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Chemical composition of grape stalks of *Vitis vinifera* L. from red grape pomaces

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ABSTRACT

The chemical composition and the structure of macromolecular components of grape stalks from red grape pomaces have been evaluated. These are composed mainly of cellulose (30.3%), hemicelluloses (21.0%), lignin (17.4%), tannins (15.9%) and proteins (6.1%). Among hemicelluloses the xylan was the most abundant (ca. 12%). The parameters of cellulose unitary cell, average diameter of nanofibrils and the degree of crystallinity (75.4%) were assessed by X-ray scattering analysis. The xylan was partially acetylated glucuronoxylan (DS = 0.49) possessing the Xyl*p*:MeGlc*p*A ratio of 25:1. The lignin of grape stalks was suggested to be of HGS type with H:G:S molar proportion of 3:71:26 as revealed by analysis of nitrobenzene oxidation products. Among alkali soluble condensed tannins procyanidins prevailed over prodelphinidins. The abnormal response of grape stalks to kraft pulping, leading to poorly delignified fibrous material, was attributed to a particular lignin structure and it structural association with other macromolecular components of grape stalks.

1. Introduction

The winemaking contributes substantially to national economics around the world. More than 60% of wine worldwide is produced by European Union (EU). In particular, Portugal, with an annual production of about 7 million hL, occupies the 10th place in the world ranking and 5th place among European countries, exceeded only by Italy, France, Spain and Germany (GPP, 2007). The utilisation of wine by-products is gaining increasing attention because of their promising eventual applications and due to the environmental concerns. Recently, in January 2007, a draft proposal urging the revision in the Organization Common Market (*wine OCM*) regulation regarding the disposal of wine by-products has been announced by European Commission. In particular, the new *wine OCM* regulation presumes measures against burying of wine by-products affecting the soil erosion/compaction and the quality of groundwater due to the loss of organic matter (CCE, 2006). Accordingly, the fulfilling of environmental requirements in this sector urges new solutions for the utilisation of winemaking by-products. In this context, both the implementation of conventional technologies and developing new approaches to the exploitation of underutilised resources, represent a fundamental step for the sector, either in economic (value added materials) or in environmental terms.

The production of 100 L of white wine gives rise to about 31.2 Kg of by-products (including 17 kg of skins and 4 kg of stalks) and about 25 kg of by-products arise from production of 100 L of red wine (including 13.2 kg of skins and 4 kg of stalks) (Costa, 1983). Grape skins and stalks are not hazardous wastes, but the high content of organic matter and their seasonal production can contribute to potential pollution problems, especially regarding the chemical and biological oxygen demand of groundwater (Spigno et al., 2008).

Unlike grape skins that are partially used in animal feeds the current applications of grape stalks are limited essentially to their use as fertilizers (Bertrán et al., 2004; Bustamante et al., 2007). A comprehensive study of grape stalk chemistry is certainly missing (Cruz et al., 2004; Spigno et al., 2008; Ping et al., 2011). This knowledge, however, is crucial to define the possible areas of grape stalks utilisation and to explain the difficulties found upon its processing. The main goal of this work was to assess the chemical composition and the main structural features of macromolecular components of grape stalk from red grape pomaces (*Vitis vinifera* L.) collected in the Dão Region of Portugal.

2. Materials and methods

2.1. Materials

Grape stalks of the variety *Vitis vinifera* L. were collected after destemming at the Cooperative Wine Cellar of Silgueiros, in the Dão Region of Portugal. The material was dried at room temperature,

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milled on a Retsch cross-beater mill SKl, and sieved to ca 1 mm particles.

2.2. Chemical analyses

The grape stalks were characterized regarding the ash content, extractives (in acetone, dichloromethane and in hot water), proteins, tannins, cellulose, lignin and hemicelluloses. The ashes content was determined by calcination of the material at 525 °C, according to the standard procedure Tappi T 211 om-93. Metal cations were analysed by ICP after ash wet digestion. The elementary analysis was made on a Leco CHNS-932 Elemental Analyzer.

