

Gas Chromatography–Mass Spectrometry (GC–MS) Characterization of Volatile Compounds in Quality Vinegars with Protected European Geographical Indication

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The volatile composition of 26 premium quality vinegars belonging to three different protected geographical indications (traditional balsamic vinegar of Modena, balsamic vinegar of Modena, and sherry vinegar) has been characterized by means of a solid-phase extraction (SPE) gas chromatography–mass spectrometry GC–MS method. Among the about 90 quantified compounds, short-chain fatty acids, furanic compounds, enolic derivatives, and some esters were found to discriminate the samples as a consequence of differences in the extent of Maillard reactions, presence of alcoholic fermentation, or duration of wood aging.

KEYWORDS: Traditional balsamic vinegar of Modena; balsamic vinegar of Modena; sherry vinegar; vinegar aroma; GC–MS; SPE; volatile compounds; furanic compounds

INTRODUCTION

In the European Union, the promotion of foods that have been recognized to be traditionally linked to a certain geographical area is based on a legislative system known as “protected denomination” (1). Nowadays, most of the member states possess a number of food specialties with a protected designation of origin (PDO) or a protected geographical indication (PGI), with each of them obtained following approved specifications related to their chemical-sensory features, their production process, and certification.

For what concern vinegars, at the European level, two different PDOs exist: (i) traditional balsamic vinegar of Modena (TBVM) and (ii) traditional balsamic vinegar of Reggio Emilia (TBVRE) (2). Two further specialties are the sherry vinegar (JVs), a product which has its own denomination of origin and has recently been accepted by the European Commission as PDO (3), and balsamic vinegar of Modena (BVM), which is under consideration of the European Commission, with its geographical indication now temporarily protected (4).

TBVM and TBVRE are Italian vinegars coming from the Emilia-Romagna region, differing almost exclusively from the production area and the grape cultivars used to obtain the starting must. They consist of a dark, syrupy sausage, in which the sour notes are well-balanced by a sweet background deriving from the initial unfermented must. Briefly, for TBVM, the process starts with the cooking of the grape must in open boilers to reduce the initial volume of about 30%. During this phase, conditions are propitious for the development of caramelization and Maillard reactions (5) that generate compounds, some of which with a

remarkable aromatic impact. Once cooked, the must is transferred to a cask, where a partial alcoholic fermentation takes place (up to 70 g/L of ethanol). After 5 or 6 months, the acetic fermentation process is promoted and the product is divided into some smaller casks (typically five) made of different kinds of wood (oak, chestnut, mulberry, or juniper) and decreasing volume. During this period, acetic acid bacteria grow on the media surface, where the oxygen concentration is higher. Vinegar oxidation is produced mainly by strains belonging to *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter* species, which oxidize ethanol to acetic acid (6). A long aging period follows during which, because of evaporation, casks are refilled using the method consisting of taking the finished product from the smaller cask (the fifth cask) and refilling it with the vinegar coming from the fourth cask. Then, the fourth cask is subsequently refilled from the third cask and so on until the larger cask (the first), which is filled with fresh cooked must. Only after at least 12 years of aging, the vinegar could be withdrawn to be marketed as TBVM, while for the older “Extravecchio” vinegar, the aging should be at least 25 years.

Balsamic vinegar of Modena (BVM) differs from the above-cited TBVM in the fact that it substantially comes from a wine vinegar, to which a minor portion of cooked must and caramel have been added, and that it has undergone a shorter aging period (from 6 months to 2 years) usually in one single oak cask. Finally, sherry vinegar (JVs) is a Spanish D.O. produced in the Jerez-Xérès-Sherry wine region (Andalucía) and derives from a wine vinegar aged in casks often following the traditional “solera” method, where a volume of older vinegar from the “solera” (placed on the ground) line of casks is bottled. This volume is refilled with younger vinegar from another line of casks and so on until the line of casks with the youngest vinegar, which is refilled

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Table 1. Principal Characteristics of the Studied Samples

vinegar	code	studied samples	raw material	aging type	aging time	caramel addition
traditional balsamic vinegar of Modena "extravecchio"	TBVM-ST	5	cooked must	dynamic	>25 years	no
traditional balsamic vinegar of Modena "affinato"	TBVM-AF	5	cooked must	dynamic	>12 years	no
sherry vinegar	VJ	4	sherry wine	dynamic	>6 months	no
sherry vinegar reserva	VR	6	sherry wine	dynamic	>2 years	no
balsamic vinegar of Modena	BVM	6	wine	static	<3 years	yes

with no aged vinegar. The complete process can take at least 6 months (Vinagre de Jerez, VJ) or 2 years (Reserva, VR). Very recently, a further category of JVs, aged at least 10 years (Gran Reserva), has been introduced (3).

Apart from the different raw material (the grape) and the geographical area, all of these vinegars share some production similarities and, at the same time, differ for specific technological phases that overall could contribute to their organoleptic complexity and diversity. For instance, all of them undergo acetic fermentation and wood aging (even, in some cases, of different duration).

On the other hand, only BVM and JVs come from an alcoholic raw material (the wine), while TBVM is produced from partially fermented musts. Moreover, caramelization and Maillard reactions only occur in the TBVM production process (during the cooking of the must), while the dynamic production method is characteristic of both TBVM and JVs.

Undoubtedly, from a sensorial point of view, one of the most important features of such vinegars, along with taste, is the aromatic impact. The volatile composition of these vinegars has been the subject of previous independent studies (7–12), with most of them carried out by means of the gas chromatography–mass spectrometry (GC–MS) technique. However, because of differences in sampling, sample extraction, and analytical procedures, bibliographic data are difficult to be compared. Although some of the studied compounds have been described previously in this kind of samples, a comprehensive assessment of the composition in volatile compounds of these premium quality vinegars is still lacking.

Solid-phase extraction (SPE) is a well-known extraction technique that can be applied directly to liquid samples to extract volatile compounds. Dependent upon the polymeric phase and solvent employed, a wide range of different compounds can be analyzed. In addition, contrary to other newer techniques, this technique is more economic and easy to use. On the other hand, this technique presents difficulties of automation and a relatively higher consumption of solvent. In a previous work, a SPE method has been developed for the analysis of volatile compounds in traditional balsamic vinegars and other kind of vinegars (13). The aim of the present work is, hence, to carry out an exhaustive characterization of the volatile composition of TBVM, BVM, and JVs by means of an SPE method applied to GC–MS analysis to establish the main volatile compound differences and its later correlation with the technological process applied and the raw material used.

