Effects of Supercritical Carbon Dioxide (SC-CO_2) Oil Extraction on the Cell Wall Composition of Almond Fruits

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Extraction of oil from almond fruits using supercritical carbon dioxide (SC-CO_2) was carried out at 50 °C and 330 bar on three sets of almonds: raw almond seeds, raw almond kernels, and toasted almond seeds. Three different oil extraction percentages were applied on each set ranging from ∼15 to 16%, from ∼27 to 33%, and from ∼49 to 64%. Although no major changes were detected in the fatty acid composition between fresh and partially defatted samples, carbohydrate analysis of partially defatted materials revealed important changes in cell wall polysaccharides from almond tissues. Thus, at low extraction percentages (up to ∼33%), pectic polysaccharides and hemicellulosic xyloglucans were the main type of polymers affected, suggesting the modification of the cell wall matrix, although without breakage of the walls. Then, as supercritical fluid extraction (SCFE) continues and higher extraction rates are achieved (up to ∼64%), a major disruption of the cell wall occurred as indicated by the losses of all major types of cell wall polysaccharides, including cellulose. These results suggest that, under the conditions used for oil extraction using SC-CO_2, fatty acid chains are able to exit the cells through nonbroken walls; the modification of the pectin−hemicellulose network might have increased the porosity of the wall. However, as high pressure is being applied, there is a progressive breakage of the cell walls allowing the free transfer of the fatty acid chains from inside the cells. These findings might contribute to providing the basis for the optimization of SCFE procedures based on plant food sources.

Keywords: *Supercritical fluid; carbon dioxide; almond fruit; cell walls; pectic polysaccharides*

INTRODUCTION

Supercritical fluid extraction (SCFE) is a new and powerful technique in separation processes. Several investigations have been made in recent years on probable industrial applications of SCFE, which offer some advantages over conventional methods, such as separation by extractive solvents or by distillation, especially in the areas of the food, pharmaceutical, chemical, and oil industries (*1*−*4*). Extraction processes with supercritical fluids involve an appreciable number of variables that should be handled simultaneously for the design of the operating conditions (*5*). Properties such as low viscosity, high difussivity, and low surface tension may enhance the solute mass transfer from inside a solid matrix. In general, this solid matrix is formed by a cell wall when SCFE processes are performed on plant-based products.

Supercritical carbon dioxide (SC-CO_2) has been the most frequently used extractant in the food and pharmaceutical industries (*6*), being nontoxic, nonflammable, inexpensive, and easily separable from the extracts.

Much effort has gone into qualitative and semiquantitative testing of the solubility of biomaterials, such as essential or volatile oils, in SC-CO_2 (*7*, *8*), whereas little attention has been focused on other fundamental aspects of SCFE processes. For instance, a key aspect when extraction is based on plant food sources is the understanding of the main effects that SCFE might produce on the cell wall matrix.

The models of Goto et al. (*9*), Sovová (*10*), and Sovová et al. (*11*) based on a physical hypothesis to explain the SC-CO_2 oil extraction procedure from different vegetable sources, represented the first attempts to introduce a description of the structure of the vegetable matter by a mathematical model. However, it required several parameters related to physicochemical properties of the vegetables, which were difficult to measured or calculate and, therefore, were adjusted by the authors to fit the experimental results.

In the case of almond oil extraction by SC-CO_2, an extraction model based on "broken" and "intact" cells has been proposed (*12*). It is clear that, from a biochemical point of view, the hypothesis of "broken" or "intact" cell walls used in the latter model could at least be considered as too simplistic. In fact, the inherent complexity of the cell wall matrix is that it varies enormously in composition and structure not only from plant to plant but also among the different tissue types which form plant organs (*13*, *14*). Knowledge of specific modifications in the different cell wall components promoted by SCFE procedures might enhance our understanding of how mass transfer processes occur, providing the basis on which to develop more accurate techniques for correlation and prediction that could be used with confidence in future investigations.

