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Study of the antioxidant power of brandies and vinegars derived from Sherry wines and correlation with their content in polyphenols

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Abstract

The protective effect on health of wine consumed in moderation has been widely studied in recent years. This has been attributed to the content in polyphenols and to the antioxidant activity of these compounds. The products derived from wine, such as vinegar and brandy, also contain polyphenols and could possess a certain antioxidant activity, similarly contributing to a protective effect for health. This paper describes the study of the antioxidant power of diverse Sherry brandies and vinegars, by means of an electrochemical method previously devised. The total polyphenolic content is studied using the method of Folin–Ciocalteu, and some polyphenols are identified and quantified by means of HPLC. A close correlation between the antioxidant power and the total polyphenolic content of the samples has been found. Considering the polyphenols individually, the compounds that are better correlated with the antioxidant power of the samples are not necessarily those that are present in higher concentrations. For brandies, the contact with wood has an important contribution to their antioxidant power.

Keywords: Antioxidant power; Polyphenols; Brandy; Vinegar; HPLC

1. Introduction

It is now widely accepted that antioxidants play a crucial role in the prevention of many diseases, thanks to their capacity for capturing, de-activating or repairing the damage caused by the free radicals that are implicated in such diseases. Fruits and vegetables, and all the foods and drinks derived from these, are rich in polyphenolic compounds, which have been demonstrated to be powerful antioxidants. Hence these protective health effects derived from the consumption of such foods have been attributed to their content in polyphenols (Huang, Ho, & Lee, 1992; Rice-Evans & Packer, 1998).

In particular, the relationship between the consumption of wine and the prevention of cardiovascular diseases and certain cancers, among others, has been widely studied (Renaud & De Lorgeril, 1992; Tomera, 1999). The great advantage of wine as a matrix for polyphenols in the diet is that, in wines, they are present in the soluble state and hence are more biologically available, whereas in fruits and vegetables, they are strongly bonded and hence less easily absorbed. In the products derived from wine, polyphenolic compounds are also present, and could thus contribute to this protective antioxidant action. Even in the byproducts from wine production, numerous polyphenols have been found, and these are now being extracted and used in pharmaceutical compositions (Henry, Pauly, & Moser, 2001; Shrikhande, 2000).

In the Jerez region of the south of Spain, there is an ancient winemaking tradition that has given rise to the world-famous Sherry wines. But other products derived from these wines have also become commercially important, particularly the Sherry brandies and vinegars, all of which are manufactured following the traditional system of dynamic aging known as the "*soleras* and *criaderas*" method. This system consists of stacking the

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casks, always made of American oak, in rows or levels called *escalas*, in function of the age of the product contained. The particular *escala* that contains the oldest wine, brandy or vinegar is termed the *solera* and traditionally it is situated at ground level, at the bottom of the stack. In successive rows or levels above the *solera* are placed the *first criadera*, *second criadera*, etc., with the highest containing the youngest, newly made product. The product to be bottled and sold is extracted from the *solera* level casks, in an operation called the *saca*. The quantity drawn off from the *solera* is replenished (by the so-called *rocío* process) from the *first criadera*, which in turn is replenished from the *second criadera*, and so on successively, in such a way that the products are continually homogenised.

The aging process allows the polyphenols, which contribute to the antioxidant power, to pass from the wood to the aged products. It should, therefore, be interesting to study these products, their polyphenolic content and their possible antioxidant activity. This paper deals with the study of different Sherry vinegars and brandies.

2. Materials and methods

2.1. Reagents

The Folin reagent (Sigma–Aldrich, Madrid, Spain) and sodium carbonate (Panreac, Barcelona, Spain) were employed for the measurement of the Folin–Ciocalteau total polyphenolic index. The calibration curve was constructed with gallic acid (Merck, Darmstadt, Germany).

A saturated solution of $Zn(CH_3COO)_2$ (Panreac) and a solution of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Sigma–Aldrich) in a phosphate buffer medium (pH 6) were used in the electrolytic system. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) supplied by Sigma–Aldrich was used to construct the calibration curve.

