DOI:10.1021/jf1029493

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Study of the Polyphenolic Composition and Antioxidant Activity of New Sherry Vinegar-Derived Products by Maceration with Fruits

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Several experiments of maceration of a sherry wine vinegar with different fruits (orange, lemon, strawberry, grapefruit, and lime) have been carried out. After optimization (only peel, no heating and seven days as maximum time of maceration), parameters such as polyphenolic content, superoxide anion scavenging ability (related to antioxidant activity) and ascorbic acid content were determined in sherry wine vinegars macerated with two amounts of peel and for two maceration times (3 and 7 days). The analysis of variance pointed to a clear relationship ($p < 0.01$) between type of fruit and amount of peel and polyphenolic content. The factor "time" was practically not significant for any polyphenol. Sherry wine vinegars macerated with different fruits exhibited higher superoxide anion scavenger ability, with the maximum values found for the vinegar macerated with lemon peel. The correlation analysis showed that the superoxide anion scavenger ability of the vinegars macerated, and thus their antioxidant activity, was highly correlated ($p < 0.01$) with several polyphenols, especially with naringin, hesperidin, neohesperidin and gentisic acid and not with the ascorbic acid content.

KEYWORDS: Sherry vinegar; maceration; fruits; polyphenols; superoxide anion scavenger ability; antioxidant activity

INTRODUCTION

In the past few years, the food industry and consumers are becoming more conscious of the nutritional value and safety of food and ingredients. Interest in the consumption of natural antioxidants (especially phenolic compounds) has increased considerably, due to their antiviral, anti-inflammatory and antihypertensive properties (*1*, *2*). Therefore, some authors affirmed that the daily ingestion of phenolic-rich food could prevent chronic, degenerative and coronary heart diseases (*3*, *4*), such as cancer and atherosclerosis (*5*).

Polyphenolic compounds are present to a large extent in vegetal products, such as vinegars, wines and several fruits (*6*−*8*). On the one hand, citrus fruits are a great source of phenolic compounds, and hydroxycinnamic acid derivatives and flavanones are the two main groups. Ferulic, *p*-coumaric, sinapic, caffeic and chlorogenic acids are the principal hydroxycinnamic acid derivatives in citrus fruits (*9*, *10*). Naringin and narirutin, together with hesperidin and neohesperidin, are the most abundant flavonoids in the edible part of many species of citrus fruits (*3*, *11*). On the other hand, the polyphenol content has been studied at length in enological products, particularly vinegars, such as red wine vinegars (*12*), traditional balsamic vinegars (*4*) and Sherry wine vinegars (*6*, *13*). Particularly, our research group has previously studied the polyphenolic composition of Sherry wine vinegars (*14*−*16*). According to these authors, *trans*-caftaric acid and *trans*-*p*-coutaric acid are the main hydroxycinnamic acid derivatives present in Sherry wine vinegars, together with (+)-catechin and gallic acid, among flavan-3-ols and benzoic acids.

Several papers suggest that phenolic compounds play an important role in the antioxidant activity of fruits, vegetables and all their derived products. Alonso et al. (*15*) studied the correlation between the antioxidant power of brandies and vinegars and their polyphenolic content. In citrus fruits, several studies have tried to establish the relationship between antioxidant activity (by means of total antioxidant determination, free radical scavenging activity and superoxide anion scavenging ability) and polyphenolic concentration (*9*, *10*, *17*, *18*). Also, several authors have studied the antioxidant capacity of some individual phenolic compounds. For instance, Gorinstein et al. (*18*) associated the lowest antioxidant activity with ferulic acid and the highest with caffeic acid, whereas Miller et al. (*19*) observed that (−)-epicatechin and gallic acid, together with hesperidin and narirutin, had a higher antioxidant capacity.

Apart from these findings, nowadays, the enological market is full of traditional products. With the aim of diversifying it, new and healthy products derived from vinegars are now starting to be developed and studied. Although only a few researches on acetic fermentation of fruits have been developed in Europe, Japan and China has got a consolidated industry in this type of products. For instance, Chang et al. (*20*) studied the physicochemical properties of different concentrated fruit vinegars, and subtropical fruit vinegars have also been analyzed by other authors (*21*, *22*). With regard to macerated vinegars with fruits, to date no European

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 Published on Web 10/29/2010

scientific data have been reported in the literature, but some studies have been found in Asia (*23*, *24*).

The aim of this research is to study the polyphenolic composition and antioxidant activity (by means of the superoxide anion scavenging ability) of Sherry wine vinegars macerated with several fruits. In this way, new vinegar-derived products with higher functional properties and optimal organoleptic characteristics have been developed. Therefore, in order to establish optimal maceration conditions (parts of the fruits, heating, maceration time and fruit quantity), several assays have been carried out. In addition, a correlation study between the polyphenolic content and the antioxidant activity has been carried out.

MATERIALS AND METHODS

Samples. A Sherry wine vinegar (nonaged, 7 acetic degrees and 1 alcoholic degree), supplied by a local winery, was employed to carry out the present research. This vinegar was individually macerated with different fruits which presented an optimal maturity and health stage: orange (*Citrus sinensis* and *Citrus aurantium*, from Valencia, Spain), lemon (*Citrus limon*, from Murcia, Spain), lime (*Citrus latifolia*, from Mexico), red grapefruit (*Citrus paradisi*, from Valencia, Spain) and strawberry (*Fragaria ananassa*, from Segovia, Spain). The employed Sherry wine vinegar without maceration was used during all the process as control vinegar, maintained under the same conditions as the macerated vinegars.

