

INFLUENCE OF ACIDIC HYDROLYSIS ON THE AROMA COMPOSITION OF GRAPE MUST

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Non-polar aroma compounds of musts from four white grape varieties ('Aligoté' and 'Muscat Ottonel' from the Iași region, Romania, and 'Muscat of Alexandria' and 'Pedro Ximénez' from Montilla-Moriles, Spain) have been analyzed using Stir Bar Sorptive Extraction/Gas Chromatography/Mass Spectrometry analytical techniques, before and after acidic hydrolysis. 51 compounds were identified, and 21 compounds were quantified and subjected to Multiple-Sample Comparison (MSC) and Principal Component Analysis (PCA). By means of MSC 13 aroma compounds were selected before acidic hydrolysis and 12 aroma compounds after acidic hydrolysis for the characterization of musts by means of PCA. The analytical platform and statistical method used provide a fast and powerful tool for the characterization of the effect of acidic hydrolysis on the contents of some aroma compounds of musts.

Keywords: acidic hydrolysis, grape-musts, aroma compounds

Einfluss der sauren Hydrolyse auf die Aromenzusammensetzung von Traubenmost. Unpolare Aromastoffe von Most aus vier weißen Rebsorten ('Aligoté' und 'Muscat Ottonel' aus der Region Iași, Rumänien, und 'Muscat of Alexandria' und 'Pedro Ximénez' aus Montilla-Moriles, Spanien) wurden mittels Stir Bar Sorptive Extraction/Gas Chromatography/Mass Spectrometry-Analysetechniken vor und nach der sauren Hydrolyse untersucht. 51 Verbindungen wurden identifiziert, und 21 Verbindungen wurden quantifiziert und einer Multiple-Sample Comparison-Testung (MSC) und Principal Component Analysis (PCA) unterzogen. Mittels MSC wurden 13 Aromaverbindungen vor und zwölf nach der sauren Hydrolyse zur Charakterisierung von Mosten mittels PCA ausgewählt. Die analytische Plattform und die statistischen Verfahren, die verwendet wurden, bieten ein schnelles und leistungsfähiges Werkzeug für die Charakterisierung der Wirkung der sauren Hydrolyse auf die Gehalte einiger Aromaverbindungen des Mostes.

Schlagwörter: saure Hydrolyse, Traubenmost, Aromastoffe

Wine aroma was classified accordingly to its origin in varietal aromas, pre-fermentative, fermentative and post-fermentative aromas (MORENO and PEINADO, 2012; VILANOVA et al., 2012; COTEA et al., 2009a). The first two are so-called primary aromas and they are present in free form, with high volatility, contributing directly to the odour of musts and wines, and also in the bound form, then non-volatile and constituting the "occult aroma" of grapes and musts.

The bound aroma can be released by acidic or enzymatic hydrolysis into free volatile compounds that enhances the aroma (VILANOVA et al., 2012; KANG et al., 2010; GENOVESE et al., 2013). The enzymatic hydrolysis is the most efficient method to measure the aroma potential of grapes and musts for winemaking, but the predictive ability of the enzymatic hydrolysis strategy is rather poor compared to acidic hydrolysis (LOSCOS et al., 2009). SEFTON et al. (1994) suggest that transformations taking place during fermentation include relevant chemical rearrangements in acid media that are better predicted by acidic hydrolysis, which is considered being a more adequate method to measure the aroma potential of grapes and musts for winemaking.

In this respect, the aim of this study is to characterize the effect of acidic hydrolysis on the content of some aroma compounds of musts obtained from four grape varieties cultivated in two European Union countries, Romania and Spain.

MATERIAL AND METHODS

In this study, grapes from 'Aligoté' (AL) and 'Muscat Ottonel' (MO) from Iași (Romania) and 'Muscat of Alexandria' (MA) and 'Pedro Ximénez' (PX) from Montilla-Moriles (Spain) were crushed with a laboratory stainless steel press (Ferrari, Parma, Italy) and the must obtained was subjected to analyses in triplicate. Total soluble solid were determined by a hand refractometer, model Master M, from Atago (Tokyo, Japan), pH by a laboratory pH-meter basic model from Crison Instruments (Barcelona, Spain) and titratable acidity was quantified by titration of the musts with a solution 0,1 M of sodium hydroxide standardized, according to the Organization International of Vine and Wine (OIV) and following European Union Official Methods.

