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Food Chemistry 100 (2007) 43–47
Food Chemistry
www.elsevier.com/locate/foodchem

Angiotensin I-converting enzyme inhibitory compounds in white and red wines

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Received 28 February 2005; received in revised form 5 September 2005; accepted 5 September 2005

Abstract

The antihypertensive activity of 41 wines has been determined by measuring the inhibitory activity of angiotensin-converting enzyme. The activity determined ranges from 10.3% to 95.4%, with significantly higher mean values in red wines than in the other wines. The fraction molecular weight below 10,000 has been obtained in one white wine and in one red wine by ultrafiltration and then fractionating by low pressure liquid chromatography in a Sephadex LH-20 column. The amino acids of the peptides of 6 fractions that presented antihypertensive activity were determined. The amino acids Asx, Glx and Val, form part of 5 of the 6 fractions studied, and Thr and Ala part of 4 of these fractions. Valine, which is not a majority amino acid in wine peptides, is the majority amino acid in 5 of the 6 fractions with antihypertensive activity studied.

Keywords: ACE-inhibitory compounds; Antihypertensive activity; Wine peptides; Amino acids

1. Introduction

The nitrogen fraction of wines is comprised of amino acids, peptides and proteins, with peptides being the majority compounds (Moreno-Arribas, Pueyo, & Polo, 1996). Not many studies have been carried out on these compounds, owing to their complexity and the difficulty of separating them from other wine compounds, such as amino acids and phenolic compounds, which makes it necessary to use long analytical protocols (Desportes, Charpentier, Duteurtre, Maujean, & Duchiron, 2000, 2001; Person et al., 2004).

It is known that low molecular weight peptides have important functional properties, such as their potential as tensioactive agents, antioxidants and antimicrobial agents and that they are responsible for sweet or bitter flavours (Polo, González-Llano, & Ramos, 2000). Their properties also include bioactivity. Bioactive peptides are among the functional compounds recently identified in food products. They are produced during enzymatic hydrolysis of food products, such as milk, meat, fish, corn, wheat, soybean and egg and also in fermented foods (Gobbetti, Minervini, & Rizzello, 2004; Gómez-Ruiz, Ramos, & Recio, 2004; Hernández-Ledesma, Amigo, Ramos, & Recio, 2004; Yamamoto, Ejiri, & Mizuno, 2003). The most studied bioactive property of peptides is their antihypertensive activity. This property has been well studied, owing to the increase in hypertension over recent years, which is now considered as one of the most common chronic diseases in developed countries (Gómez-Ruiz et al., 2004). Most antihypertensive peptides derived from food products function by inhibiting angiotensin-converting enzyme (ACE). This enzyme is responsible for the increase in blood pressure by converting angiotensin I into a strong vasoconstrictor, angiotensin II, and by degradation of bradykinin, a vasodilator. Therefore, ACE inhibition produces a hypotensive effect.

Takayanagi and Yokotsuka (1999), in the only study we have observed on ACE inhibitory activity (IACE) of wine peptides, have determined this activity in two red wines,

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doi:10.1016/j.foodchem.2005.09.007

and four white wines, in grapes of the red variety Muscat Bailey A and during fermentation of grape juice to obtain wine. They have demonstrated that red wines have a greater IACE activity than white wines. They also observed that this activity declines during fermentation without any clear reason for this decrease.

Perrot et al. (2003) have demonstrated, in a chronic study on normotense and spontaneously hypertense rats, that the extract of the low molecular weight fraction of a Champagne wine has an antihypertensive activity in hypertense rats but does not affect normotense rats. However, owing to the complexity of this fraction, the authors point out that this reduction cannot be attributed to only one compound in the fraction.

This research has been carried out because of the interest in this property of peptides and the dearth of studies on these compounds in wine. Our objective was to determine IACE activity in white, red and rosé wines obtained with different technologies and to gain more knowledge about the compounds responsible for this activity.

