‘Bitterness’ was not significantly different between samples in the Tukey’s post hoc test, differing from three-way ANOVA results. ‘Sweetness’ and ‘sourness’ were significantly different but had no clear separation between the hybrid and the commercial samples. ‘Strawberry flavour’ and ‘astringency’ were the

Table 4

Intensities (mean $\pm$ stdev, $n = 3$) of the sensory attributes from 0 (absent) to 10 (very intense) ($n = 15$) of hybrid and commercial strawberry samples ($n = 6$).

Sensory Attributes	HS2.2	HS3.1	HS4.1	HS7.1	HS10.1	'Honeoye'
Intensity	6.85	6.88	7.17	6.94	6.96 $\pm$	6.06 $\pm$
odour	± 1.25 ab	± 1.09 ab	± 1.03 b	± 1.17 b	1.07 b	1.40 a
Strawberry odour	6.41	6.16	6.08	6.18	6.10 $\pm$	5.82 $\pm$
	± 1.82 a	± 1.62 a	± 1.74 a	± 2.04 a	1.53 a	1.66 a
Sweet odour	5.01	5.28	5.38	5.10	4.69 $\pm$	4.90 $\pm$
	± 1.44 a	± 1.30 a	± 1.28 a	± 1.58 a	1.31 a	1.31 a
Sour odour	3.89	3.97	3.47	3.62	3.83 $\pm$	3.40 $\pm$
	± 2.08 a	± 2.41 a	± 2.26 a	± 2.09 a	2.17 a	1.97 a
Fruity odour	5.76	5.80	5.73	5.80	5.53 $\pm$	5.43 $\pm$
	± 1.70 a	± 1.60 a	± 1.79 a	± 1.81 a	1.97 a	2.03 a
Intensity Red	7.55	6.24	5.38	6.51	7.19 $\pm$	7.88 $\pm$
	± 1.04 d	± 0.99 b	± 1.30 a	± 1.28 bc	1.09 cd	1.17 d
Viscosity	7.67	6.59	7.28	7.04	5.50 $\pm$	4.94 $\pm$
	± 1.31 c	± 1.54 bc	± 1.16 c	± 1.27 c	1.72 ab	2.21 a
Seeds	3.97	3.63	3.77	3.57	3.37 $\pm$	3.03 $\pm$
	± 0.76 c	± 0.81 bc	± 0.73 bc	± 0.90 abc	0.72 ab	0.72 a
Intensity flavour	7.31	7.07	6.91	7.22	7.15 $\pm$	6.10 $\pm$
	± 1.13 b	± 1.26 b	± 1.22 ab	± 1.18 b	0.98 b	1.33 a
Strawberry flavour	7.03	6.53	7.00	6.75	6.69 $\pm$	6.45 $\pm$
	± 1.33 a	± 1.61 a	± 1.21 a	± 1.50 a	1.35 a	1.41 a
Sweetness	4.97	3.94	4.60	3.67	3.96 $\pm$	3.87 $\pm$
	± 1.47 b	± 1.26 a	± 0.99 ab	± 1.36 a	1.21 a	1.20 a
Sourness	3.33	4.56	3.85	4.72	5.02 $\pm$	3.79 $\pm$
	± 1.98 a	± 1.65 ab	± 1.76 ab	± 1.58 b	1.55 b	1.58 ab
Bitterness	2.00	3.09	2.15	2.39	2.10 $\pm$	2.38 $\pm$
	± 1.67 a	± 1.87 a	± 1.80 a	± 1.93 a	1.78 a	2.04 a
Astringency	1.74	2.49	2.17	2.38	2.56 $\pm$	2.16 $\pm$
	± 1.75 a	± 1.78 a	± 1.85 a	± 2.06 a	2.23 a	1.96 a
Graininess	6.93	6.59	6.75	7.07	6.58 $\pm$	5.27 $\pm$
	± 1.05 b	± 1.49 b	± 1.42 b	± 1.46 b	0.90 b	1.89 a

Different letters in the same row indicates statistical difference ($p < 0.05$) with one-way ANOVA using Tukey's test.

two flavour attributes that were not significantly different between the samples. This indicated that overall high 'total intensity of flavour' in HSs might not be fully explained by a typical strawberry flavour, but possibly unexpected flavour attributes could be present. Similarly, records of the odour attributes showed that 'Honeoye' had lower odour intensity than most of the HS samples except for HS02 and HS03, but there were no significant differences between the odour categories strawberry, sweet, sour, or fruity odours between any of the samples. Among the samples, HS04 had a high overall odour and high sweet odour values. On the other hand, HS02, being relatively low in odour intensity, had the highest sweetness flavour ($p < 0.05$) among all samples and a high flavour intensity.

