Food Chemistry 116 (2009) 29–33

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant activity of Brandy de Jerez and other aged distillates, and correlation with their polyphenolic content

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ARTICLE INFO

Article history:
Received 18 September 2008
Received in revised form 30 December 2008
Accepted 29 January 2009

Keywords:
Antioxidant activity
Brandy
Sherry
Brandy de Jerez
Aged distillates

ABSTRACT

The antioxidant activities (AA) of commercial sherry brandies (Solera, Solera Reserva and Solera Gran Reserva) and samples obtained from an experimental ageing system were measured. In the dynamic ageing system, the data show that the casks containing the youngest distillate become exhausted over time. For experimental and commercial brandies, antioxidant activity and polyphenolic content both increase with increasing age, so the correlation between the two parameters was analysed. It is concluded that there is a significant relationship between them; however, it seems that some non-phenolic compounds could also contribute to the antioxidant activity. The results revealed certain differences between commercial and experimental sherry brandies, and these differences could be due to the addition of caramel colouring. Several other commercial aged distillates of various different geographical origins (Cognacs, Armagnacs, and Spanish, French and South African brandies) were studied, and their antioxidant power was compared with that of the three categories of Brandy de Jerez. It was found that Solera Gran Reserva sherry brandies show the highest antioxidant activity of all the products considered.

1. Introduction

Reactive oxygen species, including free radicals, such as superoxide anion radicals, hydroxyl radicals, and non-free-radical species, such as H_2O_2 and singlet oxygen, play a key role in the oxidation process that can damage cells. This process is considered to be one of the initial development stages of many chronic diseases, such as cancer, cardiovascular disease, atherosclerosis, and diabetes (Abdi & Ali, 1999; Abe & Berk, 1998; Lefer & Grander, 2000). Over the last decade, considerable experimental evidence has confirmed the importance for health of following a diet rich in antioxidants, which can protect the organism against the damage caused by these radicals. Some of these antioxidants are well known, such as vitamins (particularly vitamins E and C) and carotenoids, including β-carotene. A healthy diet should provide an adequate and continuous supply of these antioxidants. Other antioxidants, like ubiquinols and antioxidant thiols, are produced in small amounts by the organism, but the levels of many of them can be increased by dietary supplements (Rice-Evans & Packer, 1998). For these reasons, there is an increasing interest in characterising foods and drinks in terms of antioxidant potential; numerous methods have been developed with this aim.

In plants like rosemary, thyme, clove and ginger, the antioxidant activity is attributed to the natural presence of phenolic compounds. Most of the natural antioxidants come from plants. These compounds are polyphenols, which are found in all parts of the plant (roots, bark, stem, leaves, fruits, flowers, etc.). Some of them can form complexes with metals, although their main antioxidant activity is based on their chain-breaking and free radical-scavenging activities. Important antioxidant properties of flavanols, a common type of polyphenol, have been widely studied (Alonso, Domínguez, Guillén, & Barroso, 2002; Arnous, Makris, & Kefalas, 2001; Brenna & Pagliarini, 2001; De Beer, Joubert, Gelderblom, & Manley, 2005).

In recent years considerable attention has been paid to wine and its derivatives as a source of polyphenols. In particular, the relationship between the consumption of wine and the prevention of cardiovascular diseases, certain cancers, and other diseases (the French paradox) has been widely studied (Renaud & De Lorgeril, 1992; Tomera, 1999).

The considerable increase in the antioxidant activity of aged distilled beverages during ageing in barrel has been attributed to contact between the distillate and the wood (Da Porto, Calligaris, Celotti, & Nicoli, 2000; Goldberg, Hoffman, Yang, & Soleas, 1999). The best correlation between antiradical activity and the chemical composition of Cognac has been observed for ellagitannins, which are extracted from wood mainly in the first year of ageing

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doi:10.1016/j.foodchem.2009.01.096

(Da Porto et al., 2000). As reported by Goldberg et al. (1999), the antioxidant activity of distillates studied presents values that are intermediate between the corresponding red and white wines. The highest values of total antioxidant activity were found in Armagnacs, Cognacs and bourbon whiskeys, which also possessed the highest contents in phenolic compounds. Rum, vodka, gin and other distillates had negative values of antioxidant activity, which was consistent with the low concentrations of phenolic compounds in these beverages. From these results, the authors concluded that the ageing process increases the content of phenolics and thus the antioxidant activity of distillates.