MATERIALS AND METHODS

Samples. A total of 10 TBVM (5 > 12 years old "affinato" and 5 > 25 years old "extravecchio") supplied by different Italian producers, 6 BVM (< 3 years old) purchased from an Italian market, and 10 JVs (4 > 6 months old "vinagre de Jerez" and 6 > 2 years old "vinagre de Jerez Reserva") purchased from a Spanish market were analyzed. The acidity of the samples (expressed in grams of acetic acid/100 g of vinegar) varied from 5 to 7 for TBVM and from 7 to 10 for JVs. The acidity of BVM was 6% for all of the samples. With this limited number of studied vinegars (26 samples), the different types of products were covered. Furthermore,

traditional Italian vinegars were relatively difficult to obtain because of their high value. **Table 1** shows the principal characteristics of the studied samples.

Sample Extraction. Sample extraction was carried out according to Durán Guerrero et al. (13) using a SPE technique. LiChrolut-EN cartridges (200 mg, Merck, Darmstadt, Germany) were conditioned with 4 mL of dichloromethane, followed by 4 mL of methanol and 4 mL of water.

A total of 7 g of sample, diluted 1:4 in Milli-Q water, was deposited at about 1.5 mL/min onto the cartridge. After this and without taking the cartridge to dryness, a process of washing with 5 mL of water was carried out. After the washing process, the cartridge was dried by air passage. The volatile compounds were recovered by elution with 10 mL of dichloromethane. The eluted samples were stored at $-18\text{ }^{\circ}\text{C}$ for at least 24 h to freeze the remaining water. Then, the samples were filtered, and 100 μL of I.S. solution were added to the filtered sample. Finally, a volume of approximately 0.5 mL was attained by evaporation using nitrogen.

GC–MS. A total of 2 μL of the extract were injected in the splitless mode (splitless time of 0.60 min) into a Thermo Finnigan Trace GC ultra gas chromatograph (San Jose, CA), equipped with a Thermo Finnigan Trace DSQ selective mass detector, a Merlin Microseal injector, and a fused silica capillary column Stabilwax (Restek, Bellefonte, PA), 30 m, 0.25 mm inner diameter, and 0.25 μm film thickness, under the following working conditions: GC-grade helium as a carrier gas at flow rate (constant flow, vacuum compensation) of 1.0 mL/min; 62 kPa; column temperature program, 35 $^{\circ}\text{C}$ heated at 3 $^{\circ}\text{C}/\text{min}$ to 100 $^{\circ}\text{C}$ and then heated at 5 $^{\circ}\text{C}/\text{min}$ to 240 $^{\circ}\text{C}$ (held for 10 min). The injection temperature was 250 $^{\circ}\text{C}$. Detection was carried out by electron ionization mass (EI) in the full scan mode, using an ionization energy of 70 eV and transfer line at 220 $^{\circ}\text{C}$. The mass acquisition range was 30–400 amu.

Peak identification was carried out using the NIST 2.0 and Wiley 7.0 libraries by analogy of mass spectra and confirmed by retention times of standards (when available). Furthermore, the retention index (RI) of each tentatively identified compound was compared to the RI found in the literature. Quantitative data from the identified compounds were obtained by measuring the relative quantifying ions peak area in relation to that of I.S. (50 μL of a 514 mg L^{-1} solution of 2-octanol were added to 10 mL of each sample) and building calibration curves for each compound for which the standard was at our disposal. For compounds lacking reference standards (34 compounds), quantification was carried out by using the calibration curves of standards with similar chemical structures, obtained in the TIC mode. These were the following: 3-ethoxy-1-propanol, vanillin, benzaldehyde, butyric acid, decanoic acid, triacetin, maltol, 2-acetyl furan, furoic acid, γ -butyrolactone, and guaiacol.

Statistical Analysis. The whole set of data was submitted to an analysis of variation (ANOVA) analysis followed by a post-hoc comparison test (Tuckey's test) and to a principal component analysis (PCA) using "Statistica 6" software package (StatSoft Italia).

RESULTS AND DISCUSSION

The volatile compounds identified in vinegars are reported in **Table 2**. Although the used method is not suitable to determine major volatile compounds (acetaldehyde, ethanol, methanol, and ethyl acetate), a total of 93 compounds were identified, 57 of which were confirmed by comparing their rT and mass spectra with authentic standards. For the remaining volatiles, identification was accomplished by matching their mass spectra with Nist 2.0 and Wiley 7 libraries and further confirmed by the comparison to linear retention indexes (RIs) found in the literature (7–12). Identified volatiles were grouped in function of the chemical class,

Table 2. Quantification and Identification Criteria for Volatile Compounds Identified in at Least One Vinegar Sample