For the determination of the aroma fraction, Stir Bar Sorptive Extraction-Gas Chromatography-Mass Spectrometry technique was used. The free aroma compounds were absorbed on a polydimethylsiloxane (PDMS)-coated stir bar or Twister®, with 0.5 mm film thickness and 10 mm length, from Gerstel GmbH (Mülheim an der Ruhr, Germany) at room temperature during 100 min at 1200 rpm. The hydrolysis of the bound fraction of aroma compounds was carried out at 70 °C for 2 hours by adding a 2 M citric acid solution to 1 ml of grape-must to a pH value of 2.5 according to the method of PEDROZA et al. (2010). After hydrolysis, the samples were cooled at room temperature and the same protocol used for the analysis of free aroma fraction was applied. The compounds were transferred to an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass detector (Agilent Technologies, Palo Alto, California, USA) and provided with a fused silica capillary column (HP-5MS stationary phase 30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness from Agilent Technologies (Delaware, USA)). The initial oven temperature was set at 50 °C (held for 2 min), then raised to 190 °C at 4 °C/min and held for 10 min. For mass spectrometry analysis, electron impact mode (EI) at 70 eV was used. The mass range varied from 35 to 550 amu and the detector temperature was 150 °C. Three replicates were carried out for each analysis.

The quantification of aroma compounds was performed using standard solutions of the studied compound. Each compound was quantified from its response factor, which was determined by using standard solutions of known concentration subjected to the same treatment as the samples, and the target ions and qualifier ions were selected for each compound by the Hewlett-Packard Chemstation (Palo Alto, California, USA).

The characterization of the effect of acidic hydrolysis on the content of some aroma compounds of musts was based on multivariate statistical analysis using Statgraphics® Centurion XVI Software Package from Stat Points Technologies, Inc. (Warrenton, Virginia, USA). The multivariate statistical methods used in this study were Multiple-Sample Comparison (MSC) and Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

In this work, musts obtained from two floral (MO and MA) and two non-floral grape varieties (AL and PX) were analyzed by their composition in free and total aroma compounds. Table 1 shows the most important enological variables for the characterization of grape musts prior to being subjected to the winemaking process. All the parameters showed in Table 1 indicate that the musts are suitable for table wine.

There were identified 51 aroma compounds before and after acidic hydrolysis of the musts obtained from each grape variety studied (Table 2). Depending on the functional groups and similarity of chemical structures, the identified compounds were classified in 6 groups, as follows: 14 compounds are classified as terpenes and norisoprenoids, 7 as aldehydes and ketones, 2 as alcohols, 7 as benzene compounds, 8 as acids and 13 are classified as ethyl esters. This classification has the advantage that the components in the same group have similar aroma descriptors (Table 2), in addition to their similar physical and chemical properties.

Of the 51 compounds identified, 21 were quantified in free form, before acidic hydrolysis (Table 3) and 20 were quantified in musts after acidic hydrolysis (Table 4). Even that there were more compounds in quantifiable levels in musts, the laboratory possibilities did not allow quantifying more compounds due to lack of pure compounds. Also, data obtained and presented in Tables 2 to 4 cannot be considered as an absolute response or content in grape musts because of the limitations of the used Twisters, which are covered with the PDMS adsorbent. This is a non-polar adsorbent and, consequently, only the non-polar compounds of the musts are preferentially adsorbed (NIE and KLEINE-BENNE, 2011).

By summing the relative area of each individual compound shown in each table for the six established groups it is possible to establish an effective comparison among the musts of studied varieties (Fig. 1). Concerning the terpenes and norisoprenoids, they were found in higher quantities in MO and MA, as expected, because Muscat and Muscat-like grapes are characterised by a high concentration of terpenes in both free and bound forms (GÜNATA et al., 1985; CORDONNIER and BAYONOVE, 1974; COTEA et al., 2009b).

