

CHARACTERISATION OF VOLATILE COMPOUNDS IN THREE ACETIFICATION BATTERIES USED FOR THE PRODUCTION OF "ACETO BALSAMICO TRADIZIONALE DI REGGIO EMILIA"

STUDIO DEI COMPOSTI VOLATILI IN TRE BATTERIE DI ACETIFICAZIONE PER
LA PRODUZIONE DI ACETO BALSAMICO TRADIZIONALE DI REGGIO EMILIA

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ABSTRACT

In contrast to common vinegar Traditional Balsamic Vinegar is produced from acetic acid fermentation of cooked grape must in a "battery", a series of 5 or 7 wooden casks called "vaselli". The aim of this work was to study the effect of the acetification battery on the volatile components of the product obtained with the same acetification process. Then the volatile fraction of the vinegar from three acetification batteries operating under the same conditions but with different wooden casks and used

RIASSUNTO

A differenza dell'aceto comune l'Aceto Balsamico Tradizionale viene prodotto mediante la bioossidazione acetica di mosto cotto in una "batteria", una serie di 5 o 7 botti di legno chiamate "vaselli". Lo scopo di questo lavoro è stato quello di studiare qual è l'effetto della batteria di acetificazione sui componenti volatili del prodotto finito mantenendo costante il processo di acetificazione. È stata quindi esaminata la frazione volatile di tre batterie di acetificazione operanti nelle stesse condizioni,

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for the production of Traditional Balsamic Vinegar (Aceto Balsamico Tradizionale di Reggio Emilia, Italy) was analysed. From among over one hundred detected compounds ketones, aldehydes, ethyl esters and acetates from alcoholic and acetic fermentation and the furan derivatives from Maillard reactions, such as furfural, 5-methyl-2-furaldehyde, 5-hydroxymethylfurfural or HMF, 5-acetoxymethylfurfural and 2-furoic acid were the most abundant compounds identified. In every battery the compound concentrations produced during alcoholic fermentation decreased from the first barrel to the last barrel and, on the contrary, the concentration of derivatives of the acetic acid fermentation, the oxidative ageing and the Maillard reactions products increased from the first barrel to the fifth barrel. Also the wood used for manufacturing the casks and their age seems to have a significant effect on the aroma components.

ma costituite da differenti "vaselli" ed utilizzate nella produzione di Aceto Balsamico Tradizionale di Reggio Emilia. Fra gli oltre cento composti isolati i più abbondanti sono risultati essere i chetoni, le aldeidi, gli esteri etilici e gli acetati provenienti dalla fermentazione alcolica e dalla biossidazione acetica ed i derivati furanici provenienti dalle reazioni di Maillard quali il furfurale, il 5-metil-2-furfurale, il 5-idrossimetilfurfurale, il 5-acetossimetilfurfurale e l'acido 2-furoico. In tutte le batterie la concentrazione dei composti formati durante la fermentazione alcolica diminuisce passando dal primo all'ultimo "vasello", mentre al contrario quella dei derivati della biossidazione acetica, dell'invecchiamento ossidativo e delle reazioni di Maillard aumentano dal primo all'ultimo "vasello". Anche il tipo di legno utilizzato per la costruzione del "vasello" e la sua età sembrano avere grande effetto sui componenti della frazione volatile.

INTRODUCTION

Traditional balsamic vinegar is a special vinegar which dates back to the X-XI century and is produced exclusively in the regions of Modena and Reggio Emilia (Italy). According to Italian laws, traditional balsamic vinegar is made from the acetic acid fermentation of cooked grape must of Trebbiano, Spermogola, Lambrusco and other local grapevine varieties. It must be aged at least 12 years in barrels of different sizes and wood, but it is possible to find vinegar aged 40-50 years and even up to 200 years. Unlike common vinegar it is not made from wine but grape must. The production process is very complex and often varies among producers. Generally after grape crushing, the seeds and the skins are separated and the must is

concentrated by boiling at atmospheric pressure directly exposed to heat and reduced 20-30% of the starting volume. This cooked must is then transferred into a cask taking care to not exceed 80% of its capacity. In these casks the must undergoes partial alcoholic fermentation with an ethanol production of 40-70 g L⁻¹. After 5 to 6 months, the must is blended with wine vinegar containing a large number of acetic acid bacteria and then divided into 5 to 7 smaller casks called *vaselli*, again taking care not to exceed 80% of its capacity. The *vaselli* are made of different types of wood (oak, chestnut, mulberry or juniper) and come in different sizes.

Due to evaporation during ageing, the volume of the liquid decreases, and the *vaselli* are refilled using the "Solera" method. For example in a battery of five

vaselli, the fifth *vasello* (the last and smallest) is refilled from *vasello* number 4, then number 4 is refilled from number 3 and so on until *vasello* number 1 is refilled with fresh cooked must. These refills must be made once a year, in the spring, for the entire life of the battery. Only from the twelfth year onward, is it possible to take the finished product from the last *vasello* for consumption.

The battery needs a long time to consistently produce a balsamic vinegar with sufficiently stable composition and sensorial characteristics. Alcoholic fermentation occurs in the cooked must before blending it into the battery, while acetic acid fermentation usually takes place in the first two *vaselli*. In the others there is no longer any microbiological activity, but only chemical and enzymatic activities favoured by temperature changes that happen during the year in the storage places, usually lofts. The aroma of the product is also influenced by the cooking of the must that causes the development of Maillard reactions or non-enzymatic browning between the amino acids and the reducing sugars (GOGUS *et al.*, 1998). These reactions include the formation of many products most of which have an impact on the flavour and appearance of the vinegar.

The first step in the amino acid-reducing sugar reaction is a sugar-amine condensation in equimolar ratios leading to N-glycosylamines or N-fructosylamines. The isomerisation of these compounds leads to 1-amino-1-deoxy-2-ketoses or 1-amino-2-deoxy-2-aldoses also known as Amadori and Heyns intermediates which are essential precursors of heterocyclic compounds (VERNIN and PARKÁNYI, 1982).

Two traditional balsamic vinegars, *Aceto Balsamico Tradizionale di Modena* (ABTM) and *Aceto Balsamico Tradizionale di Reggio Emilia* (ABTRE) are available on the market. They differ due to the area of production and the grape used for the must production.

Many studies have been carried out

on traditional balsamic vinegars (COPPINI *et al.*, 1978; TURTURA and ROSETTI, 1984; PLESSI *et al.*, 1988, 1989; STACCHINI *et al.*, 1990; TURTURA and BORTOLOTTI, 1991; GIUDICI, 1993; CORRADINI *et al.*, 1994; GIUDICI *et al.*, 1994; MASINI and GIUDICI, 1995; DEL SIGNORE, 1997; 1998; 2000; 2001a,b; MEGLIOLI *et al.*, 1997; ANKLAM *et al.*, 1998; CHIAVARO *et al.*, 1998; DEL SIGNORE *et al.*, 1998, 2000; MASCOLO and GAVIOLI, 1998; STANCHER *et al.*, 2001). These studies have shown that the chemical composition of traditional balsamic vinegar is highly variable and depends on the acetification process (type of grape must, cooking modality, temperature and humidity during aging) and the acetification battery it originates from (battery age, wood used for the *vaselli*, *vaselli* age). In fact, its acidity, expressed as acetic acid, is generally between 30 to 120 g kg⁻¹; the reducing sugars are between 100 to 500 g kg⁻¹ and the ethanol is between 0.4 to 14 mL kg⁻¹ (MEGLIOLI *et al.*, 1997; MASCOLO and GAVIOLI, 1998).

Among the volatile compounds, particular attention has been given to substances found exclusively in the finished product with the aim of determining possible commercial frauds: 5-acetoxy-methyl-2-furaldehyde, a characteristic substance present only in aged vinegars (GIACCIO *et al.*, 1996; GIACCIO and DEL SIGNORE, 1997), 5-hydroxymethylfurfural (THEOBALD *et al.*, 1998), ethyl acetate (STACCHINI *et al.*, 1990), and some principal volatile compounds (DEL SIGNORE, 2001b).

Since the chemical composition of balsamic vinegar is determined by the acetification process and the acetification battery the aim of this work was to study the effect of the acetification battery on the volatile compounds of the product obtained with the same acetification process. Then the volatile fraction of the vinegar from three acetification batteries with different wooden casks used for the production of ABTRE (*Aceto*

Balsamico Tradizionale di Reggio Emilia, Italy) was analysed.