number	RI ^a	compound	other name	quantification ^b	identification ^c	supplier ^d
1	1142	1-butanol		56	S, MS	Aldrich
2	1208	3-methyl-1-butanol	isoamyl alcohol	55 + 70	S, MS	Merck
3	1240	ethyl hexanoate		88	S, MS	Sigma
4	1246	3-hydroxy-3-methyl-2-butanone		59	S, MS	Sigma
5	1265	dihydro-2-methyl-3(2 <i>H</i>)-furanone		NQ	MS	
6	1279	hexyl acetate		43	S, MS	Aldrich
7	1286	3-hydroxy-2-butanone	acetoin	43 + 45	S, MS	Sigma
8	1348	ethyl lactate		45	S, MS	Sigma
9	1360	4-hydroxy-4-methyl-2-pentanone	diacetone alcohol	59	S, MS	Sigma
10	1379	3-ethoxy-1-propanol		59 + 71	S, MS	Aldrich
11	1389	3-acetiloxy-2-butanone		NQ	MS	
12	1407	tetradecane		85 + 71	S, MS	Aldrich
13	1443	linalool oxide isomer		59	S, MS	Fluka
14	1469	2-furancarboxaldehyde	furfural	95 + 96	S, MS	Sigma
15	1472	linalool oxide isomer		59	S, MS	Fluka
16	1497	butanediol diacetate Isomer		TIC	MS	
17	1507	2-acetylfuran	2-furylmethyl ketone	95 + 110	S, MS	Sigma
18	1521	benzaldehyde		105 + 106	S, MS	Fluka
19	1524	ethyl 3-hydroxybutirate		43	S, MS	Aldrich
20	1534	butanediol diacetate isomer		TIC	MS	
21	1547	propanoic acid		73 + 74	S, MS	Sigma
22	1555	1,2-etanediol diacetate		43 + 86	S, MS	Fluka
23	1567	5-methyl-2-furancarboxaldehyde	5-methyl furfural	109 + 110	S, MS	Sigma
24	1577	isobutyric acid		TIC	MS	
25	1594	δ -valerolactone		TIC	MS	
26	1597	dihydro-4-methyl-2(3 <i>H</i>)-furanone		NQ	MS	
27	1601	2-acetyl-5-methylfuran		TIC	MS	
28	1611	dihydro-2(3 <i>H</i>)-furanone	γ -butirolactone	42 + 86	S, MS	Fluka
29	1617	2-acetoxy-1-propanol		TIC	MS	
30	1627	butanoic acid		60	S, MS	Fluka
31	1654	propanediol diacetate Isomer		TIC	MS	
32	1661	2-furanmethanol	furfuryl alcohol	82 + 97	S, MS	Fluka
33	1667	4-hydroxy-3-pentanoic acid γ -lactone	angelicalactone	98 + 55	S, MS	Aldrich
34	1670	3-ethyl-2-hydroxy-2-cyclopenten-1-one		TIC	MS	
35	1673	isopentanoic acid	isovaleric acid	60	S, MS	Sigma
36	1673	diethyl succinate		101 + 129	S, MS	Sigma
37	1687	γ -hexalactone	caprolactone	85	S, MS	Aldrich
38	1725	benzyl acetate		TIC	MS	
39	1742	propanediol diacetate isomer		TIC	MS	
40	1748	pentanoic acid	valeric acid	60 + 73	S, MS	Aldrich
41	1768	methyl salicilate		120	S, MS	Sigma
42	1774	1-(5-methyl-2-furyl)-2-propanone		NQ	MS	
43	1780	ethyl 2-phenylacetate		164	S, MS	fluka
44	1786	5-valerolactone		TIC	MS	
45	1791	butoxyethoxyethanol		75	S, MS	Aldrich
46	1818	2-phenylethyl acetate		104	S, MS	Aldrich
47	1839	2-hydroxy-3-methyl-2-cyclopenten-1-one	cyclotene	TIC	MS	
48	1870	hexanoic acid	caproic acid	60 + 73	S, MS	Aldrich
49	1875	2-methoxyphenol	guaiacol	109 + 124	S, MS	Sigma
50	1898	<i>trans</i> -4-methyl-5-butyldihydro-2(3 <i>H</i>)-furanone	<i>trans</i> -whiskeylactone	99	S, MS	Aldrich
51	1899	benzyl alcohol		79 + 108	S, MS	Aldrich
52	1903	1,4-butanediol diacetate		TIC	MS	
53	1925	2-phenylethanol	phenylethyl alcohol	91 + 122	S, MS	Fluka
54	1948	5-ethoxymethyl-2-furaldehyde		TIC	MS	
55	1967	<i>trans</i> -4-methyl-5-butyldihydro-2(3 <i>H</i>)-furanone	<i>cis</i> -whiskeylactone	99	S, MS	Fluka
56	1975	3-hydroxy-2-methyl-4 <i>H</i> -pyran-4-one	maltol	126	S, MS	Aldrich
57	1996	2,5-furancarboxaldehyde		TIC	MS	
58	2019	1-(2-furanyl)-2-hydroxyethanone	furylhydroxymethyl ketone	TIC	MS	
59	2027	4-methyl-5,6-dihydropyran-2-one	dehydromevalonic lactone	TIC	MS	
60	2044	1 <i>H</i> -pyrrole-2-carboxaldehyde		TIC	MS	
61	2047	4-ethyl-2-methoxyphenol	<i>p</i> -ethylguaiacol	137 + 152	S, MS	Sigma
62	2051	dihydro-3-hydroxy-4,4-dimethyl- 2(3 <i>H</i>)-furanone	pantalactone	71	S, MS	Fluka
63	2061	3-acetoxypropene	allyl acetate	TIC	MS	
64	2064	diethyl malate		117	S, MS	Sigma
65	2082	5-ethoxydihydro-2(3 <i>H</i>)-furanone	solerone	TIC	MS	
66	2092	octanoic acid		60 + 73	S, MS	Sigma
67	2103	1,2,3-propanetriyl triacetate	triacetin	43	S, MS	Aldrich
68	2110	4-methylphenol		TIC	MS	

Table 2. Continued

number	RI ^a	compound	other name	quantification ^b	identification ^c	supplier ^d
69	2185	2-methoxy-4-(2-propenyl)phenol	eugenol	164	S, MS	Sigma
70	2198	4-ethylphenol		107 + 122	S, MS	Sigma
71	2215	5-acetoxymethyl-2-carboxaldehyde	acetoxymethyl furfural	126	S, MS	Aldrich
72	2244	1,2,3-propanetriol diacetate		TIC	MS	
73	2271	1,2,3-propanetriol monoacetate		TIC	MS	
74	2272	2,6-dimethoxyphenol	siringol	139 + 154	S, MS	Fluka
75	2275	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	ddmp	TIC	MS	
76	2283	decanoic acid		60 + 73	S, MS	Sigma
77	2295	3,5-dihydroxy-2-methyl-4H-pyran-4-one	5-hydroxymaltol	TIC	MS	
78	2328	4-(1-hydroxyethyl)- γ -butanolactone		NQ	MS	
79	2404	ethyl hydrogensuccinate		101	S, MS	Sigma
80	2455	benzoic acid		105 + 122	S, MS	Sigma
81	2467	2-furancarboxylic acid	2-furoic acid	95 + 112	S, MS	Sigma
82	2509	5-hydroxymethyl-2-carboxaldehyde	HMF	97 + 126	S, MS	Sigma
83	2519	5-acetyl-2-furanmethanol		NQ	MS	
84	2567	4-hydroxy-3-methoxybenzaldehyde	vanillin	151 + 152	S, MS	Sigma
85	2574	phenylacetic acid		91 + 136	S, MS	Aldrich
86	2658	4-hydroxy-3-methoxybenzoic acid ethyl ester	ethyl vanillate	196	S, MS	Aldrich
87	2667	1-(4-hydroxy-3-methoxyphenyl)ethanone	acetovanillone	151	S, MS	Aldrich
88	2722	tetradecanoic acid		TIC	MS	
89	2786	4-hydroxy-3-methoxyphenethanol	homovanillyl alcohol	TIC	MS	
90	2827	hexadecanoic acid		73 + 129	S, MS	Sigma
91	2854	4-hydroxy-3,5-dimethoxybenzaldehyde	siringaldehyde	181 + 182	S, MS	Aldrich
92	2864	4-hydroxybenzaldehyde		TIC	MS	
93	2925	4-hydroxyphenyl ethanol	tyrosol	107	S, MS	Sigma

^a Retention index. ^b Mass fragments used for quantification purposes (TIC denotes the use of the peak area as acquired in the TIC mode; NQ stands for not quantified). ^c Identification obtained by means of standard compounds (S) and/or mass spectra (MS). ^d Supplier of the standard compound.

and their amount was compared between samples, also taking into account the starting material and the production process specific for each vinegar.

