JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Pink Discoloration of Canned Pears: Role of Procyanidin Chemical Depolymerization and Procyanidin/Cell Wall Interactions

Carine Le Bourvellec,*[,†,‡] Barbara Gouble,[†,‡] Sylvie Bureau,[†,‡] Michèle Loonis,[†,‡] Yves Plé,[§] and Catherine M. G. C. Renard[†,‡]

[†]INRA, UMR408 Sécurité et Qualité des Produits d'Origine Végétale, F-84000 Avignon, France

[‡]Université d'Avignon et des Pays de Vaucluse, UMR Sécurité et Qualité des Produits d'Origine Végétale, F-84000 Avignon, France.

[§]CTCPA, ZA de l'Aéroport, F-84000 Avignon, France

ABSTRACT: After canning, pear pieces turn occasionally from whitish-beige to pink. Conditions were set up to obtain this discoloration systematically and investigate its mechanism. Canned pears showed a significantly lower L^* coordinate compared with fresh pears, and the L^* coordinate of canned pears decreased with decreasing pH. The values of the a^* and b^* coordinates increased significantly after processing, the increase being greater for the more acidic pH values, with corresponding redder colors. After canning, polyphenol concentrations decreased significantly, mainly due to loss of procyanidins. This supported the hypothesis of conversion of procyanidins to anthocyanin-like compounds. However, no soluble product was detected at 520 nm, the characteristic wavelength of anthocyanins. When purified procyanidins were treated at 95 °C at three different pH values (2.7, 3.3, and 4.0), procyanidin concentrations decreased after treatment, the more so as the pH was lower, and a pinkish color also appeared, attributed to tannin−anthocyanidin pigment. The pink color was bound to cell walls. Extraction of the neoformed pink entities was attempted by successive solvent extractions followed by cell wall degrading enzymes. The pink color persisted in the residues, and canned pears gave significantly higher amounts of residues after solvent and enzyme treatments than fresh pears. Procyanidins were the entities responsible for the appearance of pink discoloration. However, it seems that this pink discoloration also involved the formation of strong, probably covalent, bonds to the cell wall.

KEYWORDS: *Pyrus communis L., processing, condensed tannins, polyphenol, polysaccharide, covalent binding*

INTRODUCTION

Pink discoloration of canned pears is an occasional problem to the canner, as it adversely affects commercial value.[1−4] It can also appear during the processing of other fruits such as quinces,[5] apples, bananas, gooseberries, guavas, and peaches.[6] The appearance of pink discoloration depends on various factors, such as pH, temperature, treatment time, type of can used, and storage.[1−4,7] However, the main processing factors leading to increased discoloration are excessive heating and delayed cooling of the cans.[6] This phenomenon is poorly understood. A possible mechanism is chemical depolymerization of procyanidins, also referred to as condensed tannins, under hot acidic conditions during processing, and their conversion into anthocyanidins.[1,5,8−13] In hot acidic conditions, the interflavan bond, which is relatively fragile, is cleaved (Figure 1). In this process, terminal units are released as free flavan-3-ols, whereas intermediate C-4 carbocations are formed from the extension units. These carbocations are very reactive and can either give anthocyanidins by autoxidation[8,11,13] or react with nucleophilic compounds such as cell wall polymers to form covalent bonds,[14] as in the case of protein.[10]

Proanthocyanidins, also known as condensed tannins, are widely found as secondary metabolites. They are present in fruits, barks, leaves, and seeds of many plants.[15,16] Proanthocyanidins affect the physicochemical and organoleptic properties of fruits, vegetables, and foods such as purees, ciders, and wines by contributing to their color, astringency, and bitterness.[17,18] Proanthocyanidins are also unstable compounds that undergo numerous enzymatic and chemical reactions during processing. Three common flavan-3-ols, which differ in their hydroxylation patterns, are found in proanthocyanidins. Procyanidins consist exclusively of (+)-catechin and/or (−)-epicatechin subunits. Propelargonidins and prodelphinidins, less abundant than procyanidins, consist of (epi)afzelechin and (epi)gallocatechin subunits, respectively. In the pear, the polymeric flavan-3-ols, that is, proanthocyanidins, are procyanidins and are the predominant class of phenolic compounds. Pear procyanidins are oligomers or polymers, >95% of which are composed of (−)-epicatechin units, most frequently linked to one another by either C4−C8 or C4−C6 bonds.[19] Their average size differs among varieties: number-average degrees of polymerization ($\overline{\mathrm{DP}}n$) between 4 and 35 can be observed.[20−22]

It is important to understand the influence of thermal treatment on the polyphenolic compounds contained in fruits and vegetables, because we consume them daily, most often after cooking or processing. The purpose of this research was to establish the involvement of procyanidins in pink discoloration. To understand the changes caused by the heating of pears, the phenolic profile and color of fresh and canned pear samples were determined, and the mechanism causing pink discoloration was investigated. For this purpose, change in purified

Received: February 11, 2013
Revised: June 3, 2013
Accepted: June 3, 2013
Published: June 3, 2013

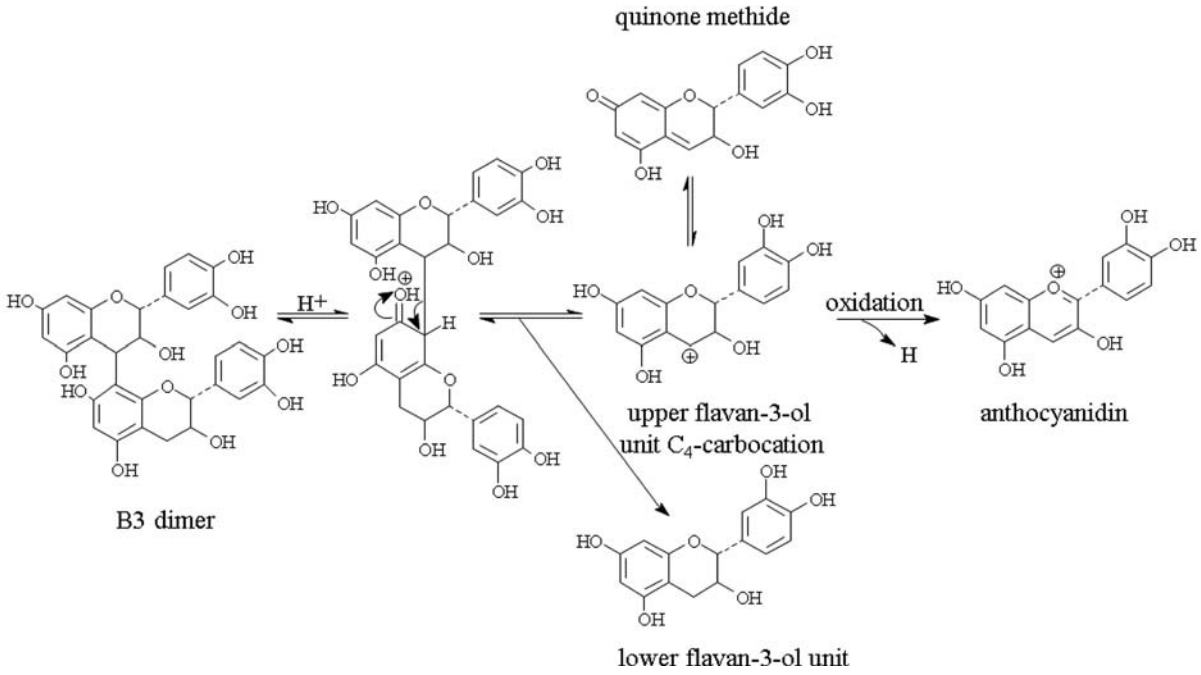


Figure 1. Acid-catalyzed depolymerization of procyanidins. Example of dimer B3 (adapted from Beart et al.[10]).

Table 1. Polyphenol Composition and Concentrations (Milligrams per Kilogram Dry Weight) and Procyanidin $\overline{DP}n$ in the Flesh of Fresh and Canned Pears[a]

		pH			flavan-3-ols						phenolic acids	
sample	origin	citric acid	citric and ascorbic acids	PV	EC	PCA	$\overline{DP}n$	% CAT_t	%EC_t	%EC_{ext}	5-CQA	tryptophan-*N*-glycoside
SO fresh	Soses				50.2	1672.6	5.8	6.0	8.6	85.6	567.9	
SO past	Soses	3.3		304		372.7	5.3	6.3	12.4	81.2	213.7	722.8
pH 2.7 SO	Soses	2.7		384.9		160.9	5.5	0.0	18.1	81.9	170.2	4189.9
pH 3.3 SO	Soses	3.3		384.9		455.2	5.2	4.3	15.0	80.7	263.7	825.8
pH 4.0 SO	Soses	4.0		384.9		843.6	6.9	3.3	11.2	85.5	254.9	329.0
citric SO	Soses	3.3		662.1		392.0	4.3	7.3	15.9	76.8	367.4	1938.8
ascorbic SO	Soses		3.3	662.1		218.1	4.2	0.0	24.0	76.0	182.4	2801.5
CA fresh	Cavaillon				67.2	1769.9	4.4	6.7	12.9	80.4	441.6	
CA past	Cavaillon	3.3		388.1		273.2	3.9	6.2	19.4	74.4	235.9	1109.5
pH 2.7 CA	Cavaillon	2.7		418.2		276.8	2.8	8.7	27.0	64.4	276.6	3079.4
pH 4.0 CA	Cavaillon	4.0		418.2		553.4	5.2	5.0	14.3	80.7	241.4	382.8
citric CA	Cavaillon	3.3		341.5		477.0	5.2	4.4	14.9	80.7	265.4	1190.3
ascorbic CA	Cavaillon		3.3	341.5		549.1	3.9	7.3	18.2	74.5	242.1	1539.7
CB fresh	Cheval-Blanc				85.9	2710.0	7.6	3.9	6.5	89.6	664.0	
CB past	Cheval-Blanc	3.3		233.9		855.2	6.0	5.2	11.5	83.3	318.0	682.4

[a]PV, pasteurization value in minutes; EC, (−)-epicatechin; PCA, procyanidins; $\overline{DP}n$, number-average degree of polymerization of flavan-3-ols (catechins + procyanidins); %CAT_t, percentage of (+)-catechin as terminal unit; %EC_t, percentage of (−)-epicatechin as terminal unit; %EC_{ext}, percentage of (−)-epicatechin as extension unit; 5-CQA, 5-caffeoylquinic acid; tryptophan-*N*-glucoside, quantified in tryptophan equivalent.

procyanidin extracts during heat treatment at different pH values were determined, successive solvent extractions and enzymatic digestions were carried out to extract pink color pigment from canned pears, and interactions between purified procyanidin fractions and cell wall material from pears were studied with and without heat treatment.

MATERIALS AND METHODS

Standards and Chemicals. Methanol, acetonitrile, acetic acid, hexane, and acetone were from Fischer Scientific (Pittsburgh, PA, USA). Chlorogenic acid, (+)-catechin, and (−)-epicatechin were from Sigma-Aldrich (Darmstadt, Germany). Sugar standards (rhamnose, fucose, xylose, mannose, galactose, and galacturonic acid) were from Fluka-Biochemica (Sigma-Aldrich, Steinheim, Germany). Inositol and glucose were from Merck (Darmstadt, Germany). Methanol-d_3 was from Acros Organics (Geel, Belgium). Arabinose, $NaBH_4$, *N*-methylimidazole, benzylmercaptan, and acetic anhydride were from Sigma-Aldrich. Enzymatic cocktails (Endozym polifruit, Endozym pectofruit Liq+, and Endozym pectofruit xl) were from Spindal groupe AEB (Gertz-Armainvilliers, France).

Plant Material. Pear fruits (*Pyrus communis* L.) of the William variety were harvested at commercial maturity during the 2009 season in Soses, Spain (SO); Cheval-Blanc, France (CB); and Cavaillon, France (CA) and were used for canning (see Industrial Processing and Sample Preparation).

For the cell wall material preparation, pears of the William variety were purchased at the local market during the 2010 season.