MATERIALS AND METHODS

Samples

The studies in this field are very difficult because an acetification battery can produce balsamic vinegar only after 12 years as established by regulation (G.U., 1986; G.U., 1987) but only after at least 20 years is the vinegar production stable. Then the lengthy production times prohibit planning repetitive experimentation and the research on the trend of acetification can only be carried out on the battery already in production. In this study three acetification batteries (A, B and C) at the Reggio Emilia firm, each with 5 *vaselli* and initiated contemporarily 50 years ago were studied (Table 1). In batteries A and C almost all the *vaselli* were made of oak, while in battery B there were also chestnut and mulberry *vaselli*. Mulberry was also present in battery C and in both the bat-

teries it was used for the 4th *vasello* required by tradition. The ages of the *vaselli* varied a lot. Batteries B and C were formed by *vaselli* that replaced the original ones in the 1980s. Battery A, instead, was formed largely by the original *vaselli* that were over 200 years old. Five 50 mL samples were obtained from each battery.

Chemical analysis

Acidity was determined according to the official Italian methods (MAF, 1986). The dry extract content was evaluated with a refractometer and is reported as °Brix.

Extraction of volatile compounds

The volatile compounds were extracted by reverse-phase Solid Phase Extraction with a C₁₈ cartridge (DI STEFANO, 1991; KARAGIANNIS *et al.*, 2000). Internal standard (1-heptanol; 50 µL of a 1.7 g L⁻¹ solution) was added to vinegar samples (10 g) and diluted up to 50 mL with deionised water. Each sample was poured into a 2 g C₁₈ cartridge (Bakerbond SPE,

Table 1 - Type of wood used and age of casks and vinegar characteristics for each cask.

Battery	Barrel (<i>vasello</i>) code	Barrel wood	Barrel age	°Brix	Titrate acidity (acetic acid g kg ⁻¹)
A	1	Oak	1980	24.5	20.2
	2	Chestnut	1700	40.1	55.1
	3	Oak	1800	47.5	63.9
	4	Oak	1960	61.1	72.8
	5	Oak	1800	64.5	70.6
B	1	Oak	1980	32.0	15.1
	2	Chestnut	1980	33.0	29.9
	3	Chestnut	1980	37.5	51.8
	4	Mulberry	1980	45.5	70.2
	5	Oak	1980	58.5	70.8
C	1	Oak	1980	27.5	21.1
	2	Oak	1990	29.1	24.2
	3	Oak	1980	35.5	39.6
	4	Mulberry	1986	45.0	68.6
	5	Oak	1985	57.1	63.6

J.T. Baker, Deventer, Holland) previously activated with methanol (20 mL) and washed with water (20 mL). After washing with 30 mL of water, volatile compounds were eluted by gravity with 40 mL of dichloromethane (Merck, Darmstadt, Germany). The solution obtained was dried with anhydrous sodium sulphate then concentrated under nitrogen at room temperature to a final volume of 0.5 to 1 mL and injected. Three extractions were performed for each sample.

Gas Chromatography-Mass Spectrometry (GC-MS)

Compound identification was achieved with a Shimadzu GC-17A gas chromatograph (GC) coupled with a Shimadzu QP-5000 quadrupole mass spectrometer (Shimadzu, Tokyo, Japan). The GC was equipped with a J&W fused silica column (DB-WAX, 30 m x 0.25 mm i.d., 0.25 μ m film thickness, J&W Scientific Inc., Folsom, CA, USA) and a split-splitless injector. The injected volume was 2 μ L. The temperature program and the operating conditions were as follow: injector temperature 250°C; injection in splitless mode for 0.30 min and then split 1:60; carrier gas helium at 1 mL min⁻¹; the column was maintained at 35°C for 5 min then ramped at 2°C min⁻¹ to 210°C and finally 10 min at 210°C.

Mass spectra were recorded in the electron impact mode at an ionisation voltage of 70 eV in the 29-250 amu mass range. The ion source and the interface were maintained at 220°C. Compound identification was carried out with the mass spectra and retention times of standard compounds, when available, or the NIST 12 and NIST62 mass spectral data base.

Quantitative measurements of each compound were carried out with a Varian GC-3400 gas chromatograph (Varian, Walnut Creek, CA, USA) equipped with a split-splitless injector and a flame ionisation detector held at 250°C. Data inte-

gration was performed with EZCHROM™ data system (Scientific Software Inc., San Ramon, CA, USA) with no consideration of extraction recoveries, i.e. calibration factors F=1.00 for all compounds. The column and the operating GC-FID chromatographic conditions were the same as GC-MS. Injections of standard compounds in the GC-FID system gave identical peak sequences as GC-MS.

RESULTS AND DISCUSSION

The increase in acidity in the three batteries was similar, but battery C had values slightly lower than batteries A and B (Table 1).

The dry extract content was very different in the three batteries reaching 64.5°Brix in battery A, but only 58°Brix in the other two. There was a noticeable increase of the °Brix values in batteries B and C from the *vaselli* made of mulberry to the *vaselli* made of oak. The mulberry, being more porous, facilitates evaporation and thus concentration of the product.

In the fifteen products studied, over one hundred volatile substances were detected (Table 2). They originated from alcoholic and acetic acid fermentations, Maillard reactions, pyrolysis reaction during cooking of the must, oxidative ageing, extraction from wooden barrels and enzymatic reactions that occur in balsamic vinegar production. In battery A, there were many more compounds than in batteries B and C and in greater quantities. The coefficient of variation of these compounds for each *vasello* varied from 2 to 19%.

In every battery, the concentrations of the compounds produced during alcoholic fermentation decreased from the first barrel (where the fresh cooked must was added) to the last barrel (where the balsamic vinegar was taken from). On the contrary, the concentration of the derivatives of the acetic acid fermenta-

Table 2 - Mean concentration ($\mu\text{g kg}^{-1}$; three injections) and retention time (min) of aroma compounds detected in the three batteries of Aceto Balsamico Tradizionale di Reggio Emilia (II) and calculated by GC-FID signals using 1-heptanol as internal standard. For the unknown and unidentified compounds the m/z value of the base peak is reported.

Battery A	Rt (min)	Vasello				
		1	2	3	4	5
ketones						
diacetyl*	5.6		2830	2289	1408	878
2-pentanone	5.8	442				
cyclopentanone	15.2	25				
3-hydroxy-3methyl-2-butanone	19.4	344	238	200	144	167
acetoin*	22.2	35799	37172	42940	24695	3089
1-hydroxy-2-propanone	22.9	45	56	50		
3-hydroxy-2-pentanone	25.8	524	392	350	249	204
3,4-dimethyl-2,5-furandione	49.5		48		169	112
1-ethoxy-2-heptanone	56.4	336	216			
dihydro-2-methyl-3(2H)-furanone	20.7		54		89	195
dihydro-2,5-furandione	71.2	481				
esters						
ethyl isobutyrate	5.5		122			
ethyl isovalerate*	9.5	188	175			
ethyl pyruvate*	21.6	1487	638	67		
ethyl lactate*	26.5	24842	13120	350	441	348
ethyl glycolate	31.4	1351	5798	1042		
ethyl 2-hydroxyisovalerate	31.8		74			
ethyl 3-hydroxybutyrate	37.8	434	262			
diethyl succinate*	47.4	8371	2847			
ethyl benzeneacetate	53.2	285	1000			
monoethyl succinate	83.1	38830	35582	9400	2005	3107
diethyl malate	67.2	15020	8408	484		521
diethyl tartrate	80.6	1817	1988	530		
acetates						
isobutyl acetate*	7.1		271			
1,3-propanediol, monoacetate	51	450	761	1625	2602	5370
1,3-propanediol, diacetate	46.2				249	592
isopentyl acetate*	12.3	517	758			15
2-ethoxyethyl acetate	23.4				232	418
meso 2,3-butanediol, diacetate	29	283	1874	2360	2200	2121
1,2-ethanediol, diacetate	34.4					394
dl 2,3-butanediol, monoacetate	41	8366	16893	30052	33364	32242
+ isobutyric acid*						
meso 2,3-butanediol, monoacetate	41.8		6828	15490	22074	21872
2-phenylethyl acetate	54.9	tr	1182	1162	1323	2040
1,2,3-propanetriol, triacetate	68.9				327	690
1,2-ethanediol, monoacetate	45.1	22	288	733	1270	689
1,2,3-propanetriol, diacetate	76.5	191	4074	17956	25809	48907
(isomer 1)						
1,2,3-propanetriol, diacetate	77.9		1210	5520	9345	15523
(isomer 2)						
1,2,3-propanetriol, monoacetate	78.6		2008	6600	7352	10304
aldehydes						
2-methyl-pentanal	6.2	688	745	747		471
benzaldehyde*	37.2		48			10
alcohols						
1-propanol*	8.3	1471	422			