2. Material and methods

2.1. Wine samples

Samples of different types of Spanish commercial wines, including white (9), rosé (3), red (9), sherry wines (5) and sparkling wines (15) were acquired in the local market. White wines were table wines made from grapes of the Airén, Verdejo and Sauvignon Blanc varieties and manufactured in Madrid and Valladolid. Rosé wines were from the Tempranillo and Garnacha varieties, produced in Madrid and Valladolid. Red wines were of the Tempranillo variety and manufactured in Rioja, Madrid and Castilla-La Mancha. Sherry wines were produced in Jerez with *flor* yeasts, according to the traditional method followed in this zone, named *Biological Aging*. Sparkling wines were of the Macabeo, Xarel.lo and Parellada varieties and produced, by the *Champenoise* method, in Barcelona and Madrid.

2.2. Chemical analysis

Total nitrogen was determined by the Kjeldahl method with a Tecator Digestion System and a Kjeltec 1030 Auto Analyzer (Tecator AB, Höganäs, Sweden). Free and total amino acids were quantified by the ninhydrin-cadmium method (Doi, Shibata, & Matoba, 1981, method C) before and after hydrolysis, respectively. Hydrolysis was carried out with 6 M HCl and thioglycolic acid under vacuum atmosphere for 24 h at 110 °C. Phenolic compounds were determined using the Folin-Ciocalteu reagent (Singleton & Rossi, 1965).

2.3. ACE-inhibitory activity

ACE-inhibitory activity was determined by the method described by Cushman and Cheung (1971), modified by Hernández-Ledesma, Martin-Álvarez, and Pueyo (2003). The method is based on the quantification of the hippuric acid formed by reaction of hippuryl–histidyl–leucine (HHL) with ACE in the presence and absence of the inhibitor. The absorbance was measured at 228 nm. The interference of compounds that absorb at this wavelength was eliminated with a sample blank. The inhibitory activity was expressed as a percentage.

2.4. Ultrafiltration

A volume corresponding to 100 ml of wine was centrifuged at 5000*g* and 10 °C for 15 min. Fifty milliliters of supernatant were ultrafiltrated through a membrane (Centricon, Amicon Inc., Beverly, MA, USA) of 10,000 Da cut-off. The filtrate was vaccum-concerntrated giving a final volume of 10 ml.

2.5. Fractionation of the ultrafiltrate by low pressure column chromatography

Five milliliters of the concentrate were applied to a Sephadex LH-20 column (Amersham Biosciences, Uppsala, Sweden) 390 mm long × 25 mm i.d. swollen and packed according to the supplier's instructions. Elution was performed at room temperature under the conditions described by Acedo, Pueyo, and Polo (1994). Eluent was 0.3 M ammonium acetate buffer, pH 4. Flow rate was 24 ml/h, and detection was at 280 nm. Sephadex LH-20 has an exclusion limit of about 5000 Da according to the supplier's information. The eluate was collected in 5 ml fractions. The void volume was calculated using dextran blue.

2.6. Analysis of amino acids

The analysis of amino acids was performed by RP-HPLC of the amino acid derivatives with *o*-phthalaldehyde after hydrolysis with 6 M HCl and thioglycolic acid under vacuum atmosphere for 24 h at 110 °C.

2.7. Statistical methods

The statistical methods used for the data analysis were one-way analysis of variance and the least significant difference (LSD) test for means comparisons. STATISTICA (1998) programme was used for data processing. This programme was run on a personal computer.

3. Results and discussion

3.1. ACE inhibitory activity of wines

The mean, maximum and the minimum values of IACE activity, expressed as a percentage, of 9 white table wines, 5 sherry wines, 15 sparkling wines, 3 rosé wines and 9 red wines are shown in Fig. 1. Figure also shows the results

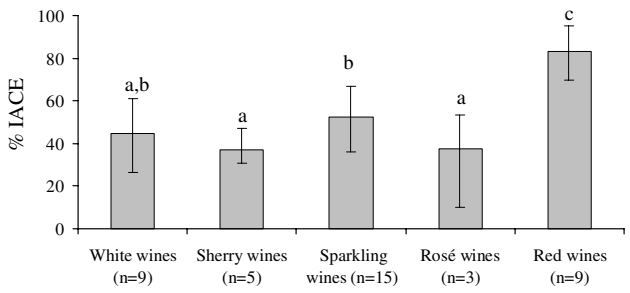

Fig. 1. Mean, maximum and minimum values of IACE activity of each of the groups of wine. The superscripts show the results of applying the LSD test to determine the existence of significant differences between the groups of wines.

