Title: CHARACTERIZATION OF ODOUR ACTIVE COMPOUNDS IN STRAWBERRY VINEGARS

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Abstract

Odour-active compounds in strawberry vinegars were determined by gas chromatography equipped with an olfactometer (GC-O) using the modified frequency (MF) technique. The initial strawberry substrate was also analysed showing that ethyl 2-methylbutyrate, mesifurane, β-damascenone, furaneol and γ-decalactone were preserved during the double fermentation process, presenting high MF values. The final aromatic profile of strawberry vinegars is formed both by compounds from the substrate and by those formed during alcoholic and acetous fermentation. Due to their high MF, a total of 12 odour zones, identified as acetic, butyric and isovaleric acids, methional, 3-nonen-2-one, 2-phenylethanol, pantolactone+furaneol, p-vinylguaiacol, sotolon, phenylacetic acid and vanillin, were considered as possible impact odorants of strawberry vinegars. Finally, all potential impact odorants with similar sensory descriptors were grouped into 8 categories, these being: fruity, sweet, grassy, spicy, butter-lactic-cheesy, chemical, empyreumatic and miscellaneous. According to the MF percentage of these categories,

grassy, fruity, sweet and spicy aroma seem to have the highest influence on the overall impression of strawberry vinegars.

Keywords: impact odorant, olfactometry, vinegar, strawberry, modified frequency.

Introduction

Because of its worldwide availability and its great array of varieties, vinegar is one of the world's most widespread and common products. In Spain, vinegar is mainly produced by the double fermentation (alcoholic and acetous) of grapes. Present, innovations in vinegar production falls into two areas: improving production processes and employing different raw materials.

Strawberries are prized for both their aroma and flavour. Spain is the world's second largest strawberry producer. Since there are surpluses of second quality strawberry, this makes it an attractive candidate for use as a raw material in producing new kinds of vinegars.

Aroma is certainly one of the most important determinants in food quality and acceptance[1]. Therefore, when introducing a new food product, the characterization of its aroma is an important aspect to be considered. Aroma is determined by a large number of compounds that are involved in different ways. Among the above, odour-active compounds play an important role in perceived aroma and especially, impact odorants, that directly influence in it.

An essential tool when characterizing the olfactory impact of an odorant (its odour descriptor and intensity) is the coupling of Gas Chromatography/Olfactometry (GC/O)[2]. Among the different types of olfactometric analysis, the three main ones are: dilution methods, perceived intensity methods and frequency of detection methods. The frequency of detection has been proved to provide quantitative estimates of the sensory importance of a compound [3] and has even been proved to be accurate enough to provide a real quantitative data[4], but its limited due to the fact that once the concentration becomes higher than the less sensitive member of the panel, the signal becomes saturated[5]. This limitation can be partly overcome by combining the frequency of detection with a simple measurement of intensity, which also has been proved to provide reliable quantitative data[6]. As concentration increases, odour intensity may continue to increase, however the frequency of detection might not increase when all members of the panel are finally able to detect the odorant[5].

The combination of intensity and frequency of detection can be done through the so called modified frequency (MF) which is the geometric mean of the detection frequency of an aromatic zone (expressed as a percentage) and the average intensity (expressed as a percentage of the maximum intensity)[7]. This concept is useful because the discriminative capabilities of the detection frequency may be improved by taking intensity into account [8] and, moreover, because the members of a tasting panel can have widely divergent sensitivities[7]. MF, therefore, provides more reliable results.

The volatile composition of strawberry has been the object of extensive study. The aroma of this fruit is generally a complex mixture of esters, furanones, and terpene alcohols with smaller amounts of lactones, aldehydes, alcohols and sulphur compounds[9-16]. Among the above, furaneol and mesifurane are considered to be the two major flavour contributors due to their low odour threshold and their high quantities[17]. These compounds have been confirmed in several studies on the odour-active compounds in different strawberry varieties. In these works, the authors have identified a considerable number of odour-active compounds and they agree on furaneol, mesifurane and γ-decalactone as being impact odorants in strawberry[18,19].

In this work we have determined odour-active compounds and the possible impact odorants by GC-O in strawberry vinegars obtained through double fermentation.

Moreover, an olfactometric analysis of the starting substrate has been also performed to ascertain whether the key odorants of raw material remain in the final products.

Experimental

Chemicals

Dichloromethane and anhydrous sodium sulphate were purchased from Merck (Darmstadt, Germany), all of them were of analytical quality.

Strawberry vinegar samples

Vinegars analyzed in this study were produced from second quality strawberries of *Camarosa* variety. For that purpose, fruit was crushed and mixed with pectolytic enzymes and sulphur dioxide. Besides, 50 g/L of sucrose were added to ensure an appropriate final acidity in the resulting vinegar. This starting substrate (F8P2) was submitted to two types of alcoholic fermentations, spontaneous and inoculated with the yeast *Saccharomyces cerevisiae* QA23 at a concentration of $2x10^6$ cells/mL. All resulting wines were spontaneously acetified by surface culture. Thus, we obtained two different vinegars: F8VI (from inoculated alcoholic fermentation) and F8VE (from spontaneous alcoholic fermentation).