Perry pears of the Fausset variety, used for procyanidin preparation, were harvested before technological maturity on October 10, 2002, in the orchard of M. Boisgontier (Orne, France). The fruits were stored at ambient temperature for 12 days and chilled by storage for 2 days at 2 °C before processing.[21]

Industrial Processing and Sample Preparation. Canned pear processing was carried out by the Centre Technique de la Conservation des Produits Agricoles (CTCPA), Avignon. Pears were peeled manually and cut into two parts. One part was frozen, freeze-dried, and stored at −20 °C for analysis. The other part was packed in 720 mL Twist Off jars, 380 g of pear per jar. A hot (70 °C) sucrose syrup was then added to obtain a Brix degree of 17 ± 0.5° and for a final composition of 54% of pear and 46% of syrup. The pH of the syrup was adjusted to obtain three different pH values: 4.0, 3.3, and 2.7. For pH 4.0, the pH of fresh pears was close to 4.0, and so no acid was added to the syrup. For pH 2.7, the acidity was adjusted with citric acid: addition of 18.2 g/L citric acid for pears from Cavaillon and 14.7 g/L of citric acid for pears from Spain. For pH 3.3, the acidity was adjusted with citric acid: addition of 5.5 g/L of citric acid for pears from Cavaillon and 5.8 g/L for pears from Spain. A pH of 3.3 was obtained by adjusting by mixing one-third citric acid and two-thirds ascorbic acid: the pH was adjusted by adding 14.6 g/L of ascorbic acid and 7.5 g/L of citric acid. Canned pears were hot-conditioned and treated at 98 °C for various times (Table 1).

For analysis, canned pears were separated from the syrup and then frozen, freeze-dried, and stored at −20 °C before analysis.

Preparation of Cell Wall Material. Alcohol-insoluble solids (AIS) from William pear cultivar were prepared according to the methods of Renard et al.[23] and Renard.[24] The sample was named cell wall material (**CWM**).

Extraction and Purification of Procyanidin Fraction. Pear procyanidins were extracted and purified from pear juice of the Fausset variety as described by Le Bourvellec et al.[21] The fraction was named **Pdp35** for purified procyanidin fraction of number-average degree of polymerization 35 from pears. The fraction was stored under argon at −20 °C before use.

Solvent Extractions and Enzymatic Digestions of Fresh and Canned Pears. Hexane, methanol, and aqueous acetone extracts of pear polyphenols were obtained by successive solvent extractions of freeze-dried fresh (fresh pear from Soses, sample **SO fresh**, Table 3) and canned pears (pear from Soses heated with a pasteurization value of 384.9 min at pH 2.7, sample **pH 2.7 SO past**, Table 3).[25] Extracts corresponding to each solvent extraction were pooled and concentrated on a rotary evaporator before freeze-drying and thioacidolysis-HPLC-DAD analysis. The insoluble residue of extraction was frozen and freeze-dried.

The freeze-dried insoluble extraction residue was then degraded using enzymes, according to a method adapted from Micard et al.[26] Enzymatic cocktails (20 mg of protein) were added to 200 mg of insoluble residues of extraction in 40 mL of citrate−phosphate buffer (pH 6). The suspensions were incubated in hermetically sealed tubes placed under nitrogen to prevent oxidation, at 37 °C for 5 days. Hydrolysis was stopped by immersion in boiling water for 15 min. The supernatants were obtained by centrifuging (6000*g* for 15 min at 4 °C), freeze-dried, and analyzed by thioacidolysis-HPLC-DAD. The residues were freeze-dried and weighed. All solvent and enzymatic extractions were carried out in triplicate.

Three enzymatic cocktails were used: Endozym pectofruit Liq+, Endozym pectofruit xl, and Endozym polifruit. They are used respectively for fruit liquefaction, for juice clarification by pectin degradation, and as a pressing aid and contain various levels of pectolytic, hemicellulolytic, and cellulolytic enzymes. However, their specific activities were not tested here.

Heat Treatment of Purified Procyanidin Fraction at Different pH Values. Procyanidin fraction **Pdp35** (2 g/L) was incubated in sealed tubes under ambient atmosphere in citrate−phosphate buffer (pH 2.7, 3.3, and 4.0; ionic strength = 0.1 M) at 95 °C. Aliquots were drawn from the tubes at 0, 30, 60, 90, 120, 240, 320, and 420 min, and procyanidins were analyzed by HPLC-DAD with or without thioacidolysis. Samples of fraction **Pdp35** heated for 420 min at pH 2.7, 3.3, and 4.0 were named **heated Pdp35 pH 2.7**, **heated Pdp35 pH 3.3**, and **heated Pdp35 pH 4.0**, respectively, and were also further used for binding with pear cell wall material.

Binding Reaction of Purified Procyanidin Fractions with Pear Cell Wall Material as a Function of Heat Treatments and pH Values. The study of the binding of purified procyanidin fraction with pear cell wall material in model suspensions was conducted according to three designs and using the methods already described by Renard et al.[27] and Le Bourvellec et al.[21]

Cell wall material was placed in contact with native purified procyanidin fraction **Pdp 35** at low temperature (25 °C) at three different pH values (control); with preheated purified procyanidin fractions, that is, **heated Pdp 35 pH 2.7**, **heated Pdp 35 pH 3.3**, and **heated Pdp 35 pH 4.0** at low temperature (25 °C) at three different pH values ("pre"-procyanidin modification); and with native purified procyanidin fraction **Pdp 35** at high temperature (95 °C) at three different pH values (processing conditions).

Native **Pdp35** or **heated Pdp35 pH 2.7**, **heated Pdp35 pH 3.3**, or **heated Pdp 35 pH 4.0** procyanidin fractions (5 g/L) were incubated with a suspension of **CWM** (20 g/L, in citrate−phosphate buffer, ionic strength = 0.1 M) in sealed tubes with planetary stirring for 1 h at 25 °C. Native purified procyanidin fraction **Pdp35** (5 g/L) was incubated with a suspension of **CWM** (20 g/L, in citrate−phosphate buffer, ionic strength = 0.1 M) in sealed tubes under planetary stirring for 8 h at 95 °C. After incubation, the procyanidin solution and the bound procyanidin-cell wall complexes were separated by centrifugation (3000*g* for 15 min at 20 °C). Free procyanidins in the supernatant were measured by absorbance at 280 nm and/or HPLC following thioacidolysis after freeze-drying as described below under Analysis Methods. Bound procyanidin−cell wall complexes were further analyzed by thioacidolysis. Adsorptions were carried out at three different pH values, 2.7, 3.3, and 4.0, and all assays were carried out in duplicate.

Samples were named low native pH 2.7 (**LN pH 2.7**), low native pH 3.3 (**LN pH 3.3**), and low native pH 4.0 (**LN pH 4.0**) for binding at low temperature (25 °C) between native purified procyanidin fraction **Pdp 35** and pear cell wall material at pH 2.7, 3.3, and 4.0, respectively.

Samples were named low heated pH 2.7 (**LH pH 2.7**), low heated pH 3.3 (**LH pH 3.3**), and low heated pH 4.0 (**LH pH 4.0**) for binding at low temperature (25 °C) between **heated Pdp35** fractions (**heated Pdp35 pH 2.7**, **heated Pdp35 pH 3.3**, **heated Pdp35 pH 4.0**) and pear cell wall material at pH 2.7, 3.3, and 4.0, respectively.

Samples were named high native pH 2.7 (**HN pH 2.7**), high native pH 3.3 (**HN pH 3.3**), and high native pH 4.0 (**HN pH 4.0**) for binding at high temperature (95 °C) between native purified procyanidin fraction **Pdp35** and pear cell wall material at pH 2.7, 3.3, and 4.0, respectively.

Analysis Methods. Polyphenols were measured by high-performance liquid chromatography (HPLC)−diode array detection (DAD) after thioacidolysis using a method described by Le Bourvellec et al.[28] Procyanidins were characterized by thioacidolysis to determine subunit composition, average molecular mass, and the number-average degree of polymerization ($\overline{DP}n$). The $\overline{DP}_n$ of procyanidins was measured by calculating the molar ratio of all lavan-3-ol units (thioether adducts plus terminal units) to (−)-epicatechin and (+)-catechin corresponding to terminal units.

UPLC-MS analyses were performed on an Acquity ultraperformance LC (UPLC) apparatus from Waters (Milford, MA, USA), equipped with a UV−visible diode array detector and coupled with a Bruker Daltonics (Bremen, Germany) HCT ultra ion trap mass spectrometer with an electrospray ionization source. Separations were achieved using a Licrospher PR-18 5 μm column (Merck, Darmstadt, Germany) with a guard column (Licrospher PR-18 5 μm column, Merck) operated at 30 °C. The mobile phase consisted of water/formic acid (99:1, v/v) (eluent A) and acetonitrile/formic acid (99:1) (eluent B). The flow rate was 1 mL/min. The elution program was as follows: 3−9% B (0−5 min); 9−16% B (5−15 min); 16−50% B (16−

50 min); 50−90% B (45−48 min); 90−90% B (48−52 min). Samples were injected at a level of 10 μL. The column effluent was monitored at 280, 320, and 520 nm. The mass spectra were generated in the Ultrascan positive ion mode in the m/z range of 100−900. Nitrogen was used as the nebulizing gas. The ion source parameters were as follows: nebulizer pressure, 70 psi; drying gas flow, 12 L/min; desolvation temperature, 365 °C; and capillary voltage, 3 kV. Helium was used as the damping gas. Data were collected and processed using Bruker Compass DataAnalysis software.

Neutral sugars were analyzed as alditol acetates after acid hydrolysis. For cell walls containing cellulose, samples (ca. 10 mg of AIS) were prehydrolyzed with 250 μL of 72% sulfuric acid for 1 h at room temperature (Saeman procedure)[29] and then diluted to 1 mol/L by addition of water and internal standard (inositol). Samples were placed in an oven at 100 °C for 3 h for hydrolysis. After hydrolysis, they were derivatized to alditol acetates.[30] They were injected on a GC-FID HP 5890 series II instrument (Agilent, Inc., Palo Alto, USA) with a 30 m × 0.25 mm i.d. capillary column coated with DB225 MS, film thickness = 0.25 μm (J&W Scientific, Agilent, Inc., Palo Alto, CA, USA). The conditions were as follows: temperature of injection, 250 °C in split mode (ratio 1:25); hydrogen as carrier gas at 45 cm/s (at 215 °C); column flow, 1.3 mL/min; and oven temperature, isothermal at 215 °C. Uronic acids were measured spectrophotometrically by the *m*-hydroxydiphenyl assay using galacturonic acid as external standard.[31] Methanol was determined by headspace-GC-MS after saponification using CD_3OH as internal standard as described by Renard and Ginies.[32] The degree of methylation (DM) was calculated as the molar ratio of methanol to uronic acid.

The color of the freeze-dried pear powder was measured by surface reflectance spectra in a Konica-Minolta CM-2300d spectrocolorimeter (Minolta Co. Ltd., Osaka, Japan). Five measurements were made for each sample. The instrument measures the reflectance spectrum between 350 and 750 at 10 nm intervals. The CIE $L^*a^*b^*$ color coordinates of the samples were determined using a CR-400 chromameter (Minolta Co. Ltd.) and a glass cell made of optical glass with a path of 10 mm (Minolta Co. Ltd.). The CIE 1976 $L^*a^*b^*$ color coordinates[33] and hue angle ($\tan^{-1}(b^*/a^*)$) were calculated on the basis of D65 illuminant, 0° view angle, and illumination area diameter = 8 mm. In the evaluation of the hue angle we used the most widely acceptable criterion of assigning angle 0° to the semiaxis $+a^*$ (redness), angle 90° to the semiaxis $+b^*$ (yellowness), angle 180° to the semiaxis $-a^*$ (greenness), and angle 270° to the semiaxis $-b^*$ (blueness).[34] Eight measurements were made for each sample. In the $L^*a^*b^*$ space, a^* represents the green−red color and b^* the blue−yellow color of the samples. The L^* coordinate represents lightness, where $L^* = 0$ is completely black and $L^* = 100$ is completely white.

Statistical Analysis. Results are presented as mean values, and the reproducibility of the results is expressed as pooled standard deviation. Pooled standard deviations were calculated for each series of replicates using the sum of individual variances weighted by the sum of the individual degrees of freedom.[35]

Analysis of variance (ANOVA) was performed using the Excelstat package of Microsoft Excel.