The solvents employed for the HPLC analysis were prepared with methanol, acetic acid (quality HPLC, Scharlau, Barcelona, Spain) and water purified in a Milli-Q system (Millipore, Bedford, MA). The solutions were filtered through cellulose acetate membranes (solvent A) and Teflon membranes (solvent B) of 0.45 μm pore size (Micron Separation, Westboro, MA) and degasified in an ultrasound bath.

Calibration curves were constructed for the identified polyphenols, supplied by Sigma–Aldrich, Merck, Fluka (Buchs, Switzerland) and Eastman Kodak (Rochester, NY). Caftaric, *cis*-*p*-coutaric, *trans*-*p*-coutaric, and *cis*-*p*-coumaric acids are not commercialized, so the former was quantified using the caffeic acid calibration curve and the rest using the *trans*-*p*-coumaric acid one (Chilla, Guillén, Barroso, & Pérez-Bustamante, 1996). The pure compounds were dissolved in a brandy-like model solution, prepared with ethanol (Panreac) (40% v/v) and purified water, or vinegar-like model solution, prepared with acetic acid (60 g/L), ethanol (0.5% v/v) (Panreac) and purified water.

2.2. Samples

All the brandy samples were supplied by the "*Consejo Regulador de la Denominación Específica Brandy de Jerez*". We studied three types of Sherry brandies: *Solera* (SB, aged in oak casks for a minimum of 6 months), *Solera Reserva* (SRB, aged for a minimum of 1 year) and *Solera Gran Reserva* (SGRB, aged for a minimum of 3 years).

The vinegar samples were supplied by wine companies of Jerez. We divided the vinegars in two groups: samples aged in oak casks (V–W, approximately 5 years) and samples without wood contact (V).

2.3. Total polyphenolic index: Folin–Ciocalteu method

Observing the sequence specified here, the following are introduced into a calibrated 25 mL flask: 250 μL of sample, 12.5 mL of distilled water, 1250 μL of Folin–Ciocalteu reagent, 5 mL of a solution of sodium carbonate at 20% and distilled water to make up the total volume of 25 mL. The solution is agitated to homogenize it and left to stand for 30 min for the reaction to take place and stabilize. The absorbance at 750 nm is determined in a cuvette of 1 cm (Singleton & Rossi, 1965). The calibration curve was prepared with gallic acid solutions ranging from 0 to 1000 mg/L, and the results are given as "gallic acid equivalents" (GAE).

2.4. Measurement of the antioxidant activity

This is carried out by means of an electrochemical method developed previously in our research group (Alonso, Guillén, Barroso, Puertas, & García, 2002). In an electrolytic device are placed: on the anode, 25 mL of ABTS 50 μM (pH 6) and aliquots of the sample, and on the cathode, 30 mL of saturated zinc acetate. Holding the intensity constant at 2 mA, the absorbance at 414 and 734 nm is recorded. As response function, the coulombs consumed in the oxidation of the sample are measured, and the antioxidant of reference for the calibration is Trolox. The results are expressed as equivalent concentration of Trolox in mM.

2.5. Analysis by high performance liquid chromatography

The analysis was performed using a Waters HPLC system (Waters/Millipore, Milford, MA) consisting of a model 616 pump, a model 600S gradient controller, a model 717 automatic sampler, and a model 996 photo-

diode detector. The separation of the polyphenols was conducted in a LiChrospher 100 RP-18 column (Merck), 5 μm, 250 mm × 3 mm i.d.

The chromatographic conditions for the brandies were: 0.4 mL/min flow rate, 50 μL injection volume, eluents: A (5% methanol, 2% acetic acid, 93% water) and B (90% methanol, 2% acetic acid, 8% water). The gradient employed is shown in Table 1 (Barroso, Rodríguez, Guillén, & Pérez-Bustamante, 1996). The chromatographic conditions for the vinegars were: 0.5 mL/min flow rate, 80 μL injection volume, eluents: A (5% methanol, 95% water) and B (95% methanol, 5% water), both adjusted at pH 2.5 with sulfuric acid (Panreac). The gradient employed is shown in Table 2.

The detection by UV absorption was conducted by scanning between 250 and 600 nm, with a resolution of 1.2 nm, and the identification and quantification were conducted at 280 and 320 nm. The data acquisition and treatment were conducted using the Millenium 2010, version 2.21 software.

