Research Article

Received: 11 December 2012 Revised: 12 September 2013 Accepted article published: 20 September 2013 Published online in Wiley Online Library: 21 October 2013

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6412

Identification of volatile compounds associated with the aroma of white strawberries (*Fragaria chiloensis*)

Loreto Prat,[a] María Inés Espinoza,[b] Eduardo Agosin[b,c] and Herman Silva[a]*

Abstract

BACKGROUND: *Fragaria chiloensis* (L.) Mill spp. *chiloensis* form chiloensis, is a strawberry that produces white fruits with unique aromas. This species, endemic to Chile, is one of the progenitors of *Fragaria x ananassa* Duch. In order to identify the volatile compounds that might be responsible for aroma, these were extracted, and analyzed by gas chromatography–mass spectrometry (GC-MS), gas chromatography–olfactometry (GC-O) and compared with sensory analyses.

RESULTS: Three methods of extraction were used: solvent-assisted evaporation (SAFE), headspace solid phase micro-extraction (HS-SPME) and liquid–liquid extraction (LLE). Ninety-nine volatile compounds were identified by GC-MS, of which 75 showed odor activity using GC-O. Based on the highest dilution factor (FD = 1000) and GC-O intensity ≥2, we determined 20 major compounds in white strawberry fruit that contribute to its aroma. We chose 51 compounds to be tested against their commercial standards. The identities were confirmed by comparison of their linear retention indices against the commercial standards. The aroma of white strawberry fruits was reconstituted with a synthetic mixture of most of these compounds.

CONCLUSION: The volatile profile of white strawberry fruit described as fruity, green–fresh, floral, caramel, sweet, nutty and woody will be a useful reference for future strawberry breeding programs.
© 2013 Society of Chemical Industry

Supporting information may be found in the online version of this article.

Keywords: solvent-assisted flavor evaporation (SAFE); volatile profiling; *Fragaria chiloensis*; gas chromatography–olfactometry

INTRODUCTION

Strawberry, a member of the Rosaceae family, is one of the most important fruits in the world. The cultivated red strawberry (*Fragaria x ananassa* Duch.) is derived from a cross between the North American strawberry (*Fragaria virginiana* Mill.) and the endemic Chilean strawberry [*Fragaria chiloensis* (L.) Mill.].[1] The resulting hybrid has large fruits derived from *F. chiloensis*, the red color derived from *F. virginiana* and a good aroma derived from both parental species.[2] Research during the last decades has been dedicated to the identification of volatile compounds present in cultivated red strawberry, whereas the identification of these compounds in the white strawberry (*F. chiloensis* spp. *chiloensis* form chiloensis) is limited.[3] Identification of these volatile compounds in white strawberry should reveal interesting consumer traits, since white strawberries have a very characteristic and pleasant aroma which leads to consumer preference for white strawberries when compared to red ones.[4]

More than 360 volatiles have been identified in *F. x ananassa* Duch.[5–8] Among them, the esters methyl butanoate, ethyl butanoate, methyl hexanoate, ethyl hexanoate, ethyl 2-methylbutanoate, hexyl acetate and (*E*)-hex-2-enyl acetates are considered to be important flavor active components that contribute to the fruity and green aroma.[9–11] The terpene linalool has been linked to the flowery and sweet aroma and the furanones, mesifuran (2,5-dimethyl-4-methoxy-3(2*H*)-furanone) and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), to the fruity and caramel aromas.[12–16] Additionally, even when the acids have small odorant impact, the 2-methylbutanoic acid (fruity and buttery aroma) and the hexanoic acid (unpleasant aroma) contributed to the aroma.[12–19] The γ-decalactone is also important since it contributes to the fresh fruity aroma.[20]

Only two reports have identified the profile of volatile compounds in *F. chiloensis*. The main compounds identified include esters, some alcohols and ketones.[3,21] However, to our knowledge,

* *Correspondence to: Herman Silva, Universidad de Chile, Facultad de Ciencias Agronómicas, Departamento de Producción Agrícola, Laboratorio de Genómica Funcional & Bioinformática. 8820808, La Pintana, Santiago Chile. E-mail: hesilva@uchile.cl*

a *Universidad de Chile, Facultad de Ciencias Agronómicas, Departamento de Producción Agrícola, Laboratorio de Genómica Funcional & Bioinformática, 8820808, La Pintana, Santiago, Chile*

b *Centro de Aromas y Sabores, DICTUC, Avda. Vicuña Mackenna 4860, Macul, Santiago, Chile*

c *Department of Chemical and Bioprocess Engineering, College of Engineering, Pontificia Universidad Católica de Chile, Casilla 306 – Correo 22, Santiago, Chile*

Table 1. Odorants detected by gas chromatography–olfactometry (GC-O) at the highest dilution factors (FD 500 and 1000) and GC-O intensity ($I > 2$)

RI	Aroma compound	Descriptor
1046	Ethyl butanoate	Sweet, sugary
1224	*trans*-2-Hexenal	Sweet
1257	Ethyl hexanoate	Tropical fruits
1294	Hexyl acetate	Tropical fruits, banana
1358	2-Hexen-1-ol acetate	Fruity, strawberry like
1541	Linalool	Floral
1567	Furfuryl acetate	Tropical fruits
1589	2,5-Dimethyl-4-methoxy-3(2*H*)-furanone (mesifuran)	Fruity, caramel
1631	Ethyl decanoate	Fruity, caramel
1863	Benzyl alcohol	Floral
1804	2-Phenylethyl acetate	Sweet, tea, floral
2021	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (DMHF)	Caramel, strawberry like
2033	Hydrocinnamyl alcohol	Floral, citric
2133	γ-Decalactone	Floral, peach
2140	Cinnamyl acetate	Floral, sweet
2154	2,6-Di(*t*-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	Caramel
2189	3,7-Dimethyloct-1-en-3,8-diol	Sweet
2252	(*E*)-2,6-Dimethylocta-2,7-dien-1,6-diol	Sweet, floral
2288	(*Z*)-2,6-Dimethylocta-2,7-dien-1,6-diol	Honey
2952	Hexadecanoic acid	Chemical, lactic

there are no reports of gas chromatography–olfactometry (GC-O) of volatile compounds in *F. chiloensis*. In this study, we tentatively identified the volatile compounds that might contribute to the aroma of fresh white strawberry fruits using three different extraction methods: solvent-assisted flavor evaporation technique (SAFE), headspace solid-phase microextraction (HS-SPME) and liquid–liquid extraction (LLE). Then, we proposed several potent volatile compounds that are involved in the aroma of *Fragaria chiloensis*. These results provide information that may be used in selection breeding programs to improve the aroma of strawberries.

