Effects of Drying Pretreatments on the Cell Wall Composition of Grape Tissues

A. Femenia, E. S. Sánchez, S. Simal, and C. Rosselló*

Enginyeria Química, Departament de Química, Universitat de les Illes Balears, Ctra. Valldemossa Km 7.5, 07071 Palma de Mallorca, Spain

Pretreatments by consecutive dipping in NaOH, citric acid, and $K_2S_2O_5$ solutions help to increase the drying rate of grapes and to reduce the darkening due to enzymic and nonenzymic browning during storage of raisins. However, such pretreatments have also an important effect on the cell wall composition of grape tissues. In both skin and pulp tissues the yield of cell wall material decreased substantially with processing, by 19.7 and 22.5%, respectively. Sodium hydroxide and citric acid solutions solubilized large amounts of pectic substances and xyloglucans, whereas potassium metabisulfite solution caused minor modifications to the composition of cell wall polysaccharides. Moreover, drying pretreatments promoted important changes in the amounts of Ca and Mg associated with cell wall components, which suggested possible structural rearrangements of polymers within the wall. All of these observations were in good agreement with the main results obtained after the application of a nondegradative technique such as FTIR spectroscopy to the cell wall preparations of fresh and processed tissues.

Keywords: *Raisins; drying pretreatments; dietary fiber; cell wall polysaccharides; FTIR*

INTRODUCTION

Grapes (*Vitis vinifera* L.) are popular seasonal and perishable fruits. Dehydration is a traditional method commonly used to extend the shelf life of grapes. This process leads to a product (raisins) that is consumed without prior rehydration and which possesses nutritional and organoleptic characteristics highly valued by the consumer (Simal et al., 1996a). In particular, raisins are considered an important source of dietary fiber (Valiente et al., 1995).

Before drying, different treatments are often used not only to increase the drying rates but also to preserve the physical, chemical, nutritional, and organoleptic characteristics of the dried product. Such treatments often include the use of sodium hydroxide to increase the drying rate by caustically creating fissures in the product's surfaces (Alvárez and Legues, 1986; Sharma et al., 1992), the use of weak acids to reduce the degree of nonenzymic Maillard browning by decreasing the pH (Nursten, 1986) and the use of sulfur dioxide to reduce the darkening due to both enzymic and nonenzymic browning during drying and storage (Bolin and Jackson, 1985). However, such treatments may have significant effects on the composition of the different grape tissues.

In fact, such pretreatments have an important effect on the texture of grape tissues, e.g. breakage of skin and overall tissue softening (Sharma et al., 1992), and since a major determinant of the textural properties of plant-based foods is the cell wall (Van Buren, 1979), the knowledge of changes regarding the cell walls resulting from processing may help us to understand how the quality of fiber derived from grapes is affected by such pretreatments.

Fruit processing (i.e. by applying chemical agents) can affect the original structure of the different polysaccharides within the fiber matrix. For example, modification of pectic polysaccharides caused by different processing treatments has been reported for many fruit and vegetable tissues (Van Buren et al., 1988; Wu and Chang, 1990; Andersson et al., 1994). According to Albersheim et al. (1960) and Desberg (1965), the pectic polymers can be broken down by the β-elimination reaction after pectins are heated at neutral or weakly acidic pH. This reaction is known to be catalyzed by several cations and anions (Keijbets and Pilnik, 1974; Ben Shalom et al., 1982). In addition, it has been observed that heat alone may also induce degradation by β-elimination (Thibault, 1983). The rate of the β-eliminative cleavage is related to the degree of methyl esterification of pectic polysaccharides (Sajjaanankatul et al., 1989); usually, a higher methyl content promotes a greater extent of pectin degradation. Methyl de-esterification of galacturonosyl residues may result in the formation of new pectic chains of higher molecular weight through the formation of calcium bridges (Plat et al., 1991). This type of rearrangement cannot be detected by carbohydrate analysis. However, it is possible to identify such modifications by determining the amount of mineral elements associated with cell wall components. Other types of polysaccharides such as xyloglucans can also be affected by the processing treatments (Jimenez et al., 1996).

A major problem in identifying both the importance of the fiber matrix in nutrition and the changes resulting during processing is that the use of methods developed to measure the fiber content of foods (Lee et al., 1992; Englyst et al., 1992) may result in the degradation of pectic polysaccharides due to heat treatment. Thus, it is difficult to resolve the effects due to processing from those owing to the method of analysis. Therefore, nondegradative methods are strongly recom-

* Author to whom correspondence should be addressed (fax 34 71 173426; e-mail dqucrm0@ps.uib.es).

S0021-8561(97)00502-5 CCC: $15.00
Published on Web 01/19/1998

mended, since the identified changes can be attributed only to processing and not to the method conditions. In this context, an important new feature of this paper is the application of a nondegradative technique such as FTIR spectroscopy to detect structural and compositional changes occurring in the cell walls of grapes during processing.

