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Liquid chromatographic fractionation of small peptides from wine

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Abstract

Peptides are difficult to isolate from wine because they are present in a complex mixture together with non-peptidic compounds. A method for the isolation, separation and purity assessing of small peptides is proposed. Small peptides (M_r<3000) were isolated from wine by hollow fibre ultrafiltration followed by column chromatography using the gel matrix Sephadex LH20. Fractions obtained by gel filtration on Sephadex LH20 were subjected to HPLC on a porous graphitic carbon column in order to isolate small peptides. Peak purity was then analysed by capillary electrophoresis.

Keywords: Peptides

1. Introduction

Peptides can be substances of great biological importance. They exhibit interesting functional properties: they can be antioxidants, antimicrobial agents or surfactants with foaming and emulsifying capabilities; they can also play a role in the development of characteristic flavours such as sweetness and bitterness in various types of food.

In wine, oligopeptides with a molecular mass (M_r) below 1000 could form an important nitrogen substrate for bacterial growth during malo-lactic fermentation [1]. Sparkling wine peptides could play a role in foam stability. In beer, polypeptides are the principal foam stabilising agents [2]. There exists a positive correlation between both polypeptides molecular mass and hydrophobicity and foam stabilising activity [3]. Finally, they could play a role in organoleptic properties of wine, like in other foodstuffs. In cheese, bitter flavour seems to be due to the accumulation of bitter-tasting peptides formed from casein. Indeed, tasty peptides have been isolated and identified in different types of cheeses [4–7].

Unlike other nitrogenous compounds in wine, peptides have received little attention in literature. Many reports have been published on the amino acids and proteins in musts and wines; only a few studies on peptides in musts and wines have been carried out; the peptidic fraction still remains little known, although it accounts for 25 to 30% of wine total nitrogen [8]. Peptides represent together with amino acids the main nitrogen fraction in wines.

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Most of the studies conducted on wine peptides have been restricted to the determination of the total amino acid composition of wine peptides; the results have been simply obtained by difference between the amino acid content before and after peptides hydrolysis [8–14].

Peptides have been rarely studied because their isolation from wine is quite difficult. On one hand, despite the total amount encountered in wine (about several hundred milligrams per litre), each of them represents a low quantity. On the other hand, wine peptides occur in an extremely complex mixture, together with proteins, amino acids, and a multitude of peptide-unrelated substances such as phenolic compounds. The latter are likely to interfere with the isolation of peptides. Indeed peptides and phenolic compounds are isolated from wine using the same chromatographic methods. They are also able to associate together in wine.

Despite these difficulties, the analysis of champagne peptides is an important step towards greater understanding of their technological role in sparkling wines. The development of a method capable of separating the small peptides from wine would be a valuable tool in characterising such beverages.

The aim of this study made on a base wine is to develop a technique for the fractionation, isolation and purification of small wine peptides (M_r<3000) despite their low quantity and the interfering presence of phenolic compounds.

2. Experimental

2.1. Production of wine

The studied wine was a base wine industrially manufactured from white grapes of the Chardonnay variety.

2.2. Ultrafiltration: elimination of the compounds with a high molecular mass

Wine was recirculated through a hollow fibre ultrafiltration apparatus Hiflow 30/FIB/L4 (Inceltech, Toulouse, France) containing a hollow fibre cartridge with a molecular mass cut-off of M_r 3000 (Inceltech).

2.3. Nanofiltration: elimination of the compounds with a low molecular mass

The wine permeate (M_r<3000) (18 l) was then recirculated through eight organic membranes with a molecular mass cut-off of M_r 200–300 (APV Baker, Evreux, France). The wine retentate (200–300<M_r< 3000) was collected and stored at −20°C.

2.4. Fractionation of peptides by low-pressure chromatography

An 8-ml volume of this solution was applied to a glass column (500 mm×16 mm) with Sephadex LH20 (Pharmacia-LKB Biotechnology, Piscataway, NJ, USA). The void volume was measured by passing a dilute aqueous solution of Blue Dextran through the gel bed. Sephadex LH20 has an exclusion limit at about M_r 4000 for peptides. Elution was performed at room temperature using 0.3 *M* acetate buffer, pH 4.0. A flow-rate of about 21 ml/h was produced using a peristaltic pump. The absorbance of the effluent was monitored at 280 nm. Fractions of 5 ml were collected. Appropriate fractions were pooled, freeze–dried, and resuspended in 0.1% (v/v) trifluoroacetic acid (TFA). They were submitted to several colorimetric methods.

