Composition of Phenolic Compounds in a Portuguese Pear (*Pyrus communis* L. Var. S. Bartolomeu) and Changes after Sun-Drying

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The composition of phenolic compounds of a Portuguese pear cultivar (*Pyrus communis* L. var. S. Bartolomeu) was determined by HPLC after thioacidolysis. The average concentration of phenolic compounds in pear harvested at commercial maturity stage was 3.7 g per kg of fresh pulp. Procyanidins were the predominant phenolics (96%), with a mean degree of polymerization (mDP) of 13−44; hydroxycinnamic acids (2%), arbutin (0.8%), and catechins (0.7%) were also present. The most abundant monomer in the procyanidin structures was (−)-epicatechin (99%), which was found as extension and terminal units; (+)-catechin (1%) was found only as a terminal unit. Sun-drying of these pears caused a decrease of 64% (on a dry pulp basis) in the total amount of native phenolic compounds. Hydroxycinnamic acids and procyanidins showed the largest decrease; the B2 procyanidin was not found at all in the sun-dried pear. Less affected were arbutin and catechins. In the sun-dried pear, the procyanidins with high mDP became unextractable in the solvents used.

KEYWORDS: Procyanidins; degree of polymerization; hydroxycinnamic acids; extractability; oxidation; thioacidolysis

INTRODUCTION

One of the traditional Portuguese food products is a sun-dried small pear of reddish brown color and unique elastic properties (*1*). The drying of fruits is a very ancient practice for food preservation still in use nowadays. After the S. Bartolomeu pear is harvested, it is peeled and allowed to dry in the sun for about 5 days. Then the pears are laid in baskets and covered with a cloth for 2 days. This treatment is necessary to give the pears the elasticity needed to be flattened without breakage. After this, the pear is submitted to a second sun-drying process. These kinds of traditional agricultural products are socially and economically important in the interior regions of Portugal, and contribute to the diversification of agriculture production.

Color, flavor, and texture are organoleptic properties that influence the end-product quality and thus its commercial value. The changes in color and specific flavors occurring during the processing of fruits are associated with the presence of phenolic compounds, in particular caffeoylquinic acid, (+)-catechin, (−)-epicatechin, and proanthocyanidins, also known as condensed tannins (*2, 3*). In pome fruits such as apples (*4−6*) and pears (*7, 8*) the proanthocyanidins are essentially procyanidins composed mainly of (−)-epicatechin units. A brown discoloration was observed during the sun-drying process of S. Bartolomeu pear. Browning during the processing of pears (*9−11*) has generally been interpreted in terms of oxidation of caffeoylquinic acid and catechins by catecholase activity of pear polyphenol oxidase (PPO) (*12−15*). Oxidation due to PPO activity occurs to an appreciable extent only when tissues are damaged as the phenolic compounds are spacially separated from PPOs in intact tissue (*16*). In apples, PPO was found mainly in chloroplasts (*11, 17*) and phenolics were localized mainly in the vacuole (*18*). The S. Bartolomeu pear gives a sensation of astringency when eaten fresh. The astringency seems to be related to a high degree of polymerization of proanthocyanidins (*19, 20*). Phenolic compounds are also involved in interactions (*21*) and cross-linking (*22*) with cell-wall polysaccharides, thus modulating the mechanical properties of the cell walls which may be determinant in industrial processing of plants (*23*).

To analyze the phenolic compounds in S. Bartolomeu pear we employed a technique designed to enable both separation and quantitative analysis of phenolics by reversed-phase HPLC

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coupled with diode array detection. This technique has been used to analyze phenolic compounds in pears (*8, 24–29*), pear juices (*30, 31*), and pear purees (*32*), and in the evaluation of authenticity of quince jellies and jams prone to be adulterated with pears (*33*). In these studies, the phenolic compounds identified in pulp were hydroxycinnamic acids (caffeoylquinic acid, dicaffeoylquinic acid, caffeic acid, coumaroylquinic acid, coumaric acid, and coumaroylmalic acid), the flavan-3-ols (+)-catechin and (−)-epicatechin, oligomeric procyanidins, and arbutin. However, the polymeric procyanidins have not been studied. Recent work of Guyot et al. (*34, 35*) has shown the benefit of the use of depolymerization of proanthocyanidin based on thioacidolysis, prior to HPLC, of apple cider phenolic extracts. The thioacidolysis reaction yields benzylthioether derivatives of (−)-epicatechin derived from the extension units of apple procyanidins, and monomeric catechins from the terminal units. This methodology enables both the quantification and the characterization of procyanidins, giving access to the nature of their constitutive units and their average degree of polymerization (*34*).

The aim of this work was to characterize the native phenolic compounds in fresh pears and to study the effect of the traditional sun-drying process on the phenolic profile. This would contribute to a better understanding of the chemical and biochemical changes that occur during the sun-drying process and that are related to changes in texture, color, and flavor. The final goal is to improve the quality of sun-dried pears through a tight control of the process parameters.