EXPERIMENTAL

Strawberry fruits

White strawberry fruits were harvested in January 2011, from 5-year-old plants grown in the fields of Contulmo in the south of Chile (38° 04′ 8.6″ S, 73° 14′ 2.96″ W) at 605 m above sea level.[22] Plants were grown under standard horticultural practices. Aroma extraction was performed on ripe stage fruits (stage 4 according Figueroa *et al.*[23]). Fruits from several plants were pooled. After harvesting, the fruits were transported on ice to the laboratory where they were immediately processed.

Chemicals

Fifty-one aroma standards were used in this study. They were obtained from the following companies: Fluka Analytical, Sigma-Aldrich and Hangzhou Imaginechem Co. (Supplementary Table 1).

Solvent-assisted evaporation and liquid–liquid extractions

For all extraction methods, fresh white strawberry fruits (250 g) were sliced and blended with 250 mL of a saturated calcium chloride solution. Two hundred and fifty microliters of an internal standard consisting in 4-nonanol (3.526 mg mL^{-1}, pH 1.1) were added to this solution. Then, the pH of the suspension was adjusted to 3.6, the pH of the natural white strawberry mature fruit, with 2 mol L^{-1} NaOH. Volatiles were isolated using the solvent-assisted flavor evaporation technique (SAFE), at 35°C under vacuum (10^{-5} mbar).[24] The aqueous distillate was extracted with diethyl ether (four times, 50 mL each time). The solvent extract was treated with aqueous 0.5 mol L^{-1} sodium bicarbonate to separate the acidic volatiles from the neutral–basic fraction. The combined aqueous solutions were adjusted to pH 2.0 with hydrochloric acid (2 mol L^{-1}) and extracted with diethyl ether (four times, 50 mL each time) to isolate the acidic compounds (acid fraction). The solutions containing either the acidic or the neutral–basic fraction were concentrated to 200 µL under a stream of nitrogen.

For the liquid–liquid extraction, the same method described for SAFE and LLE was used. The obtained solution was extracted with diethyl ether (three times with 50 mL each time) under nitrogen atmosphere with agitation at 4° C, for 20 min. After centrifugation and separation, the organic extracts were combined and dried over anhydrous sodium sulfate. Then, the sample was concentrated to 350 µL under a nitrogen stream.

Headspace solid phase micro-extraction analysis

A 2 cm 50/30 µm DVB/Carboxen/PDMS StableFlex fiber (Supelco, Inc., Bellefonte, PA, USA) was used for aroma extraction. The *Fragaria* solution (10 mL) plus sodium chloride (3 g) was placed in a 20 mL vial tightly capped with a Teflon/silicone septum (catalog number S126-0020; I-CHEM, Tennessee, USA). The sample was equilibrated at 40°C in a water bath for 15 min and extracted under stirring for 1 h at the same temperature. After extraction, the SPME fiber was inserted into the injection port of the GC (250°C) to desorb the analytes.

Analysis of volatile compounds by gas chromatography–mass spectrometry

The analyses were carried out on a HP 6890 Gas Chromatograph, coupled to a 5972A MSD Hewlett Packard mass spectrometer and equipped with a 60 m × 0.25 mm × 0.25 µm DB-WAXETR capillary column (J&W Scientific, California, USA). The original concentrated samples (2 μL) from the acid and neutral–basic fractions of SAFE, as well as from the liquid–liquid extractions were injected in a splitless mode using a constant helium flow of 1.9 mL min^{-1}. The HS-SPME sample was injected in the specific SPME port. The injector was set at 180°C and column oven temperature was held for 5 min at 40°C, then raised 3°C min^{-1} until it reached 240°C where it was held for 25 min. Mass spectra was obtained by electron impact ionization (70 eV) scanning a mass range of 40–450 *m/z*. The MS quadrupole and MS source temperatures were 150°C and 220°C, respectively. Volatiles were tentatively identified by comparing Kovats indices and the spectrometric data from NIST-EPA-NIH libraries (http://www.nist.gov/srd/nist1a.htm) that have more than 130,000 entries. We chose 51 compounds to be tested against their commercial standards. The identities were confirmed by comparison of their linear retention indices against the commercial standards (Supplementary Table 1).

 © 2013 Society of Chemical Industry

Table 2. Identification of compounds by gas chromatography–mass spectrometry (GC-MS) and detected by gas chromatography–olfactometry (GC-O) in *Fragaria chiloensis* berries

