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Effect of Storage Time, Storage Temperature and Packaging Material on the Composition of Major Volatile Compounds of White Wines from Ionian Islands (Greece).

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Abstract: *In the present study, the changes in the concentrations of the major volatile constituents of different types of Greek white wines produced in the areas of Cephalonia ("Robola"), Leucada ("Vardea") and Zante ("Pavlos" and "Moshato") during storage for 12 months in glass and PET containers under cellar and refrigeration temperature were investigated. A liquid/liquid extraction method was applied for the isolation of the studied compounds. The identification of the volatile compounds was performed by GC/MS and the quantitative determination by GC/FID analysis. Obtained data were evaluated applying techniques of multivariate statistical analysis (Cluster and Principal Component Analysis). Cluster analysis was performed to group the wine varieties and storage times. Principal Component Analysis showed that the first three components explained 83.4 % of the total variation. 1-pentanol and furfural prevailed in PC1, ethyl lactate in PC2 and ethyl acetate in PC3. Using ANOVA on the resulting principal components it was found that wine variety and storage time are statistically significant factors for all three principal components, while packaging material, storage temperature and all possible factor interactions were not. According to these results, PET containers could be at sight considered as suitable for the packaging of these types of wines under the studied storage conditions.*

Keywords: *Cluster and Principal Component Analysis, GC/MS and GC/FID analysis, storage time and temperature, packaging material, white wine volatiles.*

I. Introduction

Flavor is a combination of taste and aroma and it is of particular importance in determining food preferences. Wine flavor depends on a number of factors, the most important of which is its chemical composition. Aroma substances are important in wine as they make a major contribution to the quality of the final product. Several hundreds of different flavor compounds such as alcohols, esters, organic acids, carbonyl compounds and monoterpenes have been found in wines. It is the combined contribution of these compounds that forms the character of the wine. Since many viticulture and enological factors greatly influence the type and concentration of flavor compounds, the ability to determine each individual compound would provide an approach to optimize the operational conditions, such as canopy management of the vine, harvest parameters, juice preparation and fermentation techniques, use of yeast, lactic acid bacteria and enzymes, and wine storage and aging [1-4].

Moreover, the particular importance of each compound on the final aroma depends on the correlation between chemical composition and perception thresholds, because most of the volatile compounds are present at concentrations near or below their individual sensory thresholds [5]. If wines have been stored under proper conditions they may retain initial quality, but if they have been stored in warm or lighted areas they will have lost their best attributes [6-7].

A large number of chemical changes occur in wines during storage, which can affect their final properties. The formation of new aroma compounds together with variations in the amounts of other existing components may take place, affecting the overall quality of the wine. These changes can be related to one of the following aspects: 1) changes in the ester content (decrease in acetates and increase in mono- and dicarboxylic acid ethyl esters), 2) formation of substances from carbohydrate degradation, 3) decrease in the concentrations of monoterpene alcohols and 4) formation of unwanted products [8-9].

The self-life of a wine is defined as the period of time it remains stable from a chemical, microbiological and biochemical point of view and maintains its good sensory properties. Considering this, the packaging of wine requires special attention. The most suitable container for packaging wine is glass. Nowadays the use of containers other than glass ones has become common. The materials which can be used are multilayer carton formed as cellulose card board, aluminum, and low-density polyethylene, polyesters, bag in box, etc. Among polyesters, polyethylene terephthalate (PET) is the most usable and looks like glass to a large extent [8, 10, 11].

In the island of Cephalonia the elegant “Robola” grape variety is cultivated and used to produce a dry white wine with appellation of superior quality (VQPRD), the well-known “Robola of Cephalonia” [12].
“Vardea” is a Greek grape variety cultivated in the island of Leucada and produces a dry white wine with fine soft aroma [12]. “Moshato” is one of the most important grape varieties cultivated in Greece and is considered relative variety with the French Muscat de Frintignan. It is cultivated mainly in the islands of Zante (from where we took our samples) and Samos and in Northwest Peloponesse. The white dry wines are internationally known and characterised by freshness and vivacity [12]. “Pavlos” is a dry white wine originates from the variety “Cardinal”. It is cultivated mainly in Crete, Peloponesse, Larissa, Evia, Zante (from where we took our samples) and Cyclades [13].

