Isolation and Characterization of Individual Peptides from Wine

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Two peptide fractions with molecular weight higher and lower than 700 Da were obtained from a sparkling wine aged with yeast over 15 months. The amino acid composition of each fraction and their hydrophobicity were determined. Asp and/or Asn, Glu and/or Gln, Ser, Thr, and α-Ala were found to be the major amino acids that form both fractions. The peptide fraction <700 Da showed an hydrophobicity value higher than the fraction >700 Da. Four main peptides from the fraction >700 Da and six peptides from the fraction <700 Da were isolated by reversed-phase HPLC. The isolated peptides from the fraction >700 Da were found to contain between 13 and 25 amino acid residues, whereas the ones from the fraction <700 Da corresponded to peptides with a number of residues between 6 and 10. Hydrophobicities of the isolated peptides ranged from 0 to 1128.9 cal/amino acid residue.

Keywords: *Peptides; wine; amino acids; hydrophobicity*

INTRODUCTION

Peptides exhibit surfactant and sensory properties that can influence the organoleptic characteristics of wine. Peptides can serve as nutrients for yeast and bacteria that show peptidase activity (Feuillat et al., 1980; Manca de Nadra et al., 1997). However, in view of the few studies about wine peptides reported in the literature, the real importance of peptides in wine is still unknown.

The peptide composition of a wine supposedly depends on the grape variety and the winemaking process (i.e., yeast). Several studies have shown differences in the protein fraction of musts obtained from different grape varieties (Yokotsuka et al., 1977; Hsu and Heatherbell, 1987; González-Lara et al., 1989; Polo et al., 1989; Pueyo et al., 1993). This suggests that the same yeast protease would lead to different wine peptides (different amino acid composition and/or sequence) when acting on must from different grape varieties. Wine peptides could also come from the hydrolysis of yeast proteins, whose amino acid composition can vary slightly among yeast (Fleet, 1991).

Several authors (Yokotsuka et al., 1975; Usseglio-Tomasset and Bosia, 1990; Acedo et al., 1994) have determined the amino acid composition of the total peptide fraction in still wines elaborated from different grape varieties. Asp and/or Asn, Glu and/or Gln, Gly, and Pro were found to be the main amino acids that form wine peptides. Acedo et al. (1994) also included Ser and Ala as main amino acids. In previous studies carried out in our laboratory (Moreno-Arribas et al., 1996), peptides from different musts, still wines, and sparkling wines were separated by reversed-phase HPLC. Results indicated that the release and degradation of peptides in wine through the action of yeast enzymes occur simultaneously. It was also found that the individual peptides from sparkling wines of different varieties, but subjected to the same production process and aged with yeast over 26 months, appeared to be the same (according to their UV spectra and OPA−fluorescence response). This finding suggested that grape variety was not a determinant factor in the peptide composition of sparkling wine peptides (Bartolomé et al., 1997).

With the aim of increasing knowledge of the wine peptide fraction, we have now isolated and characterized the main peptides present in a sparkling wine. Two peptide fractions with molecular weight higher and lower than 700 Da were obtained, and their amino acid composition was determined. Peptides from these fractions were separated by reversed-phase HPLC. Ten chromatographic peaks, all with UV spectra compatible with peptide structures, were isolated. The amino acid composition of the isolated peaks was also determined, and, on the basis of these data, the number of residues that form the individual peptides was calculated. In addition, the hydrophobicity of the total wine peptide fractions as well as of the isolated peptides was calculated. Previous studies have shown a relationship between hydrophobicity of peptides (St. John Coghlan, et al. 1992) and of amino acids (Dale and Young, 1992), and foam characteristics in beer.

MATERIALS AND METHODS

Samples. Six bottles from the same batch of a sparkling wine (Parellada variety), manufactured industrially by the Champenoise method, and aged with yeast over 15 months, were mixed and homogenized before sampling.

Peptide Analysis. *Obtention of the Ethanol Soluble Fraction.* A 200-mL sample of wine was centrifuged at 5000*g* for 15 min, concentrated under vacuum to 10 mL, and then precipitated with 5 volumes of 95% ethanol in an acid medium. After centrifugation, the supernatant was concentrated under vacuum to 5 mL.