Sample extraction

The liquid-liquid extraction method was performed to obtain a representative extract of the samples[9]. The procedure followed was: 5 g of anhydrous sodium sulphate was added to 50 mL of each sample and was extracted twice during 5 minutes with 5 mL of dichloromethane using a magnetic stir bar. Then, 2.5 mL of the organic phase was concentrated 5 times under a nitrogen stream.

Gas Chromatography-Olfactometry conditions (GC-O)

Analyses were conducted using a Varian 3800 GC (Middelburg, The Netherlands) equipped with a flame ionization detector (FID) and an OP275 olfactometer (GL

Science Inc., Tokyo, Japan). Two microliters of each extract were injected in splitless mode into a DB-WAX column with 60 m x 0.25 mm x 0.22 μm film thickness (J & W Scientific, Agilent Technologies Inc., Santa Clara, USA). The oven temperature program was as follows: 40° for 1 min, increasing to 220 °C at a rate of 2°C/min and held for 30 min. The column effluent was split 2:3 into a FID and a heated sniffing port. The injector and detector temperature was 220ºC. The carrier gas was H_2 at a flow rate of 1 mL/min. The sensory panel was composed of three trained tasters, all of them sniffed each sample twice, assigning to each perceived odour an intensity level: 1, 2 or 3. Results were expressed as "modified frequency" (MF), calculated with the formula proposed by Dravnieks[20].

Gas Chromatography-Mass Spectrometry (GC-MS) conditions

Analyses were conducted using an Agilent 6890 GC system coupled to an Agilent 5975inert quadrupole mass spectrometer and equipped with a Gerstel MP2 headspace autosampler (Müllheim an der Ruhr, Germany). The analytical column was a CP-Wax 57CB column of 50 m × 0.25 mm and 0.20 μm film thickness (Varian, Middelburg, The Netherlands). The injector port was heated to 220°C and 2 μL of sample extract were injected in splitless mode with a purge flow of 70 mL/min and purge time of 2 minute. The carrier gas was He at a flow rate of 1 mL/min. The oven temperature program was the same employed in GC-O. Quadrupole, source and transfer line temperatures were 150, 230 and 250 °C, respectively. Electron ionization mass spectra data from m/z 29~350 were collected in the scan mode, with an ionization voltage of 70 eV.

Identification of Aroma Compounds

In the GC-MS analysis, volatile compounds were identified based on the comparison of the retention times of individual standard and computer matching with the reference mass spectra from the NIST 98 library.

In the GC-O analysis, compounds were identified by the comparison of their linear retention indices (LRIs) with those obtained in GC-MS analysis of extract and standards as well as with LRIs and odour qualities from Flavornet (www.flavornet.org)[21] and Pherobase (www.pherobase.com)[22] online data bases and literature[9,23-38].

Linear retention indices (LRIs) were calculated on the basis of retention times of n-alkanes (C10–C32) under identical conditions for each instrument.

Statistical analysis

All statistical analyses were performed using Statistica software version 7.0 software package (Statsoft, Tulsa, USA). A principal component analysis (PCA) was carried out as an unsupervised method in order to ascertain the degree of differentiation between samples and which compounds were involved.

Results and Discussion

In this study, we only considered those odour zones which were detected in at least half of the total sniffing trials as odour-active. The odour zones and their corresponding identification are listed in Table 1, some of them were only tentatively identified (TI). Thus, GC-O analyses evidenced 79, 55 and 49 odour-active zones in substrate, F8VI and F8VE vinegars respectively.

In the substrate, the odour zones that presented maxima MF (100) were those which were identified as methyl butyrate (LRI 990, TI), isoamyl alcohol (LRI 1208), *cis*-2-nonenal (LRI 1504, TI), γ-decalactone (LRI 2150) , γ-dodecalactone (LRI 2382) and vanillin (LRI 2564, TI). Some have been previously reported as important components in strawberry aroma. Thus, methyl butyrate is one of the major esters present in this fruit[39,40]. According to Gomes da Silva and Chaves das Neves[41], prominent lactones were γ-decalactone and γ–dodecalactone, giving fruity notes. *Cis*-2-nonenal has been described as a frequent odorant present in different strawberry varieties[42]. On the other hand, the presence of vanillin among the aroma compounds in strawberry has been scarcely observed by other authors[43,44] and until now it had been described as an odour-active compound in one variety only [45].

As expected, furaneol (LRI 2034) and its methoxy derivative[46], mesifurane (LRI 1594), the strawberry components most quoted as character impact compounds, also reached high MF; 91 and 75, respectively.

Within those odour zones which reached high MF, we can highlight those that we have tentatively identified for the first time in strawberry such as 2,6-dimethylpyrazine (LRI 1305), 2-furfurylthiol (LRI 1433), guaiacol (LRI 1881), 6,7-dyhydro-7-hydroxylinalool (LRI 1971), 4-ethylphenol (LRI 2183), sotolon (LRI 2203) and ethyl hexadecanoate (LRI 2270). The first two odorants and sotolon are typical compounds produced by the Maillard reaction[47-49]. The presence of these compounds may be due to the same formation pathway, since strawberry has reducing sugars as well as amino acids.