Within this context, this paper aims to evaluate the main effects that SCFE causes on the cell wall polysac-

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Table 1. Overall Composition of Fresh, Toasted, and Partially Defatted Almond Samples (Results Are Expressed as Grams per 100 g of Almond Sample)

	fresh seed	% extracted oil			fresh kernel	% extracted oil			toasted seed	% extracted oil		
		15.5	34.8	64.3		15.8	27.0	57.0		15.9	33.9	48.8
moisture	5.5 ± 0.2	5.1 ± 0.1	5.0 ± 0.2	5.0 ± 0.3	4.0 ± 0.2	4.2 ± 0.2	4.3 ± 0.2	4.0 ± 0.2	1.2 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	1.2 ± 0.0
fat	49.9 ± 1.4	44.7 ± 1.0	37.1 ± 1.1	25.2 ± 1.2	59.8 ± 2.1	55.6 ± 1.1	52.1 ± 1.4	39.0 ± 1.4	57.9 ± 1.4	53.6 ± 1.0	47.7 ± 1.3	41.3 ± 1.1
protein	22.2 ± 0.7	25.1 ± 0.8	28.1 ± 0.7	31.4 ± 1.4	20.2 ± 1.0	22.8 ± 0.9	24.3 ± 0.8	31.2 ± 0.9	20.3 ± 0.9	22.7 ± 0.9	26.2 ± 0.4	30.5 ± 0.4
fiber	12.9 ± 0.3	14.2 ± 0.4	17.1 ± 0.4	23.1 ± 0.7	6.11 ± 0.8	6.70 ± 0.7	7.21 ± 0.6	8.12 ± 0.7	10.2 ± 0.4	11.1 ± 1.6	12.3 ± 0.3	13.8 ± 0.3
ash	3.20 ± 0.1	3.51 ± 0.1	4.52 ± 0.3	6.13 ± 0.4	3.01 ± 0.3	3.41 ± 0.2	3.98 ± 0.1	4.78 ± 0.0	3.10 ± 0.3	3.65 ± 0.2	4.12 ± 0.1	4.51 ± 0.2
soluble sugars	6.20 ± 0.1	7.29 ± 0.4	8.26 ± 0.4	9.45 ± 0.8	6.92 ± 0.2	7.36 ± 0.4	7.95 ± 0.2	8.65 ± 0.1	7.24 ± 0.2	7.98 ± 0.3	8.67 ± 0.2	9.05 ± 0.2

charides from almond fruits after extraction of oil with SC-CO_2. This might help to identify key aspects that regulate the mass transfer process taking place during SCFE.

MATERIALS AND METHODS

Almonds. Almond (*Prunus amygdalus*) seeds (kernel with tegument) from a variety known as "Mallorca comuna" were used in this investigation. All almond fruits were purchased from a local retailer.

Three sets of almonds were used in this investigation. The first set comprised raw almond seeds (kernel and tegument); three different oil extraction percentages (given as the percentage of oil extracted from the total oil available) were tested on these samples, namely, 15.5, 34.8, and 64.3% of extracted lipids. The second set contained raw almond kernels; three different oil extraction percentages were also tested on these samples, namely, 15.8, 27.0, and 57.0% of oil extracted. The third set included toasted almond seeds (kernel plus tegument); these almonds were toasted at 190 °C for 30 min before extraction. The percentages of oil extracted from these samples were 15.9, 33.9, and 48.8%. All samples from the three almond sets were milled and passed through a 1 mm aperture sieve before SC-CO_2 extraction. Almond fruits, raw and toasted, were used as starting materials because these are the usual ways in which almonds are used for consumption.

SC-CO_2 Extraction. A TEX-5000 supercritical fluid extractor (AINIA, València, Spain) was used in the present study. The extraction vessel capacity was 6 L, and the amounts of almond introduced within the extraction vessel were ~1.5 kg (for raw seeds and kernels) and ~2.0 kg (for toasted almond seeds). The extraction pressure and temperature were 330 bar and 50 °C, respectively, in all tests performed. The solvent used was CO_2 at 99% purity (Abelló Oxígeno Linde, València, Spain).

For the first set containing raw almond seeds the amounts of CO_2 recirculated were 20 kg (15.5% extraction), 40 kg (34.8% extraction), and 90 kg (64.3% extraction); for the second set of almond kernels the amounts of CO_2 recirculated were 20 kg (15.8% extraction), 40 kg (27% extraction), and 110 kg (57% extraction). A flow rate of 20 kg/h of CO_2 was used in these experiences. For the third set composed of roasted almond seeds, the amounts of CO_2 recirculated were higher: 140 kg (15.9% extraction), 370 kg (33.9% extraction), and 460 kg (48.8% extraction). Moreover, in these latter experiments, a flow rate of 40 kg/h of CO_2 was used.

