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IDENTIFICATION OF FLAVONOL GLYCOSIDES IN WINEMAKING BY-PRODUCTS BY HPLC WITH DIFFERENT DETECTORS AND HYPHENATED WITH MASS SPECTROMETRY

IDENTIFICAÇÃO DE GLICÓSIDOS DE FLAVONÓIS EM SUBPRODUTOS DA VINIFICAÇÃO POR HPLC COM DIFERENTES DETECTORES E HIFENADO COM ESPECTROMETRIA DE MASSA

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SUMMARY

The recovery of high added-value products from waste plant material has been an important issue with economic relevance for the pharmaceutical and food industries. The recovery of antioxidants from wine industry by-products is of great importance in the nutraceutical field.

The aim of this study was to identify the main flavonol glycosides, present in winemaking by-products from two white grape varieties: 'Arinto' and 'Moscatel' using liquid chromatography with diode array and electrochemical detection. Liquid chromatography with electrospray and atmospheric pressure chemical ionization, in negative and positive modes, were also used to characterize the structures of the compounds detected by means of MS^2 and MS^3.

In the by-products analysed quercetin-3-glucoside and quercetin-3-glucuronide were identified as well as kaempferol-3-glucoside. By-products from 'Arinto' variety showed higher levels of quercetin glycosides and kaempferol-3-glucoside than the by-products of 'Moscatel variety'.

Key words: flavonol glycosides, winemaking by-products, HPLC – DAD/ED, HPLC – MS, MS^n

Palavras chave: glicósidos de flavonóis, subprodutos da vinificação, HPLC – DAD/ED, HPLC – MS, MS^n

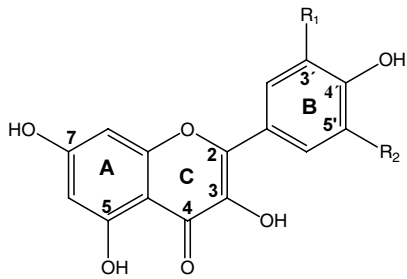
* The work was equally performed in both laboratories.

INTRODUCTION

A large amount of agricultural by-products is composed of plant tissues rich in phytochemicals, which could have valuable chemical and biological properties. The recovery of high added-value products from waste plant material has therefore been a significant issue with economic importance for the pharmaceutical and food industries (Guendez *et al*., 2005). There is currently an increasing awareness concerning the use of synthetic antioxidants, since studies have provided evidence for their role in carcinogenesis (Guendez *et al*., 2005; Louli *et al*., 2004). There is thus an interest in the isolation and study of natural antioxidants. The most important natural antioxidants commercially exploited are tocopherols, ascorbic acid and recently some plant extracts (Louli *et al*., 2004). These extracts contain mainly phenolic compounds such as flavonoids and phenolic acids that are well known for their antioxidant, anti-mutagenic, anti-inflammatory, anti-carcinogenic activities, as well as for reducing the risk of cardiovascular diseases (Louli *et al*., 2004; Pinelo *et al.*, 2005).

Flavonoids are an important group of phenolic compounds with biological properties and antioxidant activity. They are identified in plants mainly in the form of glycosides, which contribute to their absorption in the living organisms (Molnár-Perl and Füzfai, 2005).

Flavonols are one of the major subclasses of flavonoids. Flavonols have a C-ring structure with a double bond at the 2-3 position (Santos-Buelga and Williamsom, 2003), as shown in Figure 1. These molecules are present in



$R_1 = H$	$R_2 = H$	**Kaempferol**
$R_1 = OH$	$R_2 = H$	**Quercetin**
$R_1 = OH$	$R_2 = OH$	**Myricetin**

Figure 1 – General structure of flavonols

Estrutura geral de flavonóis

grapes, mainly in the skin (Wulf and Nagel, 1980; Cheynier and Rigaud, 1986) in the monoglycoside form, with the sugar residue linked to the hydroxyl group mainly in position C-3 of the O-containing ring.

Phenolic compounds play an important role in the organoleptic characteristics of wine and have been related to the benefits of wine consuming in the human diet. For instance, the flavonol quercetin has gained considerable prominence as an inhibitor of carcinogens and of cancer cell growth of human tumors (Flamini, 2003). Other biological actions of quercetin have been reported including protection of LDL cholesterol against oxidation and promotion of endothelial vasorelaxation (Careri *et al.*, 2003; Stecher *et al.*, 2001). Quercetin, myricetin and kaempferol have antioxidative and synergistic antihistamine properties (Stecher *et al.*, 2001; Vuorinen *et al.*, 2000). The glycoside flavonols of kaempferol, quercetin and myricetin can form co-pigments with anthocyanins (in red wines); together with oxidation products of tannins, they also contribute to the color of grapes and wines (Flamini, 2003).

