Research Article

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Roasting conditions for preserving cocoa flavan-3-ol monomers and oligomers: interesting behaviour of Criollo clones

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Abstract

BACKGROUND: Cocoa bean roasting is important for creating the typical chocolate aroma through Maillard reactions, but it is also a key step deleterious to the polyphenol content and profile.

RESULTS: Compared with usual roasting at 150 °C, keeping the beans for 30 min at 120 °C or for 1 h at 90 °C proved much better for preventing strong degradation of native P1, P2 and P3 flavan-3-ols in cocoa (shown for Forastero, Trinitatio and Criollo cultivars). Surprisingly, Cuban, Mexican and Malagasy white-seeded beans behaved atypically when roasted for 30 min at 150 °C, releasing a pool of catechin. Enantiomeric chromatographic separation proved that this pool contained mainly (−)-catechin issued from (−)-epicatechin by epimerisation. As the (−)-epicatechin content remained relatively constant through Criollo bean roasting, flavan-3-ol monomers must have been regenerated from oligomers. This emergence of (−)-catechin in Criollo beans only, reported here for the first time, could be due to increased flavan-3-ol monomer stability in the absence of anthocyanidin-derived products.

CONCLUSION: The degradation rate of flavan-3-ols through roasting is higher in cocoa beans containing anthocyani(di)ns. The liberation of a pool of (−)-catechin when submitted to roasting at 150 °C allows to distinguish white-seeded cultivars.

Keywords: polyphenol; flavan-3-ol; cocoa; roasting; Criollo

INTRODUCTION

The cocoa tree (*Theobroma cacao* L.) is a tropical evergreen species cultivated worldwide in a zone extending about 20° north and south of the equator.[1] Ripe pods are harvested before releasing their content, and the beans surrounded by sweet mucilage are fermented for 4–7 days, dried and then exported.[2] Transformation of the beans into chocolate involves cleaning, roasting, winnowing, grinding, milling and conching.

For decades, four cocoa 'varieties' have been distinguished by chocolate makers, namely the originating Criollo and Forastero, their first hybrid known as Trinitario, and the unique Arriba-like Nacional beans from Ecuador. However, alongside the highly diversified Trinitario group, Motamayor established in 2008 a new, genetically based classification with ten clusters, reflecting much better the potential diversity of cocoa.[3] This diversity concerns agronomic resistance, productivity, pod shape and colour and bean colour and flavour.

Cocoa polyphenols can reach 120–180 g kg^{-1} in raw beans.[4] They belong to several families: hydroxybenzoic acids (gallic/syringic/protocatechic/vanillic acids), hydroxycinnamic acids and analogues (caffeic/ferulic/*p*-coumaric/phloretic acids, clovamide, dideoxyclovamide), flavonols (quercetin), flavones (luteolin, apigenin), flavanones (naringenin) and flavan-3-ols (catechin, epicatechin, oligomers, procyanidins).[5,6] Catechins and proanthocyanidins (58 and 37% of total polyphenols) constitute the main antioxidant fraction, while anthocyanins (4%) bring the colour to unfermented Forastero, Nacional and Trinitario beans.[7] Among the catechins, (−)-epicatechin (molecular weight (Mw) = 290) is by far the most abundant,[8] but (+)-catechin, (−)-gallocatechin and (−)-epigallocatechin are also found in minor quantities.[9] Oligomers built from (−)-epicatechin subunits, from dimers (B2 and B5; Mw = 578) and trimers (C1; Mw = 866) up to dodecamers (Mw = 3458),[10] are the main proanthocyanidins found in cocoa. The mean degree of polymerisation (mDP) of this fraction is around 3.6–4.5.[11] Most oligomers exhibit β-interflavane bonds, but A-type dimers and trimers have also been reported.[12,13] Among the cocoa anthocyanins, cyanidin-3-α-arabinoside and cyanidin-3-β-galactoside constitute the major fraction,[4,14] although cyanidin-3-*O*-glucoside has also been reported.[15]