2.5. Colorimetric methods

Peptides were measured by the biuret method [15]. The biuret reaction specifically measures peptide bonds and gives a positive colour reaction with peptides and proteins. Peptides react with cupric sulfate in an alkaline medium to lead to the formation of a coloured complex. Absorbance was read at 540 nm.

The amino nitrogen was estimated using the method of Doi et al. (Method C) [16]. The α- and ϵ-amino groups react with an ethanolic solution of ninhydrin and $CdCl_2$ to develop a red colour. Absorbance was read at 507 nm.

Phenolic compounds were evaluated by molybdotungstophosphoric colorimetry using the

Folin–Ciocalteu reagent [17]. Absorbance was read at 750 nm.

2.6. Determination of total nitrogen

Total nitrogen was measured by pyro-chemiluminescence, using the Antek 7000 N automated nitrogen analyzer (Antek Instruments, Houston, TX, USA).

2.7. Amino acid analysis of low-pressure chromatography fractions

Amino acids were analysed by reversed-phase high-performance liquid chromatography (RP-HPLC), after pre-column derivatisation with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC). The AQC reagent reacts rapidly with primary and secondary amino acids to yield highly stable derivatives that strongly fluoresce at 395 nm. The amino acid derivatisation was made, according to the specifications of the Waters AccQ-Tag method: 10 μl of sample, 20 μl of AQC reagent and 70 μl of 0.2 *M* borate buffer, pH 8.8 were mixed in a small tube. This solution was heated for 10 min at 55°C.

Separation was performed using an AccQ-Tag C_{18} column (150×3.9 mm I.D., 4 μm particle size) (Waters–Millipore, Milford, MA, USA), that was thermostated at 37°C. A ternary gradient system was used. Eluent A consisted of 38.08 g sodium acetate in 2 l of Milli-Q water, 2 ml of EDTA of 1 mg/ml and finally 1.95 ml triethylamine; the eluent was titrated to pH 5.8 with phosphoric acid. Eluent B was acetonitrile (Mallinckrodt, Paris, KY, USA) and eluent C was Milli-Q water. Flow-rate was 1 ml/min. The following gradient was employed: initially, 100% A; 0–1 min, 1% B; 1–16 min, 1–3% B; 16–25 min, 3–6% B; 25–35 min, 6–14% B; 35–40 min, 14% B; 40–51 min, A–B–C (0:60:40); 51–54 min, 100% A; 54–100 min, A–B–C (0:60:40). The eluted AQC derivatives were detected by monitoring their fluorescence using 250 and 395 nm as the excitation and emission wavelengths, respectively. A standard solution of 0.1 mmol/l of amino acids was injected prior to each analysis group. 1 mmol/l of α-aminobutyric acid was used as internal standard. Total amino acid analysis was determined after hydrolysis of the samples in glass tubes with 6 *M* HCl, under vacuum, at 110°C during 24 h.

2.8. Fractionation of peptides by HPLC

The fractions likely to contain peptides, obtained by gel chromatography on the Sephadex LH20 column, were separated by HPLC. The HPLC system used consisted of two Waters 625 LC pumps (Waters–Millipore), a Rheodyne Model 9125 manual injector (Rheodyne, Cotati, CA, USA) and a Waters 990 diode array detector. A 50-μl volume was applied to a porous graphitic carbon (PGC) Hypercarb column (100×4.6 mm I.D., 5 μm particle size, 250 Å pore diameter) (Shandon Scientific, Runcorn, UK), that was thermostated at 30°C. Eluent A was 0.1% (v/v) TFA (Merck, Darmstadt, Germany) in Milli-Q water and eluent B was 0.1% (v/v) TFA in acetonitrile (Mallinckrodt). The following gradient was employed: 0–5 min, 0% B; 5–20 min, 0–10% B; 20–25 min, 10% B; 25–40 min, 10–30% B; 40–45 min, 30–50% B; 45–55 min, 50–100% B. Flow-rate was 0.8 ml/min. The absorbance of the column eluent was continuously monitored between 200 and 400 nm. The isolated peptides, collected during seven runs, were pooled, freeze–dried, and resuspended in 200 μl Milli-Q water.

Some chromatographic analyses were carried out with post-column *o*-phthalaldehyde (OPA) fluorescence detection. The effluent from the UV detector cell was mixed at a t-union with OPA–mercaptoethanol solution (400 mg OPA, 10 ml methanol, 500 ml 0.5 *M* borate buffer, pH 10.4 and 2.5 ml 2-mercaptoethanol). The mixture was allowed to react while passing through a reaction tube (2 m×0.5 mm I.D.), that was thermostated at 40°C. Fluorescence was detected using a 820FP fluorimeter (Jasco, Tokyo, Japan) (340 nm excitation filter and 425 nm emission filter).