MATERIALS AND METHODS

Phenolic Standards. Arbutin was purchased from Sigma Chemical Co. (St. Louis, MO). *p*-Coumaroylquinic acid was obtained and identified as described by Sanoner et al. (*5*). The esterification of caffeoylquinic acid and *p*-coumaroylquinic acid, with methanol, under the conditions of thiolysis was confirmed by Sanoner et al. (*5*). Caffeoylquinic acid, (+)-catechin, (−)-epicatechin, and (−)-epicatechin benzylthioether were obtained as described by Guyot et al. (*34*). Procyanidin B2 was provided by J. M. Souquet (Unité de Recherches des Polymères et des Techniques Physico-Chimiques, INRA, Montepellier, France).

Solvents and Reagents. *n*-Hexane, methanol, acetone, and acetonitrile of chromatographic grade quality was purchased from Biosolve Ltd (Netherlands). Glacial acetic acid was also purchased from Biosolve. Toluene-α-thiol and Folin–Ciocalteu reagent were purchased from Merck (Germany). Deionized water was obtained with a Milli-Q water system (Millipore, Bedford, MA).

Sample Origin. The analyses were performed on a single lot of fresh and sun-dried peeled pears (*Pyrus communis* L. var. S. Bartolomeu). Both lots were obtained from the same producer. Harvesting and processing occurred during July and August 2000. The moisture content of fresh and sun-dried pears were 85 and 35%, respectively. The number of fruits per kg was 34 for fresh pears and 105 for sun-dried pears. Cell wall material (CWM) free of intracellular polymers was prepared from fresh and dried pulps as previously described (*1, 36, 37*). The pulp was homogenized in aqueous 1.5% sodium dodecyl sulfate (SDS) containing 5 mM sodium metabisulfite ($Na_2S_2O_5$) with an Ultraturrax. The homogenate was filtered through a nylon cloth, and the residue was washed with aqueous 0.5% SDS containing 3 mM $Na_2S_2O_5$. The residue was suspended in the same 0.5% SDS solution and was ball-milled for 8 h and centrifuged. After the residue was washed with water, it was stirred overnight in phenol–HOAc–H_2O (PAW, 2:1:1, w/v/v) at 20 °C, centrifuged, and stirred again in PAW. The residue was collected, washed, dispersed in water, dialyzed, and freeze-dried to give the CWM.

Extraction of Phenolic Compounds. The methodology for extraction of pear phenolic compounds was adapted from Guyot et al. (*34*). The freeze-dried pulps, from fresh and sun-dried pears, were immersed

in liquid nitrogen and ground. To ensure complete dryness, the powder obtained was again freeze-dried. The sample (20 g) was extracted with 200 mL of hexane, at room temperature, under magnetic stirring, to remove lipids. The insoluble material was extracted 5× with 200 mL of methanol to extract low-molecular-weight phenolics and to dissolve sugars and organic acids. The methanol extract mixtures were filtered through a G3 sintered glass filter. Filtrate solutions were combined and concentrated by evaporation under reduced pressure at 40 °C, frozen at −20 °C, and freeze-dried (methanol extract). The residue from methanol extractions was treated 3× with 200 mL of acetone/water (6:4 v/v) to extract polymerized phenolic compounds. Following each extraction the suspension was centrifuged at 15000*g*, at 9 °C, for 15 min. Supernatant solutions were combined and concentrated as above, frozen, and freeze-dried (acetone extract). The insoluble residue derived from fresh pear pulp, was washed 3× with water, by centrifugation as described above, and finally frozen and freeze-dried (residue). Except for the hexane treatment, all extractions were carried out in acidic conditions (addition of acetic acid at 5 mL/L) to avoid oxidation of phenolic compounds.

For the dried pear, the extraction with aqueous acetone was followed by a treatment with acetone/8M urea (6:4 v/v) (twice) to extract the remaining noncovalently bound products. After each extraction the mixture was centrifuged, and the supernatants were combined and concentrated as above. The urea was removed by Sep Pack C18 cartridges (5 g, Waters, Milford, MA). The cartridges were preconditioned by sequential treatment with 20 mL of methanol, 20 mL of H_2O, and 20 mL of dilute acetic acid (2% v/v in water), respectively, with a very slow flow rate. The sample solution was distributed onto 4 cartridges (50 mL per cartridge). To remove the urea, each cartridge was eluted with 30 mL of dilute acetic acid (2% v/v). The phenolic fractions were eluted from each cartridge with 30 mL of acetone/2% acetic acid (in water) (6:4 v/v). The eluted solutions were pooled and then concentrated to an aqueous suspension, frozen, and freeze-dried (acetone/urea extract). The insoluble residue was treated as described for fresh pear pulp.

