Complementary Use of Hyphenated Purge-and-Trap Gas Chromatography Techniques and Sensory Analysis in the Aroma Profiling of Strawberries (*Fragaria ananassa*)

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The aroma compositions of *Fragaria ananassa* varieties are assessed by purge-and-trap high-resolution gas chromatography. Gas chromatography/mass spectrometry and gas chromatography/Fourier transform infrared spectroscopy allow the identification of 93 components from which 21 are for the first time described as constituents of strawberry aroma. Despite the complexity of the aroma, sensory properties are assigned to 40 components as perceived by three independent testers by application of a sniffing technique to the chromatographic effluent. The data are used for comparative sensory characterization of strawberry varieties by means of principal component analysis.

Keywords: *Strawberries; Fragaria ananassa; aroma; sensorial evaluation; purge-and-trap gas chromatography; hyphenated techniques*

INTRODUCTION

Different authors have used various techniques to study the volatile fractions of different varieties of strawberries in the past 30 years. More than 360 components have been identified as volatile components in varieties of *Fragaria ananassa* (Winter and Wilhalm, 1964; McFadden et al., 1965; Barron, 1991) by analysis of liquid−liquid extracts or simultaneous distillation products (Wilhalm et al., 1996; Pyysalo et al., 1979; Schreier, 1980; Douillard and Guichard, 1989). These sampling procedures require heating and, eventually, concentration steps that are likely to promote losses of more volatile components and production of artifacts. Moreover, many of the components of the extracts thus obtained are not real volatiles and are not present in the aroma itself. The introduction of a technique for the concentration of the headspace of strawberries on Tenax allowed the identification of 30 true strawberry aroma compounds (Dirinck et al., 1977). This technique requires a compromise between the large sample required and the retention capacity of the adsorbent (Polesello et al., 1993; Ventura et al., 1993; Pellizzari et al., 1976; Brown and Purnell, 1979). About 70 compounds have been described to date by the application of purge-and-trap methods to headspace analysis (Dirinck et al., 1981; Pérez et al., 1992; Ulrich et al., 1995; Gomes da Silva and Chaves das Neves, 1997).

In the present paper we describe the gas chromatographic (GC) profile of the aroma from strawberries *Fragaria ananassa* (variety Oso Grande) as assessed by purge-and-trap sampling for GC/mass spectrometric (GC/MS) and GC/Fourier transform infrared spectroscopic (GC/FTIR) techniques. From the 93 identified compounds in the aroma of the variety Oso Grande, 21 are for the first time described as components of strawberry aroma. The odorous properties of 40 individual components were assessed by three independent testers by adapting a sniffing at column outlet. These data are used for comparative sensory characterization of strawberry varieties together with previously published data for varieties Selva and Chandler by means of principal component analysis (PCA).

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MATERIALS AND METHODS

Chemicals. Furaneol [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone] was purchase from Alltech Associates (Deerfield, IL). Diazomethane was prepared according to standard methods. Mesifurane [2,5-dimethyl-4-methoxy-3(2*H*)-furanone] was prepared by methylation of Furaneol (Polesello et al., 1993) with freshly prepared diazomethane in ethyl ether.

Instruments. GC was performed with a Carlo Erba instrument, Model 6000, Vega Series, equipped with a Carlo Erba automatic purge-and-trap injector, Model TDAS 5000, a flame ionization detector, and a 30 m × 0.32 mm i.d. DB-Wax (J&W, Folsom, CA) or alternatively, a DB-5 column (J&W) $d_f = 1.0$ μm for purge-and-trap injection or $d_f = 0.25$ μm for split injection (split ratio = 10: 1). The initial linear heating program was from 40 to 50 °C at a heating rate of 3 °C/min and then up to 220 °C (DB-Wax) or 250 °C (DB-5) at 6 °C/min. Helium $P_i = 100$ kPa was used as carrier gas. The TDAS was connected to the analytical column through a 40 cm × 0.32 mm i.d. deactivated fused-silica tube. A Merck-Hitachi Model D2000 integrator was used for quantitation.

GC/FTIR assays were performed through coupling of the chromatograph to a Nicolet SXC instrument by means of a light-pipe interface equipped with an MCT detector (Nicolet Instruments, Madison, WI). The light pipe and transfer line temperatures were 250 °C. Helium (1 mL/min) was used as makeup gas. MS detection was made by connecting an ITD detector (Finnigan Mat, San Jose, CA) to the chromatograph by means of an open-split interface at 250 °C. Temperature of the exit nozzle was 240 °C and the manifold temperature, 211 °C.

