

Involvement of Flavanoids in Beer Color Instability during Storage

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Besides Maillard reactions, structural rearrangements of flavan-3-ol monomers cause color changes in beer during storage. Acetone/water-soluble fractions (70/30, v/v) of three lager beers of the same batch, differently stabilized before bottling in glass or poly(ethylene terephthalate) (PET) bottles, were monitored by normal-phase HPLC-ESI(-)-MS/MS over a 1-year period of storage at 20 °C. In parallel, beer color was monitored by the European Brewery Convention assay. The evolution of color was similar in the silica gel-filtered beer to that in identically bottled and stored poly(vinylpyrrolidone)-treated samples, despite the high flavanoid dimers content of the former. On the other hand, color evolved more rapidly in the PET bottle, suggesting a key role of oxygen. The kinetics was still increased in model media containing (+)-catechin, while no color was detected when normal-phase HPLC-fractionated dimers or trimers were investigated. (+)-Catechin emerged as the precursor of less polar products, characterized by a yellow-brown color. MS/MS enabled us to identify these products as issued from the oxidation and intramolecular additions of dehydrocatechin B4. Similar structures were found in aged beers spiked with (+)-catechin. Beer storage in the absence of oxygen and at low temperature is recommended so as to minimize the synthesis of such pigments.

KEYWORDS: Beer; proanthocyanidins; flavanoids; color aging; storage; stability

INTRODUCTION

Polyphenols, widely distributed in nature (wine, tea, fruits, vegetables, cocoa, etc.), include many classes of compounds ranging from phenolic acids to simple flavanoids, colored anthocyanidins, and tannins. Polyphenol concentration ranges from 50 to 150 mg/L in lager beers, depending on the stabilization treatment applied. Poly(vinylpyrrolidone) (PVPP)-treated (1) beers are known to be poorer in flavanoids than silica gel-filtered beers with, however, a higher monomer/dimer ratio (2). Up to 30% of beer polyphenols derive from hops, although malt is added in much larger amounts (3–8). (+)-Catechin, (-)-epicatechin, procyanidin B3, and prodelfinidins (B3–9 and two A-types) have been found in beer (1, 9–12). In the brewing field, proanthocyanidins are known to be responsible for colloidal instability during storage (13). Flavanoids are also most probably involved in beer color instability. Improving the shelf life of beer requires better knowledge of all chemicals involved.

As far as colloidal stability is concerned, proline-rich proteins and oligomeric flavanoids are known to form soluble complexes (14) which grow to form a visible haze (15, 16). Very little haze is produced with (-)-epicatechin and (+)-catechin monomers. Procyanidin B3 and especially prodelfinidin B3 are closely related to haze formation (12, 13, 16). Oxygen, agitation,

light, metals, and other factors are known to strongly accelerate the process. In order to remove haze-active materials while preserving foam-active proteins, fining agents or adsorbents such as silica gel, bentonite, PVPP, or poly(vinylpyrrolidone) (PVP)-modified silica gel are abundantly used in breweries (17–20).

Regarding color, scarce information is available in the brewing field. An almost linear increase of color was observed for samples stored at 40 °C in the absence of oxygen (12, 21). The formation of colored Maillard products under these storage conditions was likely the predominant cause (22). In the presence of oxygen, the color increase was rapid during the first days, due to oxidation and subsequent degradation of the polyphenols. Such degradation was confirmed by the decrease in total flavanoids (measured with *p*-dimethylaminocinnamaldehyde) and the constant total polyphenol concentration determined by the Bishop European Brewery Convention (EBC) assay (complexation reaction with ferric ions in alkaline solution) (21). Likewise, McMurrough et al. (12) observed a decrease in (+)-catechin, (-)-epicatechin, prodelfinidin, and procyanidin B3 concentrations at 37 °C, especially during the first 4–5 weeks. Dimers disappeared more rapidly than monomers. In contrast, after a lag period of about 5 weeks, the levels of “tannoids” (measurement based on the turbidity formed by titration with a PVP solution) were found to increase (23). According to Gardner and McGuinness (24), oxidative mechanisms may induce flavanoid polymerization.

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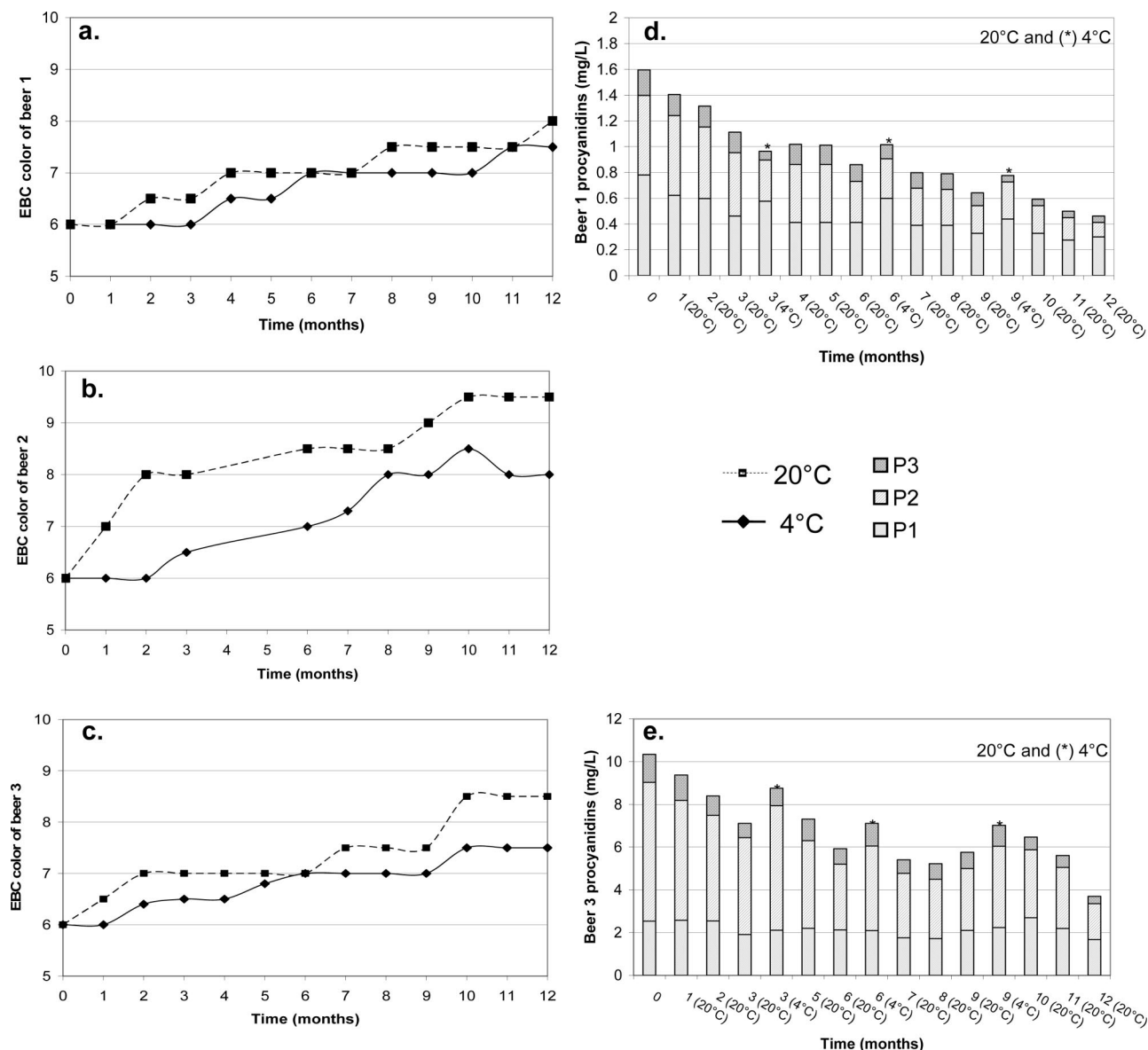