The aldehydes and ketones contents were higher after acidic hydrolysis in all the samples, as well as the alcohol contents. The contents in benzene compounds and acids decrease after acidic hydrolysis in all samples, while the content in ethyl esters slightly increases after acidic hydrolysis (Fig. 1). This can be explained by the chemical reactions that take place during the acidic hydrolysis, similar to the ones from the wine making process, reactions that release the bound aroma compounds.

From data shown in Tables 2 and 3, only 13 aroma compounds before acidic hydrolysis and 12 aroma compounds after acidic hydrolysis were selected by Multiple-Sample Comparison analysis, for further statistical analyses. There were only the compounds selected that established three or more homogeneous groups (HG), in correspondence with the four grape varieties.

The 13 selected free volatile compounds were subjected to PCA in order to reduce the dimensions and investigate which differences of the four grape varieties before and after acidic hydrolysis could be visualised. Two PC are selected explaining 77.85 % of the total variance in the data set (Fig. 2), having the eigenvalue 6,37 for PC1 and 3,75 for PC2. In the biplot obtained this way samples were separated along the PC1 and PC2, MO being separated from the other varieties by PC1 (has positive values on PC1), PC2 allowing to differentiate the other three varieties (AL has the highest value on these PC, MA has a value close to 0 and PX has a negative value) (Fig. 2). The compounds with the highest contribution for PC1 were furfural, nonanal, 1-hexanol, benzaldehyde, 2-phenethyl acetate and ethyl heptanoate having a coefficient value higher than 0.3 and showing odour type as sweet, woody; fat, citrus; green, fruity; bitter, oily; fruity and fruity, fresh, in the order as listed. For PC2 the compounds that contributed the most were limonene, n-decanoic acid, n-hexadecanoic acid, hexyl acetate and ethyl heptanoate with the same selecting criteria and with odour type as citrus, herbal; rancid, sour; waxy, fatty; fruity, fresh and fruity.

A PCA was also performed with the aroma of musts after acidic hydrolysis, using the concentration of the 12 compounds that established three or more homogeneous

groups allowing the characterization of the four musts studied (Fig. 3). The first two PC selected, explaining 82.76 % of total variance, have the eigenvalue 5,88 for PC1 and 3,7 for PC2.

From Figure 3 one can conclude that MO has the highest values on both PC1 and PC2, being differentiated from all the other samples. Actually, it seems like all the samples are separated one from the other, each sample

occupying one separate quartile of the graphic area.

The results obtained by MSC and PCA for the aroma compounds before and after acidic hydrolysis suggest that these hydrolyses can be considered as an overview of the evolution of the primary aroma compounds (varietal and pre-fermentative) through the wine making process at the acidic pH of must and wine.

Table 1: Technological characteristics of musts from four grape varieties. Mean concentrations and standard deviations (n = 3)

Must composition	Aligoté	Muscat Ottonel	Muscat of Alexandria	Pedro Ximénez
pH	3.61a ± 0.01	4.15c ± 0.02	3.923b ± 0.006	4.14c ± 0
Titrateable acidity (g/l)*	5.65d ± 0.05	3.47a ± 0.06	5.02c ± 0.07	3.78b ± 0
Solid fraction (°Bx)	19.43b ± 0.05	23.0d ± 0.1	18.93a ± 0.06	21.3c ± 0.1
Sugars (g/l)	191.9b ± 0.5	226d ± 1	179.8a ± 0.6	207c ± 1
Ethanol potential (% v/v)	11.4b ± 0.1	13.45d ± 0.07	10.68a ± 0.03	12.3c ± 0.06

* Expressed as tartaric acid

^{a, b, c, d}: Different letters in the same row indicate statistical differences at the 0.05 level according to the Fisher's least significant difference method.

