

**EFFECT OF ETHANOL LEVEL IN THE PERCEPTION
OF AROMA ATTRIBUTES AND THE DETECTION
OF VOLATILE COMPOUNDS IN RED WINE**

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ABSTRACT

Sensory profile of 23 monovarietal Malbec wines were evaluated and related to headspace composition of aroma at two alcohol levels (10.0-12.0 to 14.5-17.2 %, v/v). Twelve attributes were selected by quantitative descriptive analysis. At $P < 0.01$ two attributes showed lower aromatic intensity when alcohol level increased, and at $P < 0.05$ three attributes showed lower intensity; only one attribute showed higher intensity ($P < 0.05$). Seventeen aroma compounds were identified using solid-phase micro-extraction gas chromatography (GC). Only one identified aroma compound showed lower contribution when alcohol level increased ($P < 0.01$); another aroma was added at $P < 0.05$. Only one aroma showed higher contribution ($P < 0.05$). Ethanol influenced the relative contribution of aroma compounds in different way: some declined while and others increased. The sensorial aroma perception was also changed; when ethanol was at 14.5 to 17.2%, the odor was described just as herbaceous instead of fruitiness as was perceived at low ethanol levels.

Practical Applications

The general tendency in the wine industry is the search for stronger wines with high level of alcohol. The most common reason given for this practice is that winemakers are concerned in soften tannins in the grapes on the vine, and so they have to pick grapes later in the ripening cycle. A longer hang time also produces more fruit flavors and fewer vegetal ones, up to a certain level where alcohol produces a decrease of fruity aromas. Many of these wines are considered out of balance, and dominated by ethanol-associated attributes. The contribution of this study is to outline the changes of aroma when the alcohol in wine is raised.

Key words: Sensory evaluation; volatile compounds; wine; SPME–GC-MS; aroma; alcohol; Principal Component Analysis.

INTRODUCTION

Release of volatile flavour materials from alcoholic beverages depends not only on the concentration of the volatiles in the solution, but is affected by interactions between volatiles, by the presence of various non-volatile materials and by the ethanol concentration. Ethanol is the most abundant of the volatile compounds in wine and it could modify both, the sensory perception of aromatic attributes as the detection of volatile compounds.

With regard to the detection of volatile, one of the factors sometimes encountered when developing quantitative extraction methods, is competition effects related to other matrix components. Samples can contain percent levels of solvents (ethanol, for example) that can alter distribution coefficients between the aqueous and the headspace. For the solid-phase micro extraction (SPME) fiber, recovery of C4-C10 methyl esters is significantly reduced in the presence of 10% ethanol (Pfannkoch *et al.* 2002). The effect of wine matrix ingredients and conditions on the headspace (HS) sampling of 3-alkyl-2-methoxypyrazines was investigated with SPME and capillary gas chromatography by Hartmann *et al.* (2002). Changing the sample ethanol concentration from 0 to 20% (v/v) resulted in an exponential decrease in the recovered analytes. Ethanol increased the solubility of the pyrazine analytes in the aqueous phase, shifting the equilibrium concentration away from the headspace. Ethanol is also a volatile compound, present at several million times the concentration of the analytes that competes strongly for solubility on the SPME fiber. These two effects combine to reduce the effectiveness of SPME for pyrazine analyte extraction from aqueous ethanol solutions. Câmara *et. al* (2006) stated that there are no statistically significant differences for pH range of 1.2-5.9 on detection of monoterpenols and

norisoprenoids in Madeira wines, but a significant effect was found on the ethanol content indicating a decrease in the extraction yield.

With regard to the sensory perception, Escudero *et al.* (2007) evaluated the effect of ethanol on the perception of fruitiness from mixtures of nine fruity compounds at the maximum concentrations found in the wines. When there is no ethanol in the mixture, the smell is strong; however, the intensity of the smell decreases with the amount of ethanol present in the mixture; at 10% ethanol the intensity of the fruity odor is much less intense, while at 12% is just barely perceptible, and at 14.5% it is not longer perceived. Le Berre *et al.* (2007) studied physico-chemical and perceptual interactions between woody and fruity odors in aqueous and dilute alcohol solutions. These results demonstrate that a reduction in alcohol content in wine can affect the aromatic bouquet, especially by reinforcing perceptual interactions between woody and fruity wine odorants but also by modifying their chemical proportions.