In the winemaking process, several phenolic compounds at different degree of polymerization are extracted from grape and are detected in wines. The simplest compounds are phenolic aldehydes such as vanillin, benzoic acids such as vanilic acid and cinnamic acids such as caffeic acid (Flamini, 2003). More-complex grape phenolics contain two or more aromatic rings to form flavanols, flavonols, flavones and anthocyanins.

Vinification process used to produce different wines is very important as the flavonoid compound content in wine is related with several factors namely the maceration time of the grape solids in the fermentation process. Maceration occurred during red wine fermentation is limited in white wine production (Soleas *et al.*, 1997). So the concentration of flavonoids is significantly higher in red wine than in white wine. This situation makes white wine by-products interesting matrices to study.

The recovery of phenolic antioxidants from wine industry by-products is of great importance, not only because of the above mentioned properties (Shrikhande, 2000), but also because it could exploit a large amount of the wine industry wastes (Louli *et al*., 2004).

Phenolic compounds, like flavonols, in grape products are usually analysed by reversed-phase liquid cromatography. Most HPLC methods perform separation by gradient elution with spectrophotometric UV diode array detection. Fluorimetric and electrochemical detection have also been applied to enhance the sensitivity and selectivity of detection in HPLC. Much work has also been published on the application of HPLC coupled with mass spectrometry for the analysis of these compounds (Careri *et al.*, 2003; Molnár-

Perl and Füzfai, 2005). Some applications have involved the use of capillary electrophoresis for the determination of phenolic compounds in wine (Careri *et al.*, 2003).

In this study we intended to characterize the flavonol glycosides present in winemaking by-products of two white grape varieties: 'Arinto' and 'Moscatel'. Reversed phase liquid chromatography with diode array and electrochemical detection and hyphenation with mass spectrometry was used.

MATERIALS AND METHODS

Materials

Acetonitrile (LC-MS and gradient grade) and ethanol (analytical reagent grade) were purchased from Riedel-de-Häen (Seelze, Germany). Phosphoric and formic acids (analytical reagent grade) were purchased from Panreac (Barcelona, Spain). Water purified by means of Milli-Q from Millipore unit (Bedford, USA) was used. Folin Ciocalteau reagent was purchased from Sigma (Steinheim, Germany).

Standards including myricetin, myricetin-3-rhamnoside, kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, quercetin and quercetin-3-rhamnoside, were purchased from Extrasynthese (Genay, France). Quercetin-3-glucoside was purchased from Fluka (Steinheim, Germany). Gallic acid was purchased form Aldrich (Steinheim, Germany).

Winemaking by-products of 'Arinto' and 'Moscatel' white grape varieties were supplied by Quinta da Alorna (Portugal) and José Maria da Fonseca (Portugal), respectively.

Extraction of total polyphenols

The two winemaking by-products of 'Arinto' and 'Moscatel' white grapes were roughly milled in a blender. 140 g of each sample was extracted with 560 cm^3 with a mixture water:ethanol (50:50, v/v) during 30 minutes and at room temperature. The liquid obtained was centrifuged at 4000 rpm at 20ºC during 15 min and concentrated by rotative evaporator, until the total phenolic content reached 3000 to 5000 mg/L. The final volumes of the extracts obtained were 70 cm^3 for 'Arinto' and 50 cm^3 for the 'Moscatel' by-products. The total phenolic content measured was 3850 mg/L and 5000 mg/L for 'Arinto' and 'Moscatel' by-products, respectively. Total phenolic content was measured with Folin Ciocalteau reagent and expressed in gallic acid. The extracts were filtered through a 0.2 μm filter Sarststedt (Nümbrecht, Germany) and stored at -20ºC.

Standards

Individual stock solutions were prepared in a mixture of methanol:water (1:1, v/v) with a concentration of 333 mg/L. Individual and mixed solutions were prepared in the same solvent with the concentration of 33 mg/L. Quantification by HPLC-DAD (280 nm) of flavonol glycosides identified in samples was done by constructing individual calibration curves over the 3 – 30 mg/L range, with quercetin-3-glucoside and kaempferol-3-glucoside. Solutions were stored in the darkness at 4ºC for one week.

Methods

High performance liquid chromatography (HPLC) with diode array (DAD) and electrochemical (ED) detectors

The HPLC system consisted of a pump, an autosampler and two detectors connected in tandem: diode-array (Thermofinnigan – Surveyor, San Jose C.A, USA) and electrochemical (Dionex, ED 40). Data acquisition and remote control of the system were done by Chromquest version 4.0 (Thermofinnigan – Surveyor, San Jose C.A., USA) for the diode-array detection and for electrochemical acquisition was used the software 4880 (Unicam). HPLC was performed with a LiChrospher C18 column (5 mm, 2504 mm i.d.) with a C18 precolumn (Merck, Darmstadt, Germany). All separations were performed at 35ºC.