obtained after applying the LSD test to determine the significant differences. The individual values obtained for this activity oscillated between 10.3% and 95.4%, with the highest mean values corresponding to the red wines, 83.3% and the lowest ones to the sherry wines, 37.1%. The IACE activity of the red wines was higher and significantly different from that of the rosé of wines. The mean value of the activity of the sparkling wines was equal to that of the white table wines but different from that of the other groups of wines and the sherry wines. The fact that the red wines had higher ACE inhibitory activities than the white wines confirms the results obtained by Takayanagi and Yokotsuka (1999) when they studied the ACE inhibitory activity of two red wines and four white wines.

In order to establish which fraction of wine is associated with this activity, fractionation was performed on a white and red wine into fractions with a molecular weight larger and smaller than 10,000, respectively. Table 1 shows the results obtained for total nitrogen contents, total and free amino acids, phenolic compounds and the IACE activity of wines and of the fractions of molecular weight above and below 10,000, respectively. Most of the compounds determined in the wine were found in fractions smaller than 10,000 Da which contained, in addition to free amino acids, 97% of the total nitrogen, 68% of the total amino acids, 84% of phenolic compounds and 67% of IACE activity in the case of the white wine. The fraction smaller than 10,000 Da for the red wine contained 91% of total nitrogen, 92% of total amino acids, 57% of phenolic compounds and 78% of IACE activity.

3.2. Wine fractionation by low pressure liquid chromatography

It has been reported that some phenolic compounds present in plants (Liu et al., 2003; Zhang et al., 2003) have IACE activity. Therefore, to study the ACE inhibitory activity of wine peptides, these must first be separated from the phenolic compounds. For this purpose, an aliquot of the fractions with molecular weight smaller than 10,000 of white and red wine, respectively, was refractionated by low-pressure liquid chromatography in a Sephadex LH-20 column. The chromatograms obtained are shown in Figs. 2 and 3. For the fractions collected, free amino acids, total amino acids and phenolic compounds were determined, in order to characterize them. These figures also show the results obtained from these analyses. White wine fractions eluted compounds between 110 and 220 ml were mainly comprised of peptides. Between 220 and 255 ml, the peptides eluted with free amino acids, In this fraction,

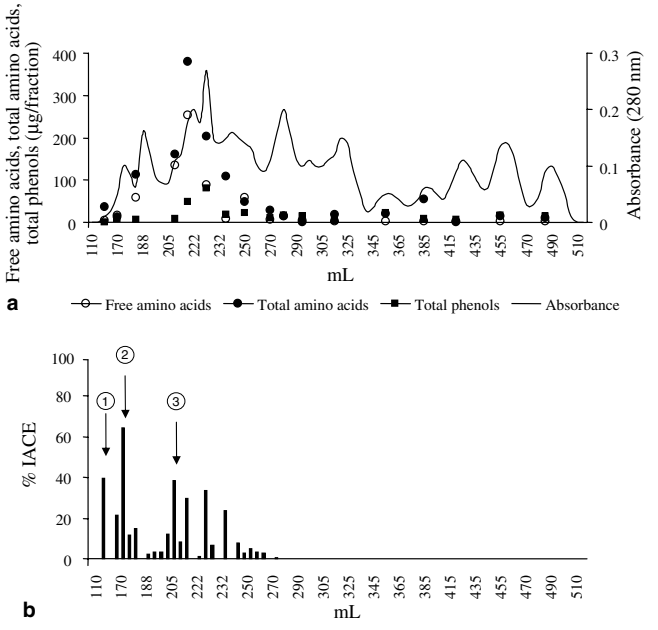

Fig. 2. (a) Chromatographic profile obtained by passing an aliquot of the fraction, smaller than 10,000 Da, of the white wine through the Sephadex LH-20 column. The contents of free and total amino acids and of total phenols are also shown and (b) ACE inhibitory activity values of the eluted fractions.