Fractionation of the Ethanol-Soluble Fraction by Low-Pressure Column Chromatography. The methodology described previously (Moreno-Arribas et al., 1996) was employed. A Sephadex G-10 column (92 × 2.5 cm i.d.) was used. Two peptide fractions containing the compounds with molecular weight higher and lower than 700 Da, respectively, were obtained.

Separation of Peptides by HPLC. Peptides from fractions higher and lower than 700 Da were separated by reversed-

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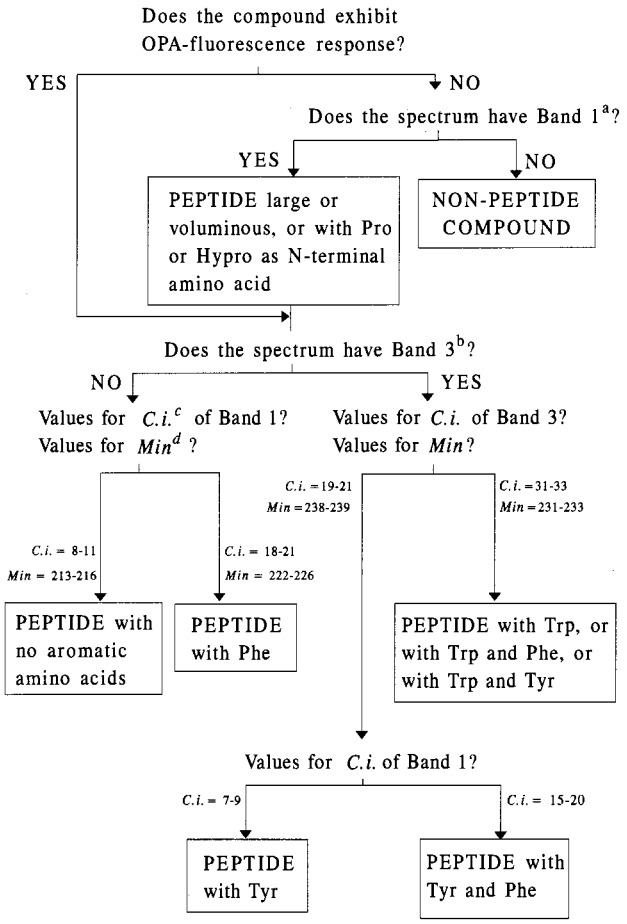

Figure 1. Flowchart for the interpretation of both spectral data and OPA-fluorescence response. It is based on data from peptide standards reported previously (Bartolomé et al., 1997). The parts of the diagram are labeled as follows: [a]band 1, spectral band (201−207 nm) due to both the peptidic bonds and the carboxy-terminal group of the peptide molecule; [b]band 3, spectral band (276−280 nm) due to the respective hydroxyphenyl and indol groups of the tyrosine and tryptophan residues; [c]*C.i.*, convexity interval (distance between the inflection points before and after the maximum in the original spectrum); and [d]*Min*, minimum in the original spectrum.

phase HPLC (Moreno-Arribas et al., 1996). Separations were performed on a Waters Nova Pak C_{18} column (150 × 3.9 mm i.d., 60 Å, 4 μm). On-line photodiode array detection and OPA (*o*-phthaldialdehyde) derivatization were used for the assessment of the peptidic nature of the chromatographic peaks according to the methodology reported previously (Bartolomé et al. 1997). The spectral parameters determined were *wavelengths of the spectrum maxima*, *convexity interval*, and *wavelengths of the second-derivative spectrum maxima*. Figure 1 shows the flowchart (based on data from Bartolomé et al. 1997) used in this study for the interpretation of both spectral data and OPA−fluorescence response of the compounds.

Peptide Isolation and Characterization. Several peptide subfractions were collected from the chromatograph outlet. To obtain sufficient amount, five consecutive chromatographic separations were performed, and the collected eluates were lyophilized. These subfractions were rechromatographed under conditions of reduced eluent polarity to purify them. Individual peptides were then collected, lyophilized and subjected to acid hydrolysis and amino acid analysis.