Samples and standards were injected (20 µL) directly on to the HPLC column and eluted with a gradient of solvent A, water/phosphoric acid (99.9:0.1, v/v) and solvent B, water/acetonitrile/phosphoric acid (59.9:40.0:0.1, v/v/v). Flow rate was 0.7 cm^3/min.

The solvent gradient started with 100% solvent A reaching 80% after 15 minutes, 30% A after 70 minutes and 0 % A after 85 minutes, followed by an isocratic step for 30 minutes and a return to initial conditions. Chromatograms at 280 nm and 360 nm were monitored in order to compare results from samples analised (scan 200 - 600 nm). For electrochemical detection the following conditions were used: linear variation from –1.0 V to 1.0 V in 1 s.

High performance liquid chromatography coupled with electrospray (ESI) and atmospheric pressure chemical ionization (APCI) mass spectrometry (MS)

The mass spectrometry system was an LCQ ion trap mass spectrometer (Thermofinnigan, San Jose C.A., USA) equipped with an ESI or APCI sources and run by Xcalibur version 1.3 software (Thermofinnigan – Surveyor, San Jose, USA). The HPLC conditions mentioned above were used but the solvents were prepared replacing phosphoric acid (0.1%) by formic acid (0.5%).

In the mass spectrometer experiments the following conditions were used for the ESI source: temperature of the heated capillary, 280ºC; electrospray voltage 3.7 kV (positive mode), 3.0 kV (negative mode); and for the APCI source: vaporizer temperature, 465ºC; discharge current, 5 μA; temperature of the heated capillary, 250ºC. Nitrogen was used as sheath gas and auxiliary gas in the experiences performed by ESI and APCI. The sheath and auxiliary gas flow rate was 80 and 20 arbitrary units, respectively.

HPLC-MS was performed in the full scan mode from *m/z* 100 to 2000.The collision energies used in MS^2 and MS^3 fragmentation experiments conducted by HPLC-MS were chosen after analyses of individual standard solutions of the flavonol glycosides studied by direct infusion in the mass spectrometer. All the fragmentation experiments were done with 35% collision energy. Exception for kaempferol assays in which was used energy of 50%. These values were applied in tandem HPLC-MS^n experiments with standards and samples.

RESULTS AND DISCUSSION

Extracts of by-products of white wine production were analised by HPLC-DAD-ED and HPLC-DAD-MS.

The ultraviolet detection (280 nm) showed there were intense peaks - A and B - with retention times between 50 and 60 minutes, as seen in Figure 2 for

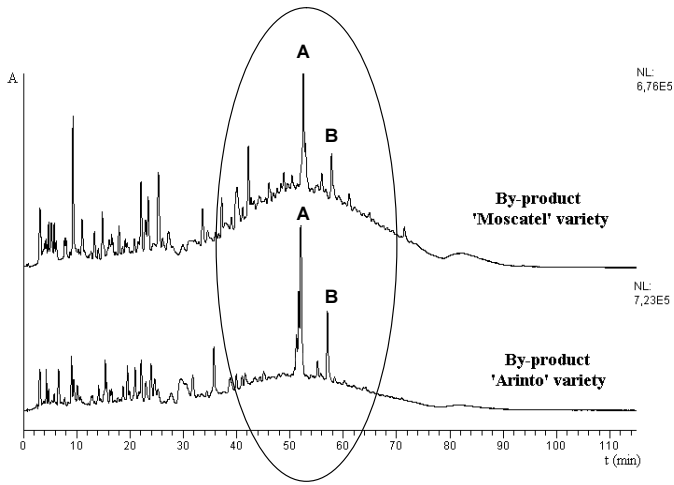


Figure 2 – Chromatograms at 280 nm of 'Arinto' and 'Moscatel' varieties by-products

Cromatogramas 280 nm de subprodutos da produção de vinho das variedades 'Arinto' e 'Moscatel'

the extracts of 'Arinto' and 'Moscatel' by-products. The absorbance of those peaks was enhanced with the change of wavelength from 280 nm to 360 nm, indicating that these compounds could belong to the flavonoid family of phenolic compounds (Stewart *et al.*, 2005), as illustrated in Figure 3 for the 'Arinto' by-product. As previously stated flavonol spectra show two absorption

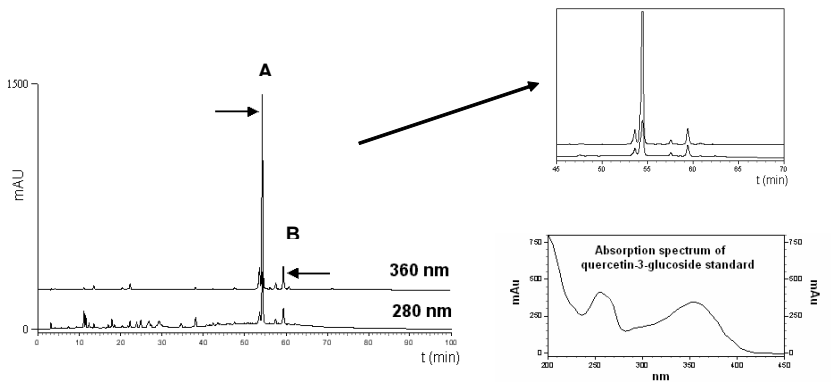


Figure 3 - Chromatograms obtained with UV detection (280 nm and 360 nm) from by-product of 'Arinto' variety; absorption spectrum of quercetin-3-glucoside in bottom right of the figure