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Table 2: Non-polar compounds identified in musts

No.	Compound name	CAS	Odour descriptor
1	Linalool ²	78-70-6	Flower, lavender, lemon and lime-like, slight herbal and floral nuance
2	Geraniol	106-24-1	Sweet, floral, fruity, rose, citrus
3	Limonene ¹	138-86-3	Citrus, herbal, terpene, camphor, sweet
4	Ocimenol	5986-38-9	Fresh citrus, lemon, lime, cologne, sweet, mace spice
5	Beta-ocimene	3779-61-1	Citrus, herb, flower, sweet
6	Ocimen quinotoxide	7416-35-5	Woody, terpy, citrus, lime
7	Gamma-Terpinene	99-85-4	Terpy, citrus, lime-like, oily, green, tropical fruity
8	(E)-Beta-Damascenone	23726-93-4	Apple, rose, honey, tobacco, sweet
9	Nerol oxide	1786-08-9	Green, vegetative, floral, leafy and waxy with an herbal, minty depth
10	Dehydroxylinalool oxide	13679-86-2	Woody, piney, spicy, minty with a green citrus nuance
11	Nerol ²	106-25-2	Lemon, bitter, green and fruity with a terpy nuance
12	(Z)-Citral ¹	106-26-3	Sweet, citral, lemon peel
13	Vitispirane	65416-59-3	Fruity, floral, earthy, woody
14	(E,E)-2,6-Dimethyl-1,3,5,7-octatetraene (Cosmene)	460-01-5	Floral. Isolated in lavender (TSCHIGGERL et al., 2010); <i>Akebia trifoliata</i> (XIAO et al., 2013); <i>Lilium</i> (ZHANG et al., 2013)
15	2-Hydroxy-2-cyclopenten-1-one	10493-98-8	Found in roasted coffee (NIEMELA, 1988); in <i>Aphanamixis grandifolia</i> (LIU et al., 2010). No descriptor available
16	Furfural ¹	98-01-1	Sweet, brown, woody, bread, caramel, with a slight phenolic nuance
17	Hexanal ³	66-25-1	Green, woody, vegetative, apple, grassy, citrus and orange
18	2-Hexenal ¹	505-57-7	Fresh green, leafy, fruity with rich vegetative nuances
19	Octanal	124-13-0	Green with a citrus, orange peel note
20	Nonanal	124-19-6	Fat, citrus, green
21	Decanal ¹	112-31-2	Waxy, fatty, citrus and orange peel with a slight green melon nuance
22	1-Hexanol ²	111-27-3	Green, fruity, apple-skin, oily
23	2-Furanmethanol	98-00-0	Alcoholic, chemical, musty, sweet, caramel, bread, coffee
24	Benzaldehyde ¹	100-52-7	Sweet, bitter, oily, almond, cherry, nutty, woody
25	Benzophenone ³	119-61-9	Balsam, rose, metallic, powdery geranium
26	Phenol ³	108-95-2	Phenolic, plastic, rubber
27	Phenol, 2,4-bis(1,1-dimethylethyl)	96-76-4	Phenolic
28	3,6-Dimethyl-2,3,3a,4,5,7a-hexahydro-benzofuran	70786-44-6	Herbal dill
29	Naphthalene, 1,2-dihydro-1,1,6-trimethyl	30364-38-6	Licorice, petroliferous
30	3-Buten-2-one, 1-(2,3,6-trimethylphenyl)	54789-45-6	Found in brandy (ZHAO et al., 2008). No descriptor available
31	Nonanoic acid	112-05-0	Waxy, dirty, cheese cultured dairy
32	n-Decanoic acid ²	334-48-5	Unpleasant, rancid, sour, fatty, citrus
33	Dodecanoic acid ¹	143-07-7	Metal, mild, fatty, coconut, bay oil
34	Tetradecanoic acid	544-63-8	Waxy, fatty, soapy, creamy, cheesy, with a good mouth feel
35	Pentadecanoic acid	1002-84-2	Waxy
36	n-Hexadecanoic acid	57-10-3	Waxy, creamy, fatty, soapy
37	cis-9-Hexadecenoic acid	373-49-9	Waxy, creamy, fatty, soapy
38	9,12-Octadecadienoic acid	60-33-3	Faint, fatty
39	1-Butanol, 3-methyl-, acetate	123-92-2	Sweet, fruity, banana, solvent
40	2-Phenylethyl acetate	103-45-7	Fruity, rose, sweet, honey, tropical
41	Hexyl acetate ²	142-92-7	fruity, green, fresh, sweet, banana peel, apple and pear
42	Hexyl butanoate ¹	2639-63-6	Fruity, green, sweet, apple, waxy
43	Ethyl heptanoate ¹	106-30-9	Fruity, pineapple, banana and strawberry with a spicy, oily nuance
44	Ethyl octanoate ²	106-32-1	Sweet, waxy, fruity and pineapple with creamy, fatty, mushroom and cognac notes
45	Ethyl decanoate ²	110-38-3	Waxy, fruity, sweet, apple
46	Octanoic acid, 2-methyl-ethyl ester	30982-02-6	Floral, sweet
47	Phenethyl butyrate ¹	103-52-6	Fruity, floral, green with a tropical winey nuance
48	Phenethyl isobutyrate ¹	103-48-0	Heavy, honey, floral, aldehydic with floral nuances
49	Phenethyl phenyl acetate	102-20-5	Honey, floral, green, rose, cocoa, hay
50	Hexyl hexanoate	6378-65-0	Sweet, fruity and green with tropical notes
51	Isoamyl butyrate ¹	106-27-4	Fruity, green, apricot, pear, banana

