Influence of Cultivar, Maturity Stage, and Storage Conditions on Phenolic Composition and Enzymatic Browning of Pear Fruits

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The phenolic composition and the degree of browning of pears were determined for nine cultivars at different stages of maturity. 5′-Caffeoylquinic acid and (−)-epicatechin were the two major phenolics found. Phenolic content and susceptibility of pears to browning were high in peel. Differences in phenolic composition and in the degree of browning were more influenced by the cultivar than by the maturity stage of the fruit. At harvest, the degree of browning was closely correlated with the initial amount of hydroxycinnamic esters and flavanols. After 4 days of storage at room temperature, the variation in enzymatic browning depended on the cultivar and the phenolic content decreased for all cultivars. Williams cultivar pears stored in air accumulated phenolics more than fruits stored under 1% CO_2/1% O_2 and 3% CO_2/3% O_2.

Keywords: *Pyrus communis; pears; enzymatic browning; hydroxycinnamic esters; flavanols; flavonols; 5′-caffeoylquinic acid; (−)-epicatechin; controlled atmospheres*

INTRODUCTION

The development of brown color in pear purées and juices is due, first, to the oxidation of the phenolic compounds by polyphenol oxidase during processing (Rivas and Whitaker, 1973) and, second, to the formation of Maillard products during heating and storage (Cornwell and Wrolstad, 1981). Enzymatic browning, which occurs very quickly, determines the suitability of fruit for processing with respect to the color of the derived products. The degree to which fruits turn brown depends on O_2 and the endogenous phenolic and polyphenol oxidase contents (Nicolas et al., 1993). As shown for apple fruits, the coloration after oxidation depends on the balance between the phenolics: hydroxycinnamics, flavanols, and flavonols (Amiot et al., 1992; Goupy et al., 1995). In addition, phenolic compositions vary greatly with cultivar, stage of maturity, and postharvest storage conditions (Herrmann, 1976; Wrolstad et al., 1988; Macheix et al., 1990; Amiot et al., 1992; Spanos and Wrolstad, 1992). In pears, significant variations in the monophenols/diphenols ratio have been observed during maturation and storage (Billot, 1983).

Pear phenolics have been characterized by several investigators as summarized by Macheix et al. (1990). The hydroxycinnamic esters were identified as chlorogenic (5′-caffeoylquinic), *p*-coumarylquinic, *p*-coumarylmalic, and dicaffeoylquinic acids (Cartwright et al., 1955; Hulme, 1958; Sioud and Luh, 1966; Challice and Williams, 1972; Billot et al., 1978; Wald et al., 1989; Oleszek et al., 1994). The presence of (+)-catechin and (−)-epicatechin has also been reported (Sioud and Luh, 1966; Ranadive and Haard, 1971; Mosel and Herrmann, 1974; Risch and Herrmann, 1988). Besides these flavanol monomers, oligomer forms were also reported (Sioud and Luh, 1966; Amiot et al., 1993). Flavonols in pears have been characterized as glycosides or malonyl glycosides of quercetin or isorhamnetin (Duggan, 1969a,b; Herrmann, 1976; Nortje and Koppen, 1965; Wald et al., 1989; Oleszek et al., 1994). Flavonols were located especially in the peel (Macheix et al., 1990). Caffeic acid derivatives, catechin and epicatechin, have been shown to be the best substrates of polyphenol oxidases in pear (Walker, 1964; Rivas and Whitaker, 1973), as in numerous other fruits (Macheix et al., 1990). In contrast, *p*-coumaric acid appears to be an inhibitor of *o*-diphenolases from pear (Rivas and Whitaker, 1973) or from apple (Janovitz-Klapp et al., 1990b). Flavonols, usually abundant in fruit, are considered to be poor substrates of *o*-diphenolases (Macheix et al., 1990; Nicolas et al., 1993). In addition, flavonols have been shown to inhibit the oxidation of ascorbic acid, a reducing agent widely used to prevent enzymatic browning (Harper et al., 1969). Furthermore, it has been shown in apples that all the phenolics present in fruit are degraded (Amiot et al., 1992; Nicolas et al., 1994), suggesting that compounds that are not substrates or are poor substrates of polyphenol oxidases could be oxidized through coupled reactions (Macheix et al., 1990; Nicolas et al., 1993).

