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Quantitative Prediction of Cell Wall Polysaccharide Composition in Grape (*Vitis vinifera* L.) and Apple (*Malus domestica*) Skins from Acid Hydrolysis Monosaccharide Profiles

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On the basis of monosaccharide analysis after acid hydrolysis of fruit skin samples of three wine grape cultivars, *Vitis vinifera* L. Cabernet Sauvignon, Merlot, and Shiraz, and of two types of apple, *Malus domestica* Red Delicious and Golden Delicious, an iterative calculation method is reported for the quantitative allocation of plant cell wall monomers into relevant structural polysaccharide elements. By this method the relative molar distribution (mol %) of the different polysaccharides in the red wine grape skins was estimated as 57−62 mol % homogalacturonan, 6.0−14 mol % cellulose, 10−11 mol % xyloglucan, 7 mol % arabinan, 4.5−5.0 mol % rhamnogalacturonan I, 3.5−4.0 mol % rhamnogalacturonan II, 3 mol % arabinogalactan, and 0.5−1.0 mol % mannans; the ranges indicate minor variations in the skin composition of the three different cultivars. These cell wall polysaccharides made up ~43−47% by weight of the skins (dry matter), the rest mainly being lignin. The predicted relative molar levels of the polysaccharide elements in the apple skins, which made up ~49−64% by weight of the skins (dry matter), appeared to be similar to those of the grape skins. The apple skins were estimated to be relatively richer than grape skins in arabinan, total levels 10−13 mol %, and relatively lower in mannan content, total levels ≤0.3 mol %. The data also demonstrate the superiority of trifluoroacetic acid to hydrochloric acid for hydrolysis of plant cell wall material to monosaccharides, notably with respect to the galacturonic acid levels and, in turn, in relation to predicting the relative contents of structural pectin elements in the plant cell wall substrates.

KEYWORDS: Plant cell wall polymers; polysaccharides; composition; pectin

INTRODUCTION

Microbially derived pectinolytic enzyme preparations are widely used for prepress fruit maceration in the industrial production of apple and berry juices, ciders, and red wines to increase juice yields (*1*). Recently, there has been an increased interest in using plant cell wall degrading enzymes to enhance the release of colored pigments and antioxidant phenolic compounds during the prepress treatment in juice and wine processing (*2*, *3*). Such enhanced release may take place via more aggressive enzyme-catalyzed degradation of the complex plant cell wall material, notably the fruit skins (*4*).

Each year the processing of grapes (*Vitis vinifera* L.) and apples (*Malus domestica*) for wine, juice, and cider production globally leaves behind an estimated amount of at least 50 million metric tons of press residues, or "pomace" (*5*). This pomace consists of fruit skins, remnants from the fruit pulp, seeds, and, in certain cases, some stems, with the skins and seeds making up the major part. Grape seeds and grape skins are rich sources of phenolic compounds and/or dietary fibers (*6*, *7*), and grape pomace from wine processing is already used for the extraction of anthocyanins on an industrial scale (*8*). Increased efforts are now directed toward more extensive valorization of the press residues from fruit juice and wine processing to obtain high-value products such as natural health remedies, food supplements, and novel nutrifunctional food ingredients or to use the material for enzyme production by solid state fungal cultivation (*8*). Apple pomace, left over from apple juice production, is used for pectin extraction, leaving the apple skin fraction behind as a secondary byproduct residue. This secondary apple byproduct residue is currently not upgraded to high-value products (*9*).

To rationally design and tailor enzymatic treatments to upgrade fruit skin residues and/or to increase the enzyme-catalyzed degradation of the fruit skins to release additional color or antioxidant phenolics during prepress treatments in juice and wine processes, a first essential step is to obtain quantitative knowledge about the structural makeup, including the glycosidic bonds, of the fruit skin polysaccharides. Several studies have already addressed certain characteristics

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of different carbohydrate polymers in grape skins (*10*−*12*), whereas surprisingly few reports have addressed the quantitative occurrence and structural features of apple skin cell wall polysaccharides (*13*, *14*). The available data on grape and apple skins either focus on unique structural characteristics of particular polysaccharides (*11*, *15*) or employ the monosaccharide composition to elucidate compositional differences among fruit tissues or of different cultivars in relation to processing or product quality (*10*, *11*, *16*). There are currently no stand-alone methods available for deducing the detailed composite structure of intact plant cell walls nor for assessing the full structures of the polysaccharides and the possible connection points that define the molecular architecture of composite plant cell walls. Hence, despite advances in, for example, electron microscopy, chromatographical methods, and mass spectrometry, combinations of different sequential extraction methods and several analytical methods are required to obtain the pieces of the puzzle and to obtain an insight into the glycosyl linkage compositions (see e.g (*11*, *12*, *16*)). The particular sequential extraction and precipitation approaches and the particular analytical methods employed may even affect the results obtained. The recovery of monosaccharides after acid hydrolysis is a widely used strategy to at least obtain an overview of the composition of the plant cell wall building blocks (*17*−*19*). The monosaccharide composition of fruit skins and other plant materials is usually determined via chromatographical analysis of the monosaccharides released after acid hydrolysis with either trifluoroacetic (TFA), hydrochloric acid (HCl), or in some cases sulfuric acid (H_2SO_4) (*17*, *18*). Monosaccharide profiles are used to provide a rough overview of the types of polysaccharides present: for example, arabinose signifies the putative presence of "arabinan", and galacturonic (or uronic) acid levels indicate the presence of "pectin"—even though, in the latter case, pectin is known to encompass several different structural elements. The monosaccharide profiles are rarely used to calculate the quantitative levels of the different types of structural polysaccharides in the plant material. An important question in relation to the rational design of enzyme treatments for efficient degradation of the cell wall polysaccharides is whether it is possible to combine the available knowledge of polysaccharide type structures, including their glycosidic bonds, in (dicot) plant materials with the now readily obtainable monosaccharide profiles and use the monosaccharide data to provide a picture of the quantitative levels of the different polymeric structural elements in plant materials—without having to include mischievous, time-consuming procedures such as polysaccharide linkage analysis. This study was undertaken to evaluate the options for calculating the most probable structural element structures from monosaccharide data. We report an iterative calculation methodology that combines current knowledge of dicot plant cell wall polysaccharide structures with the monosaccharide profile data to obtain a quantitative allocation of monomers into different structural carbohydrate polymer elements. Differences in the results obtained with different acid hydrolysis techniques are also addressed.