Alcohol Insoluble Residues (AIRs). Before preparation of the AIRs, lipids from all samples, fresh, toasted, and partially defatted almonds, were completely removed using a Soxhlet apparatus. Thus, AIRs could be obtained by immersing the deffated samples in boiling ethanol [final concentration = 85% (v/v)] as described by Waldron and Selvendran (*15*). Prior to further analysis, the AIR was milled using a laboratory type grain mill and passed through a 0.5 mm aperture sieve. All samples were free of starch and were used in subsequent analyses.

Analytical Methods. Moisture, lipids, soluble sugars, protein, lignin, and ash analyses were performed in triplicate. Average and standard deviation values are shown in Table 1.

Moisture. Moisture content was measured by drying samples overnight at 60 °C in the presence of silica gel and reweighing.

Lipids. Total content of lipids of fresh, toasted, and partially defatted samples was determined gravimetrically by extraction with diethyl ether using a Soxhlet apparatus (*16*).

Soluble Sugars. Soluble sugars were determined according to the method of Wilson et al. (*17*) based on the sum of the individual sugar contents obtained by HPLC.

Protein. The nitrogen content of samples was measured according to the method of Pearson (*18*) using a Tecator Kjeltec autosampler system 1035 analyzer. Protein content was estimated by multiplying the nitrogen value by 6.25.

Lignin. Lignin was gravimetrically determined as Klason lignin. AIRs were dispersed in 72% H_2SO_4 at room temperature for 3 h and then diluted to 1 M H_2SO_4 and heated to 100 °C for 2.5 h. Insoluble material was recovered by filtration (sinter no. 2), washed thoroughly with hot water (90 °C) until acid free, and dried at 105 °C overnight. The residue weight was recorded as Klason lignin.

Ashes. Ash contents were determined gravimetrically by overnight heating at 550 °C (*19*).

Constituent Fatty Acids. The oils were saponified after 0.1 g of oil had been dissvoled in 2 mL of *n*-heptane, and 0.2 mL of an ethanolic solution of 2 N KOH was added. The solution was stirred vigorously for 15 s. The upper phase containing the methyl esters was then ready for injection. The esters were analyzed for their respective fatty acid composition by GC on a Perkin-Elmer Sigma 3B series chromatograph using an HP-INNOWax capillary column (30 m × 0.32 mm i.d.; 0.25 μm film) using an oven temperature program of 70 °C for 1 min, 10 °C/min to 190 °C and 190 °C for 60 min. The carrier gas was helium with a flow rate of 3.5 mL/min. Injector temperature was 230 °C, and detector temperature was 250 °C. Detection was made by flame ionization detector. All analyses were performed in duplicate.

Starch. The occurrence of starch in the lyophilized fractions was tested for by staining AIR with an I_2/KI solution and examining by light microscopy.

Analysis of Carbohydrate Composition. Carbohydrate analysis was performed according to the method of Waldron and Selvendran (*15*) for neutral sugars. Sugars were released from residues by acidic 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 (*20*). A 1 M H_2SO_4 hydrolysis (100 °C for 2.5 h) was also included to estimate the cellulose content by difference. Neutral sugars were derivatized as their alditol acetates and isothermally separated by GC at 220 °C on a 3% OV225 Chromosorb WHP 100/120 mesh column (30 m × 0.25 mm i.d.; 0.25 μm film). Uronic acids were colorimetrically determined, as total uronic acid (*21*), using a sample hydrolyzed for 1 h at 100 °C in 1 M H_2SO_4. The values for carbohydrates given in this paper correspond to the means of duplicate determinations, and the variation between samples was $<3\%$. No significant differences in sugar values were observed between either type of hydrolysis except for the case of glucose.

Degree of Esterification (DE) of Pectic Substances. The DE of pectic polysaccharides was determined after the samples had been reduced with sodium borohydride (10 mg/mL) in 50% ethanol overnight (*22*). All DE determinations were performed in triplicate.