The objective of our study was to determine the effect of cultivar, maturity stage, and some storage conditions on the phenolic composition of pears in relation to enzymatic browning. The changes in phenolics during oxidation were also investigated.

MATERIALS AND METHODS

Pears. All of the cultivars were grown at the Station d'Expérimentation Arboricole de la région Provence-Alpes-Côte d'Azur (Mallemort, France) except for the cultivar Passe Crassane, which was grown at the Institut National de la Recherche Agronomique at Angers (France). For each cultivar, 50 fruits were picked at commercial maturity and for some cultivars at two or three dates pre- and postcommercial maturity. Peel and cortex were separated (in 1992) or not (in 1993), cut into small pieces, frozen in liquid nitrogen, and stored at −20 °C until use.

Extraction and Purification of Phenolic Compounds. The fresh frozen material was finely powdered, and 25 g was

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Table 1. Enzymatic Browning of the Flesh and Peel of Nine Pear Cultivars at Different Maturity Stages Picked in 1992

		flesh		peel	
cultivar	harvest date	DL^{*a} pellet	DL^* supernatant	DL^* pellet	DL^* supernatant
Williams 1	July 24	10.3 ± 2.0	5.3 ± 0.1	13.9 ± 0.5	24.4 ± 2.1
Williams 2[b]	Aug 3	11.5 ± 0.2	3.4 ± 0.2	14.1 ± 0.3	21.8 ± 2.4
Williams 3	Aug 11	9.7 ± 0.2	0.8 ± 0.3	11.9 ± 1.2	20.2 ± 1.0
Harrow Sweet 1	Aug 27	11.9 ± 2.5	2.4 ± 0.5	5.2 ± 0.5	25.0 ± 2.8
Harrow Sweet 2[b]	Sept 3	7.9 ± 0.4	2.4 ± 0.6	7.4 ± 0.4	28.0 ± 0.9
Harrow Sweet 3	Sept 7	11.6 ± 0.4	3.8 ± 0.6	7.6 ± 0.4	25.2 ± 2.1
Guyot 1	July 16	16.0 ± 0.8	5.4 ± 1.0	16.9 ± 0.3	26.5 ± 2.7
Guyot 2[b]	July 24	10.1 ± 1.1	5.1 ± 1.3	14.9 ± 0.3	23.1 ± 0.5
Guyot 3	Aug 3	10.6 ± 1.1	4.1 ± 1.0	17.4 ± 0.3	21.0 ± 1.2
P2198 1[b]	July 30	23.1 ± 0.3	5.8 ± 0.8	18.0 ± 0.5	24.9 ± 0.3
P2198 2	Aug 11	18.3 ± 1.2	2.2 ± 1.2	13.1 ± 0.1	30.6 ± 0.7
Comice	Sept 15	15.1 ± 0.5	2.5 ± 1.2	13.1 ± 0.1	30.6 ± 0.7
6.30.100	Aug 27	10.1 ± 1.0	4.3 ± 0.3	7.7 ± 0.8	21.6 ± 1.5
Conference	Aug 25	10.3[c]	4.8[c]	7.6[c]	25.5[c]
Abbe Fetel	Sept 1	11.1[c]	5.8[c]	12.6[c]	33.1[c]
Passe Crassane	Oct 18	12.7 ± 0.1	3.3 ± 0.7	7.8 ± 0.2	19.0 ± 0.5

[a] DL^* = (lightness of nonoxidized tissue) − (lightness of oxidized tissue). [b] Commerical maturity. [c] No repetitions.

immediately homogenized in 75 mL of cold EtOH (95%) for 1 min. Three successive extractions were carried out at 4 °C for 20 min. Alcohol was evaporated in vacuum at 38 °C. Phenolics were purified with ethyl acetate three times after the addition of ammonium sulfate (20%) and metaphosphoric acid (2%) to the aqueous phase. The three ethyl acetate extracts were combined, dried on Whatman paper (phase separator silicone treated), and evaporated to dryness under vacuum at 38 °C. Residue was dissolved in 2 mL of MeOH. Methanolic extracts were filtered through an Acrodisc filter (0.45 μm) before HPLC analyses.