As shown above, the influence of ethanol on the perception and the detection of wine aromas have only been studied in model solutions and not on wines.

The aim of this study was to explore the influence of alcohol in the detection of volatile compounds identified by SPME-GC and in the perception of sensory characteristics of aroma in specially designed wines having two different alcohol levels: high (14.5-17.2%) and low (10.0-12.0%).

MATERIALS AND METHODS

Wine Samples

Twenty three “non commercial” monovarietal Malbec wine samples (2004 vintage) were obtained from fermentation tanks from different wineries and

elaborated under standardized conditions without wood treatment, carbonic gas or additives. Sensory studies were first performed, and then samples were frozen at -18°C (to avoid esters hydrolysis) for chromatography determinations.

Samples were selected from a set of fifty six, studied in a previous work (Goldner and Zamora 2007), according to their alcohol level (AOAC 1990, method): high (range 14.5-17.2%; samples 1-12) and low (range 10.0-12.0%; samples 13-23).

SPME analysis of volatile compounds

A manual SPME holder (Bellefonte, PA, USA) was used for evaluation of volatile compounds. A 100 µm polydimethylsiloxane (PDMS) coated fused-silica fiber was used for absorption of volatile substances from the headspace of properly conditioned samples.

Before the extraction, the fiber was conditioned for 15min at 255°C in the injection port of the gas chromatograph. Samples (8 mL) were placed into a 20 mL glass vial and for each extraction were saturated with sodium chloride (2.0 g) and the vial was capped with a septum. Wine samples were heated at 40°C for 30 min by an ultrasonic bath (Branson 2510) with the fiber introduced into the headspace through the septum and exposed to the vial headspace.

Gas chromatography (GC) – Mass spectrometry

GC-FID-MS analysis was carried out on a Perkin Elmer Clarus 500, with one injector (split ratio 1:100) connected by a flow splitter to two capillary columns: a) polyethylene glycol PM ca. 20.000 and b) 5% phenyl-95% methyl silicone, both 60 m x 0,25 mm with 25µ of fixed phase. The whole system operated at a constant flow of 1.87 ml/min. Helium was used as gas carrier. The polar column was connected to a FID, while the non polar column was connected to a FID and a quadrupolar mass detector (70 eV) by a vent system (MSVent™). Temperature was programmed according to the following gradient: 40°C during 5 minutes,

then until 230°C at 6°C/min, and then isothermic for 6 min. Injector and both FIDs were set at 255°C and 240°C, respectively. Temperatures of the transference line and the ionic source were 180°C and 150°C, respectively; the range of masses (m/z) was 40-300 Da.

Identification of the compounds was performed from the retention indices (relative to C₈-C₂₀ *n*-alkanes) in both columns, compared with those of reference compounds, and by comparison of mass spectra using the usual libraries (Adams 2001; Mc Lafferty and Stauffer 2000) and mass spectra obtained from reference compounds. A relative percent contribution of the compounds was calculated according to the area of the chromatographic peaks (FID response), assuming all of the response factors were 1.

Sensory analysis

Ten paid not sighted assessors (4 females and 6 males, 21-55 years old) from the panel of Staffing and Training Group (S & TG), Buenos Aires consulting company, were trained in descriptive analysis of Malbec wines (Goldner and Zamora 2007). Descriptive Analysis (Stone and Sidel 1993; ASTM 1992) was made using 9-point intensity scales and the panel leader recorded the scores in oral way. All samples (50 mL) were poured from a single bottle (750 mL), presented at 18 ± 2°C in tulip-shaped transparent glasses, covered with glass petri dishes and identified by random three-digit codes. The samples were expectorated, and mineral water was provided for oral rinsing along with unsalted crackers. A randomized incomplete block design was used to evaluate all the wines. Eight samples were presented for session in the morning (2.5 hours) and the duplicate in the afternoon (2.5 hours). Training was with aroma standards over 10 hs (Goldner and Zamora 2007). The following aromas were selected for descriptive analysis: fruity, citrus, strawberry, plum, raisin, cooked fruit, floral, honey, herby, spicy and sweet pepper.