Several studies have highlighted potential health benefits of cocoa polyphenols, such as regulation of carbohydrate metabolism,[16,17] a significant decrease in both systolic and diastolic blood pressure in hypertensive, pre-hypertensive and normotensive people,[18,19] anti-inflammatory effects[20,21] and anticarcinogenic properties.[22,23]

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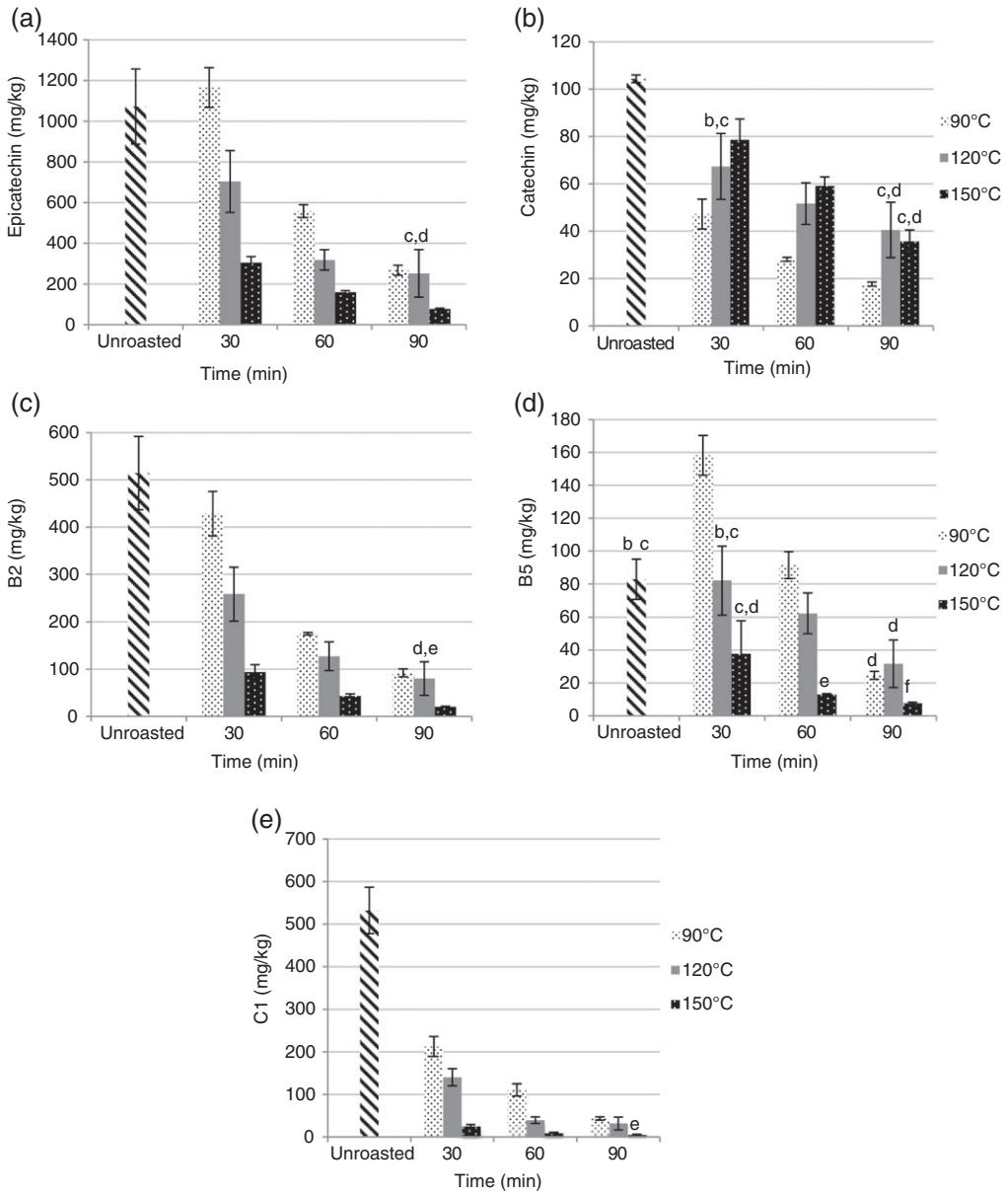