Determination of Phenolic Compounds. Separation and determination of phenolics was performed by HPLC (Varian 5500 connected to a diode array detector Waters 900) using an Adsorbosphere C_{18} 3 μm (150 mm x 4.6 mm i.d.; Alltech), column. The solvent system used was a gradient of A (water, pH 2.6 made with H_3PO_4) and B (acetonitrile−MeOH−water 1:3:1). The following gradient was applied: 0 min, 5% B; 0−5 min, 12% B; 5−10 min, 12% B isocratic; 10−44 min, 50% B; 44−70 min, 50% B isocratic. The solvent flow rate was 0.8 mL min^{-1}, and the separation was performed at 35 °C. Retention times and spectral characteristics were compared to commercial standards [(+)-catechin and (−)-epicatechin purchased from Extrasynthèse (Genay, France)] and to compounds isolated and identified previously (Oleszek et al., 1994). Quantitation was carried out by external calibration at 325 nm for hydroxycinnamics (5′-caffeoylquinic, *p*-coumarylquinic, *p*-coumarylmalic, and dicaffeoylquinic acids), at 280 nm for flavanols, and at 360 nm for flavonols. The concentrations of phenolics were expressed in milligrams per 100 g of fresh weight (FW) equivalent 5′-caffeoylquinic for hydroxycinnamics, equivalent (−)-epicatechin for flavanols, and equivalent quercetin 3-glucoside for flavonols. The variability of 7% was determined on five extractions of phenolics from cv. Williams pears. Statistical analyses on phenolic composition were performed using SAS software (SAS Institute, 1985); years 1992 and 1993 were considered as replicates.

Measurement of Browning. *(a) Year 1992.* Fresh powder (10 g) was homogenized for 2 min in 10 mL of distilled water or in 10 mL of inhibiting solution containing 2 mM NaF and 15 mM ascorbic acid. The suspensions were stirred at room temperature for 1 h and centrifuged at 15000*g* for 15 min. The pellet was rehomogenized with 2 mL of inhibiting solution. Reflectance measurements (L^* = lightness, a^* = green/red, and b^* = blue/yellow chromaticity) were determined on the supernatants and the rehomogenized pellets in a 3 mL cuvette with a chromameter Minolta CR300 apparatus. Two replicates measurements were performed.

(b) Year 1993. Two hundred grams of fresh fruit was mixed for 1 min with or without 0.1% NaF + 0.1% thiourea. The purées obtained were stirred for 1 h at room temperature. Three small Petri dishes were filled with each purée, browned or not. Reflectance measurements (L^*, a^*, b^*) were carried out with the Minolta CR300 apparatus. The susceptibility of pears to browning was determined by the difference between the tristimulus coordinates measured on browned and not-browned purées. The data were expressed in DL^*, Da^*, and Db^*. The coefficient of variation was less than 1% (data not reported in tables).

Changes in Phenolic Composition after Oxidation. Browned and not browned purées (25 g), made as previously described, were homogenized for 1 min in 75 mL cold ethanol (95%) and stirred for 20 min at 4 °C. Extraction, purification, and determination of phenolics were conducted as described above.

Controlled Atmospheres. Controlled atmospheres containing 1% O_2−1% CO_2 and 3% O_2− 3% CO_2 were produced in gastight containers (20 L) held at 4 °C and maintained during 2 months according to the method described by Chapon and Bony (1990). The gaseous composition was adjusted every day and analyzed using a Servomex gas analyzer (Servomex, La Plaine Saint-Denis, France) equipped with an oxygen analyzer (type 1420) and a CO_2 IR transducer (type 12 *x*1).