Colorimetric Quantification of Total Phenolic Compounds by the Folin–Ciocalteu Method. The experimental procedure used to quantify the total concentration of phenolics in the sun-dried pear extracts is the one adapted by Guyot et al. (*34*). For the methanol extract, a solid-phase extraction with Sep Pack C18 cartridge was done to remove the sugars that could interfere in the Folin assay. The sugars were eluted from each cartridge with 10 × 30 mL dilute acetic acid (2% v/v in water), and the phenolic fractions were eluted with 5 × 30 mL of acetonitrile/water/acetic acid (50:49:1 v/v). For the Folin assay, a solution of the purified methanol extract (1 mg/mL in dilute acetic acid, 2.5% v/v in water) and suspensions of acetone and acetone/urea extracts, 0.2 and 1 mg/mL in acetone/water/acetic acid (60:39:1 v/v), respectively, were prepared. Ultrasound was used to obtain homogenized suspensions. To 0.5 mL of solution/suspension was added 250 μL of Folin–Ciocalteu reagent. The mixture was homogenized with a vortex, then a pause of 3 min was allowed for reaction, after which 1 mL of Na_2CO_3 (200 g/L) and 3.25 mL of ultrapure water were added, giving a total volume of 5 mL, and the mixture was homogenized with a vortex. It was then incubated for 10 min at 70 °C, and then for 30 min at room temperature. The incubation gave rise to clear solutions for all the samples. The absorbance was measured at 700 nm with a Uvikon 860 UV/Vis spectrophotometer (Milan, Italy) against a blank (0.5 mL of polyphenol solvent, plus reagents) in the reference cell. The assay data were correlated to the calibration curve of caffeoylquinic acid standard for the methanol extract, and to the calibration curve of (−)-epicatechin standard for the acetone and acetone/urea extracts.

Thioacidolysis Conditions. The thioacidolysis of freeze-dried pulp, final residue of extraction, and cell wall material was performed on 40–100 mg of finely divided sample, depending on the phenolic content, with 800 μL of toluene-α-thiol (5% v/v in dry methanol) and 400 μL of 0.4 M HCl in dry methanol. A solution at approximately 200 mg/mL in dry methanol was prepared for the methanol extract, and a suspension at approximately 10 mg/L in dry methanol for acetone and acetone/urea extracts. These were then treated as described by Guyot et al. (*35*), using 150 μL of sample, 300 μL of 5% toluene-α-thiol, and 150 μL of 0.4 M HCl. Reactions were carried out at 40 °C

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for 30 min. For each sample submitted to thioacidolysis a direct RP-HPLC analysis was also performed without thioacidolysis (*34*). Each sample was mixed with 1% dilute acetic acid in methanol (v/v) and incubated for 15 min in an ultrasonic bath with ice, to avoid heating. All samples were filtered through a 0.45-μm Teflon membrane (Millipore, Bedford, MA) directly to an insert of 200 μL to be placed in the HPLC auto-sampler.

Reversed-Phase HPLC Conditions. Both with or without thioacidolysis, 10-μL samples were injected onto a Waters 2690 Separation Module HPLC equipped with an auto-sampler and a cooling system, set to 4 °C, and Waters 996 photodiode array detector. The data acquisition and remote control of the HPLC system was done by Millennium 32 version 3.20 software. The column was a 250 mm × 4 mm i.d., 5-μm endcapped Purospher RP 18 (Merck, Germany). RP-HPLC conditions were the same as those previously described by Sanoner et al. (*5*).

HPLC Characterization and Quantification of Phenolic Compounds. For (+)-catechin, (−)-epicatechin, (−)-epicatechin benzylthioether, B2 procyanidin, and arbutin the detection was performed at 280 nm, and for phenolic acids and their acid derivatives detection was performed at 320 nm. Phenolic compounds were identified on chromatograms by comparing their retention times and UV spectra with those of reference standards. The *p*-coumaroylmalic acid was purified from the methanol extract of fresh pears and identified by mass spectrometry (see below).

Quantification of the identified compounds was performed by correlating the measured peak area with the calibration curves of the corresponding standard. The esterified form of caffeoylquinic acid derived from thioacidolysis reaction was quantified as caffeoylquinic acid equivalent and *p*-coumaroylmalic acid was quantified as *p*-coumaroylquinic acid equivalent. mDP was determined by calculating the molar ratio of all the flavan-3-ol units (thioether adducts plus terminal units) to (−)-epichatechin and (+)-catechin corresponding to terminal units.

Purification of *p*-Coumaroylmalic Acid from the Methanol Extract of Fresh Pear Pulp. To remove the sugars and organic acids, the sample (14.4 g) was dissolved in 2.5% aqueous acetic acid (v/v). The suspension was stirred for 20 min in order to dissolve it, and then was filtered through a glass fiber filter (Whatman GF/C). The fractionation of the sample was performed as described by Guyot et al. (*35*) in a semipreparative reversed-phase HPLC system composed of two Dynamax SD 300 high-pressure pumps (Rainin) equipped with 100-mL WTI pump heads; a manual injection valve (Rheodyne, CA) with a 5-mL injection loop to mix the mobile phases; a radial compression module (Waters); 100 mm × 25 mm i.d., 6-μm, C18 PreNova-Pak HR chromatographic column (Waters); and a UV detector Dynamax UV1 (Rainin). Data were acquired and processed by the EZ-Chrom software (Scientific Software, Inc, CA). The solvent system used was 2.5% aqueous acetic acid (v/v) (solvent A) and acetonitrile (solvent B). The column was equilibrated during 20 min at 40 mL/min with 100% A. After injection, the sugars and highly polar compounds were eluted by 100% A for 75 min; the presence of sugars was detected by the phenol−sulfuric acid test (*38*). The phenolic fraction was recovered by elution with 50% B, and detected at 280 nm. This fraction was concentrated by evaporation then freeze-dried.