Moreover, sniffers also perceived some off-odour attributes tentatively identified as volatile phenols with high MF: guaiacol and 4-ethylphenol. Several authors point to these compounds as off-flavours in different food matrices[50,51]. Moreover, they are related to fruit contamination caused by different microorganisms[52,53].

In addition to the above-mentioned compounds, we found other important odour zones with MF higher than 70, providing fruity notes, corresponding to typical strawberry esters (ethyl butyrate (LRI 1029) and ethyl hexanoate (LRI 1234, TI)). Furthermore, we identified linalyl valerate/citronellol (LRI 1766, TI) and nerolidol (LRI 2003) around this MF value, giving grassy and citric aroma characters.

Among the 38 odour zones with a significant contribution to the aroma (MF>70), we were, even tentatively, unable to identify four of them.

The comparison of olfactometric analysis results of substrate and vinegars showed that a total of 29 odorants present in the strawberry puree were detected both in vinegars produced from wines whose substrate had been inoculated and those which had been obtained from spontaneous fermentation. These odorants were, therefore, preserved throughout the double fermentation process. Most of them, however, presented lower MF values in vinegar than in the substrate due to the losses and transformations undergone during the alcoholic and acetous fermentations[54,55]. The aromatic profile of wine vinegars is the result of the permanence of those volatile compounds from the raw material (grape varietals aroma), synthesized by yeast during alcoholic fermentation and those formed during the acetous fermentation. The results showed that after the double fermentation, some characteristic compounds of strawberry[27,56] such as: γ-decalactone, furaneol, mesifurane, β-damascenone (LRI 1821) and ethyl 2-methylbutyrate (LRI 1040) were preserved from the raw material to both vinegars. Surprisingly, moreover, they reached high MF values.

On the other hand, the impact odorants of strawberry, ethyl butyrate and γ-dodecalactone, were only perceived in vinegar produced from wine obtained by inoculation of the substrate while methyl butyrate was perceived only in vinegar from wine obtained by spontaneous fermentation.

These results indicate that the overall odour impression of vinegar would evoke aromatic notes of the raw strawberry material used.

Moreover, we observed the appearance of odour zones, or an increase in the MF scores of other odour zones corresponding to compounds usually formed in acetous fermentation[9,57]. Therefore, aromatic notes such as butter (diacetyl, LRI 970) and pungent (acetic acid, LRI 1405), appeared in the vinegars. The cheese odour, identified as isovaleric acid (LRI 1669), increased its MF (53 in the substrate), reaching the maximum value in both vinegars. Other typical compounds produced in surface acetification are the acetic esters[57], however, they were either not perceived (isobutyl acetate and isoamyl acetate) or were perceived with low MF (2-phenylethanol acetate (LRI 1824) and hydroxycinnamyl acetate (LRI 1919)) in our vinegars. The acetic esters are formed by chemical condensation that occurs slowly, especially during aging of vinegars[58]. Therefore, those vinegars which undergo a long aging process have high levels of these compounds. The vinegars analysed in this work did not have an aging process and therefore, these compounds may indeed have been present in these vinegars, but at imperceptibly low concentrations.

Within the identified compounds that underwent a large MF increase from substrate to vinegars, we found: 3-nonen-2-one (LRI 1508, TI), 2-phenylethanol (LRI 1915), p-vinylguaiacol (LRI 2196, TI), sotolon (TI) and phenylacetic acid (LRI 2556, TI). After the MF increase in the first three compounds, they then became possible impact odorants in the resulting vinegars. Among the aforementioned compounds, sotolon has already been reported as a key odorant in Sherry Vinegar[9].

Another new odour zone that appeared in vinegars, described as “baked potato”, was tentatively identified as methional (LRI: 1450). This compound is produced by *Saccharomyces cerevisiae* yeast in different fermented foods[59]. In our case, therefore, the methional was probably synthesized during alcoholic fermentation and subsequently remained in the vinegar.

It should be observed that there is a relatively high variability in the GC-O profiles between the vinegars, depending on the type of alcoholic fermentation (spontaneous or inoculated). The vinegar produced by inoculation of the substrate has a higher number of odour zones that in general are more intense than those found in the vinegar obtain by spontaneous fermentation. Several of them clearly differentiate these two vinegars, since they were only present in one of the vinegars reaching a high MF. Thus, we can highlight strawberry and liquor, identified as methyl butyrate (TI) and δ-decalactone respectively, only detected in F8VS; and liquorice/curry/spicy (unknown, LRI: 1836), burned plastic (p/m-cresol, LRI 2085, TI) and honey (acetovanillone, LRI 2644, TI) only perceived in F8VI.