Figure 1. Evolution during storage at 4 and 20 °C of the EBC color (a, beer 1; b, beer 2; c, beer 3) and the flavan-3-ol concentration (mg/L in equiv (+)-catechin; d, beer 1; e, beer 3).

In order to understand which proanthocyanidins are involved in beer color instability, three pilot plant lager beers of the same batch, differently stabilized before bottling, were investigated here. Color was monitored by the EBC assay during storage. Small flavanoids were individually quantified by NP-HPLC-ESI(-)-MS/MS from a Sephadex LH20 acetone/water (70/30, v/v) extract. To better characterize new colored products, model media containing (+)-catechin and NP-HPLC-fractionated procyanidins were also monitored by UV-vis and tandem mass spectroscopy.

MATERIALS AND METHODS

Chemicals. Acetone (99.9%), (+)-catechin (98%), (-)-epicatechin (98%), and (-)-epicatechin-4 β -8-(-)-epicatechin (B2, 90%) were from Sigma-Aldrich (Bornem, Belgium). Methanol (99.9%) and dichloromethane (99.9%) were from Romil (Cambridge, UK). Acetic acid (99.8%) was from Acros (Geel, Belgium). Ammonium acetate (99%) was from Fluka (Buchs, Switzerland). Acetonitrile (99.99%) was from Fischer Scientific (Leicestershire, UK). Formic acid (99%) was from Janssens Chemica (Geel, Belgium). Solutions were made with Milli-Q (Millipore, Bedford, MA) double-distilled water (resistance = 18 m Ω /cm²).

Beer Aging. Three pilot plant lager beers of the same batch were obtained from a Belgian brewery. Two were stabilized with PVPP (Divergan RS, BASF), one being bottled in glass (1) and the other in poly(ethyleneterephthalate) (PET) (2). The third beer was filtered on silica gel (Britesorb BK390-N, Klinger Sogofilter) before glass bottling (3). They were aged in brown bottles protected from light at 4 and 20 °C (oxygen <100 μ g/L). For beer spiked with (+)-catechin, glass bottles of beer 1 were opened and struck to produce foam. When foam reached the top, the bottle was sealed with a silicon top (vel no. 5, Merck, Belgium). Addition of (+)-catechin was done by injection through the silicon top of 0.33 mL of a 10 g/L aqueous solution. Bottles containing 10 ppm of (+)-catechin were then crown-sealed and aged for 15 days at 20 °C in a dark room.

Beer Flavanoid Solid-Phase Extraction (25). Three grams of Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO) packed in a 12-mL filtration tube with a polyethylene frit (Supelco, Bellefonte, PA) was preconditioned for 4 h with methanol/water (30/70, v/v). After loading 50 mL of degassed beer, the column was washed with 40 mL of methanol/water (30/70, v/v). Proanthocyanidins were recovered with 70 mL of acetone/water (70/30, v/v). The eluates were concentrated to dryness by rotary evaporation (30 °C) and dissolved in 2 mL of methanol. By the standard addition method, 100%