The extractives content in acetone and dichloromethane was determined by Soxhlet extraction according to the Tappi T 204 om-88. The determination of extractives in hot water was carried out with a solution of ammonium citrate (10 g/L) for 1 h under reflux (liquid-to-solid ratio 100). The proteins content in extractives-free grape stalks, after extraction with acetone, was determined by treatment with 1% pepsin solution in 0.1 M HCl at 37 °C for 16 h. The tannins content was assessed in extractives- and proteins-free stalks by the reflux with 0.3% NaOH solution (liquid-to-solid ratio 100) under nitrogen atmosphere for 1 h. The extracted material was filtered off, washed with hot water until neutral reaction of filtrate and dried at 60 °C to a constant weight. The content of tannins was assessed by the difference in weights of starting and extracted materials. The alkaline extract was precipitated by adding 3 M H_2SO_4 until pH 3. After 24 h, precipitated tannins were centrifuged and washed with water to pH 5. Finally, the tannins fraction was freeze dried. The cellulose was determined by 4 consecutive treatments of grape stalks with HNO_3:EtOH mixture (1:4, v/v) under reflux for 1 h each according to the Kürschner and Höffer method (Browning, 1967). The lignin content in grape stalks free of extractives, proteins and tannins was determined by Klason method with 72% H_2SO_4 (according to Tappi T 204 om-88). The neutral sugars were analysed by GC as alditol acetates according to previously published procedure (Selvendran et al., 1979). The chromatographic conditions were the same as described previously (Marques et al., 2010).

The lignin *in situ* was characterized by oxidation with nitrobenzene under alkaline conditions, according to the method described elsewhere (Tanahashi and Higuchi, 1988). The oxidation products were analysed on a gas chromatograph (Trace Gas Chromatograph 2000 series), equipped with a mass spectrometer (Thermo Scientific DSQII) (GC–MS). The conditions of GC analysis were as follows: column capillary DB-1 J&W (30 m × 0.32 mm *i.d.* 0.25 μm); initial temperature of the column – 100 °C; gradient of temperature – 4 °C/min; final temperature – 270 °C; injector temperature – 240 °C; detector temperature – 250 °C.

2.3. X-ray analysis of cellulose

The cellulose isolated by the Kürschner and Höffer method was analysed by X-ray scattering on a Philips X'Pert MPD diffractometer using Cu-Kα source (λ = 0.154 nm) in the 2θ range 2–40° and scanning step width of 0.02°/scan. Cellulose was analysed as textured sample and the degree of crystallinity was corrected for the presence of non-cellulosic polysaccharides based on sugars analysis (Figueiredo et al., 2010).

2.4. FTIR and ^{13}C CP/MAS NMR analyses

^{13}C CP/MAS NMR spectra were registered on a Bruker Avance 400 spectrometer (magnetic field of 9.4 T). Samples were spun in a zirconia's rotor at 7 kHz. The acquisition parameters were as follows: proton pulse of 4 μs (90°), contact time of 2 ms, recovery delay of 4 s and 7000 scans were accumulated.

The FTIR spectra were obtained on a Mattson FT-IR spectrometer Model 7300 using KBr pellets at 4 cm^{-1} resolution and acquiring 128 scans per set.

2.5. Isolation and characterization of xylan

The xylans were extracted with DMSO from the holocellulose obtained by delignification (85 °C, 30 min, 14% AcOOH) of the grape stalks with peracetic acid (Evtuguin et al., 2003). The peracetic holocellulose (ca 2 g) was milled in a vibratory ball mill for 40 min and extracted twice by dimethylsulphoxide (DMSO) at 60 °C for 24 h under a nitrogen atmosphere while being stirred (solid-to-liquid ratio 50). After extraction, the residue was filtered off and washed on a glass filter with 10 mL DMSO followed by 15 mL of water. The resulting filtrate was precipitated in 800 mL of ethanol, acidified with formic acid to pH 2 and kept for 2 days to coagulate the precipitate. After centrifugation, the residue was washed 5 times with absolute methanol and freeze dried. The isolated xylans were analysed by ^1H NMR in D_2O at 303 K on a Bruker AMX 300 spectrometer operating at 300.13 MHz. The acquisition parameters were as follows: 12.2 μs pulse width (90°), 14 s relaxation delay, and 300 scans were collected. 2D ^1H–^1H COSY spectroscopy was performed at 50 °C using a standard COSY sequence (90° pulse, relaxation delay 2 s).

2.6. Kraft pulping

The kraft pulping of grape stalks (5–10 mm particles) was carried out in 100 mL stainless steel autoclaves at liquid-to-solid ratio 4. After the load of raw material and white liquor, the autoclave was sealed and lowered into the preheated oil bath. Autoclave was occasionally shaken during the cooking. After pulping, autoclave was quickly cooled in cold water. The pulp obtained was exhaustively washed with distilled water to almost neutral reaction of filtrate. The particular conditions of cooking are presented in the text body.