Overall, the amounts of alcohols found in our samples were substantially in accordance with other works on TBV (7, 8), BVM (8, 9), and JVs (8, 10–12) (Table 3).

If compared to BVM and JVs, a general lower amount of these compounds could be seen in TBVM, likely because of the almost absent alcoholic fermentation during the production phases of this latter. This is particularly evident for 3-methyl-1-butanol and, to a lesser extent, for 2-phenyl ethanol and tyrosol, which typically derive from amino acid metabolism of yeast cells during alcoholic fermentation (14). As already found by Zeppa et al. (7), furthermore, 3-methyl-1-butanol decreases as the aging phase progresses, being the highest in BVM (where the mean aging period is shorter) and the lowest in both the JVs commercial classes. A similar trend was shown for benzyl and homovanillyl alcohols, which were lower in TBVM than in BVM and JVs.

Aldehydes may derive from alcohol oxidation or wood leakage. Significant differences between vinegars were found for benzaldehyde, syringaldehyde, and vanillin, with the latter being the highest in long wood aged TBVM and JVs. Similar amounts of vanillin were found by Callejón et al. (9), who did not consider TBVM in their work. Quite surprisingly, Zeppa et al. (7) did not find vanillin or syringaldehyde in three different batteries of TBVRE, maybe because of the extraction method employed by these authors, which used a different type of cartridge (C18 instead of LiChrolut-EN) and an higher volume of washing water (30 mL) before the elution of volatile compounds.

In vinegars, acids mainly come from alcohol oxidation acted by acetic bacteria. Their presence, hence, is expected to be somehow related to the amount of alcohols in the raw material. On the basis of our data, acids were significantly higher in VJ and VR, while both the TBVM and BVM had the lowest concentration. This finding is particularly evident for short-chain acids (C3–C6),

which were statistically the highest in VR (may be because of the longer acetification process with respect to VJ).

Excluding the nonstudied acetic acid, isovaleric acid represents by far the main acid in vinegars (up to about 80% of the total in JVs), and its concentration in VJ and VR is slightly higher than that reported by Durán Guerrero et al. (12) or other authors (9, 10). In BVM and TBVM, however, our data largely agree with Callejón et al. (9) and Zeppa et al. (7), respectively.

Acetates are formed by esterification between acetic acid and mono- or polyalcohols and are expected to increase according to aging duration (7). However, only slight differences were found between vinegars in the sum of acetates (Table 3). On the contrary, taking into account each single ester, it can be noticed that VR had the highest amount of propanediol and benzyl acetates, while TBVM contained the highest quantities of butanediol diacetate and triacetin. The latter appeared to be characteristic of TBVM, and its amount discriminated the “younger” TBVM “affinato” from the “older” TBVM “extravecchio”. Quantitatively, similar amounts of benzyl and 2-phenylethyl acetates were reported by Natera Marín et al. (11), Durán Guerrero et al. (12), and Callejón et al. (9), in JVs and BVM, respectively. Acetates coming from di- or triols were only reported by Zeppa et al. (7) in TBVM, at lower concentration ranges.

Ethyl esters are another family of compounds already reported in vinegars. Their amount has been significantly correlated with the ethanol content of the raw material (10).

As a sum, in fact, vinegars coming from an alcoholic matrix (BVM, VJ, and VR) had the highest ethyl ester amounts, while both of the TBVMs (affinato and extravecchio) had the lowest. A major part of this difference is due to ethyl hydrogen succinate, which is the main ethyl ester in all of the vinegars. However, significant differences between TBVM and all of the other vinegars were also found for ethyl lactate, diethyl succinate, methyl salicylate, and ethyl-2-phenylacetate. The latter seems to

Table 3. Concentrations ($\mu\text{g}/\text{kg}$) of Different Groups of Volatile Compounds in European Protected Denomination Vinegar Samples^a

