VOLATILE MULTIVARIATE ANALYSIS OF THREE APPLE VARIETIES

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INTRODUTION

Three apple varieties, *Bravo de Esmolfe* (BE), *Pêro Pam* (PP) and *Malápio de Vale de Açores* (VA), have been studied. These autochthonous varieties exhibit organoleptic characteristics that were very appreciated by the consumer. However, these varieties are not yet characterized which represent the major problem to their identification. The aroma is one of the most important factors in determining apple variety and quality. Solid phase microextraction-gas chromatography (SPME-GC) has been used to characterize apple volatile composition (1). The aim of the present study is to distinguish among three apple varieties using multivariate statistical techniques applied to volatile components, obtained by the application of headspace SPME-GC.

MATERIALS AND METHODS

Bravo de Esmolfe, *Malápio de Vale de Açores* and *Pêro Pam* varieties, from the 2001 harvest, were stored at 4 °C until analysis. The apples were analysed 3 months after harvesting. Two apples were introduced into a 1mL bottle. SPME (Supelco Co.) fibre coated with polydimethylsiloxane (PDMS, 100 μm thickness) was manually inserted into the headspace of the sample bottle. The sample was placed inside the flask at 25 °C, for a period of 150 min, in which the SPME fibre was kept for 30 min. The apple volatiles were desorbed from the SPME fibre on a Finnigan Trace MS gas chromatograph, equipped with a 30 m x 0.32 mm (i.d.) DB-FFAP fused silica capillary column, connected to a Finnigan mass selective detector As an exploratory technique, Principal Component Analysis (PCA) was used to establish relationships between the compounds and the varieties (2). Three extraction replicates were obtained to each variety, given a total of 12 independent measures. The data sets were previously normalized (total area equal to 1).

RESULTS AND DISCUSSION

A total of 69 volatile compounds were identified (table 1). In all the varieties, the major classes of compounds were the terpenoids, followed by the esters and the alcohols. Acids, hydrocarbons and furans were also identified. The results suggest that any compound seems to be specific of a single variety.

Table 1. Volatile compounds identified in Bravo de Esmolfe, Malápio de Vale de Açores and Pero Pam varieties. Grouped by Chemical Classes

Compound	Bravo de Esmolfe		Malápio de Vale de Açores		Pero Pam	
	Area (n=3)	CV	Area (n=3)	CV	Area (n=3)	CV
Esters						
acetic acid, ethyl ester	15561753	8.5	.....		.....	
propanoic acid, ethyl ester	450110	8.7	305196	10.1	8395046	0.6
propanoic acid, 2-methyl, ethyl ester	.....		.....		648933	9.9
butanoic acid, ethyl ester	7879201	12.0	19991260	3.9	40904136	4.9
butanoic acid, 2-methyl, ethyl ester	8208221	7.5	15658757	6.0	37811399	3.5
acetic acid, butyl ester	212953	3.2	.....		3269464	2.9
acetic acid, 2-methylbutyl ester	402053	3.0	.....		15542919	8.4
butanoic acid, propyl ester	.....		3313397	12.9	.....	
butanoic acid, 2- methylpropyl ester	.....		3315935	10.5	.....	
(*E*)-2-butenoic acid, ethyl ester	1598087	12.8	420124	8.9	1013375	0.5
acetic acid, pentyl ester	.....		.....		1923284	5.6
hexanoic acid, methyl ester	527307	12.3	1185001	9.0	.....	
butanoic acid, butyl ester			1343675	11.5	.....	
hexanoic acid, ethyl ester	20697000	0.0	117050186	5.5	145718722	10.8
butanoic acid, 3-methylbutyl ester	.....		991808	3.9	.....	
acetic acid, hexyl ester	3257805	4.2	1407113	10.2	23656997	11.1
butanoic acid, 2-methyl, 2-methylbutyl ester	.....		1888528	4.6	.....	
3-hexenoic acid, ethyl ester	.....		.....		.....	
hexanoic acid, propyl ester	448843	12.1	11411975	2.0	14508141	12.7
heptanoic acid, ethyl ester	851880	13.8	14340535	4.9	9890878	3.1
6-methyl heptanoic acid, methyl ester	321482	1.6	.....		.....	
hexanoic acid, butyl ester	880669	6.9	7616843	12.0	17048881	6.4
hexanoic acid, 2 - methyl butyl ester	3252958	6.5	87150512	10.5	12357396	10.8
octanoic acid, ethyl ester	9291056	2.1	115080157	9.4	70186618	2.9
hexanoic acid, 3-methylbutyl ester	1409671	0.0	10424812	3.4	6105147	13.2
(*Z*)-4-octenoic acid, ethyl ester	.....		2044685	12.0	.....	
2,4-hexadienoic acid, ethyl ester	2475680	0.0	.....		.....	
butanoic acid, 3 - hydroxy, ethyl ester	1776563	5.3	.....		6146209	12.2
octanoic acid, propyl ester	.....		7284303	4.0	6373610	10.7
nonanoic acid, ethyl ester	538603	0.8	987168	11.6	1475272	9.4
2-octenoic acid, ethyl ester	531517	6.9	5295804	11.6	5400418	12.1
heptanoic acid, 2 - methylbutyl ester	.....		1620824	11.9	.....	
heptanoic acid, isopentyl ester	.....		.....		800949	5.0
hexanoic acid, hexyl ester	4248395	7.1	10912426	10.9	30557902	6.3
octanoic acid, butyl ester	.....		3728591	8.4	7047048	4.8
decanoic acid, ethyl ester	2478824	3.0	5367588	6.8	5689565	3.4
octanoic acid, 2 - methylbutyl ester	.....		.....		2201902	9.3