The main aim of this work is to determine the effect of the drying pretreatments on the cell wall composition of grape tissues by combining different techniques such as analysis of carbohydrates, determination of element minerals associated with cell wall components by plasma spectroscopy (ICP/AES), and detection of structural and compositional changes by using FTIR spectroscopy.

MATERIALS AND METHODS

Fresh grapes (Red Globe variety) obtained from a local supermarket were used as the raw material in all experiments. All analyses were done on a single lot of grapes (≈25 kg). Fruits were washed and different treatments before drying were applied (A−C); these treatments were set up according to the literature (Cañellas et al., 1993; Simal et al., 1996b) and preliminary experiments. Pretreatments A−C were performed on duplicate samples of grapes: (A) immersion in a sodium hydroxide (3 g/L) solution at 100 °C for 30 s followed by washing in distilled water at 25 °C for 5 min; (B) subsequent dipping in a sodium hydroxide (3 g/L) solution at 100 °C for 30 s and washing in distilled water at 25 °C for 5 min and in a citric acid (30 g/L) solution for 5 min at room temperature (RT); (C) subsequent dipping in a sodium hydroxide (3 g/L) solution at 100 °C for 30 s followed by washing in distilled water at 25 °C for 5 min, in a citric acid (30 g/L) solution for 5 min at room temperature (RT), and in a potassium metabisulfite (40 g/L) solution for 10 min at RT.

The skins of fresh and treated grapes were carefully hand separated from the pulp by peeling the grapes with a scalpel and immediately frozen in liquid nitrogen. The pulps were cut in halves and frozen in liquid nitrogen. Seeds were removed and discarded. Skins and pulp halves were stored at −30 °C until required.

Analytical Methods. Alcohol insoluble residues (AIRs) from grape tissues (skin and pulp) were obtained by immersing frozen sample in boiling ethanol [final concentration = 85% (v/v) aqueous] as described by Waldron and Selvendran (1990). AIRs were prepared after each treatment. Prior to further analysis, the AIR was milled using a laboratory type grain mill and passed through a 0.5 mm aperture sieve.

To measure the moisture content, the AIRs were weighed, dried overnight at 60 °C in the presence of silica gel, and reweighed.

Carbohydrate analysis was performed as in Femenia et al. (1997) for neutral sugars. Sugars were released from residues by acid hydrolysis. AIRs were dispersed in 72% H_2SO_4 for 3 h followed by dilution to 1 M and hydrolyzed at 100 °C for 2.5 h (Saeman et al., 1954). A second sample of AIR was hydrolyzed only with 1 M sulfuric acid (100 °C for 2.5 h). The cellulose content was estimated by the difference in glucose obtained by Saeman hydrolysis and this more mild hydrolysis method. Neutral sugars were derivatized as their alditol acetates and isothermally separated by GC (Selvendran et al., 1979) at 220 °C on a 3% OV225 Chromosorb WHP 100/120 mesh column. Uronic acids were colorimetrically determined, as total uronic acid (Blumenkrantz and Asboe-Hansen, 1973), using a sample hydrolyzed for 1 h at 100 °C in 1 M H_2SO_4.

The nitrogen content of AIR was measured using a Tecator Kjeltec 1035 autosampler system analyzer. Protein was estimated by multiplying the nitrogen value by 6.25.

Lignin was gravimetrically determined as Klason lignin. Samples were dispersed in 72% H_2SO_4 at RT for 3 h, then diluted to 1 M H_2SO_4, and heated to 100 °C for 2.5 h. Insoluble material was recovered by filtration (sinter 2) and washed thoroughly with hot water (90 °C) until acid free before drying at 105 °C overnight. The residue weight was recorded as Klason lignin.

Ash contents were determined by overnight heating at 550 °C (AOAC, 1980). Simultaneous determination of Ca, Mg, K, Na, Fe, P, Zn, and Cu was carried out by inductively coupled plasma atomic emission spectroscopy (ICP/AES) by means of a calibration curve (Boss and Freeden, 1989).

The occurrence of starch in the preparations was tested for by staining AIR with I_2/KI solution and examination by light microscopy.

FTIR spectra were obtained on a Bruker IFS 66 instrument, at a resolution of 3 cm^{-1}, after preparing a KBr disk containing ≈2 mg of AIR. The intensity of the single beam traversing each sample was expressed as a ratio with the intensity of the single beam of the corresponding background. Equivalent samples from different experimental runs gave the same spectra in all cases. Digital subtraction of spectra by computer was based on the work of McCann et al. (1992).

The degree of esterification (DE) of pectic substances, i.e. the percentage of total uronic acids which are esterified, was determined after the samples were reduced with sodium borohydride (10 mg/mL) in 50% ethanol overnight. In this way, the esterified, but not the de-esterified, groups were reduced. The latter were colorimetrically quantified (Lurie et al., 1994).