Cromatogramas obtidos com detecção no UV (280 nm e 360 nm) do subproduto da produção de vinho da variedade 'Arinto'; espectro de absorção de quercetina-3-glucósido canto inferior direito da figura

peaks in the range of 250-295 nm (band II) and in the range of 352-385 nm (band I), thus suggesting the flavonolic nature of the components detected (Bonaccorsi *et al.*, 2005; Santos-Buelga and Williamson, 2003). In figure 3 is presented the absorption spectrum of quercetin-3-glucoside to illustrate a flavonol typical spectrum. The comparison of the chromatograms of both extracts (Figure 2) showed several detected peaks, especially until 40 minutes retention time. In 'Moscatel' extract there was also observed an increase of absorbance of the baseline between 40 and 70 minutes, which is related with the complexity of the sample indicating that a mixture of compounds is eluting between those retention times. This fact is in accordance with the total phenolic content measured in the samples: 'Moscatel' extract had more total phenolic content than 'Arinto' one, as stated in materials and methods. However, the intensity of peaks A and B was higher in 'Arinto' than in 'Moscatel' by-product.

In Figure 4 the total ion current chromatograms (TIC) of the samples are compared in negative mode, using APCI and ESI ionization. In these TIC chromatograms peaks A and B were detected for both samples, indicating

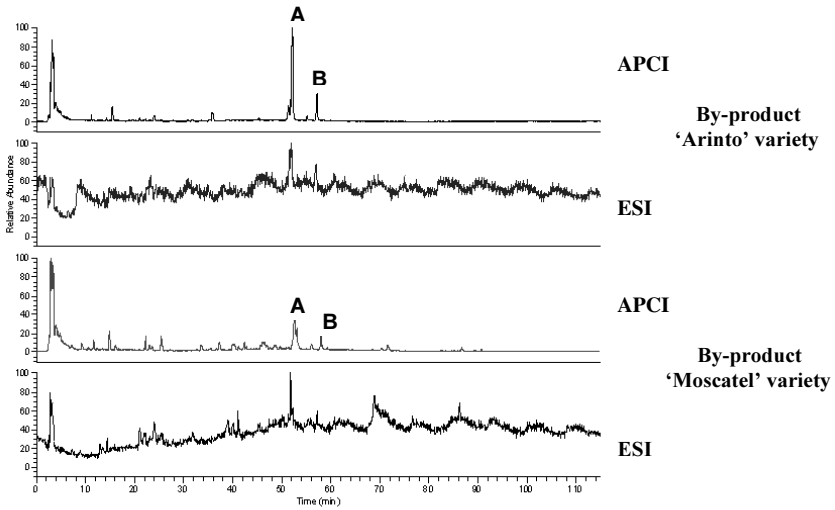


Figure 4 – Total ion current chromatograms of winemaking by-products of 'Arinto' and 'Moscatel' varieties obtained by HPLC-MS with ESI and APCI in negative mode. Ions detected in peaks A and B are characterized in Table I

Cromatogramas de corrente iónica total de subprodutos da produção de vinho das variedades 'Arinto' e 'Moscatel' obtidos por HPLC-MS com ESI e APCI em modo negativo. Iões detectados nos picos A e B estão caracterizados no Quadro I

that the conditions used for the acquisition of MS data were suitable for that type of compounds.

HPLC-MS in negative mode is more favorable in the analysis of these samples, than the positive mode, with APCI giving better signal to noise ratios than ESI. This fact is in accordance with published data (Fabre *et al.*, 2001; Cuyckens and Clayes, 2004; Santos-Buelga and Williamson, 2003) reporting the analysis of flavonoids. So the negative polarity mode was chosen in this work for further analysis.

Chromatograms obtained for samples were compared with chromatograms obtained for the standard mixture containing the eight flavonols studied. The chromatographic profiles (UV and TIC detection) of flavonol standards are compared in Figure 5; the UV absorbances at 280 and 360 nm are evidenced. In Table I are shown the *m/z* values of the ions detected in standards and in chromatographic peaks A and B of both samples.