Figure 1. Levels (mg kg^{-1}) of (a) catechin, (b) epicatechin, (c) procyanidin B2, (d) procyanidin B5 and (e) procyanidin C1 in Bahia Forastero cocoa beans after roasting (at 90/120/150 °C for 30/60/90 min). Values are expressed as means and error bars of analyses done in replicate. Values that do not share a common letter in the same graph are significantly different ($P < 0.05$).

Cocoa bean roasting is a crucial step in the synthesis of chocolate-like Maillard products. It is usually carried out for 30–45 min at 130–150 °C. Low-temperature roasting has recently been proposed as an alternative preventing excessive degradation of native polyphenols. Several studies have confirmed severe loss of total polyphenols through usual roasting.[24–26] Having shown that (−)-catechin is formed from native (−)-epicatechin by epimerisation, Cooper *et al.*[27] have proposed (−)-catechin as an indicator of the roasting strength. Epimerisation also affects dimers and trimers, which are additionally sensitive to depolymerisation.[28] In addition to epimerisation, neosynthesis of oligomers with particular interflavane bonds has also been evidenced.[29] The aim of the present study was to better assess how different roasting schemes might modulate the final content of flavan-3-ol monomers, dimers and trimers. Reverse phase high-performance liquid chromatography coupled with negative electrospray ionisation tandem mass spectrometry (RP-HPLC/ESI(−)-MS/MS) was used to quantify polyphenols in six different cocoa bean clones subjected to various heat treatments. Enantiomeric chromatography was also used to understand what occurs in white-seeded Criollo beans, where a pool of catechin seems to be released during roasting.

MATERIALS AND METHODS

Chemicals

Acetonitrile (99.99%), diethyl ether (99.9%), ethanol (97%), ethyl acetate (97%), acetone (97%) and methanol (99.9%) were supplied by VWR (Leuven, Belgium). Formic acid (99%) was obtained from Acros Organic (Geel, Belgium). (−)-Epicatechin (98%), (+)-catechin (98%) and (−)-catechin (98%) were supplied by Sigma-Aldrich (Bornem, Belgium). (−)-Epicatechin-4β-8-(−)-epicatechin (B2, 90%) and kaempferol