10.1021/jf9905121 CCC: $18.00
Published on Web 10/06/1999

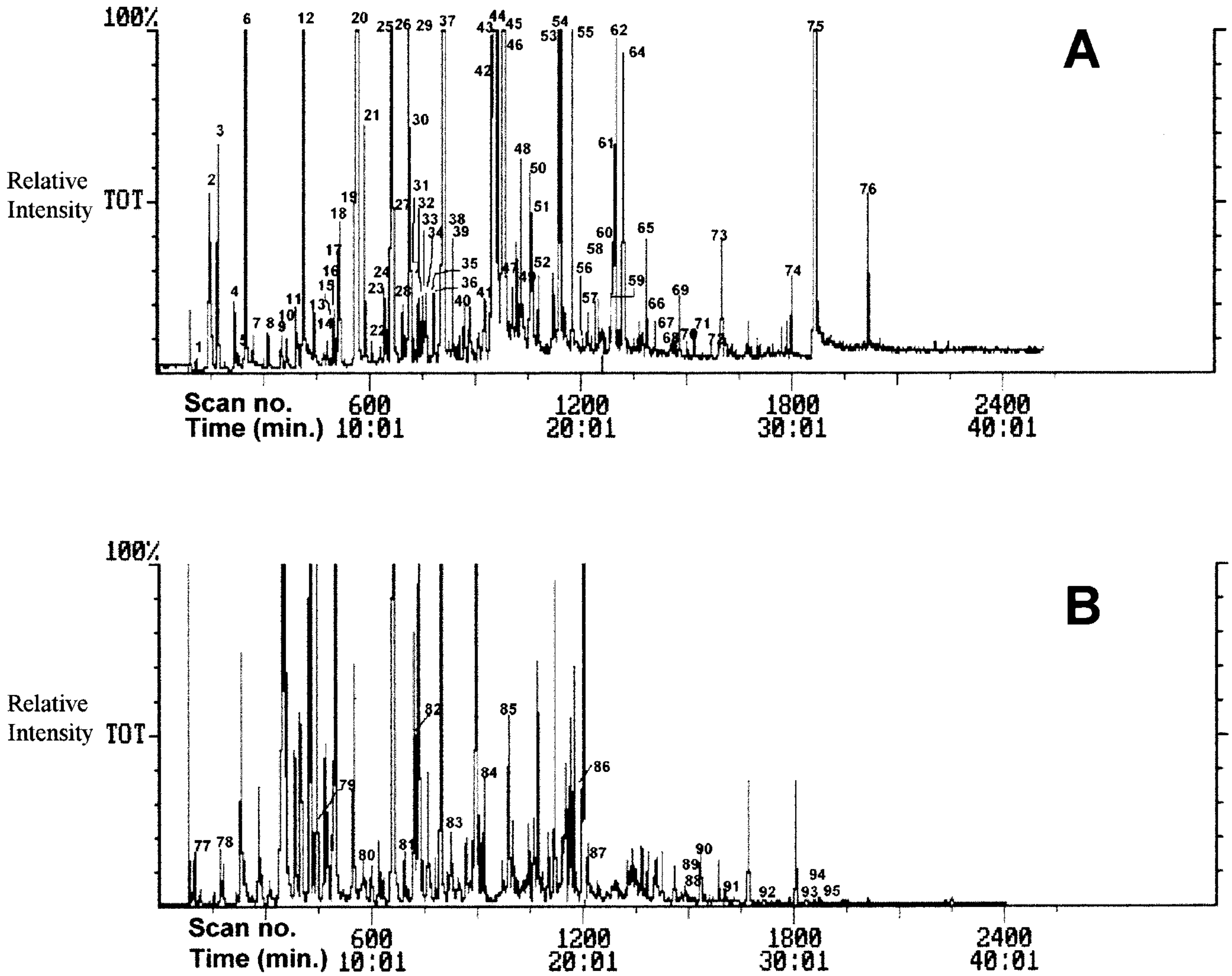

Figure 1. TIC of the GC/MS assay of the aroma from *F. ananassa* cv. Oso Grande by purge-and-trap sampling: (A) on a DB-5 capillary; (B) on a DB-Wax capillary. For details see Experimental Procedures. See Tables 1 and 2 for peak identification.