The methanolic extract solution free of high-polarity compounds was fractionated by low-pressure preparative chromatography in order to separate polymeric and oligomeric procyanidins from hydroxycinnamic acids by adsorption and size-exclusion. The column (1 × 12 cm) was packed with TSK HWF 40 gel (Merck). The sample (259 mg) was dissolved in 25 mL of 2.5% aqueous acetic acid (v/v) and introduced onto the column. It was eluted by a solution of ethanol/H$_2$O (55:45, v/v) acidified with 10% TFA (v/v) at a flow rate of 2 mL/min. The fractionated material was collected in 4-mL fractions, the absorption of which was monitored between 230 and 350 nm. Fractions that showed significant absorbance close to 320 nm were submitted to reverse-phase HPLC analysis as described above. The appropriate fractions were combined, concentrated as above, and freeze-dried.

Final purification of the *p*-coumaroylmalic acid was performed by HPLC. The freeze-dried sample (9.3 mg) was dissolved in 500 μL of 2.5% aqueous acetic acid (v/v). Several runs (injection volume of 100
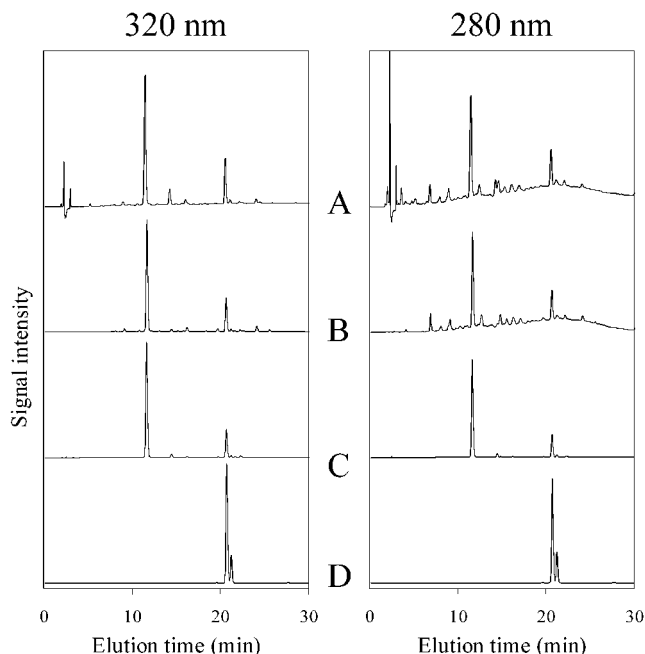

Figure 1. RP-HPLC analysis of the consecutive steps of purification of *p*-coumaroylmalic acid: A, methanolic extract of the fresh pear pulp; B, after removal of highly polar compounds by preparative HPLC; C, after removal of flavan-3-ols by affinity chromatography; D, after final purification on analytical C18 column.

μL) were performed on the analytical RP-HPLC system, with a flow rate of 1 mL/min at 30 °C and detection at 360 nm. A gradient of acetonitrile (solvent A) and 2.5% acetic acid (solvent B) was applied: initial, 3% A; 0−5 min, 9% A linear; 5−15 min, 16% A linear; 15−25 min, 18% A linear; followed by washing and reconditioning the column. The appropriate peak was collected, concentrated, and freeze-dried, and kept in a desiccator in the dark.

ESI-MS. The freeze-dried powder was dissolved in 2.5% dilute acetic acid in methanol (v/v) at 2 g/L, and the solution was introduced into the ion spray source at a constant flow rate of 10 μL/min. The mass spectrometry system was an API 2000 (Perkin-Elmer Sciex Instruments, Tornhill, Canada) equipped with an electrospray ionization source. Infusion analyses were performed in the negative mode with an ion spray voltage of approximately 4500 kV, a −60 V orifice voltage, and a nominal mass range up to *m/z* 1800.