Hydrolysis. This was carried out with 6 M HCl in the presence of thioglycolic acid, and under vacuum atmosphere for 24 h at 110 °C. Hydrolysis was done in duplicate.

Amino Acid Analysis. The amino acid composition of the hydrolyzed fractions was determined by reversed-phase HPLC analysis of OPA derivatives according to the method described by González de Llano et al. (1991). The separation of the OPA derivatives was performed on a Waters Nova Pak C_{18} column (150 × 3.9 mm i.d., 60 Å, 4 μm). Detection was by fluorescence. Variation coeficients of the amino acids determination, including the hydrolysis, were from 3.5 to 9.8%.

Table 1. Amino Acid Molar Distribution and Hydrophobicity of the Sparkling Wine Peptide Fractions with Molecular Weight Higher and Lower than 700 Da

amino acid	fraction >700 Da	fraction <700 Da
Asx (%)	13.1	11.7
Glx (%)	10.9	10.0
Ser (%)	19.8	14.4
His (%)	4.7	1.7
Gly (%)	8.5	7.8
Thr (%)	15.9	15.3
Arg (%)		1.9
β-Ala (%)		0.6
α-Ala (%)	9.8	7.9
Tyr (%)	6.8	4.6
Val (%)	5.4	6.1
Phe (%)		0.3
Ile (%)	0.2	7.0
Leu (%)	4.8	4.2
Lys (%)		7.3
hydrophobicity (cal/amino acid residue)	551.4	810.0

Calculation of Hydrophobicities. Hydrophobicities of the peptide fractions as well as of the isolated peptides were calculated from their data of amino acid molar distribution according to the method of Bigelow (1967) on the basis of the hydrophobicity coefficient values determined by Tanford (1962). Results are expressed in cal/amino acid residue.

RESULTS AND DISCUSSION

Amino Acid Composition and Hydrophobicity of the Sparkling Wine Peptide Fractions. The amino acid molar distribution of the hydrolyzed sparkling wine peptide fractions with molecular weight higher and lower than 700 Da, is shown in Table 1. 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 respectively reported as Asx and Glx. It should also be noted that Pro and Hyp are not determined in this study due to the fact that these amino acids do not react with OPA.

Asx, Glx, Ser, Thr, and α-Ala were found to be the major amino acids in both sparkling wine peptide fractions (Table 1). The peptide fraction >700 Da differed from the fraction <700 Da in the absence of Arg, α-Ala, Phe, and Lys. A higher percentage of Ile was observed in the fraction <700 Da compared to the fraction >700 Da. The main difference between these results and previously reported data about still wine peptides (Yokotsuka et al., 1975; Usseglio-Tomasset and Bosia, 1990; Acedo et al., 1994) is the occurrence of Thr as major amino acid in sparkling wine peptides. Thr, together with Ser, have been reported to be involved in the glycosidic linkages between proteins and mannans in the cell walls of yeast (Frevert and Ballou, 1985; Orlean et al., 1991). Therefore, the peptides with high percentage of Thr and Ser that seem to be present in sparkling wines, might come from the yeast mannoproteins that would be partly or totally released and degraded during the manufacture of sparkling wine. This latter is in agreement with previous studies (Moreno-Arribas et al., 1996) that suggested that peptides in sparkling wines mainly come from the yeast.

In a study for characterizing the foam positive and negative fractions in beer, St. John Coghlan et al. (1992)

Table 2. Amino Acid Molar Distribution (%), Number of Residues, and Hydrophobicity of the Peptides Isolated from the Sparkling Wine (See Figure 2)