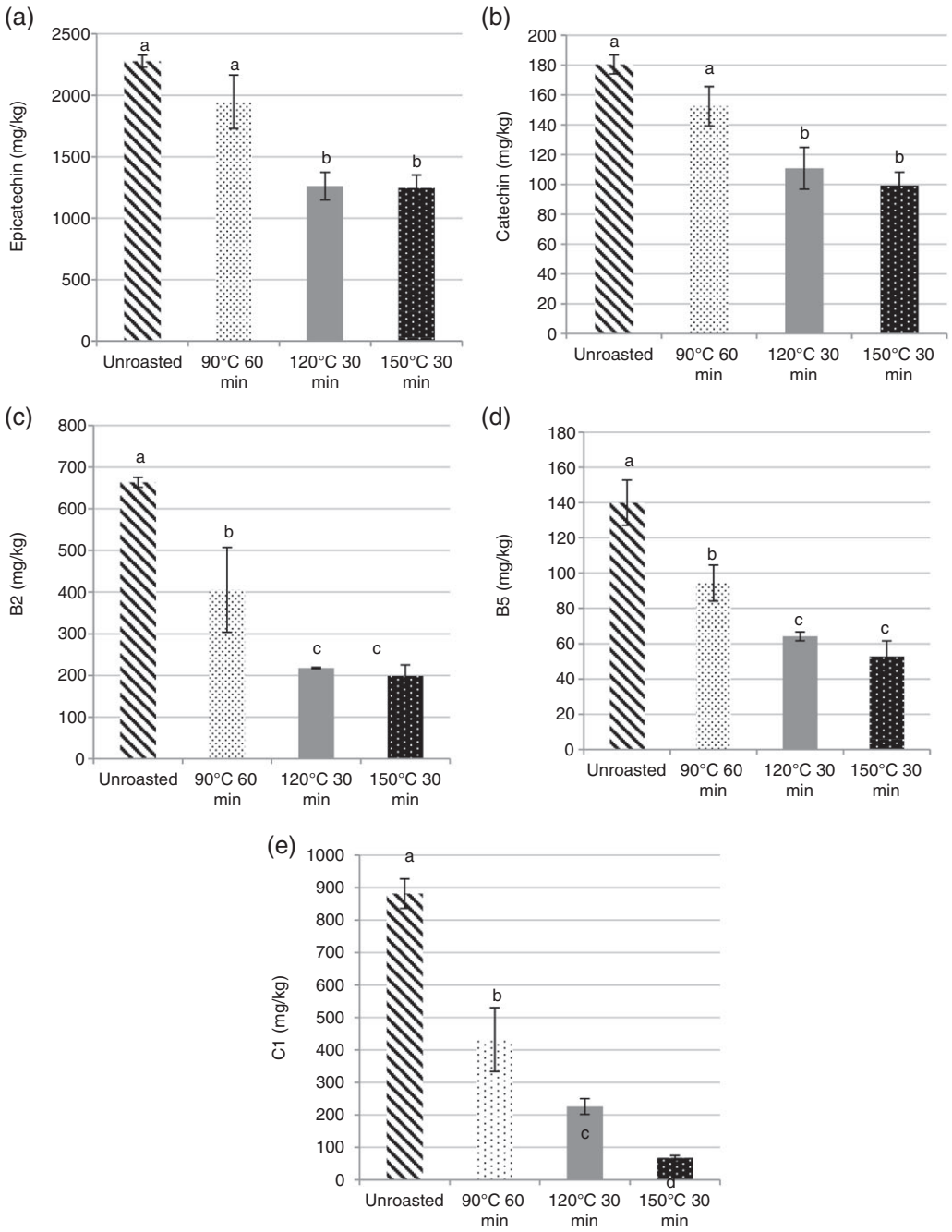

Figure 2. Levels (mg kg^{-1}) of (a) catechin, (b) epicatechin, (c) procyanidin B2, (d) procyanidin B5 and (e) procyanidin C1 in UF654 Trinitario cocoa beans before and after roasting. Values are expressed as means and error bars of analyses done in replicate. Values that do not share a common letter in the same graph are significantly different ($P < 0.05$).

(>90%) were obtained from Extrasynthese (Genay, France). (−)-Epicatechin-4β-8-(−)-epicatechin-4β-8-(−)-epicatechin (C1, 99%) was supplied by PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). Aqueous solutions were made with Milli-Q water (resistance 18 mΩ) (Millipore, Bedford, MA, USA).

Cocoa samples

Forastero beans from Bahia (Brazil) and Criollo beans from Madagascar and Mexico (Carmelo) were provided by Le Cercle du Cacao (Brussels, Belgium). Trinitario (UF654) and Criollo (C411) beans from Baracoa (Cuba) were provided by the Instituto de Investigaciones para la Industria Alimenticia (La Habana, Cuba). All beans had a moisture content below 70 g kg^{-1} and a fermentation index above 1.2, indicating well-fermented beans.

Cocoa roasting

Beans were spread in one layer on a perforated tray and roasted in a ventilated heat chamber (Serie 4000, EHRET Labor- und Pharmatechnik GmbH & Co KG, Emmendingen, Germany) at 90, 120 or 150 °C for 30, 60 or 90 min. Thirty beans were milled at room temperature and homogenised before flavan-3-ol extractions were performed in duplicate.