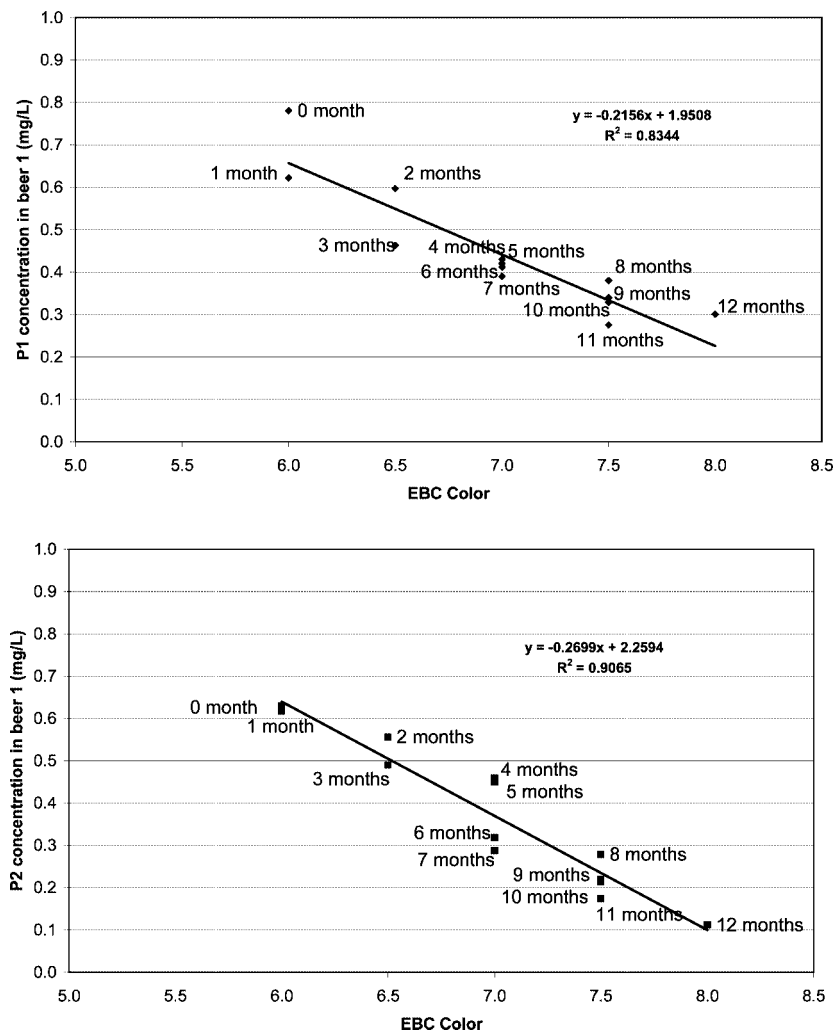


Figure 2. Correlation between P1 or P2 concentration (mg/L in equiv (+)-catechin) and the EBC color of beer 1 stored at 20 °C in the absence of light.

recovery in catechin was calculated (spike with increasing amounts of (+)-catechin before extraction). The same recovery factor was used for P2 and P3.

Preparation of Pure Oligomer Fractions (2, 26). After lipid removal by means of diethyl ether in a Soxhlet extractor, cocoa liquor (Belcolade, Erembodegem, Belgium) was extracted three times with 50 mL of acetone/water/acetic acid (70/28/2, v/v). The supernatants were pooled and concentrated by rotary evaporation (30 °C). The extract was then loaded onto a Sep-Pack cartridge (Waters, Ireland), and sugars were removed with 100 mL of deionized water. Procyanidins were eluted with 50 mL of acetone/water/acetic acid (70/28/2, v/v), concentrated, and freeze-dried. Pure fractions of procyanidins were separated on a 5- μ m, 250- \times 4.6-mm-i.d. normal-phase Luna silica column (Phenomenex, Holland). Chromatographic separation was accomplished using a flow rate of 1 mL/min with a multilinear dichloromethane (A)-methanol (B) gradient containing a constant 4% level of acetic acid/water (1/1, v/v). Gradient elution was 82–72% A, 0–20 min; 72–61% A, 20–50 min; 61–10% A, 50–55 min; 55–60 min isocratic; and return to the initial conditions for 15 min. Procyanidins were monitored at 280 nm (9 nm bandwidth). Twenty microliters of sample (10000 mg/L of procyanidin extract in methanol) was injected into the column kept at 25 °C. Fifty different HPLC runs were performed. Each minute, eluate was collected in a separate vial by the automatic collector (Pharmacia, Belgium). The contents of adequate vials (P2, from 18 to 19 min; P3, from 23 min) were pooled, concentrated to dryness, and freeze-dried.

Model Media Aging. Commercial (+)-catechin, B2, and fractions collected by NP-HPLC were prepared in non-degassed Milli-Q water (100 mg/L) and distributed in independent sealed flasks ($\text{pH}_{\text{cat}} = 5.7$,

$\text{pH}_{\text{B2}} = 5.9$, $\text{pH}_{\text{P2}} = 6.02$, and $\text{pH}_{\text{P3}} = 5.84$). They were then stored at 4 or 20 °C (with or without light). Samples were analyzed after 1, 2, 3, 5, 10, 21, 31, 60, and 90 days.

Color Analyses. Color was measured according to the official EBC method (27) comparison with a set of standardized discs from 1 to 27 EBC; Lovibond 3-field comparator (Analis, Namur, Belgium) with a light source calibrated for CIE standard illuminant D₆₅ (6500 K, daylight, UV-corrected).

HPLC Analyses (2). A SpectraSystem (Finnigan MAT, San Jose, CA) equipped with an SCM degasser, an AS3000 autosampler, and a P4000 quaternary pump was used. Proanthocyanidins were monitored from 200 to 800 nm with a UV6000LP diode array detector. Mass spectra were acquired with an LCQ ion trap mass spectrometer equipped with an ESI source. Collision-induced dissociation spectra were recorded at a relative collision energy of 30, 35, and 40% respectively for singly charged $[\text{M} - \text{H}]^{-1}$ ions of monomers, dimers, and trimers-hexamers. The system was controlled with Xcalibur software, version 1.2. Quantifications were achieved using the calibration curve of (+)-catechin. Five microliters of beer sample was injected into the column kept at 25 °C. *For NP-HPLC-DAD-ESI(-)-MS/MS analyses*, a 5- μ m, 250- \times 2.1-mm-i.d. Alltima HP silica column (Alltech, Deerfield, IL) was used at a flow rate of 0.2 mL/min. A postcolumn addition of ammonium acetate (10 mM in methanol) at 0.05 mL/min was applied. Chromatographic separation was obtained using a multilinear dichloromethane (A)-methanol (B) gradient containing a constant 4% level of acetic acid/water (1/1, v/v). Gradient elution was the same as described above for preparation of pure fractions. The ESI inlet conditions were as follows: source voltage, 4.5 kV; capillary voltage, -6 V; capillary