Statistical Analysis. Results were analyzed by means of a one-way and multifactor analysis of variance, using the LSD test with a 95% confidence interval for comparison of the test means.

RESULTS AND DISCUSSION

Tissues (skin and pulp) from deseeded grapes were separated after each pretreatment. Although the NaOH solution produced fissures throughout the skin of the grapes, it could be easily separated from the pulp after pretreatment A. Both the citric and metabisulfite solutions caused a complete discoloration of skin, probably owing to the solubilization of anthocyanins, and separation of skin was difficult after pretreatments B and C had been applied. On fresh weight basis, the pulp and skin represented ≈88 and ≈10% of the grapes, respectively. Seeds, which were discarded in this study, represented only ∼2%.

Overall Composition of AIRs. Cell wall materials (CWM) were prepared as AIRs from freeze-dried tissues. AIR prepared from fresh skin tissue accounted for 5.94% of the initial fresh material. This percentage decreased to 5.30 and 4.92% after treatments A and B, respectively, and increased to 5.05% after treatment C. In the case of fresh grapes pulp, AIR accounted for 1.01% of the fresh material and the AIR yields after each pretreatment followed the same trend as for skin tissues, decreasing after treatments A and B to 0.88 and 0.83%, respectively, and then slightly increasing after treatment C to 0.89%. All of these results were statistically significant ($p < 0.05$).

The moisture, protein, total carbohydrate, lignin, and ash contents of the AIRs corresponding to fresh and processed grapes tissues are shown in Table 1. To allow a better comparison, all results were referred to the amount of material recovered in the AIRs prepared from fresh tissues, for either skin or pulp.

The moisture values were lower for skin (4.0−5.0%) than for pulp tissues (8.1−8.8%); however, no significant differences were found ($p > 0.05$) between the moisture contents of fresh and processed samples when the same tissues were compared. Protein content exhibited a similar feature, being higher in AIRs prepared from grape pulp than those prepared from skin. Moreover,

Table 1. Overall Composition of AIRs of Grape Tissues after Each Treatment (Micrograms per Milligram of AIR of Fresh Sample)

	grape skins				grape pulp			
		pretreatment				pretreatment		
fraction	fresh	A	B	C	fresh	A	B	C
moisture	49.7 ± 2.1	45.3 ± 3.0	40.2 ± 2.7	43.4 ± 2.3	87.5 ± 3.2	81.4 ± 2.4	83.6 ± 3.6	86.5 ± 3.1
protein	68.4 ± 2.0	65.6 ± 2.1	61.3 ± 1.7	60.9 ± 1.9	128.2 ± 3.4	114.1 ± 2.3	110.5 ± 2.6	109.8 ± 2.9
carbohydrate	686.2 ± 12.2	615.1 ± 10.7	571.0 ± 11.9	551.0 ± 10.1	595.4 ± 11.3	487.0 ± 7.8	469.3 ± 9.0	461.6 ± 8.1
lignin	64.5 ± 4.1	57.8 ± 3.8	43.4 ± 3.4	90.9 ± 5.1	tr[a]	tr	2.0 ± 0.4	7.3 ± 0.9
ash	42.7 ± 6.4	30.4 ± 4.0	30.3 ± 6.4	28.5 ± 2.3	167.3 ± 2.7	174.1 ± 5.6	134.6 ± 1.9	199.6 ± 0.5
total	911.5 ± 21.5	814.2 ± 24.3	756.2 ± 19.7	774.7 ± 21.1	978.4 ± 18.9	856.6 ± 19.1	800.0 ± 17.7	864.8 ± 15.8

[a] Trace.

Table 2. Carbohydrate Analysis of Grape Tissues after Each Treatment (Micrograms of Sugar per Milligram of AIR of Fresh Sample)