3. Results and discussion

3.1. Chemical composition of grape stalks

The general chemical composition of grape stalks from red grape pomaces is presented in Table 1. The ash content (7.0%) is similar to that previously reported for grape stalks by Santos and Campos (1986) and by Spigno et al. (2008). Among more than 20 metal

Table 1
Chemical composition of grape stalks.

Composition	%, w/w
Ash	7.0
K	0.90
Ca	0.15
Mg	0.02
Zn	0.01
Na	<0.01
Cellulose (Kürschner–Höffer)	30.3
Proteins[a]	6.1
Tannins[b]	15.9
Klason lignin[c]	17.4
Hemicelluloses	21.0
Extractives	
Acetone	2.3
Dichloromethane	1.0
Hot water	23.7

[a] Corrected for extractives in acetone.
[b] Corrected for extractives in acetone and proteins.
[c] Corrected for extractives in acetone, proteins and tannins.

cations detected in ash, potassium was the major one followed by calcium, magnesium, zinc and sodium (Table 1).

The amounts of extractives soluble in acetone and dichloromethane in grape stalks was similar or even lower than usually reported for hardwoods (1–5%) or softwoods (3–8%) (Biermann, 1996). Recently reported composition of ethyl acetate and dichloromethane extractives from grape stalks refers essentially to saturated and unsaturated fatty acids followed by triterpenoids and high alcohols (Cruz et al., 2004; Ping et al., 2011).

The content of extractives soluble in hot water was surprisingly high (23.7%) and, besides dissolved inorganic salts and polysaccharides, may be explained by the presence of water-extractable tannins, which add brownish colour to extracts.

The proteins fraction was 6.1% as revealed by treatment of extractives-free grape stalks by pepsin. The detected protein content was similar to that determined by the same method in other agricultural crops such as banana tree (Oliveira et al., 2007), kenaf (Pascoal Neto et al., 1996) and reed stalks (Pascoal Neto et al., 1997).

By analogy with the analysis of barks (Laks, 1991; Fradinho et al., 2002), the content of tannins was assessed by extraction of grape stalks free of acetone-soluble extractives and proteins with NaOH solution (0.3% solution corresponded to 30% w/w of alkali load). Such an approach is also rather common for the analysis and characterization of lignin in woods containing tannins, when tannins are preliminary extracted with 0.3% NaOH thus allowing the correct lignin detection and the isolation of analytes free of tannins (Evtuguin et al., 2001). The value obtained (15.9%) was more than double to that reported for grape stalks while determined by methanol:water (1:1) extraction (Ping et al., 2011). Besides variation of grape stalks origin, such a difference may be explained by contribution of high molecular weight condensed tannins soluble in alkali solution only and of hydrolysable tannins (including polyphenolic acids) which are readily degraded under alkaline conditions.

The content of lignin was determined as acid-insoluble residue (Klason lignin) after preliminary removal of extractives in acetone, proteins and tannins from grape stalks, because these concomitants interfere the analysis due to their co-precipitation and condensation with lignin (Browning, 1967). This approach allows the detection of reliable lignin content (17.4%), which was almost half of that reported previously (ca 33–47%) by direct analysis of grape stalks (Cruz et al., 2004; Spigno et al., 2008; Ping et al., 2011).

The cellulose content determined by the Kürschner-Höffer method (30.3%) was in the range of values (30–33%) previously reported by other researches for the grape stalks (Cruz et al., 2004; Spigno et al., 2008). Cellulose represents the largest grape stalks component, the second being hemicelluloses (21%), conventionally determined by the difference in weight of oven dried (o.d.) grape stalk (100%) and the percentages of extractives soluble in acetone (2.3%), proteins (6.1%), tannins (15.9%), lignin (17.4%), cellulose (30.3%) and ash (7.0%) (Table 1).

The analysis of neutral sugars in starting raw material and in corresponding peracetic holocellulose was carried out to provide a general characterization of polysaccharides in grape stalks (Table 2). The results on glucose content indicate that the major part of glucan is certainly cellulose, taken into consideration the total yield of sugars (51.3%) and the detected cellulose content (Table 1). The second in abundance polysaccharide in grape stalks is xylan, which amounts should not be exceeded of 12%, including the eventual contribution of uronic acids (about 10% w/w). These findings are in agreement with previously reported percentages of cellulose and xylan in grape stalks (Spigno et al., 2008; Ping et al., 2011). The sugar composition (Table 2) revealed a high retaining of xylan in holocellulose, which yield was 41.0%, and the removal of arabinan, galactan and mannan upon treatment with peracetic acid. The imbalance between glucan in grape stalks and in holocellulose, taken in consideration the total yield of sugars in analysis and the holocellulose yield, indicate than at least 6–7% of glucan in grape stalks must be of non-cellulosic origin.