Sensory Evaluation. The column outlet was diverted by means of a needle valve at an approximately 1:1 ratio to a flame ionization detector and to an odor evaluation port (Savenhed et al., 1985). Three independent testers sniffed two replicates of each sample. Two testers sniffed a third replicate simultaneously. The impressions of each tester were recorded separately and in confidentiality.

Sample Preparation. The strawberry samples (*F. ananassa* var. Oso Grande, Chandler, and Selva) were randomly collected at Herdade do Brejão (Odemira, Portugal), where they were cultivated under controlled selected conditions. They were immediately frozen under liquid nitrogen for transportation and stored at −35 °C until use.

Purge-and-Trap. The chopped strawberries (100 g) were introduced in a closed two-neck round-bottom flask immersed in a water bath at 45 °C. The flask was connected by means of Teflon O-tubing to a nitrogen R cylinder (Companhia Portuguesa do Ar Liquido, Lisbon, Portugal), at the inlet, and to an adsorbing tube at the outlet. The flask contents were purged with a nitrogen flow of 45 mL/min for 2 h under constant mechanical shaking. The effluent was trapped at the outlet on calibrated 100 mm × 3 mm i.d. glass tubes packed with 0.09 g of a Tenax GC 60/80 mesh (Alltech Associates) preconditioned overnight at 250 °C with nitrogen R at 30 mL/min. After completion of the trapping period, the tubes were introduced in the TDAS sampler. The sorbates were transferred into a deactivated 100 mm × 0.32 mm i.d. fused-silica tube cooled with liquid nitrogen. Chromatography is initiated after complete transfer.

Argentation Chromatography. A glass column (100 × 10 mm) packed with silver-impregnated silica gel was prepared as described by Aubert-Mammou et al. (1994). Approximately 0.5 mL of the above extract was transferred and eluted with 10 mL of pentane (five fractions of 2 mL each), 12 mL of pentane/ethyl ether 1:1 v/v (six fractions of 2 mL each), and 14 mL of ethyl ether (seven fractions of 2 mL each). Each collected fraction was concentrated to a final volume of 100 μL and submitted to GC.

PCA. The relative percent areas of the identified peaks were used as variables for object description. The objects were collected samples of each mature *F. ananassa* variety, Selva (S), Oso Grande (OS), and Chandler (C). The data matrix was used for computerized multivariate analysis of data by the software package Statistica for Windows from StatSoft (Tulsa, OK).

RESULTS AND DISCUSSION

Figure 1 shows the total ion current trace (TIC) for the GC/MS analysis of strawberry aroma on a polar DB-Wax column and on an apolar DB-5 column. The use of two columns of different polarities is necessary for establishing compound identity and for peak deconvolution due to the complexity of the matrix. For example, Furaneol (52) and nerolidol (75) coelute on the polar column, whereas they are very well differentiated on