2.6. Statistical treatment

Correlation analysis, ANOVA and cluster analysis (CA) were performed using the Statgraphics Statistical Computer Package "Statgraphics Plus 5.0" for Windows 98.

Table 1
Elution gradient program for brandies

Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
20	90	10
60	25	75

Table 2
Elution gradient program for vinegars

Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
45	50	50
85	0	100

3. Results and discussion

The total polyphenolic index (TPI) and the antioxidant power (AP) were measured for all the samples. The results are given in Figs. 1 and 2.

In general, the *Solera Gran Reserva* brandies present a higher TPI and AP than the *Solera Reserva* brandies, and these in turn show higher value than the *Solera* brandies. This indicates an increasing polyphenolic content of the brandies with increasing age, logical finding because it is believed that the process of maturing in oak casks is the main cause of the polyphenolic content of brandy. The process of aging in wood of spirits is characterized by the diffusion of compounds from within the wood, compounds such as aromatic benzoic and cinnamic aldehydes, in particular. It has been generally recognised that these compounds are the result of the degradation of the lignin (Barroso et al., 1996).

A correlation analysis was made between both these parameters for all the brandies (Fig. 3), and for each type of samples separately. The polyphenolic content and antioxidant power are very closely correlated for the set of all the samples ($R = 0.9175$). Considering each group separately, we find that the SRB brandies are the best correlated ($R = 0.9897$), followed by the SB ($R = 0.9400$) and lastly by the SGRB (0.7044).

In the case of the vinegars, the correlation analysis (Fig. 4) shows that the coefficient of correlation between the two measures was also good ($R = 0.9201$). The coefficient of correlation was 0.9433 for the samples aged in wood and 0.9741 for the samples not aged. As for the contact with wood in vinegars, this does not seem to influence the parameters TPI and AP. Vinegars V1–W and V2 stand out from the rest by their high values (Fig. 2), and while the first was aged in wood, the other was not. It

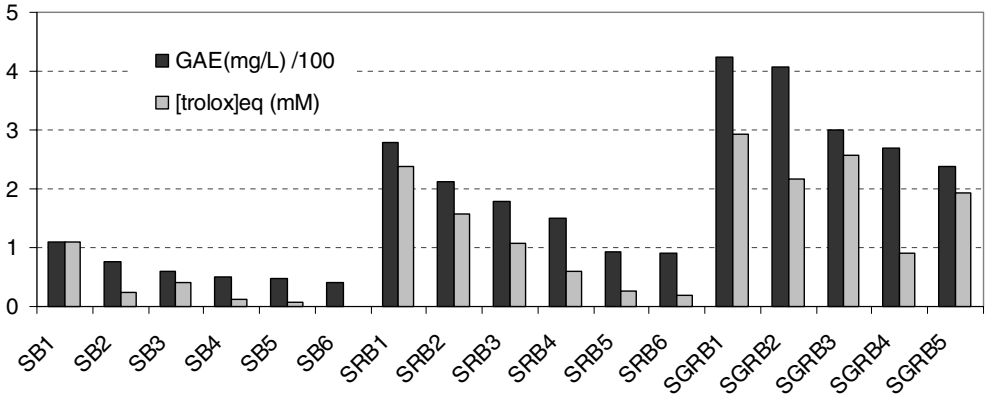

Fig. 1. Total polyphenolic index (GAE) and antioxidant power ($[Trolox]_{eq}$) of brandies.