RESULTS AND DISCUSSION

Composition of Phenolic Compounds in S. Bartolomeu Pear Pulp. On a dry pulp basis, the total concentration of phenolic compounds of fresh pear, determined by thioacidolysis, was 25 g/kg (**Table 1**). On a fresh weight basis, the total phenolic content was 3.7 g/kg in pear harvested at commercial maturity stage, a high value, comparable to that found for some bitter cider apple varieties (*5*). The phenolic compounds identified in S. Bartolomeu pear pulp can be grouped into three classes: simple phenolics, hydroxycinnamic acids, and flavan-3-ols. Arbutin (4-hydroxyphenyl-β-D-glucopyranoside) was the only simple phenolic identified. This compound is characteristic of pear, allowing the distinction between the phenolic profiles of apples and pears (*7, 33*). The predominant hydroxycinnamic acid identified was caffeoylquinic acid, in agreement with the literature (*2, 30, 31*). The second most abundant hydroxycinnamic acid was a compound with the UV profile of *p*-coumaric acid and of *p*-coumaroylquinic acid but with a different retention time. It was purified in four steps (**Figure 1**): the methanolic extract of fresh pears (**Figure 1A**, polyphenols content 9.7 mg/g) was first submitted to semipreparative HPLC

Table 1. Quantification (mg per g of dry material) of Phenolic Compounds in Pulp and Extracts of Fresh and Dried Pear Pulp, Analyzed by Reversed-Phase HPLC, after Thioacidolysis

	yield (%)[a]	arbutin	caffeoyl-quinic acid	p-coumaryl-malic acid	monomeric (+)-catechin	monomeric (–)-epicatechin	terminal (+)-catechin	terminal (–)-epicatechin	extension (–)-epicatechin	mDP	total phenolics
					Fresh						
pulp	–	0.20	0.46	0.10	0.020	0.15	0.23	0.80	23.0	24	25.0
SD (n = 3)		(0.01)	(0.02)	(0.005)	(0.001)	(0.01)	(0.02)	(0.04)	(1.3)	(1)	(1.3)
methanol	78.5	0.27	0.43	0.10	0.039	0.20	0.15	0.54	8.0	13	9.7
SD (n = 2)		(0.03)	(0.003)	(0.003)	(0.001)	(0.005)	(0.01)	(0.01)	(0.05)	(0.2)	(0.2)
acetone/water	2.1	t	0.58	t	nd	nd	3.1	10.2	575.3	44	589.2
SD (n = 2)			(0.04)				(0.1)	(0.1)	(38.8)	(2)	(38.6)
residue	18.4	nd	nd	nd	nd	nd	0.034	0.14	6.7	39	6.9
SD (n = 2)							(0.002)	(0.01)	(0.6)	(2)	(0.6)
					Dried						
pulp	–	0.19	0.021	t	0.0035	0.012	0.061	0.24	7.4	26	7.9
SD (n = 3)		(0.003)	(0.001)		(0.001)	(0.003)	(0.004)	(0.02)	(0.6)	(1)	(0.7)
methanol	84.9	0.14	0.11	t	t	t	t	t	t	–	0.25
SD (n = 2)		(0.02)	(0.02)								(0.03)
acetone/water	1.1	nd	0.54	t	nd	nd	1.3	4.3	74.7	15	80.8
SD (n = 2)			(0.03)				(0.1)	(0.1)	(0.5)	(0.1)	(0.5)
acetone/urea	0.18	nd	0.36	nd	nd	nd	t	t	1.9	–	2.3
SD (n = 2)			(0.03)						(0.1)		(0.1)
residue	15.0	nd	nd	nd	nd	nd	0.30	0.98	40.8	33	42.1
SD (n = 2)							(0.01)	(0.06)	(2.0)	(0.3)	(2.1)

[a] On a dry basis; SD, standard deviation; mDP, mean degree of polymerization; t, trace amount; nd, not detected.

to eliminate the sugars and organic acids. This purified polyphenolic fraction (**Figure 1B**, phenolic acid content 36 mg/g) still contained all flavan-3-ols, as shown by the UV chromatogram at 280 nm. These were separated from the phenolic acids by adsorption and size-exclusion chromatography on a TSK HWF 40 column, giving a fraction containing exclusively phenolic acids (**Figure 1C**, phenolic acid content 350 mg/g). The main unknown peak, separated from caffeoylquinic acid by RP-HPLC (**Figure 1D**), was identified by ESI-MS as p-coumaroylmalic acid. Two main ion peaks were observed in the mass spectrum: a peak at m/z 279 corresponding to the [M−H]⁻ molecular ion peak of p-coumaroylmalic acid, and a peak at m/z 163 corresponding to the coumaroyl moiety. The mass difference corresponded to loss of an anhydro-malic acid moiety. This p-coumaroylmalic acid has been reported to occur in some varieties of pears (*2, 24*). It represented approximately 25% of the amount of caffeoylquinic acid in the S. Bartolomeu pears. Flavan-3-ols individually identified were (+)-catechin, (−)-epicatechin, and a procyanidin dimer, B2 [(−)-epicatechin-(4β→8)−(−)-epicatechin]. Total procyanidin oligomers and polymers were completely characterized by thioacidolyis-HPLC. The catechin monomers (*25, 28, 30*) as well as the B2 procyanidin (*8, 30*), were also detected in pears of other varieties. The procyanidin polymers, with (−)-epicatechin as the major constituent unit, were similar to those found in cider apples (*5, 35*).