RESULTS AND DISCUSSION

Characterization of Fresh and Canned Pear Samples. *Changes in Color during Heat Treatment.* Figure 2 presents the William pear variety before (A) and after (B) heat treatment (412.8 min, 95 °C).

The L^* coordinate with regard to pear processing, in particular, syrup pH, is represented in Figure 3A. Canned pears showed a significantly lower L^* coordinate than fresh pears ($F = 47.7$, $P < 0.001$); also (Figure 3A), the L^* coordinate of canned pears decreased with decreasing pH. L^* coordinates were also significantly lower ($F = 47.7$, $P < 0.001$) when the pH was adjusted with a citric acid−ascorbic acid mixture (ascorbic CA) than with citric acid alone (citric CA).

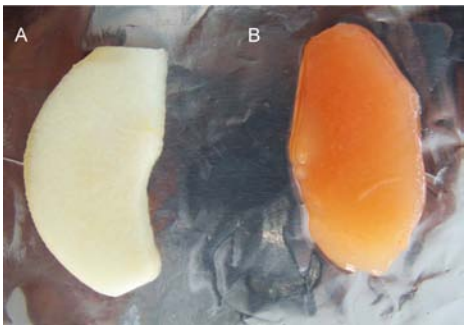
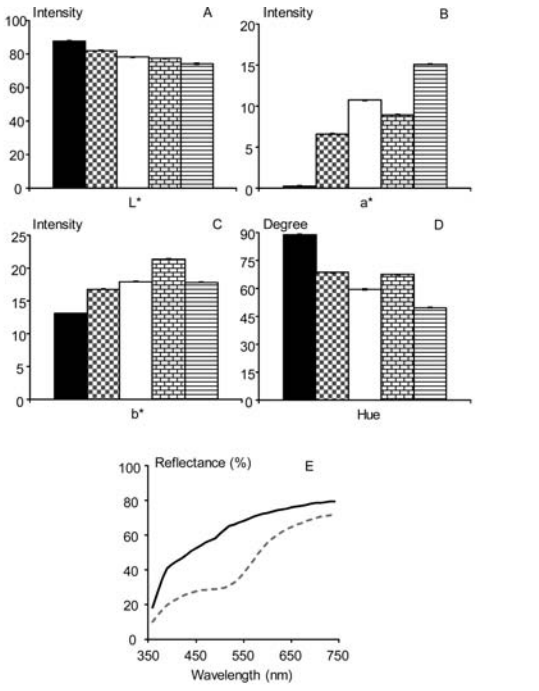


Figure 2. Photograph of fresh (CA fresh) (A) and pink canned pears (pH 2.7 CA) (B).



■ fresh pear from Cavaillon (CA fresh); ⊠ canned pear from Cavaillon heated at pH 4.0 with citric acid (pH 4 CA); □ canned pear from Cavaillon heated at pH 3.3 with citric acid (Citric CA); ▦ canned pear from Cavaillon heated at pH 3.3 with ascorbic acid (Ascorbic CA); ▤ canned pear from Cavaillon heated at pH 2.7 (pH 2.7 CA). ——— fresh pear from Cavaillon (CA fresh); ..... canned pear from Cavaillon heated at pH 2.7 (pH 2.7 CA).

Figure 3. Evolution of (A) L^* coordinate, (B) a^* coordinate, (C) b^* coordinate, and hue angle (D) as a function of pH of canned pear processing. Standard deviation intervals are represented, ddl = 35 (example of pears from Cavaillon). (E) Example of mean reflectance spectra (350−750 nm) of samples **CA fresh** and **pH 2.7 CA**.

The a^* and b^* coordinates (Figure 3B,C) increased significantly (a^* $F = 56.6$, $P < 0.0001$; b^* $F = 24.5$, $P < 0.0001$) after processing, the increase being greater for the more acidic pH values, with correspondingly redder colors. The increase in the b^* coordinate was greater when the pH was adjusted with ascorbic acid (samples **citric CA** and **ascorbic CA**). These samples turned yellow after processing.

Both coordinates a^* and b^* were used to calculate the hue angle (Figure 3D): the hue angle decreased significantly ($F = 80.0$, $P < 0.0001$) after processing. Conventionally, the hue angle measures the degree of departure from the red axis of the CIE color space, lower hue angles indicating greater visually

perceived redness. Hence, canned pear samples, which had a significantly lower (F = 80.0, P < 0.0001) hue angle, were characterized by a redder color than fresh pears.

The origin of the pears had a limited effect and was significant only for L^* (F = 19.2, P < 0.0001) and b^* (F = 11.0, P < 0.0001) coordinates.

The use of ascorbic acid to adjust the pH of the syrup did not prevent the appearance of the pink discoloration (Figure 3A–D) (**ascorbic CA** vs **citric CA**). The role of ascorbic acid was to prevent the conversion of the C_4 carbocation into anthocyanidin. Ascorbic acid only delayed the onset of color and did not prevent it. It also modified the final color, with increased yellowness in the presence of ascorbic acid. This is because ascorbic acid was consumed by oxidation; once all of the ascorbic acid had been oxidized, the oxidation reaction took over.

In Figure 3E, the average reflectance spectra between 350 and 750 nm are presented for samples **CA fresh** and **pH 2.7 CA**. A decrease in reflectance was observed across the spectrum. The **pH 2.7 CA** spectrum has a different shape from the **CA fresh** spectra, in particular, between 500 and 600 nm, which most noticeably could be associated with the significant modification of the $L^*a^*b^*$ values after processing. Also, the modification of the reflectance spectrum between fresh and processed pears reflected the appearance of new compounds absorbing at 520 nm.

Changes in Polyphenolic Profile during Heat Treatment. Table 1 summarizes the variants used in the industrial processing of canned pears and phenolic compositions of fresh and canned pears. The phenolic composition of William pear pulp was simple, with only two classes, flavan-3-ols and hydroxycinnamic acids, as confirmed by the literature.[20,22,36]

Native phenolics (determined by HPLC) ranged from 2279 mg/kg dry weight (DW) (pear from Cavaillon) to 3560 mg/kg DW (pear from Cheval-Blanc). This was in the range of previous studies on pear composition.[22,36] In fresh pears (Table 1, **SO fresh**, **CA fresh**, and **CB fresh**), polymeric flavan-3-ols, that is, procyanidins, were the predominant class. Procyanidin contents in William pears were lower than reported for Gieser Wildeman pear (4320 mg/kg DW)[22] or San Bartolomeu pear (24030 mg/kg DW).[20] Number-average degrees of polymerization ($\overline{\mathrm{DP}}n$) of procyanidins went from 4.4 (pear from Cavaillon) to 7.6 (pear from Cheval-Blanc) and were in the range reported.[22]

The flavan-3-ol monomers were only detected as (−)-epicatechin.[36] However, some studies have reported the presence of both (−)-epicatechin and (+)-catechin as flavan-3-ol monomer[20,37] whereas others report no flavan-3-ol monomer.[22] (−)-Epicatechin content in William pears was lower than reported in the literature.[20]

Hydroxycinnamic acids were mainly represented by 5-caffeoylquinic acid, which has been reported as the main hydroxycinnamic acid in pears.[20,22,36,38] Its content was comparable to literature values for San Bartolomeu pear[20] and Gieser Wildeman pear.[22]

After canning, the total polyphenol amounts detectable in the fruit decreased significantly (F = 131.8, P < 0.0001) by 51% (**pH 4.0 SO**) to 85% (**pH 2.7 SO**). The reduction observed was mainly due to the procyanidin loss. After canning, both procyanidin concentrations and $\overline{\mathrm{DP}}n$ decreased significantly (F = 112.3, P < 0.0001 and F = 8.7, P = 0.01, respectively), with a procyanidin concentration decrease more marked than the procyanidin $\overline{\mathrm{DP}}n$ decrease. At the same time, the pear sections turned from whitish-beige to pink (Figures 2 and 3A–D). This supported the hypothesis of procyanidin depolymerization and conversion into anthocyanin-like compounds[8,11,13] during processing.[5,22] However, it was not possible to detect soluble products absorbing at 520 nm, the wavelength characteristic of anthocyanins. Conventional boiling (100 °C, 20 min to 7 h) of pears[22] induces the coloration of pear section from whitish-beige to pink, and the color was limited to pear sections. The decrease in the $\overline{\mathrm{DP}}n$ of procyanidins could also be due to the formation of covalent linkages between carbocations, produced by chemical depolymerization of procyanidins,[8,9,11−13] and nucleophilic compounds of the plant matrix,[14] as reported by Baert et al.[10] for covalent binding of procyanidins to proteins. Reduction of the average procyanidin chain length ($\overline{\mathrm{DP}}n$) may arise from cleavage of the interflavanic bonds followed by nucleophilic addition of free (−)-epicatechin to the resulting intermediate carbocations.[39] Evidence of the addition of free flavan-3-ol monomers onto the intermediate carbocation was provided by the decline in free (−)-epicatechin monomer and concomitant increase in (−)-epicatechin and (+)-catechin terminal units and decrease in (−)-epicatechin extension unit (Table 3). Some 75% of the total increase in terminal subunits was due to (−)-epicatechin and the remaining 25% to catechin. As (+)-catechin was not present free in fresh pear, it may result from epimerization of (−)-epicatechin to (+)-catechin.[39] Different studies on processed fruits, including canned peach,[40] blueberry,[41] and pear,[22] reported losses in procyanidins and reduction of procyanidin $\overline{\mathrm{DP}}n$ in response to processing. Migration of procyanidin oligomer into the syrup could also contribute to the losses (procyanidin concentration and procyanidin $\overline{\mathrm{DP}}n$) observed during the canning process[42] but should result in increased $\overline{\mathrm{DP}}n$ in the fruit pieces.

No (−)-epicatechin was detected after processing, although an increase in flavan-3-ol $\overline{\mathrm{DP}}n$ would be expected in this case. Hernandez et al.[43] could not detect flavan-3-ol monomers after thermal treatments (120 °C, 20 min) of pear and apple juice. By studying the impact of thermal processing (104 °C, 10 min) on canned peach procyanidins, Hong et al.[42] have shown that there is a migration of flavan-3-ols monomers into the syrup. Also, conventional boiling (100 °C, 5 min) led to a significant loss of flavonoids (66%) from fresh raw broccoli inflorescence.[44] Thus, the decrease in (−)-epicatechin after canning may be due to migration in the syrup, thermal degradation during processing,[42−44] and addition on intermediate carbocations.[39]

Contents of 5-caffeoylquinic acid also decreased significantly (F = 56.4, P < 0.0001) after processing. However, this decrease was less pronounced than for flavan-3-ols. Conventional boiling (100 °C, 5 min) reduced total caffeoylquinic derivatives by 37% and total flavonoids by 66%. Vallejo et al.[44] have also shown that 5-caffeoylquinic acid is less affected by thermal treatment (120 °C, 20 min) than flavan-3-ol monomer. Similarly, Hernandez et al.[43] showed that reduction of 5-caffeoylquinic acid concentration in pear tissue after boiling (100 °C, 20 min) was due to both diffusion in the liquid phase and thermal degradation. Like (−)-epicatechin, the 5-caffeoylquinic acid decrease after processing may be due to leaching in the syrup, thermal degradation during processing,[22,44,45] or reaction with other compounds present in the medium.

A new compound, eluting early in the HPLC chromatograms at 280 nm, was observed in canned pears. This compound was further analyzed by HPLC-DAD/ESI-MS and ^{1}H NMR. It had

a deprotonated molecular ion $[M + H]^+$ at m/z 367. The product ion spectrum of deprotonated molecular ion $[M + H]^+$ at m/z 367 was dominated by fragment ions with m/z 205 and 188 corresponding to a typical loss of 162 mass units of an intact anhydro-sugar moiety ($C_6H_{10}O_5$) and subsequent loss of NH_3.[46,47] This fragmentation pattern is characteristic of *N*-glycosidically linked sugars.[48] This compound was identified as a tryptophan-*N*-glycoside, already detected in food samples[46,47] and particularly in pear juice.[46] Its concentration, expressed in tryptophan equivalent, ranged from 329 mg/kg DW (**pH 4.0 SO past**) to 41890 mg/kg DW (**pH 2.7 SO past**) in canned pears (Table 1). The appearance of this tryptophan-*N*-glycoside was closely correlated with the loss of procyanidin (Figure 4).