MATERIALS AND METHODS

Chemicals and Reagents. Trifluoroacetic acid 99% (TFA) and sodium azide were from Merck (Darmstadt, Germany). Monosaccharide standards D-(+)-fucose 99%, L-(+)-arabinose 99%, L-(−)-rhamnose monohydrate 99%, D-(+)-glucose 99.5%, D-(+)-galactose 99%, L-(−)-mannose 99%, D-(−)-xylose 99%, D-(−)-fructose, and D-(+)-galacturonic acid monohydrate 98% were from Sigma-Aldrich Chemical Co. (St. Louis, MO). HCl and the NaOH standard solution (HPLC grade) were from Fluka/Sigma-Aldrich Chemical Co. (St. Louis, MO).

Plant Materials and Sample Preparation. Three selected red wine grapes (*V. vinifera* L.), Cabernet Sauvignon, Merlot, and Shiraz, were obtained from the Distell Group Ltd. (Stellenbosch, South Africa). Golden Delicious (GD) and Red Delicious (RD) apple varieties (*M. domestica*) were purchased locally in Lyngby, Denmark. Fruits were peeled manually under a continuous stream of nitrogen (N_2), and the skins were carefully separated from the pulp using a scalpel. Skins were washed with cold, distilled water at 4 °C, and lyophilized immediately by use of a Lyovac GT 2 freeze-drier (Leybold-Haraeus, Germany) and then milled for 30 s under N_2 at 20000 rpm in an M 20 Universal mill (Jahnke & Kunkel GmbH, Staufen, Germany). The chamber of the M20 mill was cooled with running tap water. Fruit skin particles of 125−250 μm were collected by means of a sieves tower, nominal aperture sizes of 500, 250, and 125 μm, respectively (Endecotts Ltd., London, U.K.). The fruit skin particles were kept in tightly closed glass jars under N_2 at −20 °C until use.

Acid Hydrolysis Methods. Two different hydrolysis methods, each including five replicates of each grape and apple skin sample, were compared. In the first method, 400 μL of 2 M TFA was added to 2 mg of lyophilized sample in a screw-cap vial. Each vial was tightly sealed and heated at 121 °C for 2 h in a drying oven. In the second method, 2 M HCl was used instead of TFA. The conditions of time, temperature, and sample/acid ratio were the same as for the TFA hydrolysis [a more detailed comparison of acid hydrolysis methods for fruit skin analyses has been published previously (*19*)]. Hydrolysates were lyophilized and kept at −20 °C under N_2 until analysis. Prior to analysis by HPAEC-PAD (see below), the fruit skin hydrolysates were redissolved in 5 mL of doubly deionized water containing 0.1% of sodium azide to prevent microbial growth. Just before injection for HPAEC-PAD analysis, each hydrolysate was filtered through a 0.22 μm GH Polypro Acrodisc filter (Pall Life Sciences, Ann Arbor, MI). Recovery values of the monosaccharides were estimated as described previously (*19*) by exposing a mixture of monosaccharide standards L-(+)-fucose, L-(+)-rhamnose, D-(−)-arabinose, D-(+)-galactose, D-(+)-glucose, D-(+)-xylose, D-(+)-mannose, D-(−)-fructose, and D-(+)-galacturonic acid to the corresponding acid hydrolysis conditions.

Monosaccharides Analysis by HPAEC-PAD. Separation and quantification of monosaccharides in hydrolysates were performed by use of a BioLC system, equipped with a CarboPac PA20 (3 mm × 150 mm) analytical column, and an ED50 electrochemical detector and controlled via Chromeleon 6.60 Sp2 Build 1472 software (Dionex Corp., Sunnyvale, CA) as reported previously (*19*). Monosaccharides were expressed as micrograms per milligram of lyophilized fruit skin. The values were then translated into micromoles of monocarbohydrates per milligram of lyophilized fruit skin for easier reconstruction according to the molar ratio between different monocarbohydrates in each type of polysaccharide. The data are reported as relative molar levels in percent.

Determination of Klason Lignin. Klason lignin was determined according to the method of Theander and Åman (*20*).

Statistical Analysis. All measurements were done in triplicate except for monosaccharides analyses with HPAEC-PAD that were done in duplicate. Quadratic curve fit through the origin was used for monosaccharides standards analysis by HPAEC-PAD, and the calibration points were weighted by the factor $1/\text{response}^2$.