Table 2
Composition of monosaccharide's (% wt) in grape stalks and in corresponding holocellulose.[a]

Monosaccharides	Grape stalks	Holocellulose
Rhamnose	1.7	0.4
Fucose	<0.2	–
Arabinose	5.5	–
Xylose	20.4	22.3
Mannose	4.8	1.4
Galactose	4.9	1.0
Glucose	62.7	74.9

[a] The yield of holocellulose was 41.0%.

The structural features of major macromolecular components of grape stalks, cellulose, xylan, lignin and tannins were studied in more details.

3.2. Structural analysis of cellulose

The structure of isolated Kürschner-Höffer cellulose was assessed by wide angle X-ray scattering (WAXS) as textured sample (Figueiredo et al., 2010). WAXS analysis allowed the general dimensions of the unitary cell assessed based on characteristic reflections in diffractogram as depicted in Fig. 1. The general parameters of unitary cell were identical to those known for the polymorph cellulose I (Hon, 1994; Ioelovitch et al., 1989). The average width of cellulose crystallites, assessed in 0 0 2 lattice plane (d_{002}) employing the Scherer equation corrected to crystallite defects (Ioelovitch et al., 1989), was of 4.2 nm, which is a typical value frequently reported for woody and non-woody plants.

The degree of crystallinity (DC) of cellulose from grape stalks was 75.4%. This is much higher than DC found in wood (55–65%) and close to values reported for cotton or bacteria cellulose (Hon, 1994; Ioelovitch et al., 1989). Since crystalline cellulose is hardly accessible by hydrolytic enzymes, this fact, together with high content of tannins, can explain the difficult microbiological digestion of cellulose in grape stalks (Ben-Ghedalia, 1982; Couto et al., 2003; Ping et al., 2011). On the other hand, high DC of cellulose presumes eventual increased strength of cellulosic fibers, which may be important for the applications in papermaking or in biocomposites.

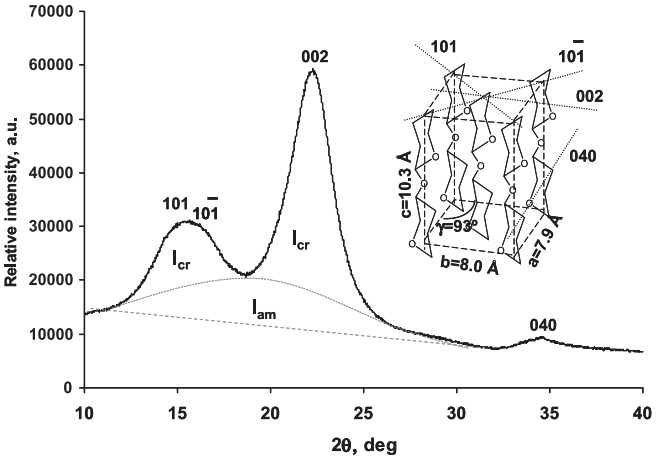

Fig. 1. X-ray scattering diffractogram of cellulose from grape stalks. The degree of cellulose crystallinity was assessed based on integral intensities of reflexions from crystalline cellulose (I_{cr}) and the amorphous halo (I_{am}). The elementary cell unit depicts basic cell dimensions and lattice plans.