Compounds were identified by linear retention index (VAN DER DOOL and KRATZ, 1963) in a HP-5MS capillary column (30 m/0.25 mm/0.25 µm, He) and MS spectrum from Wiley and NIST libraries; n.f. = not found; Superscripts indicate compounds identified also by MS spectrum of standard provided by: ¹ Sigma Aldrich, ² Fluka and ³ Merck

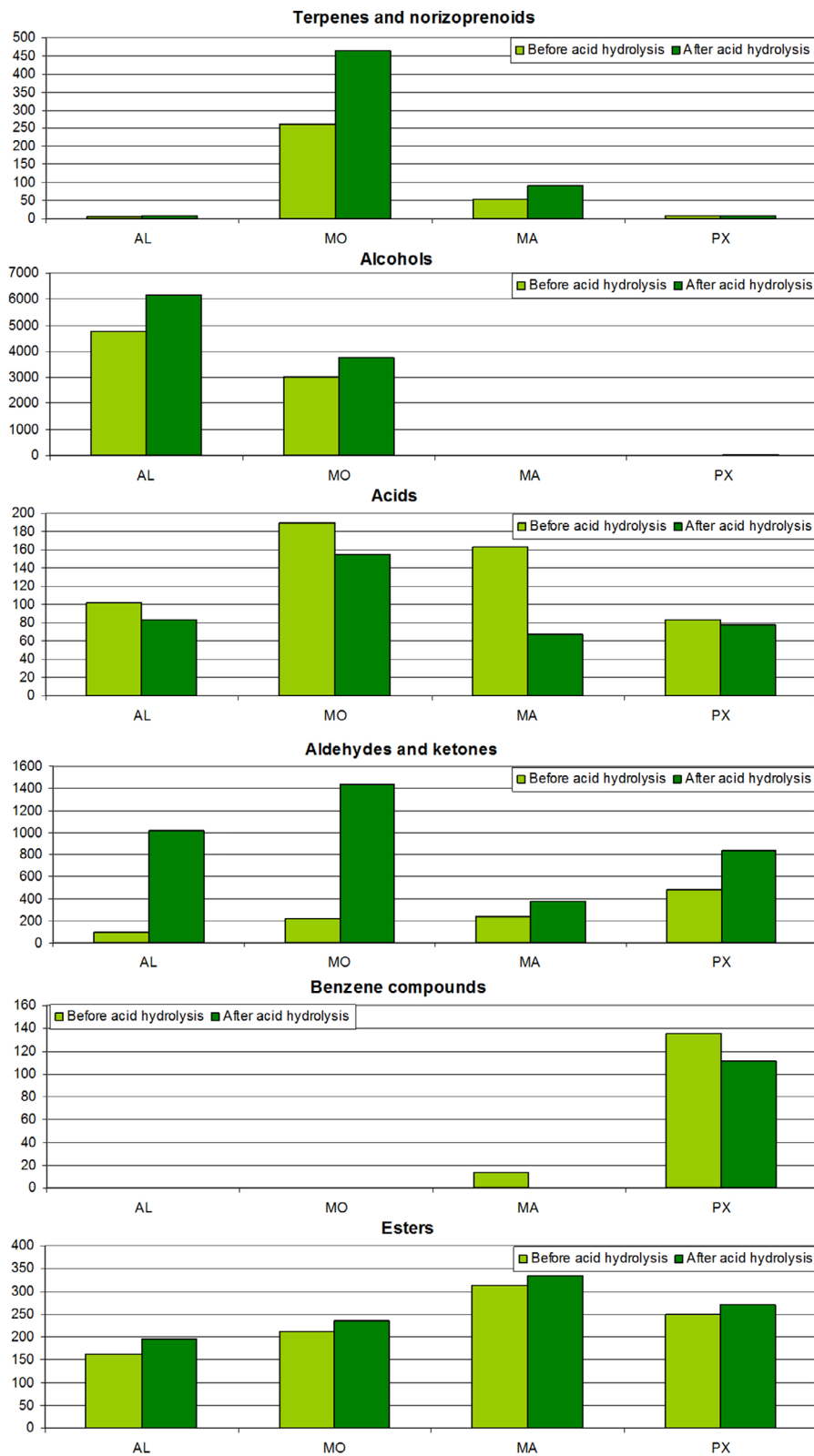


Fig. 1: Non-polar aroma compounds grouped by chemical families, before and after acidic hydrolysis

Table 3: Concentration ($\mu\text{g/l}$) of free non-polar aroma compounds of musts (mean of $n = 3$; standard deviations and homogeneous groups)

No.	Aligoté	Muscat Ottonel	Muscat of Alexandria	Pedro Ximénez
1	^a n.f.	^b 156.1±16.1	^a 9.31±1.01	^a n.f.
2	^a n.f.	^b 90.41±6.09	^a n.f.	^a n.f.
3	^b 5.67±0.26	^c 9.01±0.43	^a 43.15±6.21	^c 8.87±0.33
12	^a n.f.	^b 5.93±0.02	^a n.f.	^a n.f.
16	^a 47.55±4.08	^b 93.80±6.06	^c 124.88±5.29	^d 285.09±9.01
17	^a n.f.	^b 2.85±0.52	^a n.f.	^c 3.71±0.25