Mineral Elements Associated with Cell Wall Polysaccharides. Simultaneous determination of Ca, Mg, K, Na, Fe, P, and Cu was carried out by inductively coupled plasma atomic emission

spectroscopy (ICP/AES) by means of a calibration curve (*23*). Mineral element determinations were performed in duplicate.

Statistical Analysis. Results were analyzed by means of a one-way and multifactor analysis of variance, using the least significant difference (LSD) test with a 95% confidence interval for the comparison of the test means (*24*).

RESULTS AND DISCUSSION

Overall Chemical Composition of Almond Samples. Moisture, oil fraction, protein, dietary fiber, ashes, and soluble sugars were determined for almond preparations before and after oil extraction. The results expressed on a fresh weight basis are shown in Table 1. As can be observed, samples of nontreated fresh almond seeds, fresh almond kernels, and toasted seeds were analyzed to allow comparison with further analyses performed on partially defatted samples.

Lipids formed the predominant fraction in fresh almond samples, accounting for ~50% of fresh material for raw almond seed and up to 60% for almond kernels. The lower oil percentage obtained for whole seed was due to the presence of tegument, which has a reduced oil content (*25*). The toasted seeds exhibited a higher oil content than the raw seeds, owing mainly to water losses and also to the partial degradation of protein and fiber (probably water soluble pectins).

As expected, oil extraction through SC-CO_2 promoted an increase of the percentages of the remaining fractions. Thus, defatted almond samples contained large amounts of protein and dietary fiber. This latter fraction was particularly important in the case of whole almond seeds. From a nutritional point of view, the reduced fat content, together with the increased protein and dietary fiber fractions, might provide almond kernels as an interesting food source. In fact, dietary recommendations have called for a reduction of total calories received from fat with an equivalent increase in calories from complex carbohydrates (*26*).

An appreciable amount of water was extracted together with the oil fraction, which can be seen in Table 1, because percentages of water did not show any significant ($p > 0.05$) increase after fat extraction.

Fatty Acid Composition of Oil Fractions. Fatty acid composition of the extracted oils with SC-CO_2 was compared with that of oils either from fresh or toasted almonds extracted using the Soxhlet equipment. The results obtained are given in Table 2. All of the examined oils were very rich in unsaturated fatty acids (oleic and linoleic acids represented between 89 and 90% of total fatty acids), the saturated fatty acid content being relatively low. In all samples analyzed, palmitic acid was the major saturated fatty acid detected. The high concentration of linoleic acid in almond fruit makes this oil of high nutritional value, as linoleic acid is one of the three essential fatty acids. The ability of some unsaturated vegetable oils to reduce the serum cholesterol level may focus attention on the almond kernel oil due to its high unsaturated oil content.

The values obtained for fatty acid composition obtained for almond oils agree to a great extent with those reported by Saura-Calixto et al. (*25*). Moreover, apricot and peach kernel oils approximately verge on the almond oil composition (*27*).

The fatty acid composition of extracted oils exhibited minor differences in comparison to the oils from either fresh or toasted almonds. However, a small increase in the percentage of oleic acid was detected when ~65%

Table 2. Fatty Acid Composition of Almond Samples before and after SC-CO_2 Extraction (Results Are Expressed as Grams of Fatty Acid per 100 g of Total Fatty Acids)