If we consider those compounds with a frequency and modified frequency higher than 3 and 70 respectively as possible impact odorants, we found 12 possible impact odorants in our strawberry vinegars: acetic, butyric (LRI 1623) and isovaleric acids, methional, 3-nonen-2-one, 2-phenylethanol, pantolactone+furaneol, p-vinylguaiacol, sotolon, phenylacetic acid and vanillin, some of which have been previously reported as key odorants in Sherry Vinegar[9].

Principal component analysis (PCA) was performed using the potential impact odorants data (F>3 y MF>70) to summarize major differences and highlight potential relationships among the substrate and the vinegars obtained from the wines produced by inoculated and spontaneous alcoholic fermentation. The first two components explain 73.42% and 26.58% of the total variance, respectively (Figure 1). PC1 separated the substrate from vinegars and PC2 the two vinegars. As we can see in Figure 1, the substrate is related to a great number of potential impact odorants. As expected, odour zones which reached maxima MF (100) in the substrate are placed on the right side of the graph (Figure 1b), being more related with the strawberry substrate (Figure 1a). The aromatic zones identified as characteristic aroma compounds of vinegars such as acetic and isovaleric acids among others, appear on the left side of the plot. If we compare both vinegars, we observe that some compounds such as p-vinylguaiacol and δ-decalactone seem to be more related to those vinegars obtained from spontaneous wines and p/m-cresol with those from inoculated ones. In addition, PCA results confirm the influence of the alcoholic fermentation process (spontaneous or inoculated) in the aroma profile of vinegars. Hence, among the impact odorants of strawberry, ethyl butyrate and γ-dodecalactone are located at the bottom of the graph, and thus are closer to the vinegar produced from inoculated alcoholic fermentation. Meanwhile methyl butyrate is located nearer to those vinegars produced by spontaneous process.

Finally, the potential impact odorants of all samples with similar sensory descriptions were grouped into 8 categories on the basis of the character of their aroma: fruity, sweet, grassy, spicy, butter-lactic-cheesy, chemical, empyreumatic and miscellaneous. This last group included odour zones described as tempera, plastic or metallic. Figure 2 shows the contribution of each aroma category as a percentage of the total MF of odour zones that might be potential impact odorants in at least one sample.

In the substrate, the grassy aroma character (30.7% total MF) stood out, followed by fruity, sweet and spicy aroma categories, the remaining aroma groups accounting for approximately 25%. However, in the vinegars, the percentages of the first two categories decreased and increased, principally, the spicy and butter-lactic-cheesy percentages of total MF. For this last aroma group, the result was to be expected since the compounds responsible for this aroma category are the acids typically formed in acetous fermentation. The comparison of the total MF percentages in the vinegars showed that the miscellaneous group, related to negative aromatic nuances, had higher values in F8VI than in F8VS.

Therefore, although aromatic notes that mainly contribute to aroma samples correspond to the grassy category, the sum of aromatic characters – fruity, sweet and spicy – probably results in a major influence in the overall odour impression.

Conclusions

The final aromatic profile of strawberry vinegars was formed by compounds from the substrate and others produced during alcoholic and acetous fermentations. Thus, impact aromatic compounds characteristic of strawberry such as ethyl 2-methylbutyrate, mesifurane, β-damascenone, furaneol and γ-decalactone, were preserved throughout the double fermentation process, presenting high MF values.

The type of alcoholic fermentation (spontaneous or inoculated) had an influence on the number of odour-active compounds. Hence, vinegars from the inoculated process accounted for more odour zones with high MF than the spontaneous process and, moreover, these odour zones had pleasant aromatic nuances.

We can therefore conclude that the MF of odour zones identified as acetic, butyric and isovaleric acids, methional (TI), 3-nonen-2-one (TI), 2-phenylethanol, pantolactone+furaneol, p-vinylguaiacol (TI), sotolon (TI), phenylacetic acid (TI) and vanillin (TI) point to these compounds as possible impact odorants in these strawberry vinegars.

Considering the total MF percentage of the different aroma categories of potential impact odorants, the grassy, fruity, sweet and spicy aroma seem to have the highest influence on the overall odour impression of strawberry vinegars. Vinegars produced from spontaneous wines presented a higher total MF percentage of grassy and butter-lactic-cheese aroma categories.

All of these results indicate that inoculated alcoholic fermentation could provide vinegars with a higher aromatic quality than those produced by spontaneous process.

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Figure Captions

Figure 1. Data scores (MF) (a) and variable loadings (b) plots on the planes made up of the first two principal components (PC1 against PC2). The compound names corresponding to the number of variables are located in Table 1.

Figure 2. Contribution of each aroma category as a percentage of the total MF of odour zones that might be potential impact odorants in at least one sample.

Table 1. Odour active compounds in strawberry substrate and obtained vinegars.