compound	TBV affinato			TBV extravacchio			BVM			VJ			VR							
	median	min	max	median	min	max	median	min	max	median	min	max	median	min	max					
Alcohols																				
1-butanol		nd	nd		nd	nd	loq	nd	7.45	loq	nd	1.39	loq	nd	10.65					
3-methyl-1-butanol (mg/kg)	0.53	0.02	16.6	a	0.10	0.00	0.79	a	56.21	25.61	77.46	c	27.01	15.62	37.08	b	25.83	14.73	48.44	b
3-ethoxy-1-propanol	68.5	55.4	391		103	26.4	438		242	141	1084		239	177	307		276	163	1600	
2-acetoxy-1-propanol ^b	30.8	17.6	83.6		53.8	47.7	148		38.6	16.1	59.7		31.4	23.5	39.2		42.2	40.3	86.8	
butoxyethoxyethanol	141	72.4	276	b	120	18.8	373	b	29.1	22.1	47.3	a	25.9	21.1	46.5	a	41.3	20.0	93.0	ab
benzyl alcohol	177	81.6	292	a	342	231	510	a	576	443	1106	b	656	524	849	b	1079	848	1532	c
2-phenylethanol (mg/kg)	8.65	3.55	9.89	a	14.12	8.14	35.3	ab	18.2	13.3	28.7	ab	17.9	14.3	21.2	ab	21.8	15.9	29.8	b
homovanillyl alcohol ^c	75.9	41.4	80.6	a	87.4	84.2	110	a	138	90.2	232	ab	345	286	486	b	253	112	830	b
tyrosol (mg/kg)	8.17	5.71	16.9	a	18.0	12.0	32.9	a	11.6	7.19	27.1	ab	45.2	25.0	54.7	b	30.2	12.2	85.2	b
sum alcohols (mg/kg)	18.4	13.2	26.0	a	30.8	23.7	70.2	a	88.1	48.1	132	b	90.6	65.3	107	b	90.8	44.0	145	b
Aldehydes																				
benzaldehyde	9.47	4.82	11.2	a	8.87	4.86	15.3	a	15.8	9.77	23.4	a	61.2	17.5	110	ab	71.5	63.4	198	b
vanillin	967	399	1161	b	1476	1248	4810	b	192	43.0	625	a	1039	700	3317	b	1537	1165	2821	b
siringaldehyde	887	233	1982	ab	1568	1440	6313	ab	185	nd	792	a	3133	2984	8849	b	3409	1585	6684	b
4-hydroxybenzaldehyde ^d	45.1	20.9	80.5		82.7	54.2	119		18.5	9.88	32.3		55.8	48.1	292		89.4	64.2	226	
sum aldehydes (mg/kg)	1.94	0.70	3.05	ab	3.13	2.82	11.2	b	0.42	0.06	1.44	a	4.25	3.83	12.6	b	5.23	3.03	9.80	b
Acids																				
propanoic acid	502	128	837	a	397	309	636	a	2143	1283	4107	b	3164	2028	4296	bc	4573	3425	6337	c
isobutyric acid ^e	600	272	1606	a	737	638	1748	a	1358	816	2442	a	2775	2243	3105	b	3749	3560	4775	c
butyric acid	196	67.2	353	a	200	129	489	a	724	535	2372	a	1400	1264	4263	ab	3459	1593	8069	c
isovaleric acid (mg/kg)	8.42	0.98	35.5	a	16.0	4.01	24.9	a	17.4	11.0	36.7	a	110	75.2	154	b	151	139	218	c
pentanoic acid	24.7	4.50	54.3	a	31.5	26.1	77.0	a	74.4	44.6	142	a	132	115	161	ab	367	188	1156	b
hexanoic acid	350	117	990	a	561	307	988	a	1522	862	2452	b	2336	1871	2768	b	3255	2766	3966	c
octanoic acid	63.1	loq	382	a	210	101	1197	a	1325	518	2434	b	1748	1258	2745	b	2065	1577	2420	b
decanoic acid	loq	loq	10.3	a	10.7	loq	136	a	79.3	7.26	129	a	156	56.7	409	ab	165	97.4	386	b
tetradecanoic acid ^f	52.6	6.48	77.9		67.0	51.6	265		58.4	15.1	133		127	86.0	217		98.4	30.6	166	
hexadecanoic acid	395	85.9	669		547	286	1497		492	227	922		1835	871	10886		715	173	1890	
phenylacetic acid	2506	955	2713	ab	3537	2795	5332	ab	913	583	2999	a	3897	3172	8627	bc	6381	3607	8733	c
benzoic acid	1757	479	3646	a	4673	1650	5411	a	476	246	1183	a	3311	2376	11795	ab	8804	5530	13642	b
sum acids (mg/kg)	15.4	8.13	44.2	a	30.5	13.6	33.4	a	27.7	16.3	52.3	a	134	94.3	192	b	195	168	260	b
Acetates																				
hexyl acetate	6.87	loq	33.65		2.25	loq	25.12		16.01	9.21	109.94		25.97	12.35	76.38		50.37	19.34	75.13	
butanediol diacetate isomer ^g	loq	loq	36.6		2.73	nd	69.5		nd	nd	nd		nd	nd	nd		nd	nd	3.55	
butanediol diacetate isomer ^g	loq	loq	72.5		22.7	4.16	149		nd	nd	nd		nd	nd	nd		8.38	loq	31.6	
1,2-etanediol diacetate	loq	loq	5.75		loq	nd	33.5		nd	nd	nd		nd	nd	nd		nd	nd	nd	
propanediol diacetate isomer ^g	76.6	30.6	368	a	341	229	458	a	574	103	799	a	368	243	431	a	995	582	1589	b
benzyl acetate	6.86	loq	16.3	a	11.7	8.94	31.4	a	18.9	6.63	29.5	a	22.5	16.3	28.7	a	59.3	46.3	68.8	b
propanediol diacetate isomer ^g	349	158	1673	a	811	462	1160	a	3291	1139	4766	b	2033	1761	2270	ab	3232	2349	6335	b
2-phenylethyl acetate	693	279	2189		1599	746	4999		1812	1169	2241		1642	1123	1892		2364	2111	5086	
1,4-butanediol diacetate ^g	40.6	30.8	67.0	ab	136	111	231	b	31.3	11.1	80.4	a	29.4	19.0	67.6	a	62.4	32.3	87.6	a
allyl acetate	27.8	9.21	63.1	ab	63.2	61.2	187	b	loq	nd	19.1	a	3.93	loq	12.7	ab	18.2	2.49	61.9	ab
triacetin	15.2	loq	84.8	b	112	79.4	351	c	nd	nd	nd	a	nd	nd	nd	a	loq	nd	0.03	a
1,2,3-propanetriol diacetate ^g	670	485	1891		2465	1232	4867		681	282	1273		882	733	1155		1323	1040	2264	
1,2,3-propanetriol monoacetate ^g	43.1	19.2	169		240	97.2	519		37.5	loq	117		65.8	48.6	88.8		113	75.3	203	
sum acetates (mg/kg)	2.61	1.14	4.83	a	5.97	3.38	12.3	ab	6.82	3.62	6.99	ab	5.08	4.47	5.49	ab	8.14	6.79	13.42	b
Esters																				
ethyl hexanoate	loq	nd	18.2		nd	nd	loq		nd	nd	nd		loq	loq	6.35		6.43	loq	20.7	
ethyl lactate	126	loq	366	a	10.0	1.86	379	a	3209	770	11925	b	1698	979	19427	b	3681	427	8166	b
ethyl 3-hydroxybutyrate	51.9	10.2	368		168	113	661		99.4	89.1	145		147	65.9	168		188	138	283	
diethyl succinate	loq	loq	21.4	a	nd	nd	loq	a	635	100	1915	b	823	181	1366	b	194	61.6	689	ab
ethyl 2-phenylacetate	2.09	loq	24.0	a	loq	loq	29.2	a	3.82	loq	34.4	a	88.3	63.5	236	b	207	108	368	b
methyl salicylate	5.02	1.34	15.7	a	5.06	1.41	30.5	a	20.4	14.3	67.2	ab	32.8	19.6	37.7	ab	57.7	27.8	76.8	b
diethyl malate	nd	nd	nd		loq	loq	249		nd	nd	nd		loq	loq	78.5		nd	nd	nd	
ethyl hydrogensuccinate (mg/kg)	0.14	loq	6.94	a	loq	loq	18.6	a	85.7	42.5	193	b	145	60.0	182	b	99.0	16.1	131	b
ethyl vanillate	16.5	3.57	28.8		37.2	19.9	119		56.0	1.39	130		117	96.4	147		96.6	47.0	228	
sum esters (mg/kg)	0.71	0.04	7.02	a	0.33	0.15	20.0	a	90.8	50.9	199	b	157	60.6	185	b	103	16	140	b