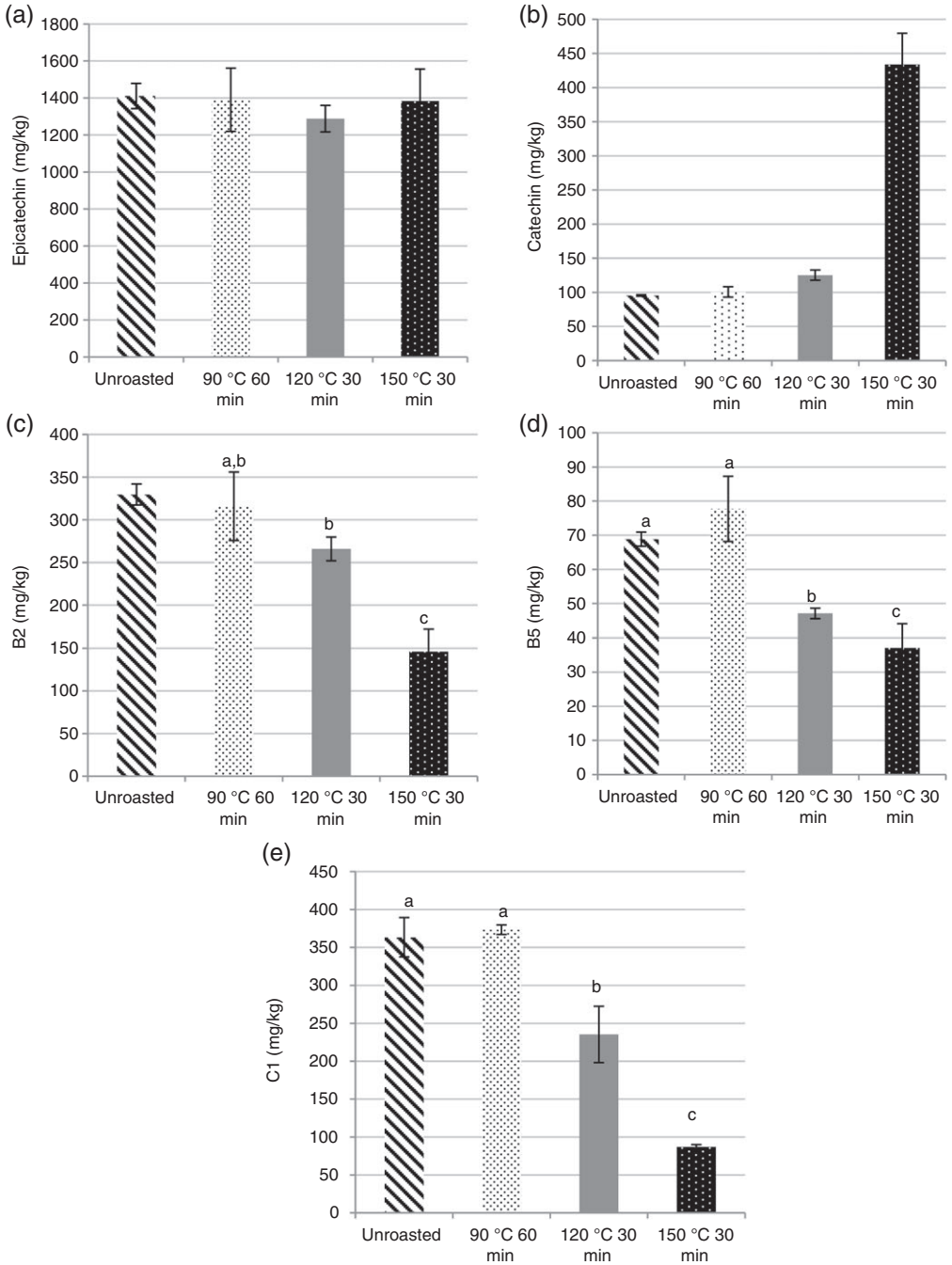

Figure 3. Levels (mg kg^{-1}) of (a) catechin, (b) epicatechin, (c) procyanidin B2, (d) procyanidin B5 and (e) procyanidin C1 in C411 Criollo cocoa beans before and after roasting. Values are expressed as means and error bars of analyses done in replicate. Values that do not share a common letter in the same graph are significantly different ($P < 0.05$).