	peptides from fraction > 700 Da								peptides from fraction < 700Da											
	3.1		3.2		3.3		4		6		7.1		7.2		7.3		8.1		8.2	
amino acid	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Asx	19.2	2	10.8	2	28.2	7	19.1	3	13.1	1	19.2	1	25.3	2	11.7	1				
Glx	13.1	2	12.4	3	155	4	20.8	4			22.4	1	13.2	1	15.6	1	14.0	1	9.7	1
Ser	24.6	3	6.8	1	3.9	1	14.9	3	15.0	1	29.4	2			18.7	2	11.7	1		
His					11.3	3														
Gly			15.9	3					17.2	2	29.0	2			14.1	1	22.7	2	20.4	2
Thr	23.7	3	13.9	3	11.7	3	11.7	2	17.1	2					12.9	1	12.6	1	13.7	1
Arg																				
β-Ala													14.7	1						
α-Ala	11.8	2	19.9	4	16.8	4	16.8	3	16.4	2			17.3	1	14.7	1	16.3	2	21.7	2
Tyr	7.7	1	7.3	2			5.7	1	10.4	1										
Val			8.3	2					10.9	1										
Phe																				
Ile													14.8	1	12.3	1	12.1	1	13.9	1
Leu			4.7	1	12.7	3	11.1	2					14.7	1			10.8	1	20.6	2
Lys															13.5	1				
total residues		13		21		25		18		10		6		7		9		9		9
hydrophobicity (cal/amino acid residue)	414.6		673.8		483.2		607.4		681.7		0		1031.8		733.9		794.1		1128.9	

[a] Amino acid molar percentage. [b] Calculated amino acid residues per mole peptide.

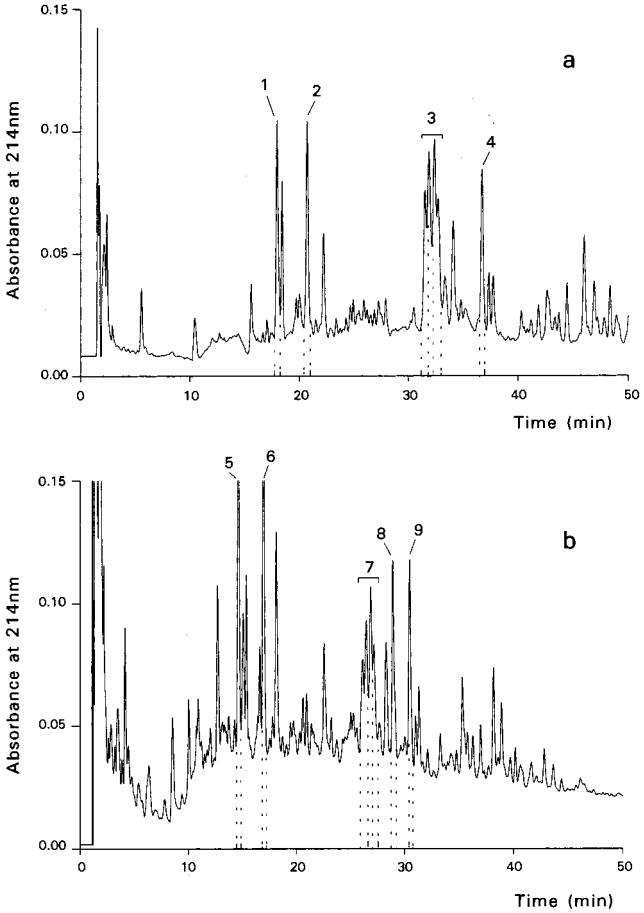

Figure 2. HPLC profiles of sparkling wine peptide fraction with molecular weight higher (a) and lower (b) than 700. Numbers refer to subfractions that were studied.

found that the foam positive fraction consisted almost exclusively of hydrophobic peptides. Onishi and Proudlove (1994) have also found a strong correlation between hydrophobicity and foam stability of beer. Foam stability is also one of the most appreciated properties of sparkling wines. To contribute to the knowledge of the hydrophobicity of the peptide fractions of the wine, this parameter has also been calculated. The peptide fraction with molecular weight higher than 700 Da showed an hydrophobicity value of 551.4 cal/amino acid residue. The hydrophobicity of the fraction with molecular weight lower than 700 Da is higher (810.0 cal/amino acid residue) (Table 1).