RESULTS AND DISCUSSION

Monosaccharide Compositions. *Grape Skins.* Acid hydrolysis with TFA consistently gave higher analytical values than HCl hydrolysis with respect to the levels of monosaccharides after HPAEC-PAD analysis (after adjustment for recovery factors) (**Table 1**). The high galacturonic acid levels in the TFA hydrolysates of the grape skins, $> 300\ \mu g\ mg^{-1}$ of

Table 1. Analyzed Monosaccharide Composition of Grape Fruit Skins

		monocarbohydrates (μg mg^{-1} of lyophilized skins)										
grape variety	hydrolysis method	L-(+)-fucose	L-(+)-rhamnose	D-(−)-arabinose	D-(+)-galactose	D-(+)-glucose	D-(+)-xylose	D-(+)-mannose	D-(+)-galacturonic acid	sum of total monocarbohydrates (μg mg^{-1})	Klason lignin (μg mg^{-1})	sum of Klason lignin and total monocarbohydrates (μg mg^{-1})
Cabernet Sauvignon	TFA	2.1	14.0	32.8	17.4	94.9	16.5	2.4	304.0	484.1	406	890
	HCl	0.9	4.7	9.3	8.4	73.6	2.4	11.2	17.6	128.1	406	534
Merlot	TFA	2.5	14.0	40.8	22.0	88.2	21.1	4.7	345.0	538.3	437	975
	HCl	1.6	6.6	11.8	12.2	67.7	2.5	9.9	20.5	132.8	437	570
Shiraz	TFA	2.4	13.8	35.6	20.3	60.8	19.9	4.6	328.8	486.2	451	937
	HCl	1.0	5.1	11.3	10.7	47.1	2.9	12.3	16.4	106.8	451	558

lyophilized skins (**Table 1**), indicated that the grape skins contained significant amounts of pectinaceous polysaccharides. When consideration of the rhamnose, arabinose, galactose, and fucose levels (**Table 1**) was included, it appeared that these pectinaceous polysaccharides included both homogalacturonan (HG) and branched rhamnogalacturonans. The higher galacturonic acid levels of the TFA-treated samples as compared to the HCl-treated samples indicated that TFA had a stronger hydrolyzing efficiency than HCl toward pectin (all values had been adjusted for recovery). The galacturonic acid values obtained from the TFA hydrolysis were somewhat higher than those reported by others using other methods of hydrolysis and on other grape varieties (**Table 2**). Previously, De Ruiter et al. (*17*) demonstrated that the method of hydrolysis has a significant influence on the monosaccharide results and in turn on the determined composition of the plant materials. Our high galacturonic acid values with TFA hydrolysis were thus proposedly a result of the superior efficiency of TFA to HCl for hydrolysis of pectin but perhaps also the extended treatment time (*19*) and the use of a highly sensitive chromatographic method of detection. Furthermore, we used recovery factors to compensate for monosaccharide losses during hydrolysis. However, with TFA hydrolysis, our values for mannose were lower than those found by others using the Saeman procedure (involving sulfuric acid hydrolysis) (**Table 2**) and lower than those obtained with HCl hydrolysis (**Table 1**), but similar to those reported previously for grape pulp cell wall material hydrolyzed by TFA combined with methylation and sonication (*16*). For grape berry skins it has previously been shown that mannose yields may be lower with TFA hydrolysis than with the Saeman hydrolysis procedure (*10*).

The analyzed levels of fucose, rhamnose, arabinose, galactose, and xylose in the TFA hydrolysates of the grape skins (**Table 1**) were similar to those reported by others, even for other grape varieties, but the glucose values of our TFA-treated grape skin samples were 2−3 fold lower than those reported previously by others (*10*, *11*, *21*) (**Table 2**). It is well-known that TFA has a relatively poor hydrolyzing capability against cellulose (*22*, *23*), but this alone cannot fully explain the lower glucose levels, because TFA was also used by others for the hydrolysis (**Table 2**). We consider it unlikely that cellulose contents will vary this significantly among different grape varieties. We therefore ascribe the relatively low glucose values of our grape skin samples (**Table 1**) to be a result of the deliberate inclusion of washing of the skins prior to the acid hydrolysis treatment in our procedure. Washing will free the skin material from intercellular liquid, rich in glucose and fructose, and remove any free monosaccharides (*24*). Hence, we presume that the high values of other samples may in fact be a result of the (artifact) presence of free glucose from disrupted pulp cells in the grape skin samples. When an anhydro correction factor of 0.88 was used, the polysaccharides together made up ∼43−47% by weight of the grape skins; the levels varying slightly among the grape cultivars.

Apple Skins. For the apple skins, hydrolysis with TFA, as opposed to with HCl, also consistently resulted in higher monosaccharide values, notably for galacturonic acid—but except for mannose, which was lower with TFA than with HCl hydrolysis, exactly as seen for the grape skins (**Table 3**). Hence, apparently the TFA hydrolysis was more capable than HCl hydrolysis of disclosing differences between monosaccharide profiles of different grape and apple skin samples (**Tables 1** and **3**). In general, the monocarbohydrate profiles of the TFA hydrolysates of the apple skins were quite similar to those of the grape skins, but the galacturonic acid level in the TFA hydrolysate of the Red Delicious apple skin was ∼30% higher than the levels found in the Golden Delicious and in the grape skin samples (**Tables 1** and **3**). Likewise, the arabinose and galactose levels and—to a lesser extent—the xylose levels were higher in the TFA hydrolysates of the apple skins than in the corresponding grape skin hydrolysates, notably for the Red Delicious (**Table 3**). Partial monosaccharide analyses of apple marc have previously indicated that arabinose and galactose are particularly abundant in apple cell wall materials, with values reaching 85 and 40 μg mg^{-1}, respectively (*13*). The relatively high arabinose levels found in the apple skin hydrolysates were consistent with the presence of highly branched α-L-arabinofuranans ("arabinans") linked to rhamnogalacturonan I in apple cell walls (*25*, *26*). When an anhydro correction factor of 0.88 was used, the polysaccharides made up ∼64% of the skin weight of the Red Delicious skins and ∼47% by weight of the Golden Delicious fruit skins.