Battery A	Rt (min)	Vasello				
		1	2	3	4	5
2-methyl-1-propanol	10.9	17326	3644	121		
1-butanol*	13.9	445	131	tr		
<i>trans</i> 3-penten-2-ol*	15.5			65		
3-methyl-1-butanol*	17.9	60603	12372	1257	284	240
2-ethoxy-ethanol	18.4		102	270	2153	2175
2-hexanol	24	1295	1137	1018	1171	834
2-heptanol	25.5	100				
1-hexanol*	27.5	732				
3-ethoxy-1-propanol*	28.8	1839	1464	277	122	164
<i>cis</i> -3-hexen-1-ol*	29.4	39				
2-butoxyethanol + unknown (<i>m/z</i> 54)	30.3				219	263
<i>dl</i> 2,3-butanediol*	39.6	10219	13128	20554	20063	9260
1-acetoxy-2-propanol	41.6	147	1703	15490	7513	8179
<i>meso</i> 2,3-butanediol*	42	2501	3688	4772	5922	4185
2-acetoxy-1-propanol	44.2		785	1624	3047	4236
butoxyethoxyethanol	54.1				732	
benzyl alcohol*	58.4	347				
2-phenyl-ethanol*	60.3	19113	14721	13749	9339	10550
furan derivatives						
furfural* + unknown (<i>m/z</i> 45)	33.9		4262	5411	8253	11763
5-methyl-2-furfural	40.7	47	903	724	743	1223
methyl 3-furoate	64.6	266	2158	4720	5120	9078
5-acetoxy-methyl-2-furaldehyde	74.3		3920	7068	10294	28755
2-furoic acid	85.2	122	985	216	979	1559
5-(hydroxymethyl)-2-furaldehyde	88.8	11349	164529	357213	305083	287233
2-furyl methyl ketone + unknown (<i>m/z</i> 44)	36.4	15385	8332	2453	2534	
2-furyl methyl ketone	36.4					2025
enolic derivatives						
cyclotene*	62.3	405	1770	3264	4748	2953
maltol*	62.8		194	358		1021
5-hydroxy-maltol	78.7	138	6314	14335	9383	11482
lactones						
α -angelica lactone	31.6		49	59	61	114
γ -methyl- γ -butyrolactone	42.4	721	560	92		435
γ -butyrolactone*	43.5	13484	12933	18679	17728	9498
γ -caprolactone	47.9				136	tr
γ -crotonolactone	50.8		182	392	711	302
γ -ethoxybutyrolactone	49.4	18				
α -methyl- γ -crotonolactone	58		56	100	104	163
<i>cis</i> - β -methyl- γ -octalactone	62	77			222	
dehydromevalonic lactone	64.8	tr	2070	8415	9509	21763
pantolactone	66.1	764	1384	1670	1674	1699
solerone	67.4	tr	54	442	353	816
miscellaneous						
ethyl 4-hydroxyronanoate	68.4	167				
ethyl 4-hydroxydecanoate	73	361	169			
2-methyl-1,3-dioxane	9.1	191				
2,2,4,5-tetramethyl-1,3-dioxolane	37.4				79	129
1,3,5-trioxane	14.6	10				
<i>trans</i> furan linalool oxide*	32.8	149	207	223	153	223

Battery A	Rt (min)	Vasello				
		1	2	3	4	5
cis furan linalool oxide*	34.7		108	106	78	122
cis pyran linalool oxide + unknown (<i>m/z</i> 95)	52.5				185	128
nitrogen compounds						
N-ethylacetamide	45.6	186	308	286	288	
N-amylacetamide	58.4			844		1097
N-amylacetamide + benzyl alcohol*	58.4		734		700	
2-pyrrolidinone	66.5		149	207	204	367
1-methyl-1H-pyrrole-2- carboxaldehyde	69.8		168			66
1H-pyrrole-2-carboxaldehyde + allomaltol	65.6		tr		tr	848
hydrocarbons						
undecane	11			74		669
dodecane	17		46	148		
tridecane	23.6	240	195	456		
fatty acids						
acetic acid*	33.4	773	2328	9400	2282	1906
propanoic acid*	39	584	1067	1436	1564	250
butanoic acid*	44.6	1197	1016	1103	1418	1571
isovaleric acid*	47	9741	18926	24517	17787	19972
2-butenic acid	52.8	35	101	153	580	213
hexanoic acid*	57	1589	1677	1820	1201	1599
2-ethylhexanoic acid	62.6				701	
octanoic acid*	68.1	130	700	430	189	523
benzoic acid*	64.8	62	490	223		935
sulfur compounds						
methionol	49.7	33				
phenols						
2-methoxy-phenol	57.4				142	122
phenol	65.1	96				
4-ethyl-phenol*	73.5	83				
syringol	77.5		260			7974
tyrosol	83.9	963	4219	4081	13431	2187
unidentified compounds						
<i>m/z</i> 75	7.8	8869	10498	24844	26315	17398
<i>m/z</i> 43	16.4	388	476			
<i>m/z</i> 45	27		439	290	238	
<i>m/z</i> 73	31.3				433	295
<i>m/z</i> 43	33	1072	1305	808	783	816
<i>m/z</i> 43	33.2	1081	1827	1840	1346	1283
<i>m/z</i> 45	33.7	1489				
<i>m/z</i> 43	36				232	444
<i>m/z</i> 43	38.4			131	118	598
<i>m/z</i> 43	38.8					82
<i>m/z</i> 45	42.4	12581	6882			
<i>m/z</i> 45	44.8				93	99
<i>m/z</i> 43	45.6					823
<i>m/z</i> 43	47.3				573	399
<i>m/z</i> 45	46.6					2038
<i>m/z</i> 43	53.2			740	580	845
two compounds with <i>m/z</i> 45 and <i>m/z</i> 41	55.7	10551	4389	1550	2176	3299

Battery A	Rt (min)	Vasello				
		1	2	3	4	5
<i>m/z</i> 76	55.1	392	1387	1216	936	1191
<i>m/z</i> 43	57.3	658	1076	354		
<i>m/z</i> 43	58.8				556	1037
<i>m/z</i> 183	59.1		85	253	207	399
<i>m/z</i> 97	61		351	278	175	482
<i>m/z</i> 124	63.3		97	571	233	270
<i>m/z</i> 45	67.9					778
<i>m/z</i> 45	68.3					675
<i>m/z</i> 43	69.3	2129	2864	928		
<i>m/z</i> 45	71	8607	4382	237		
<i>m/z</i> 111	72.8	3571	708			
<i>m/z</i> 43	74.1	1260				
<i>m/z</i> 55	71.9				168	349
<i>m/z</i> 55	72.2				115	313
<i>m/z</i> 126	73.8				162	673
<i>m/z</i> 85	76	139				
<i>m/z</i> 43	75.3				1123	7595
<i>m/z</i> 45	75.1	19273	15502			
<i>m/z</i> 97	82.6	321	68			
<i>m/z</i> 140	89	42			145	8844
<i>m/z</i> 45 (compound A)	80	1550	5661	12012	13856	17434
<i>m/z</i> 45 (compound B)	82.4	1522	2360	2740	2465	3283
Battery A						
ketones						
2-pentanone	5.8	526				
cyclopentanone	15.2	44				
3-hydroxy-3-methyl-2-butanone	19.4	207	174	164	70	86
acetoin*	22.2	13611	39252	29409	130	143
3-hydroxy-2-pentanone	25.8	156	320	383		
1-hydroxy-2-butanone	27.9		27	24		
4-cyclopentene-1,3-dione	41.2	102	18			
1-ethoxy-2-heptanone	56.5	539	537	292		
esters						
ethyl isovalerate*	9.5	50	88	55		
ethyl caproate*	19.2	27				
ethyl pyruvate*	21.6	1112	789	272		
ethyl lactate*	26.5	10366	7509	4987	344	79
ethyl glycolate	31.4	2083	4471	3047		
ethyl 2-hydroxyisovalerate	31.8		34			
ethyl 3-hydroxybutyrate	37.8	370	208			
ethyl laevulinate	43.3	362	547			
diethyl succinate*	47.4	5150	3180	821		
diethyl malate	67.2	14414		1727	200	
diethyl malate + solerone	67.2		13184			
diethyl tartrate	80.6	1457	2158	496	384	149
monoethyl succinate	83.1	42325	35052	16629	5486	2059
monoethyl malate	87.2	992	958	403		
ethyl benzeneacetate + unknown (<i>m/z</i> 45)	53.2		482			