Data analysis

One way analysis of variance (ANOVA) was carried out to assess attributes and volatile compounds significantly different among wines from two level of alcohol. The variability among assessors was studied using an ANOVA model where assessor and wine were considered as random factors, and replication as fixed factor (SPSS version 13.0, Inc., Chicago, IL). Pearson correlation was calculated between sensory and GC data. Principal Component Analysis (PCA) of average panel and GC data was evaluated to compare the relationship among sensory attributes and volatile compounds. Covariance matrix was used and the minimum eigenvalue was set at 1. Partial least-squares regression (PLS, Infostat v. 2007, Universidad Nacional de Córdoba, Argentina) was used to explore relationships between GC (X-variables, regressors: predicting) and sensory data (Y-variables, regressands: predicted) at two levels of ethanol.

RESULTS AND DISCUSSION

Applying one-way ANOVA to sensory data, six aroma attributes (four at $P < 0.05$ and two at $P < 0.01$; Table 1) were significant at both ethanol range. Aroma intensity decreased when increasing ethanol level, except for herby attribute. It is interesting to highlight the reduction in fruity/strawberry scores and the increase in herby ones when alcohol level was taking into account. These results are in agreement with Escudero *et al.* (2007) who found that ethanol exerts a strong suppression effect on fruitiness.

The assessors showed a good reproducibility (replication factor was not significant) and a good consensus (wine x assessor interactions were not significant).

Seventeen aroma compounds were identified by HS-SPME-GC-MS: eight esters (ethyl acetate, ethyl isovalerate, isoamyl acetate, ethyl hexanoate, diethyl succinate, ethyl octanoate, ethyl phenyl acetate and ethyl decanoate), six alcohols (isobutanol, n-pentanol, 2-methyl butanol, 3-methyl butanol, hexanol and 2-phenyl ethanol), one hydrocarbon (toluene), aldehyde (furfural) and norisoprenoid (vitispirane) (Table 2). Applying one-way ANOVA to GC data, five compounds (four at $P < 0.05$ and one at $P < 0.01$; Table 2) were significant between ethanol levels. The effect was similar to that found in the sensory analysis: the relative contribution of compounds decreased when increasing the level of ethanol, except for ethyl phenyl acetate (PhetAc). This is in accordance with the results of *Hartman et al.* (2002), who found that when ethanol concentration was raised from 0 to 20% (v/v) an exponential decrease in the recovered analytes was observed.

The Pearson coefficients also showed a positive correlation ($P < 0.05$) between herby attribute and PhetAc, but herby displayed a negative correlation with furfural and ethyl isovalerate (Table 3). These compounds and ethyl octanoate correlated with fruity attributes; it is noteworthy the interpretation of alcohol effect in suppressing fruity aromas and enhancing herbaceous notes in wine samples.

Principal component analysis of sensory and CG data

For sensory data two principal components accounted for 70.5% of variance (PC1, 48.1%; PC2, 22.4%) and the biplot (Fig. 1) showed clustering of fruity attributes, scored positively on PC1 and contained samples (13, 14, 15, 16, 17, 19, 20, 21, 22 and 23) with low alcohol level. Herby, spicy and sweet pepper were clustered positively on PC2 and linked to great proportion of samples (2, 3, 4, 5, 6, 8 and 11) with high alcohol level; these samples were also negatively scored on PC1. The samples negatively scored on PC2 (7, 8, 12, 18) with a

mixture of high and low alcohol levels, were linked to honey and strawberry. The Pearson correlation values among sensory descriptors (Table 4) were high ($r > 0.69$) in some cases (fruity/strawberry/plum; raisin/cooked fruit/plum; spicy/sweet pepper); therefore, these compounds were considered redundant (Koussissi *et al.* 2007) and were grouped in subsequent analysis.