				FD = 1		FD = 500		FD = 1000	
RI on DB WaxETR*	Aroma compound†	Extraction methods‡	Descriptor§GC-O	Aroma[a]	Int.[b]	Aroma[a]	Int.[b]	Aroma[a]	Int.[b]
	Esters								
841	Ethyl acetate	SAFE A, SAFE B, SPME, LLE	—	ND	0[c]	ND	0	ND	0
993	Methyl butanoate	SPME, LLE	Sweet, fruity	+	1	+	1	+	1
997	Isobutyl acetate	LLE	—	ND	0	ND	0	ND	0
1010	Methyl 2-methylbutanoate	SPME, LLE	Caramel, sweet, fruity	+	1	+	1	+	1
1046	Ethyl butanoate	SPME, LLE	Nutty	+	2	+	2	+	2
1035	Ethyl 2-methylbutanoate	SPME,LLE	Fruity, woody	+	1	+	1	+	1
1098	Butyl acetate	SPME, LLE	Sweet, fruity	+	0.5	ND	0	ND	0
1096	2-Methylbutanoate	SPME, LLE	—	ND	0	ND	0	ND	0
1206	Methyl hexanoate	SPME,LLE	—	ND	0	ND	0	ND	0
1257	Ethyl hexanoate	SAFE A, SPME, LLE	Tropical fruits	+	3	+	3	+	3
1276	3-Methyl-2-butenyl acetate	SPME, LLE	—	ND	0	ND	0	ND	0
1294	Hexyl acetate	SPME, LLE	Tropical fruits, banana	+	2	+	2	+	2
1358	2-Hexen-1-ol acetate	SPME	Fruity, strawberry jam	+	2	+	2	+	2
1384	Methyl octanoate	LLE	Caramel, soy sauce	+	2	+	1	+	1
1430	Ethyl octanoate	SPME, LLE	Fruity, sweet	+	1	+	1	+	1
1490	Methyl 3-hydroxybutanoate	SAFE A	Sweet, berries	+	1.5	ND	0	ND	0
1512	Ethyl 3-hydroxybutanoate	SAFE A, SAFE B, LLE	Fruity	+	1	+	1	+	0.5
1567	Furfuryl acetate	SPME	Tropical fruit	+	2	+	2	+	2
1631	Ethyl decanoate	SPME LLE	Fruity, caramel	+	2	+	2	+	2
1668	Ethyl 3-hydroxyhexanoate	SAFE A, SAFE B, SPME, LLE	—	ND	0	ND	0	ND	0
1741	Benzyl acetate	SAFE A, LLE	Lactic alcohol, sweet	+	1	ND	0	ND	0
1782	Methyl dodecanoate	SAFE B, SPME, LLE	—	ND	0	ND	0	ND	0
1804	2-Phenylethyl acetate	SPME, LLE	Sweet, tea, floral	+	2	+	2	+	2
1881	Ethyl dodecanoate	SAFE B	Sweet	+	0.5	ND	0	ND	0
1930	Hydrocinnamyl acetate	SPME, LLE	Sweet	+	2	+	1	+	1
2010	Butyl laurate	LLE	Strawberry jam	+	3	+	3	+	1
2036	Ethyl tetradecanoate	LLE	Strawberry jam, tropical fruit	+	3	+	3	ND	0
2140	Cinnamyl acetate	SAFE B, SPME, LLE	Floral, sweet	+	3	+	3	+	3
2302	Ethyl hexadecanoate	SAFE B	Caramel	+	2	+	2	ND	0
2618	Benzyl benzoate	LLE	chemical	+	1.5	+	2	+	1
2646	Ethyl octadecanoate	LLE	—	ND	0	ND	0	ND	0
2733	(*EoZ*) Ethyl-*p*-hydroxy cinnamate	SPME	—	ND	0	ND	0	ND	0
2962	(*EoZ*) Ethyl-*p*-hydroxy cinnamate	SPME	—	ND	0	ND	0	ND	0
	Alcohols								
1030	2-Methyl-3-buten 2-ol	LLE	—	ND	0	ND	0	ND	0
1094	Isobutyl alcohol	SAFE A, LLE	Pungent	+	1	ND	0	ND	0
1136	1-Butanol	SAFE A, SAFE B, LLE	Fruity	+	1	+	1	+	1
1197	3-Methyl-1-butanol	LLE	Berries, candy	+	1.5	+	2	+	1
1344	1-Hexanol	SPME, LLE	Fresh, green	+	1	+	1	+	1
1376	3-Hexen-1-ol	LLE	—	ND	0	ND	0	ND	0
1513	2-Ethyl-1-hexanol	SPME, LLE	Green, can	+	1.5	+	1.5	+	1
1568	2,3-Butanediol	SAFE A, LLE	—	ND	0	ND	0	ND	0
1863	Benzyl alcohol	SAFE A, SAFE B SPME, LLE	Floral	+	2	+	2	+	2
1941	2-Phenyl ethanol	SPME	Tea floral	+	1	+	1	+	1
2005	1-Dodecanol	SAFE B, SPME	Berries jam	+	2	+	2	ND	0
2033	Hydrocinnamyl alcohol	SAFE A, SPME, LLE	Floral, citric	+	3	+	3	+	3