	fresh seed	% extracted oil			fresh kernel	% extracted oil			toasted seed	% extracted oil		
		15.5	34.8	64.3		15.8	27.0	57.0		15.9	33.9	48.8
palmitic (16:0)	8.42 ± 0.21	8.46 ± 0.12	8.38 ± 0.18	7.99 ± 0.12	8.40 ± 0.19	8.40 ± 0.21	8.48 ± 0.71	7.87 ± 0.43	8.76 ± 0.51	8.80 ± 0.08	8.49 ± 0.52	8.13 ± 0.05
palmitoleic (16:1)	0.61 ± 0.04	0.63 ± 0.02	0.62 ± 0.04	0.57 ± 0.05	0.62 ± 0.03	0.63 ± 0.08	0.62 ± 0.05	0.56 ± 0.07	0.59 ± 0.02	0.59 ± 0.03	0.57 ± 0.05	0.57 ± 0.02
stearic (18:0)	1.42 ± 0.11	1.45 ± 0.09	1.51 ± 0.12	1.74 ± 0.08	1.50 ± 0.07	1.58 ± 0.11	1.58 ± 0.21	1.68 ± 0.20	1.49 ± 0.12	1.48 ± 0.06	1.35 ± 0.09	1.53 ± 0.11
oleic (18:1)	69.23 ± 1.24	69.15 ± 1.45	69.19 ± 1.52	69.87 ± 1.78	69.10 ± 2.12	69.33 ± 1.35	69.25 ± 1.56	70.31 ± 1.72	68.76 ± 1.65	68.79 ± 1.32	69.3 ± 1.21	69.91 ± 1.87
linoleic (18:2)	20.31 ± 0.82	20.32 ± 0.61	20.36 ± 0.43	20.01 ± 0.67	20.20 ± 0.52	20.16 ± 0.54	20.15 ± 0.48	19.65 ± 0.92	20.4 ± 0.32	20.37 ± 0.76	20.16 ± 0.31	19.86 ± 0.32

Table 3. Cell Wall Composition of Almond Samples before and after SC-CO_2 Extraction (Results Are Expressed as Milligrams of Sugar[a] per Gram of Fresh Non-defatted Almond)

	fresh seed	% extracted oil			fresh kernel	% extracted oil			toasted seed	% extracted oil		
		15.5	34.8	64.3		15.8	27.0	57.0		15.9	33.9	48.8
Rha	1.17 ± 0.11	1.14 ± 0.11	1.07 ± 0.09	1.02 ± 0.07	0.78 ± 0.10	0.74 ± 0.05	0.63 ± 0.09	0.63 ± 0.21	1.22 ± 0.03	1.20 ± 0.10	1.20 ± 0.15	1.12 ± 0.21
Fuc	0.75 ± 0.05	0.74 ± 0.08	0.72 ± 0.07	0.69 ± 0.05	0.53 ± 0.05	0.53 ± 0.08	0.63 ± 0.06	0.44 ± 0.03	0.83 ± 0.12	0.83 ± 0.07	0.71 ± 0.08	0.62 ± 0.11
Ara	17.38 ± 0.36	15.30 ± 0.85	14.00 ± 1.51	12.18 ± 0.42	11.93 ± 0.56	10.53 ± 0.32	8.72 ± 0.41	8.88 ± 0.15	19.79 ± 0.32	19.45 ± 0.42	18.01 ± 0.67	15.26 ± 0.45
Xyl	7.67 ± 0.24	7.57 ± 0.42	6.64 ± 0.35	6.24 ± 0.23	3.77 ± 0.08	3.77 ± 0.12	3.43 ± 0.15	3.24 ± 0.12	7.75 ± 0.10	7.64 ± 0.12	6.87 ± 0.16	6.09 ± 0.21
Man	2.09 ± 0.10	2.04 ± 0.05	1.96 ± 0.10	1.92 ± 0.09	0.88 ± 0.02	0.88 ± 0.06	0.98 ± 0.08	0.94 ± 0.08	1.89 ± 0.09	1.82 ± 0.11	1.87 ± 0.12	1.80 ± 0.14
Gal	5.56 ± 0.08	5.54 ± 0.42	5.29 ± 0.24	4.98 ± 0.13	2.85 ± 0.11	2.85 ± 0.15	2.96 ± 0.12	2.82 ± 0.05	4.97 ± 0.11	4.89 ± 0.09	3.98 ± 0.07	4.10 ± 0.05
Glc	24.52 ± 0.56	24.36 ± 1.25	23.88 ± 1.35	21.59 ± 1.12	12.48 ± 1.09	12.48 ± 0.43	12.52 ± 0.52	9.95 ± 0.32	22.80 ± 0.45	22.00 ± 0.87	21.55 ± 0.78	20.94 ± 0.89
(Glc 1 M)	(2.33 ± 0.11)	(2.19 ± 0.15)	(2.06 ± 0.12)	(1.98 ± 0.11)	(1.33 ± 0.11)	(1.26 ± 0.13)	(1.22 ± 0.12)	(1.04 ± 0.11)	(1.96 ± 0.12)	(1.89 ± 0.15)	(1.79 ± 0.12)	(1.68 ± 0.11)
UA	51.18 ± 1.35	45.52 ± 1.89	42.28 ± 1.32	41.59 ± 1.09	26.54 ± 1.01	24.47 ± 0.98	23.22 ± 1.12	21.67 ± 1.23	39.05 ± 0.78	38.10 ± 0.65	33.57 ± 1.15	32.23 ± 1.34
total	112.64	106.72	103.34	90.36	61.91	56.24	53.09	48.56	100.25	95.93	87.75	82.17
DE (%)	57.24 ± 1.87	56.21 ± 1.34	55.90 ± 1.76	56.10 ± 1.21	52.15 ± 0.98	53.15 ± 1.45	54.10 ± 2.10	50.18 ± 1.10	33.20 ± 1.10	32.10 ± 1.01	33.15 ± 1.21	29.25 ± 0.89
lignin	21.12 ± 0.87	21.05 ± 0.78	20.95 ± 1.10	20.21 ± 0.76	4.12 ± 0.32	4.01 ± 0.20	3.98 ± 0.23	3.87 ± 0.31	24.35 ± 1.10	23.89 ± 0.96	23.67 ± 0.92	23.87 ± 0.99