2.9. Capillary electrophoresis of collected peptides

Capillary electrophoresis (CE) was performed on a P/ACE-5500 automated instrument (Beckman Instruments, Fullerton, CA, USA) with full spectrum UV–Vis diode array detection. Computer control of the detector was performed with Gold Software (version 8.10). Applied voltage for all separations

shown was 30 kV. Voltage was kept within the linear range of an Ohm's law plot of voltage/current at 20°C. Fused-silica capillaries (Beckman) of 57 cm (50 cm separation length)×75 μm I.D. were used. The running buffer used for all CE separations was: 25 m*M* phosphoric acid, adjusting pH to 2.5 by the addition of 1 *M* NaOH. All experiments were carried out at 20°C. The samples were injected at the cathode hydrodynamically by pressure at 0.03 bar during 10 s. The capillary was rinsed sequentially with 0.1 *M* NaOH and buffer for 1 min each, between the individual electrophoretic runs. The electrophoretic buffer was changed every five injections. Samples, buffers, and wash solutions were filtered through a 0.2-μm nylon filter unit (Gelman Sciences, Ann Arbor, MI, USA) and carefully degassed by sonication for 10 min before use.

3. Results and discussion

3.1. Ultrafiltration and nanofiltration

Peptides, which may have a flavour impact, are generally low-molecular-mass peptides. Therefore, this study was made on peptides with an M_r below 3000. These peptides were isolated according to the method summarised in Fig. 1.

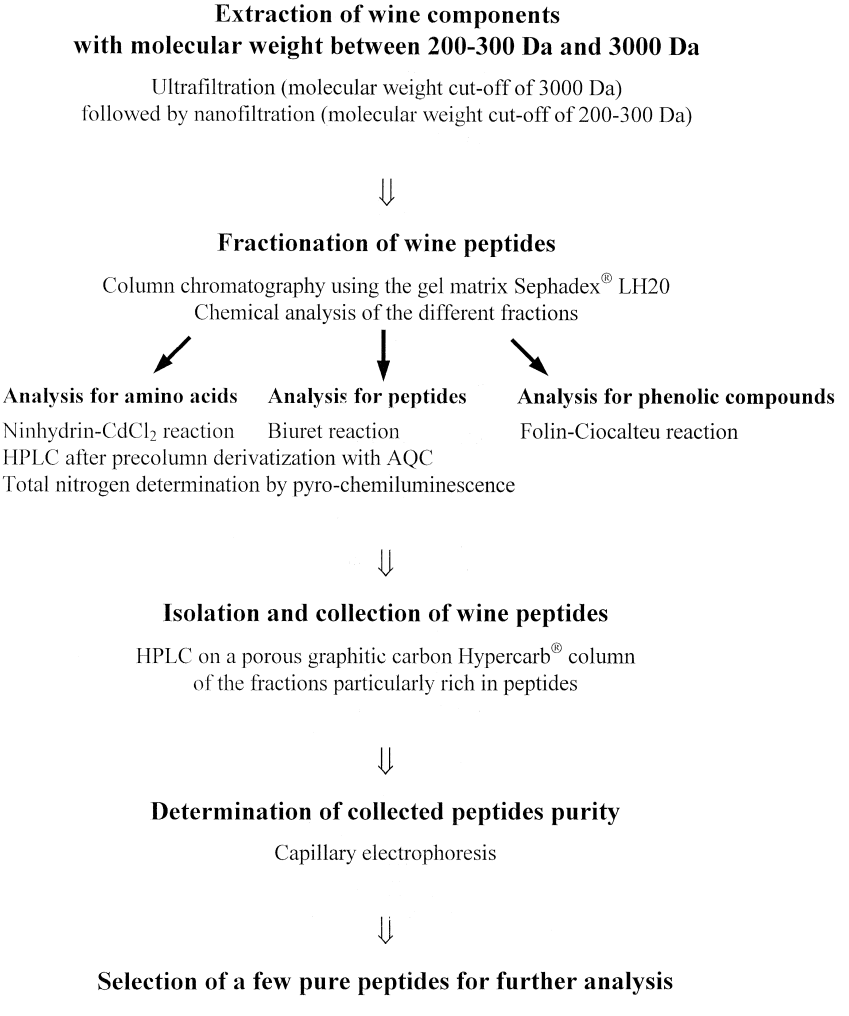

Fig. 1. Extraction and fractionation of wine peptides.