Development of Optimal Maceration Conditions. First, in order to establish the optimal conditions of the maceration process, three assays were carried out. For this approach, orange fruit was selected as representative of the rest of the studied fruits, because it is one of the most consumed, extended and previously studied ones.

Three different parameters were taken into account: part of the fruit, maceration time and heating. Three glass containers were filled with two liters of Sherry wine vinegar in each one. With the aim of establishing the most appropriate part of the fruit, 3 × 3 cm pieces of orange were added, both peel and pulp to one container (400 g + 250 g), and only peel to other two containers (400 g). In order to study the temperature effect, heating (40 °C) was applied in one of the containers with peel. The experiences with heating and pulp plus peel were discarded. The authors thought that the joint use of both variables, high temperature and pulp, could produce different and numerous reactions which could deteriorate vinegar's sensorial profile. Sampling was done at 7 and 14 days of maceration. All the containers were continuously stirred at 300 rpm, and every 12 h they were completely mixed to facilitate a closer contact of the upper material with the vinegar. Each experiment was carried out in duplicate.

Maceration with Different Fruits. A second scheme of maceration conditions with different fruits was carried out. The fruits selected were orange, lemon, lime, grapefruit and strawberry. Two different maceration times (3 and 7 days) and two different amounts of peel (200 and 400 g) were studied. In the case of strawberry, 200 and 400 g of the entire fruit was employed. No temperature was applied in this second scheme of maceration. All the experiments were carried out in duplicate.

Analysis of Polyphenols and Furanic Compounds. UPLC separation, identification and quantification of phenolic compounds were performed on a Waters Acquity UPLC system, equipped with a diode array detector (DAD), according to the method proposed by Schwarz et al. (*25*). An Acquity UPLC BEH C18 column (100 × 2.1 mm/ID, with 1.7 μm particle size), also from Waters, was used. The identification of each compound was carried out by comparing retention times and UV−vis spectra with those provided for commercial standards. When commercial standards were not available, the calibration curves of compounds with similar chemical structures were used. This was the case of eriocitrin and neohesperidin, which were quantified with the calibration curves of naringin and hesperidin, respectively. All the analyses were carried out in duplicate.

Several phenolic compounds (such as benzoic acids, hydroxycinnamic acid derivatives and glycosylated flavanones, the last ones specific to citrus fruits) and furanic derivatives have been identified in Sherry wine vinegars macerated with fruits. The identification and quantification of benzoic acids, furanic derivatives and glycosylated flavanones were made using the DAD chromatograms obtained at 280 nm, whereas a wavelength of 320 nm was used for the hydroxycinnamic acid derivatives.

Table 1. Study of the Optimal Maceration Conditions[a]

time (days)	heating	peel/peel + pulp[b]	superoxide anion scavenger ability (mmol/L ± SD)
7	yes	P	3.273 ± 0.063
7	no	P	3.058 ± 0.005
7	no	PP	2.756 ± 0.063
14	yes	P	3.586 ± 0.017
14	no	P	3.045 ± 0.121
14	no	PP	2.820 ± 0.040

[a] Mean values (n = 4). Fruit: orange. [b] P, peel; PP, peel + pulp; SD, standard deviation.

Analysis of Antioxidant Activity. In order to determine the antioxidant activity of water-soluble samples by means of superoxide anion scavenging ability, a method based on photochemiluminescence (PCL) was used. The photosensitized chemiluminescence was measured with a Photochem apparatus (Analytik Jena AG, Germany).

An optical excitation of a photosensitizer (luminol) produces free radicals (superoxide anion radicals), which are partially eliminated from the sample by reaction with the antioxidants present in the sample. In the measuring cell, the remaining radicals are detected by chemiluminescent reaction with luminol, and antioxidant activity is determined. Unlike others, this method is not tied to specific pH values, so it is adequate for vinegar samples. Results are presented in equivalent concentration units of ascorbic acid.

This analytical method has been previously employed in blood plasma, drugs and cosmetics (*26*−*28*), plants and several fruits (*29*, *30*). However, no previous data about antioxidant activity in vinegars have been reported by this technique.

Ascorbic Acid Determination. In order to check the possible influence of the ascorbic acid content on the antioxidant activity, it was separately quantified. The vitamin C content in the final vinegars macerated with fruits was determined by direct titration with iodine (*31*). Briefly, 25 mL of the macerated Sherry vinegar and 25 mL of 2 N sulfuric acid (Scharlau, Barcelona, Spain) was transferred into a 250 mL Erlenmeyer flask. The mixture was diluted with 50 mL of water and 3 mL of starch, and 1% (Panreac, Barcelona, Spain) was added as an indicator. The solution was directly titrated with 0.1 N iodine previously standardized (Panreac, Barcelona, Spain).

Sensory Analysis. The sessions were carried out in a standard tasting room (UNE 87-004). All evaluations, exclusively orthonasal, were carried out at 22 °C. Fifteen milliliters of macerated sample was presented in blue glasses, generally used for olive oil sensory analysis, and covered with a glass top in order to minimize the possible loss of aroma. The panel judges (number of judges: 15), all of whom were laboratory personnel, were submitted to a training period about general and specific sensorial aspects.