[a] Abbreviations: Rha, rhamnose; Fuc, fucose; Ara, arabinose; Man, mannose; Gal, galactose; Glc, glucose; UA, uronic acids; DE, degree of esterification.

Table 4. Mineral Elements Associated with AIR Preparations (Milligrams of Mineral Element per Gram of AIR)

	fresh seed	% extracted oil			fresh kernel	% extracted oil			toasted seed	% extracted oil		
		15.5	34.8	64.3		15.8	27.0	57.0		15.9	33.9	48.8
Ca	12.42 ± 0.31	10.46 ± 0.42	10.18 ± 0.28	8.59 ± 0.12	11.40 ± 0.29	10.20 ± 0.21	9.48 ± 0.51	7.97 ± 0.48	11.76 ± 0.51	10.84 ± 0.48	9.39 ± 0.42	8.43 ± 0.05
Mg	5.61 ± 0.74	4.63 ± 0.72	4.12 ± 0.94	2.57 ± 0.25	3.62 ± 0.53	3.33 ± 0.48	2.52 ± 0.55	1.56 ± 0.37	4.59 ± 0.62	3.99 ± 0.63	2.57 ± 0.35	2.59 ± 0.42
Na	1.32 ± 0.71	1.35 ± 0.89	1.51 ± 0.92	1.74 ± 0.88	1.50 ± 0.97	1.58 ± 0.91	1.58 ± 0.81	1.68 ± 0.90	0.49 ± 0.82	0.48 ± 0.66	0.35 ± 0.99	0.53 ± 0.31
K	19.13 ± 1.44	22.13 ± 1.55	22.29 ± 1.32	28.77 ± 1.28	14.12 ± 1.18	12.23 ± 1.18	11.25 ± 1.96	9.41 ± 1.46	18.96 ± 1.45	18.79 ± 1.52	24.3 ± 1.24	23.91 ± 1.57
P	1.23 ± 0.14	1.15 ± 0.05	1.19 ± 0.02	1.37 ± 0.08	1.10 ± 0.12	1.31 ± 0.05	1.27 ± 0.06	133 ± 0.12	1.36 ± 0.15	1.29 ± 0.12	1.89 ± 0.21	1.91 ± 0.27
Fe	0.31 ± 0.04	0.35 ± 0.05	0.39 ± 0.05	0.28 ± 0.03	0.26 ± 0.02	0.33 ± 0.05	0.28 ± 0.03	0.31 ± 0.02	0.36 ± 0.05	0.29 ± 0.02	0.33 ± 0.03	0.41 ± 0.02
Cu	0.25 ± 0.02	0.31 ± 0.01	0.35 ± 0.03	0.21 ± 0.07	0.20 ± 0.02	0.16 ± 0.01	0.15 ± 0.02	0.28 ± 0 02	0.41 ± 0.03	0.39 ± 0.06	0.28 ± 0.02	0.25 ± 0.02