LRI DB-Wax	LRI CP-Wax or/and literature	Odour Descriptor	Odorant[d]	F[a]	FM[b]	F[a]	FM[b]	F[a]	FM[b]	Identification[c]
				Substrate		Inoculated		Spontaneous		
934	-	strawberry acid	unknown	-	-	-	-	3	41	-
963	961[e]	plastic, synthetic	ethyl propionate	-	-	3	41	3	41	MS, OD
970	969[9];970[23,24]	butter	diacetyl	-	-	4	67	4	67	LRI, OD
990	990[21]	strawberry	methyl butyrate[1]	6	100	-	-	6	100	LRI, OD
1012	1012[e]; 1014[25]	medicinal	isobutyl acetate	4	47	-	-	-	-	LRI, MS, OD
1020	1022[21]	fruit, strawberry, pineapple	methyl isovalerate[2]	6	71	-	-	-	-	LRI, OD
1029	1029[e];1032[24]	banana, strawberry, soap	ethyl butyrate[3]	5	75	5	53	-	-	LRI, MS, OD
1040	1040[e],1041[26]	banana, fruit	ethyl 2-methylbutyrate[4]	6	82	6	58	6	82	LRI, MS, OD
1055	1055[e],1056[26]	fruit, strawberry, banana	ethyl isovalerate	4	67	3	41	3	41	LRI, MS, OD
1066	1067[22];1069[27]	metallic, rubber, sweat	hexanal[5]	5	75	-	-	-	-	LRI
1069	-	toasted bread, coffee	unknown[6]	5	75	-	-	-	-	-
1083	1083[e],1084[9,28]	rubber, sweat, latex	isobutanol	4	67	-	-	-	-	LRI, MS, OD
1105	1105[e]	banana	isoamyl acetate	3	41	-	-	-	-	MS, OD
1144	1146[e],1145[21] /1145[21]	boiled potato, vegetable, grass	1-butanol/myrcene[7]	6	82	3	58	3	41	LRI, MS, OD/ LRI, OD
1166	1169[29]	green, plastic, rubber,	limonene	3	41	-	-	-	-	LRI, OD
1183	1181[30]/1185[25]	sweet, medicinal, aspirin	methyl hexanoate/ethyl 3-methylpentanoate	4	47	-	-	-	-	LRI, OD
1208	1205,1208[21]; 1212[e]	rancid, rubber, chemical	isoamyl alcohols(2-methyl-1-butanol+3-methyl-1-butanol)[8]	6	100	4	47	6	58	LRI, MS, OD
1220	1220[21]	plastic	2-hexenal	4	67	-	-	-	-	LRI, OD
1224	-	chicken, cooked, synthetic, vegetable	unknown	-	-	3	41	-	-	-

1234	1230[e],1240[30]	strawberry, blackberry, citrus, violet	ethyl hexanoate[9]	5	75	-	-	-	-	LRI, MS, OD
1247	-	vegetable, plastic, flower	unknown	3	41	-	-	-	-	-
1264	1269[31]	boiled potato, boiled vegetable	α-terpinolene	4	58	-	-	-	-	LRI,OD
1289	-	metallic, paint	unknown	-	-	-	-	3	41	-
1302	1303[25];1306[24]	plastic, cooked vegetable, metallic	1-octen-3-one	5	53	-	-	-	-	LRI, OD
1305	1308[21]	toasted corn, boiled potato	1-octen-3-one+2,6-dimethylpyrazine[10]	5	75	4	47	5	53	LRI, OD/ LRI, OD
1317	1315[25]	barbecue	2-methyl-3-furanthiol	-	-	3	58	5	53	LRI, OD
1327	1331[33]	toasted, chicken soup, dairy product, plastic	2-acetyl-1-pyrrolidone	-	-	-	-	6	58	LRI, OD
1347	1345[21]/1345[21]	vegetable, grass	2-octenal/3-nonenal	3	41	-	-	-	-	LRI, OD
1350	1350[28]; 1355[34]; 1357[e]	plastic	1-hexanol	3	58	-	-	-	-	LRI, MS, OD
1373	1368[24];1377[34]/ 1377[21]	green leaf , fish	dimethyl trisulfide/*trans*-2-hexenol[11]	6	71	-	-	-	-	LRI, OD/LRI, OD
1391	-	baked potato, vegetable	unknown	3	58	-	-	-	-	-
1396	1394[25, 30]; 1396[e]	baked potato, mushroom	*cis*-3-hexenol[12]	5	75	3	41	3	41	LRI, MS, OD
1405	1405[e]	pungent	acetic acid[13]	-	-	6	82	6	82	MS, OD
1419	1424[25]	anise, sweet, plastic doll	ethyl cyclohexanoate	5	53	-	-	-	-	LRI, OD
1433	1436[25]	toast, coffee	2-furfurylthiol[14]	6	82	-	-	-	-	LRI, OD
1435	1432[e];1436[21]	fruit, strawberry, lemon	ethyl octanoate	3	58	3	41	-	-	LRI, MS, OD
1450	1449[35]/1452[25]	baked potato	methional[15]	-	-	6	100	4	82	LRI, OD
1463	1568	strawberry, banana, vanilla, sweet	benzaldehyde	-	-	3	41	-	-	MS, OD
1500	-	toast, burned, hot iron	unknown	3	41	-	-	-	-	-
1502	-	vegetable, baked potato, orange	unknown	3	58	-	-	-	-	-
1504	1502[35]	toasted, vegetable, river water	*cis*-2-nonenal[16]	6	100	4	47	3	41	LRI, OD
1508	1511[36]	river water, vapour	3-nonen-2-one[17]	4	67	5	91	5	91	LRI, OD
1535	1534[e]	plastic, rancid	2-(methylthio)ethanol	3	58	-	-	-	-	MS, OD
1560	1560[30]	grass, boiled green beans, flower	linalool	4	67	-	-	-	-	-
1563	1563[21]; 1565[e]	cheese, vomit	isobutyric acid	-	-	-	-	4	58	LRI, MS,OD