Battery B	Rt (min)	Vasello				
		1	2	3	4	5
acetates						
isobutyl acetate*	7.1	118	157			
butyl acetate*	9.7		26		56	
isopentyl acetate*	12.3	438	399	200		
2-ethoxyethyl acetate	23.4					420
2,3-butanediol, diacetate	29	158	947	1919	2426	2549
1,2-ethanediol, diacetate	34.4				81	121
<i>dl</i> 2,3-butanediol, monoacetate	42	1119	tr		15327	24205
<i>dl</i> 2,3-butanediol, monoacetate + isobutyric acid*	41			16538		
<i>meso</i> 2,3-butanediol, monoacetate	41.8	4759	2599	6290	10293	18351
1,2-ethanediol, monoacetate	45.1		161	424		
1,3-propanediol, monoacetate	51	278	285	713		
1,3-propanediol, diacetate	46.2				99	
2-phenylethyl acetate*	54.9		119	795	1289	1544
1,2,3-propanetriol, triacetate	68.9				371	
1,2,3-propanetriol, diacetate	76.5	938	1013	5123	9455	15577
(isomer 1)						
1,2,3-propanetriol, diacetate (isomer 2)	77.9	242	321	1571	3789	6914
1,2,3-propanetriol, monoacetate	78.6	215	1098	3100		
alcohols						
<i>tert</i> -pentyl alcohol	7.3		1000	1039		
1-propanol*	8.3	1552	869	106		
2-methyl-1-propanol	10.9	8640	4392	658		
1-butanol*	13.9	471	159			
<i>trans</i> -3-penten-2-ol*	15.5	138	142	142	949	1469
3-methyl-1-butanol*	17.9	38855	15977	2917	420	173
2-ethoxy-ethanol	18.6	106	41	197		
2-hexanol	24	1451	1210	1188	1060	1371
3-methyl-2-buten-1-ol	25.2				164	243
1-hexanol*	27.5	293	151			
3-ethoxy-1-propanol*	28.8	868	1339	744	162	83
2-butoxyethanol	30.3	62	133	164		301
<i>dl</i> 2,3-butanediol*	39.6	8098	12237	13323	538	637
1-acetoxy-2-propanol	41.6	447	698	1851		
<i>meso</i> 2,3-butanediol*	42	1511	3202	3606		
2-acetoxy-1-propanol	44.2		271	732		
α -terpineol*	48.7	tr				
butoxyethoxy ethanol	54.1	727	918	1020		
benzyl alcohol*	58.4	186				
2-phenyl-ethanol*	60.3	12406	11700	10616	11089	10092
1-dodecanol	63.7				83	
octadecanol	71.5				1056	1035
aldehydes						
hexanal*	10				22	
2-methyl-crotonaldehyde	10.4		35			
heptanal*	15.8				34	
octanal*	22.6				91	
nonanal*	29.5				69	
benzaldehyde*	37.2		24	32		
4-hydroxy-benzaldehyde	86.8			197		

Battery B	Rt (min)	Vasello				
		1	2	3	4	5
furan derivatives						
furfural*	33.9	100	929	3608	1020	1744
2-furaldehyde ethylmethyl acetal	54.3	317				
methyl 3-furoate	64.6	794	1675	2237		
2-furyl methyl ketone	36.4				395	448
2-furyl methyl ketone + unknown (m/z 44)	36.4	6848	8690	2573		
5-methyl-2-furfural	40.7	368	1071	1126	633	
5-acetoxy-methyl-2-furaldehyde	74.3			4270	5948	10857
2-furoic acid	85.2	114	925	933		
5-(hydroxymethyl)-2-furaldehyde (HMF)	88.8	51286	150464	197559	4476	7164
enolic derivatives						
cyclotene*	62.3		1537	2668	21	36
maltol*	62.8				497	645
allomaltol	65.6				208	113
5-hydroxy-maltol	78.7	621	3439	11952	7930	6509
lactones						
5-methyl-2(3H)-furanone (α -angelica lactone)	31.6	32	40	41		
γ -methyl-g-butyrolactone	42.4	605	582	307		
γ -butyrolactone*	43.5				87	90
γ -butyrolactone* + unknown (m/z 43)	43.5	7084	9730	12047		
γ -crotonolactone	50.8		85	240		
α -methyl- γ -crotonolactone	58			75		
cis- β -methyl- γ -octalactone	62					106
solerone	67.4			355		
pantolactone	66.1	559	1343	1521		
miscellaneous						
1,3,5-trioxane	14.6			28		
trans furan linalool oxide*	32.8	127	142	187	249	251
cis furan linalool oxide*	34.7	39	32	66		
ethyl 4-hydroxydecanoate (m/z 85)	73	313	221			
nitrogen compounds						
N-ethylacetamide	45.6	349	138	301		
N-amyacetamide + benzyl alcohol*	58.4		363	580	1785	1862
phenyl carbamate	65.1			682	151	13
hydrocarbons						
undecane	11				50	
dodecane	17	88	36	136		
tridecane	23.6	241	142	406	130	136
fatty acid						
acetic acid*	33.4	820	1925	3663	903	1504
propanoic acid*	39	274	958	1278		
isobutyric acid*	41	4360	9748			
butanoic acid*	44.6	649	729	1044	97	154
isovaleric acid*	47	2239	12179	21122	22755	24346
valeric acid*	51				245	
2-butenic acid	52.8		48	128		
hexanoic acid*	57	1161	1203	1795	2059	1668
2-ethylhexanoic acid	62.6	606	814	974		
octanoic acid*	68.1	456	551	484	505	332
homovanillic acid	81	150				
benzoic acid*	84.8	86	288	703	697	644

Battery B	Rt (min)	Vasello				
		1	2	3	4	5
phenols						
syringol	77.5		148		1151	2005
tyrosol	83.9	1439	59	1511		
<i>p</i> -guaiacol	57.3				71	22
unidentified compounds						
<i>m/z</i> 75	7.8	6258	9962	10255	10532	36828
<i>m/z</i> 43	16.4	1262	1147	490		
<i>m/z</i> 43	33.1	177	771	572	2304	1045
<i>m/z</i> 43	33.2	352	1024	1047	903	2009
<i>m/z</i> 45	33.8	435	379			
<i>m/z</i> 47	36.7	54	76			
<i>m/z</i> 97	51.4	2073	257			
<i>m/z</i> 87	55	906				
<i>m/z</i> 76	55.2	222	535	753	720	599
<i>m/z</i> 45	55.8	4293	4061	1309		
<i>m/z</i> 43	57.4	798		947		
<i>m/z</i> 97	61.1	111	371	408	343	246
<i>m/z</i> 124	63.3	44	104	463		
<i>m/z</i> 43	69.6	2105		3435		
<i>m/z</i> 45	71	1202	5518	840		
<i>m/z</i> 111	72.9	3962	659			
<i>m/z</i> 45	75.2	33826	21692	4610		
<i>m/z</i> 85	75.7	626				
<i>m/z</i> 45 (compound A)	80	806	2248	6481		
<i>m/z</i> 45 (compound B)	82.4	1177	2601	2020		
<i>m/z</i> 43	87.4	1582	2712			
<i>m/z</i> 41	88.7	114			144	395
Battery C						
ketones						
3-hydroxy-3-methyl-2-butanone	19.4	88	105	19		84
acetoin*	22.2	316	300	173	527	105
1-ethoxy-2-heptanone	56.4	599	492	451		
4-hydroxy-2-methyl-acetophenone	74.2	921	tr			
esters						
ethyl isobutyrate*	5.5		256	164		
ethyl isovalerate*	9.5		161	148		
ethyl lactate*	26.5	3114	3237	3752	3231	230
ethyl 2-hydroxyisovalerate	31.8		42	23		
ethyl 3-hydroxybutyrate	37.8		438	126		
ethyl laevulinate	43.3	108	492	456		
diethyl succinate*	47.4	1976	3833	1660	450	
ethyl benzeneacetate + unknown (<i>m/z</i> 45)	53.2			394	324	
diethyl malate	67.2	8040	19841	9841	1432	133
diethyl tartrate	80.6	762	2446	1476	439	
monoethyl succinate	83.1	19763	39746	31820	9477	3598
acetates						
isobutyl acetate*	7.1		267	236		
isopentyl acetate*	12.3	147	632	586	212	