The wine was first ultrafiltered to eliminate compounds with an M_r over 3000, mainly represented in wine by proteins and phenolic compounds. Small molecules (mainly represented in wine by amino acids, organic acids, alcohols, salts) were then partially eliminated by nanofiltration (molecular mass cut-off of M_r 200–300). After both filtrations, the studied wine only contains, in theory, molecules with an M_r between 200–300 and 3000.

The first step of filtration, especially nanofiltration, represents a concentration phase of small peptides (M_r<3000) and enables the elimination of a great part of undesirable components. The peptide nitrogen represents 58.5 mg/l N in the ultrafiltered wine and becomes, after nanofiltration, of 168 mg/l N in the studied wine. Nevertheless, after nanofiltration, amino acids were not totally eliminated; the studied wine still contains 105 mg/l N amino nitrogen.

The amino acid analysis was carried out by RP-HPLC after pre-column derivatisation with AQC. It was performed before hydrolysis (free amino acids) and after hydrolysis (total amino acids), with the difference indicating amino acids bound in peptide linkage. The retention times of derivatised amino acids were very stable with standard deviations between 0.01 and 0.11. The data presented in Table 1 indicate that in the studied wine, about 62% of the total amino acid fraction consists of peptides. Acid hydrolysis results in the destruction of tryptophan and in the deamidation of the amide groups present on asparagine and glutamine to yield aspartic acid, glutamic acid and ammonia. It also often results in severe losses of cysteine. Moreover, several unknown peaks did interfere with quantitation of some amino acids; it is suspected that some of these unknown peaks are small peptides or nonprotein amino acids, such as α-aminoadipic acid, homoserine and α-aminobutyric acid, which react with AQC.

Proline, which is not usually metabolised by yeasts during fermentation, is by far the major amino acid of the studied wine. It accounts for 49% of the total amino acid content. Lysine, leucine, glutamic acid and glutamine, alanine, aspartic acid and asparagine, arginine and finally γ-aminobutyric acid are present in relatively large amounts.

Peptides are mainly composed of glutamic acid and glutamine, proline and finally aspartic acid and asparagine; they are exactly the same amino acids as those previously described as major in the composition of wines peptides [8–10,12–14,18]. These studied peptides contain also high levels of threonine, alanine, serine and lysine, which are reported as very abundant in recently studied Spanish wine peptides [12–14]. Except proline and alanine, the major amino acids which make up these peptides are hydrophilic.

Table 1
Amino acid analysis of the base wine after ultrafiltration and nanofiltration, obtained by RP-HPLC after pre-column derivatisation with AQC

Amino acid	Free (mg/l)	Total (mg/l)	Linked (mg/l)
Ala	25.7	113.9	88.2
Arg	20.7	57.5	36.8
Asx[a]	42.3	172.4	130.1
Cys–Cys[b]	19.7	24	4.3
GABA[c]	21.6	22.8	1.2
Glx[d]	41.4	216.3	174.9
Gly	6	113.2	107.2
His	14	36.8	22.8
Ile	6.7	79.4	72.7
Leu	34.5	102.2	67.7
Lys	60.1	140.5	80.4
Met	16.1	25.4	9.3
Orn	3.2	2.8	–
Phe	17.5	56.7	39.2
Pro	382.3	545.7	163.4
Ser	15.3	102.4	87.1
Thr	13.1	110.8	97.7
Trp	ND[e]	ND[e]	ND[e]
Tyr	19.4	58.7	39.3
Val	17.7	89	71.3
Total	777.3	2070.5	1293.2

[a] Asx: aspartic acid and asparagine.
[b] Cys–Cys: cystine.
[c] GABA: γ-aminobutyric acid.
[d] Glx: glutamic acid and glutamine.
[e] No determination was performed.

3.2. Fractionation of peptides by low-pressure chromatography on Sephadex LH20

In order to fractionate peptides, 8 ml of the studied wine was passed through a Sephadex LH20 column. This technique was firstly described by Dale and Young [19], who separated low-molecular-mass compounds from beer. Sephadex LH20 has both hydrophilic and lipophilic properties. It enables the

separation of compounds within a wide range of M_r mainly by adsorption chromatography. A typical separation pattern of the studied wine on Sephadex LH20 is presented in Fig. 2. The absence of the void volume fraction at 25 ml in the elution profile shows the effective removal of high-molecular-mass compounds by ultrafiltration.