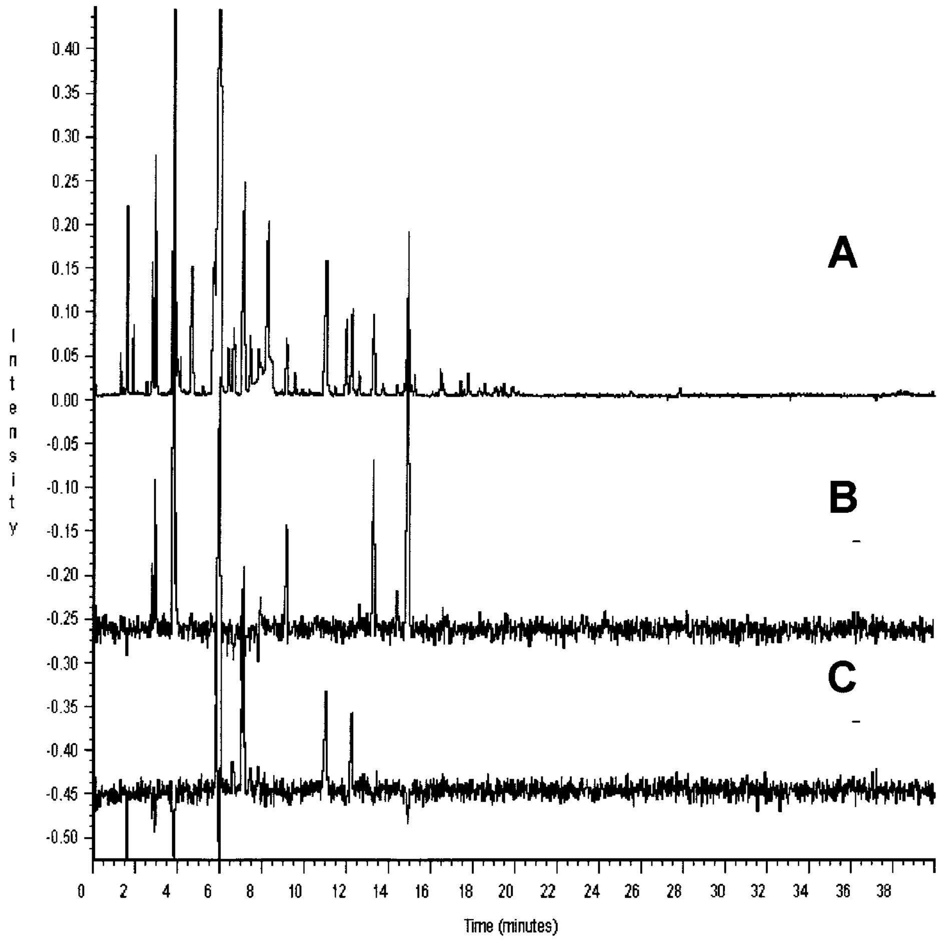

Figure 2. GC/FTIR profiling of the aroma from *F. ananassa* cv. Oso Grande on a DB-Wax capillary by purge-and-trap sampling: (A) total Gram−Schmidt reconstruction; (B) selected wavelength chromatogram within the range 1250−1210 cm^{-1} (esters); (C) selected wavelength chromatogram within the range 1200−1150 cm^{-1} (acetates). For details see Materials and Methods.

the apolar column. Figure 2 illustrates the Gram−Schmidt reconstruction from the GC/FTIR analysis of the aroma on a DB-Wax column. The corresponding window chromatograms at adequate wavenumbers show that esters constitute the most important group of compounds, to which acetates are major contributors. These compounds constitute >80% of the total aroma fraction. The spectral information obtained from both GC/MS and GC/FTIR techniques is an invaluable tool in assessing chemical identification of individual components because GC/FTIR has over GC/MS the advantage of allowing distinction between isomers, which is not always easy with GC/MS data alone. However, the lower sensitivity of GC/FTIR demands that comparison with authentic samples be performed in compounds of lower relative concentration. Table 1 lists the identified compounds that are common to strawberry aromas. Table 2 lists the compounds that have not been previously described as components of strawberry aromas. Whenever sensory impressions could be unequivocally assigned to a particular peak, these are also indicated. Esters in general and acetates in particular contribute the floral and fruity notes to the overall aroma (Figure 2). Alcohols and aldehydes are mainly responsible for the green and pungent impressions. The odorous properties of some compounds are a valuable complement for their identification when the small amounts present are not enough to obtain unequivocal spectral data. The sweet nuts odor of β-ionone (92), the caramel-like odor of ethyl cinnamate (93), and the cooked sugar odor of cinnamyl acetate (95) are examples of how the sensory properties could be used to confirm the identity suggested by mass spectral and retention data. These examples illustrate well the importance of sensory data in aroma analysis (Hirvi and Hokanen, 1982; Hirvi, 1983). Identically, the typical caramel-like odor threshold of mesifurane [2,5-dimethyl-4-methoxy-3(2*H*)-furanon] (51) is high enough (Hirvi and Hokannen, 1982) to allow its location in the GC/FTIR chromatogram, where it is badly detected due to weak infrared absorption bands and usual very low concentration (Douillard and Guichard, 1989). It is a known that the human nose is able to recognize aroma active compounds at levels that remain undetected by highly sensitive analytical techniques (Mosandl, 1992).