In fully ripe stage, strawberries of *F. chiloensis* ssp. *chiloensis* are big and their colour remains white-pink, whereas strawberries from ssp. *pacifica* and ssp. *lucida* are light or dark red on their surface; all subspecies are internally variably but weakly coloured (Hancock et al., 2003; Morales-Quintana & Ramos, 2019). Among the samples selected for sensory panel evaluation, HS02 was the only one with admixed history, possibly explaining its highest intensity of red colour among HSs. Two samples obtained lower intensities of red colour than HS07,

being these HS03 and HS04, pointing at the influence of other factors in this attribute. The HSs having higher number of seeds might be associated with the smaller size of berries for most of non-commercial strawberries. Other authors evaluated the number of seeds in the sample for strawberries, however this was not significantly different between cultivars (Scott et al., 2021), which evaluated commercial varieties.

Fruit weights ranged between 1.4 g/fruit (HS07.1) and 9.1 g/fruit ('Honeoye') (Table 1). The weights of HS progenies were smaller compared to commercial strawberries, which was not observed in the sensory analysis since the samples were analysed in the pureed form. The biggest fruit size among the HS progenies was HS12 with weight of 5.0 g/fruit, notably smaller than commercial strawberries.

3.5. Associations between chemical and sensory properties

The intensity of flavour is a complex mixture of attributes, and it is related to many different compounds. A PCA model was constructed with the sensory attributes ($n = 15$) as variables and the hybrid strawberry (HS) progenies and 'Honeoye' ($n = 6$) evaluated by the trained sensory panel as samples (Fig. 2E and F). The first two principal components together explained a total of 81% of the variance in the sensory data. The two PCs clearly indicated separation of the samples to different groups: HS02, HS04, HS07, and HS03 are separate from 'Honeoye' and HS10 in PC1. In PC2, HS02 and 'Honeoye' are separate from the rest of the samples. In the PCA, strawberry odour positively correlated with HS02. Indeed, the volatile compound heatmap indicates that HS02 had a high level of many volatile compounds, such as ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, furaneol, acetic acid, and 2-methyl butanoic acid (Fig. S2), which are previously reported important for a characteristic strawberry odour (Schieberle & Hofmann, 1997). On the other hand, mesifuran and methyl anthranilate are both reported to be important for strawberry odour (Schieberle & Hofmann, 1997; Ulrich et al., 1997). However, HS02 had low levels of mesifuran and methyl anthranilate (Fig. 1). HS02 also correlated positively with the anthocyanins pelargonidin and kaempferol glycosides (Fig. 2A and B).

As indicated earlier, the results from the analyses of volatile compounds indicated HS02 to belong to the expectedly poor-flavoured DHF aroma type (Ulrich et al., 1997): no detectable methyl anthranilate, the highest contents of 2-methyl butanoic acid, furaneol and total lactones (Table S2). However, flavour attribute evaluations given to HS02 by the sensory panel indicated the opposite (Table 4). According to Schwietzman et al. (2014), strawberry odour and sweetness intensities have the greatest influence on overall liking by consumers. HS02 was evaluated as the sweetest and least sour sample (Table 4), indicating high possibility to be liked by consumers. However, HS02 negatively correlated with all detected sugars in a PCA constructed with the polar metabolites (Fig. 2C and D), indicating that in addition to the fruit's sugar/acid ratio some volatile compound may have affected the perception of the high sweetness in HS02 (Fan et al., 2021). Fan et al. (2021) studied sweetness of strawberry and how volatile compounds enhance the sweetness perception independently of sugars. They reported γ -dodecalactone and γ -decalactone, among other compounds, to highly correlate with the sweetness perception of strawberries. In addition, they reported that γ -dodecalactone affected consumers liking of strawberries independently from sugars. Therefore, high levels of γ -dodecalactone, together with mid-chain ethyl esters and 6-methyl-5-hepten-2-one, was suggested as a main target for breeding and quality control in strawberry (Fan et al., 2021). Indeed, notably higher level of γ -dodecalactone was detected in HS02 compared to the other strawberry progenies (Fig. 1).

According to the PCA model (Fig. 2E and F), intensity of odour, sweet odour, and fruity odour positively correlated mostly with HS04, but also with HS07 and HS03. According to the analyses results of volatile compounds (Fig. S2), linalool, total terpenes, methyl 3-methylbutanoate, methyl 2-methylbutanoate, methyl acetate, and γ -decalactone were observed in high levels in HS07 and HS03 while HS4 had high

levels of ethyl hexanoate and ethyl butanoate. Of these compounds, linalool, methyl 3-methylbutanoate, and methyl 2-methylbutanoate have been reported to have a significant positive contribution to flavour intensity of strawberry (Schieberle & Hofmann, 1997; Schwieterman et al., 2014) and γ -decalactone, ethyl hexanoate and ethyl butanoate associated with sweet odour (Nuzzi et al., 2008).