Lignin. Lignin constituted 40−45% by weight of the grape skins (**Table 1**) and 34−39% by weight of the apple skins (**Table 3**). These relatively high levels of lignin agree well with previously published data for grapes (*21*, *27*), whereas the lignin values for the apple skins were a little higher than those previously reported for apple pomace (*28*). The relatively high values may be because in our study the skins were carefully separated from visible pulp material prior to acid hydrolysis.

The high levels of lignin found in both grape and apple skins are noteworthy and categorize the grape and apple

Table 2. Previously Reported Monosaccharide Compositions of Grape Fruit Skins

		monocarbohydrates[a] (μg mg^{-1} of skins dry matter)										
grape variety	hydrolysis method	L-(+)-fucose	L-(+)-rhamnose	D-(−)-arabinose	D-(+)-galactose	D-(+)-glucose	D-(+)-xylose	D-(+)-mannose	D-(+)-galacturonic acid	sum of total monocarbohydrates (μg mg^{-1})	Klason lignin (μg mg^{-1})	sum of Klason lignin and total monocarbohydrates (μg mg^{-1})
Carignan Noir (*10*)	2 M TFA, 121 °C, 1 h/Saeman procedure [a]	2[b]	10[b]	42[c]	20[c]	180[c]	22[c]	20[c]	195	491	ND[d]	ND
Grenache Blanc (*11*)	extraction of CWM by HEPES + treatment with glycosyl hydrolases	2.9	4.7	32.2	17.6	168.0[c]	19.4[c]	17.0[c]	224.0	485.8	ND	ND
Red Globe (*21*)	Saeman procedure and 1 M H_2SO_4 100 °C, 2.5 h; or 1 M H_2SO_4 100 °C, 2.5 h on AIRs[e]	7.3	13.4	59.4	34.2	280.7	31.4	32.4[c]	227.4	686.2	ND	ND

[a] Cell wall material (CWM) (μg mg^{-1}) was obtained by Selvendran's method (*10*). [b] By TFA hydrolysis. [c] By Saeman hydrolysis. [d] ND, not determined. [e] AIRs, alcohol insoluble residues (*21*).

Table 3. Analyzed Monosaccharide Composition of Apple Fruit Skins

		monocarbohydrates (μg mg^{-1} of lyophilized skins)										
apple variety	hydrolysis method	L-(+)-fucose	L-(+)-rhamnose	D-(−)-arabinose	D-(+)-galactose	D-(+)-glucose	D-(+)-xylose	D-(+)-mannose	D-(+)-galacturonic acid	sum of total monocarbohydrates (μg mg^{-1})	Klason lignin (μg mg^{-1})	sum of Klason lignin and total monocarbohydrates (μg mg^{-})
Red Delicious	TFA	2.9	18.5	92.7	32.0	95.1	25.3	0.90	456.8	724.2	386	1110
	HCl	0.60	5.20	14.6	11.7	49.0	1.90	5.50	21.10	109.6	386	496
Golden Delicious	TFA	3.3	11.8	56.9	23.2	90.6	22.1	1.5	348.4	557.8	344	902
	HCl	0.9	4.0	11.2	8.8	47.1	1.6	6.0	15.9	95.5	344	440

skins as lignin-rich biomass materials with respect to enzymatic attack and degradation.

Quantitative Calculation of Predicted Polysaccharide Profiles: Methodology. During the development of the iterative calculation method to quantitatively predict the cell wall polysaccharides and, implicitly, the bonds building the structural elements from monosaccharide profiles, we first allocated monosaccharides to a relatively simple and well-defined, homopolymeric molecular structure, namely, mannan (**Figure 1**). In grape pericarp, mannan is basically composed of chains of mannose, linear chains made up of β-1,4-linked mannose units. Mannose is not a constituent of any other plant cell wall polysaccharides when it is assumed that mannose is not a side-chain substituent of rhamnogalacturonan I (RG-I) (*29*). Then, the galacturonic acid residues were distributed among the three main pectin structural units: HG, made up of a backbone of α-1,4-linked galacturonic acid residues; RG-I, made up of a backbone of alternating α-1,2-linked rhamnose and α-1,4-linked galacturonic acid residues (the rhamnose residues may be substituted with neutral side chains of galactan, arabinan and/or different arabino-galactan side chains); and rhamnogalacturonan II (RG-II), which consists of a backbone of HG with clusters of four different side chains containing apiose, aceric acid, 2-*O*-methylfucose, 3-deoxy-lyxo-2-heptulosaric acid (DHA), and 3-deoxy-manno-2-octulosonic acid (KDO). The distribution between the three main structural pectin units was done by keeping in mind a relative molar ratio of HG:RG-I:RG-II in grape skins of approximately 16:3:1 (80:15:5) (*11*). To distribute the galacturonic acid as correctly as possible, we found it expedient to first reconstruct RG-II, the least abundant of the structural pectin units. This was done by using all of the available fucose monomers and then allocating the required galacturonic acid in a fucose:galacturonic acid ratio of 1:6 (**Figure 1**). This ratio was determined from the available data for RG-II structures in dicots (*30*, *31*). Fucose is uniquely present in the side chain of RG-II (*31*), which is why the quantitative prediction of RG-II could be based on allocating the total fucose to RG-II and then allocating the required galacturonic acid proportionally to the fucose level (**Figure 1**). The total level of fucose thus established the corresponding consumption of galacturonic acid to RG-II as well as the predicted molar amount of RG-II. Other unique constitutents of RG-II such as apiose, DHA, and KDO were not detected in the hydrolysates by our HPAEC-PAD method. By first allocating galacturonic acid to RG-II, that is, to the minor pectin unit, any marginal differences between the available galacturonic acid monomers and the correct distribution among the different pectin elements would have less impact on the subsequent galacturonic acid allocation to HG and RG-I, whereas if the distribution of the galacturonic acid to the major pectin components first is accomplished first, the potential relative error on the estimated amounts of the minor component, in this case RG-II, would be maximized.