In the fresh pulp, the hydroxycinnamic acids and monomeric flavan-3-ols occurred in minor amounts (85 and 25 mg/kg of fresh pulp, respectively) when compared with procyanidins (3.6 g/kg) (cf. **Table 1**). The caffeoylquinic acid content (70 mg/kg of fresh pulp) was in the range of values reported for d'Anjou (40−70 mg/kg) (*39*), Guyot, Comice, Conference, Williams, and Abbe Fetel pear varieties (40−141 mg/kg of fresh pulp) (*25*). It was also comparable with values for apple dessert varieties, such as Granny Smith (28−71 mg/kg), Red Delicious (63−106 mg/kg) (*40*), Golden Delicious (132 mg/kg), and Avrolles cider apple (154 mg/kg) (*5*). However, it was much lower than the amounts measured for 14 other cider apples (338−1195 mg/kg of fresh pulp) (*5*) and Reineta apple (266−357 mg/kg) (*40*). The p-coumaroylmalic acid, as far as we know,

has not yet been quantified in pears, as it had been reported to occur only in trace quantities in different pear varieties (*2*). In S. Bartolomeu pear, a value of 15 mg/kg of fresh pulp was found. The amount of monomeric flavan-3-ols was in the range of that found in Guyot, Comice, Conference, and Williams pear varieties (13−26 mg/kg) but much lower than that in Abbe Fetel pears (92 mg/kg) (*25*) and some cider apples varieties (102−1464 mg/kg) (*5*). The most abundant monomer in S. Bartolomeu pear was (−)-epicatechin (88%), in accordance with the results reported for other pear varieties (*25, 28, 41*) and some cider apples varieties (*5*).

A low content in caffeoylquinic acid (23 mg/kg) in pear pulp has been related to a large amount of sclereids and minor susceptibility to browning of pear pulp (*42*). A large amount of sclereids has been associated with a lack of pulp softening upon ripening (*42*) as is verified with S. Bartolomeu pear.

The low concentration in monomeric catechins and hydroxycinnamic acids in S. Bartolomeu pear might be related to the very slow change of color during the process of sun-drying. After 1 day the fruit was only slightly brown, in contrast to what usually is observed with apples and other varieties of pears (personal communication from producers). Monomeric catechins are also known to be partly responsible for high browning in apple products (*43*). The presence of caffeoylquinic acid in pears enhanced the degradation rates of epicatechin (*14*). o-Quinones derived from oxidation of o-diphenols, such as caffeoylquinic acid (*44*) are able to oxidize flavan-3-ols by nonenzymatic coupled reaction mechanisms (*45, 46*). The polymerization of o-quinones leads to the brown pigments (*17*).

Thioacidolysis-HPLC of the pulp showed the presence of (−)-epicatechin benzylthioether, which allowed the presence of proanthocyanidins to be inferred (*47*). On the basis of the data obtained before and after thiolysis, the relative amounts of 1% (+)-catechin terminal residues, 3% (−)-epicatechin terminal residues, and 96% (−)-epicatechin residues as extension units allowed a mean degree of polymerization (*48*) of 24 residues to be estimated (**Table 1**). These mDP values were 4−5-fold higher than those of cider apple, except for the varieties Avrolles (mDP 50) and Guillevic (mDP 40) (*5*). The S. Bartolomeu pear showed a polymeric procyanidin composition similar to

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Table 2. Quantification of Phenolic Compounds in Purified Methanol Extracts of Fresh and Dried Pear Pulp[a]

	fresh	dried
yield (%)	1.8	1.8
arbutin	14.8 (0.8)	7.5 (0.4)
caffeoylquinic acid	27.4 (0.3)	6.1 (0.7)
p-coumarylmalic acid	6.4 (0.1)	0.7 (0.1)
monomeric (+)-catechin	2.4 (0.4)	2.6 (0.5)
monomeric (−)-epicatechin	11.1 (0.6)	4.1 (0.8)
terminal (+)-catechin	8.3 (0.6)	t
terminal (−)-epicatechin	29.8 (1.1)	1.6 (0.3)
extension (−)-epicatechin	445.6 (5.3)	10.1 (0.5)
mDP	13 (0.4)	7 (1)
total phenolics	545.8 (8.6)	32.7 (2.5)

[a] Results are given on a dry basis in mg/g. Standard deviations ($n = 2$) are given in parentheses. mDP, mean degree of polymerization; t, trace amount.

that of these apple varieties. The phenolic profile of S. Bartolomeu pear pulp was composed, in weight %, of 0.8% arbutin, 2% hydroxycinnamic acids, 0.1% (+)-catechin, 0.6% (−)-epicatechin, and 96% procyanidins. B2 procyanidin only accounted for 1% of the total amount of procyanidins.

Fractionation of Fresh Pear Phenolic Compounds. To compare the physicochemical state of phenolics in fresh and dried pear, phenolic compounds were sequentially extracted from the freeze-dried pulp with methanol and aqueous acetone to leave a final residue.