 © 2013 Society of Chemical Industry

Table 2. Continued

RI on DB WaxETR*	Aroma compound†	Extraction methods‡	Descriptor§GC-O	FD = 1		FD = 500		FD = 1000	
				Aroma[a]	Int.[b]	Aroma[a]	Int.[b]	Aroma[a]	Int.[b]
2267	Cinnamic alcohol	SAFE B, SPME, LLE	Floral, sweet	+	1	+	1	+	1
2232	1-Hexadecanol	SPME, LLE	Spicy	+	2	+	2	ND	0
2564	1-Heptadecanol	SAFE B, SPME	Fruity	+	0.5	ND	0	ND	0
	Aldehydes and ketones								
953	2-Pentanone	LLE	Caramel, sweet	+	0.5	+	0.5	ND	0
970	3-Methyl-3-buten-2-one	LLE	—	ND	0	ND	0	ND	0
1095	Hexanal	SPME, LLE	Asparagus, green	+	2	+	2	+	1
1097	2-Methyl-2-butenal	SAFE A	—	ND	0	ND	0	ND	0
1155	2-Methylpentenal	SAFE A, SAFE B, LLE	Green	+	1	+	1	+	1
1200	2-Heptanone	SPME	—	ND	0	ND	0	ND	0
1179	Heptanal	SAFE B, LLE	Humidity	+	1	+	1	+	1
1224	*trans*-2-Hexenal	SAFE A, SPME , LLE	Sweet	+	1.5	+	2	+	2
1288	Acetoin	SAFE A, SAFE B, LLE	Lactic	+	1	+	1	+	1
1389	Nonanal	SAFE A, SAFE B, LLE	Green olive	+	1.5	+	1	+	1
1402	2,4-Hexadienal	SPME	Green tea	+	1	+	1	ND	0
1493	Furfural	SPME	—	ND	0	ND	0	ND	0
1526	Benzaldehyde	SAFE A, SPME, LLE	Caramel, milk-like	+	1	+	1	+	1
1644	Acetophenone	SPME, LLE	—	ND	0	ND	0	ND	0
2154	2,6-Di(*t*-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	SPME	Caramel	+	3	+	3	+	3
2545	Benzophenone	SAFE B	Berries jam	+	1.5	+	1	+	1
2548	Vanilline	LLE	Sweet	+	1.5	ND	0	ND	0
	Acids								
1443	Acetic acid	SAFE A, SAFE B, SPME, LLE	Vinegar	+	1	ND	0	ND	0
1533	Propanoic acid	SAFE A, LLE	Smoky, can	+	1.5	+	1	+	1
1561	Isobutyric acid	SAFE A	Milk like, cheesy	+	2.5	ND	0	ND	0
1621	Butanoic acid	SAFE A, SPME, LLE	Lactic	+	2	ND	0	ND	0
1674	2-Methylbutanoic acid	SAFE A, LLE	Vinegar, yeast	+	2	+	2	ND	0
1724	Valeric acid	SAFE A, SPME, LLE	Yeast, mushroom	+	1	+	1	+	1
1849	Hexanoic acid	SAFE A, LLE	Rusty, can, oxide	+	1	ND	0	ND	0
2191	Nonanoic acid	LLE	—	ND	0	ND	0	ND	0
2276	3-Methylthiopropanoic acid	LLE	Spicy, pepper	+	2	+	1	+	1
2735	Tetradecanoic acid	LLE	Lactic	+	1.5	+	1.5	ND	0
2749	(*EoZ*)-Cinnamic acid	LLE	—	ND	0	ND	0	ND	0
2840	(*EoZ*)-Cinnamic acid	LLE	Fruity, floral	+	1	+	0.5	+	0.5
2952	Hexadecanoic acid	SAFE A	Chemical, lactic	+	3	+	2	+	2
	Lactones and furanones								
924	2-Ethylfuran	SPME	Fruity	+	2.5	+	2.5	ND	0
1589	2,5-Dimethyl-4-methoxy-3(2*H*)-furanone(mesifuran)	SAFE A, SAFE B, SPME, LLE	Fruity, caramel	+	2	+	2	+	2
1627	5-Ethyl-2(5*H*)-furanone	SPME	Caramel, sweet	+	2	ND	0	ND	0
1690	γ-Hexalactone	SAFE A, SAFE B, SPME, LLE	Fruity, sweet	+	1	+	1	ND	0
1778	δ-Hexalactone	LLE	—	ND	0	ND	0	ND	0
2022	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (DMHF)	SAFE A, LLE	Caramel, strawberry like	+	3	+	3	+	3
2133	γ-Decalactone	LLE	Floral, peach	+	3	+	2.5	+	3
2362	γ-Dodecalactone	SAFE B, LLE	Berries, sweet, tea	+	2.5	+	1	+	1
	Nonisoprenoids and terpenes								
1280	Styrene	SPME, LLE	—	ND	0	ND	0	ND	0
1541	Linalool	SPME, LLE	Floral	+	2.5	+	2	+	2
1720	α-Terpineol	SPME, LLE	—	ND	0	ND	0	ND	0
1712	α-Muurolene	SAFE B, LLE	Nuts	+	1.5	+	1	+	1
1737	α-Farnesene	SAFE B, LLE	Green	+	1	+	1	ND	0
2189	3,7-Dimethyloct-1-en-3,8-diol	LLE	Sweet	+	2	+	2	+	2

 © 2013 Society of Chemical Industry

Table 2. Continued

RI on DB WaxETR*	Aroma compound†	Extraction methods‡	Descriptor§GC-O	FD = 1		FD = 500		FD = 1000	
				Aroma[a]	Int.[b]	Aroma[a]	Int.[b]	Aroma[a]	Int.[b]
2252	(*EoZ*)-2,6-Dimethylocta-2,7-dien-1,6-diol	LLE	Sweet, floral	+	2.5	+	2	+	2.5
2288	(*EoZ*)-2,6-Dimethylocta-2,7-dien-1,6-diol	SAFE B, LLE	Honey	+	2	+	2	+	2
2518	Farnesol	SAFE B, LLE	Sweet	+	3	+	1	+	1
2618	3-Oxo-α-ionol + unknown	LLE	Tropical fruits, floral	+	2.5	+	3	+	3
3045	Squalene	SPME, LLE	Nutty	+	1.5	+	2	+	1
Not determined	Vomifoliol	SAFE B, LLE	—	ND	0	ND	0	ND	0
	Others								
1205	Diethyl disulfide	LLE	Pungent	+	1	+	1	ND	0
	Unknown 1	—	Green	+	2	+	1.5	+	1.5
	Unknown 2	—	Eucalyptus	+	1	+	0.5	+	0.5
	Unknown 3	—	Chemical	+	1	+	1	+	1
	Unknown 4	—	Sweet	+	3	+	3	+	3
	Unknown 5	—	Fruity, berries	+	1.5	+	2	+	2
	Unknown 6	SAFE B, LLE	Floral	+	3	+	2	+	1
	Unknown 7	LLE	Sweet, tropical fruit	+	2	+	2	+	0.5
	Unknown 8	LLE	Fruity	+	1	+	1	+	1
	Unknown 9	LLE	Sweet, fruity	+	3	+	2.5	+	3

*RI, retention index on DB WaxETR column.
†Aroma compounds.
‡SAFE A, acid fraction compounds from SAFE were identified by MS spectra; SAFE B, neutral/basic fraction compounds from SAFE were identified by MS spectra; SPME, compounds detected by SPME were identified by MS spectra; LLE, compounds detected by liquid–liquid extraction were identified by MS spectra.
§Aroma descriptors attributed by the sensory panel.
[a]Aroma detected; + = detected by sensory panel, ND, not detected by sensory panel.
[b]Intensity (Int.); 0 = not perceivable, 1 = slightly perceivable, 2 = perceivable, 3 = strongly perceivable.
[c]Average of eight trained sensory panelists.