For CG data two principal components accounted for 93.2 % of variance (PC1, 77.6; PC2, 15.6%) and the biplot (Fig. 2) showed, for some of the samples, a similar distribution observed from sensory data. Two pairs of samples 14/16 and 9/19 were opposite with extreme values in both plots (Fig. 1 and Fig. 2). Sample 14 linked to compounds EtAc, 2Phet and EtDec and to attributes spicy, sweet pepper and herby. Sample 19 linked to compounds EtVal, FUR and Hex and attributes fruity, strawberry and honey. Samples 1, 4, 5, 10 and 11 perceived as low fruitiness and low herbaceous with high alcohol level also were clustered in both plots: linked to Tol, 3MetBut, 2MetBut and IsBut, but not linked to any sensory attribute. A correlation between these components and sensory attributes (Table 3) was not observed; probably the sensory attributes that characterized these samples were not correctly identified or not defined. Finally, samples 7, 8 and 12 were clustered in both plots, and associated to nPen and strawberry and honey aromas.

Pearson correlation values between chemical compounds (Table 5) identified by CG were high ($r > 0.69$) in three cases (EtHex/EtOc/EtDec), and therefore these compounds were considered redundant and were grouped in subsequent analysis.

Partial least squares analysis (PLS)

The influence of alcohol level in the detection of volatile and in the perception of sensory characteristics of wine aroma was further confirmed by

PLS. It has been reported that substances with similar flavour are additives (Williams 1994) and combining compounds with similar aroma shrinks the data set and reduces the problem of over-fitting by the PLS model (Schulbach *et al.* 2004). PLS2 was performed after grouping the attributes and chemical compounds with high Pearson coefficients using a model where the total intensity is equal to $(A^2+B^2+C^2)^{1/2}$ and A, B, C represent the aroma intensity of each attribute or compound (Schulbach *et al.*, 2004). Alcohol level of the samples also was included as predicting variable. PLS2 explained 90% of X- (GC data) and 41% of Y- (sensory data) variance in two first factors (Fig. 3). The compounds 2MeBut, EtAc, Vit, EtHex/EtOc/EtDec, nPen and TOL were clustered and related to attributes Sweet/Spicy, Citrus and Floral. Another cluster was formed by compounds Hex, Succ and 2Pheth and related to attribute Cooked/Raisin. EtVal was related with Fruity/Straw/Plum and FUR with Honey. Finally, PhetAc, Ethanol and IsAc were clustered and related to Herby. The samples were grouping by alcohol levels: wines 1 to 12 were clustered and linked with Herby-PhetAc, IsAc, 3MeBut and IsBut and were in opposite of fruity aromas. Wines with low alcohol level (except samples 16, 20 and 22) were clustered and linked with fruitiness attributes. IsAc, 3MetBut and IsBut did not correlate with any sensory attribute; probably the sensory attribute that characterized these compounds was not well defined and possibly it could be solvent or alcohol because they was in the same group of ethanol.

CONCLUSIONS

Red wines with alcohol content higher than 14.5% modified the headspace composition and also changed the sensory perception, as compared with wines having 10.0-12.0% alcohol. The effect of ethanol was not the same for all aroma compounds; it resulted in the decline of some chemical compounds and the increase of others. In addition, the sensory perception of the aroma changed dramatically, depending on ethanol content in the wine. At high ethanol levels the odor was described just as herbaceous altering its status as fruitiness as it has been perceived at low ethanol levels.

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TABLE 1. MEAN AROMA ATTRIBUTES FROM TWO ETHANOL RANGE OF 23 WINES

Attribute	Mean aroma attribute from ethanol range (%) \pm SEM	
	10.0-12.0	14.5-17.2
Fruity	2.6 \pm 0.2	1.8 \pm 0.2**
Citrus	1.5 \pm 0.1	1.8 \pm 0.2
Strawberry	3.2 \pm 0.3	2.1 \pm 0.1**
Plum	3.2 \pm 0.2	2.4 \pm 0.2*
Raisin	2.6 \pm 0.4	1.8 \pm 0.2
Spicy	2.6 \pm 0.3	2.6 \pm 0.2
Cooked fruit	2.9 \pm 0.3	1.9 \pm 0.3*
Floral	2.2 \pm 0.2	2.3 \pm 0.3
Honey	2.5 \pm 0.3	1.6 \pm 0.2*
Herby	2.0 \pm 0.3	3.2 \pm 0.3*
Sweet pepper	2.5 \pm 0.3	2.2 \pm 0.2