Table 1
Contents of total nitrogen, free and total amino acids, total phenols and ACE inhibitory activity of the white and red wines and of fractions with molecular weights above and below 10,000, respectively

	White wine			Red wine		
	Total	Fraction		Total	Fraction	
		>10,000 Da	<10,000 Da		>10,000 Da	<10,000 Da
Total nitrogen (mg/l)	181	14.3	175	543	26.3	493
Free amino acids (mg leucine/l)	128	–	108	303	–	277
Total amino acids (mg leucine/l)	282	10.6	193	570	41.3	525
Total phenols (mg galic acid/l)	251	8.3	212	3112	1190	1786
%IACE	61.1	11.8	41.1	69.6	14.9	54.1

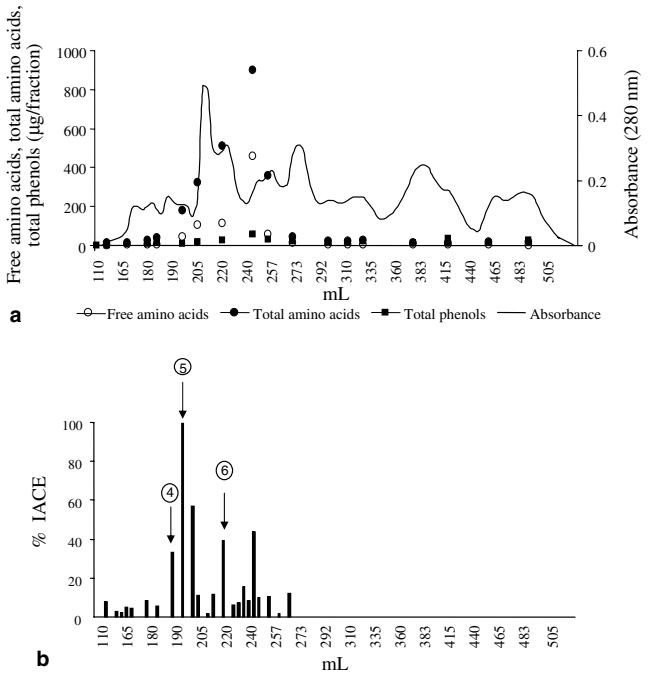

Fig. 3. (a) Chromatographic profile obtained by passing an aliquot of the fraction, smaller than 10,000 Da, of the red wine through the Sephadex LH-20 column. The contents of free and total amino acids and of total phenols are also shown and (b) ACE inhibitory activity values of the eluted fractions.

there were also some compounds, in low concentrations, that reacted with the Folin Ciocalteu reagent. These could be phenols or peptides with aromatic ammo acids (Zoecklein, Fugelsang, Gump, & Nury, 1990). ACE inhibitory activity was determined in all the eluated fractions. The highest activity was detected in some of the fractions that contain mainly peptides (Fig. 2b).

Similar results were obtained by passing an aliquot of the fraction smaller than 10,000 Da of the red wine through the Sephadex LH-20 column (Fig. 3a) in this case mainly peptides eluted in the fractions between 190 and 220 ml, and up to approximately 250 ml, peptides together with free amino acids eluted. Only small amounts of compounds were detected that reacted with the Folin Ciocalteu reagent in these eluates in spite of the fact that the fraction of molecular weight below 10,000 of the red wine possessed more phenolic compounds (1786 mg gallic acid/l) than the same fraction in the white wine (212 mg gallic acid/l). From this observation, it can be deduced that the compounds that react with Folin Ciocalteu reagent are mainly peptides. The antihypertensive activity of each of the fractions collected from the red wine eluates was determined and the highest percentages of ACE activity, as for the white wine, were detected in the fractions containing the peptides.