Battery C	Rt (min)	Vasello					
		1	2	3	4	5	
	2-ethoxyethyl acetate	23.6					78
	2,3-butanediol, diacetate	29	99	353	1036	1878	2421
	1,2-etanediol, diacetate	34.4					58
	<i>dl</i> 2,3-butanediol, monoacetate	41	1625	3113	6425	17685	22745
	<i>meso</i> 2,3-butanediol, monoacetate	41.8	667	1249	2969	9041	16048
	1,3-propanediol, diacetate	46.2					163
	2-phenylethyl acetate*	54.9			740	790	1142
	1,2,3-propanetriol, triacetate	68.9					193
	1,2,3-propanetriol, diacetate (isomer 1)	76.5	891	tr	1189	6746	14720
	1,2,3-propanetriol, diacetate (isomer 2)	77.9	190		238	1671	5838
alcohols							
	<i>tert</i> -pentyl alcohol	7.3			97		
	2-methyl-1-propanol	10.9	3218	2675	1197		
	1-butanol*	13.9	151	160	43		
	<i>trans</i> -3-penten-2-ol*	15.5	1527	1077	1384	1582	1196
	3-methyl-1-butanol*	17.9	51602	33280	15598	3838	263
	2-hexanol	84	1489		1426	1334	1195
	2-heptanol	25.5	235				
	3-methyl-2-buten-1-ol	25.2	228				
	1-hexanol*	27.5	285	175			
	3-ethoxy-1-propanol*	28.8	635	798	767	480	137
	<i>dl</i> 2,3-butanediol*	42	465	641	428	736	514
	1-acetoxy-2-propanol	41.6					160
	2,5-hexanediol	57.5	60	145			
	2-phenyl-ethanol*	60.3	11870	13212	11580	9936	9607
aldehydes							
	4-hydroxy-benzaldehyde	86.8	tr				
	2,4-dihydroxy-benzaldehyde	85.6				11359	
furan derivatives							
	furfural*	33.9	57	21	563	1533	1817
	2-furyl methyl ketone	36.4	109		278	281	268
	5-methyl-2-furfural	40.7	351	128	1112	763	567
	2-acetyl-5-methyl-furan	41.4		31			
	5-acetoxy-methyl-2-furaldehyde	74.3			1676	3237	7284
	2-furaldehyde ethylmethyl acetal	54.3	487				
	5-(hydroxymethyl)-2-furaldehyde	88.8	4131	4305	7720	16845	12067
enolic derivatives							
	maltol*	62.8					475
	allomaltol	65.6					153
	5-hydroxy-maltol	78.7	95	1202	2527	5635	7047
lactones							
	γ -methyl- γ -butyrolactone	42.4		20			
	δ -valerolactone	42.6		82			74
	<i>cis</i> - β -methyl- γ -octalactone	62		85	114		124
	dehydromevalonic lactone	64.8				210	659
miscellaneous							
	ethyl 4-hydroxydecanoate (<i>m/z</i> 85)	73	186	372	118		
	<i>trans</i> furan linalool oxide*	32.8		158	175	185	197
	<i>cis</i> furan linalool oxide*	34.7	35	75	59	98	146

Battery C	Rt (min)	Vasello				
		1	2	3	4	5
nitrogen compounds						
N-acylacetamide	58.4		401	715	1028	1426
phenyl carbamate + (amine) <i>m/z</i> 44	65.1			94		
phenyl carbamate	65.1					117
hydrocarbons						
tridecane	23.6	166	108	97		79
fatty acids						
2-hydroxy-2-methyl propanoic acid	22.7					125
acetic acid*	33.4	178	165	475	3328	766
butanoic acid*	44.6	91	146	152	246	147
isovaleric acid*	47	3462	7537			
isovaleric acid* + 2-methyl butanoic acid	47			15722	24103	20776
hexanoic acid*	57	902	1043	1200	1311	1465
octanoic acid*	68.1	147	318	388	213	281
benzoic acid*	84.8	441	50	225		
phenols						
syringol	77.5		98	72		1689
tyrosol	83.9		11514	3265	11468	9843
unidentified compounds						
<i>m/z</i> 75	7.8	41763	15953	26228	57312	20511
<i>m/z</i> 43	33	406	796	618	604	967
<i>m/z</i> 43	33.2	583	1107	992	1254	2015
<i>m/z</i> 45	42.6	881	1464	559	260	
<i>m/z</i> 97	51.4	1379	1420	149		
<i>m/z</i> 87	54.9	608	1110			
<i>m/z</i> 76	55.1	15	311	355	488	633
<i>m/z</i> 69	55.8					954
<i>m/z</i> 76	59.8					95
<i>m/z</i> 97	61.1		183	467	263	160
<i>m/z</i> 44	65.1		139			
<i>m/z</i> 109	66		469			
<i>m/z</i> 43	66.6		283			
<i>m/z</i> 45	67.9					444
<i>m/z</i> 45	68.4					337
<i>m/z</i> 85	68.3	222	268			
<i>m/z</i> 45	71	3309	6451	3163	494	
<i>m/z</i> 111	72.8	11447	4198	325		
<i>m/z</i> 45	75.1	5209	8294	3912		
<i>m/z</i> 85	75.6	318	563	384		
<i>m/z</i> 125	89				6233	1121
<i>m/z</i> 120	89.2	4321	451	889		

(tr - trace level $\leq 10 \mu\text{g kg}^{-1}$; * identified also by comparing the retention time with those of the standard compound).

tion, the oxidative ageing and the Maillard reaction products increased from the first to the fifth barrel. This was particularly evident in battery A.

The volatile compounds were subdivided into 15 groups: ketones, ethyl esters, acetates, aldehydes, alcohols, furan de-

rivatives, enolic derivatives, lactones, nitrogen compounds, hydrocarbons, fatty acids, sulphur compounds, phenols, miscellaneous and unidentified compounds.

There were few ketones and they generally decreased from the first to the fifth barrel. Only acetoin was very

abundant and increased until *vasello* 3. This compound is initially produced by alcoholic fermentation (DUBOIS, 1994), it then increases due to the transformation of acetic acid bacteria of lactate, α -acetolactate and 2,3-butanediol (ASAI, 1968).

3-hydroxy-2-pentanone has already been found in vinegars and may be derived from the reduction of 2,3-pentanedione by yeasts (CHARLES *et al.*, 2000).

Diacetyl was present only in battery A. This compound may be produced either by the fragmentation of rearranged Amadori and Heyns intermediate compounds derived from the Maillard reactions (VERNIN and PÁRKÁNYI, 1982) or by the acetic acid bacteria from acetoin (ASAI, 1968). The fragmentation of rearranged Amadori and Heyns intermediate compounds leads to other α -dicarbonyl compounds such as 1-hydroxy-2-propanone (acetol) (VERNIN and PÁRKÁNYI, 1982) which are also present in the volatile fraction of ABTRE.

The ethyl esters were abundant only in the first three *vaselli* and were generally absent in the fourth *vasello* due to the shift in esterification equilibrium caused by the small amount of ethanol and probably to volatilisation. Monoethyl succinate was an exception since it was also present in *vasello* n. 5.

The acetates increased from the first to the fifth *vasello* due to the increase in esterification during ageing, by reaction of acetic acid with mono-, di- and polyhydroxy alcohols, which then generally decreased from the first to the fifth *vasello*, and which had already disappeared in barrel n. 3 (Fig. 1a).