In this work, a combined solvent extraction-GC/FID method for the quantitative analysis of the major volatile constituents of different types of Greek white wines produced in the areas of Cephalonia ("Robola"), Leucada ("Vardea") and Zante ("Pavlos" and "Moshato") is reported, during their storage under cellar and refrigeration temperature conditions for a period of 12 months in two types of containers (glass and PET). Identification of compounds was accomplished by GC/MS analysis. Dichloromethane was used to extract the volatiles and the extracts were subjected to GC analysis without further concentration [14]. Obtained data were evaluated applying techniques of multivariate statistical analysis (Cluster and Principal Component Analysis).

II. Materials And Methods

The wine samples were from 2005 vintage and were bottled in 2006. The fermentation temperature was 18 ^oC. Samples were stored in a cellar (with temperature ranged from 12 ^oC to 16 ^oC) and in refrigeration in two types of containers (glass and PET) and analyzed at specific time intervals (0, 3, 6, 9 and 12 months). Measurements were carried out in triplicate. The storage time was taken to be the time elapsed from the exact date of bottling to the date of analysis. High purity chromatographic standards, substances and solvents were obtained from Merck and Aldrich (Germany). All reagents were used without further purification.

A liquid/liquid extraction method was applied for the extraction of the volatile compounds of the wine samples: wine (100 ml), CH_2Cl_2 (10 ml) as extraction agent, 1 ml of 2-octanol solution (72.04 mg/l in CH_2Cl_2) as internal standard and sodium chloride (20 g) to reduce the degree of emulsification at the wine/CH_2Cl_2 interface, were added in a 300ml-flask. The flask was cooled in melting ice and the wine/ CH_2Cl_2 mixture was stirred at 200 rpm for 2h. The wine/CH_2Cl_2 emulsion formed during stirring was separated from the aqueous layer and frozen at -20 ^oC. The flask was then allowed to reach room temperature, and the CH_2Cl_2 layer, progressively separated from the remaining wine, was transferred without concentration into screw-capped vial and stored at -20 ^oC for further GC/FID and GC/MS analysis [14]. A model wine solution was prepared, composed of tartaric acid at 6.5 g/L in 11.5% (v/v) aqueous ethanol. The synthetic working standard solution was prepared by dissolving appropriate amounts of each of the volatile compounds listed in Table 1 in the model wine solution. This solution was used to evaluate the extraction recovery of the flavor compounds [1, 15, 16].
Recovery tests were performed by extraction of the synthetic working standard solution according to the proposed method. Calibration curves for GC quantification were constructed by dissolving known amounts of the flavor compounds, including internal standard (2-octanol), in aqueous ethanol solution having an alcohol content similar to that of the analyzed wines.

The GC unit was a Fisons 9000 series gas chromatograph equipped with a flame ionization detector. The separation column was 30 m long X 0.32 mm internal diameter fused silica DB-Wax capillary (J&W Scientific) with film thickness of 0.25 μm. The following GC parameters were kept constant: detector temperature, 240 ^oC; injector temperature, 200 ^oC; carrier gas (He) flow rate, 1.5 ml/min; injection mode split with split ratio 1:50; injection volume 2 μl. The applied column temperature program was 40 ^oC (7 min), from 40 ^oC, at a rate of 15 ^oC/min, to 160 ^oC (1 min) and from 160 ^oC, at a rate of 30 ^oC/min, to 230 ^oC (5 min). The GC/MS system consisted of a Hewlett-Packard 6890 (Wilmington, DE, USA) gas chromatograph coupled to an HP-5973 mass selective detector. The GC was equipped with a DB-Wax capillary column. The GC conditions were the same as above. The transfer line was held at 260 ^oC. Ionization was carried out with electron energy of 70 eV. Identification of compounds was accomplished by comparing retention times and mass spectra (SCAN mode, 28-550 amu) with those of reference standards using the GC/MS Willey 275L workstation.

Cluster analysis (Ward linkage method, correlation coefficient) was performed on the data in order to group the wine varieties and storage times. Principal Component Analysis was also used to determine which volatile compounds contribute to the variability between wine types, storage times, storage temperature and packaging material. ANOVA was used on the resulting principal components in order to determine if they are influenced significantly by these factors and their two-way interactions.

III. Results And Discussion

Thirty compounds were detected in the characteristic volatile profile of wine samples. Most of them participate in the configuration of the aroma of the wine. Fig. 1 shows a representative GC/MS chromatogram. The major volatile compounds, which were determined quantitatively in the wine samples and their retention times, are given in Table 1. The cited retention times are the average of at least three injections.