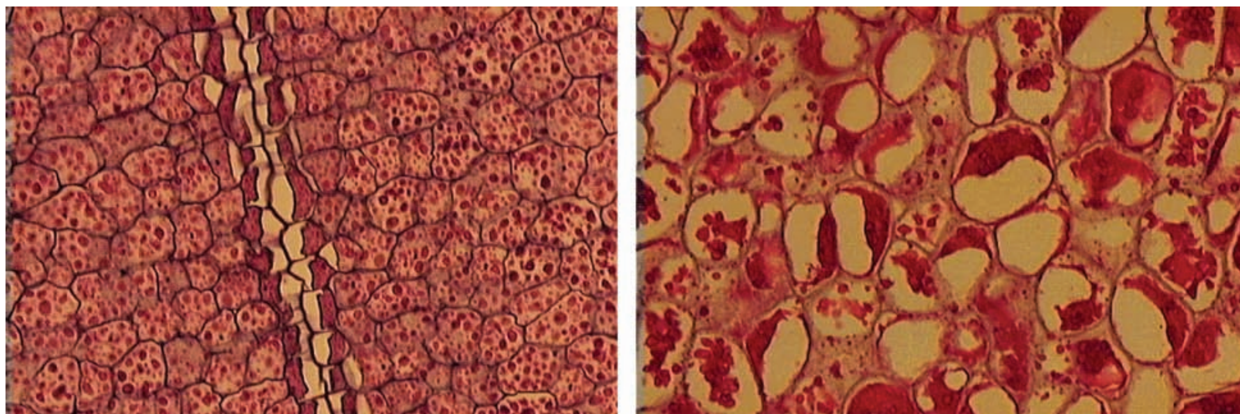

Figure 1. Light micrographs of fresh almond cells (left) and partially defatted almond cells (right) after extraction of 35% of total oil using SC-CO_2.

of oil was extracted in all three sets of samples. To a lesser extent, an increase in stearic acid was also detected for the above almond samples.

Nevertheless, it seems clear from these results that SC-CO_2 extraction promoted minor modifications of the fatty acid profile of the extracted oils.

Carbohydrate Composition. Sugars in the AIRs obtained after defatting completely all almond samples were released using two hydrolytic procedures, which helped to distinguish the sugars from noncellulosic polysaccharides and cellulose. All AIR preparations were shown to be effectively free of starch, by I_2/KI staining and from the low recovery of glucose following hydrolysis in 1 M sulfuric acid. The results for almond fruit cell wall polysaccharides from the AIRs of fresh, toasted, and partially defatted almonds are shown in Table 3.

Cell Wall Polysaccharides. The cell walls from raw almond fruit were mainly composed by pectic polysaccharides and cellulose, accounting for 65 and 21%, respectively, of the total polysaccharides, in addition to considerable amounts of xylose, indicating the occurrence of hemicelluloses (∼7−8% of total polysaccharides). The occurrence of galacturonic acid and arabinose units indicates that pectic polymers rich in arabinose neutral side chains were present in large amounts; galactose and rhamnose, which were also detected, are also characteristic of pectins, whereas the presence of cellulose could be inferred from the fact that the bulk of glucose could be released only after Saeman hydrolysis. The results shown in Table 3 for fresh almond are in broad agreement with those reported by Englyst et al. (*28*).

Toasting did not cause major modifications on the overall cell wall composition. However, the significant decrease ($p < 0.05$) detected in the DE (see Table 3) might be indicative of structural changes occurring within the cell wall matrix during exposure to high temperature (*29*). The presence of a high level of free carboxylic acid groups might contribute to the formation of new pectin chains of higher molecular weight (*30*), although the removal of water during toasting might also decrease the mobility of pectins within the wall matrix, thus hindering the latter effect.