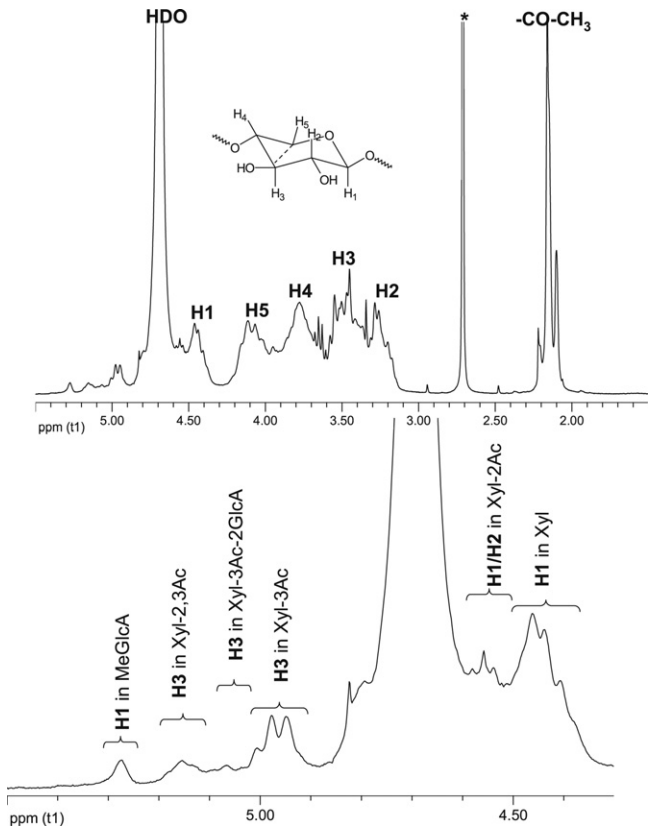

Fig. 2. ^{1}H NMR spectrum (D_2O, 30 °C) of xylan from grape stalks (top image) and the expanded region of anomeric protons (bottom image). The designations are the same as presented in Table 3.

3.3. Structural analysis of xylan

The xylan was isolated from peracetic holocellulose by extraction with DMSO (Evtuguin et al., 2003) and subjected to sugar analysis, which confirmed it purity (xylose – 89.0%; glucose – 5.5%; uronic acid – 4.9%; rhamnose – 0.5%; arabinose and galactose – traces). The presence of glucose in isolated xylan may be explained by it structural association with xylan or by simple sorption from solution during precipitation in ethanol. This point needs more detailed study.

The general structural features of xylan were assessed by liquid-state ^{1}H NMR in D_2O (Fig. 2). Although the spectra were identical to those usually reported for partially acetylated β(1→4)-linked heteroxylans (Teleman et al., 2002; Evtuguin et al., 2003), the single bond proton–proton correlation NMR spectrum (COSY) was additionally acquired to confirm the proton assignments that are presented in Fig. 2. The expanded spectrum region at 4.3–5.5 ppm depicts anomeric protons and protons at carbons with acetylated hydroxyls, which integrals were used for calculations on the frequency of occurrence of acetyl groups in xylan backbone. All calculations were performed according to previously established methodology per 100 anhydro-β-D-xylopyranosyl (Xyl*p*) units (Teleman et al., 2000; Evtuguin et al., 2003). The total balance of acetyl groups was checked by integral of its $\underline{CH_3}$– moieties at 2.05–2.30 ppm and integral of characteristic protons attached to carbons with acetylated hydroxyls (Fig. 2). The results on acetyl groups' distribution per 100 Xyl*p* units are presented in Table 3. The obtained degree of acetylation (average number of acetyl groups per Xyl*p*) was 0.49, which is lower to that reported, for example, for the xylan from sisal (0.70) (Marques et al., 2010) or from plantation eucalypt (Evtuguin et al., 2003) and paulownia (Gonçalves et al., 2008) woods. The frequency of occurrence of acetyl groups at *O*-3 in Xyl*p* residue (0.29) was remarkably higher than at *O*-2 (0.20).

The abundance of uronic groups (mostly 4-*O*-methyl-D-glucuronic acid, MeGlc*p*A) was assessed based on characteristic resonance of anomeric proton in corresponding residue at 5.28 ppm (Fig. 2). This amount of detected MeGlc*p*A (4 mol.%) corroborated with the percentage of uronic acids detected by wet chemistry analysis (4.9%), taken into consideration other uronic moieties that could be present in the xylan. Thus, weak resonance at ca 5.36 ppm in ^{1}H NMR spectrum may be assigned to H1 of galacturonic acid residue (Evtuguin et al., 2003), which is an integral part of heteroxylans (Shimizu, 1991). It may be proposed that, like in all known xylans, MeGlc*p*A is attached to *O*-2 of Xyl*p* unit. This proposition is corroborated with similar frequency of occurrence of MeGlc*p*A and *O*-3 acelylated Xyl*p* units ramified at *O*-2 with MeGlcA in xylan (Table 3).

Overall the heteroxylan from grape stalks may be considered as *O*-acetyl glucuronoxylan with relatively low proportion of MeGlc*p*A residues attached to xylan backbone (Xylp:MeGlcA = 25:1). Relatively low degree of substitution of grape stalks heteroxylan with acetyl groups and MeGlc*p*A residues may pre-determine it strong binding to cellulose and be one of the reasons for highly difficult removal upon chemical processing both in alkaline and in acidic media (Spigno et al., 2008; Ping et al., 2011) and for the particular resistance in degradation by rumen microorganisms (Ben-Ghedalia, 1982).