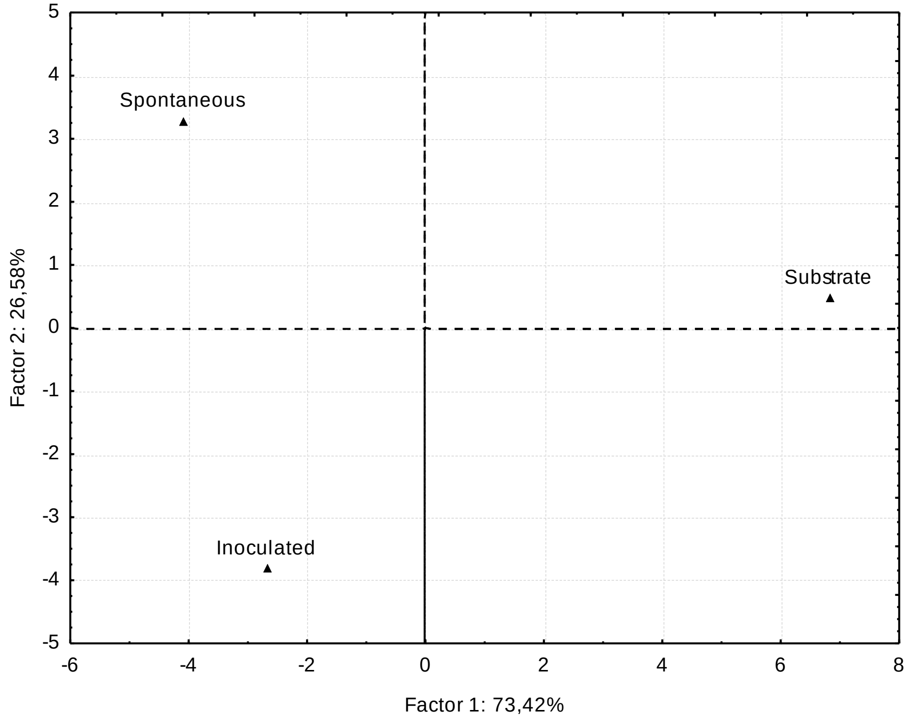
1581	1575[21]	violet, flower, vegetable, soap	E,Z-2,6-nonadienal[18]	6	82	4	75	3	71	LRI, OD
1594	1584[22]; 1592[e]	caramel, sweet, cotton candy	mesifurane[19]	5	75	3	41	6	71	LRI, MS, OD
1611	-	river water, rancid	unknown	3	58	-	-	-	-	-
1616	-	cut grass, river water	unknown	3	50	-	-	-	-	-
1619	1621[25]	toasted, peanut, rancid	2-acetylpyrazine	3	58	-	-	-	-	LRI, OD
1623	1623[e]; 1632[35]	cheese, vomit	butyric acid	6	82	6	82	6	82	LRI, MS, OD
1648	1645[21];1647[e]	river water, fruit compote, sweet, plastic	acetophenone	3	41	-	-	3	41	LRI, MS, OD
1669	1665[21]	cheese	isovaleric acid[20]	5	53	6	100	6	100	LRI, OD
1692	1692[e]	fruit, flower, plastic, vapour	γ-hexalactone	3	41	-	-	-	-	MS, OD
1700	1702[e]; 1711[22]	cut grass, soap, plastic, flower	α-terpineol	4	67	4	47	-	-	LRI, MS, OD
1715	1718[e];1706[37]	plastic doll, anise	benzyl acetate[21]	5	75	-	-	-	-	LRI, MS, OD
1721	1720[21]	mint, lemon, vegetable	carvone	5	65	5	53	3	58	LRI, OD
1731	1731[e];1738[38]	plastic, river water	methionol	3	58	4	67	-	-	LRI, MS, OD
1740	-	plastic, bitumen, pneumatic	unknown	4	67	-	-	4	58	-
1766	1765[21]/1762[21]; 1769[34]	rancid, flower, citric, fresh	linalyl valerate/citronellol[22]	5	75	-	-	3	41	LRI, OD/ LRI, OD
1785	1784[21]	mint, plastic	ethyl salicylate	3	58	-	-	-	-	LRI, OD
1810	-	plastic, peanut, barbecue	unknown[23]	3	58	5	75	4	67	-
1821	1818[25]	fruit preserve, quince compote, roast apple	β-damascenone[24]	6	82	5	75	3	58	LRI, OD
1824	1823[e];1829[21]	mint, flower, jasmine	2-phenylethanol acetate	-	-	4	58	-	-	LRI, MS, OD
1836		licorice, curry, spicy	unknown[25]	-	-	6	82	-	-	-
1844	1838[e];1842[21]	plastic, sweat, dung, rancid	ethyl dodecanoate[26]	5	75	-	-	-	-	LRI, MS, OD
1861	1864[25]	river water, olive, clove, barbecue	guaiacol[27]	6	82	4	67	4	67	LRI, OD
1877	1880[e]	boiled potato, metallic, mint, violet	benzyl alcohol	4	58	-	-	4	58	MS, OD
1915	1916[25]	rose, hyacinth	2-phenylethanol[28]	5	53	6	71	5	75	LRI, MS, OD
1919	1920[e]	coconut, sweet	hydroxycinnamyl acetate	-	-	3	41	-	-	MS, OD
1926	-	cut grass, lima beans, gasoline	unknown [29]	5	75	-	-	-	-	-
1941	-	chamomile, urine	unknown	-	-	4	67	-	-	-
1957	-	lemon, baked potato	unknown	3	41	-	-	-	-	-