19	^a n.f.	^b 9.50±0.22	^a n.f.	^a n.f.
20	^a 36.4±0.3	^c 110.30±10.55	^b 86.14±2.04	^d 168.72±2.57
21	^b 6.68±0.26	^a 1.90±0.13	^d 28.10±2.03	^c 21.76±2.37
22	^c 4792.9±111.3	^b 3018.8±167.7	^a n.f.	^a n.f.
24	^a n.f.	^a n.f.	^b 13.56±0.38	^c 135.47±7.49
32	^a n.f.	^c 113.7±7.5	^b 81.5±4.3	^a n.f.
36	^c 102.85±3.55	^a 75.37±4.02	^b 82.50±2.93	^b 83.92±2.42
40	^b 8.74±0.77	^a n.f.	^a n.f.	^c 33.26±3.72
41	^d 21.24±0.38	^b 51.75±0.12	^a n.f.	^c 6.13±0.33
43	^b 84.4±4.3	^a n.f.	^a n.f.	^c 200±14.6
44	^a 7.10±0.28	^a 66.86±0.46	^c 162.48±0.97	^b 11.25±0.27
45	^b 0.77±0.19	^b 40.94±0.02	^a n.f.	^a n.f.
46	^b 37.82±0.81	^a n.f.	^a n.f.	^a n.f.
49	^a n.f.	^a n.f.	^a n.f.	^b 29.93±2.77
50	^b 1.82±0.12	^b 51.58±0.13	^a 150.99±0.18	^a 1.24±0.12

For compound names, see Table 2; n.f. = not found; ^{a, b, c, d}: Different letters in the same row indicate statistical differences at 0.05 level according to Fisher's least significant difference method.