3.3. Amino acid composition of the fractions of white and red wine, with antihypertensive activity

Amino acid analysis, after hydrolysis, was carried out in some of the fractions with highest activity (fractions #1–3 from the white wine and #4–6 from the red wine). The results of the amino acid molar distribution of these fractions are shown in Table 2. Due to the partial conversion of asparagine and glutamine into aspartic acid and glutamic acid, respectively, during hydrolysis, the data from asparagine and/or aspartic acid and glutamine and/or glutamic acid are reported, respectively, as Asx and Glx. It should also be noted that proline and tryptophan were not determined in this study. The amino acid proline does not react with OPA and tryptophan is destroyed during acid hydrolysis.

Fraction #1 contained Asx and Glx in a 4/1 ratio. Fraction #2 was more complex. The peptides from this fraction contained the amino acids Asx, Glx, Ser, Gly, Thr, α-Ala, Val and Lys. The most abundant amino acid was Val. This amino acid was also the most abundant in fraction #3 that also contained Asx, Glx, Ser, Gly, Thr, Arg, α-Ala, Ile and Leu. In the #4 fraction of the red wine, only valine was detected. This corresponded to a peptide of valine and/or valine and/or proline and/or tryptophan since, as mentioned previously, proline and tryptophan were not determined by the analytical method used here. The #5 fraction was comprised of peptides with Asx, Glx, His, Thr, Arg, α-Ala, Met, Val Ile, Leu and Lys, and valine was also the majority amino acid in this fraction. Peptides of the #6 fraction had Asx, Glx, Gly, Thr, Arg, α-Ala, Met, Val, Ile and Leu.

The amino acids Asx and Glx were detected in all the fractions with antihypertensive activity studied. These amino acids also formed part of all the peptide fractions isolated by Acedo et al. (1994) from a white wine, of the peptides isolated by Moreno-Arribas, Bartolomé, Pueyo, and Polo (1998) in a sparkling wine and of peptides from

Table 2
Amino acid molar distribution of isolated fractions of white and red wines (for identification of the fractions see Figs. 2 and 3)

Amino acid	Fractions from white wine			Fractions from red wine		
	#1	#2	#3	#4	#5	#6
Asx	4	3	5		3	5
Glx	1	4	2		2	3
Ser		4	2			
His					2	
Gly		3	4			3
Thr		1	1		2	1
Arg			3		1	2
β-Ala						
α-Ala		2	6		2	2
GABA[a]						
Tyr						
α-Aba						
Met					1	1
Val		8	46	1	11	26
Phe						
Ile			2		1	1
Leu			2		2	1
Orn						
Lys		4			4	

[a] GABA, γ-aminobutyric acid.

French flor-sherry wines analysed by Dos Santos, Feuillat, and Charpentier (2000). The majority amino acids detected in these fractions were also Asx and Glx, Val, Ser, Lys, Gly and α-Ala. All these amino acids, except for valine, were detected as majority amino acids in wine peptides by different authors (Acedo et al., 1994; Desportes, Charpentier, Duteurtre, Maujean, & Duchiron, 2001; Moreno-Arribas et al., 1998; Person et al., 2004; Takayanagi & Yokotsuka, 1999; Usseglio-Tomasset & Bossia, 1990; Yokotsuka, Aihara, Umehara, & Kushida, 1975). However, valine, the majority amino acid of five of the six fractions studied, was not a majority amino acid of the peptides identified in wine by the previously cited authors, in spite of this, it has been shown that this amino acid frequently forms part of peptides with IACE activity in hydrolyzed milk proteins (Gómez-Ruiz et al., 2004; Hernández-Ledesma et al., 2004; Yamamoto et al., 2003) and in sake lees (Saito, Nakamura, Kawato, & Imayasu, 1994).

Wine is a product rich in phenols and peptides and both families of compounds may include individual compounds with antihypertensive activity (Gómez-Ruiz et al., 2004; Liu et al., 2003; Zhang et al., 2003). This study has demonstrated the contribution of peptides to this activity. However, the fact that red wines had the highest IACE activity reflects an important participation of phenolic compounds in this property.

Acknowledgements

This study was supported by the Comunidad Autónoma de Madrid, Project 07B/0023/2002. J.M.A. is the recipient of a predoctoral grant from Ministerio de Educación y Ciencia, Spain.

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