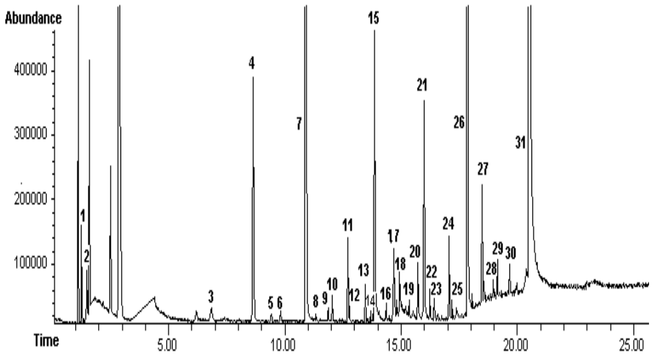

Figure 1.GC/MS chromatogram of dry white wine "Robola" stored in glass container for 12 months.

(1) acetaldehyde, (2) ethyl acetate, (3) 1-propanol, (4) isobutanol, (5) isoamyl acetate, (6) 1-butanol, (7) amyl alcohols, (8) ethyl hexanoate, (9) 1-pentanol, (10) 3-hydroxybutanone or acetoin, (11) ethyl lactate, (12) 1-hexanol, (13) ethyl octanoate, (14) furfural, (15) acetic acid, (16) L-linalol, (17) 2-octanol (I.S.), (18) 2, 3-butanediol, (19) butanoic acid, (20) γ-butyrolactone, (21) butanedioic acid, (22) a-terpineol, (23) 2-methylthiopropanol or methionol, (24) 4-hydroxy-ethylbutanoate, (25) hexanoic acid, (26) 2-phenylethanol, (27) benzyl alcohol, (28) octanoic acid, (29) decanoic acid, (30) ethyl decanoate, (31) monoethyl succinate.

The relative recovery (RR) values for liquid/liquid extraction of flavor compounds ranged from 93.20 to 99.65% and are generally comparable to those reported by other investigators [1, 15,17].

Cluster analysis (Fig. 2) showed that there are five different groups of wine types according to their composition and storage time. The first group includes all wine types before they are stored for any period of time (storage time = 0). A group for each one of the wine types for storage times ranging from 3 to 12 months is formed. Hence, the composition of the four wine types is similar before storage, but changes significantly and in a different manner for all wine types over the first three months of storage

Table 1. Volatile compounds identified in wine samples and their retention times

Peak no.	*A. Volatile compound*	**Retention time (min)**
1	Acetaldehyde	1.36
2	Ethyl acetate	1.67
3	1-Propanol	3.32
4	Isobutanol	4.35
5	Amyl alcohols	8.38
6	1-Pentanol	9.40
7	Ethyl lactate	10.81
8	1-Hexanol	11.06
9	Ethyl octanoate	12.02
10	Furfural	12.20
I.S^a	2-Octanol	14.00
11	2-Phenylethanol	16.56
^{a}, internal standard		

Principal Component Analysis results showed that the first three components explained 83.4 % of the total variation (Table 2). 1-Pentanol and furfural were the prevailing volatile compounds for the first principal component, ethyl lactate for the second principal component and ethyl octanoate together with ethyl acetate for the third principal component.

From the loading plot between the first two principal components (Fig. 3) and the corresponding score plot for wine types (Fig. 4) it can be concluded that "Moshato" wines exhibit higher concentrations of 1-pentanol and furfural, "Robola" wines exhibit higher concentrations of ethyl lactate and "Pavlos" and "Vardea" wines higher concentrations of ethyl octanoate compared to other wines.

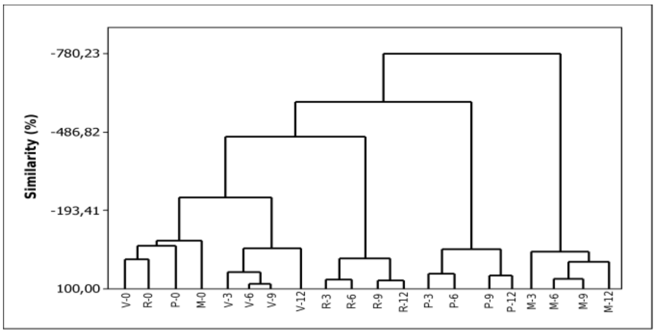

Figure 2. Cluster analysis dendrogram (Ward linkage, correlation coefficient). V=Vardea, R=Robola, P=Pavlos, M=Moshato, 0-3-6-9-12: Storage time (months)