The 20 fractions, collected from the chromatographic pattern, were rapidly analysed for free amino acids with the ninhydrin–$CdCl_2$ reaction, for peptides with the biuret reaction and for phenolic compounds with the Folin–Ciocalteu reagent. Absorbance differences measured with the biuret reaction (Fig. 2) show that peptides elute early, essentially in fractions 5 to 7. The colorimetric dosage of amino acids show that, despite nanofiltration, there are still numerous free amino acids in collected fractions. Free amino acids are essentially localised in fractions 4 to 8. They are somewhat found in the beginning of the elution profile. It shows that size exclusion is not the only rule of the separation.

The results of the Folin–Ciocalteu reaction show that a wide number of fractions contain phenolic compounds: from fractions 4 to 16. But this reagent also reacts with tryptophan, tyrosine and cysteine and peptides containing such amino acids. Despite this lack of selectivity, this colorimetric reaction allows to recognise fractions which are particularly rich in phenolic compounds. They are fractions 7 to 11 with a maximum in fraction 8, which corresponds to the highest peak of the elution profile. It seems consistent with the fact that phenolic compounds such as flavanols and simple phenols without conjugation are characterised by an absorbance maximum at 280 nm.

These first results were confirmed by a total nitrogen determination by pyro-chemiluminescence and an analysis for amino acids before and after acid hydrolysis. Total nitrogen determination by pyro-chemiluminescence presents several advantages over the Kjeldahl method: it has a better sensitivity, requires less analysis time and a low sample quantity (sample volume: 5 μl). Fractions 4 to 9 are particularly rich in nitrogen compounds (Fig. 2). Indeed, 87% of the total wine nitrogen was recovered in these fractions.

The comparison of the amino acid content of gel permeation fractions, before and after hydrolysis (Fig. 2), indicates that peptides are found mainly in fractions 4 to 9. Fractions 6 to 8 contain the whole measured free amino acids, except phenylalanine and

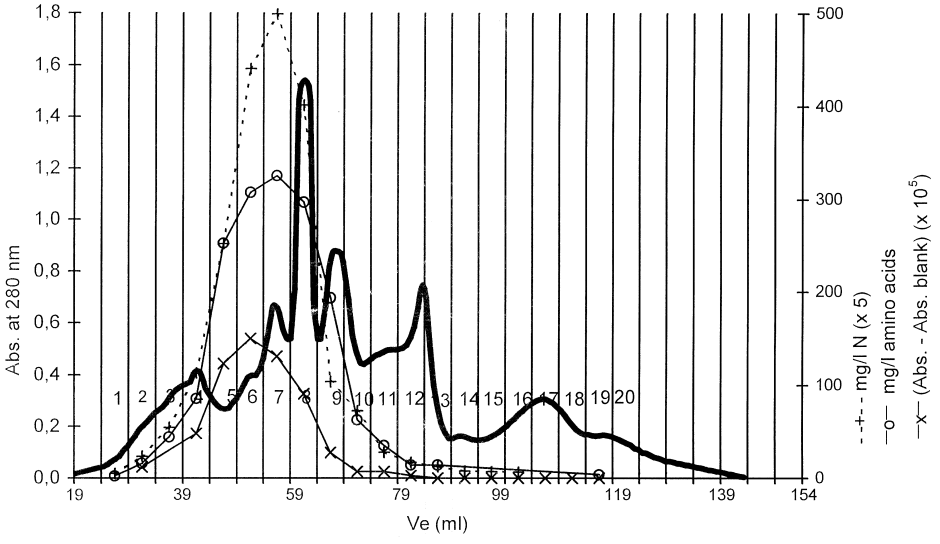

Fig. 2. Elution profile of the fraction obtained after ultrafiltration and nanofiltration of the base wine. Sephadex LH20 column (50 cm×1.6 cm I.D.). Eluent: 0.3 *M* sodium acetate, pH 4. Flow-rate: 21 ml/h. (——) Absorbance at 280 nm. (×–×) Peptides (biuret method). (o–o) Peptides (RP-HPLC after derivatisation with AQC). (+- - -+) Total nitrogen (pyro-chemiluminescence). (1) . . . (20) Collected fraction numbers.

tyrosine, which have higher elution volumes. Phenylalanine is essentially localised in fractions 9 to 11 and tyrosine in fractions 11 to 13. This delayed elution is related to the Sephadex LH20 properties, which have a strong affinity for aromatic and cyclic compounds.

The results of colorimetric methods and the results of HPLC after pre-column derivatisation with AQC are consistent concerning the elution fractions of peptides even if the result curves are not strictly superimposable: peptides are found mainly in fractions 4 to 9. Amino acids are found mainly in fractions 6 to 9. Finally, phenolic compounds are found mainly in fractions 7 to 10. These results show that peptide collection will be easier in the case of fraction 5 than in the case of fraction 8, because fraction 5 is mainly made up of peptides whereas fraction 8 contains peptides in large amounts but also free amino acids and especially phenolic compounds.