After the initial fermentation and distillation processes, "Brandy de Jerez" does not reach its organoleptic equilibrium until it has been aged in American oak (*Quercus alba*) casks (Quiros & Carrascal, 1992). "Brandy de Jerez" is aged by the traditional dynamic system ("Solera and Criaderas"), and sometimes additionally by the static system ("Añadas"). During the ageing period, slow physico-chemical changes involving both brandy and wood take place (Mosedale & Puech, 1998). These changes result in the radical modification of the product, producing well-known changes in colour, taste and flavour. This evolution comprises changes in the composition and concentration of compounds related to the sensorial characteristics of the brandy.

In this work, the antioxidant activities of brandies from an experimental system, "Brandies de Jerez" and other commercial distillates, were measured and correlated with the total polyphenolic content of these beverages.

2. Materials and methods

2.1. Reagents

Folin–Ciocalteau 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 using gallic acid (Merck, Darmstadt, Germany).

In the electrolytic system for the measurement of the antioxidant activity, 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. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), supplied by Sigma–Aldrich, was dissolved in methanol of HPLC grade (Scharlau, Barcelona, Spain) and used to construct the calibration curve (0–25 mM).

2.2. Samples

The experimental ageing system set up in the Jerez Centre of Viticulture and Enology for the analytical monitoring of brandy ageing consisted of 15 casks of 500 l capacity each. Twelve of the casks were used to age brandy according to the dynamic system of "Solera and Criaderas" traditionally used in the Denomination of Origin area, in groups of three casks for each ageing scale. The Solera scale, designated S, was the oldest; the First Criadera, the next oldest; the Second Criadera, the next; and the Third Criadera, the youngest. The other three casks held the same new distillate but for static ageing, according to the system of Añadas. All of the casks had previously contained sherry wine for at least 3 years, as established in the regulations for the manufacture of Brandy de Jerez.

The four scales contained distillate with a 40% v/v graduation. Programmed decantings every 3 months involved first drawing off one-fourth (125 l) of the total volume of brandy held in each of the casks of the oldest Solera scale. Then, a similar proportion of the volume held in the First Criadera casks was drawn off; these amounts (from the First Criadera casks) were then mixed together, divided into three parts, and used to refill the three Solera casks to their original volume. This procedure of partially refilling older casks with younger brandy drawn off the preceding scale is repeated for each of the ageing scales of the system. In the final decanting, the youngest scale (the Third Criadera in this system) is replenished with new distillate from the distillery. The periodic partial extraction of brandy and consequent replenishing is known as "sacas y rocíos". Sampling of each scale was carried out every 3 months, taking advantage of the operations of "sacas y rocíos". Small sample volumes (20 ml) were obtained from the product after the mixing of the partial volumes drawn off the three casks belonging to the same scale, which thus constitutes a representative mean of the whole scale. A total of 76 samples (4 scales × 19 "sacas y rocíos") were analysed during the 5 years of the study.

The three casks used for the Añadas were filled with the same new distillate at the same time as the casks of the dynamic system. The only product drawn off from these static system casks was that needed for sampling (20 ml every 3 months). A total of 19 samples were obtained by mixing the aliquots from the three casks of the static system in every sampling.

64 commercial sherry brandies were studied: 21 Solera (S), 18 Solera Reserva (SR) and 25 Solera Gran Reserva (SGR). The sampling covered the products of all of the companies registered with the Regulatory Commission of the Specific Denomination of "Brandy de Jerez".

Distillates of different geographical origin included 12 Spanish brandies without a Denomination of Origin (D.O.), 4 South African brandies, 11 Cognacs, 4 Armagnacs, and 4 French distillates without a D.O.

Aromatic macerations and infusions analysed were made from raisins, prunes, oak wood fibres (toasted – T, and not toasted – A and C), sheaths of vanilla, shells of nut, and pericarp of almonds. Colouring caramels analysed were made from glucose, sucrose and grape must.

All samples were supplied by the Regulatory Commission of the Specific Denomination of "Brandy de Jerez".

2.3. Measurement of the total phenolic content

Total phenols were determined using the Folin–Ciocalteau reagent. The reaction mixture contained 250 μl of sample, 1250 μl of Folin–Ciocalteau reagent and 5 ml of 20% sodium carbonate. Dilutions were carried out in duplicate, and the absorbance was measured at 750 nm. The calibration curve was prepared with gallic acid solutions ranging from 0 to 1500 mg/l, and the results are given as gallic acid equivalents (GAE).