Gas chromatography–olfactometry analysis

These analyses were carried out using a HP series 6890 gas chromatograph equipped with a flame ionization detector (FID) and a sniffing port (9000 sniffer; Brechbuhler AG, Texas, USA). The samples from acid and neutral–basic fractions of SAFE were analyzed on a DB-WAXETR capillary column (J&W Scientific) (60 m × 0.25 mm × 0.25 µm). The column effluent was split 1:1 (v/v) into the FID and a heated sniffing port with a fused silica outlet splitter (Alltech Associates, Illinois, USA). The injector and detector temperatures were 180°C and 280°C, respectively. The helium column flow rate was 1.9 mL min^{-1}, and 4 μL of the sample was injected in splitless mode. The oven temperature was programmed for 5 min at 40°C, then raised 3°C min^{-1} until it reached 240°C and held isothermally for 25 min at this temperature.

The sensory panel was composed of eight females, 35–50 years of age, all of them belonging to the staff of the Center of Aromas and Flavors from Catholic University of Chile. All of them had more than 4 years experience in quantitative descriptive analysis (QDA), with more than 400 h of GC-O analysis. This panel has been trained under ISO 8586 and controlled by the National Standards Institute. In the GC-O sessions the panelists recorded the retention times and sensorially described the volatile compounds. Aroma intensities were ranked on a four-point scale ranging from 0 to 3, where the value 0 represents no perception and the value 3 is strongly perceivable. The concentrated basic fraction obtained from SAFE was diluted to a dilution factor (FD) of 1:500 and 1:1000 and the eight panelists performed a GC-O with these three samples.

Preliminary aroma reconstitution experiment

The odorants with the highest dilution factor (FD = 1000) and GC-O intensity ≥ 2, were positively identified by comparing mass spectra, aroma and RIs with commercial standards. The RIs were calculated according to their Kovats retention indices. The panelists attended a specific training session, where they generated the descriptive terms required to define the white strawberry fruit aroma. Different aroma standards were presented to the panel and discussed. From these discussions, nine aroma descriptors (apple, green, lactic, pineapple, citric–grapefruit, cherimoya, banana, pear and caramel) were selected for further descriptive analysis. The aroma reference standards employed are listed in Table 1. These standards, when available, were used to reconstitute the model solutions. The relative quantification was carried out by comparing the areas of the peaks of three different replicas for each extraction method and then compared with an internal standard (4-nonanol) in the GC-MS for SAFE, SPME or LLE. The results are expressed in µg kg^{-1} fresh weight. The standards were added to 1 L of Milli-Q water in concentration levels previously determined in the fresh juice and adjusted to pH 3.6, the natural pH of the white strawberry fruit. The reconstituted model solution was carried out as duo-trio tests, which were repeated twice. The solutions were placed in glass vessels with screw caps and then were presented to the eight members of the sensory panel for orthonasal evaluation. The best-reconstituted model solutions were compared to the white strawberry fresh fruits, in order to determine if there were any detectable aromatic differences.

 © 2013 Society of Chemical Industry

RESULTS AND DISCUSSION

Identification of volatiles by gas chromatography–mass spectrometry

We identified 99 volatile compounds in white strawberry fruits extracted by SAFE, HS-SPME and LLE extraction methods; Table 2 summarizes the compounds tentatively identified using a MS library search and compared with the retention indices available in the literature. The volatile compounds were classified into esters, alcohols, aldehydes and ketones, acids, C_{13}-norisoprenoids and terpenes, as well as lactones and furanones. All these families of volatile compounds have been reported in other *Fragaria* species.[25,26] Three different methods for aroma extraction were used in order to maximize the possibilities to obtain and identify the widest variety of volatile/odorant compounds present in white strawberry. Seventy-nine different compounds were extracted by LLE, 50 compounds by HS-SPME and 54 compounds with the neutral–basic fraction and acid fraction of SAFE. Based upon the number of diverse compounds extracted, it may be suggested that LLE is the most complete extraction method. However, 22 compounds not detected in the LLE extracts, were extracted using the other methods. Therefore, these results demonstrated that the largest number of volatile compounds are extracted using the LLE method; even though the other methods extracted additional compounds than the LLE method. Therefore, in order to have a more complete volatile profile of the white strawberry, a combination of all these extractive methods should be performed.

Of the 99 volatile compounds identified, only 13 were identified previously in *Fragaria chiloensis*: ethyl acetate, ethyl butanoate, butyl acetate, methyl hexanoate, ethyl hexanoate, hexenyl acetate, hexyl acetate, 2-hexenyl butanoate, benzyl acetate, phenylethyl acetate, 1-butanol, 1-hexanol and 2-heptanone.[3,21] It is worth mentioning that methyl anthranilate (MA) has been described as a discriminative key compound among *Fragaria* species. It has been found in high levels in some wild species such as *Fragaria vesca*, *Fragaria x vescana* and *F. moschata* and low or undetectable levels in *Fragaria x ananassa* and *F. virginiana*.[27] MA has also been reported in some accessions of *Fragaria chiloensis* subsp. *lucida*,[27,28] although we were unable to detect it. This may be because we evaluated a different subspecies (*Fragaria chiloensis* spp. *chiloensis*). Alternatively, the extract methods we used might not be able to extract MA or may have led to modifications of MA (i.e. oxidation).