Fractions 4 to 9, mainly containing peptides, were further analysed by HPLC. They were separated on a PGC column.

3.3. Fractionation of peptides by HPLC

The chosen column for HPLC is not a traditionally used octadecyl column (C_{18}) but a PGC column. The graphitic sheets, which form the stationary phase, are flat and homogeneous. The primary factor, which governs overall retention, is adsorption of the analyte to the surface from the eluent solution. The strength of adsorption is proportional to the molecular area. It is generally found that molecules which can readily adopt a planar configuration are more strongly retained on PGC. Therefore, aromatic molecules appear to fit well to the surface of graphite and are strongly retained [20].

The chromatograms of the collected fractions on PGC are highly complex; peaks appear thin and relatively high resolved. The retention times of peptides on the PGC column were relatively stable with standard deviations between 0.08 and 0.25. These chromatograms show the presence of numerous peptides with a wide range of polarity, but also of peptide-unrelated substances with $M_r < 3000$ such as amino acids, organic acids, nucleic compounds and phenolic compounds.

But this stationary phase presents the advantage of separating peptides from phenolic compounds. On this column, phenolic compounds are eluted together at the end of the elution gradient whereas peptides are eluted at the beginning and in the middle of the elution gradient (Fig. 3). This separation is related to the fact that PGC strongly retains aromatic compounds. This property enables one to avoid the masking of peptides peaks by phenolic compounds peaks, that have a larger ϵ at the given wavelength.

In order to avoid collecting amino acids, it was necessary to know their retention behaviour on the PGC column. Because most amino acids hardly absorb radiation of wavelength 214 nm, a mixture of standard amino acids was subjected to HPLC on this

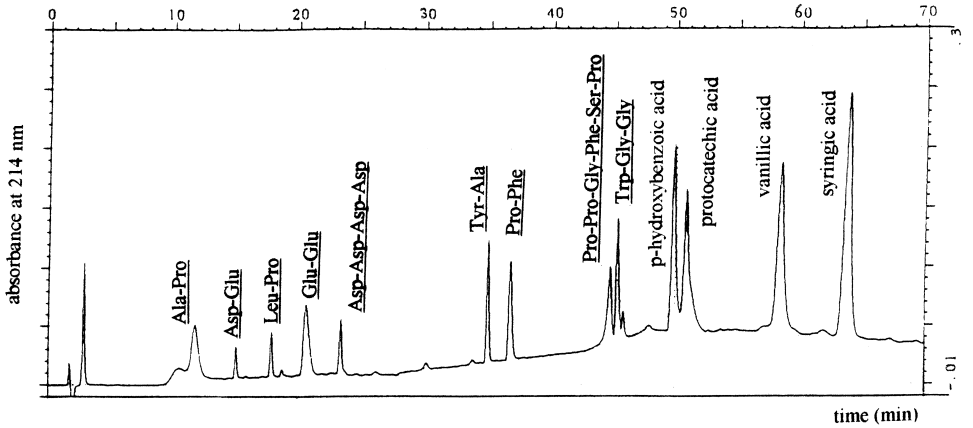

Fig. 3. HPLC profile at 214 nm of a standard solution of peptides and phenolic compounds Hypercarb PGC, 5 μm column (100 mm×4.6 mm I.D.). Eluent A: 0.1% TFA in water. Eluent B: 0.1% TFA in acetonitrile. Flow-rate: 0.8 ml/min. Peptides are underlined.

column with post-column derivatisation with OPA, to determine their retention time. Only histidine, arginine, phenylalanine, tyrosine and tryptophan produce peaks, that are easily identified by their UV spectra and retention times. The other amino acids should not interfere with the wine peptides collection because they are eluted together in the first fraction which appears to pass straight through the column. Thanks to these observations, collection of amino acids and phenolic compounds should be easily avoided.

A group of peptides were collected in fractions 4 to 9. The retention times of the peptides collected in fractions 5 to 7 are noted on the chromatograms of Fig. 4. These peaks were selected after the observation of their UV spectra obtained between 200 and 400 nm; only compounds which have peptide-type-spectra were collected.