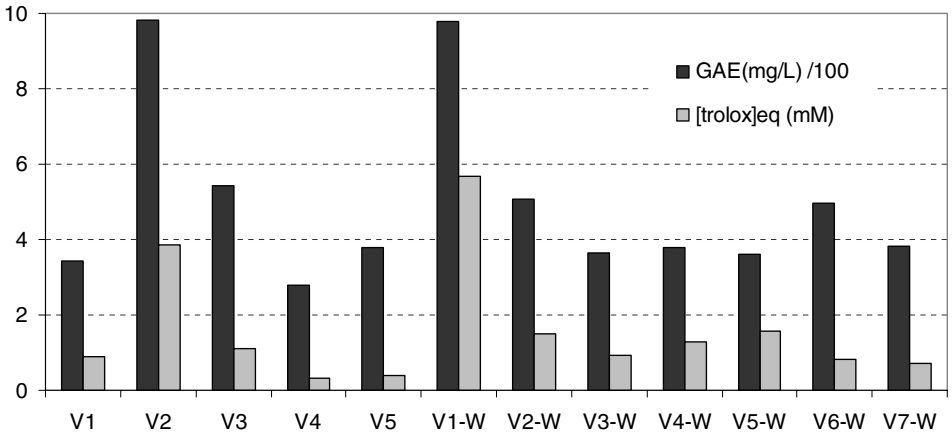

Fig. 2. Total polyphenolic index (GAE) and antioxidant power ($[Trolox]_{eq}$) of vinegars.

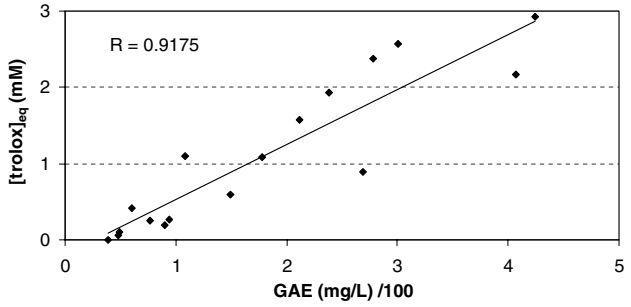

Fig. 3. Correlation line between total polyphenolic index (GAE) and antioxidant power ($[Trolox]_{eq}$) of brandies.

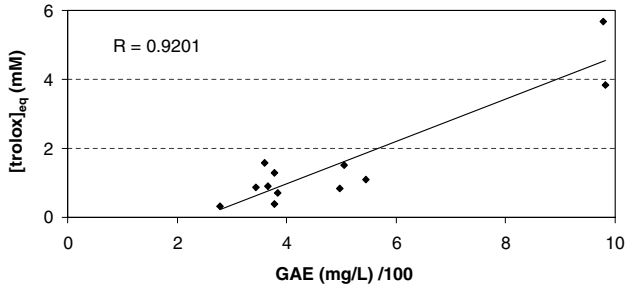

Fig. 4. Correlation line between total polyphenolic index (GAE) and antioxidant power ($[Trolox]_{eq}$) of vinegars.

is not surprising that wood contact does not influence the polyphenols content of vinegar because for this type of product, the main source of polyphenols is the base-wine. So, the differences in GAE (200–1000 mg/L, Fig. 2) found among vinegar samples, are probably due to the different wines of origin used by each company.

These findings could indicate that, in general, the antioxidant power in brandies and vinegars is determined or highly influenced by their polyphenolic content.

In order to discover if a particular compound has more influence than any other in determining the antioxidant power, a new correlation analysis was made between the measures of AP and the results of the chromatography (Tables 3 and 4).

In the case of the brandies, most of the compounds presented a low correlation (Table 3). Some compounds exhibited a good correlation for SB (sculetin, followed by vanillic acid, coniferylaldehyde and scopoletin) and for SRB (coniferylaldehyde, followed by vanillic and syringic acids). In general, for all the types of brandies, coniferylaldehyde was significant.

For the particular case of vinegars, the correlation analysis could just be done for compounds identified in more than two samples. In general, it was found that most of polyphenols identified presented a high correlation with the AP measured (Table 4). For the vinegars without aging, gallic acid was especially important; this polyphenol was also one of the most abundant in the samples. For the vinegars aged in wood, *cis-p*-coumaric, ferulic, i-ferulic, and syringic acids together with vanillin and *p-hydroxy* benzaldehyde exhibited high correlation values.

In relation to the polyphenolic content found in the samples studied, the values obtained are shown in Tables 5 and 6. For the three types of brandy, the same compounds were found, although in lower quantity in the *Solera* brandies. The most important were vanillin and siringic acid, followed by sculetin and vanillic acid. This finding is in agreement with the wood origin of the polyphenols commented before.