Sensory evaluation, gas chromatography–olfactometry

GC-O results of the concentrated and diluted extracts (1:500 and 1:1000 fold dilutions) led to 84 odor-active zones that were detected by the panelists during the gas olfactometric analyses. From these zones, nine could not be identified by GC-MS (unknown compounds) (Table 2). The olfactometric analysis allowed the trained panel to define at least nine sensory descriptors of white strawberry: fruity, tropical fruits (like pineapple, guavas, and banana odors), green–fresh, floral, caramel, sweet, nutty, woody and unpleasant. The fruity notes, mainly derived from esters, are represented by 2-ethylfuran, methyl butanoate, butyl acetate, methyl 2-methylbutanoate, ethyl 2-methylbutanoate, 2-hexen-1-ol acetate, ethyl octanoate, methyl 3-hydroxybutanoate, ethyl 3-hydroxybutanoate, ethyl decanoate, mesifurane, furaneol, butyl laurate, gamma decalactone, isoamyl alcohol, 1-dodecanol and seven unknown compounds. The tropical fruit notes belong to six odorant zones containing only esters: ethyl hexanoate, hexyl acetate, acetyl acetate, furfuryl acetate, 1-methylethyl dodecanoate and ethyl tetradecanoate. The esters are key compounds of commercial strawberry aroma responsible for fruity impressions.[5,12,17] Green–fresh notes appeared in seven odorant zones, characterized by aldehydes like 2-methyl-2-pentenal, hexanal, nonanal, 2,4 hexadienal; alcohols (1-hexanol and 2-ethyl-1-hexanol) and the sesquiterpene α-farnesene. Among these, hexanal, trans-2-hexenal and hexanol have been already reported as contributors to green and fresh notes.[29] C_6-compounds are known to decrease with maturity, lowering green notes, whereas esters and furanones, responsible for fruity and caramel notes, respectively, increase with maturity.[30]

The floral zones are represented by seven odorants: benzyl alcohol, linalool, 2-phenylethyl acetate, hydrocinnamyl alcohol, cinnamyl acetate, cinnamic acid and gamma decalactone. The cinnamic compounds were detected previously in *Fragaria chiloensis*.[31] The caramel odor is reported as one of the most important notes in *Fragaria x ananassa*, the furaneol [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone] and mesifuran [2,5-dimethyl-4-methoxy-3(2*H*)-furanone] are considered to be the two most important furanones in strawberries that contribute to caramel and fruity descriptors.[9,12,32] In our study, both compounds were detected and contributed to the caramel odor; but others also contributed to caramel note, (i.e. methyl octanoate, ethyl hexadecanoate, 5-ethyl-2-(5*H*)-furanone and 2,6-di(*t*-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one. In general, sweet notes were produced by: 2-pentanone, γ-hexalactone, ethyl dodecanoate, ethyl butanoate and α-muurolene. The alcohols and acids contribute little to the flavor and they are irrelevant as odorant impact compounds. They are, however, responsible for five odorant zones detected in our study with unpleasant notes, such as pungent, and chemical aromas: isobutyl alcohol, acetic acid, propanoic acid, 2-methylbutyric acid and valeric acid. Among sulfur compounds, we identified diethyl disulfide, as well as several others with unpleasant notes, such as hydrogen sulfide, methanethiol, diethyl disulfide, dimethyl disulfide, methyl thioacetate and methyl thiobutanoate, already reported in strawberries.[33,34] Some of the latter could be important to the aroma of cultivars, even though they are present at very low concentrations.[27]

Among all the compounds detected by GC-O, 75 were detected by the sensory panel only in the concentrated sample (1:1), 63 in the 1:500 dilution and 51 in the 1:1000 dilution. The detection for each compound is different based on its detection threshold (Table 2).

Preliminary aroma reconstitution

Despite the numerous compounds present in the white strawberry extract (Table 2), only those with a dilution factor of 1:1000 and an intensity $\geq$2 were used for a preliminary reconstitution experiment (Table 1). From the 23 selected odorant zones, five compounds were not used in the reconstitution experiment because three were unknown and the other two – 2,6-di(*t*-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one and 3,7-dimethyloct-1-en-3,8-diol, respectively – were not commercially available. The 18 remaining odorants were mixed at the same relative concentrations (expressed as 4-nonanol equivalent) as they occur in white strawberry juice, using commercial compounds and modifying their concentrations according to the sensorial panel's suggestions. Similarities or differences were obtained by comparing the aroma of reconstitution models with the original white strawberry juice. The results of this experiment are illustrated as a spider diagram (Fig. 1).

 © 2013 Society of Chemical Industry

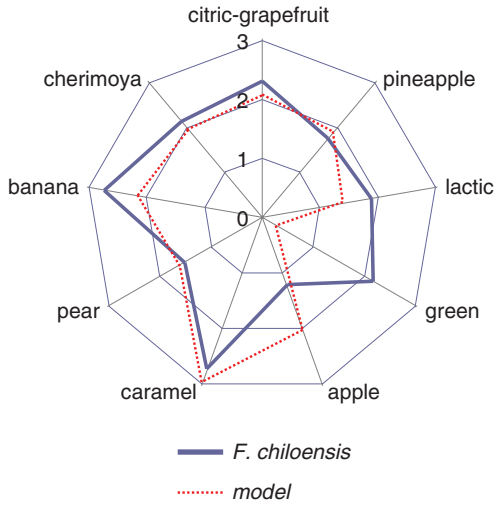

Figure 1. Aroma reconstitution of *Fragaria chiloensis* fruits. The dashed red line (thin) shows the model based on white strawberry juice. The solid blue line (thick) shows the reconstitution model for *Fragaria chiloensis* fruits using the compounds suggested by the sensory panel. 0, 1, 2 and 3 represent the gas chromatography–olfactometry intensity. The names on the figure represent descriptive terms to define the *Fragaria* fruits according to the sensory panel.