RESULTS

Effect of Cultivar and Maturity Stages on the Enzymatic Browning and the Phenolic Composition of Pear Flesh and Peel. The data on browning and phenolic content obtained in 1992 are presented in Tables 1 and 2. The degree of enzymatic browning was measured and is expressed as DL^* (Table 1). The values of DL^* measured on supernatants were higher in peel than in flesh. In peel, more soluble pigments have been extracted than in flesh, where the main brown pigments either remained bound to membranes or were precipated by polymerization. The more intense browning in peel could be explained by the higher content of phenolics (Table 2). This higher content was characteristic for all classes of phenolics (hydroxycinnamics, flavanols, and flavonols) except for cv. P2198, for which fruit flesh had a high content of hydroxycinnamics. Pear flesh contained mainly hydroxycinnamics and flavanols, while peel was rich in flavanols and flavonols.

Great variations in the susceptibility of pears to browning and in phenolic composition were apparent among the cultivars studied (Table 1). The level of browning was high for cv. Abbe Fetel, Comice, P2198, and Guyot and low for cv. 6.30.100 and Harrow Sweet. In some cultivars, i.e. cv. Abbe Fetel, Comice, and P2198, higher browning could be related to a higher phenolic content (Table 2). In contrast, cv. Guyot, characterized by high browning data, had fruits with a low content of phenolics compared to the other cultivars studied.

Table 2. Phenolic Composition (Milligrams per 100 g of Fresh Weight) of the Flesh and Peel of Nine Pear Cultivars Harvested in 1992 at Different Maturity Stages (See Table 1 for Harvest Dates)

	hydroxycinnamics			flavanols			flavonols			total amount		
cultivar	flesh	peel	fruit	flesh	peel	fruit	flesh	peel	fruit	flesh	peel	fruit
Williams 1	3.4	21.9	7.1	1.0	44.1	9.6	1.1	41.2	9.1	5.5	107.2	25.8
Williams 2	0.9	24.0	5.5	2.6	65.9	15.3	0.6	37.1	7.9	4.1	127.0	28.7
Williams 3	1.9	24.2	6.9	1.1	50.1	10.9	0.1	20.6	4.2	3.1	94.9	21.4
Harrow Sweet 1	2.7	31.5	8.5	1.2	23.1	5.6	0.5	34.5	11.5	4.4	89.1	25.6
Harrow Sweet 2	1.8	11.3	3.7	2.4	25.8	7.1	0.2	35.2	7.2	4.4	72.3	18.0
Harrow Sweet 3	1.9	10.2	3.6	1.5	27.2	6.6	0.4	34.8	7.3	3.8	72.2	17.5
Guyot 1	3.4	9.2	4.6	0.2	27.0	5.6	0.1	23.6	4.8	3.7	59.8	15.0
Guyot 2	3.2	14.9	5.5	0.3	32.3	6.7	0.3	22.6	4.7	3.8	69.8	16.9
Guyot 3	3.7	15.0	5.9	1.7	25.0	6.4	0.3	25.5	5.3	5.7	65.5	22.7
P2198 1	27.2	23.8	26.5	2.3	54.5	16.2	1.8	44.2	10.3	31.3	122.5	53.0
P2198 2	4.1	14.8	6.2	4.4	59.7	15.6	0.2	29.2	6.0	8.7	103.7	27.8
Comice	0.8	23.7	5.3	0.7	64.3	13.4	0.2	55.4	11.3	1.7	143.4	30.0
6.30.100	3.7	10.1	5.0	0.3	6.8	1.6	0.0	30.9	6.2	4.0	47.8	12.8
Conference	3.9	17.4	6.6	0.4	32.0	6.7	0.2	43.1	14.9	4.5	92.5	28.2
Abbe Fetel	3.8	34.4	9.9	2.6	76.2	17.3	1.1	50.3	10.3	7.5	160.9	37.5
Passe Crassane	2.3	22.1	6.3	3.9	23.0	15.4	0.2	33.9	6.9	6.4	79.0	18.6