	grape skins				grape pulp			
		pretreatment				pretreatment		
sugar	fresh	A	B	C	fresh	A	B	C
rhamnose	13.4 ± 0.4	13.1 ± 0.2	12.8 ± 0.4	11.8 ± 0.1	15.0 ± 0.2	11.5 ± 0.1	8.4 ± 0.1	8.1 ± 0.2
fucose	7.3 ± 0.1	4.2 ± 0.0	3.0 ± 0.0	2.9 ± 0.1	6.0 ± 0.1	4.3 ± 0.0	3.2 ± 0.0	3.0 ± 0.1
arabinose	59.4 ± 1.2	55.6 ± 1.0	49.2 ± 0.7	44.0 ± 0.4	52.6 ± 0.3	43.8 ± 0.4	42.3 ± 0.0	40.9 ± 0.2
xylose	31.4 ± 0.5	25.2 ± 0.6	20.8 ± 0.4	20.5 ± 0.2	38.2 ± 0.2	24.4 ± 0.1	24.3 ± 0.2	24.0 ± 0.1
mannose	32.4 ± 1.1	27.3 ± 0.7	23.9 ± 0.4	21.8 ± 0.8	9.5 ± 0.1	6.9 ± 0.0	4.8 ± 0.1	4.5 ± 0.1
galactose	34.2 ± 0.9	31.6 ± 0.7	26.4 ± 0.5	21.3 ± 0.6	48.7 ± 0.3	32.6 ± 0.2	30.9 ± 0.4	28.7 ± 0.2
glucose	280.7 ± 3.4	271.1 ± 2.5	251.5 ± 4.1	250.2 ± 3.9	217.2 ± 2.3	175.2 ± 2.7	173.3 ± 3.2	170.4 ± 3.9
(Glc 1M)	(26.3 ± 0.7)	(21.4 ± 0.8)	(16.8 ± 0.2)	(16.5 ± 0.5)	(34.2 ± 0.6)	(26.8 ± 0.1)	(25.9 ± 0.5)	(24.7 ± 0.3)
uronics	227.4 ± 6.7	187.0 ± 5.1	183.4 ± 4.9	178.5 ± 4.8	208.2 ± 3.5	188.3 ± 5.1	182.1 ± 6.4	182.0 ± 5.1
total	686.2 ± 12.2	615.1 ± 10.7	571.0 ± 11.9	551.0 ± 10.1	595.4 ± 11.3	487.0 ± 7.8	469.3 ± 9.0	461.6 ± 8.1
DE[a] (%)	63.2 ± 3.1	41.7 ± 0.9	30.6 ± 2.1	29.7 ± 1.8	37.5 ± 3.2	34.3 ± 4.3	33.2 ± 2.7	32.9 ± 3.1

[a] Degree of esterification.

a slight decrease in protein content was observed after each drying pretreatment step in both tissues. Klason lignin was mainly detected in skin tissue; however, different compounds are likely to be associated with this residue. According to Rebolé et al. (1989) components such as tannins (condensed tannins) and proteins (as protein−tannin complexes or as Maillard products) are in the Klason lignin fraction. The presence of tannins, as proanthocyanidins, in the CWM of grape berry skins has been reported by Lecas and Brillouet (1994), which could explain the increase in Klason lignin after treatment C. Ash represented an important fraction of AIR from grape pulp; this value was significantly lower ($p < 0.05$) for skin tissues. Carbohydrate was the most important fraction for all AIRs in both tissues, accounting for ∼60 and ∼69% of the weight of AIR in fresh pulp and skin tissues, respectively. Each pretreatment removed wall carbohydrate to a greater extent than any other material isolated within the AIRs. Thus, the yield of CWM decreased substantially with processing in both tissues. Approximately 19.7 and 22.5% of nonstarch polysaccharides (NSP) from skin and pulp, respectively, were solubilized during drying pretreatments. This represented ∼21.3% of the total NSP present in the grapes.

Light microscopic examination of the preparations after treatment with I_2/KI showed the absence of starch. Therefore, from the obtained amounts of carbohydrate, it can be inferred that the dietary fiber content of grapes was 0.92 g of NSP/100 g of fresh weight. Although skin represented only ∼10% of the grapes, it contained ≈43% of total NSP present in the fruit.

Carbohydrate Composition of AIRs. Hydrolysis of AIRs was performed using 1 M H_2SO_4 and by the Saeman method (Saeman et al., 1954). The former hydrolyzes the bulk of the neutral sugars from noncellulosic polysaccharides of the cell wall, whereas the latter also hydrolyzes cellulose. The values given in Table 2 correspond to the means of duplicate determinations, and the variation between duplicates was <3%. In a previous work (Selvendran et al., 1979) it has been shown that the standard deviation corresponding to quadruplicate analysis is of the same order. For fresh and processed preparations, no significant differences in sugar values were observed between either type of hydrolysis except in the case of glucose.

The results in Table 2 indicated that pectic polysaccharides and cellulose were the main types of polysaccharide constituents of the CWM isolated from grape tissues. The presence of pectic polysaccharides was inferred from the relatively large amounts of uronic acids, arabinose, galactose, and rhamnose. Rhamnose, although present in small amounts, is usually diagnostic of pectins (Waldron and Selvendran, 1990). The occurrence of cellulose was inferred from the fact that the bulk of glucose could be released only after Saeman hydrolysis.

Fresh skin and pulp tissues showed a similar composition, although skin contained a larger amount of cellulosic glucose. In both tissue types, the small but significant presence of xylosyl, fucosyl, and noncellulosic glucose residues was indicative of the occurrence of hemicellulosic polysaccharides such as xyloglucans. In addition, skin tissue contained higher amounts of mannosyl residues (mannans) than pulp tissue.