Retention times and the mass determination for the molecular ions detected suggested that peaks A and B could correspond to flavonols quercetin-3-glucoside and quercetin-3-rhamnoside, respectively. This situation lead us to fragment the ions referred and to do further comparative analysis with

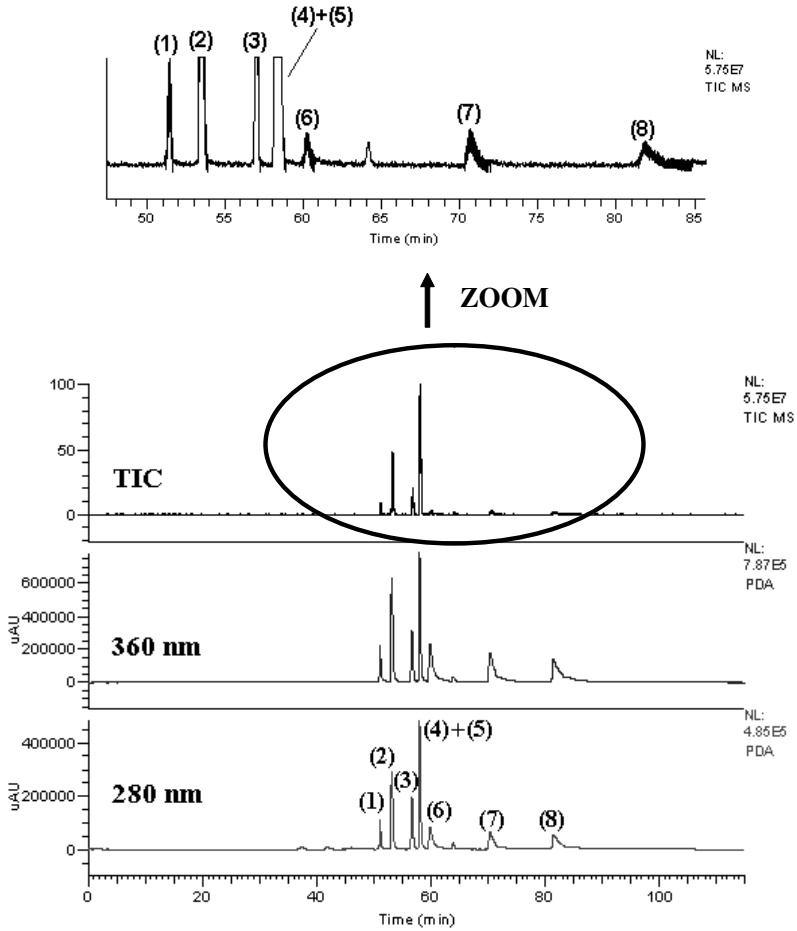


Figure 5 – UV chromatograms (280 and 360 nm) and total ion current chromatograms (APCI ionization in negative mode) obtained with a standard mixture; zoom of the TIC chromatogram is presented in top of the Figure (for peak identification see Table I)

Cromatogramas com detecção UV (280 e 360 nm) e cromatogramas de corrente iónica total (APCI em modo negativo) de solução de padrões; ampliação do cromatograma TIC no topo da figura (identificação dos picos na tabela 1)

standards. The use of fragmentation in mass spectrometry allowed us to observe the corresponding flavonol aglycones as fragments of the molecular ions detected. MS^n results obtained in negative mode for compounds detected in peaks A and B with those from flavonol standards are presented in Table I.

Table I

Ions detected (*m/z*) in the mass spectrum by HPLC-MS in negative mode and main fragments obtained by MS^2 and MS^3 for samples and standards

Iões detectados (m/z) no espectro de massa por HPLC-MS e principais fragmentos obtidos por MS^2 e MS^3 para amostras e padrões

Compound (molecular mass)	**Retention time (min)**	**$[M-H]^-$**	**MS^2**	**MS^3**
peak A	*53*	*463*	*300,301**	*151, 179*
		477	*300,301**	*151, 179*
peak B	*58*	*447*	*284*,285*	*227, 255, 267*
(1) myricetin-3-rhamnoside (M= 464)	51	463	316*,317	151, 179, 271
(2) quercetin-3-glucoside (M= 464)	*53*	*463*	*300, 301**	*151, 179*
(3) kaempferol-3-rutinoside (M=594)	57	593	284, 285*	229, 257
(4) quercetin-3-rhamnoside (M=448)	58	447	300, 301*	151, 179
(5) kaempferol-3-glucoside (M=448)	*58*	*447*	*284*, 285*	*227, 255, 267*
(6) myricetin (M=318)	60	317	151, 179	-
(7) quercetin (M=302)	71	301	151, 179	-
(8) kaempferol (M=286)	82	285	229, 257, 267	-

* most intense ion detected in the mass spectrum.

Chromatographic peak A

Two intense molecular ions $[M-H]^-$ with *m/z* values of 463 and 477 were detected for both samples. Attending to the retention time of the ion with *m/z* 463 and its respective fragmentation pattern we concluded that it corresponded to quercetin-3-glucoside.