A structured five-point scale (UNE 87020 equivalent to ISO 4121:1987) was used to quantify the general impression: bad (0), mediocre (1), acceptable (2), good (3) and very good (4).

Statistical Analysis. Analysis of variance (ANOVA), Student's t test, correlation analysis (CA) and principal component analysis (PCA) using the statistical computer packages Statgraphics Centurion, version 15.0 (Statpoint Inc., USA), and SPSS Statistics version 15.0 (SPSS Inc., IL, USA) for Windows XP were performed.

RESULTS AND DISCUSSION

Optimal Maceration Conditions. After preference evaluation of a panel of expert assessors in sensory analysis, and taking also into account superoxide anion scavenging ability values (antioxidant activity) (**Table 1**), optimal maceration conditions were selected. As can be seen, Sherry wine vinegars macerated only with peel presented higher antioxidant activity values than those macerated with both peel and pulp. In addition a longer maceration time (14 days) did not significantly increase antioxidant activity.

In relation to the general impression determined by sensory analysis, for each sample, both the average value and the standard deviation for all the tasters were calculated: peel without heating after 7 days, 3.5 ± 0.4; peel without heating after 14 days, 2.8 ± 0.6;

peel with heating after 7 days, 3.2 ± 0.3; peel with heating after 14 days, 2.3 ± 0.4; peel and pulp without heating after 7 days, 2.5 ± 0.6; peel and pulp without heating after 14 days, 1.7 ± 0.7. Among the vinegars macerated with only peel, the one without heating after one week reached the highest value (3.5 ± 0.4), while that elaborated by using heating after 2 weeks was the worse valued (2.3 ± 0.3). As can be seen, shorter periods of time (7 days) were preferred, and heating deteriorated the aromatic profile of macerated samples.

Therefore, and taking into account that the final product was mainly focused on the consumers, the following conditions were fixed for subsequent macerations: employing only the peel, without heating and with a maximum time of maceration of 7 days. The fact of not heating during the maceration process provides a wider scope to be proposed as a new development method of vinegar-derived products for the industrial sector.

Vinegars Macerated with Different Fruits. Polyphenolic Composition. Taking into account the previous results, later macerations using the achieved optimal conditions were carried out with different fruits (orange, lemon, lime, grapefruit and strawberry). Two different maceration times (3 and 7 days) and two different amounts of peel (200 and 400 g) were studied.

Eighteen polyphenolic compounds and furanic derivatives were determined in the studied samples, and their concentrations in the different macerated vinegars as well as in the control vinegar (without maceration) are presented in **Table 2**. As can be seen, in general terms, all the vinegars macerated with fruits increased their individual polyphenolic concentration. Moreover, as it is logical, some new compounds, not found in the control Sherry vinegar, were transferred to it during the maceration process. On the one hand, some compounds were ceded to the vinegars in a large extent (on the order of g/L). Hesperidin was the main compound detected in vinegars macerated with lemon, which was in agreement with Grandi et al. (*32*), and it was also found in those vinegars macerated with orange, lime and grapefruit (*33*−*35*). Naringin was detected in vinegars macerated with grapefruit, being the polyphenolic compound with the highest concentration when this fruit was used. This fact was previously reported by Hsu et al. (*35*) who described this compound as the main one in grapefruit juices. It was also present, but in a lower level, in vinegars macerated with orange and lime. Finally, neohesperidin was found in vinegars macerated with orange, lemon and grapefruit in a high level of concentration. On the other hand, some compounds not present in the control vinegar were detected in the macerated vinegars, but in a minor level of concentration (on the order of mg/L). These were gentisic acid and ferulic acid, found in vinegars macerated with orange, lemon, and grapefruit; eriocitrin, which was detected in vinegars macerated with lime and grapefruit; and narirutin, which was in those macerated with orange, grapefruit and strawberry.

In addition, typical polyphenolic compounds from Sherry vinegar (e.g.: gallic acid, protocatechuic acid, *p*-hydroxybenzaldehyde, caffeic acid, etc.) were also determined in the macerated vinegars with concentration values similar to those found in the literature (*15*, *16*). However, some compounds from the control vinegar were not found in some of the macerated vinegars such as furfural in orange, lemon, lime and grapefruit macerations or protocatechualdehyde and tyrosol in vinegars macerated with lime and grapefruit. This fact could be explained by taking into account possible interference of other nonidentified compounds derived from the fruits that could affect the identification of the studied compounds and/or possible losses by adsorption of these compounds to the solids during maceration. Further studies could be done in order to clarify this point.

In order to study statistically the differences in the polyphenolic content of all the samples, polyphenol data were submitted to analysis of variance. In this case, three independent factors were considered: fruit, amount of peel and maceration time. As can be seen in **Table 3**, the most significant factor was fruit ($p < 0.01$) followed by amount of peel, significant for protocatechuic acid, hesperidin, naringin, *cis*-*p*-coutaric acid, and ferulic acid. Hesperidin and naringin exhibited clear increases as the amount of peel increased. The factor time was practically not significant for any polyphenol. So, a less time-consuming process for the development of this new type of vinegar could be proposed.