Table 2. Loading factors for the first three Principal Components

Volatile compound	**PC1**	**PC2**	**PC3**
2-Phenylethanol	0,352	-0,542	0,318
Acetaldehyde	0,481	0,743	0,227
Amyl alcohols	0,612	0,575	-0,235
Ethyl lactate	-0,025	0,909	0,376
1-Hexanol	0,762	-0,150	0,389
Isobutanol	0,765	-0,101	-0,588
Ethyl octanoate	-0,017	-0,577	0,754
Ethyl acetate	-0,614	-0,030	-0,706
1-Pentanol	0,922	-0,058	-0,160
1-Propanol	0,755	-0,461	-0,413
Furfural	0,868	0,080	0,144
Variance	40,4 %	23,6 %	19,4 %

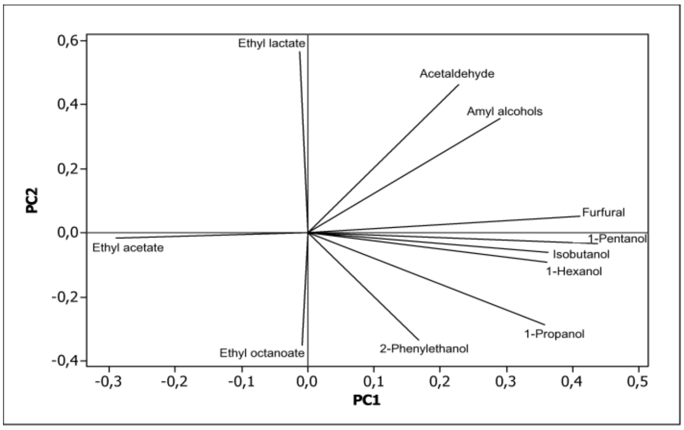

Figure 3. Loading plot for the first two principal components

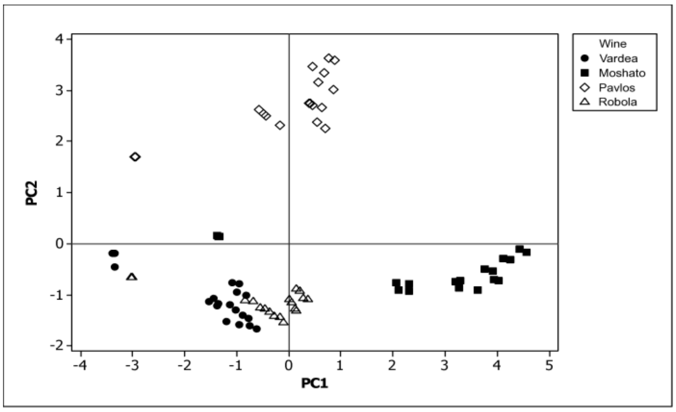

Figure 4. Score plot between the first two principal components categorised by wine type

The score plot for storage time (Fig. 5), when examined together with the loading plot (Fig. 3) and also the score plot for wine types (Fig. 4), shows that wines of any type not yet put in storage show high concentrations of ethyl acetate and low concentrations of 1-pentanol, furfural, isobutanol and 1-hexanol. Wine types stored for 3 months or more have decreased concentrations of ethyl acetate and increased concentrations of 1-pentanol, furfural, isobutanol and 1-hexanol, effects which are amplified with storage time.

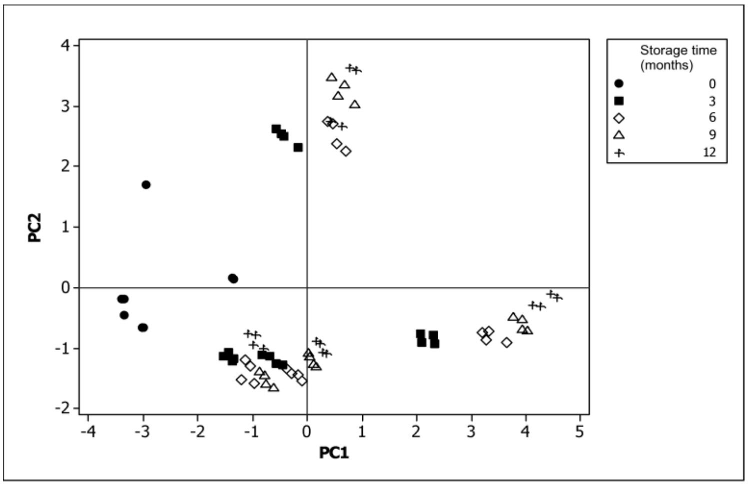

Figure 5. Score plot between the first two principal components categorised by storage time

Performing 4-way ANOVA on the resulting principal components shows that wine type and storage time are significant factors while packaging material and storage conditions are not for all three principal components, while the only significant two-way interaction is the wine type with storage time interaction (Table 3).