Drying pretreatments mainly solubilized pectic polysaccharides. In skin tissues, pectins accounted for ∼60% of the total polysaccharides removed during processing, while in pulp tissues pectins represented ∼50%. In both tissues there was also a significant decrease in the amounts of xylose, fucose, and noncellulosic glucose, suggesting that xyloglucans were also

Table 3. Mineral Elements Associated with AIR Preparations (Micrograms per Milligram of AIR)

mineral element	grape skins				grape pulp			
		pretreatment				pretreatment		
	fresh	A	B	C	fresh	A	B	C
Ca	11.19 ± 0.35	6.57 ± 0.35	8.76 ± 0.42	14.19 ± 0.43	18.96 ± 0.54	16.47 ± 0.21	10.74 ± 0.56	17.25 ± 0.76
Mg	nd[a]	nd	nd	nd	11.46 ± 0.21	6.75 ± 0.19	2.10 ± 0.23	9.48 ± 0.21
Na	5.70 ± 0.11	2.31 ± 0.23	6.54 ± 0.21	3.93 ± 0.21	15.93 ± 0.35	16.86 ± 0.11	13.53 ± 0.21	21.60 ± 0.23
K	8.25 ± 0.09	nd	nd	nd	94.38 ± 2.21	106.38 ± 1.78	55.14 ± 0.76	181.26 ± 1.27
P	1.47 ± 0.23	0.87 ± 0.32	1.26 ± 0.08	2.01 ± 0.06	3.96 ± 0.21	6.72 ± 0.21	2.40 ± 0.10	6.78 ± 0.08
Fe	0.66 ± 0.08	0.33 ± 0.02	0.81 ± 0.03	0.57 ± 0.04	1.59 ± 0.08	1.38 ± 0.08	0.93 ± 0.06	2.16 ± 0.11
Cu	0.21 ± 0.02	0.09 ± 0.00	0.21 ± 0.02	0.18 ± 0.02	0.33 ± 0.04	0.33 ± 0.02	0.27 ± 0.04	0.54 ± 0.02
Zn	0.42 ± 0.06	0.06 ± 0.00	0.12 ± 0.01	0.42 ± 0.05	0.60 ± 0.03	0.48 ± 0.04	0.30 ± 0.02	0.51 ± 0.06
total	28.49 ± 1.26	11.15 ± 0.78	18.47 ± 0.97	22.11 ± 0.77	150.88 ± 2.87	158.01 ± 2.54	87.39 ± 1.34	242.32 ± 2.13

[a] Not detected.

affected by processing. In particular, these types of polysaccharides accounted for 35 and 40% of the total carbohydrate solubilized during processing in skin and pulp tissues, respectively. Small amounts of cellulose and mannose-containing polysaccharides were also extracted by drying pretreatments in both tissues.

From a comparison of treatments A−C, it was inferred that most of the carbohydrate material was solubilized by the NaOH solution. In skin tissues, ≈53% of the total carbohydrate removed was solubilized by the former solution and up to 81% in the case of grape pulp. Pectic polysaccharides and hemicellulosic xyloglucans comprised most of the material extracted from both tissues by the NaOH solution. The citric acid solution solubilized a significant amount of carbohydrate in skin tissues (∼33% of total polysaccharides extracted), whereas the amount of carbohydrate removed from grape pulp was appreciably lower (∼13% of total). In skin tissues, the carbohydrate solubilized was composed of a mixture of pectic polysaccharides and xyloglucans, while in pulp tissues the carbohydrate removed was mainly formed from pectins. Minor amounts of pectic and hemicellulosic polysaccharides were solubilized by pretreatment with potassium metabisulfite in both tissues.

Moreover, the NaOH solution also caused a major decrease in the DE of pectic substances, especially in the case of skin tissues (Table 2). This modification may result in structural modifications within the fiber matrix, which cannot be detected by simple carbohydrate analysis.

Determination of Mineral Elements Associated with AIR Preparations. The amounts of Ca, Mg, Na, K, P, Fe, Cu, and Zn detected in the AIRs of fresh and processed samples are shown in Table 3. A significant decrease in the amounts of calcium associated with the AIR of skin and pulp can be observed after treatments A and B. This could be attributed to a solubilization of pectic polysaccharides since galacturonic acid units have the capacity to bind certain cations such as calcium or magnesium (Alonso et al., 1995). However the increase in calcium after treatment C suggested the possible formation of new calcium bridges between galacturonic acid units belonging to adjacent pectic chains, which could help the insolubilization of pectic material and inhibit its degradation by polygalacturonase (Burns and Pressey, 1987) and, in turn, increase the shelf life of dehydrated grapes. The former facts could be related to the minor effect that treatment with metabisulfite had on the carbohydrate composition of both grape tissues.