Figure 1 shows the UPLC chromatograms at 280 nm of the Sherry wine vinegars macerated with all studied fruits and the control vinegar without any maceration. As can be seen, vinegar macerated with strawberry presented the most similar polyphenolic profile to the control vinegar, i.e., it was the fruit that provided less polyphenolic compounds to the macerated vinegar (only narirutin). Vinegar macerated with orange was the vinegar with more additional compounds, and, in addition, some polyphenols already present in the control vinegar, such as *p*-hydroxybenzaldehyde, *cis*-*p*-coutaric acid and caffeic acid, increased their concentrations with this maceration as well (**Table 2**).

This fact could be corroborated with the principal component analysis (PCA) that was carried out on polyphenols. When the set of data was subjected to PCA, five significant PCs arose according to Kraiser's criterion (eingenvalues > 1). **Figure 2** shows the score plot of the first two PCs, which explained 57.12% of the total variance of the polyphenols. As can be seen, almost all samples are clearly grouped according to the fruit employed in the maceration. The group of the most similar samples to the control vinegar was the one formed by the samples macerated with strawberry, as it was previously noticed. Orange maceration provided the most different vinegars to the control vinegar, and samples macerated with lime, lemon and grapefruit were placed in an intermediate position. The variables with a higher weight for the principal component 1 (39.53% of total variance) mainly were protocatechualdehyde, tyrosol and *t*-caftaric acid. For the second factor, principal component 2 (17.59% of total variance), *p*-hydroxybenzaldehyde, naringin, narirutin, *cis*-*p*-coutaric acid and gentisic acid were the main constituents.

Vinegars Macerated with Different Fruits. Antioxidant Activity. Antioxidant activity of all macerated vinegars, and of the control vinegar without maceration, was measured by means of superoxide anion scavenging ability (**Table 4**). It is worth mentioning that antioxidant activity of Sherry wine vinegars significantly increased when they were macerated with the majority of the fruits in the studied conditions, with the exception of strawberry, according to Student's *t* test (**Table 4**). So, in most cases, more healthful products were developed by macerating fruits and vinegar. Analysis of variance (**Table 4**) revealed, in most cases, as significant factors for antioxidant activity the three factors considered: fruit, amount of peel and maceration time.

By far, the maximum values were found when the vinegar was macerated with lemon peel, especially with 400 g of peel. These values increased significantly (more than 100% of increase) in relation to those obtained employing a minor amount of peel (200 g). Miller et al. (*19*) reported very high levels of antioxidant activity in fruit juices due to the presence of hesperidin, which is the main polyphenolic compound in the vinegars macerated with lemon, as it was previously stated. In general for the rest of the fruits, significant increases of antioxidant activity were also found when higher amount of peel and maceration time were used, with the exception of maceration time for lime.

The high amount of ascorbic acid that is present in the studied fruits is well-known (*36*), and it has been related to the antioxidant activity of fruit juices by Miller et al. (*19*). In order to check if the final ascorbic acid content of the macerated vinegars could have influence on their antioxidant activity, it was quantified in

Table 2. Concentrations of Polyphenolic Compounds and Furanic Derivatives (mg/L, with Standard Deviations in Brackets) of the Studied Vinegars[a]