Group-oriented chromatographic fractionation on argented silica gel allows one to globally assess the overall odor contributions of two major component groups. The ester fraction obtained from the pentane/ethyl ether eluate (Figure 3) has an intense floral-to-fruity odor, as anticipated. The aldehyde-dominated fraction is characterized by intense an "green" or pungent odor. This is not surprising because it was previously established that aldehydes are the main characterizing compounds of strawberries in the green maturation stage [16]. The latter eluted fraction of the ethyl ether elution step is enriched in lactones (Figure 4). The major lactone components are γ-decalactone (94) and γ-dodecalactone

Table 1. Peak Identification (Figures 1−4) for Common Strawberry Aroma Compounds Identified in *F. ananassa* Variety Oso Grande by Purge-and-Trap Sampling and High-Resolution GC

peak	compound	odor perception
2	acetic acid	
3	methyl acetate	
4	2,3-butanedione	
5	2-butanone	
6	ethyl acetate	chemical
7	methyl propanoate	
8	3-pentanone	
9	pentanal	green
10	lsopropyl acetate	
11	ethyl propanate	sweet-caramel
12	methyl butyrate	floral/pineapple
14	ethyl isobutyrate	fruity
16	isobutyl acetate	
17	methyl isovalerate	
18	methyl 2-methy butyrate	cooked apple
19	*n*-hexanal	green
20	ethyl butyrate	vanilla-like
21	butyl acetate	
22	methyl valerate	
24	ethyl 2-butenoate (isomer)	cooked apple
25	ethyl 2-methylbutirate	
26	ethyl isovalerate	cooked apple
27	hex-2(*E*)-enal	sweet/pungent
28	ethyl benzene	
29	isoamyl acetate	fruity
30	2-methylbutyl acetate	
31	3-methyllbutenyl acetate (isomer)	
33	propyl butyrate	
86	benzaldehyde	lecherous
35	butyl propanoate	
36	amyl acetate	fruity
37	methyl caproate	fruity/pineapple
38	methyl 2-hexenoate (isomer)	
42	ethyl caproate	fruity/acid
44	*n*-hexyl acetate	banana/pineapple
45	*n*-hexyl acetate	cooked apple
47	2-hexenyl acetate (isomer)	
48	isopropyl caproate	
49	amyl butyrate	
51	ethyl 2(*E*)-hexenoate	
52	2,5-dimethyl-4-methoxy-3(2*H*)-furanone	caramel-like
55	methyl caprylate	green/fatty
57	benzyl acetate	coriander
60	*n*-hexyl butyrate	
62	ethyl caprilate	
63	decanal	
64	octyl acetate	perfumed
65	amyl caproate	
66	isoamyl caproate	
67	dec-2-enol	
68	2,4-decadienal	
71	methyl decanoate	
72	*n*-hexyl caproate	
73	decyl acetate	
75	nerolidol	green-fruity,
76	γ-dodecalactona	strawberry-like
77	acetaldehyde	
78	propanone	
79	toluene	
80	pent-2(*E*)-enal	
81	isoamyl alcohol	apple jam
82	butyl butyrate	
84	hex-3(*Z*)-en-1-ol	chemical
85	nonanal	green-pungent
87	linalool	floral
89	methyl salicylate	
91	hexanoic acid	fatty
92	β-ionone	nuts/sweet
93	ethyl cinnamate	caramel-like
94	γ-decalactone	sweet/caramel-like
95	cinnamyl acetate	cooked sugar

Table 2. Components Identified for the First Time in Strawberry Aroma by Purge-and-Trap Sampling and High-Resolution GC (*F. ananassa* Variety Oso Grande)

peak	compound	odor perception
1	1,3-butanediol	
13	4-methyl-2-pentanone	
15	methyl 2-butenoate (isomer)	
23	isopropyl isobutyrate	
32	styrene[a]	
34	3-methyl-2-heptanol	parsley
39	ethyl 3-methyl-2(*E*)-butenoate	
40	propyl isovalerate	
41	2-pentylfurane	
43	2,5-dimethyl-3-hydroxy-4-methoxy-2,3-dihydrofurane	
46	cyclohexyl acetate	fruity
50	2-pentyl isobutyrate	
53	nona-2(*E*),4(*E*)-dienal	tissue
54	non-2-en-1-ol (isomer)	cooked milk
56	non-2,6-dienal (isomer)	
58	ethyl benzoate	
59	hex-4-enyl isobutyrate (isomer)	
61	*n*-hexyl isobutyrate	
69	*n*-nonyl acetate	gardenia
70	pulegone	
74	di-*tert*-butyl-4-hydroxytoluene[a]	
83	octanal	chemical/green
88	naphthalene[a]	
90	myrtenal	nuts/sweet

[a] Artifacts originated from the adsorbent.