Kårlund et al. (2015) found that the total intensity of flavour is correlated positively to the bitterness and astringency of strawberry puree. They also reported quercetin glycosides to be related to the intensity of flavour. Bitterness and astringency are not desirable attributes for strawberries, being inversely proportional to consumer liking (Laaksonen et al., 2016). Different studies identified many volatiles responsible for the intensity of the sweetness in strawberry samples (Fan et al., 2021; Schwieterman et al., 2014). The content of sugar is associated with sweetness, and the fraction of different sugars influence this sensory attribute in different intensities (Kårlund et al., 2015; Schwieterman et al., 2014). Schwieterman et al. (2014) found that and both sucrose and fructose influence the sweetness, and sucrose contributed the most to liking in strawberries. Fan et al. (2021) reported low results for liking related to lower concentration of total sugars. Furthermore, the sugar/acid ratio is a major contributor to the perceived sweetness of fruits and berries. Although there was a negative correlation of HS02 (evaluated as the sweetest sample) and sucrose, the low acid content may have an impact on the overall sweetness.

The intensity of red colour observed from the sensory evaluation results correlates directly to the anthocyanin content, as discussed previously. Commercial sample 'Honeoye' had higher concentrations pelargonidin glycosides and the highest intensity of red colour compared to hybrid samples used in the sensory evaluation (Fig. 2E and F). In general, the main anthocyanin in commercial strawberries is pelargonidin-3-O-glucoside, but in *F. chiloensis* ssp. *chiloensis* it is cyanidin-3-O-glucoside (Salvatierra et al., 2010). This can be seen clearly in the PCA for sensory attributes, where HS02, admixed with *F. × ananassa*, and 'Honeoye' positively correlate with "Intensity Red" on PC2, and HS02 positively correlate with all pelargonidin glycoside variables in the PCA for phenolic compounds (Fig. 2A and B). Furthermore, all other HS progenies are negatively with "Intensity Red" on PC2 of the PCA for sensory attributes, as expected based on their low levels of pelargonidin glycosides.

4. Conclusions

Significant differences between the reconstructed strawberries and the commercial cultivars, and among the progenies of reconstructed strawberries were detected both in chemical and in sensory analyses. This is consistent with the introduction of new chemical and sensory properties from diverse sources of the progenitor species. Although being smaller in size, the hybrid strawberries contained interesting sensory and chemical characteristics, which was illustrated by the different volatile compound, phenolic, and polar metabolite profiles, and the intensities of sensory attributes compared to the commercial strawberry samples (Table S6).

Hybrid strawberries were associated with higher relative concentration of total aldehydes, acetates, volatile acids, and esters. In addition, they had several volatile compounds that were not observed in commercial strawberries. In general, hybrid strawberries had lower intensity of red colour, associated with the lower concentration of anthocyanins. HS02 was one of the most potential reconstructed hybrid progenies due to its early ripening and pleasant sensorial attributes, such as high intensity of red colour and sweetness and lower intensity of bitterness, sourness, and astringency. In addition, its high γ -decalactone concentration may contribute to perceived sweetness and, potentially, to pathogen resistance due to antifungal activity of this compound (Chambers et al., 2013). However, more research on quantification with GC-O and the odour active values of volatile compounds detected in strawberries is needed to make more accurate conclusions on their

aroma properties. The future work should also cover the characterization of ellagitannins.

This study clearly showed how the reconstruction of strawberries by crossing *F. virginiana* and *F. chiloensis* parents allows for the introduction of new chemical composition, colour, and flavour properties into garden strawberry. Hence, aroma compounds characteristic to wild octoploid strawberries are possible to reintroduce to garden strawberry through de novo species reconstruction, and through a careful selection of reconstruction parents. However, more thorough research on within-progenies variation is needed to reveal the genetic factors behind the interesting sensory properties.

CRedit authorship contribution statement

Niina M. Kelanne: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carla Vecenâncio da Silva:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Oskar Laaksonen:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Tuuli Haikonen:** Writing – review & editing, Writing – original draft, Resources, Methodology, Funding acquisition, Data curation, Conceptualization. **Baoru Yang:** Writing – review & editing, Resources, Funding acquisition. **Maaria Kortensniemi:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2025.144233>.

Data availability

Data will be made available on request.

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