Table 4: Concentration ($\mu\text{g/l}$) of non-polar aroma compounds of musts after acidic hydrolysis (mean of $n = 3$; standard deviations and homogeneous groups)

No.	Aligoté	Muscat Ottonel	Muscat of Alexandria	Pedro Ximénez
1	^a n.f.	^b 181.13±0.91	^c 14.32±0.23	^a n.f.
3	^a 8.56±0.32	^d 282.5±5.6	^c 78.33±0.18	^b 10.23±0.41
16	^b 202.65±7.72	^c 355.7±2.9	^a 128.6±3.2	^d 488.7±5.7
17	^a n.f.	^a n.f.	^a n.f.	^b 9.46±0.21
19	^d 39.61±3.33	^c 29.18±2.87	^b 15.7±0.86	^a 4.40±0.04
20	^c 753.69±7.17	^d 1006.27±0.81	^a 203.75±4.22	^b 310.38±1.84
21	^a 24.16±4.25	^b 44.72±1.18	^a 23.81±0.16	^a 25.56±1.91
22	^c 6189.6±50.6	^b 3764.9±36.7	^a n.f.	^a n.f.
23	^a n.f.	^a n.f.	^a n.f.	^b 40.89±1.83
24	^a n.f.	^a n.f.	^a n.f.	^b 110.85±0.94
32	^a n.f.	^b 38.51±1.08	^a n.f.	^a n.f.
33	^a n.f.	^B 73.7±11.8	^a n.f.	^a n.f.
34	^c 8.28±0.15	^c 8.18±0.13	^a 4.34±0.06	^b 6.8±0.8
36	^c 75.2±2.5	^a 34.3±3.3	^a 62.4±1.2	^b 70.5±1.8
40	^a n.f.	^a n.f.	^c 39.5±0.5	^b 22.4±1.9
41	^a n.f.	^b 85.9±0.9	^c 6.8±0.2	^b 4.52±0.08
43	^b 185.2±3.4	^a n.f.	^d 239.5±0.9	^c 230.43±1.07
44	^a 10.9±0.7	^c 93.7±0.6	^{ab} 11.53±0.04	^{bc} 12.52±1.06
45	^a n.f.	^b 56.8±0.2	^a n.f.	^a n.f.
46	^a n.f.	^a n.f.	^b 36.3±1.9	^a n.f.

For compound names, see Table 2; n.f. = not found; ^{a, b, c, d}: Different letters in the same row indicate statistical differences at 0.05 level according to Fisher's least significant difference method.