3.4. Lignin characterization

The isolated acid-insoluble residue (Klason lignin) was subjected to analysis by FTIR and ^{13}C NMR in order to confirm it lignin nature. The FTIR spectrum (Fig. 3) showed typical for lignin bands at 1606, 1509 and 1423 cm^{-1} assigned to aromatic skeletal vibrations (Faix, 1992). The skeletal vibrations of syringyl (S) and guaiacyl (G) units were detected at 1317 and 1267 cm^{-1} together with aromatic C–H in-plan deformation vibrations at 1118 and 1031 cm^{-1} in corresponding structures. The relative intensity of band at 1461 cm^{-1}, including the deformation vibrations of C–H in methoxyl groups (T_{1509}/T_{1461}), is typical for lignins constituted by *p*-hydroxyphenyl (H), S and G units (Faix, 1992; Oliveira et al., 2009). The band at 1714 cm^{-1} showed the presence of non-conjugated ketone groups in Klason lignin (Herbert, 1971).

The analysis of acid-insoluble residue by solid-state ^{13}C NMR spectroscopy confirmed, in general, it lignin origin (Fig. 4). The tertiary aromatic carbons (=CH–) showed resonances at both 102–110 and 110–125 ppm regions, assigned to S and G lignin units, respec-

Table 3
Relative content of acetyl groups in structural units of heteroxylan from grape stalks.

Structural fragment and short designation	Relative abundance (per 100 Xyl*p* units)
→4)-β-D-Xyl*p*-(1→(Xyl)	57
→4)[2-*O*-Ac]-β-D-Xyl*p*-(1→(Xyl-2Ac)	14
→4)[3-*O*-Ac]-β-D-Xyl*p*-(1→(Xyl-3Ac)	19
→4)[3-*O*-Ac][2-*O*-Ac]-β-D-Xyl*p*-(1→(Xyl-2,3Ac)	6
→4)[3-*O*-Me-α-D-Glc*p*A-(1→2)][3-*O*-Ac]-β-D-Xyl*p*-(1→(Xyl-3Ac-2GlcAc)	4
4-*O*-Me-α-D-Glc*p*A-(1→(GlcA)	4

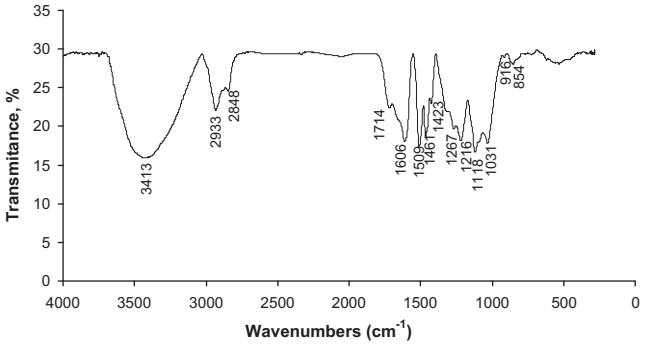

Fig. 3. FTIR spectrum of Klason lignin from grape stalks.

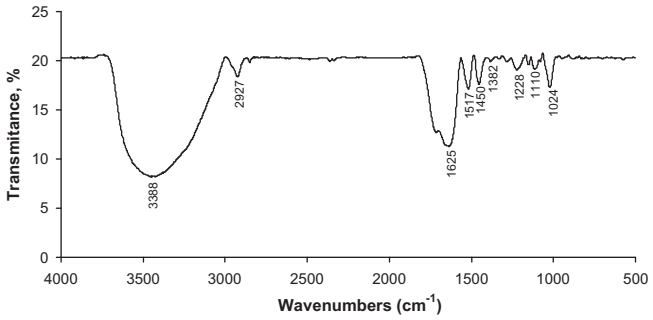

Fig. 5. FTIR spectrum of tannins from grape stalks.

tively (Hawkes et al., 1993). The unusually strong signals centered at ca 173 ppm and a series of resonances at 25–45 ppm belongs to carboxyl groups and to unsaturated (–CH_2– and –CH<) moieties, respectively, and may arise from polyphenolic acids, proteins or fatty matter that were not removed from grape stalks for some reasons or structurally associated with lignin.

The quantitative assessment of lignin structural units was carried out by analysis of nitrobenzene oxidation products (NOPs) derived from extractives-free grape stalks. This *in situ* lignin oxidation provided the molar ratio between *p*-hydroxybenzaldehyde, vanillin and syringaldehyde of 3:71:26. A relatively low yield of NOPs detected in this study (ca 12% wt.) may be tentatively explained by significant amounts of condensed structures in lignin that did not release the aforementioned NOPs.