The absorption spectra between 200 and 400 nm of the peaks detected at 214 nm were analysed in the presence of phenylalanine (absorbance maximum at 250–260 nm), tyrosine (absorbance maximum at 280 nm, low absorbance at 290 nm), and tryptophan (absorbance maximum at 280 nm and relatively high absorbance at 290 nm). UV spectra of peptides with an aromatic side-chain appear to be easily recognisable. The peptides containing tyrosine or phenylalanine seem to be relatively specific whereas the spectrum of peptide containing tryptophan is similar to that of a phenolic compound such as gallic acid and to that of nucleic compound such as cytosine. Spectra of non-aromatic peptides do not have any specificity. They exhibit an absorbance maximum at 200–220 nm.

The analysis of peak spectra enabled the collection of nearly 60 peaks. At this stage of purification, we had no idea if the HPLC peaks contain one or several peptides. It was therefore necessary to check the purity of collected peptides before identifying them.

3.4. Capillary electrophoresis of collected peptides

Frequently, one HPLC separation is insufficient to obtain pure peptides. Therefore, testing purity is a prerequisite for further structural investigations. Free-solution capillary electrophoresis (FSCE) has proven to be a valuable method to determine the peptide purity and can be used as a quick screening tool for sample purity subsequent to HPLC fraction isolation and prior to peptide sequencing. Indeed, FSCE uses a separation mechanism different from that of HPLC. Also it has very small sample and buffer requirements and finally is a fast and simple technique which possesses a high resolution potential [21].

Optimum separation of wine peptides was achieved in 25 m*M* phosphate buffer, pH 2.5. At pH 2.5, the capillary wall is nearly uncharged, resulting in a very low, if any, electroosmotic flow. Thus, peptides migrate towards the cathode according to their charge-to-mass ratios. At pH 2.5, peptides have one positive charge because their N-terminal residue is ionised. Other positive charges can exist due to the presence of arginine, lysine and histidine residues.

FSCE enabled one to test the purity of all the collected peaks. Fig. 5 shows examples of electropherograms obtained for a few collected peaks. For the first two examples, both studied peptides, collected by HPLC in fractions 6 and 7 with respective retention times of 36.9 and 31.5 min, look pure; there is only one peak on each electropherogram. But, the second type of electropherogram with several peaks is usually obtained; the injected peptides are definitely not pure. Here, the injected peptide appears as one main peak with a migration time of 5.8 min and several minor peaks. The use of another HPLC separation method using distinctly different conditions is probably the preferred route for further purification of these contaminated peaks.

The separations reported here demonstrate that practical peptide separations may be carried out by FSCE in relatively short times and enable assessing rapidly the purity of collected peptides. It can also give some prior indication on peptide identity: injection of synthetic peptides, in the same separation conditions, shows that peptides, with relatively short migration time, usually possess more than one positive charge and then contain arginine, histidine or lysine residues. Peptides with long migration time (above 8 min) were usually peptides with high M_r and only one positive charge. The peptide with a short migration time of 4.7 min (Fig. 5) does probably contain arginine, histidine or lysine.

FSCE seems to be a good technique subsequent to HPLC in order to select pure peptides for further analysis. Indeed, purity is a prerequisite for peptides