Treatment B lowered the pH of grape tissues. This may promote the cation exchange capacity of pectic polysaccharides, exchanging polyvalent metal ions such as K, Na, Ca, and Mg and other multivalent cations for hydrogen (Torre et al., 1991). The former effect could explain the overall decrease in the amounts of cations detected in pulp tissues after treatment B. However, the opposite effect in the case of skin tissues was observed after the same treatment was applied.

An overall increase in the individual amounts of mineral elements was observed after treatment C. While an increase in calcium and magnesium may be related to an increase in firmness, an increase in sodium and potassium has a double effect: it improves texture by reducing the electrostatic repulsion of acidic groups, but it has the opposite effect on texture by competing with calcium (Van Buren, 1979).

Although other elements such as P, Fe, Cu, and Zn were present in very small amounts, they may have an important influence on the textural properties derived from cell wall carbohydrates; for example, complexes formed by Fe^{3+} with three carboxylic groups have been identified at low pH by Deiana et al. (1989) after studying the interactions of Fe^{2+} and Fe^{3+} with D-galacturonic acid.

It seems clear that although the overall carbohydrate compositions of skin and pulp tissues were similar, the compositions of mineral elements associated with the AIRs are fairly different.

Determination of Chemical and Structural Changes Using FTIR Spectroscopy. Although the carbohydrate compositions of fresh pulp and skin tissues were rather similar, their FTIR spectra (Figures 1A and 2A) were different. Nevertheless, both samples have peaks in common at 1015, 1070, and 1105 cm^{-1} in the carbohydrate region. These are characteristic of pectins (McCann et al., 1992). An ester band at 1740 cm^{-1} is evident in both samples, although for grape pulp the peak seems to contain a second ester band at 1725 cm^{-1}. This could simply reflect the different local molecular environment of this bond, but it is also possible that a different ester is formed in the case of pulp tissues, since phenolic esters absorb at 1720 cm^{-1} while saturated esters absorb at 1740 cm^{-1}. The fact that the carboxylic acid stretch at 1414 cm^{-1} is more prominent in pulp tissues indicates the occurrence of a much higher proportion of unesterified pectins in these walls, which agrees with the determined values of the DE of pectins corresponding to both tissues (Table 2). In both spectra, the absorption between 1550 and 1650 cm^{-1} may be attributed to amide-stretching bands of contaminating protein in the AIRs. In the spectrum of skin tissues, the peaks observed between 1500 and 1600 cm^{-1} suggest the presence of coprecipitated proanthocyanidins in the AIR.

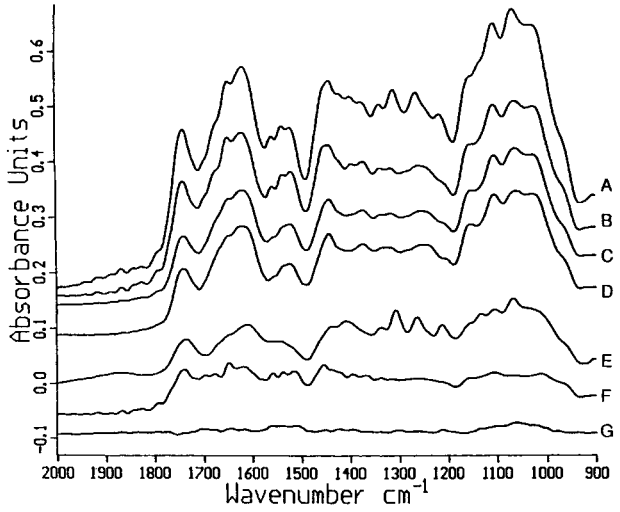

Figure 1. FTIR spectra of fresh grape skin (A), after treatment A (B), after treatment B (C), and after treatment C (D) and digital subtraction spectra of successive treatment steps (E) spectra of fresh skin minus spectra of treatment A (F), treatment A minus treatment B (G), and treatment B minus treatment C.

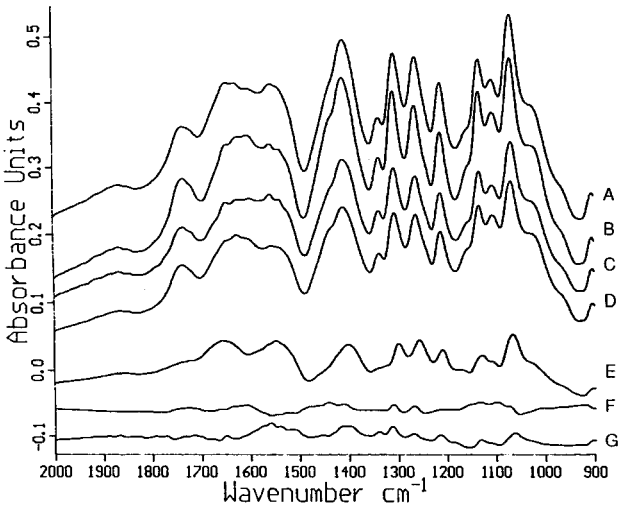

Figure 2. FTIR spectra of fresh grape pulp (A), after treatment A (B), after treatment B (C), and after treatment C (D) and digital subtraction spectra of successive treatment steps (E) spectra of fresh skin minus spectra of treatment A (F), treatment A minus treatment B (G), and treatment B minus treatment C.