In the case of the vinegars, the most abundant compounds, in general, were gallic acid and tyrosol. For the vinegars in process of production (not aged), caftaric acid, the major phenolic compound found in recently prepared musts, is also significant. Those that were aged in wood show a high content of catechin. Other authors (García-Parrilla, Heredia, & Troncoso, 1999) have found that gallic acid is concentrated during aging, which is confirmed in this study. Previous research works (Carrero, Barroso, & Pérez-Bustamante, 1999;

Table 3
Correlation matrix between the chromatographic results and the antioxidant power in brandies

	All	*Solera* (*SB*)	*S. Reserva* (*SRB*)	*S.G. Reserva* (*SGRB*)
p-OH-Benzaldehyde	0.5918	0.4765	0.6673	−0.3146
Vanillic acid	0.7624	**0.8361**	**0.8212**	−0.1484
Siringic acid	0.6690	0.3492	**0.8465**	−0.0197
Vanillin	0.6038	−0.1995	0.5378	0.4734
p-Coumaric acid	0.6551	0.1184	0.4407	0.5513
Coniferilaldehyde	**0.8363**	**0.8463**	**0.9500**	0.4737
Sinapaldehyde	0.4677	0.5298	−0.0831	0.2788
Sculetin	0.4629	**0.9401**	0.6489	−0.7716
Scopoletin	0.4219	**0.8354**	0.7889	−0.1193

The highest coefficients are indicated in bold.

Table 4
Correlation matrix between the chromatographic results and the antioxidant power in vinegars

	All	Without wood	With wood
Gallic acid	0.9070	**0.9780**	0.9358
Protocatechuic acid	0.7978	**0.9437**	0.8565
Protocatechualdehyde	0.4899		0.3723
Tyrosol	0.8307	0.7059	0.9284
p-OH-Benzoic acid	0.9453		0.9400
Catechin	0.8673		0.9478
p-OH-Benzaldehyde	**0.9629**	0.7505	**0.9604**
Siringic acid	0.9122	0.8902	**0.9741**
Vanillin	**0.9683**		**0.9673**
Caftaric acid	−0.0246	0.3124	−0.2436
cis-p-Coutaric acid	0.5019	0.6133	0.1682
trans-p-Coutaric acid	0.4358	0.3454	0.7778
Clorogenic acid	0.8062	0.6443	0.9305
Caffeic acid	0.8514	0.8564	0.9075
cis-p-Coumaric acid	**0.9740**	**0.9405**	**0.9929**
trans-p-Coumaric acid	0.8388	0.7718	0.9484
i-Ferulic acid	0.9265	0.1977	**0.9955**
Ferulic acid	0.7935	−0.0963	**0.9962**

The highest coefficients are indicated in bold.

Garcia-Parrilla et al., 1999) have found that the aromatic aldehydes and their derivatives, produced by alcoholysis of the lignin of the wood, are present at higher levels in aged vinegars. In this case, it is confirmed that compounds such as vanillin, *p*-hydroxybenzaldehyde and protocatechualdehyde, are present at higher levels in the samples that have been aging in oak casks.

In order to determine the possible influence of the aging in wood on the antioxidant power and polyphenolic variables studied, an ANOVA was carried out. Fisher's weight was calculated to establish the discriminant capacity of each variable. For vinegars, only vanillin was a significant variable at $p < 0.05$. For brandies, these ones were sculetin, siringic acid, vanillic acid, *p*-OH-benzaldehyde, antioxidant power and total polyphenolic index.

PCA is a good statistical tool to investigate associations between variables, and moreover, to detect natural groups among samples. For brandies, when data matrix was subjected to PCA, two significant PCs arose according to Kraiser's criterion (eingenvalues >1). With