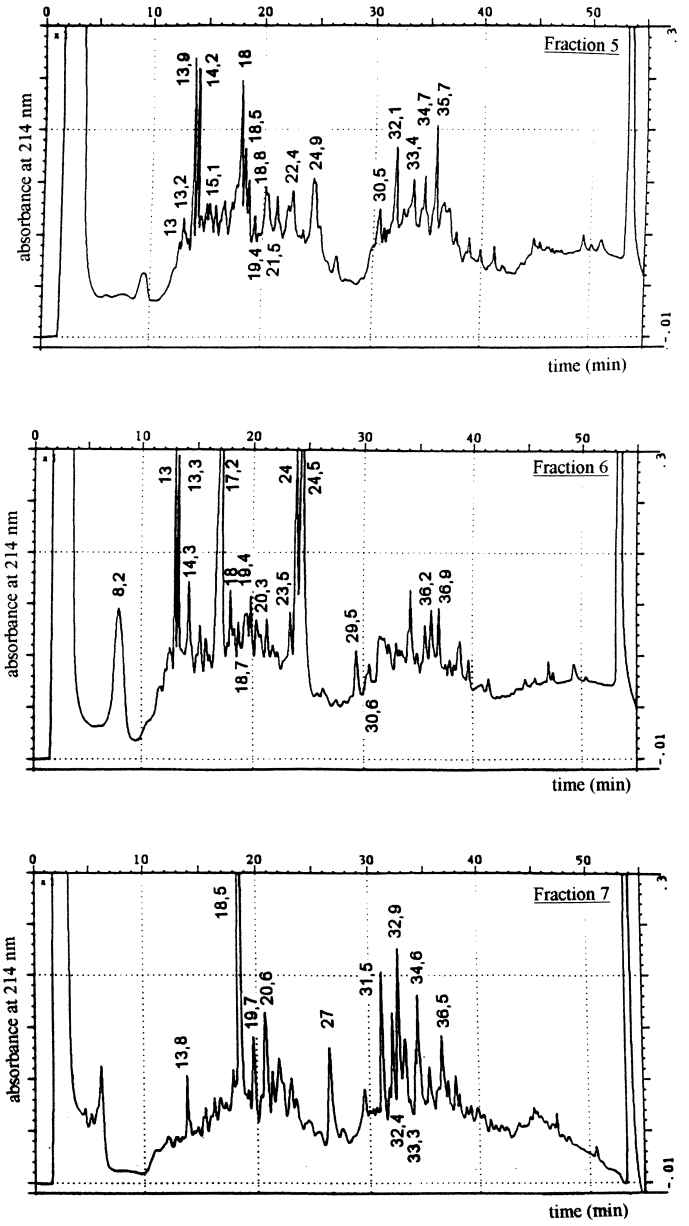

Fig. 4. HPLC profiles at 214 nm of fractions 5, 6 and 7 from a Sephadex LH20 column. Hypercarb PGC, 5 μm column (100 mm×4.6 mm I.D.). Eluent A: 0.1% TFA in water, Eluent B: 0.1% TFA in acetonitrile. Flow-rate: 0.8 ml/min.

structural investigations. Apparently, the observation of FSCE results could help to select a few peptides likely to be pure in order to be identified by determination of their amino acid sequence.

4. Conclusions

Wine peptides were hardly studied because it is difficult to isolate them from wine and particularly

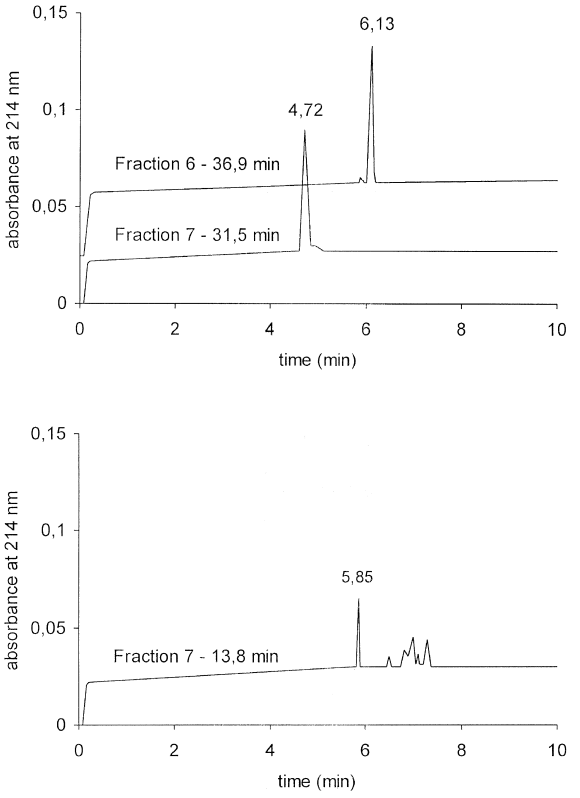

Fig. 5. Electropherogram examples at 214 nm of three peaks collected by HPLC in fractions 6 and 7. Buffer: 25 m*M* phosphoric acid, pH 2.5. Voltage: 30 kV. Temperature: 20°C.

from numerous other compounds such as amino acids, proteins and phenolic compounds.

A procedure for the isolation of wine peptides was developed using ultrafiltration, nanofiltration, low-pressure chromatography on Sephadex LH20, HPLC on a PGC column and FSCE.

Ultrafiltration resulted in the elimination of compounds with an M_r above 3000. Nanofiltration enabled one to concentrate the fraction of small peptides. Low-pressure chromatography on Sephadex LH20 led to a first fractionation of wine compounds; this step enabled to divide the small peptides into different fractions. HPLC of the fractions collected from the previous step enabled the isolation of small peptides. The use of PGC as a stationary phase facilitated this step, because peptides are separated from phenolic compounds. FSCE offered a separation mechanism different from that of HPLC and represented a simple, fast and effective method to test peptide purity.

These first results demonstrated the complexity of the peptide composition of this base wine. These peptides mainly contain glutamic acid and glutamine, proline and finally aspartic acid and asparagine.

Further analysis of the pure collected peptides may enable the sequencing of individual small peptides from wine. This identification should help to specify the origin of these peptides, their evolution during aging, their organoleptic properties: sensory and foaming capacities.

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