2.4. Measurement of the antioxidant activity

Most of the methods used for determining antioxidant activity are based on the study of a reaction in which a free radical is generated and how this reaction is inhibited by the addition of the compound or sample whose antioxidant power is being measured. The method used in this work (Alonso, Guillén, & Barroso, 2003) has been developed and validated by the research group from a previous design (electrochemical test of accelerated browning) taken as a template. The method used here is based on the electrochemical oxidation of 2,2″-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS). This is a compound which, when oxidised, generates a coloured cation-radical, easily detectable by visible spectrophotometry. When an antioxidant compound or sample is added to the solution, the oxidation of the ABTS is delayed, and the reaction consumes a greater quantity of electrical current,

which is related to the degree of antioxidant activity of the sample added.

The device (Fig. 1) consists of an 80 ml beaker (cathode) inside which a 30 ml filtering crucible, pore size 4 (anode) is set. A flat platinum electrode (30 × 60 mm) is introduced in the cathode and a cylindrical platinum mesh (22 × 22 mm) is introduced in the anode. To facilitate contact between the electrodes and the solutions, they are kept in continuous agitation by a magnetic agitator fitted to each cell. The feed source used (FAC-307C from Promax) allows the working conditions to be set in constant intensity mode. The reaction was monitored using a UV–Vis transmission probe coupled to a PC 2000 miniaturized spectrophotometer from Oceans Optics, Inc. (Eerbeek, The Netherlands) with a DH-2000 halogen–deuterium light source from Top Sensor System (Eerbeek, The Netherlands).

The test consists of the oxidation, by means of the electrolytic system described, of a solution of ABTS (50 μM), to which the sample to be tested is added. Sample volumes were adjusted in the range 0.5–2 ml, to optimise the analysis times. To start the experiment, a constant intensity of 2 mA is applied, and the absorbance at 414 and 734 nm (the two wavelengths at which $ABTS^{\cdot+}$ presents its maximum principal values) is continuously recorded. As response function, the coulombs consumed in the oxidation of the sample added are measured. This quantity of coulombs is then compared to a calibration curve prepared with solutions of known concentrations of Trolox.

3. Results and discussion

3.1. Experimental ageing system

Fig. 2 shows the evolution of the antioxidant activity at successive numbers of "sacas y rocíos". Points are experimental data, and lines were drawn using the moving average method. Young distillates showed no antioxidant activity; activity increases in line with time spent in contact with the wood. This is a logical finding because it is believed that the process of maturing in oak cask is characterised by the diffusion of compounds from within the wood.

The profile of the antioxidant activity curve in the dynamic system is affected by several variables: the content of the cask in compounds that can be extracted from it; the dilution effect of mixing with younger distillates; the difference in the extractive power of the distillates (the lower the compound concentration, the greater the extractive power), and the different extraction kinetics of the various compounds. The initial rise in the antioxidant activity is produced by the predominance of the extraction phenomenon over the dilution effects produced by the addition of younger distillate.

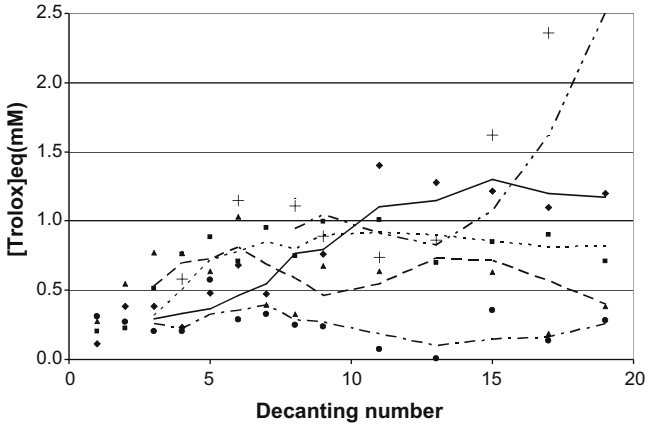

Fig. 2. Evolution of the antioxidant activity (as [Trolox] mM) in the experimental ageing system of sherry brandy. ♦ —— Solera; ■ - - - - - - First Criadera; ▲ — — — Second Criadera; ● — - - - — Third Criadera; + — - - — - - — Añada.

This situation changes after a certain number of decantings, when it stabilises and then decreases slightly.