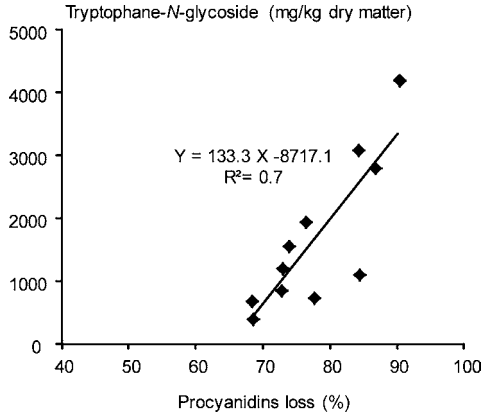


Figure 4. Tryptophan-*N*-glycoside concentration quantified in tryptophan equivalent as a function of procyanidin loss.

High temperature and acidic pH are prerequisites for the formation of tryptophan-*N*-glycosides.[46] Both procyanidin loss and appearance of tryptophan-*N*-glycosides could serve as markers of thermal processing of pear product.

Pear pieces became pink upon heat treatment, and the appearance of this pink discoloration was a function of both the pH of the syrup and the presence or absence of ascorbic acid. However, the origin of the pears had a limited effect on the pink discoloration. Pear processing was accompanied by the appearance of pink discoloration and a loss of procyanidins, but no pink soluble product could be detected at 520 nm.

Changes in Purified Procyanidin Fraction during Heat Treatment at Different pH Values. The appearance of pink discoloration coincided with the disappearance of procyanidins, and a potential causative link has been proposed. To test this hypothesis, procyanidins extracted from pears (**Pdp35**) were submitted to heat treatments at the same pH values (2.7, 3.3 and 4.0).

The Fausset variety of Perry pears was selected because of its high procyanidin $\overline{DP}$ of 35. In a typical procyanidin dimer such as B3 (Figure 1), only the upper unit may yield a carbocation and therefore be able to produce cyanidin. Oligomer and polymer chains are built up by addition of further "upper' units, and so the yield of cyanidin should increase as the chain length increases.[11] The **Pdp35** fraction was chosen to maximize the possibility of cyanidin formation and to study the impact of heat treatment on procyanidins.

The composition and the characterization of the pear procyanidin fractions **Pdp35** and **heated Pdp35** are summarized in Table 2. **Heated Pdp35** fractions correspond to the final point of the heat treatment (480 min at 95 °C) at different pH values (2.7, 3.3, and 4.0), and were further used for interaction with pear cell wall material.

Table 2. Composition (Milligrams per Gram) of the Pear Procyanidin Fraction Pdp35 before and after Treatment at 95 °C for 480 min (Fraction Heated Pdp35)[a]

		flavan-3-ols			phenolic acids
fraction	pH	EC	PCA	$\overline{DP}n$	5-CQA
Pdp35	2.7	5.1	599	34	118
heated Pdp35	2.7	6.0	282	22	96
variation (%)		*+18*	*−53*	*−36*	*−18*
Pdp35	3.3	5.4	616	35	121
heated Pdp35	3.3	6.0	328	18	105
variation (%)		*+11*	*−47*	*−48*	*−13*
Pdp35	4.0	3.9	605	35	118
heated Pdp35	4.0	4.7	369	22	99
variation (%)		*+22*	*−34*	*−37*	*−16*
SD		0.1	2.1	0.1	0.3

[a]EC, (−)-epicatechin; PCA, procyanidins; $\overline{DP}n$, number-average degree of polymerization of flavan-3-ols (catechins + procyanidins); 5-CQA, 5-caffeoylquinic acid; variation, polyphenol difference between native fraction and heated fraction in percent; SD, standard deviation (degrees of freedom = 6).

The thioacidolysis yield for the purified fraction **Pdp35**, calculated according to weight, ranged from 72 to 75%, consistent with previous results.[21,49] The purified procyanidins presented a $\overline{DP}n$ of 35. The most abundant monomer in the procyanidin structure was (−)-epicatechin (99%), found as extension and terminal units; (+)-catechin (<1%) was found only as a terminal unit.[19−21]

During heat treatment, an increase in (−)-epicatechin was observed (Table 2; Figure 5A). The (−)-epicatechin concentration rapidly increased, to more than twice the initial concentration after 2 h of heating at pH 3.3 and 2.7 (Figure 5A). At pH 4.0 the increase was much less pronounced than at pH 3.3 and 2.7. The (−)-epicatechin concentration subsequently decreased to slightly above the initial concentration, whatever the pH. This trend, that is, increasing during the first 2 h and then decreasing, has already been observed in the heat treatment of Chinese quince phenolics.[50] The (−)-epicatechin increase during the first 2 h may be due to chemical depolymerization of procyanidins under hot acidic conditions.[5,8,11,9,12,13,50] Evidence of release of the (−)-epicatechin terminal unit from chemical depolymerization of procyanidins onto the intermediate carbocation was given by the decline in (−)-epicatechin as terminal unit (Figure 5E) and concomitant increase in extension units (Figure 5F). However, after 2 h of treatment, production of (−)-epicatechin due to procyanidin depolymerization, although it continued (Figure 5C), no longer compensated for its thermal degradation, whence the (−)-epicatechin concentration decrease.[42−44] The increase in (−)-epicatechin concentration between **Pdp35** and **heated Pdp35** fractions ranged from 11 to 22% according to pH (Table 2). Although (+)-catechin was found as a terminal unit of the **Pdp35** fraction and despite the fact that isomerization from (−)-epicatechin to (+)-catechin might occur in these heated conditions,[39,51] none was detected. The percentage of (+)-catechin as terminal unit increased during the heat process

dx.doi.org/10.1021/jf4005548 | J. Agric. Food Chem. 2013, 61, 6679−6692

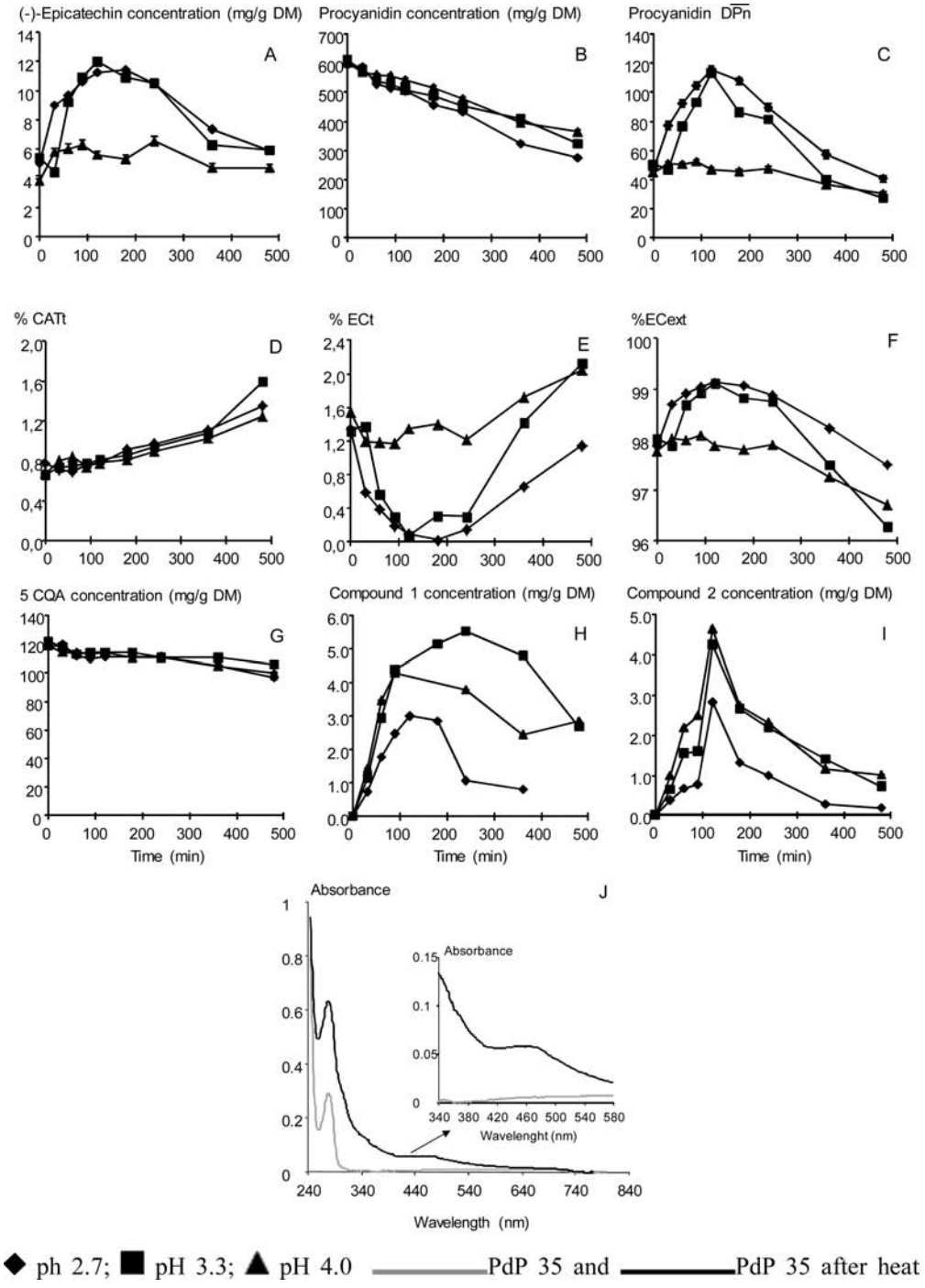


Figure 5. Change in (−)-epicatechin (A), procyanidin concentrations (B), $\overline{DP}n$ of procyanidins (C), percentage of (+)-catechin as terminal unit (% CATt, D), percentage of (−)-epicatechin as terminal unit (%ECt, E), percentage of (−)-epicatechin as extension unit (%ECext, F), 5-caffeoylquinic acid (G), and new compounds **1** (H) and **2** (I) concentrations during the heating of procyanidin **Pdp35** fraction at three different pH values for 480 min. Compounds **1** and **2** were quantified as (−)-epicatechin equivalent. Bars indicate standard errors (n = 2). (J) Absorption spectrum of the fraction **Pdp35** before and after treatment for 480 min at 95 °C and pH 2.7.

(Figure 5D). This increase could also be a statistical effect of release of (−)-epicatechin as terminal unit.