Digital subtraction spectra generated by subtracting the spectrum of the remaining material after each pretreatment from the spectrum corresponding either to the fresh (Figures 1E and 2E) or to the previous pretreatment (Figures 1F,G and 2F,G) showed, in both tissues, changes owed to the caustic treatment (Figures 1E and 2E) and, to a lesser extent, by the citric acid treatment (Figures 1F and 2F). In particular, the presence of the band at 1740 cm^{-1} in Figures 1E and 1F is indicative of the decrease in the DE of pectins in skin tissues undergone after the NaOH and citric acid treatments. The $K_2S_2O_5$ treatment caused a minor disruption to cell wall components (Figures 1G and 2G), which is in agreement with the main results obtained by carbohydrate analysis of AIR preparations.

Conclusions. These results show how the combined application of three different techniques, such as carbohydrate analysis, determination of mineral elements by ICP/AES, and FTIR spectroscopy of isolated CWM (as AIRs), can provide useful information about the main compositional and structural changes that cell wall components of plant-based food products undergo during processing.

ACKNOWLEDGMENT

We thank M. Forcén for suggestions on the English syntax of the manuscript.

LITERATURE CITED

Albersheim, P.; Neukom, H.; Deuel, H. Splitting of pectin chain molecules in neutral solution. *Arch. Biochem. Biophys.* **1960**, *90*, 46−51.

Alonso, J.; Rodríguez, T.; Canet, W. J. Effect of calcium pretreatments on the texture of frozen cherries. Role of pectinesterase in the changes in the pectic materials. *J. Agric. Food Chem.* **1995**, *43*, 1011−1016.

Alvárez, P. I.; Legues, P. A. A semi-theoretical model for the drying of Thompson seedless grapes. *Drying Technol.* **1986**, *4* (1), 1−17.

Andersson, A.; Gekas, V.; Lind, I.; Oliveira, F.; Oste, R. Effect of preheating on potato texture. *Crit. Rev. Food Sci. Nutr.* **1994**, *34* (3), 229−251.

AOAC. *Official Methods of Analysis*; Association of Official Analytical Chemists: Arlington, VA, 1990.

Ben-Shalom, N.; Shomer, I.; Pinto, R.; Kanner, J. Optimum conditions for determining depolymerization of pectic substances with the Sumner reagent. *Food Chem.* **1982**, *9*, 205−212.

Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.

Bolin, H. R.; Jackson, R. Factors affecting sulfur dioxide binding in dried apples and apricots. *J. Food Process. Preserv.* **1985**, *9*, 25−34.

Boss, C. B.; Freeden, K. J. *Concepts, Instrumentation and Techniques in ICP/AES*; Perkin-Elmer Corp.: Norwalk, CT, 1989; pp 15−57.

Burns, J. K.; Pressey, R. Ca^{2+} in cell walls of ripening tomato and peach. *J. Am. Soc. Hortic. Sci.* **1987**, *112* (5), 783−787.

Cañellas, J.; Rosselló, C.; Simal, S.; Soler, L.; Mulet, A. Storage conditions affect quality of raisins. *J. Food Sci.* **1993**, *58* (4), 805−809.

Deiana, S.; Gessa, C.; Solinas, V.; Piu, P.; Seeber, R. Analytical study of the interactions of D-galacturonic acid with iron(III) and iron(II) in solution and with iron(III)-bentonite. *Anal. Chim. Acta* **1989**, *226*, 315−324.

Desberg, J. J. *Pectic Substances in Fresh and Preserved Fruits and Vegetables*; IBVT Communication 25; Institute of Research on Storage and Processing of Horticultural Produce: Wageningen, The Netherlands, 1965; pp 43−47.

Englyst, H. N.; Quigley, M. E.; Hudson, G. J.; Cummings, J. H. Determination of dietary fiber as non-starch polysaccharides by gas-liquid chromatography. *Analyst* **1992**, *117*, 1707−1714.

Femenia, A.; Lefebvre, A. C.; Thebaudin, J. Y.; Robertson, J. A.; Bourgeois, C. M. Defined vegetable fibers as supplements in food systems: effects on physical and organoleptic properties. *J. Food Sci.* **1997**, *62*, 635−639.