Some acetates which have already been found in wine vinegars (CHARLES *et al.*, 2000) are derived from the reaction between acetic acid and some diols (1,3-propanediol, 2,3-propanediol and 2,3-butanediol). Due to the long ageing of traditional balsamic vinegar, esterification is however more forced than in wine vinegar and forms large amounts of mono-, di- and tri-acetates.

Two isomers of 1,2,3-propanetriol diacetate were also found. As indicated by CHARLES *et al.* (2000), they are formed by reaction between glycerol and acetic acid. It may be hypothesized that the compound labelled "isomer 1" in Table 2 is the 1,3-diacetate isomer, while "isomer 2" is the 1,2-diacetate isomer. In fact, isomer one is more abundant in the three batteries, with respect to the second because it has less steric hindrance. Some aldehydes present in battery A could be derived from the corresponding alcohols by oxidation.

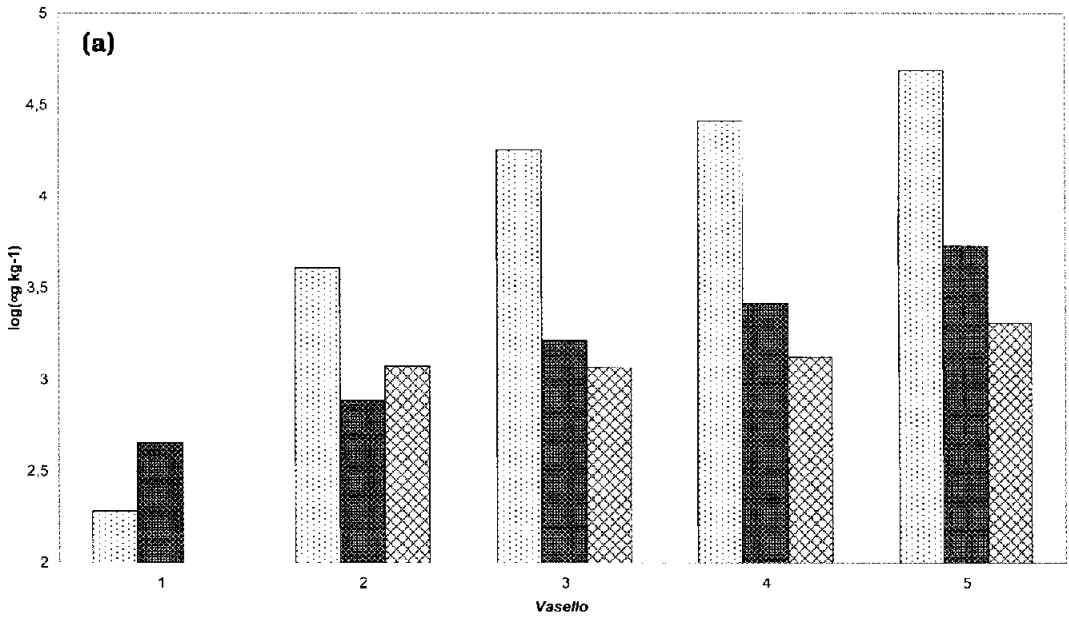
The alcohols generally decreased from the first to the fifth *vasello* in all batteries (Fig. 1b). 2-methyl-1-propanol and 3-methyl-1-butanol have already been identified in wine vinegars (CHARLES *et al.*, 2000) and their concentration decreases as acetic acid fermentation progresses. It is interesting to note the presence of phenylethyl alcohol also found in wine vinegars (CHARLES *et al.*, 2000) and in many products obtained from fermentation (wine, beer, cider, port, sherry, etc.) as by-products of alcoholic fermentation. Its production is due to amino acid transformation and mainly to phenyl-alanine deamination (Ehrlich reaction).

2,3-butanediol was very abundant and increased until *vasello* n. 3 due to the alcoholic fermentation of sugar by yeasts.

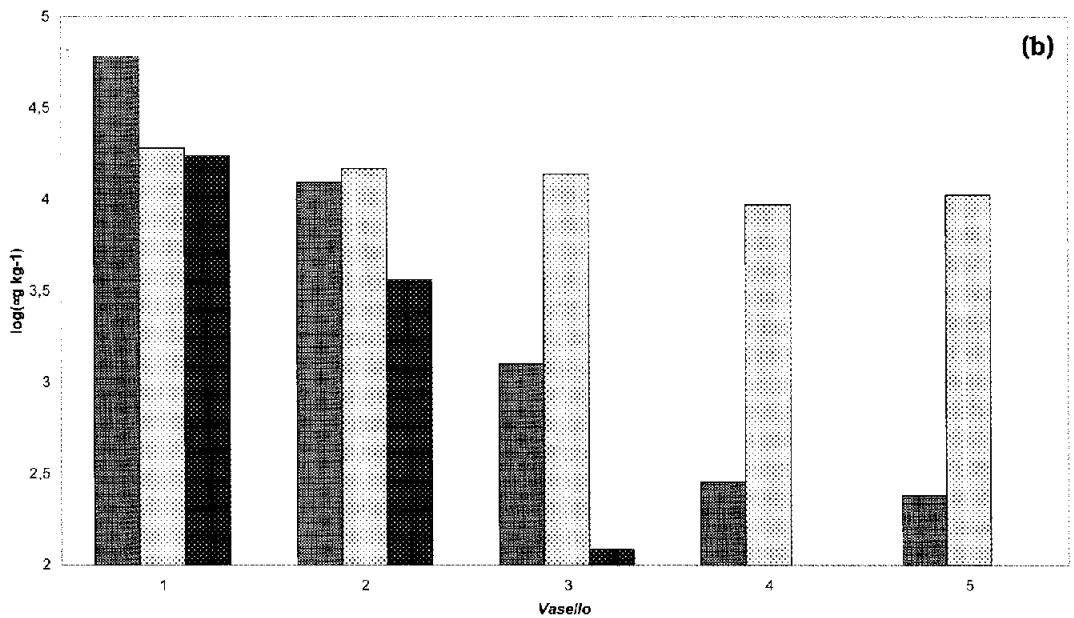
1-acetoxy-2-propanol and 2-acetoxy-1-propanol have not yet been reported. The former is more common in esterification due to steric action.

Furan derivatives partially produced during the Maillard reactions are characteristic of these vinegars, produced with cooked must (Fig. 1c). The most important ones are furfural from pentose sugars, 5-methyl-2-furaldehyde and 5-hydroxymethylfurfural (HMF) from hexose sugars, 5-acetoxymethylfurfural from the esterification between acetic acid and HMF and 2-furoic acid from ascorbic acid (VERNIN and PÁRKÁNYI, 1982). The most important is HMF (THEOBALD *et al.*, 1998), also found in sweet

□ 1,2,3-propanetriol, diacetate ■ 1,3-propanediol, monoacetate ▨ 2-phenylethyl acetate



▨ 3-methyl-1-butanol ▨ 2-phenylethanol ■ 2-methyl-1-propanol



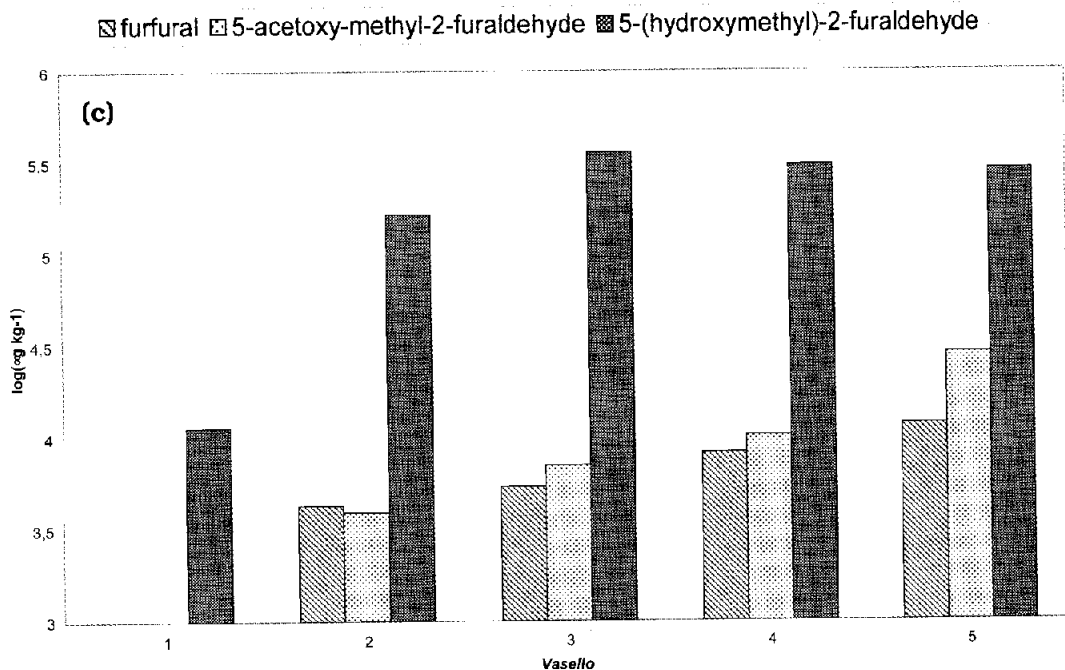


Fig. 1 - Evolution of some acetates (a), alcohols (b) and products of Maillard reactions (c) in battery A. The concentrations are reported in logarithmic form.