During heat treatment, procyanidin concentrations decreased by one-third to half: this trend was more pronounced when the pH was low (Table 2; Figure 5B). Such a decrease has already been observed extensively.[22,40−42,50] It is linked in particular to the chemical depolymerization of procyanidin,[8,9,11−13,50] leading to the formation of positively charged carbocations. These carbocations are very reactive and can further react by intermolecular or intramolecular addition with nucleophilic compounds.[10,13,15,52,53] At pH 4.0, the $\overline{DP}n$ of procyanidins decreased. This was confirmed by Hamauzu et al.,[5] who showed a decrease in thioacidolytic products of procyanidins with increasing heating time, resulting in a decreased $\overline{DP}n$ of procyanidin. However, at pH 2.7 and 3.3 the $\overline{DP}n$ of procyanidins increased during the first 2 h of treatment and then decreased (Figure 5C). The intermediate cleavage products, namely, the positively charged carbocations, may

dx.doi.org/10.1021/jf4005548 | J. Agric. Food Chem. 2013, 61, 6679−6692

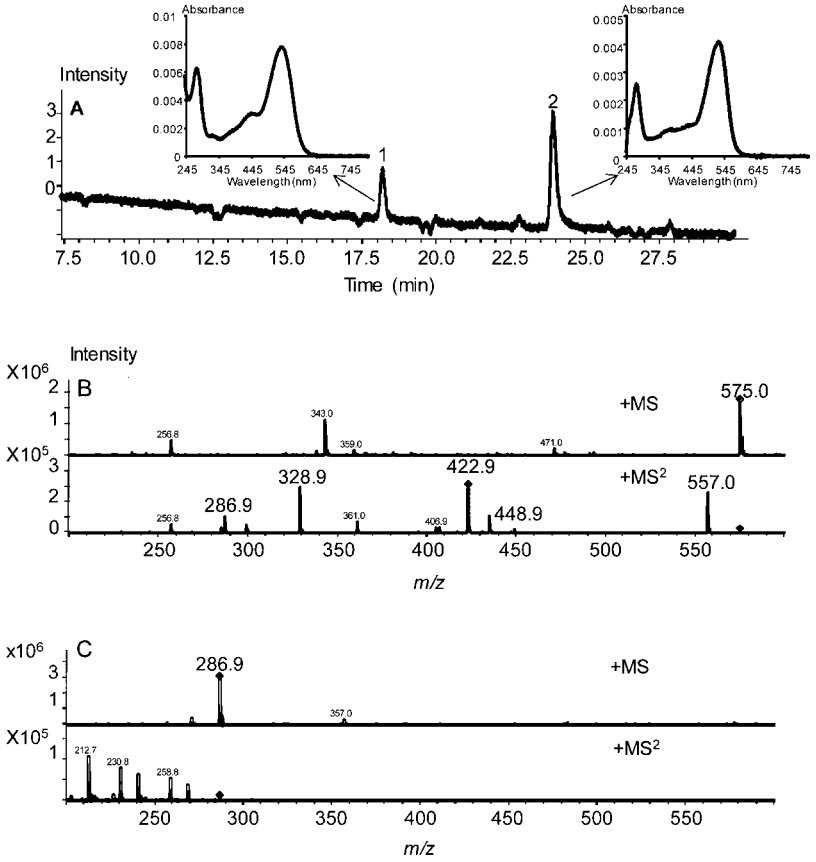


Figure 6. (A) Thioacidolysis HPLC-DAD analysis at 520 nm of **Pdp 35** purified procyanidin fraction after treatment for 120 min at 95 °C and pH 2.7; compound **1** with a deprotonated molecular ion $[M + H]^+$ at m/z 575; compound **2** with a deprotonated molecular ion $[M + H]^+$ at m/z 287. (B, C) Fragmentation pattern of ion peaks at m/z 575 (B) and m/z 287 (C).

have further reacted with nucleophilic groups of procyanidins present in the medium, leading to an increase in $\overline{DP}n$ of procyanidins.[10,15,18,39,52−55] This increase could also be a statistical effect of release of (−)-epicatechin as terminal unit (Figure 5E). After 2 h, the $\overline{DP}n$ decrease could be attributed, as in the case of pH 4.0, to thermal degradation[22,40−42,50] and chemical depolymerization of procyanidins.[5] At the final point of heating, the procyanidin $\overline{DP}n$ had decreased from 35 (**Pdp35**) to 22 (**heated Pdp 35 pH 2.7** and **heated Pdp35 pH 4.0**) and 18 (**heated Pdp35 pH 3.3**) (Table 2).

During heat treatment, whatever the pH, 5-caffeoylquinic acid concentration decreased slightly (Table 2; Figure 5G) to 82, 87, and 84% of the initial concentration for pH 2.7, 3.3, and 4.0, respectively. This confirms that 5-caffeoylquinic acid is relatively stable during heating at 100 °C.[56] The loss of 5-caffeoylquinic acid was interpreted as the result of thermal degradation[22,45] and/or of intermolecular addition with procyanidins[10] during heat treatment.

Thioacidolysis of **Pdp 35** after hot acidic treatment yielded two new compounds with an adsorption maximum at 525 nm. The change in these two compounds was the same as for (−)-epicatechin and $\overline{DP}n$, that is, an increase in the first 2 h and then a decrease (Figure 5H,I). Their appearance was enhanced at higher pH. These compounds were further analyzed by HPLC-DAD/ESI-MS after thioacidolysis. Compound **1** had a deprotonated molecular ion $[M + H]^+$ at m/z 575. This compound was identified as (epi)catechin−cyanidin and named F-A^+ adduct. Assuming that the flavan-3-ol moiety was (epi)catechin and the anthocyanidin was cyanidin derived from procyanidin depolymerization, the mass spectrum of the (epi)catechin−cyanidin must contain the specific ions of cyanidin and epicatechin.[57] The MS/MS fragmentation (Figure 6B) gave the specific ion of cyanidin, namely, m/z 287 (cyanidin), m/z 329 (cyanidin + 42) corresponding to the partial loss of the flavanol moiety,[58] and m/z 423 (cyanidin +136) corresponding to the retro-Diels−Alder reaction,[58] the two ions for the whole aglycon, m/z 557, which correspond to the loss of a water molecule ($[M - 18]^+$), and m/z 449 ($[M - 126]^+$), which can be interpreted as resulting from loss of a fragment $C_6H_6O_3$ corresponding to the loss of the A ring in the flavanol.[57] This fragmentation scheme indicates that the flavanol is bound by its C-4 and the anthocyanidin by its C-6 or C-8, because the fragment ion at m/z 449 (−126 amu) cannot arise from the anthocyanin A-ring, and, according to Freidrich et al.,[59] it is characteristic of the upper unit of dimers. The retention time and UV−visible absorbance spectrum of compound **2** correspond to those of cyanidin. HPLC-DAD/ESI-MS (Figure 6C) of the extract after thioacidolysis confirmed that compound **2** had a deprotonated molecular ion $[M + H]^+$ at m/z 287, which corresponds to the signal expected for cyanidin.[57]

Release of cyanidin only after thioacidolysis, whereas sample **Pdp35** did not contain cyanidin, implied the autoxidation, during treatment, of a flavan-3-ol unit of a procyanidin to give an F_n-A^+ pigment.[8,10,11] We postulate that the mechanism of formation of this tannin−anthocyanidin compound began with the acid-catalyzed cleavage of the interflavan bond of a procyanidin (F_n-F-F) (Figure 7A, step 1). In the process, the

dx.doi.org/10.1021/jf4005548 | J. Agric. Food Chem. 2013, 61, 6679−6692

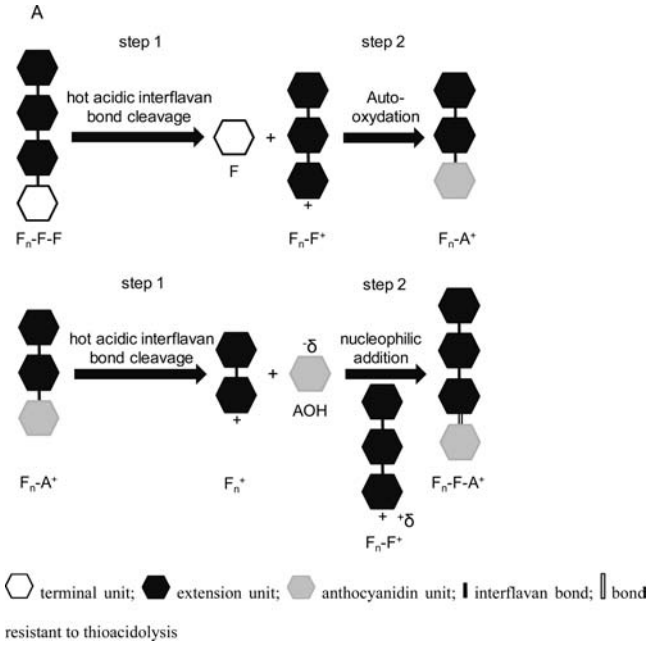


Figure 7. Postulated mechanism of formation of cyanidin (A^+, compound **2**) (A) and of (epi)catechin−cyanidin adduct ($F\text{-}A^+$ adduct, compound **1**) (B).

terminal unit was released as free flavan-3-ol (F), and the intermediate C-4 carbocation $F_n\text{-}F^+$ was formed from extension units.[12] $F_n\text{-}F^+$ was further autoxidized (Figure 7A, step 2) to give the $F_n\text{-}A^+$ pigment.[8,10,11,13] Basically, during thioacidolysis, benzylthioether derivatives are formed from residues linked by their C-4 position (extension units), whereas unchanged monomeric units are provided by residues linked only by their C-6 or C-8 of the A-ring in the original tannin structure. The fact that cyanidin was released by thioacidolysis in underivatized form prompts us to postulate two linkage positions in the tannin−anthocyanidin adducts. Cyanidin could be linked by its C-6 or C-8 as terminal unit in the tannin−anthocyanidin pigment.[18,60]

The fact that the $F\text{-}A^+$ adduct was released only after thioacidolysis implies, during the heat treatment, the formation of a tannin−anthocyanidin ($F_n\text{-}F\text{-}A^+$) pigment[60] in two steps: (ii) formation of an anthocyanidin A^+ after acid-catalyzed cleavage of an $F_n\text{-}A^+$ pigment, because anthocyanidins were not detected in the **Pdp 35** fraction before hot acid treatment (Figure 7B, step 1); (ii) nucleophilic addition of the C-8 or C-6 of this anthocyanidin moiety, in its hydrated hemiketal form (AOH), to an electrophilic intermediate C-4 carbocation ($F_n\text{-}F^+$), first released by acid-catalyzed cleavage of the interflavanic bond of a procyanidin molecule (Fn-F-F). This yielded the colorless compound (F_n-F-AOH), which dehydrates to the red flavylium form $(F_n\text{-}F\text{-}A)^+$ (Figure 7B, step 2).[18,54,55] The impact of pH on formation of the F_n-F-AOH product also supports this hypothesis, higher pH favoring formation of the hemiketal.

The $F\text{-}A^+$ adduct was still present after thioacidolysis, implying that it was hydrolyzed from an $F_n\text{-}F\text{-}A^+$ structure[60] and that the bond between flavan-3-ol and anthocyanidin moieties was resistant to acid-catalyzed cleavage. This may be due to the presence of the anthocyanidin in its flavylium form in the structure of the $F\text{-}A^+$ adducts, under the thioacidolysis conditions, which is expected to increase resistance to acid-catalyzed cleavage, as it cannot undergo the proton addition necessary for initiating acidic cleavage.[55] This resistance has been demonstrated in the case of ethyl-linked flavanol−anthocyanin adducts[61] and flavanol−anthocyanin adducts.[54,55]

The formation of new products on heating in an acidic medium could also be observed by the appearance of a large number of peaks at the end of the chromatogram (data not shown) that could not be degraded by thioacidolysis. This appearance of new peaks reflects the fact that procyanidins have been cleaved and then repolymerized to give novel compounds with formation of new bonds resistant to thioacidolysis.[39,50] This resistance to thioacidolysis reaction was also reflected in the reaction yields, which were much lower in the case of heated fractions, ranging from 37 to 53% (Table 2) less than for the initial fractions.

In addition, the adsorption spectra of the **Pdp35** fraction changed after heat treatment (Figure 5J). Absorbance at 280 nm increased, as also did adsorption in visible wavelengths (400−550 nm) as observed for quince procyanidins.[5,50]

Isolation of Pink Color Pigment by Successive Solvent Extractions and Enzymatic Digestions of Canned Pears.

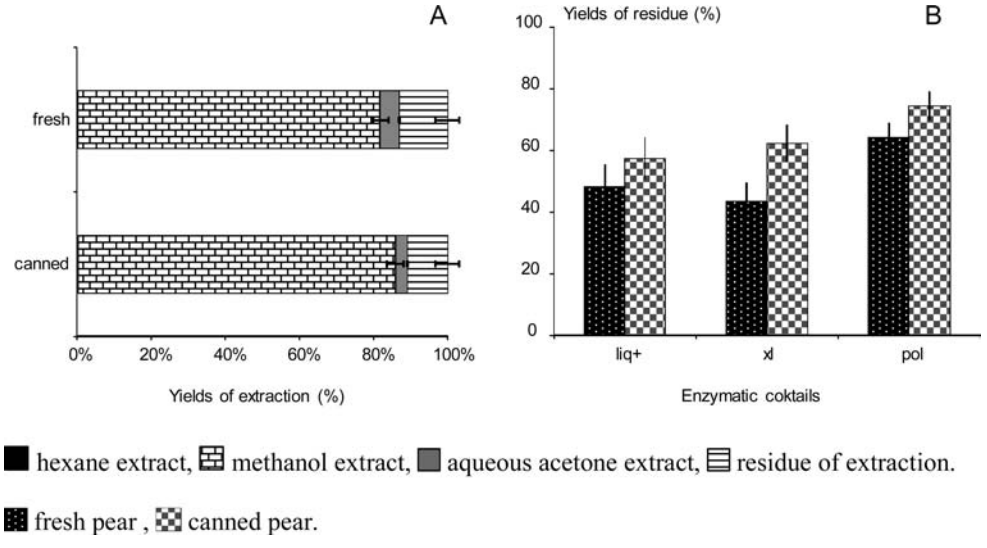


Figure 8. Yields of solvent extraction (A) and enzymatic digestion (B) of fresh (**SO fresh**) and canned pear (**pH 2.7 SO**).