Table 3. Continued

compound	TBV affinato			TBV extravecchio			BVM			VJ			VR							
	median	min	max	median	min	max	median	min	max	median	min	max	median	min	max					
Enolic Derivatives																				
3-ethyl-2-hydroxy-2-cyclopenten-1-one ^h	21.6	15.3	97.3	ab	42.3	21.1	126	b	6.7	nd	9.6	a	nd	nd	nd	a	nd	nd	nd	a
cyclotene ^h	198	100	269	bc	300	249	457	c	181	loq	514	b	loq	loq	76.9	a	6.86	loq	36.5	a
maltol	462	317	611	ab	1003	791	1417	b	111	73.8	275	a	83.9	45.4	123	a	178	99.1	311	a
5-hydroxymaltol ^h	585	174	1989		1003	743	2654		360	169	1533		96.6	20.9	1025		234	175	2050	
sum enolic derivatives (mg/kg)	1.36	0.69	2.72	ab	2.55	2.19	3.85	b	0.61	0.25	1.96	a	0.18	0.07	1.22	a	0.40	0.31	2.35	a
Furanic and Pyranic Derivatives																				
5-methyl-2-furaldehyde	4436	2499	5224	bc	3894	2150	6664	c	1229	716	3081	ab	loq	loq	962	a	51.6	loq	487	a
furfural (mg/kg)	75.2	40.0	76.7	b	54.3	38.5	90.2	b	9.12	2.74	41.7	a	1.82	1.38	5.88	a	5.12	3.66	21.6	a
2-acetylfuran	802	396	1790	ab	767	428	2422	b	631	238	842	ab	60.1	13.4	254	a	164	110	519	a
2-acetyl-5-methylfuran ⁱ	loq	loq	1092		33.8	12.1	246		loq	nd	13.1		nd	nd	nd		nd	nd	3.38	
2-furanmethanol	118	94.4	149	ab	147	122	248	ab	264	90.8	713	b	44.3	7.78	88.2	a	1.65	1.65	198	a
5-ethoxymethyl-2-furaldehyde ^j	loq	nd	122	ab	153	loq	2647	b	loq	loq	165	a	nd	nd	nd	a	nd	nd	nd	a
2,5-furandicarboxaldehyde ^j	1778	680	2439	c	1934	1404	3586	c	227	153	680	b	nd	nd	nd	a	nd	nd	nd	a
furylhydroxymethyl ketone ⁱ	2544	loq	6362	ab	4178	2406	9504	b	1888	loq	7985	ab	nd	nd	nd	a	nd	nd	nd	a
1H-pyrrole-2-carboxaldehyde ^j	283	155	518	b	433	180	567	b	351	216	555	b	nd	nd	nd	a	nd	nd	nd	a
5-acetoxymethyl-2-furaldehyde (mg/kg)	89.6	54.8	97.4	b	215	103	271	b	loq	nd	9.29	a	nd	nd	nd	a	nd	nd	nd	a
ddmp ^h	273	125	292	b	298	131	331	b	392	259	1052	b	10.5	10.5	43.2	a	10.5	10.5	39.1	a
2-furanicarboxylic acid	1167	901	2339	ab	3854	2889	4679	b	858	262	2328	a	1504	1228	2154	ab	2112	1766	2907	ab
5-hydroxymethyl-2-furaldehyde (mg/kg)	1614	728	2308	b	2055	1456	2782	b	646	427	931	ab	11.5	4.46	51.8	a	12.9	0.69	46.3	a
sum furanic compounds (mg/kg)	1773	886	2466	c	2388	1689	3117	c	659	430	1001	b	15.1	7.51	60.2	a	19.9	6.86	70.9	a
Ketones																				
acetovanillone	102	63	173		267	161	431		124	107	157		263	247	607		336	180	765	
3-hydroxy-3-methyl-2-butanone	19.8	10.3	28.0		18.0	12.0	32.2		38.3	19.6	186		30.8	25.0	91.1		75.3	26.6	270	
3-hydroxy-2-butanone (acetoin)	322	loq	1702	a	102	loq	623	a	5960	1786	14997	b	6992	1789	11042	b	5039	2399	9654	b
4-hydroxy-4-methyl-2-pentanone	241	38	522		217	196	304		198	66	335		181	167	249		244	130	527	
sum ketones (mg/kg)	0.77	0.43	2.06	a	0.66	0.59	1.25	a	6.42	1.98	15.6	b	7.66	2.32	11.5	b	6.150	2.972	10.00	b
Lactones																				
5-valerolactone ^k	43.7	34.4	61.7	ab	70.2	41.5	99.3	b	23.0	16.9	34.7	a	18.4	13.8	32.7	a	33.3	18.5	76.7	ab
caprolactone	14.9	4.72	33.0	b	54.8	29.2	172	c	14.9	loq	26.6	a	18.1	7.41	31.6	b	64.1	30.6	99.3	c
trans-whiskeylactone	loq	loq	7.12	a	2.23	loq	428	a	1.22	loq	12.6	a	139	124	167	b	115	22.6	157	b
cis-whiskeylactone	17.0	7.04	43.0	ab	46.7	35.1	551	b	loq	loq	0.1	a	22.3	6.88	26.5	ab	27.2	loq	33.5	ab
dehydromevalonic lactone ^k	412	291	1033	ab	1588	1356	3406	b	181	97.8	367	a	193	109	382	a	470	320	828	a
pantalactone	1461	420	1911		2569	2454	2895		1189	637	2433		592	397	1418		1122	740	2297	
solerone ^k	234	105	286	ab	403	305	622	b	101	80.6	178	a	97.7	65.0	155	a	122	91.0	171	a
δ-valerolactone ^k	127	85.5	257		268	168	711		63.4	45.4	463		62.8	60.6	76.1		107	79.4	234	
γ-butyrolactone	713	422	858		1368	946	2325		1086	569	2043		666	269	713		616	462	1188	
angelicalactone ^k	567	391	635	bc	924	702	1377	c	612	89.2	1100	bc	160	64.3	424	ab	69.1	4.7	107	a
sum lactones (mg/kg)	4.50	2.91	5.22	a	8.35	7.67	12.8	b	4.49	3.13	7.27	a		3.37	3.18	4.04	4.29	6.50	a	
Phenols																				
siringol	22.5	19.5	26.5		25.7	24.8	30.4		24.6	22.6	27.6		20.9	18.3	21.5		18.8	18.0	24.7	
guaiacol	6.1	3.2	6.5		6.8	5.7	12.43		7.50	4.86	9.85		6.37	5.47	9.38		9.84	6.31	14.1	
p-ethylguaiacol	8.31	loq	22.8	a	8.93	3.82	37.1	a	92.6	30.8	137	b	149	75.2	237	b	141	105	176	b
4-methylphenol ^l	12.0	8.55	14.6		21.5	15.1	38.6		5.53	5.02	7.34		12.9	11.0	21.0		14.5	11.7	82.0	
eugenol	5.02	2.67	8.13		8.09	2.41	23.5		2.67	1.44	3.70		6.49	3.77	7.22		6.30	5.17	7.40	
4-ethylphenol	47.1	38.9	89.7	a	89.0	57.4	206.3	ab	190	17.0	283	b	237	143	249	b	199	142	249	b
sum phenols	105	82.3	160	a	162	127	320	ab	321	116	452	ab	430	266	542	b	401	318	471	b
Terpenes																				
linalool oxide isomer 1	5.35	loq	7.49	a	7.20	loq	21.0	a	35.1	12.7	50.3	b	12.8	10.9	18.9	a	28.3	25.1	40.5	ab
linalool oxide isomer 2	4.90	loq	8.60	a	5.98	1.89	9.54	a	18.1	6.45	23.4	b	7.56	5.09	9.30	a	19.1	14.3	22.3	b
sum terpenes	10.01	loq	17.21	a	13.18	2.01	30.5	a	55.3	19.2	73.7	b	20.4	16.2	28.3	a	47.4	39.4	62.9	b