fortified wines (CUTZACH *et al.*, 1999). This molecule seems to be stable in the absence of oxygen, but in the presence of oxygen it may produce 5-methyl-2-furfural (CUTZACH *et al.*, 1999). It was also found in the volatile compounds of traditional balsamic vinegar. 5-ethoxymethyl-furfural was not found since it is formed from HMF only in the absence of oxygen.

The same furfural found in white wine also comes from the toasting of the wooden barrel (FEUILLAT, 1999). With furfural there is a co-elution of a not yet identified compound (relative abundance in brackets) [m/z: 29(16), 43(57), 44(87), 45(100), 57(8), 58(10), 71(7), 88(12), 117(3), 131(2)]. This unknown compound was present only in *vassello* n. 1, in the absence of furfural and its concentration decreased in the other *vassello*, while the furfural concentration increased. The situation is analogous for 2-furyl-methyl-ketone. In fact, together

with this compound, there is a co-elution of a compound that has not yet been identified but may be attributed to a cyclic acetal (relative abundance in brackets) [m/z: 29(28), 31(13), 43(97), 44(100), 45(96), 57(17), 88(6), 103(17), 117(5)] whose quantity decreased from the first to the fourth *vassello*. In the fifth *vassello* only 2-furyl methyl ketone was present.

Among the enolic derivatives it is interesting to note the presence of cyclotene, maltol and 5-hydroxy-maltol also found in sweet wines (CUTZACH *et al.*, 1998a,b). Cyclotene can be formed by the Maillard reaction and also from lactic acid or lactic aldehyde formed from fructose (CUTZACH *et al.*, 1999). Instead maltol and 5-hydroxy-maltol are related to the use of red grape and the presence of oxygen. These molecules have been reported to be responsible for a slight "toasty" aroma of wines aged in the cask (CUTZACH *et al.*, 1997). Maltol and 5-hy-

droxy-maltol were also found, due to the thermal degradation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one, one of the most important intermediates of Maillard reactions in model systems (KIM and BALTES, 1996).

Considerable quantities of lactones were detected. γ -Lactones and solerone come from cyclization of hydroxylated fatty acids during alcoholic fermentation (DUFOSSÉ *et al.*, 1994). Pantolactone has also been identified in aged sweet wines (CUTZACH *et al.*, 1998b) and sherry (WEBB *et al.*, 1967). Dehydromevalonic lactone which was quantitatively very important in battery A has never been reported for wine and vinegar aroma.

α -Angelica lactone from sugar degradation (VERNIN and PÁRKÁNYI, 1982) and whiskey lactone (β -methyl- γ -octalactone) from wooden oak barrels (FEUILLAT, 1999) were also present.

γ -Nonalactone and γ -decalactone were not found in the balsamic vinegars although they have been found in wines (DUFOSSÉ *et al.*, 1994; CUTZACH *et al.*, 1998b). On account of ring cleavage these compounds may have given origin to γ -hydroxyacid. The corresponding esters come from the esterification between γ -hydroxyacid and ethanol. In fact the presence of two compounds identified as ethyl 4-hydroxynonanoate (relative abundance in brackets) [m/z: 29(60), 57(18), 73(7), 85(100), 102(3), 131(20)] and ethyl 4-hydroxydecanoate (relative abundance in brackets) [m/z: 29(52), 57(11), 73(3), 85(100), 102(4), 131(25), 159(5)] were found in the first barrels.

The other identified compounds were the nitrogen-containing heterocyclic products (e.g. pyrroles and 2-pyrrolydinone) produced from Strecker oxidative degradation of amino acids in the presence of α -dicarbonyl compounds (VERNIN and PÁRKÁNYI, 1982).

The small quantities of hydrocarbons found may come from the wooden barrel (SEFTON *et al.*, 1990), and they were more abundant in batteries B and C.

Considerable quantities of acids were also found. Their concentrations generally increased until the third *vasello*, after which they decreased due to esterification. Syringol and tyrosol were among the phenols found in all the batteries. Tyrosol may come from tyrosine transformation due to the action of yeast during alcoholic fermentation or from the wooden barrel (FABIOS *et al.*, 2000). Volatile phenols are more easily leached from the younger *vaselli* (CUTZACH-BILLARD, 2000) and so greater amounts were found in vinegars from battery C and in particular in *vaselli* n. 2 and n. 4.

4-ethyl-phenol was practically absent as there was no lactic bacteria action nor *Brettanomyces* to explain the production of this phenol (FLANZY, 1998).

Linalool oxides produced by the oxidation of diols or linalool (VERNIN, 1997), normally present in grape varieties used for vinegar production, were present in all batteries.

Oxygenated compounds such as glycol acetates and cyclic acetals were among the numerous unidentified compounds.

An unknown compound (relative abundance in brackets) [m/z: 29(95), 31(15), 39(16), 43(67), 45(100), 57(33), 58(30), 85(76), 86(80), 87(4), 88(4)], present in two isomer structures (labelled as "Compound A" and "Compound B" in Table 2), increased from *vasello* n. 1 to *vasello* n. 5 and was found mostly in battery A. This compound probably contains a sulphur atom as shown by the characteristic isotopic ratio between ions m/z 86 and m/z 88.

CONCLUSIONS

Balsamic vinegar flavour is very complex and is still largely unknown. However the products of alcoholic and acetic fermentation such as ketones, aldehydes, ethyl esters and ethyl acetates are very important. Also the products of the

Maillard reactions are very interesting and probably formed during cooking of the must and the long aging in warm places and with oxygen. Of particular importance are furfural, 5-methyl-2-furaldehyde, 5-hydroxymethylfurfural or HMF, 5-acetoxymethylfurfural and 2-furoic acid. The acetification techniques and acetification battery (*vaselli* number, wood, *vaselli* age, their position within the battery) are important for their formation. In fact although the three batteries examined were started simultaneously and handled in the same manner, the volatile composition was very different. There was a small number of volatile compounds in the products of the younger *vaselli* with respect to those present in the older battery and also their concentration was less. The volatile compounds from the wooden barrel were absent in the battery with older *vaselli*. Although it was not possible to study the effect of the wood on the composition of the balsamic vinegars, the effect of mulberry is evident because its porosity leads to concentration of the product and oxidation.