		orange				lemon				lime				grapefruit				strawberry			
		200 g/2 L		400 g/2 L		200 g/2 L		400 g/2 L		200 g/2 L		400 g/2 L		200 g/2 L		400 g/2 L		200 g/2 L		400 g/2 L	
compd	CV	3 d	7 d	3 d	7 d	3 d	7 d	3 d	7 d	3 d	7 d	3 d	7 d	3 d	7 d	3 d	7 d	3 d	7 d	3 d	7 d
gallic acid	19.31 (0.200)	20.46 (0.133)	20.83 (0.128)	20.31 (0.488)	19.11 (0.508)	18.26 (0.020)	20.02 (0.579)	18.22 (0.111)	19.15 (0.702)	17.36 (0.031)	18.71 (1.116)	17.01 (0.029)	18.28 (0.280)	19.80 (0.266)	19.58 (0.122)	19.02 (0.044)	19.32 (0.063)	7.817 (0.318)	17.04 (0.596)	8.086 (0.075)	8.563 (0.091)
5-OH-methylfurfural	11.13 (0.002)	12.32 (0.059)	12.59 (0.126)	12.68 (0.173)	11.94 (0.032)	11.22 (0.045)	11.37 (0.505)	11.37 (0.015)	11.76 (0.331)	10.98 (0.010)	10.88 (0.528)	11.00 (0.072)	11.54 (0.110)	10.97 (0.154)	10.89 (0.068)	10.93 (0.099)	10.96 (0.053)	9.632 (0.339)	9.569 (0.317)	9.036 (0.065)	8.995 (0.048)
protocatechuic acid	15.14 (0.213)	14.80 (0.348)	14.89 (0.140)	12.81 (1.480)	12.99 (0.531)	14.12 (0.252)	16.17 (1.262)	13.79 (0.030)	14.24 (0.249)	13.65 (0.037)	14.37 (0.689)	13.16 (0.240)	14.05 (0.044)	14.33 (0.371)	14.44 (0.218)	13.38 (0.188)	13.28 (0.063)	14.15 (0.416)	14.24 (0.423)	14.20 (0.161)	14.24 (0.040)
furfural	4.168 (0.025)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	4.098 (0.139)	2.786 (0.070)	3.760 (0.033)	2.786 (0.070)
p-OH-benzaldehyde	2.517 (0.066)	4.197 (0.062)	4.224 (0.091)	6.677 (0.011)	1.562 (0.005)	2.704 (0.037)	3.045 (0.055)	2.946 (0.003)	3.039 (0.041)	2.590 (0.017)	2.648 (0.234)	2.711 (0.112)	2.923 (0.079)	2.548 (0.122)	2.916 (0.157)	1.905 (0.004)	2.037 (0.004)	2.258 (0.105)	2.280 (0.051)	2.205 (0.002)	2.216 (0.025)
protocatechualdehyde	3.246 (0.091)	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	nd	nd	nd	nd	nd	nd	nd	nd	3.125 (0.011)	3.185 (0.148)	3.098 (0.094)	3.025 (0.055)
narirutin	nd	76.56 (1.190)	72.84 (7.520)	75.93 (0.791)	79.88 (0.646)	nd	nd	nd	nd	nd	nd	nd	nd	131.1 (3.052)	154.0 (4.067)	226.2 (0.246)	277.2 (0.782)	15.93 (0.791)	15.52 (0.041)	17.92 (0.579)	18.10 (0.426)
tyrosol	18.27 (0.684)	<QL	<QL	<QL	<QL	<QL	<QL	<QL	<QL	nd	nd	nd	nd	nd	nd	nd	nd	17.04 (0.785)	16.57 (0.652)	16.29 (0.216)	16.10 (0.088)
eriocitrin[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	84.66 (0.389)	97.40 (7.811)	160.9 (0.168)	195.2 (5.852)	56.74 (1.332)	52.15 (4.168)	60.56 (2.842)	44.74 (0.041)	nd	nd	nd	nd
hesperidin	nd	173.0 (14.81)	185.8 (10.71)	365.5 (2.096)	443.2 (6.650)	736.0 (1.904)	202.4 (0.487)	1539 (3.941)	1672 (8.848)	216.6 (0.783)	200.2 (3.817)	324.7 (5.177)	407.1 (1.886)	nd	nd	nd	nd	nd	nd	nd	nd
naringin	nd	69.65 (2.082)	69.36 (1.639)	111.6 (0.819)	93.70 (0.284)	nd	nd	nd	nd	22.32 (1.535)	26.14 (1.655)	33.08 (0.193)	41.70 (0.633)	904.8 (22.46)	949.0 (19.49)	1056 (0.279)	1027 (13.42)	nd	nd	nd	nd
neohesperidin[c]	nd	601.4 (7.104)	670.5 (9.493)	1015 (2.905)	802.8 (4.370)	511.2 (1.171)	962.2 (5.192)	735.9 (1.186)	737.3 (8.946)	nd	nd	nd	nd	217.0 (14.36)	255.6 (8.640)	360.9 (0.048)	407.4 (1.547)	nd	nd	nd	nd
t-caftaric acid[d]	30.99 (0.022)	36.01 (0.207)	35.88 (0.362)	38.68 (0.017)	35.81 (0.083)	28.70 (0.130)	29.11 (2.123)	29.01 (0.032)	28.93 (0.721)	27.33 (0.174)	28.25 (1.296)	27.29 (0.283)	28.24 (0.270)	30.94 (0.783)	30.15 (0.120)	30.93 (0.061)	30.08 (0.087)	25.39 (0.851)	25.24 (0.740)	24.10 (0.187)	23.30 (0.121)
t-*p*-coutaric acid[d]	11.81 (0.091)	12.59 (0.136)	12.62 (0.080)	12.23 (0.529)	11.49 (0.073)	11.25 (0.080)	11.67 (0.792)	11.62 (0.164)	11.75 (0.304)	11.23 (0.095)	11.75 (0.676)	11.44 (0.189)	11.94 (0.115)	11.99 (0.608)	4.504 (0.098)	12.03 (0.065)	4.480 (0.037)	10.30 (0.340)	10.38 (0.326)	9.845 (0.006)	9.562 (0.090)
c-*p*-coutaric acid[d]	4.689 (0.059)	6.079 (0.027)	6.641 (0.103)	4.260 (0.033)	4.044 (0.007)	4.522 (0.102)	4.897 (0.104)	4.432 (0.219)	4.361 (0.103)	4.194 (0.036)	4.273 (0.259)	4.119 (0.126)	4.157 (0.032)	4.296 (0.427)	4.300 (0.146)	4.490 (0.056)	1.118 (0.011)	4.124 (0.138)	4.064 (0.103)	3.926 (0.000)	3.807 (0.040)
gentisic acid[d]	nd	14.66 (0.033)	14.97 (0.144)	15.07 (0.301)	15.93 (0.267)	15.14 (0.102)	14.76 (1.672)	16.46 (0.081)	16.90 (0.184)	nd	nd	nd	nd	27.42 (3.105)	31.64 (0.606)	35.47 (0.335)	41.70 (0.189)	nd	nd	nd	nd
caffeic acid[d]	6.515 (0.264)	10.89 (0.026)	11.14 (0.367)	17.65 (0.121)	8.318 (0.071)	6.541 (0.008)	8.469 (0.483)	7.414 (0.006)	7.944 (0.154)	6.334 (0.147)	8.361 (0.565)	8.359 (1.092)	9.867 (0.731)	7.807 (0.372)	8.127 (0.419)	6.448 (0.208)	7.849 (0.034)	5.920 (0.154)	6.252 (0.152)	5.665 (0.064)	5.349 (0.044)
ferulic acid[d]	nd	7.564 (0.119)	8.293 (0.343)	13.33 (0.451)	13.59 (0.017)	0.959 (0.013)	1.019 (0.102)	1.694 (0.058)	1.735 (0.010)	nd	nd	nd	nd	5.722 (0.204)	6.912 (0.543)	20.47 (0.694)	13.42 (0.020)	nd	nd	nd	nd
sum of compds	127.8	1060	1141	1722	1554	1361	1285	2392	2529	417.2	423.0	613.8	745.0	1445	1544	1859	1901	119.8	127.1	118.1	116.0