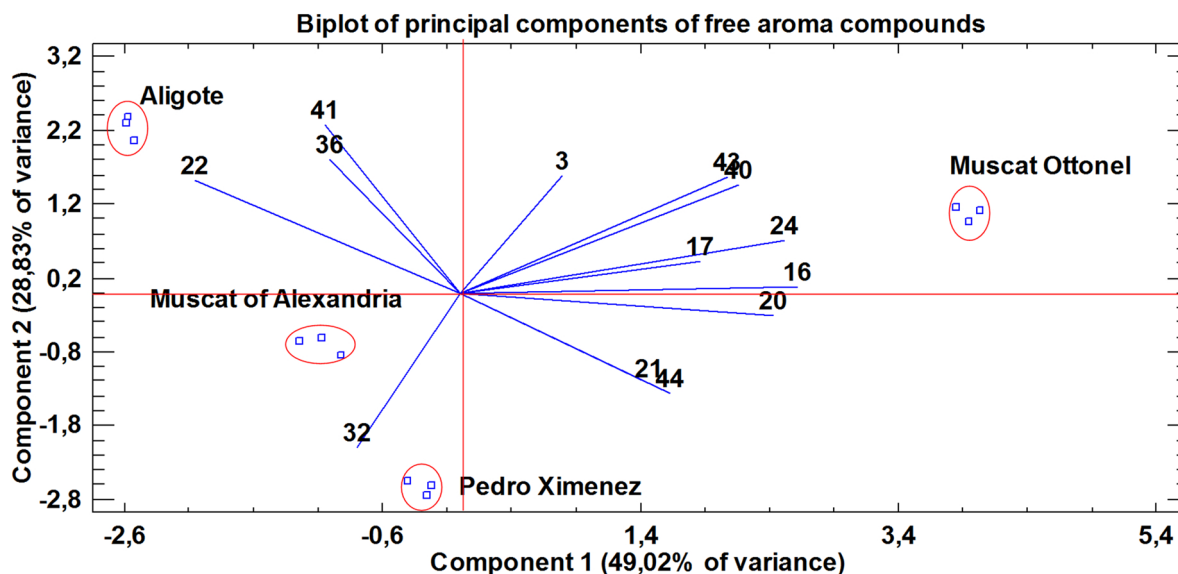


Fig. 2: Principal component analyses based on the concentration ($\mu\text{g/l}$) of selected 13 free aroma compounds in musts from four grape varieties; for compound name see Table 2

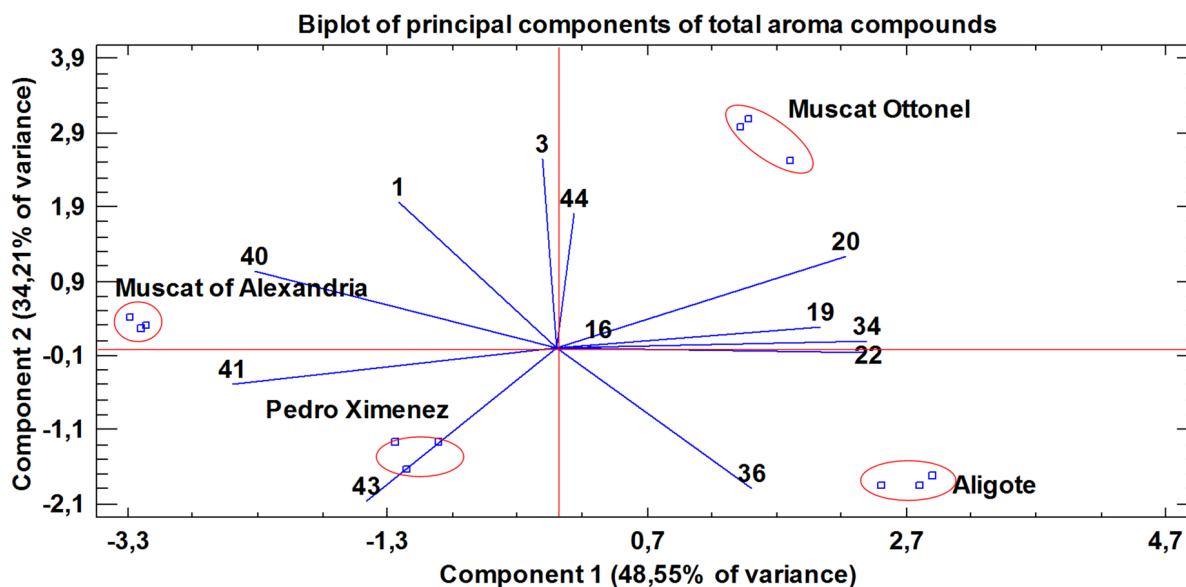


Fig. 3: Principal component analyses based on the concentration ($\mu\text{g/l}$) of selected 12 aroma compounds in musts from grape varieties after acidic hydrolysis; for compound name see Table 2

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