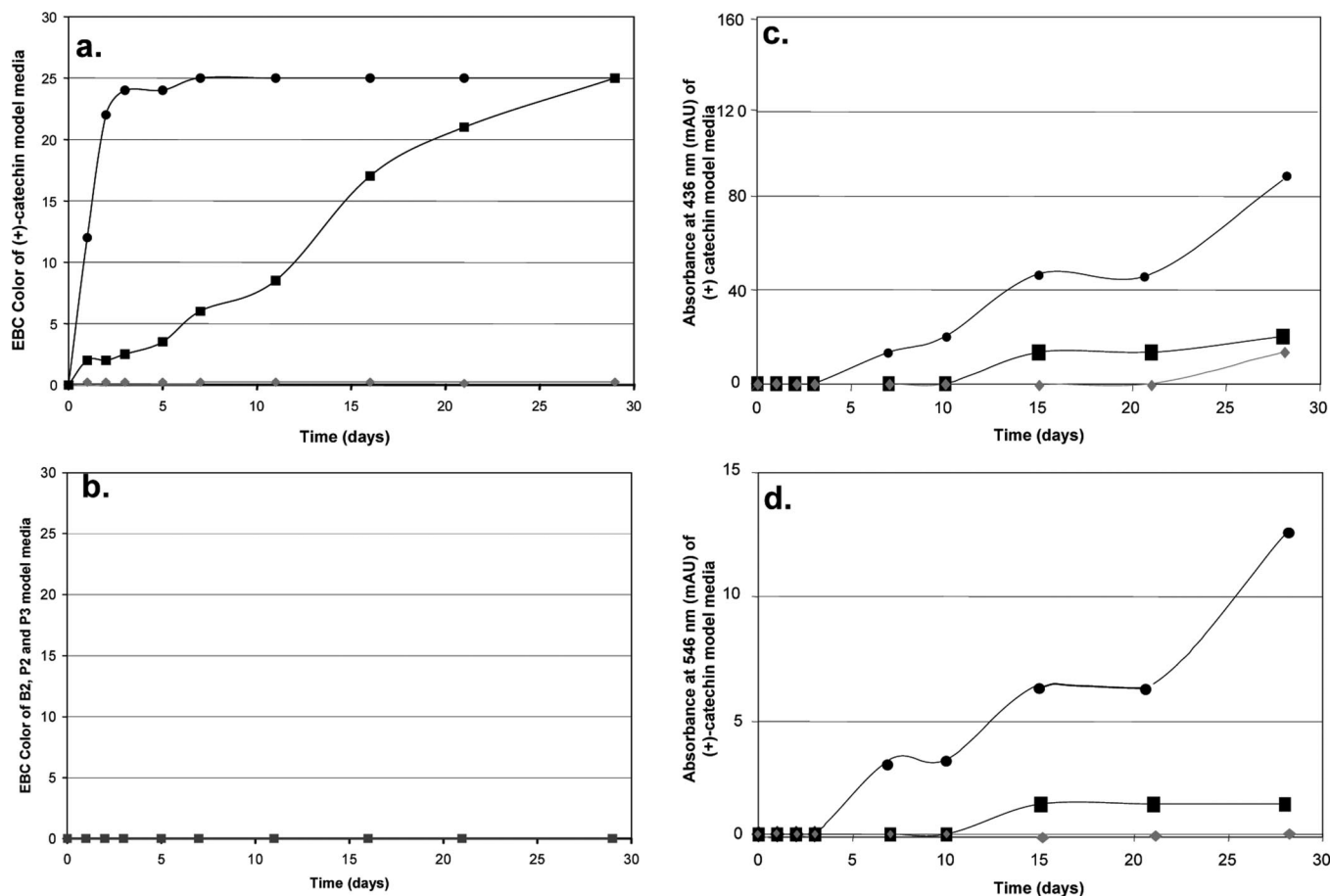


Figure 3. Evolution during storage at 4 and 20 °C, with (L) and without light (D), of the EBC color of a commercial (+)-catechin (a) and of various procyanidins (P2, P3, or B2) (b). Absorbance at 436 (c) and 546 nm (d) of the commercial (+)-catechin model medium. All solutions were at a concentration of 100 mg/L. ●, 20 °C, L; ■, 20 °C, D; ◆, 4 °C, D.

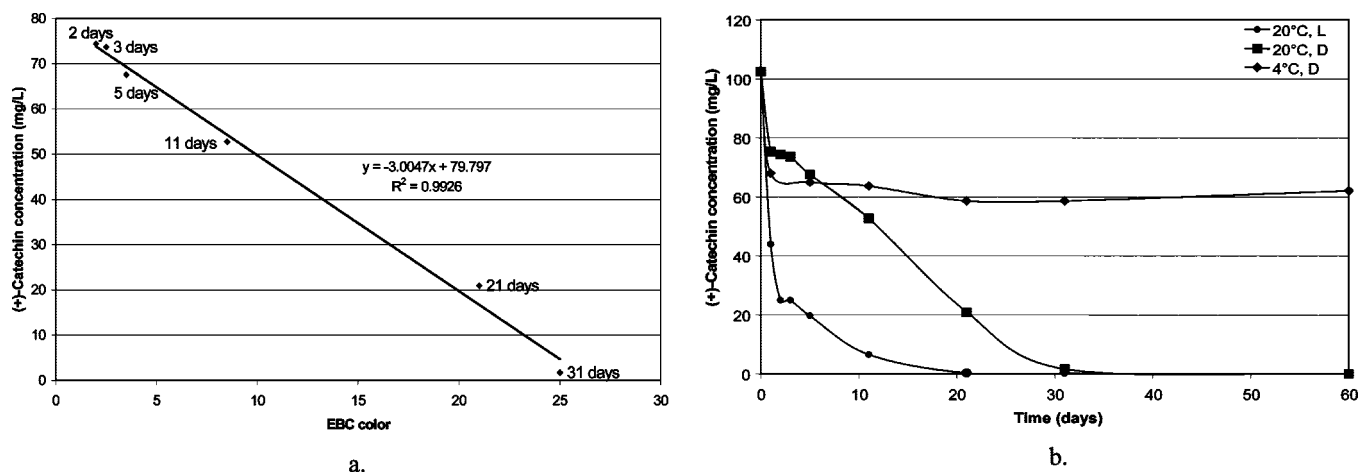


Figure 4. (a) Correlation between (+)-catechin concentration (mg/L) and the EBC color of aqueous solutions stored at 20 °C in the absence of light. (b) Evolution of (+)-catechin concentration during storage at 4 and 20 °C, with (L) or without light (D).

temperature, 200 °C; and sheath gas, 20 psi. For RP-HPLC-DAD-ESI-MS/MS analyses, a 2- μ m, 150- \times 2.1-mm-i.d. C18 Prevail column (Alltech) was used at a flow rate of 0.2 mL/min. A multilinear gradient of water containing 1% acetonitrile and 0.1% formic acid (A) and acetonitrile (B) was applied. Gradient elution was 97–91% A, 0–5 min; 91–84% A, 5–15 min; 84–50% A, 15–45 min; 50–10% A, 45–48 min; 48–51 min isocratic; and then return to the initial conditions for 15 min. For the ESI source, the following inlet conditions were applied: source voltage, 4.9 kV; capillary voltage, –4 V; capillary temperature, 200 °C; and sheath gas, 40 psi.