Heat treatment of purified pear procyanidins resulted in the appearance of two new compounds, namely (epi)catechin-cyanidin adduct (compound **1**) and cyanidin (compound **2**), that could be detected only after thioacidolysis. To confirm the implication of these compounds in the pink discoloration of canned pear, we therefore set out to isolate them from the canned pink pear pieces by successive solvent extractions (samples **SO fresh** and **pH 2.7 SO**) and enzymatic digestions.

The freeze-dried tissues were successively fractionated into hexane, methanol, and aqueous acetone extracts (Figure 8A). Hexane, dissolving lipid constituents, extracted <0.5% of the initial dry matter. Most of the constituents were extracted by methanol, such as simple sugars, oligosaccharides, and organic acids.[25] Methanol extracted from 82% (**SO fresh**) to 87% (**pH 2.7 SO**) of the initial dry matter. Aqueous acetone dissolved between 3% (**pH 2.7 SO**) and 5.5% (**SO fresh**) of the initial dry matter. Finally, a significant portion was not extractable; the insoluble residue represented between 11% (**pH 2.7 SO**) and 13% (**SO fresh**) of the initial dry matter. These results were consistent with those of Guyot et al.[25] for successive solvent extractions on freeze-dried tissues of apple. In all cases, the differences in extraction yields observed between fresh pears and canned pears were not significant ($F = 0.7$, $P < 0.4$). After thioacidolysis-HPLC analysis of methanol and aqueous acetone extracts, no structure like (epi)catechin-cyanidin adduct (compound **1**) or cyanidin (compound **2**) could be detected, and pink color substances persisted in the residues. Luh et al.[7] have also shown that in canned pears the pink pigment adheres to the surface of the pear pieces.

As the pink color persisted in the residues even after successive solvent extractions, they were treated with cell wall degrading enzymes. Three enzymatic cocktails were used: Endozym pectofruit Liq+, Endozym pectofruit xl, and Endozym polifruit. They are used respectively for fruit liquefaction, for juice clarification by pectin degradation, and as a pressing aid, with various levels of pectolytic, hemicellulolytic, and cellulolytic enzymes. However, the different enzymatic activities were not tested here. Yields of residue of enzymatic digestion obtained for fresh and processed pears (**SO fresh** and **pH 2.7 SO**, respectively) are represented in Figure 8B. Yields of residue, for each enzymatic cocktail, were representative of the degrees of cell wall degradation. The differences in yields observed between the different digestion cocktails used were significant ($F = 12.7$, $P < 0.007$) and can be explained by their composition. However, what should be emphasized is the difference in yield between fresh and canned pears. In the case of sample **SO fresh**, enzymatic cocktails left a residue comprising from 43% (cocktail Endozym pectofruit xl) to 64% (cocktail pol) of the initial mass. In the case of canned pears, residues represented from 57% (cocktail Endozym pectofruit Liq+) to 74% (cocktail Endozym polifruit) of the initial mass. They were significantly higher ($F = 17.3$, $P < 0.006$) than those obtained during the enzymatic digestion of fresh pears, which meant that in the case of canned pears, enzymes were unable to access and hydrolyze cell wall polysaccharides as in the case of fresh pears. The positively charged carbocations produced by chemical depolymerization of procyanidins[8,9,11−13] during the process, that is 95 °C for 385 min at pH 2.7, which are highly reactive, might have formed covalent linkages with plant matrix[14] as reported for covalent binding of procyanidins to proteins.[10] Such covalent linkages could be resistant to degradation and/or reduce the accessibility of polysaccharide to enzymes by steric hindrance, leading to an increase in the mass of the digestion residues, as observed when procyanidins are oxidized in the presence of cell wall.[62] Evidence of the formation of covalent bonds between procyanidin and cell wall polysaccharides lies in the fact that it was not possible to re-extract the pink discoloration, which persisted in the canned pear residues even after successive solvent extractions and enzymatic digestions and concomitant to the nondetection of (epi)catechin−cyanidin adduct (compound **1**) or cyanidin (compound **2**) in the supernatants of enzymatic digestions.

Procyanidin Cell Wall Interactions. (Epi)catechin−cyanidin adduct (compound **1**) and cyanidin (compound **2**), that is, pink color substances, can be obtained from purified pear procyanidins upon heat treatment. However, they could not be extracted from canned pear pink segments even by successive solvent extractions and enzymatic digestions, and the color remained in the insoluble residue. This being so, we hypothesized that the products from procyanidin hydrolysis would react with the cell walls by both noncovalent and covalent binding.

To test the hypothesis of binding of products from procyanidin hydrolysis to cell wall, procyanidins extracted from pears were submitted or not to heat treatments at different pH values (2.7, 3.3, 4.0) in the presence of purified cell walls.

Cell Wall Characterization. Cell wall material from pear was isolated as AIS. The yields of AIS from William pears (28 ± 0.1 mg/g fresh weight) was close to the 30 mg/g fresh weight reported by Martin Cabrejas et al.[63] and Isherwood and Jermyn,[64] but higher than the yield obtained by Renard[22] (23 mg/g fresh weight for Gieser Wildeman pears), whereas Ferreira et al.[20] showed extremely low yields, at 10.7 mg/g fresh weight, for San Bartolomeu pears. The main sugars were galacturonic acid (223 ± 31.7 mg/g DW), glucose (187 ± 15.0 mg/g DW), and arabinose (156 ± 15.5 mg/g DW). Galactose (59 ± 4.6 mg/g DW), xylose (44 ± 5.2 mg/g DW), and mannose (24 ± 18.2 mg/g DW) were present in lower amounts, whereas rhamnose (9 ± 1.3 mg/g DW) and fucose (7 ± 0.8 mg/g DW) were minor sugars. Pectic substances were present in high amount as shown by the high galacturonic acid, arabinose, and galactose contents and were highly methylated (66% ± 8.0).[63] This composition is close to those reported earlier.[22,63,65,66] It reflects the macromolecular composition of the pear cell wall: cellulose, highly methylated pectins (DM 66%) rich in arabinan and galactan side chains, and xylans, presumably concentrated in the stone cells.[63]

Binding of Purified Procyanidin Fraction with Pear Cell Wall Material as a Function of Heat Treatment at Different pH Values. To maximize interaction phenomena, a ratio of 1/5 between procyanidins and cell walls (higher than in the fruit) and procyanidins with a high $\overline{DP}n$ (from Perry pear) were chosen.[21]

At low temperature (Table 3), pH (between 2.7 and 4.0) had no significant influence ($F = 0.2$, $P < 0.8$) on interactions between procyanidins (heated or not) and cell walls (samples **LN pH 2.7**, **LN pH 3.3**, and **LN pH 4.0** and samples **LH pH 2.7**, **LH pH 3.3**, and **LH pH 4.0**). As interactions were conducted in low-temperature conditions, we presumed that the association between procyanidins and cell wall induced noncovalent binding.[14,21,27,67] Renard et al.[27] and Le Bourvellec et al.[21] have also shown the absence of influence of pH on noncovalent binding between procyanidins and cell walls from apple. However, the amounts of procyanidin bound to the pear

Table 3. Binding Reaction of Purified Procyanidin Fractions with Cell Wall Material: Quantification and Characterization[a]

	procyanidins bound			
sample	g/g of cell wall	% total	$\overline{DP}n$ of bound procyanidins	$\overline{DP}n$ of free procyanidins
LN pH 2.7	0.097 (0.008)	41	43.2 (4.4)	10.7 (1.3)
LN pH 3.3	0.100 (0.008)	45	47.4 (4.4)	10.2 (1.3)
LN pH 4.0	0.098 (0.008)	42	37.4 (4.4)	10.3 (1.3)
LH pH 2.7	0.132 (0.008)	54	26.3 (5.4)	6.0 (0.4)
LH pH 3.3	0.114 (0.008)	49	23.2 (5.4)	6.0 (0.4)
LH pH 4.0	0.126 (0.008)	54	20.3 (5.4)	5.0 (0.4)
HN pH 2.7	0.129 (0.006)	58	11.6 (2.8)	4.6 (0.6)
HN pH 3.3	0.152 (0.006)	61	17.1 (2.8)	5.8 (0.6)
HN pH 4.0	0.144 (0.006)	63	16.4 (2.8)	3.8 (0.6)

[a]**LN pH 2.7**, low temperature (25 °C) native procyanidin pH 2.7; **LN pH 3.3**, low temperature (25 °C) native procyanidin pH 3.3; **LN pH 4.0**, low temperature (25 °C) native procyanidin pH 4.0; **LH pH 2.7**, low temperature (25 °C) heated procyanidin pH 2.7; **LH pH 3.3**, low temperature (25 °C) heated procyanidin pH 3.3; **LH pH 4.0**, low temperature (25 °C) heated procyanidin pH 4.0; **HN pH 2.7**, high temperature (95 °C) native procyanidin pH 2.7; **HN pH 3.3**, high temperature (95 °C) native procyanidin pH 3.3; **HN pH 4.0**, high temperature (95 °C) native procyanidin pH 4.0. Initial polyphenol concentration = 1 g/L, CWM concentration = 5 g/L, buffer citrate/phosphate, pH 3.8; ionic strength = 0.1 M. Values in parentheses correspond to the standard deviation (n = 3).

cell wall material were lower than reported for the same procyanidin fraction on apple cell wall.[21] Between 41 and 45% of the **Pdp35** fraction was retained in pear cell wall material, whereas Le Bourvellec et al.[21] reported that 59% of the **Pdp35** fraction was bound to apple cell wall material. The differences observed might be explained by differences in both structure (polysaccharide composition) and a physical characteristic (surface area) between apple and pear cell walls.[27,68,69] Heating the procyanidins before interactions with cell wall increased their ability to bind to pear cell wall material (Table 3). This was consistent with Le Bourvellec et al.,[62] who obtained higher weight gains when cell walls interacted with preoxidized procyanidins than with native procyanidins. Heating procyanidins led to new tannin−anthocyanidin structures with possibly higher affinities for the cell wall than native fractions. Kennedy and Bindon[70] also showed that anthocyanin−tannin products had a higher affinity for cell walls, without the heat effect, than tannin.

At high temperature, pH (between 2.7 and 4.0) had a significant influence (F = 14.8, P < 0.02) on interactions between procyanidins and cell walls in high-temperature conditions (samples **HN pH 2.7**, **HN pH 3.3**, and **HN pH 4.0**; Table 3). In these acidic high-temperature conditions, we can presume that the intermediate C-4 carbocations, released from interflavan bond cleavage, which are very reactive, can react with nucleophile compounds of the cell wall polymers to form covalent bonds.[14] Also, the complexes formed between procyanidins and cell walls were colored. The amounts of procyanidin bound on cell wall in these acidic high-temperature conditions were higher than in noncovalent conditions. Le Bourvellec et al.[62] also report weight gains after procyanidin−cell wall interactions increase as using native procyanidins, preoxidized procyanidins, and simultaneously oxidized and bound procyanidins to apple cell walls. Heating in an acidic medium will also induce the formation of new products such as pyranoanthocyanins[71] or tannin−anthocyanin pigment.[60] These new structures may have higher affinity toward the cell wall than the native fractions from which they are derived.[62]

A selective partition of the procyanidins between the soluble phase and the insoluble material was observed: the $\overline{DP}n$ values of the bound procyanidins (Table 3) were higher than those of the initial fraction ($\overline{DP}n$ 35) and of the free procyanidins, whether the binding was induced in low- or high-temperature acidic conditions. The modification of binding type did not change the selective partition of the procyanidins between the soluble phase and the insoluble cell wall material, which enables the cell wall to selectively adsorb highly polymerized procyanidins.[21,27,62,67,68] However, the $\overline{DP}n$ of bound procyanidins increased in the order high-temperature acidic conditions < low temperature with heated procyanidins < low temperature with native procyanidins. The differences observed here could be due to chemical depolymerization of procyanidins[8,9,11−13] and formation of new bonds, resistant to thioacidolysis during the thermal treatment.[22,40−42,50] The same effect has already been reported by Le Bourvellec et al.[62] for chemical oxidation and binding of procyanidins on cell wall material.