Table 5
Polyphenols quantified (mg/L) in brandies

Brandy type	Samples	*p*-OH-ben	vanc	sir	van	*p*-cou	con	sin	scu	sco
Solera	SB1	0.04	0.24	0.15	0.32	n.d.	0.16	0.06	0.69	0.03
	SB2	0.05	0.06	0.07	1.85	n.d.	0.10	0.05	n.d.	n.d.
	SB3	0.07	0.25	0.58	0.60	0.09	0.05	0.02	0.05	0.03
	SB4	0.05	n.d.	0.04	4.08	n.d.	n.d.	n.d.	n.d.	n.d.
	SB5	n.d.	n.d.	n.d.	0.04	n.d.	0.03	0.02	n.d.	n.d.
	SB6	n.d.	n.d.	n.d.	0.06	n.d.	0.04	0.03	n.d.	n.d.
Solera Reserva	SRB1	0.33	1.85	4.42	4.89	0.23	0.48	n.d.	2.10	n.d.
	SRB2	0.51	0.50	1.23	1.46	1.17	0.19	0.08	1.61	0.08
	SRB3	0.31	0.42	0.56	2.45	n.d.	0.12	n.d.	n.d.	0.02
	SRB4	0.08	0.35	0.53	0.67	n.d.	0.07	0.16	0.38	0.07
	SRB5	0.25	0.55	0.87	3.70	0.10	0.07	n.d.	1.35	0.06
	SRB6	0.12	0.22	0.46	1.00	0.07	n.d.	n.d.	0.38	0.02
Solera Gran Reserva	SGRB1	0.78	1.38	3.95	4.87	0.88	0.24	0.15	1.42	0.14
	SGRB2	0.97	2.90	7.24	4.57	0.70	0.24	0.22	2.73	0.29
	SGRB3	0.26	1.34	0.07	2.53	0.52	0.21	0.03	2.53	0.11
	SGRB4	0.98	1.74	2.85	2.90	0.38	0.10	n.d.	5.68	0.09
	SGRB5	0.20	1.30	3.45	2.81	n.d.	0.35	0.29	0.91	n.d.

Key. *p*-OH-ben: *p*-OH-benzaldehyde, vanc: vanillic acid, sir: siringic acid, van: vanillin, *p*-cou: *p*-coumaric acid, con: coniferilaldehyde, sin: sinapaldehyde, scu: sculetin, sco: scopoletin.

Table 6
Polyphenols quantified (mg/L) in vinegars

Vinegar type	Samples	gal	proc	proe	tyr	*p*-OH-benc	cat	*p*-OH-ben	sir	van	caft	*c*-*p*-cout	*t*-*p*-cout	fert	caf	*c*-*p*-coum	*t*-*p*-coum	i-fer	fer
Not aged in wood	V1	10.97	9.67	n.d.	29.01	7.52	n.d.	0.96	4.55	n.d.	19.57	4.73	8.27	3.74	3.12	1.67	3.04	1.64	2.08
	V2	62.70	16.24	n.d.	39.19	n.d.	21.92	n.d.	15.17	n.d.	20.89	6.50	8.68	5.64	5.34	2.44	3.86	n.d.	1.80
	V3	12.55	6.67	0.36	37.35	n.d.	n.d.	2.04	11.18	n.d.	19.71	6.47	7.35	5.67	3.10	1.92	2.67	1.24	1.19
	V4	12.29	n.d.	n.d.	30.54	n.d.	n.d.	0.79	3.46	3.94	23.41	4.46	9.35	3.49	2.75	1.32	2.45	1.26	2.01
	V5	9.45	4.82	n.d.	20.81	4.54	11.31	1.11	3.50	n.d.	2.39	1.34	1.39	1.07	0.69	n.d.	1.10	n.d.	n.d.
Aged in wood	V1–W	95.01	n.d.	9.73	97.96	33.47	60.02	8.27	17.43	17.03	8.46	n.d.	8.47	8.22	6.60	2.98	7.59	3.37	5.10
	V2–W	43.74	n.d.	3.13	34.86	9.22	21.19	2.37	5.72	6.06	10.64	2.73	5.67	2.91	4.77	1.97	4.24	1.04	1.71
	V3–W	22.47	3.96	n.d.	26.87	5.48	12.11	n.d.	4.71	4.13	14.24	3.84	5.53	3.30	2.97	1.68	2.96	0.98	0.93
	V4–W	27.14	4.63	n.d.	25.17	4.78	9.06	1.24	5.65	4.72	17.31	3.99	6.47	3.32	3.60	1.84	3.51	1.13	1.10
	V5–W	48.90	13.69	n.d.	53.80	9.15	17.16	1.83	6.37	6.07	9.23	2.18	3.46	1.74	3.90	1.82	3.87	n.d.	1.51
	V6–W	44.41	n.d.	9.81	42.75	12.83	22.04	2.68	7.09	7.05	9.05	1.83	4.18	3.01	4.06	n.d.	4.34	n.d.	n.d.
	V7–W	27.23	n.d.	n.d.	35.13	8.01	15.57	1.46	4.29	4.21	7.10	2.01	3.13	2.42	2.81	n.d.	2.79	n.d.	n.d.