* p< 0.05 ** p<0.01

TABLE 2. IDENTIFIED COMPOUNDS, RETENTION TIME (RT), RELATIVE MEAN AREA FROM TWO ETHANOL RANGE OF 23 WINES

n°	Compound	RT	Relative mean area from two ethanol range (%) \pm SEM	
			10.0-12.0	14.5-17.2
1	Ethyl acetate (EtAc)	6.86	16.90 \pm 3.02	21.10 \pm 2.76
2	Isobutanol (IsBut)	7.39	2.10 \pm 0.33	2.22 \pm 0.33
3	n-pentanol (nPen)	10.07	0.90 \pm 0.24	1.40 \pm 0.40
4	3 methyl butanol (3MeBut)	10.39	45.4 \pm 4.72	46.9 \pm 3.33
5	2 methyl butanol (2MeBut)	10.45	6.16 \pm 0.51	6.33 \pm 0.77
6	Toluene (TOL)	10.93	0.88 \pm 0.38	0.71 \pm 0.39
7	Furfural (FUR)	13.18	0.06 \pm 0.02	0.01 \pm 0.00*
8	Ethyl isovalerate (EtVal)	13.68	0.05 \pm 0.01	3E-03 \pm 0.00**
9	Hexanol (Hex)	14.30	0.61 \pm 0.11	0.34 \pm 0.11
10	Isoamyl acetate (IsAc)	14.42	1.02 \pm 0.21	1.53 \pm 0.26
11	Ethyl hexanoate (EtHex)	17.99	2.09 \pm 0.51	1.96 \pm 0.44
12	2 phenyl ethanol (2Pheth)	21.36	1.23 \pm 0.31	1.22 \pm 0.39
13	Diethyl succinate (Succ)	22.67	1.90 \pm 0.38	1.22 \pm 0.19
14	Ethyl octanoato (EtOc)	23.10	5.83 \pm 1.47	2.89 \pm 0.53*
15	Ethyl phenyl acetate (PhetAc)	24.64	5E-03 \pm 0.00	9E-03 \pm 0.00*
16	Vitispirane (Vit)	25.39	0.09 \pm 0.04	0.03 \pm 0.02*
17	Ethyl decanoate (EtDec)	27.56	0.86 \pm 0.19	0.84 \pm 0.12

* p< 0.05 ** p<0.01

TABLE 3. CORRELATIONS BETWEEN CHEMICAL COMPONENTS AND SENSORY DESCRIPTORS

	EtAc	IsBut	nPen	3MeBut	2MeBut	TOL	FUR	EtVal	Hex	IsAc	EtHex	2Pheth	Succ	EtOc	PhetAc	Vit	EtDec
Fruity	-0.121	-0.176	0.229	-0.156	0.079	0.233	0.409	0.535**	0.611**	-0.359	-0.065	0.125	0.558**	0.104	-0.323	0.177	-0.202
Citrus	0.244	-0.261	0.583*	-0.327	-0.059	0.230	-0.181	0.085	0.483*	0.145	-0.005	0.336	0.309	-0.056	0.032	0.135	-0.144
Strawberry	-0.309	-0.046	0.011	-0.031	0.061	0.115	0.376	0.335	0.417*	-0.263	-0.073	0.008	0.382	0.086	-0.391	0.175	-0.178
Plum	0.134	-0.257	0.348	-0.377	-0.024	0.185	0.421*	0.427*	0.674**	-0.286	0.097	0.272	0.647**	0.440*	-0.308	0.334	0.116
Raisin	-0.023	-0.155	0.237	-0.311	0.198	-0.042	0.609**	0.325	0.699***	-0.286	0.304	0.196	0.642**	0.337	-0.080	0.233	0.306
Spicy	0.062	-0.155	0.180	-0.281	0.103	0.146	-0.144	-0.128	0.176	-0.100	0.323	-0.156	0.154	0.580**	0.272	0.496*	0.533**
Cooked fruit	0.129	-0.025	0.264	-0.324	0.178	0.112	0.220	0.073	0.522*	-0.335	0.057	0.182	0.447*	0.274	-0.209	0.275	0.140
Floral	0.441*	-0.311	0.448*	-0.493*	-0.178	0.039	0.058	-0.002	0.480*	-0.076	-0.048	0.268	0.471*	0.181	0.012	0.277	0.081
Honey	0.025	-0.100	0.168	-0.031	0.053	0.170	0.432*	-0.007	0.295	-0.348	-0.157	0.090	0.217	0.007	-0.405	0.079	-0.106
Herby	-0.070	0.056	0.098	0.076	0.227	0.113	-0.478*	-0.418*	-0.186	-0.122	0.035	-0.318	-0.330	-0.044	0.416*	0.178	0.237
Sweet pepper	-0.167	-0.104	-0.238	-0.164	0.121	0.075	-0.093	-0.135	0.164	-0.143	0.377	-0.338	0.000	0.435*	0.266	0.441*	0.520*