From the comparison between scales, it is concluded that the highest antioxidant activity for decanting number beyond 10, when a clear divergence is observed, corresponds to the Solera, followed by the First Criadera and Second Criadera, with the Third Criadera having the lowest value. The explanation for this is that, in the dynamic ageing system, the Solera is the scale containing the brandy that has spent the most time in the ageing system and, therefore, has been able to extract the largest amounts of compounds from the casks. These data clearly indicate the exhaustion shown by the Third Criadera: when the decanting number increases above 10, antioxidant activity decreases progressively. This will therefore affect the antioxidant activity of the oldest scale (Solera). To maintain the levels of antioxidant compounds in the Solera scale, and in the final product, we suggest the replacement of the casks of the youngest Criadera with new casks. In the static ageing system (Añadas), the antioxidant activity increases rapidly after decanting number 13, and beyond 15 it is higher than those of the Solera, the last stage in the dynamic system.

Fig. 3 shows the evolution of total phenolic content, measured as Folin–Ciocalteau Index (FCI). It can be seen that the curves for dynamic and static systems are similar to those of antioxidant activity, so the relationship between these two variables was studied. The regression models for Solera and Añada samples give coefficients of 0.71 and 0.79 respectively, confirming the role of the phenolic compounds in the antioxidant activity of aged distillates.

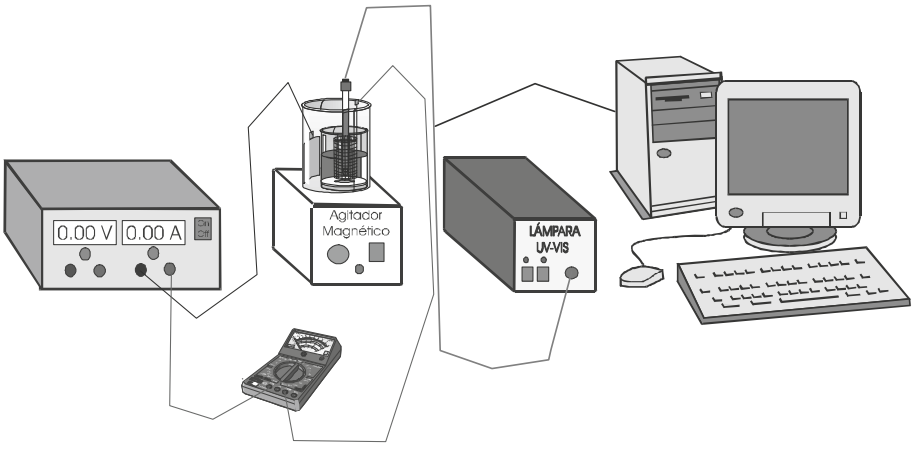

Fig. 1. Diagram of the device used for the measurement of antioxidant activity.

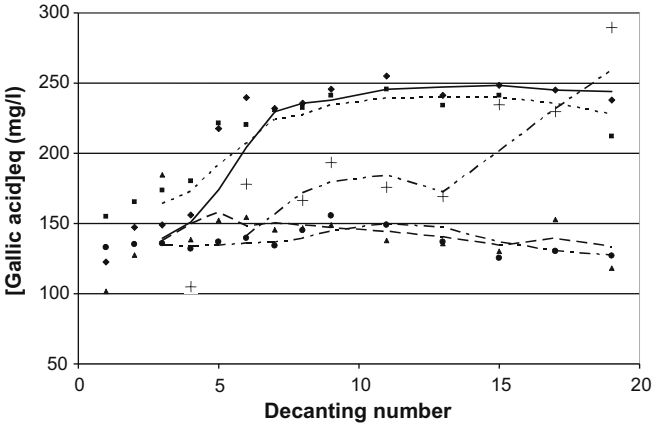

Fig. 3. Evolution of the total phenolic content (as mg/l of gallic acid) in the experimental ageing system of Sherry brandy. ♦ —— Solera; ■ - - - - - - First Criadera; ▲ — — — Second Criadera; ● — - - - - Third Criadera; + — - - — - - — Añada.

3.2. Commercial sherry brandies

Averages values of antioxidant activity of sherry brandies corresponding to the three commercial qualities are presented in Table 1, which also summarises the experimental system data. The average time of ageing of the samples from the experimental system was calculated considering the number of decantings in a year, the total system volume, the volume of each decanting, and the number of scales. This allowed us to classify 17 samples as Solera (aged for more than 6 months), 44 as Solera Reserva (aged for more than 1 year), and 8 as Solera Gran Reserva (aged for more than 3 years).