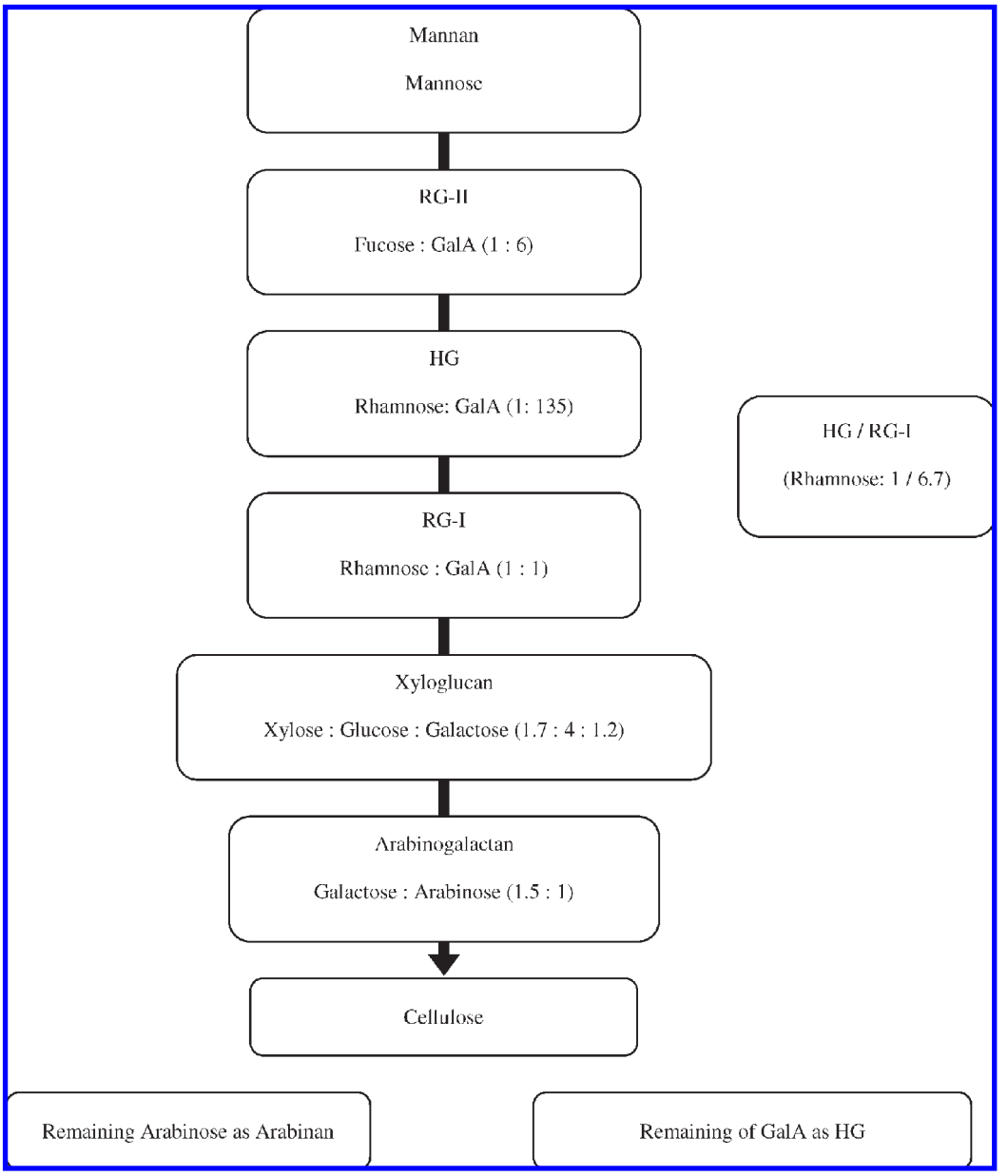

Figure 1. Schematic diagram of the iterative steps and the ratios used to calculate the major polysaccharides contents in apple and grape skins.