* p< 0,05 ** p<0,01 *** p<0,001

TABLE 4. CORRELATIONS BETWEEN SENSORY DESCRIPTORS

	Fruity	Citrus	Strawberry	Plum	Raisin	Spicy	Cooked fruit	Floral	Honey	Herby
Citrus	0.457*									
Strawberry	0.712***	0.089								
Plum	0.790***	0.434*	0.471*							
Raisin	0.629**	0.278	0.402	0.783**						
Spicy	-0.091	0.020	-0.049	0.105	0.137					
Cooked fruit	0.557**	0.296	0.483*	0.761***	0.757***	0.133				
Floral	0.563**	0.647**	0.195	0.686***	0.602**	0.024	0.606**			
Honey	0.384	-0.022	0.449*	0.578**	0.591**	-0.217	0.677***	0.394		
Herby	-0.443*	-0.015	-0.411	-0.435*	-0.234	0.664**	-0.238	-0.137	-0.466*	
Sweet pepper	-0.066	-0.225	-0.081	-0.125	0.030	0.707**	-0.111	-0.166	-0.387	0.592**

* p< 0,05 ** p<0,01 *** p<0,001

TABLE 5. CORRELATIONS BETWEEN CHEMICAL COMPONENTS

	EtAc	IsBut	nPen	3MeBut	2MeBut	TOL	FUR	EtVal	Hex	IsAc	EtHex	2Pheth	Succ	EtOc	PhetAc	Vit
IsBut	-0.066															
nPen	0.170	-0.493*														
3MeBut	-0.637**	0.464*	-0.200													
2MeBut	-0.448*	0.196	0.074	0.230												
TOL	-0.216	-0.178	0.580**	0.151	0.330											
FUR	-0.169	0.020	-0.218	-0.068	0.178	-0.196										
EtVal	-0.174	-0.192	-0.157	-0.138	0.061	0.014	0.609**									
Hex	0.102	-0.089	0.043	-0.501*	0.048	-0.095	0.416*	0.552**								
IsAc	0.262	-0.404	0.071	-0.262	-0.506*	-0.256	-0.310	-0.305	-0.221							
EtHex	-0.072	-0.021	-0.290	-0.355	0.030	-0.378	0.254	0.172	0.483*	0.181						
2Pheth	-0.049	-0.427*	0.193	-0.066	-0.209	-0.212	0.019	0.062	0.066	0.379	0.200					
Succ	0.095	-0.339	0.108	-0.346	-0.164	-0.278	0.397	0.430*	0.677**	0.053	0.404	0.411				
EtOc	0.269	-0.391	-0.019	-0.619**	-0.252	-0.185	0.240	0.333	0.442*	0.049	0.605**	0.182	0.527**			
PhetAc	-0.016	-0.324	0.185	-0.177	0.049	-0.244	-0.405	-0.478*	-0.226	0.433*	0.299	0.284	-0.022	0.088		
Vit	0.285	-0.513*	0.240	-0.672**	-0.218	0.078	-0.005	0.311	0.472*	-0.121	0.136	-0.047	0.301	0.657**	0.029	
EtDec	0.107	-0.129	-0.303	-0.460*	-0.063	-0.428*	0.128	-0.135	0.332	0.224	0.816**	0.187	0.327	0.700***	0.401	0.262