In general, the Solera Gran Reserva brandies possess a higher antioxidant activity and total polyphenolic content than the Solera Reserva brandies, and these in turn show higher values than Solera brandies, for both experimental and commercial brandies. It can therefore be concluded that antioxidant activity and polyphenolic content both increase with increasing age. To confirm this observation, a study was done to detect any significant difference between the three groups; a Kruskal–Wallis test was applied, because data of antioxidant activity showed a non-normal distribution and a different variance within the groups. It was concluded that a significant difference exists for both experimental ($p < 0.0056$) and commercial ($p < 0.0055$) samples, although this difference is exclusively attributable to the S-SGR distance. The least and the most aged samples are separated into their respective groups, while most brandies of the SR category are situated between these two groups. The explanation for this overlapping between categories is that commercial brandies are classified on the basis of minimum average times of ageing, according to the official definitions of Solera, Solera Reserva and Solera Gran Reserva brandies (for instance, a Solera Reserva brandy may be included in the Solera category because it has been aged for at least 6 months); this could partially explain the higher variation coefficients in commercial brandies. Moreover, as the dynamic ageing system involves the dilution with younger distillates, and oak casks tend to become exhausted of their compounds over time, decanting numbers (ageing time) beyond a certain value do not relate to a higher concentration in compounds extracted. This finding is clear in brandies with medium ageing, with a significant dilution factor and an extractive capacity that is rather limited.

In addition, the results revealed certain differences between brandies from the experimental system and commercial brandies. These differences could be attributable to the addition of several substances (macerations, infusions and caramel colourings), which had been made to the commercial brandies after the ageing and before bottling and which contribute to the distinctive properties of "Brandy de Jerez", whereas these substances had not been added to the brandies from the experimental system. Table 2 summarises the results for the antioxidant activity of these alcoholic extracts, macerations and caramel colourings. As can be observed, at usual addition concentrations, most of the extracts except oak C (0.21 mM Trolox) show no antioxidant activity. These concentrations can be modified by each producer, so the effect of higher additions on the antioxidant activity of aged brandies was studied. Extracts of prune, almond, oak A, oak C and vanilla showed a higher AA, but it is not clear that this can explain the higher values of commercial samples. In the case of caramels, used to adjust the final colour of the commercial brandy, glucose caramel showed the

Table 2
Antioxidant activity ($[Trolox]_{eq}$) of alcoholic extracts and caramel colourings. DL: Limit of detection.

Extract	Addition (in 40% ethanol)	$[Trolox]_{eq}$ (mM)
Prune	8 ml/l	<DL
	20 ml/l	0.33
Almond	4 ml/l	<DL
	10 ml/l	0.19
Nut	8 ml/	<DL
	l20 ml/l	<DL
Oak A	2 ml/l	<DL
	5 ml/l	0.24
Oak C	2 ml/l	0.21
	5 ml/l	1.45
Oak T	2 ml/l	<DL
	5 ml/l	0.04
Raisin	8 ml/l	<DL
	20 ml/l	<DL
Vanilla	1 ml/l	<DL
	2.5 ml/l	0.13
Must caramel 1	6.62 g/l	<DL
Must caramel 3	5.64 g/l	0.08
Glucose caramel 3	5.84 g/l	0.89
Sucrose caramel 1	5.05 g/l	0.20

Table 1
Statistics of antioxidant activity ($[Trolox]_{eq}$) and total phenolic index ($[Gallic\ acid]_{eq}$) of sherry brandies. Commercial and experimental samples are classified according to categories: Solera (S), Solera Reserva (SR) and Solera Gran Reserva (SGR).

	Experimental ageing system				Commercial sherry brandies			
	Average	Min	Max	Variation coefficient (%)	Average	Min	Max	Variation coefficient (%)
AA: $[Trolox]_{eq}$ (mM)								
S	0.39	0.01	0.77	58.1	0.61	0.04	2.58	127.2
SR	0.66	0.07	1.40	49.8	2.00	0.02	7.49	97.0
SGR	0.89	0.51	1.22	32.7	2.23	0.09	9.95	118.7
TPI: $[Gallic\ acid]_{eq}$ (mg/l)								
S	145.3	127.3	184.6	11.6	277.9	199.0	384.6	19.5
SR	190.9	117.9	255.1	25.3	486.6	265.1	1334.6	54.1
SGR	204.3	173.4	248.4	14.3	517.7	319.6	709.6	21.1

Table 3
Statistics of antioxidant activity ([Trolox]$_{eq}$) and total phenolic index ([Gallic acid]$_{eq}$) of aged spirits of different geographical origins.