Table 3. Enzymatic Browning of the Fruits of Seven Pear Cultivars at Harvest Time (1993) and after 4 Days at Room Temperature

		at harvest			after 4 days at room temperature		
cultivar	harvest date	DL^{*a}	Da^{*a}	Db^{*a}	DL^*	Da^*	Db^*
Williams	Aug 20	−13.3	11.3	−1.3	−19.0	11.8	−6.4
Guyot	Aug 17	−9.9	8.5	−3.9	−11.8	7.1	−2.2
P2198	Aug 17	−15.3	13.3	−0.6	−15.8	13.3	−3.3
Comice	Sept 28	−15.1	12.3	−2.5	−18.1	11.8	−2.9
6.30.100	Sept 28	−4.8	5.6	−1.3	−7.7	6.2	−3.2
Conference	Sept 10	−14.1	11.9	−5.3	−13.3	11.2	−5.3
Abbe Fetel	Sept 14	−16.2	13.8	−3.1	−14.7	12.2	−1.0

[a] DL^*, Da^*, Db^* = differences in lightness and in chromaticity between oxidized and nonoxidized pears.

Table 4. Phenolic Composition (Milligrams per 100 g of Fresh Weight) of Seven Pear Cultivars at Harvest in 1993 (See Table 3 for Harvest Dates) and after 4 Days of Storage at Room Temperature

	hydroxycinnamics		flavanols				
cultivar	5′CQ[a]	total	CAT[a]	EPI[a]	total	flavonols	total amt
Williams	10.0	14.2	0.5	2.1	3.4	5.8	23.4
stored	8.0	11.5	0.4	1.2	2.1	4.8	18.5
Guyot	4.3	6.5	0.1	1.2	3.2	6.2	15.9
stored	3.9	5.6	nd[b]	1.1	3.4	4.2	13.2
P2198	11.5	15.2	0.3	3.2	5.5	2.5	23.2
stored	10.5	14.0	nd	2.5	5.3	2.8	22.1
Comice	8.0	10.1	0.2	1.7	6.1	6.9	23.1
stored	6.1	7.1	nd	2.1	5.9	3.7	16.7
6.30.100	2.7	3.8	nd	0.6	1.7	1.5	7.0
stored	1.8	2.5	nd	0.1	1.2	1.2	4.9
Conference	8.5	10.5	0.1	2.0	5.3	4.8	20.6
stored	8.7	10.2	nd	1.0	2.4	7.1	19.7
Abbe Fetel	14.1	16.8	0.5	8.7	17.3	7.1	41.2
stored	11.3	13.9	0.4	5.6	12.7	7.4	34.0

[a] 5′CQ, 5′-caffeoylquinic acid; CAT, (+)-catechin; EPI, (−)-epicatechin. [b] nd, not detected.

For fruits picked at dates close to the commercial maturity stage and for a given cultivar, the susceptibility of pears to browning and the phenolic content were not greatly different (Tables 1 and 2). However, the level in phenolic content tended to decrease with delayed harvest time. In the fruit flesh of cv. Williams (Table 2), the total phenolic content dropped from 5.5 to 3.1 mg 100 g^{-1} of FW for fruits picked on July 27 and August 11, respectively. As it was previously described by Macheix et al. (1990) for many fruit species, the maximum of hydroxycinnamic and catechin content occurs during growth. Then, there is a rapid decrease, and the fluctuations are usually low during fruit maturation, as it appeared in our results.

Effect of Cultivar on the Enzymatic Browning and the Phenolic Composition of Pears (Whole Fruit) at Commercial Maturity and after 4 Days of Storage at Room Temperature. The data obtained in 1993 are presented in Tables 3 and 4. It has to be stressed that in 1993 the commercial maturity occurred later than in 1992 (see Table 1). However, the variation of the susceptibility of pears to browning and of the phenolic content in 1993 was similar to that obtained in 1992. Cv. Abbe Fetel, P2198, and Comice were characterized again by a high degree of browning, while cv. 6.30.100 had low browning (Table 3). In addition, the purées from cv. Abbe Fetel, P2198, and Comice were more reddish (higher Da^*) than that of the 6.30.100 cultivar. The cv. Guyot appeared to have a lower degree of browning in 1993 than in 1992 (Table 3).