After canning, the pear sections turned from whitish-beige to pink, and polyphenol concentrations decreased significantly, mainly due to procyanidin loss, supporting the hypothesis that the conversion of procyanidins to anthocyanin-like compounds could occur after chemical depolymerization and oxidation.[1,5,8−13] However, no soluble product was detected at 520 nm, the wavelength characteristic of anthocyanins. In a model solution, purified procyanidin concentrations also decreased after treatment (95 °C for 480 min) all the more so as pH decreased and a pinkish color also appeared. After thioacidolysis, two new pigments were detected. The first was identified as (epi)catechin-cyanidin and the second as cyanidin. A mechanism involving acid-catalyzed cleavage of procyanidin[8,9,11−13] followed by nucleophilic addition of the anthocyanidin moiety, in its hemiketal form, to the resulting C-4 carbocation[54,55] or oxidation of the resulting C-4 carbocation was proposed.[8,9,11−13]

Extraction of the neoformed pink substance from canned pears was attempted by successive solvent extractions and enzymatic degradation, but the pink color persisted in the residues. During the heat degradation of procyanidins in the presence of cell wall, a pinkish color also appeared and was bound to the cell wall particles. An explanation could be that the carbocations, which are highly reactive, and tannin−anthocyanidin structures formed by chemical depolymerization of procyanidins may lead to the formation of covalent linkages with the plant matrix.[14] Such covalent linkages could be resistant to degradation and reduce the accessibility of enzyme to polysaccharide by steric hindrance, as re-extraction of the pink compounds was not possible and pink insoluble residues were obtained.

Such a mechanism may affect the release, bioavailability, and biological activity of procyanidins from heat-processed fruits. It is becoming increasingly clear that the biological effects should not be attributed to the native procyanidins present in foods, but rather to their metabolite products in the colon.[72,73] These colonic metabolites are produced during fermentation by the colonic microflora of native substances and are strongly absorbed. Procyanidins associated with cell wall are poorly

bioavailable in the upper intestine[74] and reach the colon, where they become fermentable substrates for bacterial microflora, which convert them into active metabolites.[75] Evidence of the effect of food matrix and noncovalent interactions between procyanidin and cell wall on the metabolism of procyanidins by human microflora has been recently found.[75,76] The conversion rate of procyanidins to known microbial metabolites was much lower with isolated procyanidins than with whole fruit.[75] It seems that cell wall acts as a nutrient for colonic microorganisms responsible for the conversion of procyanidins into active metabolites. Nevertheless, it should be emphasized that we do not yet know the effect of covalent bonds formed between procyanidins and cell wall in the production of microbial metabolites of procyanidins. Presumably, the type and amount of microbial metabolites produced may depend on the nature of the bonds (noncovalent vs covalent) formed between procyanidin and cell wall. If microbial metabolites are produced, we do not know whether or not they will have the same activity (+ or −) as those produced from native procyanidins and from noncovalent bound procyanidin−cell wall complexes.

Further work to isolate and characterize bound proanthocyanidin−cell wall covalent adducts is in progress.

AUTHOR INFORMATION

Corresponding Author

*(C.L.B.) Postal address: INRA, UMR 408 Sécurité et Qualité des Produits d'Origine Végétale, Domaine St-Paul, Site Agroparc, 84914 Avignon cedex 9, France. Phone: +33 (0) 4.32.72.25.28. Fax: +33 (0)4.32.72.24.92. E-mail: carine.lebourvellec@avignon.inra.fr.

Funding

The research leading to these results received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement FP7-222-654-DREAM.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Line Tichit, Marielle Bogé, Aimée Monique Nguini Dzama, and Solenn Serrand for their technical assistance.

ABBREVIATIONS USED

AIS, alcohol-insoluble solids; CA, Cavaillon; CB, Cheval-Blanc; CWM, cell wall material from William variety pears; $\overline{DP}n$, number-average degree of polymerization; Pdp 35, purified procyanidin fraction of average degree of polymerization 35 from pears; heated Pdp 35 pH 2.7, purified procyanidin fraction of average degree of polymerization 35 from pears heated at 95 °C for 480 min at pH 2.7; heated Pdp 35 pH 3.3, purified procyanidin fraction of average degree of polymerization 35 from pears heated at 95 °C for 480 min at pH 3.3; heated Pdp 35 pH 4.0, purified procyanidin fraction of average degree of polymerization 35 from pears heated at 95 °C for 480 min at pH 4.0; HN pH 2.7, binding reaction at high temperature (95 °C) between native purified procyanidin fraction Pdp 35 and cell wall material at pH 2.7; HN pH 3.3, binding reaction at high temperature (95 °C) between native purified procyanidin fraction Pdp 35 and cell wall material at pH 3.3; HN pH 4.0, binding reaction at high temperature (95 °C) between native purified procyanidin fraction Pdp 35 and cell wall material at pH 4.0; LN pH 2.7, binding reaction at low temperature (25 °C) between native purified procyanidin fraction Pdp 35 and cell wall material at pH 2.7; LN pH 3.3, binding reaction at low temperature (25 °C) between native purified procyanidin fraction Pdp 35 and cell wall material at pH 3.3; LN pH 4.0, binding reaction at low temperature (25 °C) between native purified procyanidin fraction Pdp 35 and cell wall material at pH 4.0; LH pH 2.7, binding reaction at low temperature (25 °C) between heated Pdp 35 pH 2.7 procyanidin fraction and cell wall material at pH 2.7; LH pH 3.3, binding reaction at low temperature (25 °C) between heated Pdp 35 pH 3.3 procyanidin fraction and cell wall material at pH 3.3; LH pH 4.0, binding reaction at low temperature (25 °C) between heated Pdp 35 pH 4.0 procyanidin fraction and cell wall material at pH 4.0; SO, Soses

REFERENCES

(1) Chandler, B. V.; Clegg, K. M. Pink discoloration in canned pears. I. Role of tin in pigment formation. *J. Sci. Food Agric.* **1970**, *21* (6), 315−319.

(2) Chandler, B. V.; Clegg, K. M. Pink discoloration in canned pears. II. Measurement of potential and developed color in pear samples. *J. Sci. Food Agric.* **1970**, *21* (6), 319−323.

(3) Chandler, B. V.; Clegg, K. M. Pink discoloration in canned pears. III. Inhibition by chemical additives. *J. Sci. Food Agric.* **1970**, *21* (6), 323−328.

(4) Czerkaskyj, A. Pink discoloration in canned Williams' Bon Chretien pears. *J. Food Sci.* **1970**, *35* (5), 608−611.

(5) Hamauzu, Y.; Kume, C.; Yasui, H.; Fujita, T. Reddish coloration of Chinese quince (*Pseudocydonia sinensis*) procyanidins during heat treatment and effect on antioxidant and antiinfluenza viral activities. *J. Agric. Food Chem.* **2007**, *55* (4), 1221−1226.

(6) Adams, J. B.; Brown, H. M. Discoloration in raw and processed fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* **2007**, *47* (3), 319−333.

(7) Luh, B. S.; Leonard, S. J.; Patel, D. S. Pink discoloration in canned Bartlett pears. *Food Technol.* **1960**, *14*, 53−56.

(8) Bate-Smith, E. C. Leuco-anthocyanins. 1. Detection and identification of anthocyanidins formed leuco-anthocyanins in plant tissues. *Biochemical J.* **1954**, *58* (1), 122−125.

(9) Thompson, R. S.; Jacques, D.; Haslam, E.; Tanner, R. J. N. Plant proanthocyanidins. Part I. Introduction: the isolation, structure and distribution in nature of plant procyanidins. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1387−1399.

(10) Beart, J. E.; Lilley, T. H.; Haslam, E. Polyphenol interactions.2. Covalent binding of procyanidins to proteins during acid-catalysed decomposition, observation on some polymeric proathocyanidins. *J. Chem. Soc., Perkin Trans. 2* **1985**, No. 9, 1439−1443.

(11) Porter, L. J.; Hrstich, L. N.; Chan, B. G. The conversion of of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1986**, *25* (1), 223−230.

(12) Haslam, E. In vino veritas: oilgomeric procyanidins and the aging of red wines. *Phytochemistry* **1980**, *19*, 2577−2582.

(13) Haslam, E. *Plant Polyphenols. Vegetable Tannins Revisited*; Cambridge University Press: Cambridge, UK, 1989.

(14) Le Bourvellec, C.; Renard, C. M. G. C. Interactions between polyphenols and macromolecules: quantification methods and mechanisms. *Crit. Rev. Food Sci. Nutr.* **2012**, *52* (1−3), 213−248.

(15) Esatbeyoglu, T.; Wray, V.; Winterhalter, P. Dimeric procyanidins: screening for B1 to B8 and semisynthetic preparation of B3, B4, B6, and B8 from a polymeric procyanidin fraction of white willow bark (*Salix alba*). *J. Agric. Food Chem.* **2010**, *58* (13), 7820−7830.

(16) Serrano, J.; Puupponen-Pimia, R.; Dauer, A.; Aura, A.-M.; Saura-Calixto, F. Tannins: current knowledge of food sources, intake, bioavailability and biological effects. *Mol. Nutr. Food Res.* **2009**, *53*, S310−S329.