Extraction of flavan-3-ols from cocoa

Cocoa beans (7 g) were defatted with diethyl ether (3 × 50 mL) at room temperature under gentle stirring. After centrifugation (2900 × *g*), the samples were dried for 24 h under vacuum (1 × 10^{-6} bar). Defatted samples (~4.5 g) spiked with 500 µL of kaempferol at 10 000 mg L^{-1} in methanol (used as internal standard; amounting to 714 mg kg^{-1} non-defatted beans) were extracted with 3 × 50 mL of acetone/water/acetic acid (70:28:2 v/v/v) and purified on a 10 g C18 Sep-Pak® cartridge (Waters, Millipore) preconditioned with 200 mL of methanol and 300 mL of water. The samples were finally eluted with 50 mL of acetone/water/acetic acid (70:28:2 v/v/v), concentrated by rotary evaporation and freeze-dried. All extraction steps were done in duplicate.

RP-HPLC/ESI(−)-MS/MS quantitation of flavan-3-ols

Quantitations were performed on a C18 Prevail column (150 mm × 2.1 mm, 2.7 µm) (Grace, Deefield, IL, USA) eluted with a linear gradient of A (water/acetonitrile/formic acid, 97:1:2 v/v/v) to B (acetonitrile/formic acid, 98:2 v/v). Gradient elution was as follows: from 97 to 91% A in 5 min, from 91 to 85% A in 25 min, from 85 to 64% A in 35 min, from 64 to 10% A in 10 min and isocratic for 20 min at a flow rate of 200 µL min^{-1}. Samples (5 µL) were