The fragmentation, in negative mode, of the ion $[M-H]^-$ with *m/z* 463 detected in the mass spectrum of peak A resulted in a fragment with *m/z* 301 (Table I), by the loss of 162 m.u correspondent to the loss of a glucose moiety. Fragmentation, by MS^3, of the aglycone obtained (*m/z* 301) originated fragments common to those obtained from the fragmentation of quercetin.

Figure 6 shows an example of the MS fragmentation of quercetin-3-glucoside, in negative mode by HPLC-MS obtained by ESI (the fragments obtained were the same as for APCI). In the full MS spectrum is detected the molecular deprotonated ion, with *m/z* 463, but also the ion with *m/z* 508 which corresponds to the formate adduct and a dimer of quercetin-3-glucoside with *m/z* 927.

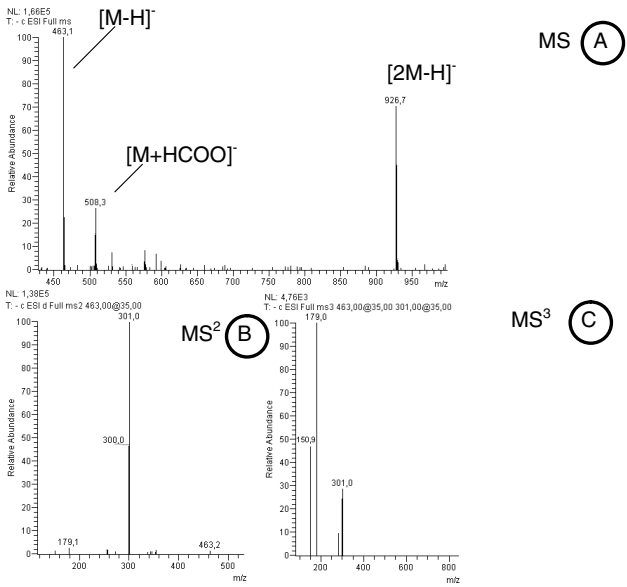


Figure 6 – Mass spectra of quercetin-3-glucoside in negative mode (HPLC-MS): A – full MS spectra; B – MS^2 from precursor ion *m/z* 463; C – MS^3 fragmentation of *m/z* 301 obtained from precursor ion *m/z* 463

Espectro de massa da quercetina-3-glucosido em modo negativo (HPLC-MS): A – espectro MS; B - MS^2 do ião precursor m/z 463; C – MS^3 do ião m/z 301 obtido a partir do ião precursor m/z 463

In both winemaking by-products were identified quercetin-3-glucoside (*m/z* 463) and quercetin-3-glucuronide (*m/z* 477) that co-elute (peak A) in the chromatographic conditions used. The identification of quercetin-3-glucuronide at this retention time is in accordance with the elution pattern described for quercetin derivatives (Santos-Buelga and Williamson, 2003). The detection of this compound in red wine (Gutiérrez *et al.*, 2004) also supports this hypothesis.

Identification of quercetin-3-glucoside was easily achieved by comparison of retention time and fragmentation pattern with the standard data showed. The identification of this compound in the samples was also supported by electrochemical detection comparing the retention times of glycoside quercetin

standards, as exemplified in Figure 7 for the by-product of 'Arinto' variety. For quercetin-3-glucuronide, and since there is no commercially available standard, the identification was achieved by the use of fragmentation in mass spectrometry and comparison with data from literature as above mentioned. After MS^2 of the precursor ion $[M-H]^-$ with *m/z* 477 it was possible to detect the ion of quercetin (*m/z* 301) obtained by the loss of the glucuronide unit (176 m.u.), and after MS^3 the detection of typical fragments of quercetin.

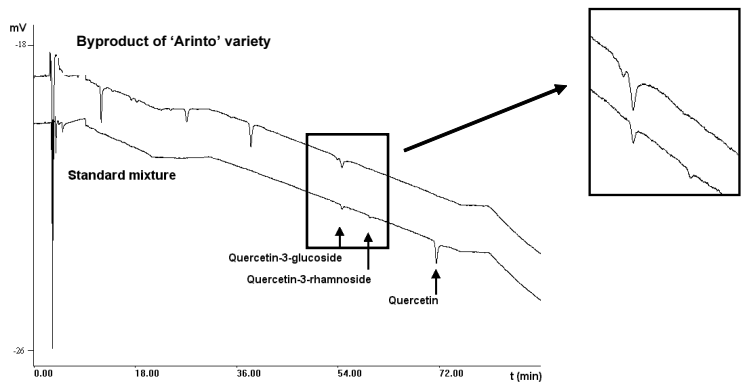


Figure 7 – Chromatograms obtained with electrochemical detection: by-product of 'Arinto' variety and standard mixture composed by quercetin-3-glucoside, quercetin-3-rhamnoside and quercetin

Cromatogramas obtidos com detecção electroquímica da variedade 'Arinto' e mistura padrão composta por quercetina-3-glucósido, quercetina-3-ramnósido e quercetina

Chromatographic peak B

The fragmentation, in negative mode, of the ion $[M-H]^-$ with *m/z* 447 detected in the mass spectrum of peak B resulted in a fragment with *m/z* 285 (Table I). The 162 m.u difference can correspond to the loss of a glucose moiety leading us to conclude that the flavonoid detected was not quercetin-3-rhamnoside but a kaempferol glycoside, since the ion of the kaempferol aglycone has an *m/z* value of 285. Kaempferol-3-glucoside has been reported to be present in grapes and wines (Flamini, 2003; Gutiérrez *et al.*, 2004) and has the same retention time and molecular mass as quercetin-3-rhamnoside. The identification was only possible by MS^n experiments, as shown in Table I.