	AA: [Trolox]$_{eq}$ (mM)				TPI: [Gallic acid]$_{eq}$ (mg/l)			
	Average	Min	Max	Variation coefficient (%)	Average	Min	Max	Variation coefficient (%)
Armagnacs	1.17	0.65	1.17	35.2	309.7	246.8	356.8	14.9
Cognacs	2.16	0.70	4.89	44.1	378.4	256.2	457.3	16.6
Spanish brandies	1.94	0.28	5.17	91.2	550.8	301.2	954.0	38.3
French brandies	0.70	0.31	1.09	78.3	277.1	231.2	322.9	16.5
South African brandies	0.90	0.16	1.85	96.7	308.7	214.0	447.9	28.5

highest AA, over the sucrose type, whereas must caramels analysed presented very low values.

A correlation analysis was made between the two parameters for all the commercial brandies, and it was confirmed the polyphenolic content and antioxidant activity are closely correlated ($r = 0.82$). The phenolic contents possess a lower coefficient of variation than AA for all the samples, so this seems to indicate that there are non-phenolic compounds contributing to the antioxidant activity. Several studies have found that Maillard reaction products (MRPs) known as melanoidins (soluble high molecular weight fraction, >10 kDa) show high antioxidant activity. As glucose caramels possess a deeper colour and because manufacturers prefer these over other types, this could also account for the high AA of commercial brandies, but this finding needs further investigation.

3.3. Aged distillates of other geographical origins

Table 3 summarises the results obtained with aged distillates of different geographical origins. Cognacs possess the highest AA, very similar to those of Solera Gran Reserva Sherry brandies (non-significant difference, p-value = 0.1406). At lower levels of AA, in decreasing order, Spanish brandies, Armagnacs, and South African brandies show no significant difference from Solera Reserva Sherry brandies; finally, French brandies show values close to those of Solera Sherry brandies. Fig. 4 shows these comparisons.

The correlation coefficient obtained for antioxidant activity and total phenolic index for these other aged spirits was 0.76. As can be seen, the relationship between the two variables is similar to that for the sherry brandy samples, as well as the lower variation coefficients of total phenolic content compared with antioxidant activity. Again, non-phenolic compounds seem to contribute to the antioxidant activity of the samples.

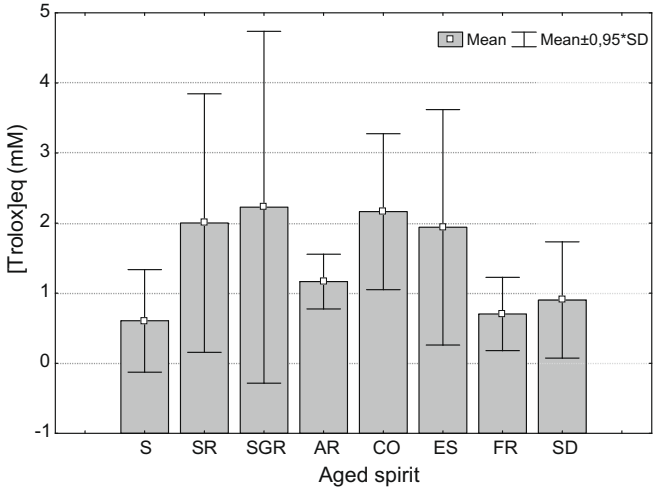

Fig. 4. Antioxidant activities of sherry brandies and aged spirits of various different geographical origins. S: Solera sherry brandies; SR: Solera Reserva sherry brandies; SGR: Solera Gran Reserva sherry brandies; ES: Other Spanish brandies; AR: Armagnacs; CO: Cognacs; FR: Other French brandies; SD: South African brandies.

4. Conclusions

The antioxidant activity of "Brandy de Jerez" has been studied, using both commercial and experimental samples. It is concluded that this activity is correlated with the total phenolic content. The results also suggest there is an additional contribution to antioxidant activity that could be related to the addition of caramel colouring, but this finding needs further investigation. Several other aged distillates of various different geographical origins were also studied, and their antioxidant power was compared with that of the three categories of "Brandy de Jerez"; it was found that Solera Gran Reserva shows the highest antioxidant activity of all the products considered.

Acknowledgement

This study has been supported by the *Junta de Andalucía* (P05-AGR-00767), National Resources and Food Technology Plan, Project Ref. RTA2006-00036-C02-02.

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