The methanol extract solubilized 79% of the pear tissue material (**Table 1**). However, the phenolic compounds accounted only for 9.7 mg/g of this extract. The major phenolic compounds in the methanol extract were the procyanidins (89 wt % of the total phenolic compounds). Hydroxycinnamic acids represented 5%, arbutin 3%, (−)-epicatechin 2%, and (+)-catechin 0.4 wt % of the phenolic compounds. Caffeoylquinic and *p*-coumaroyl-malic acids, in a proportion of 4:1, were the major hydroxy-cinnamic acids present. This proportion was also verified after purification of the extract by semipreparative HPLC (**Table 2**). This step showed the presence of additional minor, unidentified hydroxycinnamic acids, representing approximately 7% of hydroxycinnnamic acids.

Extraction with acetone solubilized 2% of pulp tissue. This extract was rich in phenolic compounds (59%), all of them procyanidins (**Table 1**). The final residue of the extractions contained 0.7% of procyanidins. These results showed that, in the extracts and residue, the predominant phenolic constituent was the polymeric form of procyanidins.

The methanol extract contained 100% of the arbutin, 97% of the hydroxycinnamic acid derivatives, and 33% of the procyanidins of the pulp. The majority of procyanidins were solubilized in the acetone extract (61%) and only 6% were left in the residue (**Figure 2a**). The average degree of polymerization of procyanidins in the different extracts indicated that, with methanol, there was a solubilization mainly of those with low mDP. The highest mDP values were estimated for acetone extract (mDP 44) and residue (mDP 39).

Effect of Sun-Drying on the Phenolic Profile of S. Bartolomeu Pear. For the sun-dried pear the total amount of phenolics quantified by HPLC, after thiolysis, was 8 g/kg, on a dry pulp basis. This value was 32% of the amount present in the pulp of the fresh pears. Comparing the phenolic composition of dried and fresh pears (**Table 1**) the results showed an overall decrease in concentration for all phenolic compounds in sun-dried fruit, except for arbutin. The hydroxycinnamic acids and monomeric catechins were the most affected (96 and 91% decrease, on a dry weight basis, respectively) and procyanidins
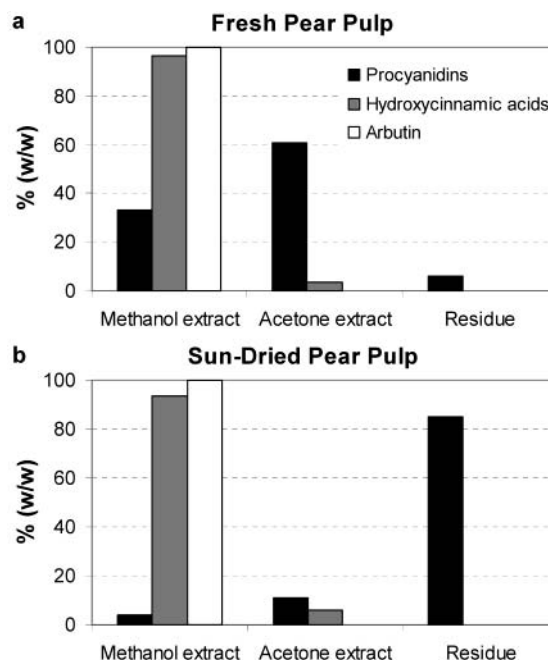
Figure 2. Distribution (%, w/w) of procyanidins, hydroxycinnamic acids, and arbutin in the methanol and aqueous acetone extracts, and in the residue of (a) fresh and (b) dried pear pulp.

decreased by 68%. As observed for the fresh fruits, the most abundant flavan-3-ol monomer was (−)-epicatechin (77%). The B2 dimer was not detected in sun-dried pear pulp. The procyanidins of dried and fresh pears presented similar composition and mDP (**Table 1**).

The resistance of arbutin to processing has been shown for pear juice (*30*). These authors gave evidence that PPO had only a minor influence on arbutin concentration. Also, the levels of arbutin were less affected than those of catechins and caffeoylquinic acid during long-term cold storage of d'Anjou and Bosc pears (*39*).

The decrease in the concentrations of caffeoylquinic acid and catechins observed after sun-drying was very probably due to the enzymatic degradation by PPO. This enzyme has higher affinity for caffeoylquinic acid than for catechins (*12, 13, 49, 50*) but caffeoylquinic acid can be regenerated by coupled oxidation notably with catechins, as has been observed for apple juice (*51*). Catechins, as well as caffeoylquinic acid, have been involved in enzymatic browning of pear (*14, 52*) and these compounds may be responsible for the browning of the S. Bartolomeu dried pear.

The absence of procyanidin B2 and the decrease in the amount of polymeric procyanidins in sun-dried pear may also be explained by the involvement of the procyanidin compounds in coupled oxidative reactions. Oxidation of procyanidins has been shown to occur in apple juice where highly reactive oxidized intermediates react irreversibly with each other and with proteins to form insoluble complexes (*53*).