RESULTS AND DISCUSSION

Evolution of Color during Beer Storage. As depicted in **Figure 1a–c**, the color intensity increased during the 1-year storage, especially at 20 °C, even though the samples were protected from light. For beer 2, which was stored in a PET bottle (known to exhibit higher oxygen permeation), color evolved more quickly, reaching 9.5°EBC after 1 year (**Figure 1b**). On the other hand, the color of beer 3, which was stabilized with silica gel instead of PVPP (beers 1 and 2), evolved similarly to that of beer 1 (**Figure 1a** vs **c**), despite

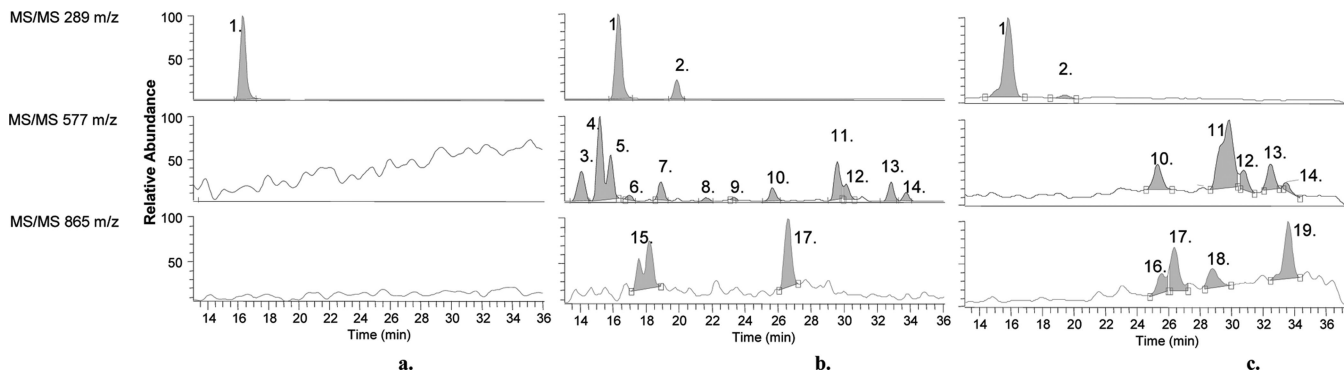


Figure 5. RP-HPLC-ESI(-)-MS/MS of (+)-catechin aqueous model media (100 mg/L) stored at 20 °C for 0 day (a), 5 days (b), and 31 days (c) in the absence of light with (1) (+)-catechin, (2) (+)-epicatechin, (4) dehydrocatechin B4, (7) dehydrocatechin B, (11 and 13) dehydrocatechin A, and (17–19) dehydrotricatechin A (the other peaks are unknowns).

Table 1. Retention Times on the RP-HPLC column, UV-Visible Maximum Absorbance, M_w , and Mass Spectra of “More Polar” (a) and “Less Polar” (b) Compounds Formed after Storage of a (+)-Catechin Model Medium

peak no.	t_R (min) ^a	UV max	$[M - H]^{-1}$	DP ^b	MS/MS ions m/z (%)	proposed identification (M_w)
1	16.36	280	289.1	1	245.2 (100), 205.2 (35), 246.2 (15), 179.1 (13), 289.1 (4)	(+)-catechin (290)
2	19.37	280	289.1	1	245.2 (100), 205.2 (35), 246.2 (15), 179.1 (13), 289.1 (4)	(+)-epicatechin (290)
3 a	14.10	280	577.1	2	393.2 (100), 425.1 (82), 439.1 (80), 269.1 (52), 559.1 (51), 533.2 (48)	
4 a	15.22	280	577.1	2	392.1 (100), 425.1 (79), 439.1 (72), 559.1 (60), 533.2 (49), 269.2 (48)	dehydrocatechin B4 (578) with C _{8D} -C _{6'B}
5 a	15.88	280	577.1	2	393.2 (100), 439.1 (86), 425.1 (79), 269.2 (55), 533.2 (51)	
6 a	17.01	280	577.1	2	559.2 (100), 439.1 (51), 533.1 (44), 425.1 (42), 393.2 (40)	
7 a	18.91	280	577.1	2	393.2 (100), 425.1 (94), 439.2 (80), 269.1 (60), 533.2 (52)	dehydrocatechin B (578) with O _{4'B} -C _{8D}
8 a	21.61	280	577.1	2	533.1 (100), 559.1 (82), 425.1 (56), 439.1 (54), 329.1 (45)	
9 a	23.29	280	577.1	2	439.1 (100), 533.1 (71), 559.2 (46), 425.1 (33), 329.1 (30)	
10 b	25.64	400	575.1	2	449.1 (100), 437.1 (40), 394.1 (28), 287.1 (22), 407.1 (20)	
11 b	29.58	400	575.1	2	449.2 (100), 394.1 (58), 287.1 (42), 437.1 (35), 407.1 (28)	dehydrocatechin A (576) with C _{8D} -C _{6'B} and O _{7D} -C _{1'B}
12 b	30.02	400	575.1	2	449.2 (100), 394.1 (58), 287.1 (42), 437.1 (35), 407.1 (28)	
13 b	32.84	400	575.1	2	449.2 (100), 394.1 (75), 287.0 (45), 407.1 (31), 437.1 (27)	dehydrocatechin A (576) with C _{8D} -C _{6'B} , O _{7D} -C _{1'B} , and O _{3C} -C _{3'B}
14 b	33.83	400	575.1	2	449.1 (100), 437.1 (40), 287.1 (38), 394.1 (36), 407.1 (24)	
15 a	18.32	280	865.2	3	727.2 (100), 713.2(35), 847.1 (33), 821.0 (31), 681.1 (30)	dehydrotricatechin B (866) with C _{8D} -C _{6'B} and C _{8G} -C _{6'E}
16 b	25.91	400	863.2	3	725.1 (100), 425.1 (19), 701.0 (17), 712.2 (17), 413.1 (14)	dehydrotricatechin A (864) with C _{8D} -C _{6'B} , C _{8G} -C _{6'E} , and O _{7D} -C _{1'B} or O _{7G} -C _{1'E}
17 b	26.89	400	863.2	3	725.1 (100), 682.1 (55), 711.3 (30), 737.4 (24), 587.1 (19)	dehydrotricatechin A (864) with C _{8D} -C _{6'B} , C _{8G} -C _{6'E} , and [O _{7D} -C _{1'B} and O _{3C} -C _{3'B}] or [O _{7G} -C _{1'E} and O _{3F} -C _{3'E}]
18 b	28.25	400	863.2	3	725.1 (100), 818.9 (21), 711.2 (18), 443.1 (17), 701.2 (15)	dehydrotricatechin A (864) with C _{8D} -C _{6'B} , C _{8G} -C _{6'E} , and [O _{7D} -C _{1'B} and O _{3C} -C _{3'B}] or [O _{7G} -C _{1'E} and O _{3F} -C _{3'E}]
19 b	33.17	400	861.4	3	681.1 (100), 724.0 (43), 530.1 (22), 574.1 (18), 736.1 (13)	dehydrotricatechin A (862) with C _{8D} -C _{6'B} , C _{8G} -C _{6'E} , O _{7D} -C _{1'B} , O _{3C} -C _{3'B} , O _{7G} -C _{1'E} , and O _{3F} -C _{3'E}