1967	1967[34]	ripe fruit, quince compote, roast apple	δ-octalactone	-	-	4	47	-	-	LRI, OD
1971	1972[e]	lemon, washing powder, green beans, grass	6,7-dihydro-7-hydroxylinalool[30]	6	82	-	-	-	-	MS
1988	-	caramel, metallic, sweet, honey	unknown	3	41	-	-	4	58	-
2003	2009[21]/2006[27]; 2010[22]	soap, vegetable	*trans*-nerolidol/*cis*-nerolidol[31]	5	75	3	41	-	-	LRI, MS, OD
2034	2034[e];2033[21]/ 2033[33]	cotton candy, caramel, quince	pantolactone+furaneol[32]	6	91	6	100	5	75	LRI, MS, OD
2054	-	sweat, grass, stagnant water	unknown [33]	5	75	-	-	-	-	-
2064	-	green beans, baked potato, rancid oil	unknown[34]	5	75	-	-	-	-	-
2085	2084[35]/2091[25]	tempera, burned plastic	p/m-cresol [35]	-	-	5	75	-	-	LRI, OD
2150	2157[e];2165[38]	fruit, blackberry, peach	γ-decalactone[36]	6	100	6	100	4	67	LRI, MS, OD
2155	-	mentholated	unknown	-	-	3	58	2	47	-
2167	2170[e]; 2159[28]; 2176[25]	clove, sponge cake	eugenol[37]	6	82	3	41	-	-	LRI, MS, OD
2183	2182[35]; 2185[25]	tempera, cucumber	4-ethylphenol[38]	4	75	5	75	3	58	LRI, OD
2196	2198[21]	coconut, clove, toasted	p-vinylguaiacol[39]	4	75	6	82	5	91	LRI, OD
2203	2203[22]; 2204[25]	licorice, curry	sotolon[40]	3	71	6	82	4	82	LRI, OD
2219	2216[22]	liquor	δ-decalactone[41]	-	-	-	-	3	71	LRI, MS, OD
2230	2223[21]/2234[38]	grass, banana, fruit, honey	o-aminoacetophenone	-	-	3	41	3	58	LRI, OD
2241	2247[21]	spice, coconut, flower, roast vegetable	abhexone[42]	4	67	5	75	3	58	LRI, OD
2270	2269[e]	tempera, barbecue, burned plastic	ethyl hexadecanoate[43]	4	75	5	75	-	-	MS, OD
2319	-	mint, lemon, toothpaste, fruit	unknown	4	47	3	58	3	58	-
2382	2384[21]; 2385[e]	apple, apricot, strawberry, sweet, milk	γ –dodecalactone [44]	6	100	4	58	-	-	LRI,MS, OD
2396	-	banana, flower, clove	unknown	4	47	-	-	3	58	-
2437	2426[21]	river water, clove, spicy, barbecue	δ –dodecalactone[45]	4	82	6	58	-	-	LRI, OD
2455	2452[e]	sweet, cinnamon	coumaran	-	-	4	58	-	-	MS, OD
2490	-	plastic, bleach, rancid	unknown	4	67	-	-	-	-	-
2545	-	sweet, coconut	unknown	-	-	4	67	-	-	-
2556	2251[21];2553[e][37]	rose, honey	phenylacetic acid[46]	4	75	6	100	5	91	LRI, OD
2564	2569[22]	vanilla, caramel	vanillin[47]	6	100	5	75	4	75	LRI, OD

2600	2598[21]	honey, vanilla	methyl vanillate	-	-	3	58	-	-	LRI, OD
2636	-	Compote, banana, strawberry	unknown	3	41	-	-	-	-	-
2644	2640[21]	honey	acetovanillone	-	-	3	71	-	-	LRI, OD