In addition to the aglycone fragment, obtained from glycoside flavonols standards studied by means of MS^2, a radical aglycone product ion was also formed. In the conditions used the radical aglycone was more abundant than

the aglycone fragment in the case of myricetin-3-rhamnoside and kaempferol-3-glucoside. The radical aglycone product ion is formed by a homolytic cleavage, while the aglycone ion is formed by a heterolytic cleavage, as illustrated in Figure 8 for kaempferol-3-glucoside. The radical cleavage of flavonoid O-glycosides resulting in odd-electron aglycone product ions has been examined using negative ion ESI-MS/MS (Hvattum and Ekeberg, 2003).

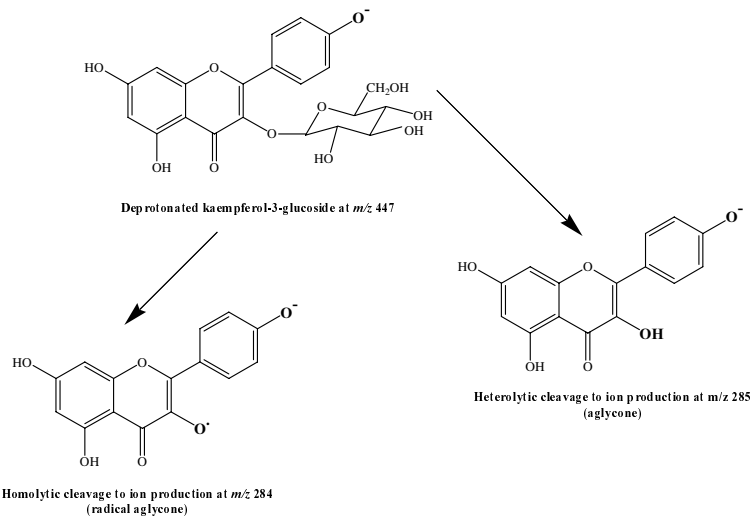


Figure 8 – Proposed collision induced dissociation of deprotonated kaempferol-3-glucoside to the radical aglycone (*m/z* 284) and aglycone (*m/z* 285) product ions

Proposta de colisão induzida do kaempferol-3-glucosido desprotonado originando os iões produto do radical aglícona (m/z 284) e aglícona (m/z 285)

Its abundance for flavones and flavonols depends strongly on the hydroxylation of the B-ring and the nature and position of the sugar residues. The abundance ratio of the radical aglycone to the regular aglycone product ion originating from cleavage at the 3-*O*-glycosidic bond increases with increasing OH substitution on the B-ring (Cuyckens and Claeys, 2004). This fact may explain that the radical aglycone was found more abundant for myricetin-rhamnoside than for quercetin-3-glucoside. However fragmentation of kaempferol-3-glucoside, in standard and samples, gave higher amount of radical aglycone (*m/z* 284) than the regular aglycone (*m/z* 285). This situation was not verified for the fragmentation of the standard kaempferol-3-rutinoside (intensity of *m/z* 285 ion was higher than for *m/z* 284 ion), leading us to confirm that the nature of the sugar residue has also an influence on the formation of the radical aglycone.

The fragmentation of peak B in samples was similar with the fragments obtained with kaempferol-3-glucoside standard. But the fragmentation of

kaempferol standard was not equal to fragments obtained by MS^3 of kaempferol-3-glucoside. This situation is due the formation of radical aglycone in MS^2 mass spectra of peak B and kaempferol-3-glucoside. Since MS^3 was done with the most intense ion detected in MS^2, the fragments obtained are from precursor ion *m/z* 284 and not 285, like in kaempferol standard.

Quantification of chromatographic peaks A and B

Peak A was identified as being a mixture of quercetin-3-glucoside and quercetin-3-glucuronide. Since we had not a standard for quercetin-3-glucuronide it was decided to quantify both compounds identified as quercetin-3-glucoside, using a linear calibration curve prepared in quercetin-3-glucoside. For peak B, and using data obtained with mass spectrometry we concluded that for this retention time there was only kaempferol-3-glucoside eluting. So, the quantification was done using a linear calibration curve prepared with the standard of this component. The results of the quantification are shown in Table II. 'Arinto' by-product showed higher levels of quercetin-3-glucoside and kaempferol-3-glucoside than 'Moscatel' by-product. Calibration curves yielded the following equations: $y = 233351\ x - 120602\ (r^2 = 0.9999)$, for quercetin-3-glucoside, and $y = 198181\ x - 144994\ (r^2 = 0.9911)$, for kaempferol-3-glucoside.