^a Retention time on a reversed-phase column eluted with water containing 1% of acetonitrile and 0.1% of formic acid to acetonitrile at a flow rate of 0.2 mL/min. ^b Degree of polymerization.

a much higher proanthocyanidin content (about 10 mg/L before aging vs 1.6 mg/L, **Figure 1e** vs **d**). All these results tend to prove that oxygen could be a limiting factor in color development. Beer bottling in glass or enhanced antioxidant activity minimize the degradation reactions accountable for color.

Stability of Flavanoids during Beer Storage. After a 1-year storage at 20 °C, 75% of small flavanoids were degraded in beer 1, leading to <0.5 mg/L P1–P3 (**Figure 1d**). As depicted in **Figure 2**, the appearance of color during storage correlated well with residual P1 ($r^2 = 0.83$) or P2 ($r^2 = 0.90$). In beer 3 (**Figure 1e**), degradation was obvious only for the dimer (the major fraction in this silica gel-filtered beer (2)) and, to a minor extent, for the trimer. The reduction power of the oligomers may explain why the monomer fraction stayed relatively

unchanged in this case. To understand which procyanidin fractions are involved in beer color instability, model media were studied.

Stability of Flavanoids in Model Media. The EBC color was measured in model media during storage at 4 and 20 °C, with and without light (**Figure 3a,b**). The model media tested were aqueous solutions containing one of the following: commercially available (+)-catechin, commercially available B2 dimer, or a pure procyanidin fraction extracted from cocoa containing either procyanidin dimers (P2) or procyanidin trimers (P3).

In the pure (+)-catechin solution (**Figure 3a**) (100 mg/L; colorless at the beginning), the color quickly evolved. After only 1 day at 20 °C under light, more than 20°EBC was measured. Significant differences were observed between (+)-catechin samples exposed to light or not. Nevertheless, even without light,