* p< 0,05 ** p<0,01 *** p<0,001

Legend for figures

FIG. 1. PRINCIPAL COMPONENT ANALYSIS OF SENSORY ATTRIBUTES OF 23 WINES AT TWO ETHANOL LEVEL

FIG. 2. PRINCIPAL COMPONENT ANALYSIS OF CHEMICAL COMPOUND OF 23 WINES AT TWO ETHANOL LEVEL

FIG. 3. PARTIAL LEAST SQUARES REGRETION (PLS2) FACTORS FOR SENSORY ATTRIBUTES AND CHEMICAL COMPOUNDS

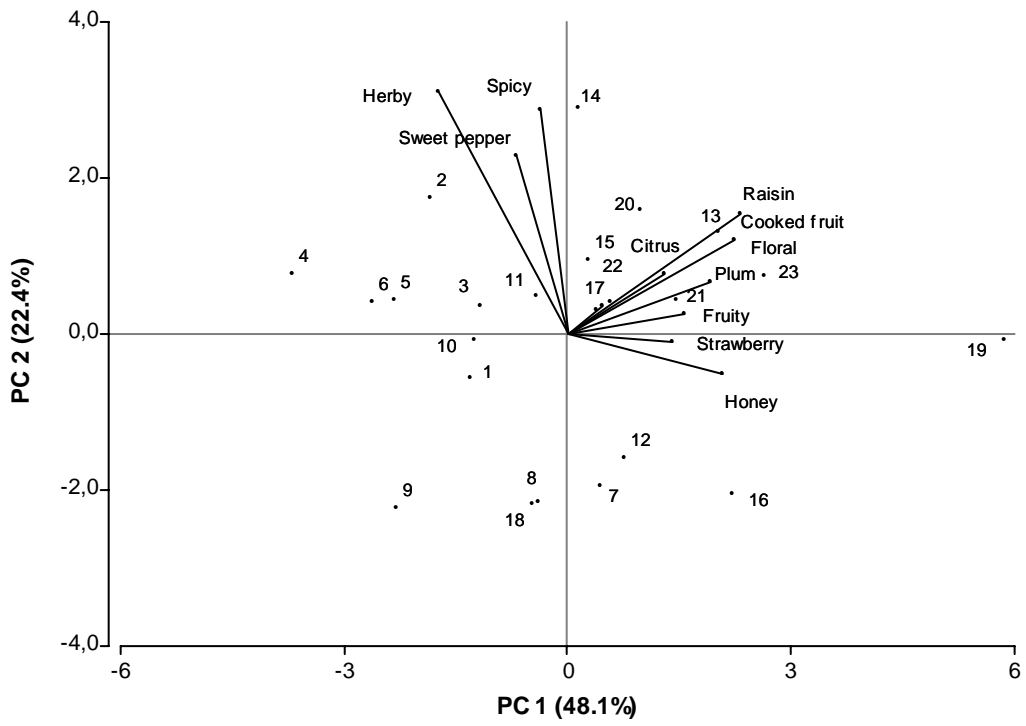


Fig. 1

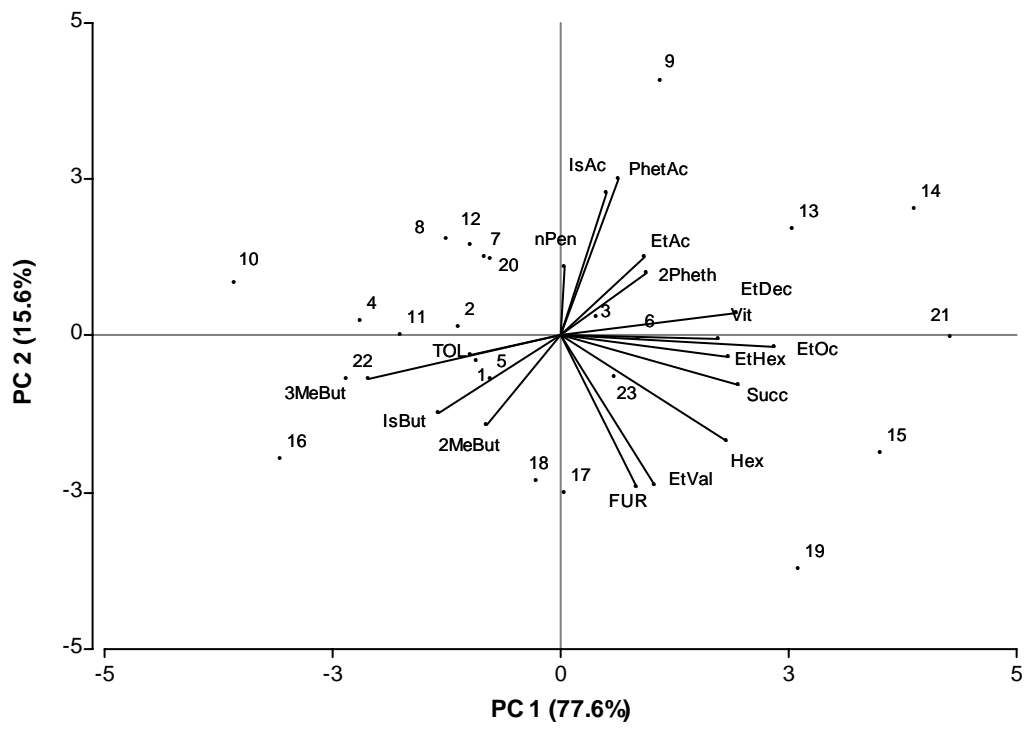


Fig. 2

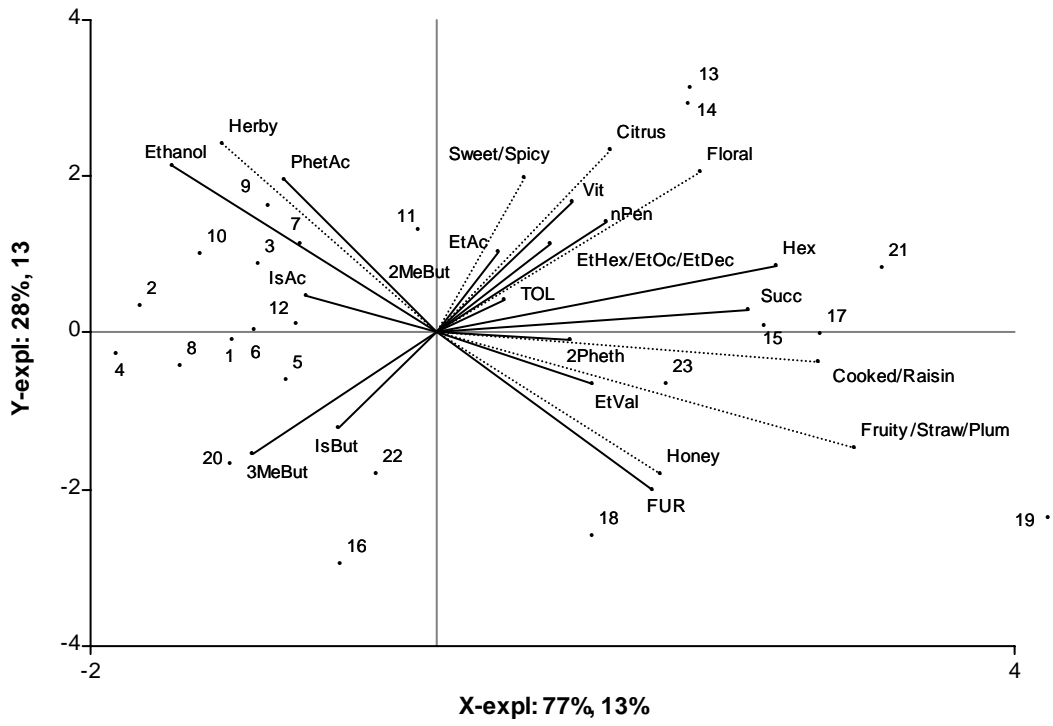


Fig. 3