Postharvest storage of fruits for 4 days at room temperature (Table 3) significantly influenced browning characteristics, with apparent differences noticed for the cultivars studied. The storage increased browning for cv. Williams, Guyot, Comice and 6.30.100, while browning decreased for cv. Conference and Abbe Fetel. For the cv. P2198, there was no significant change in the susceptibility of pears to browning . The Da^* values decreased slightly or were not different after 4 days at room temperature as compared to freshly harvest fruits. For all of the pear cultivars studied, 5′-caffeoylquinic acid and epicatechin were the two main compounds (Table 4). 5′-Caffeoylquinic acid represented between 70% and 85% of the total hydroxycinnamoyl derivatives. The other hydroxycinnamics were *p*-coumarylquinic, *p*-coumarylmalic, and dicaffeoylquinic acids. These results were in agreement with the findings of Risch and Herrmann (1988). The presence of these four cinnamics in pear fruits has been reported previously (Macheix et al., 1990). (−)-Epicatechin represented between 27% and 60% of the total flavanol content. (+)-Catechin remained very low and not detected in the fruits of the cv. 6.30.100. Besides these monomers, there were some oligomers (procyanidins) showing the same spectral characteristics as monomers, but their stuctures remain unknown.

After storage for 4 days at room temperature (Table 4), the phenolic content decreased for all of the cultivars studied. These results are in agreement with those recently reported by Siddiq et al. (1994). All three

Table 5. Effect of Storage Conditions on Enzymatic Browning and Phenolic Composition (Milligrams per 100 g of Fresh Weight) of Pear Fruit, Cv. Williams

					hydroxycinnamics		flavanols				
storage lag	storage conditions	DL^*	Da^*	Db^*	5′CQ[a]	total	CAT[a]	EPI[a]	total	flavonols	total amt
after 1 month	air	−16.0	10.7	−1.3	9.0	12.6	0.7	2.8	4.8	5.4	22.8
	1% CO_2−1% O_2	−16.4	11.8	−2.1	10.9	15.3	0.6	4.0	6.9	4.8	27.0
	3% CO_2−3% O_2	−13.1	10.9	−2.5	11.1	15.3	0.9	2.9	4.7	5.9	25.9
after 2 months	air	−20.2	10.8	−3.9	13.3	18.8	0.9	5.0	12.0	9.5	40.3
	1% CO_2−1% O_2	−16.2	10.1	−1.3	9.9	13.5	0.8	3.6	6.8	7.1	27.4
	3% CO_2−3% O_2	−16.5	10.5	−2.0	11.5	15.9	0.8	4.2	8.3	7.5	31.7

[a] 5′CQ, 5′-caffeoylquinic acid; CAT, (+)-catechin; EPI, (−)-epicatechin.

Table 6. Residual Phenolic Composition (Milligrams per 100 g of Fresh Weight) Stored under Various Conditions

		hydroxycinnamics		flavanols				
storage lag	storage conditions	5′CQ[a]	total	CAT[a]	EPI[a]	total	flavonols	total amt
after 1 month	air	2.1	4.5	0.0	0.5	0.9	5.0	10.4
	1% CO_2−1% O_2	1.8	4.6	0.0	0.6	1.0	4.1	9.7
	3% CO_2−3% O_2	2.3	4.9	0.0	0.6	1.1	5.9	11.9
after 2 months	air	1.8	3.6	0.0	0.5	0.9	6.8	11.3
	1% CO_2−1% O_2	1.9	3.8	0.0	0.6	0.9	5.7	10.4
	3% CO_2−3% O_2	1.0	1.4	0.0	0.4	0.7	5.4	7.5

[a] 5′CQ, 5′-caffeoylquinic acid; CAT, (+)-catechin; EPI, (−)-epicatechin.

phenolic classes were affected. However, the cv. P2198 appeared to have a profile after 4 days similar to that at harvest, while cv. Conference showed an increase in flavonols roughly equivalent to the observed drop in flavanols.