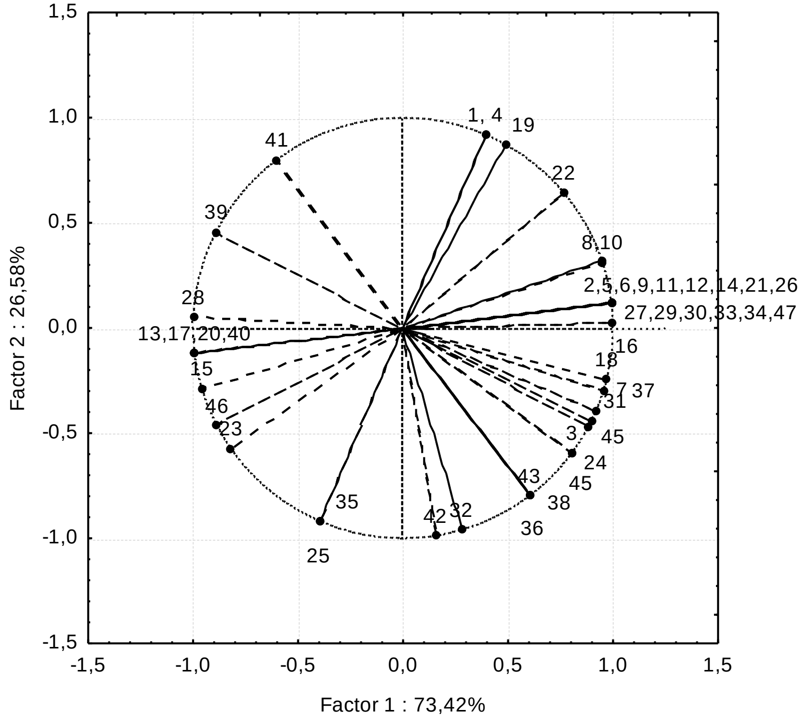
[a]F: Frequency of occurrence
[b]MF: Modified frequency.
[c]LRI: Indentified by Lineal Retention Index; MS: Identified by matching mass spectra of GC-MS analysis of extract with those from the NIST 98 library; OD: Odour descriptor.
[d]Corresponding number of this compound in Figure 1.
[e]LRI in column CP-Wax 57CB of compound identified by GC-MS.

Figure 1a



5
4
3
2
1
0
-1
-2
-3
-4
-5
Factor 2: 26,58%
Spontaneous
Substrate
Inoculated
-6
-4
-2
0
2
4
6
8
Factor 1: 73,42%

Figure 1b



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0,0
-0,5
-1,0
-1,5
-1,5
-1,0
-0,5
0,0
0,5
1,0
1,5
Factor 1 : 73,42%
Factor 2 : 26,58%
1, 4
19
41
22
39
8,10
2,5,6,9,11,12,14,21,26
27,29,30,33,34,47
28
13,17,20,40
16
15
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7 37
31
46
23
3
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24
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32
36
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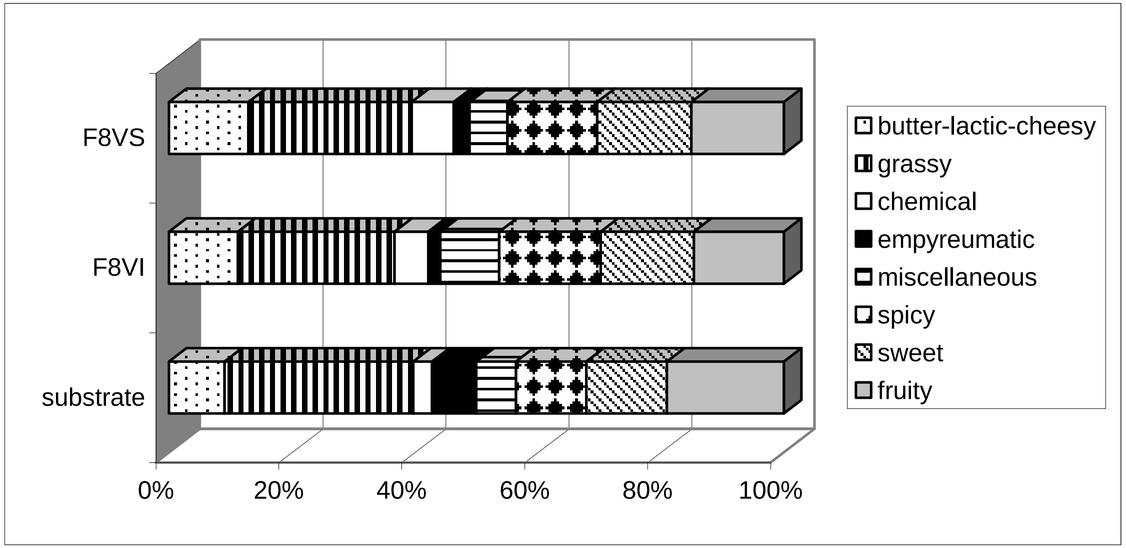
Figure 2


F8VS
F8VI
substrate
0%
20%
40%
60%
80%
100%
butter-lactic-cheesy
grassy
chemical
empyreumatic
miscellaneous
spicy
sweet
fruity