Main effects plots for all examined factors for each of the first three principal components are shown in Figs. 6, 7 and 8. “Moshato” wines show significantly higher values of PC1, “Pavlos” wines higher values of PC2 and “Robola” wines higher values of PC3. This means that “Moshato” wines have significantly higher values of 1-pentanol and furfural than all other wines, “Pavlos” wines have higher concentrations of ethyl lactate than all other wines and “Robola” wines exhibit higher concentrations of ethyl octanoate while having lower concentrations of ethyl acetate compared to other wines.

Table 3. ANOVA P-values for main factors and two-way interactions of the first three principal components.

	ANOVA P-Values		
Factor	**PC1**	**PC2**	**PC3**
Wine	<0.001	<0.001	<0.001
Packaging material	0.833	0.782	0.897
Storage time	<0.001	<0.001	<0.001
Storage temperature	0.812	0.653	0.722
Wine-Packaging material	0.476	0.432	0.244
Wine-Storage time	<0.001	<0.001	<0.001
Wine-Storage temperature	0.699	0.545	0.652
Packaging material-Storage time	0.512	0.398	0.421
Packaging material-Storage temperature	0.358	0.448	0.596
Storage time-Storage temperature	0.350	0.226	0.128

By examining the interaction between wine type and storage time on the first three principal components it can be observed that PC1, which represents mainly 1-pentanol and furfural, continues to increase for the duration of the 12 months for “Moshato” wines while it increases significantly during the first three months and remains at the same levels after 6 months of storage for “Vardea”, “Pavlos” and “Robola” wines (Fig. 9). The two-way interaction for PC2 (Fig. 10), which represents mainly ethyl lactate, shows that values for “Pavlos” wines increase during the first three months of storage, while remaining at the same levels after nine months of storage, in contrast with all other wines, which exhibit a significant decrease during the first three months and a significant increase of PC2 after nine months of storage. From the interaction plot of PC3 (Fig. 11), it can be observed that there exists a slight decrease of its values after six months and until the twelfth month of storage for “Moshato” wines, while for all other wines PC3 values do not change significantly after six months of storage. That means that ethyl octanoate concentrations decrease slightly but significantly after six months of storage together with a slight increase of ethyl acetate concentrations for “Moshato” wines.