Jiménez Araujo, A.; Guillén, R.; Sánchez, C.; Fernández-Bolaños, J.; Heredia, A. Molecular weight and ionic characteristics of olive cell wall polysaccharides during processing. *J. Agric. Food Chem.* **1996**, *44*, 913−918.

Keijbets, M. J. A.; Pilnik, W. β-elimination of pectin in the presence of anions and cations. *Carbohydr. Res.* **1974**, *33*, 359−362.

Lecas, M.; Brillouet, J. M. Cell wall composition of grape berry skins. *Phytochemistry* **1994**, *35* (5), 1241−1243.

Lee, S. C.; Prosky, L.; DeVries, J. W. Determination of total, soluble, and insoluble dietary fiber in foods—enzymatic-gravimetric method, MES-TRIS buffer: Collaborative study. *J. AOAC Int.* **1992**, *75* (3), 395−416.

Lurie, S.; Levin, A.; Greve, L. C.; Labavitch, J. M. Pectic polymer changes in nectarines during normal and abnormal ripening. *Phytochemistry* **1994**, *36*, 11−17.

McCann, M. C.; Hammouri, M.; Wilson, R.; Belton, P.; Roberts, K. Fourier transform infrared microspectroscopy is new way to look at plant cell walls. *Plant Physiol.* **1992**, *100*, 1940−1947.

Nursten, H. E. Maillard browning reactions in dried foods. In *Concentration and Drying of Fruits*; McCarthy, D., Ed.; Elsevier Applied Science: New York, 1986; pp 53−68.

Plat, D.; Ben-Shalom, N.; Levi, A. Changes in pectic substances in carrots during dehydration with and without blanching. *Food Chem*. **1991**, *39*, 1−12.

Rebolé, A.; Alvira, P.; González, G. Variations of chemical composition data of agricultural and forest fibrous by-products as determined by the two detergent systems of analysis. *J. Sci. Food Agric.* **1989**, *48*, 141−153.

Saeman, J. F.; Moore, W. E.; Mitchell, R. L.; Millett, M. A. Techniques for the determination of pulp constituents by quantitative paper chromatography. *TAPPI* **1954**, *34*, 336−365.

Sajjaanantakul, T.; Van Buren, J. P.; Downing, D. L. Effect of methyl ester content on heat degradation of chelator-soluble carrot pectin. *J. Food Sci.* **1989**, *54*, 1272−1277.

Selvendran, R. R.; March, J. F.; Ring, S. G. Determination of aldoses and uronic acid content of vegetable fiber. *Anal. Biochem*. **1979**, *96*, 282−292.

Sharma, P. C.; Sharma, K. D.; Prashar, R. S. Prospects of raisins production in tribal areas of Himachal Pradesh. *Indian Food Packer* **1992**, *46* (3), 16−19.

Simal, S.; Mulet, A.; Catalá, P. J.; Cañellas, J.; Rosselló, C. Moving boundary model for simulating moisture movement in grapes. *J. Food Sci.* **1996a**, *61* (1), 157−160.

Simal, S.; Rosselló, C.; Sánchez, S.; Cañellas, J. Quality of raisins treated and stored under different conditions. *J. Agric. Food Chem*. **1996b**, *44*, 3297−3302.

Thibault, J. F. Enzymatic degradation and β-elimination of the pectic substances in cherry fruits. *Phytochemistry* **1983**, *22*, 1567−1571.

Torre, M.; Rodriguez, A. R.; Saura-Calixto, F. Effects of dietary fiber and phytic acid on mineral availability. *Crit. Rev. Food Sci. Nutr.* **1991**, *1* (1), 1−22.

Valiente, C.; Arrigoni, E.; Estebán, R. M.; Amado, R. Grape pomace as a potential food fiber. *J. Food Sci.* **1995**, *60* (4), 818−820.

Van Buren, J. P. The chemistry of texture in fruits and vegetables. *J. Text. Stud.* **1979**, *10*, 1−23.

Van Buren, J. P.; Kean, W. P.; Wilkinson, M. J. Influence of salts and pH on the firmness of cooked snap beans in relation to the properties of pectin. *J. Text. Stud.* **1988**, *19*, 15−19.

Waldron, K. W.; Selvendran, R. R. Composition of cell walls from different asparagus (*Asparagus officinalis*) tissues. *Physiol. Plant.* **1990**, *80*, 568−575.

Wu, A.; Chang, W. H. Influence of precooking on the firmness and pectic substances of three stem vegetables. *Int. J. Food Sci. Technol.* **1990**, *25*, 558−563.

Received for review June 16, 1997. Revised manuscript received October 8, 1997. Accepted October 10, 1997.[⊗] This research work was supported by the Comisión Interministerial de Ciencia y Tecnología (Project ALI94-0565-CO3).

JF9705025

[⊗] Abstract published in *Advance ACS Abstracts*, December 15, 1997.