Compound	Bravo de Esmolfe		Malápio de Vale de Açores		Pero Pam	
	Area (n=3)	CV	Area (n=3)	CV	Area (n=3)	CV
octanoic acid, 3 - methylbutyl ester	.....		3523362	8.7	.....	
(*E*)-4-decenoic acid, ethyl ester	3360111	3.5	5268672	12.0	6417684	10.6
decadienoic acid, methyl ester	.....		908953	13.9	.....	
mixture containing a methyl ester	219257	9.4	.....		.....	
(*E,Z*)-2,4-decadienoic acid, ethyl ester	1579750	4.6	6675530	11.7	9993966	0.3
dodecanoic acid, ethyl ester	1775242	11.2	1742043	4.9	.....	
dodecanoic acid, 3 - hydroxy, ethyl ester	.....		801897	11.9	3941614	5.8
hexanoic acid, 3- hydroxy, ethyl ester	.....		3125017	14.1	8225449	11.8
benzoic acid, hexyl ester	.....		.....		550195	11.5
unidentified ester (m/z: 40; 44; 55; 88)	.....		.....		.....	
tetradecadienoic acid, methyl ester	965624	1.0	1181524	13.8	.....	
1,2-benzenedicarboxylic acid, *bis*(2-methylpropyl)ester	.....		.....		205164	10.3
Subtotal (Area)	**95200615**		**473364201**		**504008282.5**	
Alcohols						
ethanol	9952486	8.7	1308113	8.8	.....	
1-butanol	.....		1290938	10.3	2013049	4.8
2-methyl-1-butanol	.....		4284497	9.8	2623554	1.3
1-pentanol	.....		.....		.....	
1-hexanol	325988	7.0	5021380	13.1	6280255	7.4
2-methyl-6-hepten-1-ol	.....		2105758	8.9	.....	
1-octanol	.....		.....		.....	
phenol	.....		703751	12.3	.....	
Subtotal (Area)	**10278474**		**14714437**		**10916857**	
Acids						
acetic acid	.....		.....		.....	
benzoic acid	.....		.....		230999	7.9
tetradecanoic acid	133868	11.7	236741	13.0	244395	4.4
pentadecanoic acid	91886	7.6	208497	13.7	130811	4.8
hexadecanoic acid	825338	9.9	1410243	6.7	1151389	1.2
(*Z*)-9-hexadecenoic acid	275895	8.9	.....		432832	7.5
Subtotal (Area)	**1326987**		**1855480**		**2190426**	
Hydrocarbons						
O-xylene	1463216	13.3	.....		.....	
M-xylene	4074414	8.7	.....		5445535	1.6
unidentified aromatic compound (69; 101; 43; 44)	.....		.....		.....	
(*Z,E*)-3,7,11-trimethyl-1,3,6,10-dodecatetraene	2236576	7.5	.....		.....	
Subtotal (Area)	**7774206**		**0**		**5445535**	
Terpenoids						
(*Z,E*)-α-farnesene	.....		7409569	10.7	.....	
(*E,E*)-α-farnesene	171140241	5.3	432936206	6.3	661581117	3.7
trans-farnesol	420473	1.8	8107931	3.6	2653392	10.6
cis - farnesol	331952	1.6	350599	13.5	273573	6.9
Subtotal (Area)	**171892666**		**448804305.3**		**664508082**	
Furans						
unidentified furan (m/z: 69, 101, 72, 157))	428952	6.5	.....		.....	
(*E*)-3-(4,8-dimethyl-3,7-nonadienyl) furan	721028	6.6	4049940	4.9	2981658	5.4
Subtotal (Area)	**1149981**		**4049940**		**2981658**	
TOTAL (Area)	**287622929**		**493984058**		**686042558**	