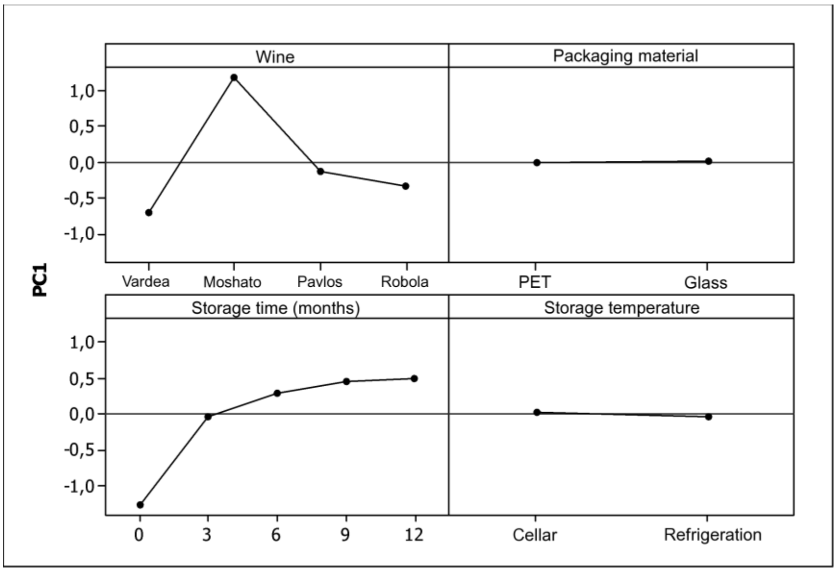

Figure 6. Main effects plot for the first principal component

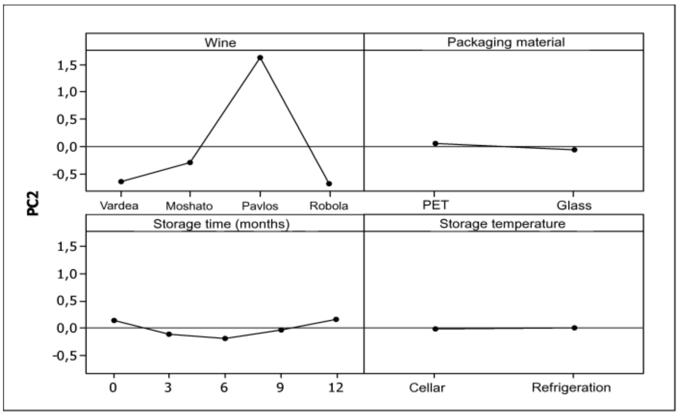

Figure 7. Main effects plot for the second principal component

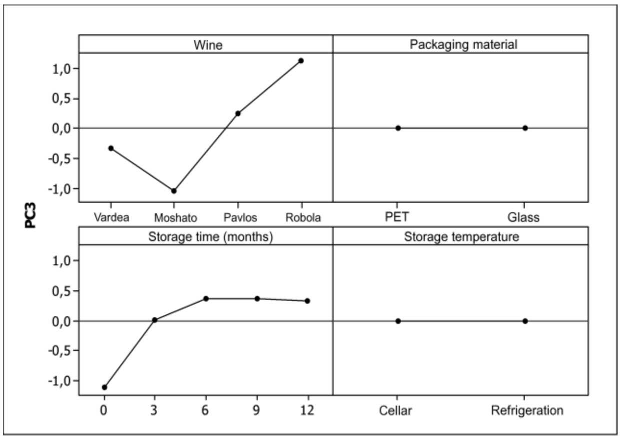

Figure 8. Main effects plot for the third principal component

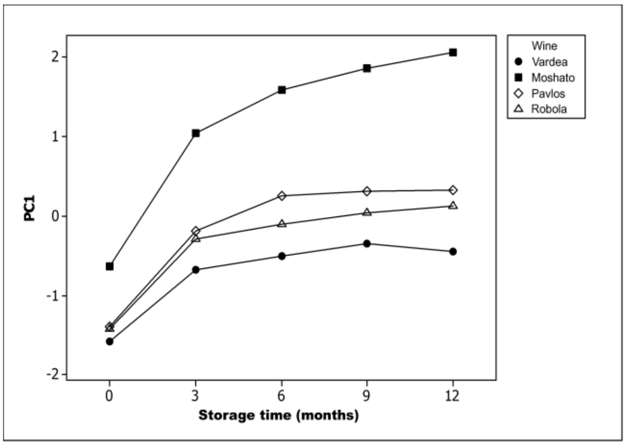

Figure 9. Interaction plot between wine type and storage time for the first principal component

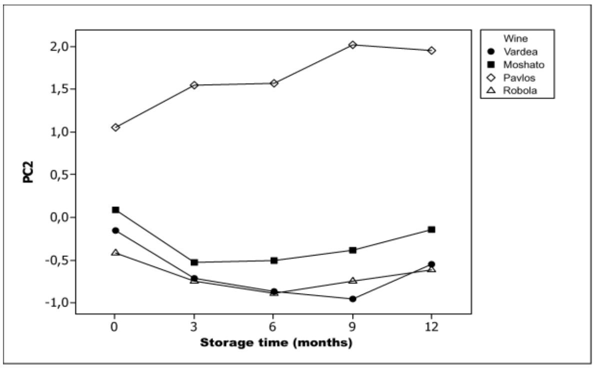

Figure 10. Interaction plot between wine type and storage time for the second principal component

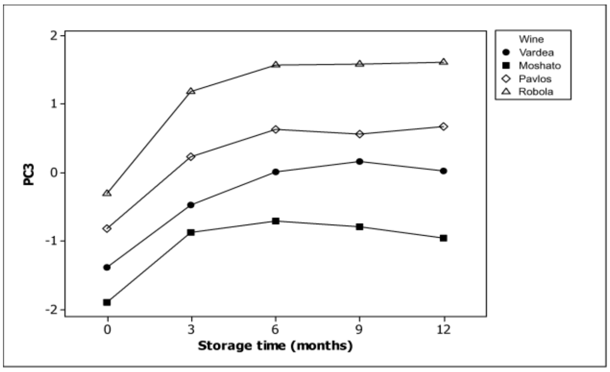

Figure 11. Interaction plot between wine type and storage time for the third principal component

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