[a] Maceration with different fruits, amount of peel and times. Mean values ($n = 4$). CV, control vinegar; g, grams; d, days; L, liters; <QL, compound detected but below quantification limit; nd: compound not detected. All compounds were quantified at 280 nm unless otherwise noted. [b] Quantified using naringin calibration curve. [c] Quantified using hesperidin calibration curve. [d] Quantified at 320 nm.

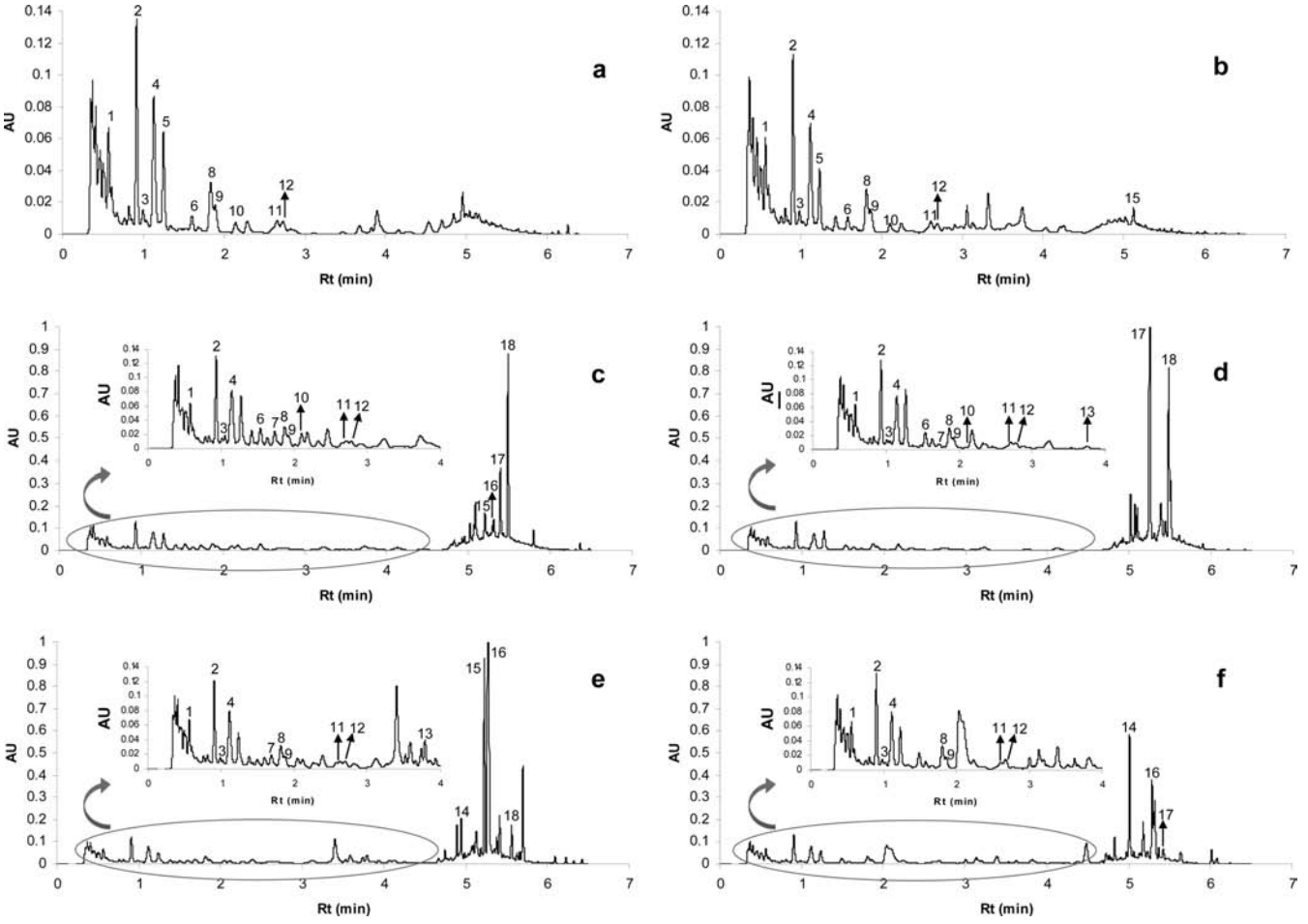

Figure 1. UPLC chromatograms at 280 nm of Sherry vinegars macerated with fruits (400 g of peel, 7 days). Legend: Control Sherry vinegar (**a**); Sherry vinegar macerated with strawberry (**b**), orange (**c**), lemon (**d**), grapefruit (**e**) and lime (**f**).Identified compounds: 1, gallic acid; 2, 5-hydroxymethyl furfural; 3, protocatechuic acid; 4, *trans*-caftaric acid; 5, furfural; 6, protocatechualdehyde; 7, gentisic acid; 8, *trans-p*-coutaric acid; 9, *cis-p*-coutaric acid; 10, tyrosol; 11, *p*-hydroxy-benzaldehyde; 12, caffeic acid; 13, ferulic acid; 14, eriocitrin; 15, narirutin; 16, naringin; 17, hesperidin; 18, neohesperidin.

Table 3. ANOVA Analysis Applied to Polyphenolic Compounds and Furanic Derivatives

	fruit		amount		time	
polyphenols and furanic derivatives	F	*p*	F	*p*	F	*p*
gallic acid	42.94	0.0000[a]	5.54	0.0248	6.88	0.0131
5-OH-methylfurfural	104.52	0.0000[a]	0.07	0.7993	0.15	0.6994
protocatechuic acid	2.07	0.1066	19.56	0.0001[a]	4.96	0.0329
furfural	269.21	0.0000[a]	0.17	0.6808	7.75	0.0088[a]
p-OH-benzaldehyde	5.96	0.0010[a]	0.17	0.6808	1.85	0.1825
protocatechualdehyde	864.13	0.0000[a]	1.97	0.1695	0.01	0.9100
narirutin	65.53	0.0000[a]	3.39	0.0748	2.12	0.1548
tyrosol	75.45	0.0000[a]	2.59	0.1173	0.76	0.3905
eriocitrin	65.86	0.0000[a]	6.89	0.0130	0.17	0.6836
hesperidin	64.11	0.0000[a]	18.64	0.0001[a]	0.78	0.3833
naringin	33.69	0.0000[a]	9.96	0.0034[a]	0.23	0.6368
neohesperidin	104.01	0.0000[a]	6.42	0.0162	1.41	0.2436
t-caftaric acid	182.77	0.0000[a]	0.05	0.8217	1.31	0.2614
t-p-coutaric acid	3.92	0.0103	2.50	0.1237	4.28	0.0465
c-p-coutaric acid	8.92	0.0001[a]	12.97	0.0010[a]	0.32	0.5780
gentisic acid	34.39	0.0000[a]	6.66	0.0145	4.35	0.0447
caffeic acid	11.94	0.0000[a]	0.72	0.4025	0.05	0.8200
ferulic acid	40.94	0.0000[a]	16.73	0.0003[a]	0.33	0.5675

[a] Values are significant at $p < 0.01$.