As with the fresh pear, the methanol solubilized most of the material in the sun-dried pear tissue (85%), in an extract very poor in phenolic compounds (**Table 1**). The purified methanol extract (**Table 2**) contained 3% of phenolics, composed of arbutin (23%), caffeoylquinic acid (19%), cou-maroylmalic acid (2%), (+)-catechin (8%), (−)-epicatechin (12%), and procyanidins (36%). This step gave a higher amount of unidentified hydroxycinnamic acids (16%) than in fresh pear.
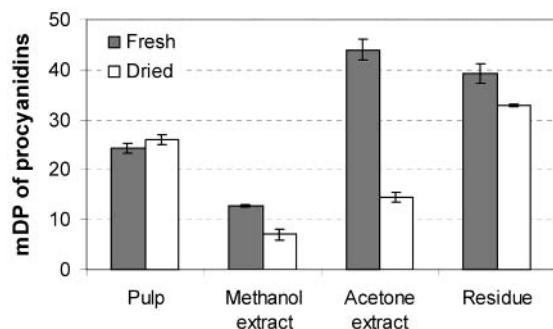
Figure 3. Mean degree of polymerization (mDP) of procyanidins of fresh and dried pear pulp. Bars represent the standard deviation of 3 replicates in the pulp and 2 replicates for the extracts and residue.

Table 3. Comparison of the Total Amount of Phenolic Compounds of Dried Pear Pulp (mg per g dry pulp) by Two Independent Methods

	Folin	thiolysis
methanol purified	1.3	0.50
standard deviation ($n = 2$)	(0.1)	(0.04)
acetone/water	1.8	0.86
standard deviation ($n = 2$)	(0.1)	(0.01)
acetone/urea	0.048	0.004
standard deviation ($n = 2$)	(0.007)	(0.001)

The acetone extraction solubilized only 1% of the sun-dried pear pulps. The extract was poor in native phenolic compounds (8%), and all of them were procyanidins (**Table 1**). On a dry weight basis, the amount of procyanidins extracted was 14× lower than that obtained in fresh fruits. In an attempt to remove more procyanidins from the acetone insoluble residue, an acetone/urea extraction step was introduced. However, the material extracted was very low in procyanidins (**Table 1**). In contrast, the final residue was composed of 4% procyanidins, which accounted for 6.3 mg/g initial dry weight, 5× more than that obtained in the final residue of fresh fruit (1.3 mg/g initial dry weight).

Taking into account the different extracts and final residue of the sun-dried pear pulp, the majority of procyanidins (86%) were insoluble in the solvents used, 12% were solubilized in the acetone extract, and 2% were solubilized in the methanol extract (**Figure 2b**). The mDP of procyanidins was 7 in methanol extract, 15 in the acetone extract, and 33 in the residue. These values were of the same order of magnitude as those for the fresh pear for the methanol extract and the residue; for the acetone extract, the procyanidins had a lower mDP. Generally, the mDP values estimated for the procyanidins of fresh and dried pears were very similar (**Figure 3**). There was an overall decrease of 64%, on a dry pulp basis, in the total concentration of native phenolic compounds after sun-drying. The most affected compounds were the hydroxycinnamic acids and the procyanidins.

The Folin−Ciocalteu analysis of the phenolic extracts derived from sun-dried pears (**Table 3**) showed that the total concentration of phenolics was 57% higher than the concentration measured by the HPLC methodology. This was of the same magnitude as the observed decrease in the total concentration of phenolic compounds in pears after sun-drying. It seems that the structure of these molecules has been modified and consequently they could no longer be identified by our HPLC methodology. Indeed, there is evidence that oxidation of caffeoylquinic acid, procyanidin B2, and (−)-epicatechin, catalyzed by PPO, leads to *o*-quinone derivatives

in model solutions designed to mimic oxidation in musts of cider apples (*51*).

Analysis of cell wall material (CWM) of fresh and sun-dried pear pulp by RP-HPLC, after thiolysis, showed the presence of polymeric procyanidins, representing 1.4 and 0.8% of the CWM, and with a mDP of 43 and 30 for fresh and dried pears, respectively. These mDP values were of a similar order of magnitude to those estimated for residue (39 and 33, for fresh and dried pears, respectively). The highly polymerized procyanidins were not totally extracted during the cell wall preparation procedure, due to interactions with the cell wall polysaccharides, as previously reported (*21*, *54*). The CWM thus contained nonwall (initially intracellular) phenolic compounds, the amounts and further extraction of which should be carefully considered and taken into account during cell wall fractionation procedures.

The results of this study give evidence that the traditional processing of S. Bartolomeu pear has caused a modification of its phenolic compounds that could result from oxidation and from reaction with other components such that these polyphenols became irreversibly bound. They could no longer be extracted by the standard methods, using methanol, aqueous acetone, or acetone/urea solvents. The observed insolubility of high mDP procyanidins and the decrease of their content might explain the sensorial loss of astringency of the sun-dried pear. Their reaction with other components, e.g, the cell wall polysaccharides, might explain the physical characteristics of the sun-dried pears, as the polyphenols form an additional, less hydrophilic network in these fruits.

ABBREVIATIONS USED

mDP, Mean degree of polymerization; RP-HPLC, reversed-phase high-pressure liquid chromatography; PPO, polyphenol oxidase.

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