^a In the same row, different letters indicate significant difference ($p < 0.01$). Median, median value; min, minimum value; max, maximum value; nd, below the detection limit; loq, below the quantification limit. Flagged compounds are expressed as follows. ^b 3-Ethoxy-1-propanol. ^c Vanillin. ^d Benzaldehyde. ^e Butyric acid. ^f Decanoic acid. ^g Triacetin. ^h Maltol. ⁱ 2-Acetyl furan. ^j Furoic acid. ^k γ-Butyrolactone. ^l Guaiacol.

be characteristic of Spanish vinegars (Table 3). Our data confirm the finding of other authors (15), who, in a stepwise linear discriminant analysis (SLDA) study aimed at differentiating the

aroma of balsamic, Jerez, wine, and cider vinegars, reported that ethyl-2-phenylacetate and methyl salicylate are among the most discriminating variables. In quantitative terms, again, our data

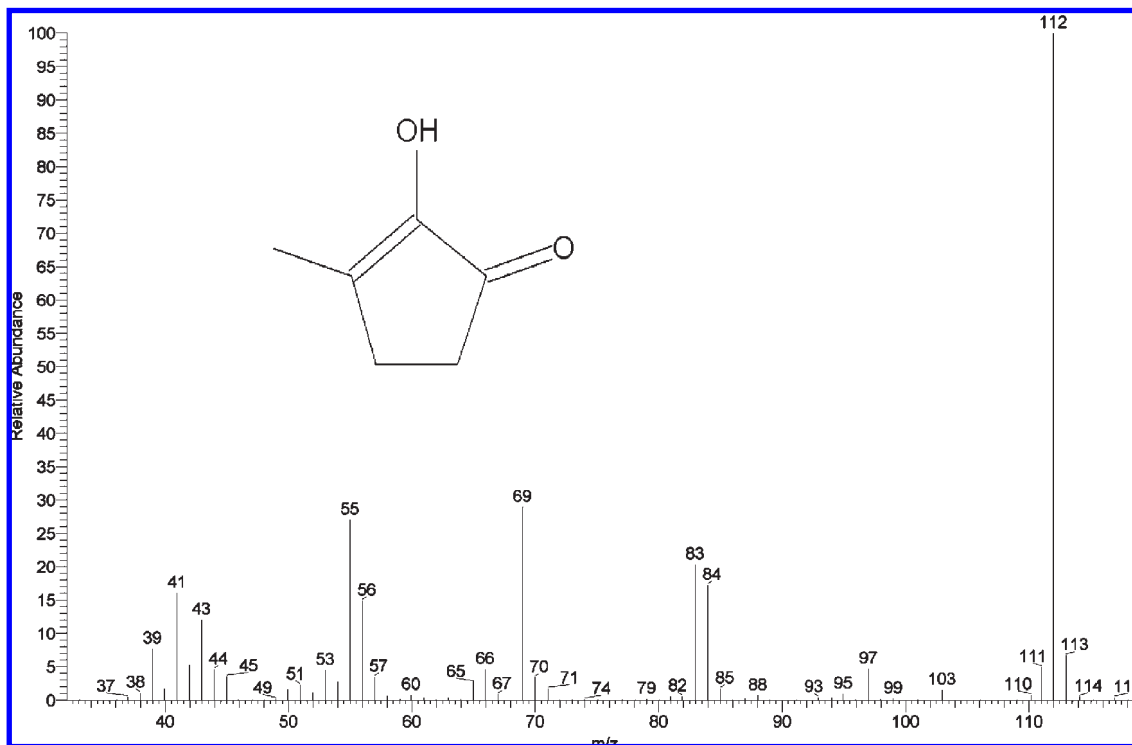


Figure 1. MS spectrum and formula of cyclotene.

agree with previous reports (7, 9–12) for TBVM, BVM, and JVs, respectively, and confirm that those esters strongly related to alcoholic fermentation, such as diethyl succinate and ethylmalate, are almost exclusively present in BVM, VJ, and VR.

It has been suggested that alkyl-substituted 5- or 6-membered cyclic α -diketones, such as maltol and related compounds, exhibit a typical burnt sugar, sweet maple flavor and that this feature could be due to their stable keto–enolic form (16, 17). These compounds typically arise from hexose thermal or acid-catalyzed degradation (17), and some of them have been already reported in foods or beverages, such as coffee (18) or sherry wines (19). Furthermore, their presence was also noticed in charred woods used in wine and vinegar aging (20) as a consequence of pyrolytic degradation of cellulose. For what concern vinegars, Zeppa et al. (7) reported the presence of some enolic derivatives (maltol, cyclotene, and 5-hydroxymaltol) in TBVRE, while a furanone with an enol-carbonyl structure (sotolon) was identified in long aged JVs by Callejón et al. (9, 10).

As shown in **Table 3**, our data suggest that TBVM possesses the highest amounts of enolic derivatives in comparison to BVM and VJ. 5-Hydroxymaltol was the main contributor to this class of compounds followed by maltol and cyclotene. To the best of our knowledge, this is the first report on the presence of cyclotene in BVM and JVs. **Figure 1** shows its formula and MS spectrum. It is worth mentioning that, for this latter compound, the odor threshold in water is around 0.3 mg/L (21) and may well-contribute to the aromatic complexity of TBVM. None of our samples revealed the presence of sotolon. This can be explained by the relatively low recovery shown by this compound during the extraction process. This low recovery could come from the extremely high solubility of sotolon in water, and therefore, its amount could be reduced during the washing step of the extraction process.