Amino Acid Composition of the Isolated Peptides. HPLC chromatographic profiles of fractions with molecular weight higher and lower than 700 Da are shown in Figure 2. More peaks and with higher absorbance at 214 nm were seen in the fraction <700 Da. OPA−fluorescence response and UV spectrum characteristics were studied for the main chromatographic subfractions: 1, 2, 3, and 4 in the fraction >700 Da (Figure 2a), and 5, 6, 7, 8, and 9 in the fraction <700 Da (Figure 2b). Subfractions 1, 2, 5, and 9 gave no fluorescence response with OPA (chromatograms not shown). These fractions showed non-peptide-type spectra, which confirmed that we were dealing with non-peptide compounds (Figure 1). Spectra of subfractions 3, 4, 6, 7, and 8 exhibited a main band at 195−200 nm, which indicated their pectidic nature. The two first peaks in subfraction 3 and the only peak in subfraction 4 (both subfractions from the peptide fraction >700 Da) and the only peak in subfraction 6 (from fraction <700 Da) showed spectrum maxima and convexity interval values proper of peptides containing Tyr (see Figure 1). The third peak in subfraction 3 (from fraction >700 Da) and the peaks in subfractions 7 and 8 (from fraction <700 Da) showed spectra proper of peptides not containing aromatic amino acids (see Figure 1).

Those subfractions that were confirmed to correspond to peptide compounds (this is to say, subfractions 3, 4, 6, 7, and 8; Figure 2) were collected and rechromatographied as described in Materials and Methods. Three separated peaks (3.1, 3.2, and 3.3) were obtained from subfraction 3 (chromatograms not shown). Subfractions 4 and 6 led to one peak each, which confirmed their purity. Three separated peaks (7.1, 7.2, and 7.3) were obtained from subfraction 7, whereas subfraction 8 led to two separated peaks (8.1 and 8.2). All these separated peaks were collected and subjected to amino acid analysis.

Table 2 reports the results of the amino acid analysis of the isolated peptides. The ones from the fraction >700 Da (peptides 3.1, 3.2, 3.3, and 4) were found to contain a number of residues between 13 and 25. The major amino acids that composed the total peptide fraction >700 Da (Asx, Glx, Ser, Thr and α-Ala) were also detected in the hydrolyzates of all the individual peptides 3.1, 3.2, 3.3, and 4. Leu and Tyr were also present in all these peptides, except Leu in peptide 3.1 and Tyr in peptide 3.3. Only peptide 3.2 was found to contain Gly and Val, and peptide 3.3 was the only one that contained His. It can be said, therefore, that almost all the amino acids detected in the total peptide fraction >700 Da (Table 1) were found to be present in each of the isolated peptides.

The isolated peptides from the fraction <700 Da (peptides 6, 7.1, 7.2, 7.3, 8.1, and 8.2) were found to contain a number of amino acid residues between 6 and 10 (Table 2). Asx, Glx, Ser, Thr, and α-Ala were found in most of the isolated peptides from this fraction. Val and Tyr were only detected in peptide 6. Gly residues were observed more often in peptides from fraction <700 Da than in those from fraction >700 Da. Ile and Lys were only detected in some isolated peptides from fraction <700 Da, whereas His was only detected in some isolated peptides from fraction >700 Da. Nevertheless, when the isolated peptides from fraction <700 Da are considered as a whole, their amino acid distribution is similar to that of the total peptide fraction <700 Da.

Hydrophobicities of the Isolated Peptides. The hydrophobicity values of the isolated peptides ranged from 0 to 1128.9 cal amino acid/residue (Table 2). Regarding the fraction >700 Da, peptide 3.2 was found to be the most hydrophobic (673.2 cal/amino acid residue), which is mainly due to the high percentages of α-Ala, Tyr, Val, and Leu in its composition. Peptide 4 also showed high hydrophobicity (607.4 cal/amino acid residue). The other two peptides isolated from fraction >700 Da exhibited low hydrophobicities. The highest hydrophobicity values were found for peptides 7.2 and 8.2, both isolated from fraction <700 Da. These peptides are much more hydrophobic (1031.8 and 1128.9 cal/amino acid residue, respectively) than the whole fraction (810.0 cal/amino acid residue). However, peptide 7.1, also from fraction <700 Da, was assigned a zero value for hydrophobicity since it is formed by amino acids with a hydrophobicity coefficient of zero (Tanford, 1962).

It can be inferred, therefore, that the different peptides isolated in this study (with hydrophobicities from 0 to 1128.9 cal/amino acid residue) might contribute in a different way to the foam properties of sparkling wine. In this sense, the isolation and characterization of wine peptides may be of great value in the determination of the real influence of peptides on wine characteristics.

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