In the next step, the rhamnose was used to reconstruct HG and RG-I. This was done by first setting the rhamnose distribution ratio between HG and RG-I to 1:6.7 (13:87%) and then distributing galacturonic acid to all of the available rhamnose by using ratios of rhamnose:galacturonic acid of 1:135 for the HG unit and 1:1 for RG-I (**Figure 1**). These latter ratios were defined from the available data for pectinaceous polymers in cell wall materials of dicots (*16*, *26*, *32*, *33*). The influence on the predicted profile of structural pectin units of employing different ratios for rhamnose: galacturonic between HG and RG-I and within the slightly rhamnose-interrupted HG will be discussed below.

To our knowledge there are no literature reports on the quantitative or qualitative occurrence of xylogalacturonans in grape and apple skins, and the literature data on xylogalacturonan in dicots do not imply that xylogalacturonan is significantly abundant when present (*34*, *35*). Hence, for the calculations we presumed that xylogalacturonan might constitute only a negligible fraction of the pectin in grape and apple skins—if present at all. Consequently, we decided to allocate all of the analyzed xylose to the xyloglucan in the fruit skins and in turn calculated that any surplus galacturonic acid—not assigned to RG-I, RG-II, or HG according to the calculations outlined above—was part of a homopolymeric HG structural unit composed of galacturonic acid only (**Figure 1**).

Reconstruction of xyloglucan was based on assuming a xyloglucan structure composed of a glucan backbone made up of β-1,4-bonded glucose units with intermittent substitutions at C6 with single α-1,6-xylopyranosyl residues or with disaccharide β-galactose(1→2)-α-1,6-xylopyranosyl substitutions and a set ratio between xylose, glucose, and galactose of 1.7:4:1.2 (*12*) (**Figure 1**). We opted for this ratio of glucose relative to xylose instead of the ratio suggested for an acidic xyloglucan fraction found previously in grape skins, which was proposed to be a linear β-1,4 xylose−glucose backbone structure in hemicellulose A-I in the grape skins with a molar xylose:glucose ratio of 1:0.1 (*15*). The rest of the glucose was then allocated to cellulose, and arabinogalactan was reconstructed from galactose and arabinose using a galactose: arabinose ratio of 1.5:1 (*32*) (**Figure 1**). Finally, the eventual "surplus" of arabinose was allocated as a pure arabinan

(side) chain in a similar way as done for galacturonic acid (**Figure 1**). The grape arabinan structure is assumed to be made up of an α-1,5-linked backbone of L-arabinofuranosyl residues to which side chains of L-arabinose are attached in the 3-position (*16*).

Predicted Polysaccharide Profiles from Monosaccharide Analyses. *Grapes*. For the skin samples from different red wine grape varieties the iterative calculation method resulted in a predicted relative molar profile (mol %) of polysaccharides in the skins of red wine grapes of 55−61 mol % homogalacturonan, 1−10 mol % cellulose [the true levels may rather be in the range of ∼5−10 mol %, as the predicted cellulose levels in the Merlot skin samples were very low (**Table 4**)], 16−20 mol % xyloglucan, ∼7 mol % arabinan, ∼5 mol % rhamnogalacturonan I, 3.5−4.0 mol % rhamnogalacturonan II, ∼0.3 mol % arabinogalactan, and 0.5−1.0 mol % mannan; the ranges indicate minor variations among the three different grape cultivars (**Table 4**). The data thus indicated that homogalacturonan and xyloglucan were by far the most dominant polysaccharides of the grape skin cell wall polysaccharides. This comprehension is consistent with previous data that have elucidated the structures and tissue distribution of pectic polysaccharides in grapes (*11*) (**Table 5**).

The defined ratio for the fucose:galacturonic acid in RG-II, and in turn the distribution of rhamnose:galacturonic acid in slightly rhamnose-interrupted HG, obviously determined the predicted relative molar levels of HG and RG-I. In this way the rhamnose monomer level in effect defined the relative levels of HG and RG-I. A scenario analysis of the consequences of changing the rhamnose distribution in RG-I:HG from, for example, 80:20 to 87:13 for Merlot grape skins, while keeping the rhamnose:galacturonic acid set at 1:60 in the slightly rhamnose-interrupted HG, clearly illustrated how such a change in the RG-I:HG ratio resulted in a decrease in the relative percentage level of the slightly rhamnose-interrupted HG and concomitantly increased the relative mole percent levels of RG-I and RG-II, whereas the surplus galacturonic acid to be allocated in smooth homogalacturonan went up (**Table 6**). Oppositely, when the rhamnose:galacturonic acid ratio in the HG was changed from 1:60 to 1:135, the estimated relative level of this HG went up, whereas RG-I, RG-II, and surplus galacturonic acid for smooth, homopolymeric HG all went down (**Table 6**)—all as a result of the rhamnose being the limiting monomer. The final fit of the galacturonic acid:fucose ratio in RG-II, the rhamnose distribution between RG-I and HG, and the rhamnose:galacturonic acid ratio in this slightly rhamnose-interrupted homogalacturonan were chosen by iteration to result in the minimal amount of "surplus" galacturonic acid (**Table 6**). The choice of a low rhamnose:galacturonic acid ratio is of course, in effect, almost the same as leaving more surplus galacturonic acid to be distributed in smooth, homomeric galacturonic acid, but the relative distribution among HG, RG-I, and RG-II will vary (**Table 6**). In grape mesocarp cells, that is, grape pulp cell wall material, cellulose has previously been reported to make up ∼30 mol % of the cell wall polysaccharides (*29*); in this light, the here estimated relative levels of cellulose seemed low (**Tables 4** and **5**). The lower hydrolysis efficiency of TFA toward cellulose relative to the efficiency of H_2SO_4 as used in the Saemann procedure (*36*) could be a factor explaining this difference. Alternatively, the lack of washing of the skins prior to acid hydrolysis in other studies might have contributed to artifacts causing higher analyzed glucose levels and in turn, perhaps, relatively high cellulose estimates.