(17) Lea, A. G. H.; Arnold, G. M. Phenolics of ciders − bitterness and astringency. *J. Sci. Food Agric.* **1978**, *29* (5), 478−483.
(18) Ribéreau-Gayon, P. The anthocyanins of grapes and wines. In *Anthocyanins as Food Colors*; Academic Press: New York, 1982.
(19) Es-Safi, N.-E.; Guyot, S.; Ducrot, P.-H. NMR, ESI/MS, and MALDI-TOF/MS analysis of pear juice polymeric proanthocyanidins with potent free radical scavenging activity. *J. Agric. Food Chem.* **2006**, *54* (19), 6969−6977.
(20) Ferreira, D.; Guyot, S.; Marnet, N.; Delgadillo, I.; Renard, C. M. G. C.; Coimbra, M. A. Composition of phenolic compounds in a Portuguese pear (*Pyrus communis* L. var. S. Bartolomeu) and changes after sun-drying. *J. Agric. Food Chem.* **2002**, *50* (16), 4537−4544.
(21) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part I. Effect of some environmental parameters. *Biochim. Biophys. Acta* **2004**, *1672* (3), 192−202.
(22) Renard, C. M. G. C. Effects of conventional boiling on the polyphenols and cell walls of pears. *J. Sci. Food Agric.* **2005**, *85* (2), 310−318.
(23) Renard, C.; Vanleeuwen, M.; Voragen, A. G. J.; Thibault, J. F.; Pilnik, W. Studies on apple protopectin. 2. Apple cell wall degradation by pure polysaccharidases and their combinations. *Carbohydr. Polym.* **1990**, *14* (3), 295−314.
(24) Renard, C. M. G. C. Variability in cell wall preparations: quantification and comparison of common methods. *Carbohydr. Polym.* **2005**, *60* (4), 515−522.
(25) Guyot, S.; Marnet, N.; Laraba, D.; Sanoner, P.; Drilleau, J. F. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety (*Malus domestica* var. Kermerrien). *J. Agric. Food Chem.* **1998**, *46* (5), 1698−1705.
(26) Micard, V.; Renard, C. M. G. C.; Thibault, J. F. Enzymatic saccharification of sugar-beet pulp. *Enzym. Microb. Technol.* **1996**, *19* (3), 162−170.
(27) Renard, C. M. G. C.; Baron, A.; Guyot, S.; Drilleau, J. F. Interactions between apple cell walls and native apple polyphenols: quantification and some consequences. *Int. J. Biol. Macromol.* **2001**, *29* (2), 115−125.
(28) Le Bourvellec, C.; Bouzerzour, K.; Ginies, C.; Regis, S.; Ple, Y.; Renard, C. M. G. C. Phenolic and polysaccharidic composition of applesauce is close to that of apple flesh. *J. Food Compos. Anal.* **2011**, *24* (4−5), 537−547.
(29) Seaman, J. F.; Moore, W. E.; Mitchell, A. E.; Millett, M. A. Techniques for the determination of flesh constituents by quantitative paper chromatography. *Tappi, J. Tech. Assoc. Pulp Paper Ind.* **1954**, *37*, 336−343.
(30) Englyst, H.; Wiggins, H. S.; Cummings, J. H. Determination of the non-starch polysaccharides in plant foods by gas−liquid chromatography of constituent sugars as alditol acetates. *Analyst* **1982**, *107*, 307−318.
(31) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54* (2), 484−489.
(32) Renard, C. M. G. C.; Ginies, C. Comparison of the cell wall composition for flesh and skin from five different plums. *Food Chem.* **2009**, *114* (3), 1042−1049.
(33) CIE, International Commission on Illumination. *Recommendations on Uniform Colour Spaces, Colour, Difference Equations, Psychometric Colour Terms*; Bureau Central de la CIE: Paris, France, 1978; CIE Publication 15 (Suppl. 2), (E-1.31) 1971/(TC-1.3).
(34) McGuire, R. G. Reporting of objective color measurements. *HortScience* **1992**, *27* (12), 1254−1255.
(35) Box, G. E. P.; Hunter, J. S.; Hunter, W. G. *Statistics for Experimenters, An Introduction to Design, Data Analysis and Model Building*; Wiley: New York, 1978.
(36) Galvis Sanchez, A. C.; Gil-Izquierdo, A.; Gil, M. I. Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *J. Sci. Food Agric.* **2003**, *83* (10), 995−1003.
(37) Oleszek, W.; Amiot, M. J.; Aubert, S. Y. Identification of some phenolic in pear fruit. *J. Agric. Food Chem.* **1994**, *42* (6), 1261−1265.
(38) Spanos, G. A.; Wrolstad, R. E. Influence of variety, maturity, processing, and storage on the phenolic composition of pear juice. *J. Agric. Food Chem.* **1990**, *38* (3), 817−824.
(39) Vidal, S.; Cartalade, D.; Souquet, J. M.; Fulcrand, H.; Cheynier, V. Changes in proanthocyanidin chain length in winelike model solutions. *J. Agric. Food Chem.* **2002**, *50* (8), 2261−2266.
(40) Asami, D. K.; Hong, Y. J.; Barrett, D. M.; Mitchell, A. E. Processing-induced changes in total phenolics and procyanidins in clingstone peaches. *J. Sci. Food Agric.* **2003**, *83* (1), 56−63.
(41) Brownmiller, C.; Howard, L. R.; Prior, R. L. Processing and storage effects on procyanidin composition and concentration of processed blueberry products. *J. Agric. Food Chem.* **2009**, *57* (5), 1896−1902.
(42) Hong, Y. J.; Barrett, D. M.; Mitchell, A. E. Liquid chromatography/mass spectrometry investigation of the impact of thermal processing and storage on peach procyanidins. *J. Agric. Food Chem.* **2004**, *52* (8), 2366−2371.
(43) Hernandez, T.; Ausin, N.; Bartolome, B.; Bengoechea, L.; Estrella, I.; Gomez Cordoves, C. Variations in the phenolic composition of fruit juices with different treatments. *Z. Lebensm. Unters. -Forsch. A (Food Res. Technol.)* **1997**, *204* (2), 151−155.
(44) Vallejo, F.; Tomas-Barberan, F. A.; Garcia-Viguera, C. Phenolic compound contents in edible parts of broccoli inflorescences after domestic cooking. *J. Sci. Food Agric.* **2003**, *83* (14), 1511−1516.
(45) O'Connell, J. E.; Fox, P. F. Proposed mechanism for the effect of polyphenols on the heat stability of milk. *Int. Dairy J.* **1999**, *9* (8), 523−536.
(46) Diem, S.; Bergmann, J.; Herderich, M. Tryptophan-*N*-glucoside in fruits and fruit juices. *J. Agric. Food Chem.* **2000**, *48* (10), 4913−4917.
(47) Gutsche, B.; Grun, C.; Scheutzow, D.; Herderich, M. Tryptophan glycoconjugates in food and human urine. *Biochem. J.* **1999**, *343*, 11−19.
(48) Frear, D. S.; Swanson, H. R.; Mansager, E. R. Picloram metabolism in leafy spurge − isolation and identification of glucose and gentiobiose conjugates. *J. Agric. Food Chem.* **1989**, *37* (5), 1408−1412.
(49) Guyot, S.; Marnet, N.; Drilleau, J. F. Thiolysis-HPLC characterization of apple procyanidins covering a large range of polymerization states. *J. Agric. Food Chem.* **2001**, *49* (1), 14−20.
(50) Hamauzu, Y.; Takedachi, N.; Miyasaka, R.; Makabe, H. Heat treatment of Chinese quince polyphenols increases rat plasma levels of protocatechuic and vanillic acids. *Food Chem.* **2010**, *118* (3), 757−763.
(51) Guyot, S.; Doco, T.; Souquet, J. M.; Moutounet, M.; Drilleau, J. F. Characterization of highly polymerized procyanidins in cider apple (*Malus sylvestris* var kermerrien) skin and pulp. *Phytochemistry* **1997**, *44* (2), 351−357.
(52) Koehler, N.; Wray, V.; Winterhalter, P. New approach for the synthesis and isolation of dimeric procyanidins. *J. Agric. Food Chem.* **2008**, *56* (13), 5374−5385.
(53) Esatbeyoglu, T.; Winterhalter, P. Preparation of dimeric procyanidins B1, B2, B5, and B7 from a polymeric procyanidin fraction of black chokeberry (*Aronia melanocarpa*). *J. Agric. Food Chem.* **2010**, *58* (8), 5147−5153.
(54) Salas, E.; Fulcrand, H.; Meudec, E.; Cheynier, V. Reactions of anthocyanins and tannins in model solutions. *J. Agric. Food Chem.* **2003**, *51* (27), 7951−7961.
(55) Salas, E.; Atanasova, V.; Poncet-Legrand, C.; Meudec, E.; Mazauric, J. P.; Cheynier, V. Demonstration of the occurrence of flavanol-anthocyanin adducts in wine and in model solutions. *Anal. Chim. Acta* **2004**, *513* (1), 325−332.
(56) Murakami, M.; Yamaguchi, T.; Takamura, H.; Matoba, T. Effects of thermal treatment on radical-scavenging activity of single and mixed polyphenolic compounds. *J. Food Sci.* **2004**, *69* (1), FCT7−FCT15.
(57) Sentandreu, E.; Navarro, J. L.; Sendra, J. M. LC-DAD-ESI/MS^n determination of direct condensation flavanol-anthocyanin adducts in

pressure extracted pomegranate (*Punica granatum* L.) Juice. *J. Agric. Food Chem.* **2010**, *58* (19), 10560−10567.
(58) Macz-Pop, G. A.; Gonzalez-Paramas, M.; Perez-Alonso, J. J.; Rivas-Gonzalo, J. C. New flavanol-anthocyanin condensed pigments and anthocyanin composition in Guatemalan beans (*Phaseolus* spp.). *J. Agric. Food Chem.* **2006**, *54* (2), 536−542.
(59) Friedrich, W.; Eberhardt, A.; Galensa, R. Investigation of proanthocyanidins by HPLC with electrospray ionization mass spectrometry. *Eur. Food Res. Technol.* **2000**, *211* (1), 56−64.
(60) Remy, S.; Fulcrand, H.; Labarbe, B.; Cheynier, V.; Moutounet, M. First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. *J. Sci. Food Agric.* **2000**, *80* (6), 745−751.
(61) Es-Safi, N. E.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Studies on the acetaldehyde-induced condensation of (−)-epicatechin and malvidin 3-*O*-glucoside in a model solution system. *J. Agric. Food Chem.* **1999**, *47* (5), 2096−2102.
(62) Le Bourvellec, C.; Guyot, S.; Renard, C. M. G. C. Interactions between apple (*Malus* × *domestica* Borkh.) polyphenols and cell walls modulate the extractability of polysaccharides. *Carbohydr. Polym.* **2009**, *75* (2), 251−261.
(63) Martin Cabrejas, M. A.; Waldron, K. W.; Selvendran, R. R.; Parker, M. L.; Moates, G. K. Ripening-related changes in the cell-walls of Spanish pear (*Pyrus communis*). *Physiol. Plant.* **1994**, *91* (4), 671−679.
(64) Isherwood, F. A.; Jermyn, M. A. Changes in the cell wall of the pear during ripening. *Biochem. J.* **1956**, *64* (1), 123−132.
(65) Ferreira, D.; Barros, A.; Coimbra, M. A.; Delgadillo, I. Use of FT-IR spectroscopy to follow the effect of processing in cell wall polysaccharide extracts of a sun-dried pear. *Carbohydr. Polym.* **2001**, *45* (2), 175−182.
(66) Ahmed, A. E.; Labavitch, J. M. Cell-wall metabolism in ripening fruit. 1. Cell-wall changes in ripening Bartlett pears. *Plant Physiol.* **1980**, *65* (5), 1009−1013.
(67) Bindon, K. A.; Smith, P. A.; Kennedy, J. A. Interaction between grape-derived proanthocyanidins and cell wall material. 1. Effect on proanthocyanidin composition and molecular mass. *J. Agric. Food Chem.* **2010**, *58* (4), 2520−2528.
(68) Le Bourvellec, C.; Renard, C. M. G. C. Non-covalent interaction between procyanidins and apple cell wall material. Part II: Quantification and impact of cell wall drying. *Biochim. Biophys. Acta* **2005**, *1725* (1), 1−9.
(69) Le Bourvellec, C.; Watrelot, A. A.; Ginies, C.; Imberty, A.; Renard, C. M. G. C. Impact of processing on the noncovalent interactions between procyanidin and apple cell wall. *J. Agric. Food Chem.* **2012**, *60* (37), 9484−9494.
(70) Bindon, K. A.; Kennedy, J. A. Ripening-induced changes in grape skin proanthocyanidins modify their interaction with cell walls. *J. Agric. Food Chem.* **2011**, *59* (6), 2696−2707.
(71) Rentzsch, M.; Schwarz, M.; Winterhalter, P. Pyranoanthocyanins − an overview on structures, occurrence, and pathways of formation. *Trends Food Sci. Technol.* **2007**, *18* (10), 526−534.
(72) Williamson, G.; Clifford, M. N. Colonic metabolites of berry polyphenols: the missing link to biological activity? *Br. J. Nutr.* **2010**, *104*, S48−S66.
(73) Del Rio, D.; Costa, L. G.; Lean, M. E. J.; Crozier, A. Polyphenols and health: what compounds are involved? *Nutr. Metab. Cardiovasc. Dis.* **2010**, *20* (1), 1−6.
(74) Donovan, J. L.; Manach, C.; Rios, L.; Morand, C.; Scalbert, A.; Remesy, C. Procyanidins are not bioavailable in rats fed a single meal containing a grapeseed extract or the procyanidin dimer B-3. *Br. J. Nutr.* **2002**, *87* (4), 299−306.
(75) Bazzocco, S.; Mattila, I.; Guyot, S.; Renard, C. M. G. C.; Aura, A. M. Factors affecting the conversion of apple polyphenols to phenolic acids and fruit matrix to short-chain fatty acids by human faecal microbiota in vitro. *Eur. J. Nutr.* **2008**, *47* (8), 442−452.
(76) Aura, A.-M.; Mattila, I.; Hyotylainen, T.; Gopalacharyulu, P.; Cheynier, V.; Souquet, J.-M.; Bes, M.; Le Bourvellec, C.; Guyot, S.; Oresic, M. Characterization of microbial metabolism of Syrah grape products in an in vitro colon model using targeted and non-targeted analytical approaches. *Eur. J. Nutr.* **2013**, *52* (2), 833−846.