3.5. Characterization of tannins

According to results of previously published studies, grape stalks contains both condensed and hydrolysable tannins (Cruz et al., 2004; Makris et al., 2007). These may represent significant interest for food additives or in various technical applications such as adhesives, polymers, tanning agents, etc. (Pizzi, 2008). The value of tannins depends on their composition and purity. Accordingly, the fraction of grape stalks soluble in hot weak alkaline solution, and conditionally assigned to tannins, was structurally characterized. The acidification of extract yielded a dark brown precipitate of about 60% yield based on dissolved matter. This residue was subjected to analysis by FTIR and solid-state ^{13}C NMR.

Fig. 5 shows the FTIR spectrum of precipitated tannins. The aromatic nature of precipitate was evidenced by predominance of bands at 1625 and 1517 cm^{-1} characterizing the skeletal vibrations of aromatic rings and band at 1450 cm^{-1} assigned to deformation vibrations of C–H in aromatic ring of procynidins and prodelphinidins (Oo et al., 2009; Laghi et al., 2010). The bands at 1328 cm^{-1} and at 1024 cm^{-1} correspond to symmetrical and asymmetrical stretching in C–O, respectively (Laghi et al., 2010). The band at 1230 cm^{-1} can be assigned to the asymmetric deformation of C–O–C in ether groups (Peña et al., 2009). The unusually strong for condensed tannins band at ca 3400 and ca 1720–1740 cm^{-1} may indicate the presence of incompletely saponified hydrolysable gallotannins and ellagotannins presenting in grape stalks. Since catechin/gallocatechin units in the alkaline solutions undergo epimerisation and rearrangement reactions with formation of catechinic acid type structures containing ketone moieties, these can also contribute to the band at 1720–1740 cm^{-1} (Porter, 1989).

The structural features revealed by FTIR analysis were confirmed while analyzing the ^{13}C CP-MAS NMR spectrum of tannins fraction (Fig. 6). Most of carbon resonances are coincident with known signal assignments in procyanidins and prodelphinidins (Martínez-Richa and Joseph-Nathan, 2003; Zhang et al., 2010), which are depicted in Fig. 6. Looking for the intensity of resonances centered at 153 ppm and at 145 ppm and the corresponding assignments (Fig. 6), it may be proposed that procyanidins containing catechin (CAT) moieties were predominant over prodelphinidins containing gallocatechin (GCAT) units. This assumption is in agreement with results of previous works where procyanidins were found as

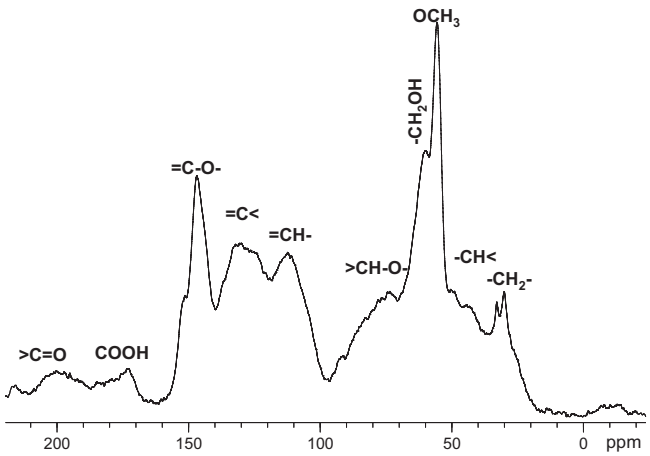

Fig. 4. ^{13}C CP-MAS NMR spectrum of Klason lignin from grape stalks.