Table 4. Theoretical Percentage of Major Polysaccharides in Lyophilized Grape Skin Cell Walls

		% to the total skin cell wall polysaccharides matrix								
grape variety	hydrolysis method	mannan	HG	RG-I	RG-II	xyloglucan	arabinogalactan	arabinan	surplus GalA[a] balanced as HG	cellulose
Cabernet Sauvignon	TFA	0.9	54.9	5.0	3.8	18.9	0.3	7.5	3.5	5.3
	HCl	7.4	104.7	9.5	9.9	9.0	11.4	4.9	−102.4	45.7
Merlot	TFA	1.0	60.0	5.4	4.0	19.7	0.2	7.3	1.6	0.9
	HCl	11.5	101.0	9.1	7.9	12.8	11.5	6.4	−96.9	36.6
Shiraz	TFA	0.5	60.8	5.5	3.5	16.4	0.3	6.7	−3.8	10.0
	HCl	8.7	164.8	14.9	5.4	8.9	7.3	4.7	−162.9	52.3

[a] GalA, galacturonic acid.

Table 5. Previously Reported Percentages of Major Polysaccharides in (Dried) Grape Skin Cell Wall Material

		% to the total skin cell wall polysaccharides matrix								
grape variety	hydrolysis method	mannan	HG	RG-I	RG-II	xyloglucan	arabinogalactan	arabinan	surplus GalA[a] balanced as HG	cellulose
Carignan Noir (*10*)	2 M TFA, 121 °C, 1 h; or Saeman procedure[a]	4.1	43.0	3.9	3.3	21.6	−0.6	8.8	−8.0	24.0
Grenache Blanc (*11*)	buffer extraction of CWM + glycosyl hydrolase treatment	3.5	20.4	1.8	4.9	19.2	−0.5	6.8	20.5	23.3
Red Globe (*23*)	μg mg^{-1} of AIRs; Saeman procedure: 1 M H_2SO_4 100 °C, 2.5 h; or 1 M H_2SO_4 100 °C, 2.5 h	4.7	41.2	3.7	8.7	22.0	0.7	8.4	−17.5	28.0

[a] GalA, galacturonic acid.

Table 6. Example of Iterative Steps To Calculate the Contribution of Different Pectinaceous Structures, HG, RG-I, RG-II, and the Surplus of GalA, in the Total Pectic Moiety after TFA Hydrolysis in Merlot Grape Skin

				contribution of different pectinaceous structures to grape skin (μg mg^{-1}) (relative mol % of the total pectic moiety)				
iterative step	RG-II Gal:Fuc	RG-I:HG Rha distribution	HG Rha: GalA	HG	RG-I	RG-II	sum (μg mg^{-1})	surplus GalA[a]
1	1:6	80:20	1:60	203.7 (81.9)	24.6 (9.9)	20.5 (8.2)	248.8	20.9
2	1:6	87:13	1:60	132.4 (73.7)	26.6 (14.9)	20.5 (11.4)	179.5	33.8
3	1:6	87:13	1:135	295.6 (86.2)	26.8 (7.8)	20.5 (6.0)	342.9	3.5

[a] Surplus galacturonic acid levels given as relative % mole to the total 100% level of the other major polysaccharides (see **Table 3**).

Table 7. Theoretical Percentage of Major Polysaccharides in Lyophilized Apple Skin Cell Walls

		% to the total skin cell wall polysaccharides matrix								
apple variety	hydrolysis method	mannans	HG	RG-I	RG-II	xyloglucan	arabinogalactan	arabinan	GalA[a] as extended chain in HG	cellulose
Red Delicious	TFA	0.1	54.0	4.9	3.3	16.8	1.5	12.3	3.9	3.2
	HCl	5.1	99.2	9.0	4.3	8.4	13.9	8.3	−88.0	39.8
Golden Delicious	TFA	0.3	44.5	4.0	4.8	19.1	0.4	10.1	11.8	5.1
	HCl	6.3	87.9	8.0	7.5	8.1	11.7	7.5	−81.6	44.7

[a] GalA, galacturonic acid.

Apples. The calculated relative molar distributions of the polysaccharides in the apple skins (**Table 7**) appeared to be similar to those obtained for the grape skins. However, in agreement with available knowledge on apple cell wall pectin, the apple skins were predicted to be relatively richer in arabinan (10−13 mol %) and to have a relatively lower mannan content than grape skins (0.1−0.3 mol %) (**Table 7**). The cellulose levels of 3.2−5 mol % in the apple skins were slightly lower than the cellulose levels of 5.3−10 mol % in the grape skins except for the level in Merlot (**Tables 5** and **7**).