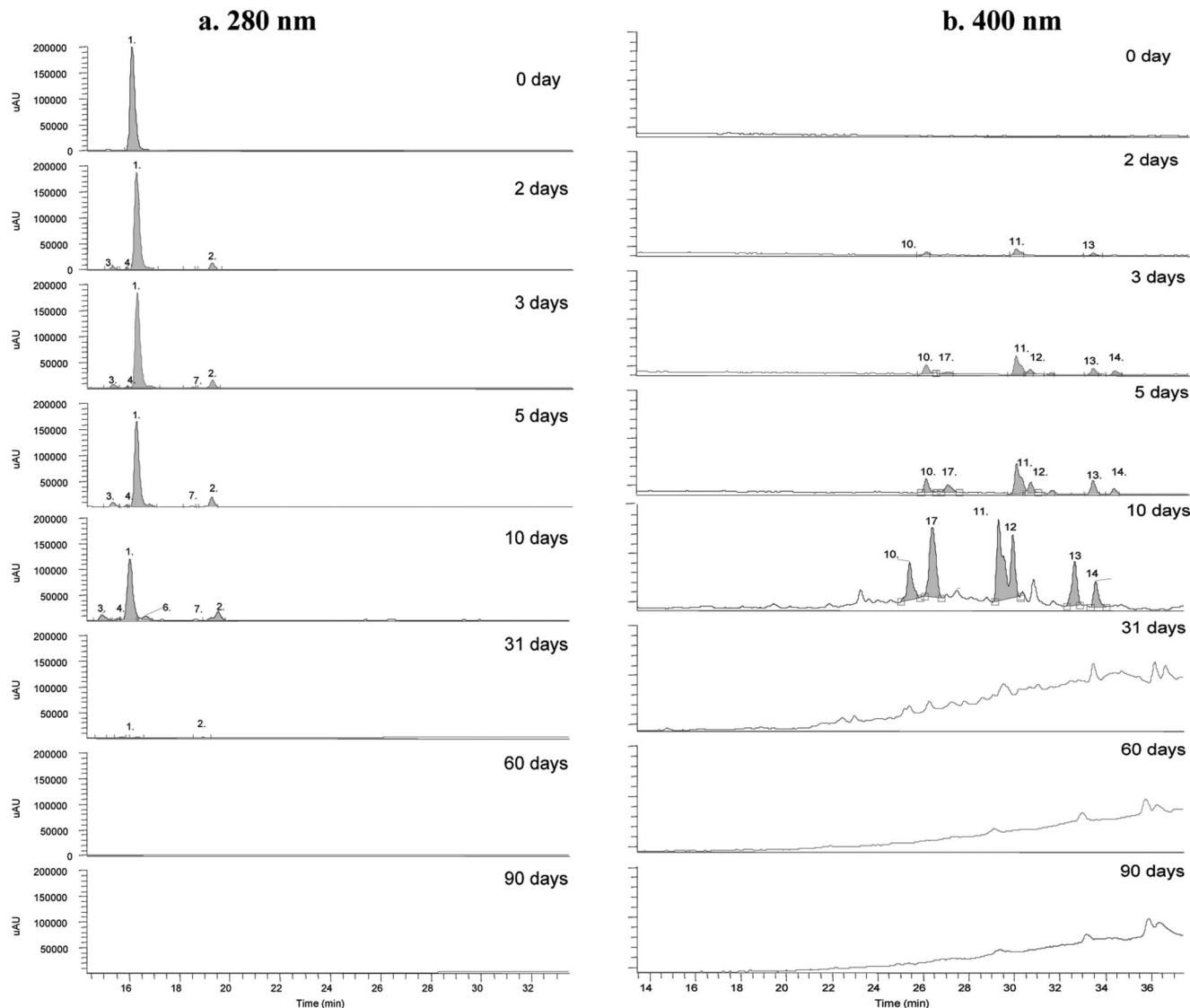


Figure 6. RP-HPLC-DAD at (a) 280 and (b) 400 nm of (+)-catechin aqueous model media (100 mg/L) stored at 20 °C in the absence of light for up to 90 days with (1) (+)-catechin, (2) (+)-epicatechin, (4) dehydrodicathechin B4, (7) dehydrodicathechin B, (11 and 13) dehydrodicathechin A, and (17) dehydrotricatechin A (the other peaks are unknowns). All the chromatograms are on the same scale.

the kinetics was raised in this oxygen-unprotected model medium, compared to beer sample (oxygen <math>< 100 \mu\text{g/L}</math>). This again highlights the key role of oxygen in color development. Absorbance measurements (Figure 3c,d) confirmed that the color of (+)-catechin solutions tended to be more yellow (high absorbance of blue light at wavelength 436 nm) and slightly red-purple (small absorbance of green light at wavelength 546 nm). The EBC color change of (+)-catechin was compared to that observed with three oligomer solutions: commercial B2 and collected dimer (P2) and trimer (P3) fractions. All samples were also stored at room temperature for 1 month. None of the oligomeric fractions showed a significant color change over this period (Figure 3b vs a). As shown in Figure 4a, the appearance of color in the catechin model medium correlated with (+)-catechin degradation ($r^2 = 0.99$). The catechin concentration quickly decreased at 20 °C (Figure 4b, loss of 34% within 5 days, 80% within 21 days, and 100% within 1 month) for the dark storage. The absence of light strongly improved the stability.

Identification of Colored Compounds. To identify the structures giving rise to the yellow-brown color, the model media were analyzed by NP- and RP-HPLC-DAD-ESI(-)-MS/MS. From the normal phase there emerged no new peak, but

RP-HPLC made it possible to investigate a series of compounds synthesized during storage (Figure 5). Table 1 gives the mass spectra of the newly formed compounds. Some of the new compounds (nos. 3–9, 15) eluted before or close to (+)-catechin (no. 1) and (+)-epicatechin (no. 2), suggesting the presence of relatively polar (labeled “a” in Table 1) dimers ($M_w = 577.1$, nos. 3–9) and trimers ($M_w = 866.2$, no. 15). No corresponding absorbance was measured at 400 nm (Figure 6). On the other hand, peaks of dimers ($M_w = 576.1$, nos. 10–14) and trimers ($M_w = 864.1$ and 862.1 , nos. 16–19) eluting after the monomers (probably less polar compounds, labeled “b” in Table 1) absorbed strongly at 400 nm (Figure 6). Identification was tentatively achieved by reference to the pathways proposed by Guyot et al. (28). The colorless polar dimers and trimers (e.g., nos. 4 and 7 in Figure 7) might be formed during the first days of storage from (+)-catechin and *o*-quinone catechin. Dehydrodicathechin B4 (no. 4) could arise through nucleophilic attack by carbon C_{8D} of an unmodified catechin unit on carbon C_{6B} of the quinone. This pathway is known to be enhanced at higher pH, because a high pH increases the nucleophilic character of (+)-catechin (28). The higher acidity of beer (pH = 4.6) could contribute to the difference in reaction rates between beer samples and model media (pH = 5.7–6.0). The O_{4B}–C_{8D} bond