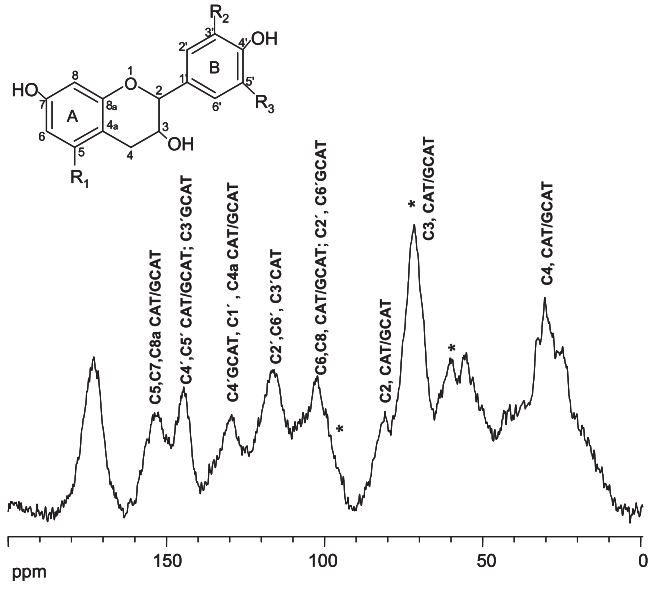

Fig. 6. ^{13}C CP/MAS NMR spectrum of tannins from grape stalks and the signal assignments to flavonoid unit that forms the basic repeating blocks in condensed tannins (catechin (CAT): R1 = R3 = OH, R2 = H; gallocatechin (GCAT): R1 = R2 = R3 = OH). Carbohydrate contaminations are marked by asterisks.

Table 4
Results on kraft pulping of grape stalks.

Pulping conditions				Pulp yield, % wt.	Residual lignin, % wt.[a]
Active alkali, %	Sulfidity, %	Temperature, °C	Time, min		
20	28	165	120	48.1	33.3

[a] Residual lignin is assessed as acid insoluble residue; acid insoluble residue in initial grape stalks was 35.4% wt.

major condensed tannins in grape stalks (Makris et al., 2007; Ping et al., 2011). At the same time the intensive resonance centered at ca 174 ppm that belongs to carboxyl groups certainly does not belong to condensed tannins and may be tentatively assigned to hydrolysable tannins saponified upon alkaline extraction (gallotannins and ellagotannins) and co-precipitated after acidification of extract. The contribution of catechinic acid type structures to aforementioned resonances cannot be completely excluded also. The presence of contaminations of carbohydrate origin is evidenced by characteristic resonances of structurally associated carbohydrates at ca 95–101 (C1), 70–78 (C2, C3, C5) and at ca 60 (C6) ppm (Maunu, 2009).

The signal at ca 55 ppm may belong to carbon in methoxy groups of lignin, which small fraction could be removed under mild alkaline conditions. Remarkable signals at 10–45 ppm indicate the eventual presence of fatty matter that was not removed by acetone extraction before the alkaline treatment of grape stalks. Resuming, isolated tannins fraction contained notable amounts of impurities and require substantial cleanup before specific applications. These tannins may find applications for the adhesives production by analogy with tannins from barks or as antioxidants in various technical areas (polymers, animal foods, etc.).

3.6. Kraft pulping of grape stalks

The grape stalks were subjected to kraft pulping aiming to evaluate the potential of this agricultural by-product for the production of cellulosic pulp. The cooking conditions were adopted from pulping of *E. urograndis* wood containing high amount of tannins (Pinto et al., 2005). These results are summarized in Table 4. Despite of a fairly good yield of pulp (48.1%), it contained an unacceptably high amount of polyphenolics detected as acid-insoluble residue (33.3%). The prolongation of pulping and reasonable increase in active alkali loads up to 22–24% fail to significantly change the delignification results (results are not present). Low selectivity in delignification of grape stalks under alkaline conditions with unacceptably high content of residual polyphenolics, even in the presence of oxidative reagents, was reported previously (Ben-Ghedalia, 1982; Ping et al., 2011). It seems that solely the amount of lignin, which is rather moderate in grape stalks (17.4%), or the presence of tannins easily soluble in alkaline solution, not explain the abnormal behavior of this agricultural residue upon processing under alkaline conditions. Knowledge about the structure of lignin and it eventual structural association with other macromolecular components (primarily with tannins and proteins) and the interaction with them under alkaline conditions is certainly missing for better understanding of difficulties related to the processing of grape stalks.

4. Conclusions

The chemical composition and the general structural features of macromolecular components of grape stalks from red grape pomaces have been studied aiming to expand the areas of eventual valorisation of this agricultural waster. The grape stalks contained rather significant amount of ash (7.0%), mainly potassium salts, and extractives soluble in hot water (ca 23%). The cellulose content in grape stalks was relatively low (ca 30%) with unusually high degree of it crystallinity (75.4%). The heteroxylan was the second most abundant polysaccharide in grape stalks, after cellulose, possessing relatively low degree of ramification with uronic moieties. The grape stalks lignin is of HGS type with predominance of G units (over 70 mol%). This lignin is apparently highly condensed and structurally associated with other macromolecular components of grape stalks. Major tannins of grape stalks are procyanidins and prodelphinidins, the former being predominant. The kraft pulping of grape stalks showed serious limitations to obtain well-delignified fibers. This fact was attributed to specific lignin structure and eventual interaction with other macromolecular components upon kraft pulping.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.indcrop.2011.06.035.

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