Significance of the Acid Hydrolysis Method. TFA was consistently more efficient than HCl in hydrolyzing the pectin fraction in both grape and apple fruit skins (**Tables 1** and **3**). This finding was in accordance with previous data for other plant materials (*37*, *38*) and resulted in the prediction of relatively abundant amounts of pectin, HG, RG-I, or RG-II, in the fruit skins and availability of galacturonic acid levels to match the presented ratio distributions. For the TFA hydrolysates, there was even a galacturonic acid "surplus", which we calculated to be part of extended chains of HG made up exclusively of galacturonic acid (**Figure 1** and **Table 6**). In contrast, after HCl hydrolysis, the deficit in "required" galacturonic acid for the structural pectin elements exceeded more than 100% of grape skin samples and ∼82−88% in the apples skin samples (**Tables 5** and **7**). These differences in the levels of galacturonic acid reflected the strong influence of the acid hydrolysis method on the quantitative prediction of pectinaceous structures. In other words, the acid hydrolysis method has a strong influence on the comprehension of the different structural elements in plant cell wall matrix and, in this particular case, especially with respect to predicting the levels of the different pectinaceous polysaccharides.

In dicotyledonous plants, the plant cell walls are mainly made up of pectin polysaccharides, xyloglucan, cellulose, mannan, lignin, and glycoproteins (*29*). In dicots, including grapes and apples, the structural elements of pectin are known to be mainly made up of three structural units: homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II)—with HG and RG-I generally being the quantitatively most dominant (*39*). Xylogalacturonan is a fourth structural unit of pectin, which has been identified in cell wall extracts from, for example, soybean and watermelon (*34*, *35*), and which was recently presumed to be present in modified hairy regions of apple pectin, as a xylogalacturonan−RG-I linkage [the oligomer structure $GalA_6Rha_3Xyl_1$ was proposed (*40*)]. However, no indications of the quantitative levels of xylogalacturonan have been given in any of these papers. Because xylogalacturonan has only been rarely identified, we have presumed that xylogalacturonan might only make up a negligible amount of the plant cell wall material in the here studied fruit skin samples.

Homogalacturonans are helical homopolymers of α-1,4-linked galacturonic acid monomers, which may be methylesterified and/or acetylated. Single α-1,2-linked rhamnose residues may interrupt the long homogalacturonan chains, resulting in a bend, a "pectic elbow", in the polygalacturonic acid structure (*1*), but the galacturonic acid:rhamnose levels vary in different plant materials; in apple cell wall materials a typical ratio is 100 galacturonic acids to 1 rhamnose (*41*).

The backbone structure of RG-I is a heteropolymer consisting of an array of repeating disaccharides of alternating α-1,2-linked rhamnose and α-1,4-linked galacturonic acid residues (1:1) (*39*). The rhamnose residues in RG-I may be substituted with neutral side chains of galactan, arabinan, and/or different arabino-galactan side chains. It was previously found that the molar ratio of galactose and arabinose in the side chains of RG-I of grape pulp cell walls is 1.5:1 and that minor amounts of mannose and glucose might also be present (*42*). However, the presence of mannose and glucose in RG-I structures is not generally recognized, which is why in the present work we decided not to include mannose and glucose when reconstructing the relative levels of RG-I from the cell wall monomers. Despite its name, RG-II has a homogalacturonan (HG) backbone rather than one of alternating galacturonic acid and rhamnose (as in RG-I), and RG-II uniquely has complex side chains attached to the galacturonic acid residues (*39*). RG-II acts as bridge to covalently cross-link two chains of HG in the cell walls of dicots and predominantly exists as a dimer

(*30*) that makes up ~5% of the weight of buffer-soluble grape mesocarp polysaccharides (*16*). The RG-II-HG network supports and stabilizes the cellulose microfibrils network that appears to be non-covalently bound to xyloglucan (*39*). Our quantitative data for the structural elements of pectin in the grape and apple skins are in full agreement with the recognition that HG typically accounts for about 80% by weight of the pectin (*11*), whereas RG-I and RG-II together account for about 10% by weight of total grape skin material (*11*).

Conclusions. The data obtained demonstrated that it was possible to quantitatively predict the abundance of structural polysaccharide units in fruit skins from monosaccharide profiles obtained by acid hydrolysis. For both the grape and apple skin samples, including three different wine grape cultivars and two different apple cultivars, the skin cell wall polysaccharides matrix appeared to be mainly made up of pectins presented mainly by homogalacturonans, rhamnogalacturonan I, and rhamnogalacturonan II, then xyloglucan and cellulose. The hydrolysis method, and thus in turn the resulting monosaccharide profiles, strongly affected the prediction and the reconstruction of the cell wall polysaccharides, notably with respect to the estimated levels of the different pectin structures versus cellulose. At this point in time no methods are available to examine the intact plant cell wall polysaccharides present in the true cell wall matrix, and the compositional analyses available thus only provide a picture of the building blocks—and the analytical, quantitative determination of the cell wall polysaccharides in fibrous, lignified plant materials such as fruit skins is complicated, if not impossible, to obtain with the currently available methods. The presented iterative calculation method may provide an important starting point for obtaining a better quantitative understanding of the polysaccharide structures in fibrous cell wall matrices. This may be useful for rationally designing enzymatic treatments, that is, selecting the relevant enzymes and estimating the expected yields, for obtaining maximal cell wall degradation in prepress treatments in fruit juice and wine processes. In addition, the quantitative prediction approach may provide a primary tool for designing efficient valorization of the skins in fruit juice and wine press residues or in novel processes such as enzymatic peeling of fruits.

ABBREVIATIONS USED

GD, Golden Delicious apple; HCl, hydrochloric acid; HG, homogalacturonan; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; RD, Red Delicious apple; RG-I, rhamnogalacturonan I; RG-II, rhamnogalacturonan II; TFA, trifluoroacetic acid.

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