the samples macerated during 7 days with 400 g of fruit peel (**Figure 3**). As can be seen, all macerated vinegars presented higher levels of ascorbic acid than control vinegar (11.50 mg/L), all of them very similar and ranging between 13.53 and 17.25 mg/L. These values were statistically different from those corresponding to the control vinegar ($p < 0.01$).

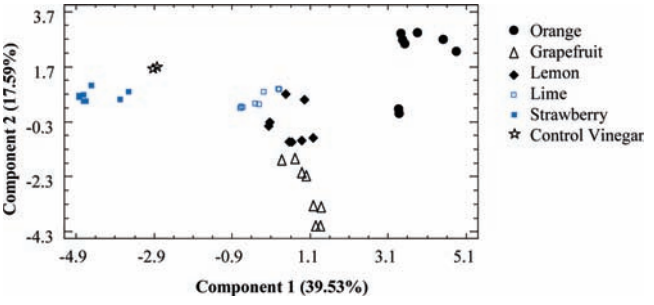

Figure 2. Plot of the studied vinegar samples in the space defined by the first two principal components (PC) with regard to polyphenolic compounds and furanic derivatives.

On the one hand, the maximum value was achieved by the vinegar macerated with strawberry, just the one which had the lowest antioxidant activity. On the other hand, vinegar macerated with lemon, which had by far the highest superoxide anion scavenging ability value, and thus antioxidant activity, presented values of ascorbic acid similar to those vinegars macerated with other fruits.

So, it is logical that, when these data were submitted to correlation analysis (CA), the results obtained (Pearson correlation coefficient = 0.230; $p = 0.236$) revealed that these two parameters,

Table 4. Superoxide Anion Scavenger Ability (mmol/L) of Sherry Wine Vinegars Macerated with Different Fruits and with Different Conditions[a]

amount of peel (g):	0	200			400		
time (days):	0	3	7		3	7	
control vinegar	2.230 ± 0.000						
orange		3.167[b] ± 0.002	3.685[c] ± 0.005	*d*	3.650[b] ± 0.001	4.163[c] ± 0.032	*d*
lemon		5.123[b] ± 0.053	6.542[c] ± 0.013	*d*	11.63[b] ± 0.250	14.07[c] ± 0.083	*d*
lime		2.750[b] ± 0.037	3.092[c] ± 0.001		3.848[b] ± 0.022	4.146[c] ± 0.107	
grapefruit		2.386[b] ± 0.007	2.951 ± 0.036	*d*	2.804[b] ± 0.021	3.152 ± 0.010	*d*
strawberry		2.184[e] ± 0.045	2.121[c,e] ± 0.041		2.306[e] ± 0.008	2.085[c] ± 0.025	*d*

[a] Fixed parameters: fruit peel and without heating. Mean values ± SD ($n = 4$); g, grams. [b] Significant differences according to the amount of peel for 3 days of maceration ($p < 0.01$). [c] Significant differences according to the amount of peel for 7 days of maceration ($p < 0.01$). [d] Significant differences according to the maceration time ($p < 0.01$). [e] No significant differences between each macerated vinegar and control vinegar ($p < 0.01$).