Key. gal: gallic acid, proc: protocatechuic acid, proe: protocatechualdehyde, tyr: tyrosol, *p*-OH-benc: *p*-OH-benzoic acid, cat: catechin, *p*-OH-ben: *p*-OH-benzaldehyde, sir: siringic acid, van: vanillin, caft: caftaric acid, *c*-*p*-cout: *cis*-*p*-coutaric acid, *t*-*p*-cout: *trans*-*p*-coutaric acid, fert: fertaric acid, caf: caffeic acid, *c*-*p*-coum: *cis*-*p*-coumaric acid, *t*-*p*-coum: *trans*-*p*-coumaric acid, i-fer: i-ferulic acid, fer: ferulic acid.

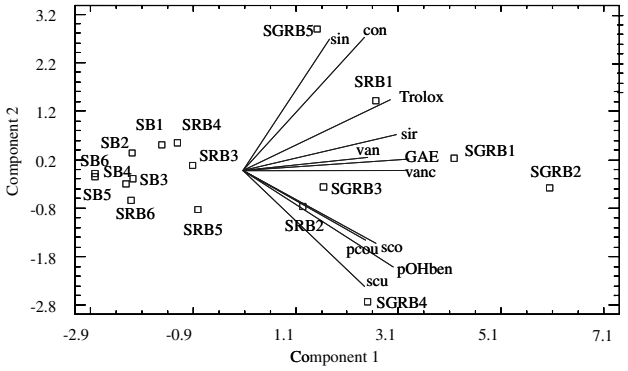

Fig. 5. Principal component analysis. Biplot representation of brandies and statistical variables (polyphenols and antioxidant power). Codes in Table 5.

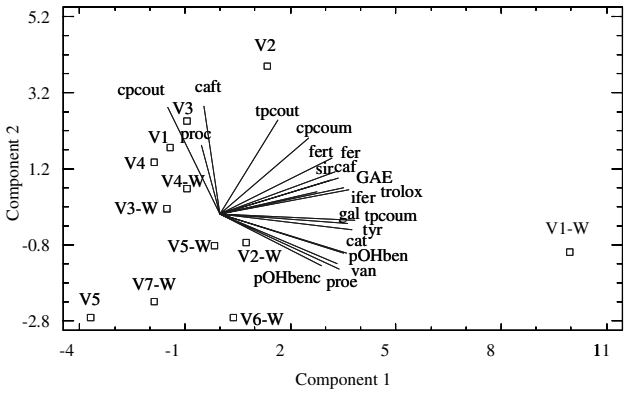

Fig. 6. Principal component analysis. Biplot representation of vinegars and statistical variables (polyphenols and antioxidant power). Codes in Table 6.

these factors, 75.95% of total variance is explained. Fig. 5 shows the score plot for the studied brandies obtained by selecting the first two PCs as axes. As can be seen, the first component (PC1) allows us to differentiate between SGRB brandies and the other two groups, SB and SRB. Taking into account that all variables considered contributed with a positive sign to this component, SGRB brandies exhibit a higher content in these parameters than the other two groups.

For vinegars, three significant components were obtained. With these factors, 89.44% of total variance is explained. Fig. 6 shows the score plot for the studied vinegars obtained by selecting the first two PCs as axes. As can be seen, no differentiation between vinegars with and without aging in wood was obtained.

4. Conclusions

From these observations, it can be concluded that the antioxidant power is very closely correlated with the total polyphenolic content of the samples. In respect of

the compounds considered individually, each polyphenol has a different antioxidant power in function of its chemical structure, with the result that the compounds which are present in greater concentrations are not necessarily those better correlated with the antioxidant power of the samples.

In the case of brandies, when the products have been in contact with wood, a significant amount of polyphenols is taken up from this source, with a corresponding important contribution to the antioxidant power. As for the vinegars, the contact with wood is only slightly significant for their phenolic content, having the polyphenols present in the base wine a higher influence.

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