In order to obtain information to allow the identification of each variety, PCAwas used to establish relationships between the compounds and the varieties. As shown Fig. 1a), two Principal Components (PCs) described almost all the variability (98%) present in the data set. This plot shows a separation of the three apple varieties. Along PC1 one could separate BE variety (positive) from VA variety (negative), with PP variety having a null influence in this axis, this variety is separate in PC2 axis. The loadings (Fig. 1b) show a relationship between acetic acid ethyl ester and ethanol to variety BE, hexanoic acid ethyl ester to variety PP, and hexanoic acid 2-methyl butyl ester, octanoic acid ethyl ester to variety VA. All the remaining compounds seem to have only a minor influence for the observed clusters.

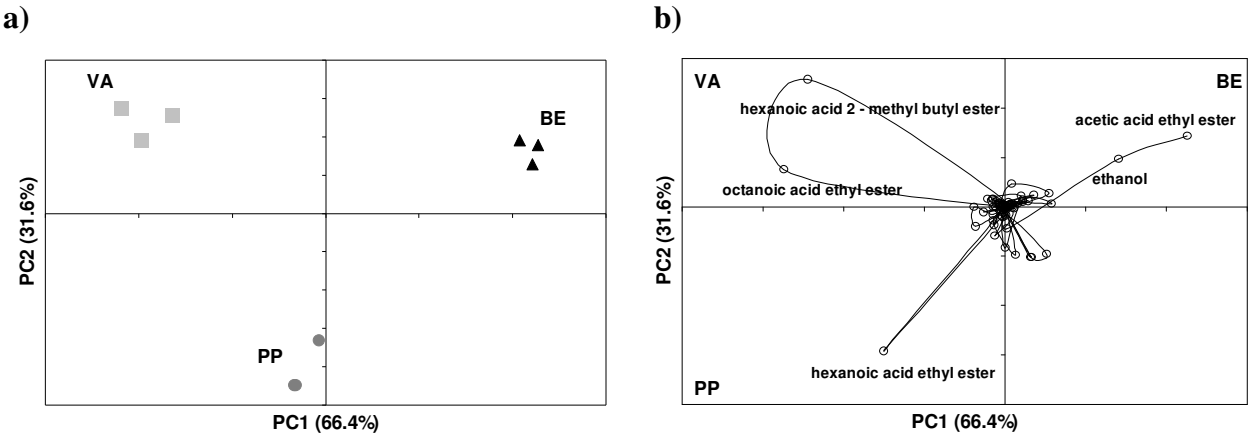

Figure 1. a) Scores scatter plot (PC1 *vs*. PC2) for data set 1. **b)** Loadings scatter plot (PC1 *vs*. PC2) for data set 1.

This preliminary analysis shows that from the PCA one can distinguish the three apple varieties based on the SPME data and several compounds were identified as been related to the observed clusters.

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