Effect of Storage Conditions on Enzymatic Browning and Phenolic Composition of Pears (Cv. Williams). The data obtained in 1993 are reported in Table 5. Both enzymatic browning (DL^*) and total phenolic content increased under all of the storage conditions studied. However, the rates in accumulation of phenolics were different among the storage conditions used. During the first month of storage in air, total phenolic content slightly increased from 18.5 at harvest to 22.8 mg 100 g^{-1} of FW at the end of month. A second month of storage resulted in a further large increase up to 40.3 mg 100 g^{-1} of FW (Table 5). Most affected were flavanols, of which the concentration increased from 4.8 to 12.0 mg 100 g^{-1} of FW, followed by flavonols (5.4 to 9.5 mg of 100 g^{-1} of FW) and cinnamics (12.6 to 18.8 mg 100 g^{-1} of FW). Controlled atmospheres strongly reduced the ability of pears to synthesize phenolics. The concentration of cinnamics did not change under controlled atmosphere, while the amount of flavanols slightly increased under 1% CO_2−1% O_2 but not under 1% CO_2−1% O_2 after 2 months of storage. Flavonol concentration increased under the two controlled atmospheres studied to the same level, but not so much as occurred in air. An increase in chlorogenic acid was previously found in d'Anjou pears during 170 days at 1 °C (Meadows, 1983).

Changes in Phenolic Content during Browning. The data obtained for 1993 are reported in Table 6. The residual phenolic content can be compared to the initial content of the unstored fruits of cv. Williams, reported in Table 5. All of the phenolic compounds were affected during oxidation. However, 5′-caffeoylquinic acid and flavanols were more degraded than flavonols. Among hydroxycinnamics, the monophenols (i.e. coumaroyl derivatives) were less affected than the *o*-diphenols. After oxidation, 5′-caffeoylquinic acid represented 50% of the hydroxycinnamic esters except for in pears stored under 3% CO_2−3% O_2, for which it made up 70% of the total and remained very low. For the six treatments performed, the flavanol content, of both monomers and oligomers, decreased strongly. In contrast, flavonols were less affected than hydroxycinnamics and flavanols.

DISCUSSION AND CONCLUSION

Great differences in the phenolic composition of the pears studied were found. More quantitive differences were due to the cultivar than to the stage of maturity at which fruits were harvested. In 1993, the whole fruit was taken. In fact, fruit-derived products (purées and juices) are usually processed from the whole fruit without peeling. Comparison of data obtained for 1992 (Table 2) with those for 1993 (Table 4) shows that the trends in phenolic content were very similar. The seven cultivars studied for the two years did not show a significant difference at level 5% (Tukey's honest significant difference test) in the mean of the phenolic composition of 1992 and 1993. There was a slighty significant effect ($P < 0.1$) of the cultivar on the hydroxycinnamic content and on the total amount. The classification (from the total highest content to the total lowest content) was for the two years: Abbe Fetel > P2198 > Comice > Williams > Conference > Guyot > 6.30.100.

In addition, the storage of cv. Williams pears under low concentrations of O_2 and CO_2 seemed to be successful at reducing the susceptibility of the pears to browning. These data support previous studies, which reported the diminution of the risk of brown core development in pears stored under low-oxygen conditions (Yoshida et al., 1986; Chen et al., 1986). While the phenolic content decreased for all of the cultivars studied, the susceptibility of pears to browning after 4 days at room temperature (Table 3) depends on the cultivar. This suggests that other factors have to be taken into account, especially the changes in polyphenol oxidase activity.