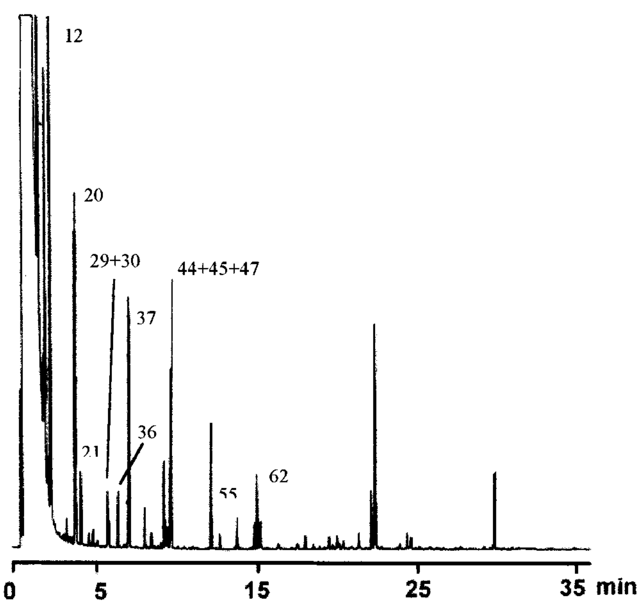

Figure 3. GC profile (10 mV full scale output) of the pentane ether fraction from argented chromatography of an aroma sample of *F. ananassa* cv. Oso Grande on a DB-5 capillary. The *y*-axis indicates response and the *x*-axis, time. Peak numbering is as in Tables 1 and 2. For details see Materials and Methods.

(76). γ-Dodecalatone is present in this fraction in high relative amounts and unequivocally is described as possessing an intense strawberry-like odor. From Table 1 it can be seen that it is the only compound to which the characteristic strawberry odor is ascribed. In the food industry γ-dodecalactone is used as a flavor

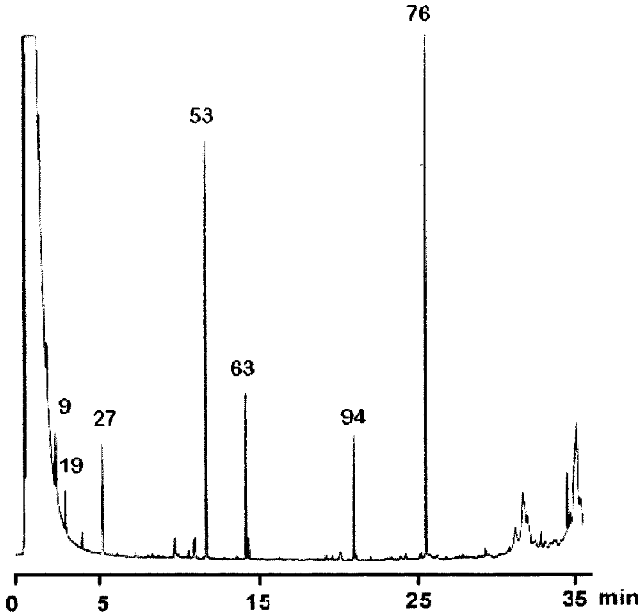

Figure 4. GC profile (10 mV full scale output) of the ethyl ether fraction from argented chromatography of an aroma sample of *F. ananassa* cv. Oso Grande on a DB-5 capillary. The *y*-axis indicates response and the *x*-axis, time. Peak numbering is as in Tables 1 and 2. For details see Materials and Methods.

additive in the manufacturing of strawberry jams (Artho and Grob, 1990).

The compounds in Table 3 were recognized in a previous study (Gomes da Silva and Chaves das Neves, 1997) as the most important set of chemical descriptors in the differential typification of aroma profiles from at least three different varieties of *F. ananassa* in different maturation stages. They are also adequate descriptors for the sensory characterization and differentiation among the strawberry varieties. Figure 5 shows the distribution of different samples of the strawberry varieties Oso Grande (O), Chandler (C), and Selva (S) plotted together with variable loadings in a plane defined by the first and second principal components in a PCA in which those compounds were used as variables. The results show that the variety Oso Grande is strongly associated with floral and fruity odors and the Chandler variety is more on the floral side. The Selva variety, on the other hand, is strongly dominated by chemical and sweet-pungent impressions. These results are in accordance with the fact that Oso Grande is a variety of great popularity in European markets, whereas the production of the Selva variety was discontinued by the producer of our samples due to the lack of commercial acceptance. However, it must be noted that the aroma properties alone are not the only factor that determines public acceptance of a given strawberry crop, other properties being taste, color, size, and form among others. However, these results seem to indicate that unfavorable aroma properties may be associated with a lower commercial value. The results of this work also suggest that analytical control of the aroma may become an important part of quality control procedures because it can be automated by the judicious operation of a recent generation of instrumental "sniffers".