The sensory profiles of white strawberry juice and the reconstitution model showed a good agreement, with the exception of the green note, which is perceived in the natural juice but not in the model. This difference may be because the concentration were estimated and not absolutes and/or because we did not include all compounds. The overall similarity between the white strawberry juice and the model was marked 2.4 on a scale from 0 to 3. Although the panel was able to discriminate between the fruit and the reconstituted formula, their aroma was considered qualitatively to be similar to the juice from the fresh fruits.

It has been reported previously that *Fragaria vesca*, a diploid strawberry, has more volatile compounds than the octoploid *Fragaria ananassa*.[2] The volatile profile of *Fragaria chiloensis* spp. *chiloensis*, reported here, revealed 99 volatile compounds, significantly more than what has been reported for *Fragaria ananassa* and *Fragaria vesca*.[2] However, there are reports that indicate that environment is important in the aroma of strawberries.[35–37] Furthermore, some octoploids would have compounds that are not present in diploids and vice versa. Also, as described in this work, the methods used for the determination can make differences. Further insight into these possibilities may be revealed by comparing the volatile profiling of *Fragaria ananassa*, *Fragaria chiloensis*, *Fragaria virginiana* and *Fragaria vesca* under the same conditions, as well as a comparative genomic analysis of the genes that regulate the production of these compounds.

CONCLUSION

We identified 99 different volatile compounds in *F. chiloensis* fruit. Only 75 of these compounds were described as odorants by the training sensorial panel. These odorants have notes such as fruity, green, floral, caramel, sweet, and some unpleasant odors that play important roles in the complete aroma (Table 2). Twenty were potent aroma compounds detected with a dilution factor of 1:1000 and an intensity $\geq$2: ethyl butanoate (nutty odor), 2-hexenal (fresh odor), ethyl hexanoate (tropical fruits odor), hexyl acetate (tropical fruits odor), 2-hexen-1-ol acetate (fruity, strawberry jam odor), furfuryl acetate (tropical fruits odor), linalool (sweet odor), mesifuran (caramel odor), ethyl decanoate (fruity and caramel odors), benzyl alcohol (spice, floral odor), 2-phenylethyl acetate (sweet, tea and floral odors), DMHF (caramel, strawberry like odor), hydrocinnamyl alcohol (floral, citric aroma), γ-decalactone (floral, peach aroma), cinnamyl acetate (floral, sweet), (*E*)-2,6-dimethylocta-2,7-dien-1,6-diol (sweet, floral aroma), (*Z*)-2,6-dimethylocta-2,7-dien-1,6-diol (caramel, honey odor) and hexadecanoic acid (chemical, lactic odor) were identified in *F. chiloensis*. Additional studies must be carried out to improve the reconstitution of the aroma of *Fragaria chiloensis*. Recently, the genome of *Fragaria vesca*[38] was sequenced, opening the door to new genes involved in metabolic pathways related to aroma. In any case, the results obtained in this work will be a useful reference for future strawberry breeding programs.

ACKNOWLEDGEMENTS

Loreto Prat is a member of the Biotechnology PhD Program at Universidad Andres Bello. This research was supported by Millennium Nucleus in Plant Cell Biotechnology (PCB) ICM P06-065-F. We would like to thank Dr Lee Meisel from INTA, Universidad de Chile, for her critical reading of the manuscript.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Darrow GM, *The Strawberry: History, Breeding, and Physiology*. Holt, Reinehart and Winston, New York, p. 447 (1966).
2 Ulrich D, Komes D, Olbricht K and Hoberg E, Diversity of aroma patterns in wild and cultivated *Fragaria* accessions. *Genet Resour Crop Evol* **54**:1185–1196 (2007).
3 Drawert F, Tressl R, Staudt G and Köppler H, Gaschromato graphisch–massenspektrometrische differenzierung von erdbeer-arten. *Z Naturforsch* 28c:488–493 (1973).
4 Adasme C, Spiller A and Diaz J, Determinacion de preferencias del consumidor de la Region Metropolitana hacia la frutilla Blanca (*Fragaria chiloensis*). Un analisis conjunto y una prueba sensorial. *Econ Agraria* **10**:1–10 (2006).
5 Latrasse A, Fruits III, in *Volatile Compounds in Foods and Beverages*, ed. by Maarse H. Dekker, New York, pp. 333–387 (1991).
6 Larsen M and Poll L, Odour thresholds of some important compounds in strawberries. *Z Lebensm Unters Forsch* **195**:120–123 (1992).
7 Larsen M, Poll L and Olsen C, Evaluation of the aroma composition of some strawberry (*Fragaria ananassa* Duch) cultivars by use of odour threshold values. *Z Lebensm Unters Forsch* **195**:536–539 (1992).
8 Schieberle P and Hofmann T, Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J Agric Food Chem* **45**:227–232 (1997).
9 Hirvi T and Honkanen E, The volatiles of two new strawberry cultivars, 'Annelie' and 'Alaska Pioneer', obtained by backcrossing of cultivated strawberries with wild strawberries, *Fragaria vesca*, *rugen* and *Fragaria virginiana*. *Z Lebensm Unters Forsch A* **175**:113–116 (1982).
10 Perez A, Olias R, Luances P and Sanz C, Biosynthesis of strawberry aroma compounds through amino acid metabolism. *J Agric Food Chem* **50**:4037–4042 (2002).
11 Azodanlou R, Darbellay C, Luisier J, Villettaz J and Amado R, Quality assessment of strawberries (*Fragaria* species). *J Agric Food Chem* **51**:715–721 (2003).
12 Ulrich D, Hoberg E, Rapp D and Kecke S, Analysis of strawberry flavour – discrimination of aroma types by quantification of volatile compounds. *Z Lebensm Unters Forsch A* **205**:218–223 (1997).