A further enolic compound (3-ethyl-2-hydroxycyclopenten-2-one) was identified exclusively in Italian balsamic vinegars. This volatile, which could be considered the ethyl homologue of cyclotene, possesses a strong burnt-sugar sweet aromatic impact, with an odor threshold notably lower than this latter

compound (17). The larger amounts of enolic derivatives in TBVM with respect to JVs and BVM may derive from the initial cooking of the must and the prolonged wood aging of this sugar-rich product. On the other side, the weak amounts of 3-ethyl-2-hydroxycyclopenten-2-one in BVM could be due to the admitted practice of caramel addition.

Other classes of volatiles, which originate from sugar thermal degradation, are furanic and pyranic compounds. A wide number of them, in fact, were reported after the heating of glucose solutions, mostly with the presence of amino acids (22, 23). It is known, on the other hand, that TBVM shows a relevant amount of HMF and furfural, together with other furanic derivatives, such as 2-acetylfuran and furoic acid (24).

As expected, both the TBVM type of vinegars had the highest total amount of furanic compounds (**Table 3**), independent from the age of the vinegar. BVM revealed a minor, even if relevant, content of total furans (likely coming from caramel addition), while JVs had the lowest content of these sugar derivatives. HMF was, by far, the major representative (up to 93.5% in BVM “affinato”), followed by furfural and 5-methylfurfural. In TBVM, 5-acetoxymethylfurfural is a further major furanic compound, which comes from the esterification between acetic acid and HMF. This volatile, already reported in TBVM at a concentration varying from 7.28 mg/kg (7) to 188 mg/kg (24) seems to be characteristic of Italian TBVM and could represent a discriminant parameter between “affinato” and “extravecchio” TBVMs, as already suggested by Giacco et al. (25).

Three further volatiles were found exclusively in Italian TBVM and BVM. They are 2,5-furandicarboxaldehyde, furylhydroxymethyl ketone, and 1*H*-pyrrole-2-carboxaldehyde. All of them may derive from Maillard reactions produced by heating proline or phenylalanine mixtures (26) and have already been reported in toasted oak extracts (20, 27). Their sensory impact has been described as “honey” and “toasty caramel” (27) and, in TBVM and BVM, may derive from the cooking of the must and the caramel addition, respectively. Other furanic derivatives somewhat relevant are 2-acetyl-5-methylfuran, with a nutty

reminiscence, and DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one), the role of which in the Maillard pathway has been described as pivotal for the generation of enolic derivatives (5-hydroxymaltol, maltol, and cyclotene in particular) (22).

Acetoin is a wine constituent initially produced during alcoholic fermentation. When wines are submitted to acetic fermentation, the acetoin amount increases because of the transformation of α -acetolactate and 2,3-butanediol acted by acetic bacteria (28). Despite the low recovery that this compound showed by the extraction method that we used (13), our data suggest that vinegars coming from wine tend to have a higher amount of this compound, which is characterized by a buttery odor.

Considerable amounts of lactones have been found in our samples, which may come from cyclization of hydroxyacids during fermentation or from sugar degradation (7). On the other hand, the presence of whiskey lactones is in relationship with wood aging, and the ratio between the two isomers is thought to depend upon the oak species used (29). Quite surprisingly and diversely from the finding of other authors (9), the cis/trans ratio of JVs was found to be < 1 (may be because of the prolonged use of old American oak barrels), while in TBVM, that ratio spans between about 2 and 20, probably as a consequence of the use of several wood species (chestnut, juniperus, or mulberry), the cis/trans ratio of which is largely unknown.

Volatile phenols were somewhat higher in JVs. Their presence in vinegars is due to wood aging (this is the case of guaiacol, siringol, and eugenol) or to the biological decarboxylation of cinnamic acids acted by malolatic or *Brettanomyces* bacteria (ethylphenol and ethylguaiacol). As shown in this work, significant differences in ethylphenol were found between wine-based vinegars and TBVM, likely because of the intervention of the bacterial fermentation on the initial wines. For what concern wood-derived phenols, their amounts were almost the same whatever the vinegar type. This finding is partially in contrast with other authors (9) who found the highest concentration of guaiacol in BVM (up to 300 $\mu\text{g/L}$) but similar amounts of eugenol between JVs, BVM, and red wine vinegars and suggests that the presence of these compounds is independent from the duration of wood aging.

PCA. To obtain a comprehensive representation of the main volatiles that could differentiate the vinegar samples, a PCA was carried out on the whole set of data. In **Figure 2**, the localization of the 25 samples in the space formed by the first two components (56% of the variance) is shown. Four groups of samples are

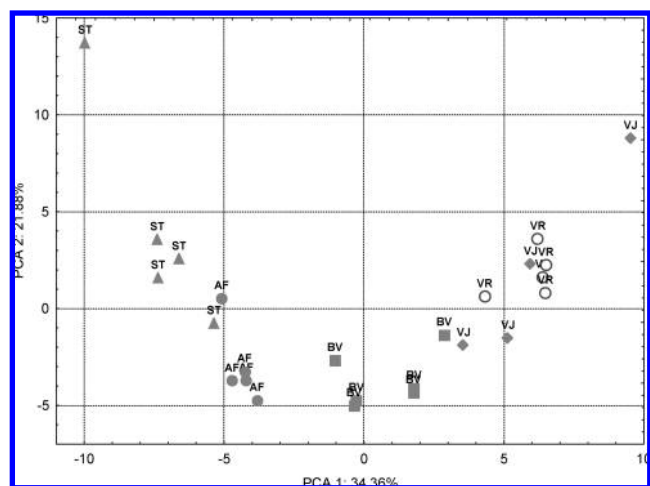


Figure 2. PCA plot of the first two principal components. AF, affinato TBVM; ST, extravecchio TBVM; BV, BVM; VJ, vinagre de Jerez; VR, vinagre de Jerez reserva.

clearly discriminated on the component 1, representing “extravecchio” TVBM, “affinato” TBVM, BVM, and JVs, respectively. It appears, hence, that the two categories of JVs (vinagre de Jerez and reserva) were almost indiscriminated from each other on this factor for which the principal parameters were propanoic and hexanoic acids, *p*-ethylguaiacol, and benzyl alcohol (with a positive sign) and 5-hydroxymethyl-2-furaldehyde, 5-methylfurfural, furfural, and 5-acetoxymethyl-2-furaldehyde (with a negative sign). Interestingly, the only discriminated sample of vinagre de Jerez (on the upper right side of the graph) was obtained from sherry vinegar with *Pedro Ximenez* grape must addition. Component 2 discriminated both the “extravecchio” TBVM and JVs from “affinato” TBVM and BVM. Parameters that mainly accounted for this variance were caprolactone, ethyl-3-hydroxybutyrate, and 1,2,3-propanetriol diacetate.

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