Table 3. Compounds Used as Aroma Variables (Percent Relative Peak Areas) in PCA of Odoriferous Properties of *F. ananassa* Varietes

peak	compound	odor
6	ethyl acetate	chemical
12	methy butyrate	floral/pineapple
14	ethyl isobutyrate	fruity
18	ethyl 2-methylbutyrate	cooked apple
19	*n*-hexanal	green
20	ethyl butyrate	fruity/vanilla
26	ethyl isovalerate	cooked apple
37	methyl caproate	fruity/pineapple
27	hex-2(*E*)-enal	sweet
42	ethyl caproate	fruity/acid
54	non-2-en-1-ol	cooked milk
53	nona-2(*E*),4(*E*)-dienal	tissue

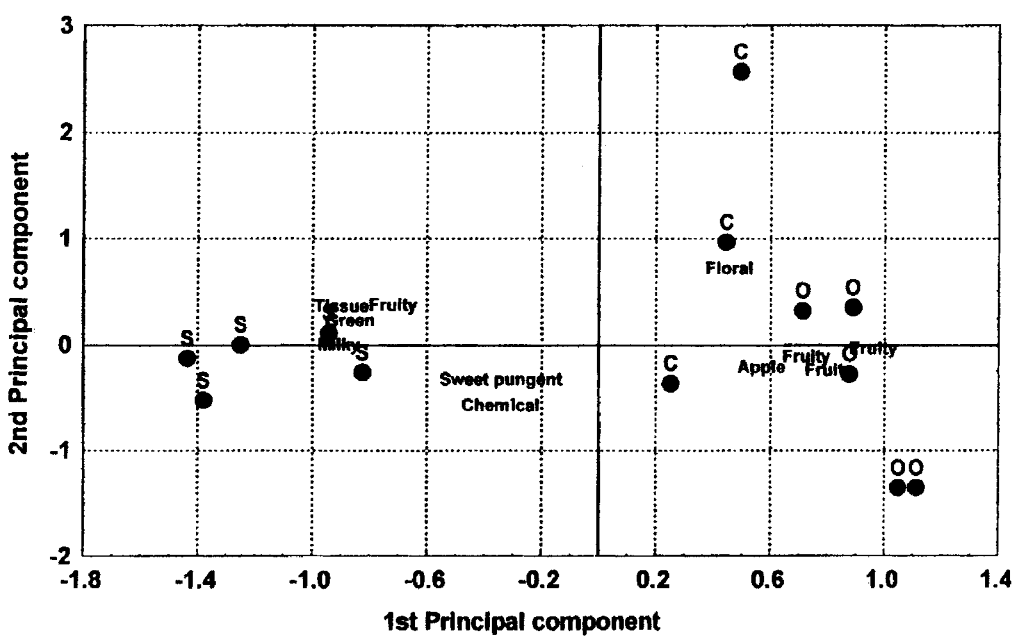

Figure 5. Extracted principal components for samples of *F. ananassa* cv. Selva (S), Oso Grande (O), and Chandler (C) as a function of the variables in Table 3, projected in the planes defined by the first and second principal components. Variable loading is labeled according to the corresponding sensory descriptions.

LITERATURE CITED

Artho, A.; Grob, K. Nachweis der Aromatisierung von Lebensmitteln mit γ-Lactonen. Wie identisch mit der Natur muessen "naturidentische" Aromen sein? *Mitt. Gebiete Lebensm. Hyg.* **1990**, *81*, 544−558.

Aubert-Mammou, C.; Farines, M.; Soulier, J. Application de la RMN à l'identification de constituents minoritaires de l'huile de sisymbre. *Etud. Rech.* **1994**, *4*, 73−78.

Barron, D. HPLC Using diol-bonded silica, an alternative to silica gel in the prefractionation of aroma extracts. *Z. Lebensm. Unters. Forsch.* **1991**, *193*, 454−459.

Brown, R. H.; Purnell, C. J. Collection and analysis of trace organic vapour polluants in ambient atmospheres. The performance of Tenax-GC adsorbent tube. *J. Chromatogr.* **1979**, *178*, 79−90.