The most relevant changes affecting the cell wall components were detected after extraction of oil with supercritical fluid. Thus, raw partially defatted almond samples (kernel and seed) with ∼15% of oil extracted exhibited significant ($p < 0.05$) decreases affecting sugars related to pectic polysaccharides, whereas hemicelluloses and cellulose did not present important modifications in comparison to the control sample (fresh samples with 0% of oil extracted). This could be inferred from the significant decreases ($p < 0.05$) observed in galacturonic acid and arabinose, whereas the overall amounts of other sugars such as xylose or glucose remained almost unchanged. Then, when extraction reached 35% of total oil present in either raw almond seed or kernel, marked changes could be observed not only in pectic polysaccharides but also in hemicelluloses, as could be inferred from the significant decreases ($p < 0.05$) observed in the amounts of uronic acids, arabinose, and rhamnose (pectins) and xylose and 1 M glucose (hemicelluloses). Furthermore, samples with 57−64% of oil extracted exhibited a major disruption of the cell walls because all main types of cell wall polymers were affected; even a significant decrease ($p < 0.05$) was detected in the amounts of glucose released under Saeman conditions (cellulose). In general, most of the latter observations were also detected for toasted seed samples except for the total glucose content, which seemed to remain almost constant during the extraction procedure. The exposure at high temperature, during toasting, might have produced significant changes in the cell wall matrix; in fact, the removal of water might have promoted new links among cell wall polymers, reducing cell wall porosity and strengthening the wall. Both effects would contribute to the reduced ability of SC-CO_2 to extract oil from the inner cells. This could explain the major difficulties detected during SCFE with toasted samples (need for higher amounts of CO_2) and the lower extraction percentages that could be achieved on these samples.

These results give some indications of the possible mechanisms involved in the SCFE procedure. Thus, at lower percentages of oil extraction (up to 35%), minor modifications were apparently promoted on cell wall polymers; however, the disruption of pectic polysaccharides and also hemicelluloses, probably xyloglucans, might result in a significant increase of the porosity of the walls (*14*), which could explain the capacity of the oil to be transferred from the inner cell to the extracted solution. At higher rates of oil extraction (e.g., up to 65%), a major disruption of the cell wall network seems to occur, probably due to the longer exposure to the high pressure applied. Thus, the broken walls would enable the fatty acid chains to be easily solubilized and transferred to the extracted oil solution.

As can be observed in the light micrographs shown in Figure 1, corresponding to fresh almond seed (left)

and partially defatted (35% extraction) almond (right), the cell walls seem to remain unbroken after 35% of the oil has been extracted. This would support the hypothesis that fatty acid chains might exit the cells through the porous walls without need of broken walls. Further and more detailed investigation on pectic polysaccharides is being carried out to confirm this observation.

Mineral Elements Associated with the AIRs. Changes in the concentration of the mineral elements associated with cell wall components might provide useful information related to structural changes in the wall (*31*). The significant decrease ($p < 0.05$) of Ca and Mg detected in all samples (Table 4), in particular after extraction of 65% of the oil, might have contributed to the overall breakage of the cell wall structure. The calcium−pectin complex acts as an intracellular cement giving firmness to the tissues (*32*); therefore, the removal of divalent cations such as Ca and Mg might reflect the destruction of cross-links between galacturonic acid units of adjacent pectic chains or between pectins and other polymers. This may have a marked influence on the textural characteristics and the porosity of the tissues.

Conclusions. A review by Carpita and Gibeaut (*33*) sets out models of the possible interactions between the components of the primary cell wall. Plant cell walls are recognized as a composite of two phases: the microfibrillar phase, consisting mainly of cellulose microfibrils forming an insoluble framework; and the matrix phase, which surrounds and embeds the framework. Hemicelluloses and pectic polysaccharides may regulate wall strength and porosity by filling the gaps and controlling the separation of the microfibrils.

High-pressure treatments do not cause the formation and destruction of covalent bonds but have effects on hydrogen, ionic, and hydrophobic bonds (*34*). The results presented in this study suggest that the application of SC-CO_2 to extract oil from almonds modifies, initially, the cell wall matrix, in particular pectic polymers and hemicelloses. At this initial phase of extraction, the breakage of ionic links within the pectin structure might increase the porosity of the wall, regulating the transfer of oil from the inner cell. Pectins are among the cell wall components with the ability to contain the turgor pressure of the cell (*35*). The breakage of hydrogen bonds between hemicelluloses and cellulose might also contribute to increase the porosity of the wall. While high pressure is applied to enhance the percentage of extracted oil, a major disruption of the cell wall network seems to occur; thus, the presence of broken walls allows the oil to flow freely and exit the cells.

These results seem to shed some light over key aspects that, from a biochemical point of view, might regulate the SCFE procedure applied on plant-based products. More research is being carried out to fully characterize and understand SCFE processes.

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