5′-Caffeoylquinic acid and (−)-epicatechin, the two major compounds found in pears, have been shown to be the best endogenous substrates for pear polyphenol oxidases (Halim and Montgomery, 1978; Rivas and Whitaker, 1973; Siddiq et al., 1994; Tale et al., 1964). Beside catechins, some oligomers (procyanidins) have been found to be particularly important in enzymatic browning. In the present work, flavanols were strongly

degraded during oxidation and certainly contributed to a large extent in the development of the brown pigments (Table 6). The degradation of hydroxycinnamic derivatives was also quite strong, so their contribution to brown coloration was also substantial. A considerable loss of hydroxycinnamic esters and a total loss of procyanidins have been previously reported in pear juices processed without SO_2 (Spanos and Wrolstad, 1990; Wrolstad et al., 1988). In pears, flavonols were identified as isorhamnetin and quercetin glycosides (Oleszek et al., 1994). They were less affected by oxidation than hydroxycinnamic esters and flavanols (Table 6), however, suggesting that pear flavonol glycosides are not substrates or are poor substrates of pear polyphenol oxidases. Flavonols are degraded by coupled reactions. They could be an important factor in enzymatic browning. First, due to the coupled oxidation, the oxidized amount of flavonols could decrease the amount of quinones resulting from other phenolics, such as hydroxycinnamics and flavanols (Goupy et al., 1995). Second, the pigments resulting from flavonols lead to widely different values in L^*, a^*, and b^* (Rouet-Mayer et al., 1990; Richard-Forget et al., 1992; Goupy et al., 1995).

A high correlation has been established between Da^* of purées obtained with freshly harvested fruits and the initial content in 5′-caffeoylquinic acid ($r = 0.925$) (Tables 3 and 4). There was no correlation, however, between browning values and catechin content, as was previously found for five cultivars (Vamos-Vigyázó and Nádudvari-Márkus, 1982). The correlation between Da^* and 5′-caffeoylquinic acid remained strong after 4 days of storage at room temperature ($r = 0.905$). 5′-Caffeoylquinic acid, considered to be the best substrate of polyphenol oxidase in pears (Rivas and Whitaker, 1973), appeared to be the major determinant in the development of reddish pigments. This correlates well with the data on apple phenolics mixtures (Goupy et al., 1995), for which it was shown that an increase in 5′-caffeoylquinic acid content caused the increase in a^* value, whereas epicatechin had almost no effect. In pears, the correlation between Da^* and the total amount of hydroxycinnamic esters and flavanols was strong at harvest ($r = 0.848$). Thus, hydroxycinnamic esters and flavanols appeared to play the major role in the enzymatic browning of pears also.

The degradation of phenolic compounds in pears could be the result of the direct oxidation by polyphenol oxidases and of coupled oxidation, as was described in other fruits, such as grapes (Cheynier et al., 1988; Cheynier and Ricardo da Silva, 1991) and apples (Oleszek et al., 1989; Richard-Forget et al., 1992). Nonenzymatic reactions depend on the amount of *o*-quinones initially formed enzymatically. The amount of *o*-quinones depends mainly on the amount of the best substrates (5-caffeic derivatives and catechins) and on the polyphenol oxidase activity.

Further studies are needed for a better control of enzymatic browning in pear-derived products, in relation to the initial phenolic content and the polyphenol oxidase activity. Experiments on storage under controlled atmospheres should be done to confirm the regulation of phenolic biosynthesis in pears before processing. The effect of CO_2 on polyphenol oxidase has to be investigated.

ACKNOWLEDGMENT

We thank C. Sautel for technical assistance, J. F. Chapon and Ph. Bony from the Centre Technique Interprofessionnel des Fruits et Légumes at St Rémy de Provence, France, for controlled atmospheres, and M. le Lezec from the Institut National de la Recherche Agronomique at Angers, France, and M. Chartier from the Station Expérimentale Arboricole de la région Provence-Alpes-Côte d'Azur at Mallemort, France, for providing pears and their advice in the selection of cultivars. We also thank Dr. R. J. Robins from the Institute of Food Research at Norwich, England, for help in proofreading the manuscript.

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Received for review August 1, 1994. Revised manuscript received February 6, 1995. Accepted March 7, 1995.[⊗] This study was supported by the grants of the Institut National de la Recherche Agronomique ("AIP Matural") and of the Ministère de la Recherche et de l'Espace to the last author.

JF940434B

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1995.