© 2013 Society of Chemical Industry

13 Lavid N, Schwab W, Kafkas E, Koch-Dean M, Bar E, Larkov O, *et al*, Aroma biosynthesis in strawberry: *S*-adenosylmethionine:furaneol *O*-methyltransferase activity in ripening fruits. *J Agric Food Chem* **50**:4025–4030 (2002).
14 Urruty L, Giraudel J, Lek S, Roudeillac P and Montury M, Assessment of strawberry aroma through SPME/GC and ANN methods. *Classification and discrimination of varieties. J Agric Food Chem* **50**:3129–3136 (2002).
15 Hakala M, Lapvetelainen T and Kallio H, Volatile compounds of selected strawberry varieties analyzed by purge-and-trap headspace GC-MS. *J Agric Food Chem* **50**:1133– 1142 (2002).
16 Zabetakis I and Holden M, Strawberry flavour: Analysis and biosynthesis. *J Agric Food Chem* **74**:421–434 (1997).
17 Fischer N and Hammerschmidt FJ, A contribution to the analysis of fresh strawberry flavour. *Chem Mikrobiol Technol Lebensm* **14**:141–148 (1992).
18 Gomes da Silva M and Chaves das Neves H, Complementary use of hyphenated purge-and-trap gas chromatography techniques and sensory analysis in the aroma profiling of strawberries (*Fragaria ananassa*). *J Agric Food Chem* **47**:4568–4573 (1999).
19 Fukuhara K, Li X, Okamura M, Nakahara K and Hayata Y, Evaluation of odorants contributing to 'Toyonoka' strawberry aroma in extracts using an adsorptive column and aroma dilution analysis. *J Jpn Soc Hort Sci* **74**:300–305 (2005).
20 Douillard C and Guichard E, The aroma of strawberry (*Fragaria ananassa*): Characterization of some cultivars and influence of freezing. *J Sci Food Agric* **50**:517–531 (1990).
21 Gonzalez M, Gaete-Eastman C, Valdenegro M, Figueroa C, Fuentes L, Herrera R, *et al*, Aroma development during ripening of *Fragaria chiloensis* fruit and participation of an alcohol acyltransferase (FcAAT1) gene. *J Agric Food Chem* **57**:9123–9132 (2009).
22 Simirgiotis MJ, Theoduloz C, Caligari P and Schmeda-Hirschmann G, Comparison of phenolic composition and antioxidant properties of two native Chilean and one domestic strawberry genotypes. *Food Chem* **113**:377–385 (2009).
23 Figueroa CR, Pimentel P, Gaete-Eastman C, Moya M, Herrera R, Caligari PDS, *et al*, Softening rate of Chilean strawberry (*Fragaria chiloensis*) fruits reflects the expression of polygalacturonase and pectate lyase genes. *Postharvest Biol Technol* **49**:210–220 (2008).
24 Engel W, Bahr W and Schieberle P, Solvent assisted flavour evaporation ± a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur Food Res Technol* **209**:237–241 (1999).
25 Kaskas E, Kaskas S, Koch-Dean M, Schwab W, Larkov O, Lavid N, *et al*, Comparison of methodologies for the identification of aroma compounds in strawberry. *Turk J Agric For* **29**:383–390 (2005).
26 Zabetakis I, Gramshaw JW and Robinson D, S. 2,5-Dimethyl-4-hydroxy-2*H*-furan-3-one and its derivatives: analysis, synthesis and biosynthesis – A review. *Food Chem* **65**:139–151 (1999).
27 Olbricht K, Grafe C, Weiss K and Ulrich D, Inheritance of aroma compounds in a model population of *Fragaria x ananassa* Duch. *Plant Breed* **127**:87–93 (2008).
28 Ulrich D, Hoberg E and Olbricht K, Flavour control in strawberry breeding by sensory and instrumental methods. *Acta Hortic ISHS* **708**:579–584 (2006).
29 Jetti RR, Yang E, Kurnianta A, Finn C and Qian MC, Quantification of selected aroma-active compounds in strawberries by headspace solid-phase micro extraction gas chromatography and correlation with sensory descriptive analysis. *J Food Sci* **72**:487–496 (2007).
30 Menager I, Jost M and Aubert C, Changes in physicochemical characteristics and volatile constituents of strawberry (cv. Cigaline) during maturation. *J Agric Food Chem* **52**:1248–1254 (2004).
31 Cheel J, Theoduloz C, Rodriguez J, Saud G, Caligari P and Schmeda-Hirschmann G, (*E*)-Cinnamic acid derivatives and phenolics from Chilean strawberry fruits, *Fragaria chiloensis* spp. *chiloensis*. *J Agric Food Chem* **53**:8512–8518 (2005).
32 Pyysalo T, Honkanen E and Hirvi T, Volatiles of wild strawberries, *Fragaria vesca* L., compared to those of cultivated berries, *Fragaria x ananassa* cv. Senga Sengana. *J Agric Food Chem* **27**:19–22 (1979).
33 Dirinck P, De Pooter H, Willaert G and Schamp N, Flavor quality of cultivated strawberries: The role of the sulfur compounds. *J Agric Food Chem* **29**:316–321 (1981).
34 Schulbach KF, Rouseff RL and Sims CA, Changes in volatile sulfur compounds in strawberry puree during heating. *J Food Sci* **69**:268–272 (2004).
35 Forney CF, Kalt W and Jordan MA, The composition of strawberry aroma is influenced by cultivar, maturity, and storage. *Hortscience* **35**:1022–1026 (2000).
36 Pelayo-Zaldivar C, Ebeler SE and Kader, AA, Cultivar and harvest date effects on flavour and other quality attributes of California strawberries. *J Food Qual* **28**:78–97 (2005).
37 Forney CF, Horticultural and other factors affecting aroma volatile composition of small fruit. *HortTechnology* **11**:529–538 (2001).
38 Shulaev V, Sargent DJ, Crowhurst RS, Mockler T, Veilleux RE, Folkerts O, *et al*, The genome of woodland strawberry (*Fragaria vesca*). *Nat Genet* **43**:109–118 (2011).

 © 2013 Society of Chemical Industry