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REFERENCES

- Anklam E., Lipp M., Radovic B., Chiavaro E. and Palla G. 1998. Characterisation of Italian vinegar by pyrolysis-mass spectrometry and a sensor device (electronic nose). *Food Chem.* 61: 243.
- Asai T. (Ed.). 1968. "Acetic Acid Bacteria" University Tokyo Press, Tokyo.
- Charles M., Martin B., Ginies C., Etlevant P., Coste G. and Guichard E. 2000. Potent aroma compounds of two red wine vinegars. *J. Agric. Food Chem.* 48: 70.
- Chiavaro E., Caligiani A. and Palla G. 1998. Chiral indicators of ageing in balsamic vinegars of Modena. *Ital. J. Food Sci.* 10: 329.
- Coppini D., Plessi M. and Monzani A. 1978. Ulteriore contributo allo studio dell'aceto balsamico. *Riv. Vitic. Enol.* 31: 351.
- Corradini F., Marcheselli L., Marchetti A., Preti C. and Biancardi C. 1994. Analysis of heavy metals in Aceto Balsamico Tradizionale di Modena by flame atomic absorption spectroscopy. *J. AOAC Int.* 77: 714.
- Cutzach-Billard I. 2000. Évolution de l'arôme des vins doux naturels non muscatés au cours de leur élevage et de leur vieillissement. *Revue Fr. Oenol.* 27: 13.
- Cutzach I., Chatonnet P., Henry R. and Dubourdieu D. 1997. Identification of volatile compounds with a "toasty" aroma in heated oak used in barrelmaking. *J. Agric. Food Chem.* 45: 2217.
- Cutzach I., Chatonnet P. and Dubourdieu D. 1998a. Study on sweet natural non muscat wine aroma. 1st part: qualitative analysis of sweet natural wines aroma found during ageing. *J. Int. Sci. Vigne Vin* 32: 99.
- Cutzach I., Chatonnet P., Henry R., Pons M. and Dubourdieu D. 1998b. Study in aroma of sweet natural non muscat wines. 2nd part: quantitative analysis of volatile compounds taking part in aroma of sweet natural wines during ageing. *J. Int. Sci. Vigne Vin* 32: 211.
- Cutzach I., Chatonnet P. and Dubourdieu D. 1999. Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *J. Agric. Food Chem.* 47: 2837.
- Del Signore A. 1997. Il colore degli aceti Balsamici. *Riv. Merceol.* 36: 137.
- Del Signore A. 1998. Differentiation of balsamic vinegars using physical indexes. *J. Commodity Sci.* 37: 51.
- Del Signore A. 2000. Infrared spectra (Mid-IR) classification of balsamic vinegars. *J. Commodity Sci.* 40: 159.
- Del Signore A. 2001a. Chemometric analysis and chemical and physical parameters of Traditional Balsamic Vinegars from Modena. *J. Commodity Sci.* 41: 33.
- Del Signore A. 2001b. Chemometrics analysis and volatile compounds of traditional balsamic vinegars from Modena. *J. Food Eng.* 50: 77.
- Del Signore A., Campisi B. and Di Giacomo F. 1998. Characterization of balsamic vinegar by multivariate statistical analysis of trace element content. *J. AOAC Int.* 81: 1087.
- Del Signore A., Stancher B. and Calabrese M. 2000. Characterisation of balsamic vinegars by amino acid content using a multivariate statistical approach. *Ital. J. Food Sci.* 12: 317.
- Di Stefano R. 1991. Proposition d'une méthode de préparation de l'échantillon pour la détermination des terpènes libres et glycosides des raisins et des vins. *Bull. OIV* 64: 219.
- Dubois P. 1994. Les arômes des vins et leurs défauts. *Revue Fr. Oenol.* 145: 27.

- Dufossé L., Latrasse A. and Spinnler H.E. 1994. Importance of lactones in food flavours: structure, distribution, sensory properties and biosynthesis. *Sci. Aliments* 14: 17.
- Fabios M., Lopez-Toledano A., Mayen M., Merida J. and Medina M. 2000. Phenolic compounds and browning in sherry wines subjected to oxidative and biological aging. *J. Agric. Food Chem.* 48: 2155.
- Feuillat M. 1999. Élevage des vins blancs: incidence aromatique des fermentations en fût et de l'autolyse des levures. *Revue Fr. Oenol.* 174: 18.
- Flanzy C. (Ed.) 1998. "Oenologie. Fondements scientifiques et technologiques" Lavoisier Ed. Tec DOC, Paris.
- Giaccio M. and Del Signore A. 1997. Il contenuto di 5-acetossimtil-2-furaldeide nell'Aceto Balsamico Tradizionale di Modena e di Reggio Emilia. *Riv. Merceol.* 36: 91.
- Giaccio M., Del Signore A., Cichelli A. and Morgante A. 1996. Differenziazione dell'Aceto Balsamico Tradizionale dall'aceto balsamico comune mediante una sostanza caratteristica. *Riv. Merceol.* 35: 17.
- Giudici P. 1993. Acido gluconico: criterio di genuinità dell'aceto balsamico tradizionale. *Industrie Bevande* 22: 123.
- Giudici P., Barbagallo R.N., Altieri C. and Masini G. 1994. Origine ed evoluzione degli acidi organici durante l'invecchiamento dell'Aceto Balsamico Tradizionale. *Industrie Bevande* 23: 569.
- Gogus F., Bozkurt H. and Ercin S. 1998. Kinetics of Maillard reactions between the major sugars and amino acids of boiled grape juice. *Leben. Wissen. Technol.* 31: 196.
- G.U. 1986. Law n. 93, April 3, 1986. Riconoscimento della produzione dell'Aceto Balsamico Tradizionale di Modena o di Reggio Emilia. *Italian Official Journal* April 9, 1986.
- G.U. 1987. Law n. 191, March 3, 1987. Disciplina della produzione dell'Aceto Balsamico Tradizionale di Reggio Emilia e delle modalità di controllo. *Italian Official Journal* May 18, 1987.
- Karagiannis S., Economou A. and Lanaridis P. 2000. Phenolic and volatile composition of wines made from *Vitis vinifera* cv. Muscat Lefko grapes from the island of Samos. *J. Agric. Food Chem.* 48: 5369.
- Kim M.O. and Baltes W. 1996. On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one in the Maillard reaction. *J. Agric. Food Chem.* 44: 282.
- MAF (Ministero dell'Agricoltura e delle Foreste). 1986. Metodi ufficiali di analisi per i mosti, i vini e gli aceti. *Gazzetta Ufficiale della Repubblica Italiana* n. 161 del 14.07.1986. Istituto Poligrafico dello Stato, Roma.
- Masini G. and Giudici P. 1995. Ricerca e innovazione nella produzione di Aceto Balsamico tradizionale. *L'Enotecnico* 31: 73.
- Mascolo A. and Gavioli G. 1998. Valutazione soggettiva ed obiettiva dell'Aceto Balsamico Tradizionale di Modena. *L'Enotecnico* 34: 77.
- Meglioli G., Parisini G., Tedeschi M. and Pesenti G. 1997. L'aceto balsamico tradizionale di Reggio Emilia. Studio delle caratteristiche chimiche e chimico-fisiche. *Industrie Bevande* 26: 41.
- Plessi M., Monzani A. and Coppini D. 1988. Determination of the monosaccharide and alcohol content of balsamic and other vinegars by enzymatic methods. *Agric. Biol. Chem.* 52: 25.
- Plessi M., Monzani A. and Coppini D. 1989. Quantitative determination of acids and derivatives in balsamic and other vinegars. *Sci. Aliments* 9: 179.
- Sefton M.A., Francis I.L. and Williams P.J. 1990. Volatile norisoprenoid compounds as constituents of oak woods used in wine and spirit maturation. *J. Agric. Food Chem.* 38: 2045.
- Stacchini A., Draisci R. and Lorusso S. 1990. Tecnologia e caratterizzazione degli aceti balsamici. *Industrie Bevande* 19: 212.
- Stancher B., Calabrese M. and Saccù D. 2001. Caratteristiche di qualità dell'aceto balsamico e dell'Asperum. *Industrie Bevande* 30: 233.
- Theobald A., Müller A. and Anklam E. 1998. Determination of 5-Hydroxymethylfurfural in vinegar samples by HPLC. *J. Agric. Food Chem.* 46: 1850.
- Turtura G.C. and Rosetti A.M. 1984. Caratterizzazione dell'aceto balsamico naturale. *Industrie Bevande* 13: 197.
- Turtura G.C. and Bortolotti P.P. 1991. Caratteristiche microbiologiche e chimiche dell'aceto balsamico naturale. II. Relazioni con le tecniche di preparazione. *Ann. Microbiol.* 41: 169.
- Vernin G. and Párkányi C. 1982. Mechanisms of formation of heterocyclic compounds in Maillard and pyrolysis reactions. In "Chemistry of Heterocyclic Aroma Compounds in Flavours and Aromas" Vernin G. (Ed.) p. 151. Ellis Horwood, Chichester, U.K.
- Vernin G. 1997. Terpénoides et norisoprenoides dans les arômes des baies de raisins et dans les vins. 1. Origines et formation. *Riv. Ital. EP-POS* 22: 19.
- Webb A.D., Kepner R.E. and Maggiora L. 1967. Sherry aroma. VI. Some volatile components of flor sherry of Spanish origin. Neutral substances. *Am. J. Enol. Vitic.* 18: 190.

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