Dirinck, P. J.; Scheyen, L.; Schamp, N. Aroma quality of tomatoes, apples and strawberries. *J. Agric. Food Chem.* **1977**, *25*, 759−763.

Dirinck, P. J.; De Pooter, H. L.; Wiliaert, G. A.; Schamp, N. M. Flavor quality of cultivated strawberries: The role of the sulfur compounds. *J. Agric. Food. Chem.* **1981**, *29*, 316−321.

Douillard, C.; Guichard, E. Comparison by multidimensional analysis of concentrations of volatile compounds in fourteen frozen strawberry varieties. *Sci. Aliments* **1989**, *9*, 53−76.

Gomes da Silva, M. D. R.; Chaves das Neves, H. J. Differentiation of strawberry varieties through purge-and-trap HRGC-MS, HRGC−FTIR and principal component analysis. *J. High Resolut. Chromatogr.* **1997**, *20*, 275−283.

Hirvi, T. Mass fragmentographic and sensory analyses in the evaluation of the aroma of some strawberry varieties. *Lebensm. Wiss. Technol.* **1983**, *16*, 157−161.

Hirvi, T.; Honkanen, E. The volatiles of two new strawberry cultivars, "Annelie" and "Alaska Pioneer", obtained by backcrossing of cultivated strawberries with wild strawberries, *Fragaria vesca*, Ruegen and *Fragaria virginiana*. *Z. Lebensm. Unters. Forsch.* **1982**, *175*, 113−116.

McFadden, W. H.; Teranishi, R.; Corse, J.; Black, D. R.; Mon, T. R. Volatiles from strawberries. II. Combined mass spectrometry and gas chromatography on complex mixtures. *J. Chromatogr.* **1965**, *18*, 10−19.

Mosandl, A. Capillary gas chromatography in quality assessment of flavours and fragances. *J. Chromatogr.* **1992**, *624*, 267−292.

Pellizzari, E. D.; Bunch, J. E.; Berkley, R. E.; McRae, J. Collection and analysis of trace organic polluants in ambient atmospheres. The performance of a Tenax GC cartridge sampler for hazardous vapors. *Anal. Lett.* **1976**, *9*, 45−63.

Pérez, A. G.; Rios, J. J.; Sanz, C.; Olias, J. M. Aroma components and free amino acids in strawberry variety Chandler during ripening. *J. Agric. Food Chem.* **1992**, *40*, 2232−2235.

Polesello, S.; Lovati, F.; Rizzolo, A.; Rovida, C. Supercritical fluid extraction as a preparative tool for strawberry aroma analysis. *J. High Resolut. Chromatogr.* **1993**, *16*, 555−559.

Pyysalo, T.; Honkanen, E.; Hirvi, T. Volatiles of wild strawberries, *Fragaria vesca* L., compared to those of cultivated berries, *Fragaria ananassa* cv. Senga Sengana. *J. Agric. Food Chem.* **1979**, *27*, 19−22.

Savenhed, R.; Borén, H.; Grimvall, A. Identification of odorous compounds in water. *J. Chromatogr.* **1985**, *312*, 219−231.

Schreier, P. Quantitative composition of volatile constituents in cultivated strawberries, *Fragaria ananassa* cv. Senga Sengana, Senga Litessa and Senga Gourmella. *J. Sci. Food Agric.* **1980**, *31*, 487−494.

Ulrich, D.; Eunert, S.; Hoberg, E.; Rapp, A. Analysis of strawberry aroma by solid-phase microextraction (SPME). *Dtsch. Lebensm. Rundsch.* **1995**, *1*, 349−351.

Ventura, K.; Dostal, M.; Churdcek, J. Retention characteristics of some volatile compounds on Tenax GR. *J. Chromatogr.* **1993**, *642*, 379−382.

Wilhalm, B.; Palluy, E.; Winter, M. Recherches sur les arômes. Identification des acids volatils et de quelques autres composés. *Helv. Chim. Acta* **1966**, *9−10*, 65−67.

Winter, M.; Wilhalm, B. Recherches sur les arômes. Sur l'arôme des fraises fraîches. Analyse des composés carbonylés, esters et alcohols volatils. *Helv. Chim. Acta* **1964**, *47*, 1215−1227.

Received for review May 14, 1999. Revised manuscript received July 23, 1999. Accepted July 27, 1999.

JF9905121