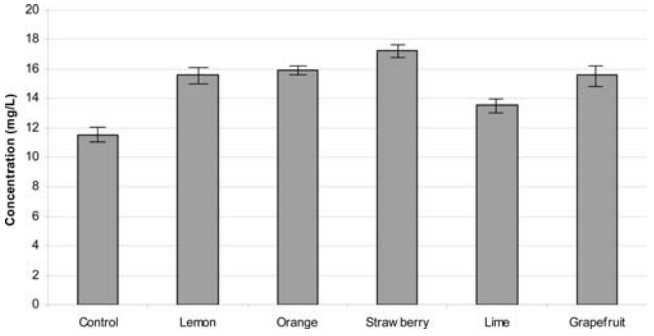

Figure 3. Ascorbic acid concentration (mg/L) in Sherry wine vinegars macerated with different fruits. Mean values with standard deviations ($n = 3$).

antioxidant activity and ascorbic acid content, in the samples studied, were not correlated.

Therefore, all of this let us conclude that the increase in ascorbic acid content after maceration did not affect antioxidant activity of macerated vinegars. This fact is in concordance with Rapisarda et al. (*9*), who pointed out that ascorbic acid only played a minor role in the antioxidant efficiency of orange juices, and with Tabart et al. (*37*), whose work revealed that all studied phenolic compounds, such as gallic acid and hesperidin, showed a greater antioxidant capacity than ascorbic acid. However, other authors (*38*) observed that ascorbic acid played a high role for the antioxidant capacity of citrus juices, higher than that played by certain glycosylated flavanones. These authors explained these contradictory results on the basis of factors such as citrus variety, maturity, material preparation and methodology used to determine the antioxidant activity.

Correlation Study. Correlation coefficients of superoxide anion scavenging ability (antioxidant activity) and individual polyphenols are shown in **Table 5**. The antioxidant activity of the vinegars macerated was highly correlated ($p < 0.01$) with several polyphenols, especially with naringin, hesperidin, neohesperidin and gentisic acid. The rest of the polyphenols, most of them phenolic acids, showed a nonsignificant correlation with antioxidant activity. Xu et al. (*38*) found that the flavanone glycosylates narirutin, naringin, hesperidin and neohesperidin showed a higher correlation with the antioxidant activity of citrus juice than certain phenolic acids (caffeic acid, ferulic acid, protocatechuic acid and *p*-coumaric acid).

In summary, the results obtained indicate that several phenolic compounds are extracted from the fruit employed during the vinegar maceration process and that this enrichment implies a higher antioxidant activity of the macerated vinegars. Among the phenolic compounds found in the macerated vinegars, those with higher antioxidant activity were several glycosylated flavanones (hesperidin, neohesperidin and naringin). So, it can be concluded that the optimal maceration conditions (fruit peel, no heating and only 3 days) produced new Sherry wine vinegars with higher functional properties and optimal organoleptic characteristics. This new proposed process could be easily employed by any vinegar maker company, and it will allow diversification of its production and to develop a final vinegar derivative that could have many beneficial effects on the final consumers, regularly employed as food dressing.

Table 5. Correlation Study between Studied Compounds (Polyphenolic Compounds and Furanic Derivatives) and their Superoxide Anion Scavenger Ability

	superoxide anion scavenger ability correlation	
studied compounds	Pearson correlation coefficient	*p* value
gallic acid	0.399	0.056
5-OH-methylfurfural	0.069	0.378
protocatechuic acid	0.325	0.065
furfural	0.052	0.406
p-OH-benzaldehyde	0.122	0.022
protocatechualdehyde	−0.075	0.367
narirutin	−0.086	0.348
tyrosol	0.107	0.313
eriocitrin	−0.078	0.362
hesperidin	0.561	0.003[a]
naringin	0.932	0.000[a]
neohesperidin	0.732	0.000[a]
t-caftaric acid	0.044	0.421
t-*p*-coutaric acid	−0.331	0.061
c-*p*-coutaric acid	−0.095	0.333
gentisic acid	0.445	0.009[a]
caffeic acid	0.081	0.357
ferulic acid	−0.079	0.360

[a] *p* values <0.01.

ACKNOWLEDGMENT

The authors would like to thank Exvina, S. L. (Jerez, Spain), for providing us with the vinegars.

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Received for review July 29, 2010. Revised manuscript received October 7, 2010. Accepted October 13, 2010. We thank Consejería de Educación y Ciencia de la Junta de Comunidades de Castilla-La Mancha, y del Fondo Social Europeo as financial institutions.