Table II

Quantitative analysis of flavonols glycosides in winemaking samples

Quantificação de glicósidos de flavonóis nos subprodutos de vinho analisados

Sample	**Total content of quercetin glycosides (mg/kg)***	**Kaempferol-3-glucoside (mg/kg)***
'Arinto' variety	50.4	13.5
'Moscatel' variety	22.0	6.6

* mg of flavonol glycoside by kg of winemaking by-product.

In the literature there is a reference concerning quercetin content in winemaking by-products (Careri *et al.*, 2003), indicating that the concentrations of flavonoid found in this type of samples was about 104 µg/g, which is in accordance with our results, considering that the higher or lower content depends on the vinification process characteristics: more or less maturation.

CONCLUSIONS

Quercetin-3-glucoside, quercetin-3-glucuronide and kaempferol-3-glucoside were identified and quantified as quercetin and kaempferol-3-glucoside in winemaking by-products of 'Arinto' and 'Moscatel' white grape varieties.

By-products from 'Arinto' variety presented higher quantities of quercetin glycosides than kaempferol-3-glucoside when compared with the extracts obtained from 'Moscatel variety'.

Chromatograms obtained after analysis of extracts by liquid chromatography with diode array and electrochemical detection showed these samples are complex and some compounds present electrochemical activity in conditions of analysis used.

HPLC-MS results, in particular MS^n data, shown to be a valuable tool for qualitative analysis of flavonol glycosides.

Further work is necessary in order to identify other compounds with bioactivity in winemaking by-products samples.

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RESUMO

Identificação de glicósidos de flavonóis em subprodutos da vinificação por HPLC com diferentes detectores e hifenado com espectrometria de massa

Uvas, vinho e subprodutos da vinificação contêm diversos compostos fenólicos pertencentes ao grupo dos flavonóides, em elevadas concentrações. Os glicósidos de flavonóis estão entre os compostos fenólicos presentes nas uvas. A sua actividade biológica é de grande interesse para as indústrias farmacêutica, cosmética e alimentar, não só pela actividade antioxidante, mas também pelas suas propriedades anti-inflamatórias e anti-cancerígenas.

O objectivo deste estudo foi identificar glicósidos de flavonóis maioritários em subprodutos da vinificação em branco das variedades 'Arinto' e 'Moscatel'. Foram utilizadas metodologias analíticas que incluiram a cromatografia líquida acoplada a detectores de díodos e electroquímico. Cromatografia líquida com espectrometria de massa foi ainda utilizada com duas fontes de ionização à pressão atmosférica: electrospray (ESI) e ionização química (APCI), nas polaridades positiva e negativa no sentido de comparar resultados. Foram também efectuados ensaios MS^2 e MS^3 para caracterizar as estruturas químicas dos glicósidos de flavonóis detectados

Nas amostras analisadas foi identificada a quercetina-3-glucósido e a quercetina-3-glucuronido e também o quempferol-3-glucósido.

Os subprodutos da variedade 'Arinto' mostraram, proporcionalmente, maior teor de glicósidos de quercetina que quempferol-3-glucósido, quando comparados com os resíduos da variedade 'Moscatel'.

RÉSUMÉ

Identification de glycosides de flavonol dans les sous-produits de la vinification par HPLC avec différents détecteurs et couplé par spectrométrie de masse

Le rétablissement de produits de haute valeur obtenue de la matière végétale est une question importante avec la pertinence économique pour les industries pharmaceutique, cosmétique et alimentaire, à cause de son activité antioxydant mais aussi pour ces propriétés anti-inflammatoire et anti-cancérigène. Le rétablissement des antioxydants des sous-produits d'industrie vinicole est de grande importance dans le domaine nutraceutique.

Le but de cette étude était d'identifier les principaux glycosides de flavonol dans les sous-produits de la vinification de deux variétés blanches de raisin: 'Arinto' et 'Moscatel'. La chromatographie liquide avec la detection par diode et électrochimique on été employées en tandem. La chromatographie liquide était également employée pour caractériser les structures des composés détectés par MS^2 et MS^3 en utilisant electrospray et l'ionisation chimique de pression atmosphérique, en modes négative et positive.

Dans les sous-produits analysés quercétine-3-glucoside, quercétine-3-glucuronide et kaempférol-3-glucoside ont été identifiés.

Les sous-produits de la variété d' 'Arinto' ont des proportions plus élevée des glycosides de quercétine et kaempférol-3-glucoside en comparaison avec les sous-produits de variété 'Moscatel '.

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