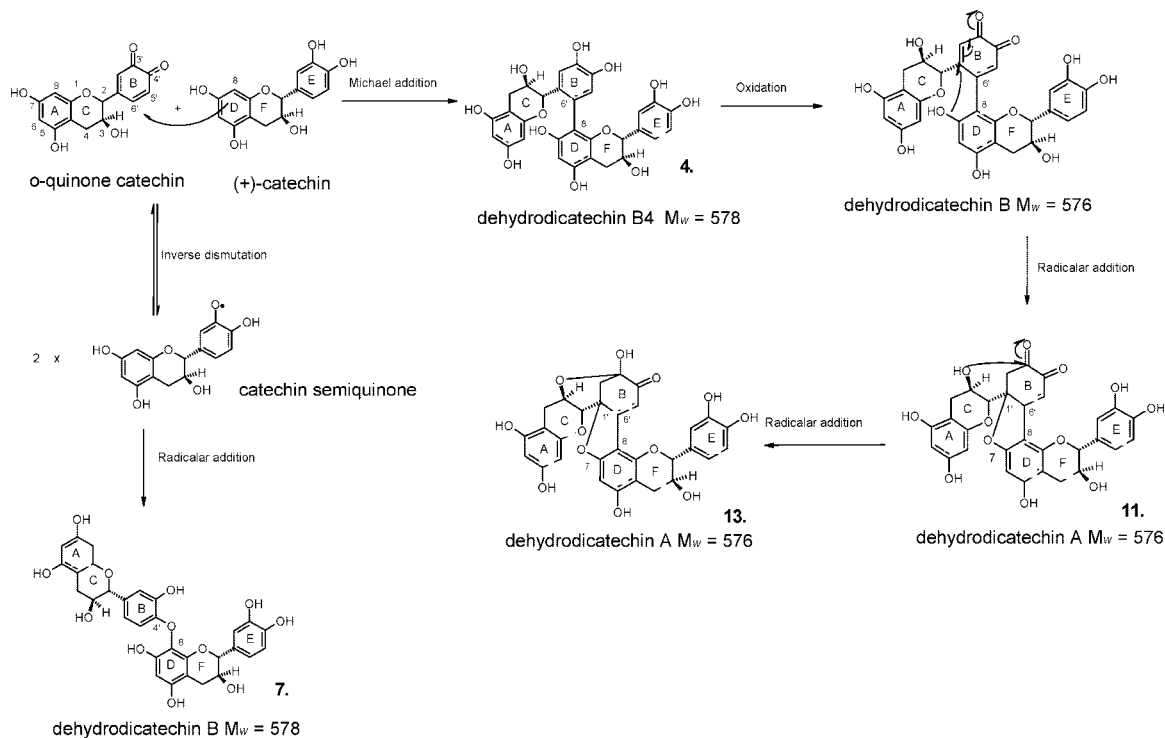


Figure 7. Scheme of (+)-catechin degradation into colorless compounds ($M_w = 578$) and yellow compounds ($M_w = 576$).

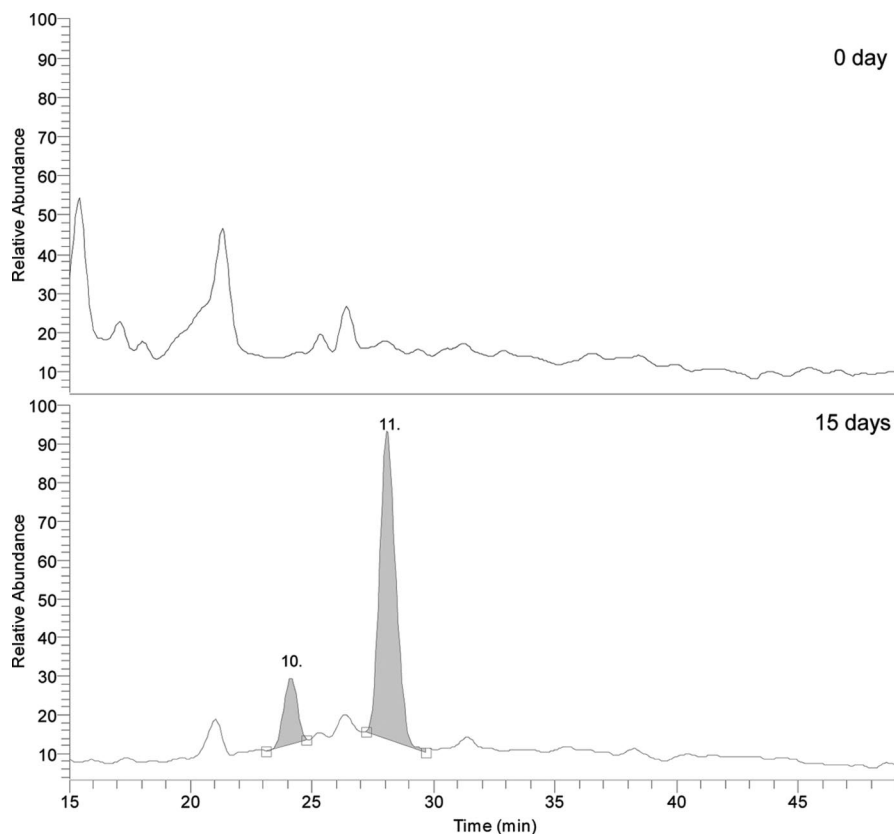


Figure 8. RP-HPLC-ESI(-)-MS/MS at m/z 575.1 of the PVPP-filtered beer 1 spiked with 10 ppm (+)-catechin: (a) fresh beer and (b) after 15 days of storage at 20 °C in a dark room.

(no. 7) probably results from a coupling reaction involving a semiquinone radical. While these polar structures disappeared (**Figure 5c**), less polar colored compounds continued to be formed between days 5 and 31 (**Figure 5c** vs **b**). Colorless dimers (nos. 3–9, especially dehydrodicatechin B4, no. 4) and trimers (no. 15) are most probably precursors of these yellow pigments (e.g., nos. 11 and 13 in **Figure 7**), yielding them

through oxidation and two subsequent intramolecular radical additions. Yet as depicted in **Figure 6**, degradation continued for over 1 month, leading to very apolar molecules that were not well resolved in RP-HPLC. Light-induced mechanisms distinct from the nucleophilic attack likely occur as well in the presence of light. Their identification will require in-depth studies.

Evolution of Color and Degradation Products in a Beer Spiked with (+)-Catechin. The color of a PVPP-treated beer spiked with 10 ppm (+)-catechin evolved up to 9°EBC after 1 month of storage (vs 6°EBC in the control), confirming that the color is generated from the monomer fraction. Moreover, RP-HPLC-ESI(-)-MS/MS revealed the presence of pigments nos. 10 and 11 in the spiked aged-beer samples (**Figure 8**). Structural rearrangements of polyphenols then cause beer color changes during storage. (+)-Catechin emerges as the precursor of those less polar products, characterized by a yellow-brown color. Storage without oxygen and at low temperature is recommended so as to minimize the synthesis of pigments.

ABBREVIATIONS USED

RP, reversed phase; NP, normal phase; ESI, electrospray ionization; MS/MS, tandem mass spectroscopy; HPLC, high-performance liquid chromatography; P1 to P3, procyanidins from monomers to trimers; PET, poly(ethylene terephthalate); PVPP, poly(vinylpyrrolidone); PVP, poly(vinylpyrrolidone); EBC, European Brewery Convention.

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