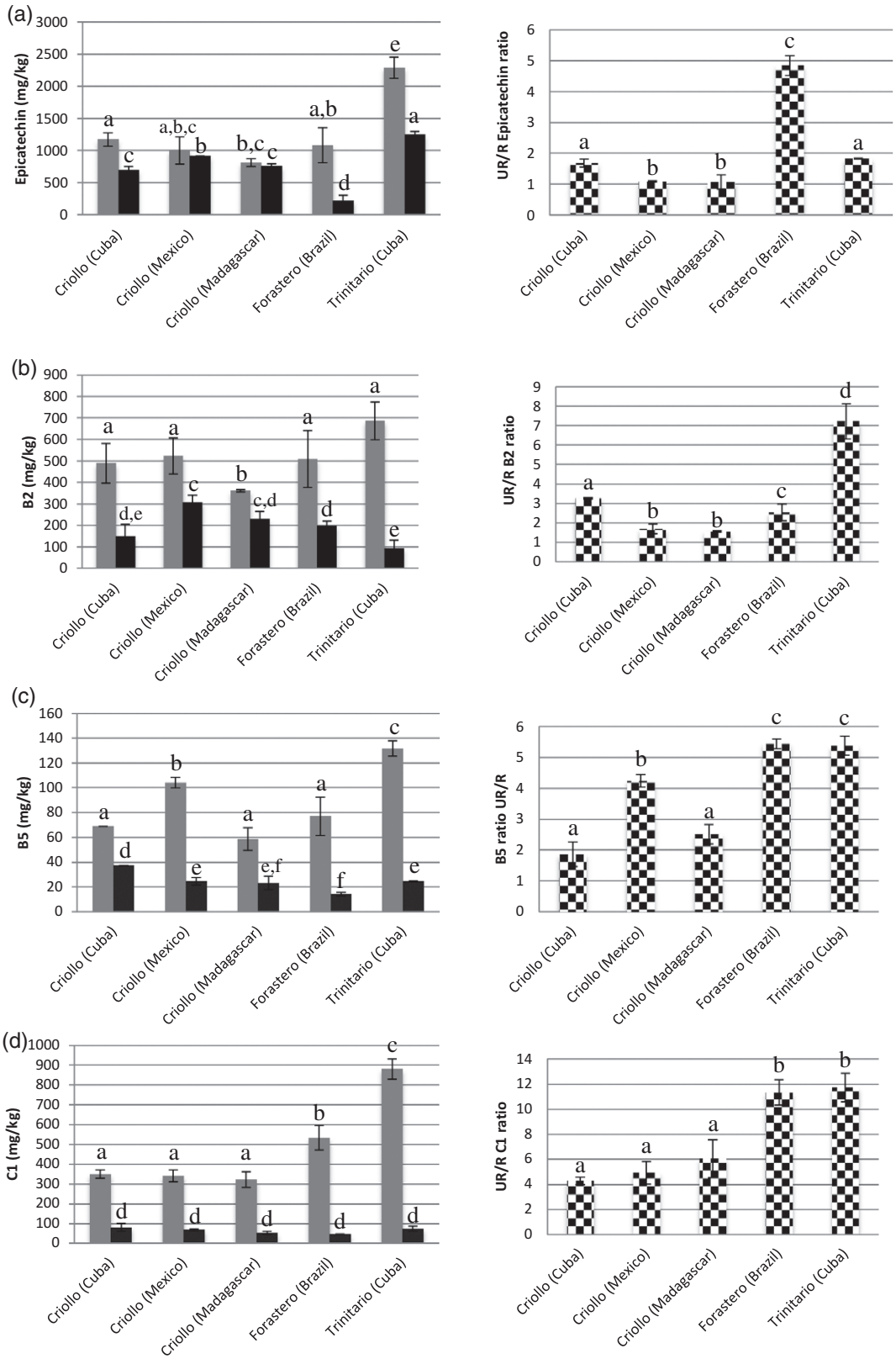

Figure 4. (Left) Levels (mg kg^{-1}) of (a) epicatechin, (b) procyanidin B2, (c) procyanidin B5 and (d) procyanidin C1 in Cuban, Mexican and Malagasy Criollo beans, Brazilian Forastero beans and Cuban UF654 Trinitario beans before (light grey) and after (dark grey) roasting for 30 min at 150 °C. (Right) Corresponding unroasted/roasted (UR/R) concentration ratios in each sample. Values are expressed as means and error bars of analyses done in replicate. Values that do not share a common letter in the same graph are significantly different ($P < 0.05$).

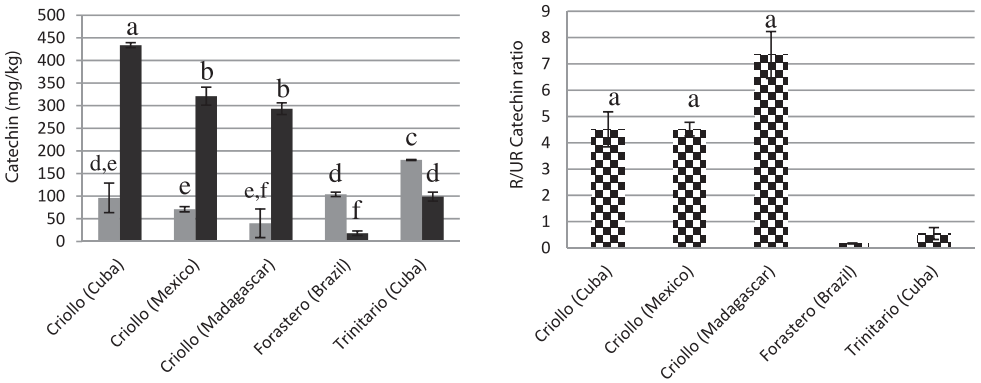

Figure 5. (Left) Levels (mg kg^{-1}) of catechin in Cuban, Mexican and Malagasy Criollo beans, Brazilian Forastero beans and Cuban UF654 Trinitario beans before (light grey) and after (dark grey) roasting for 30 min at 150 °C. (Right) Corresponding roasted/unroasted (R/UR) concentration ratios in each sample. Values are expressed as means and error bars of analyses done in replicate. Values that do not share a common letter in the same graph are significantly different ($P < 0.05$).

injected in duplicate onto the column kept at 20 °C. A SpectraSystem (Finnigan Mat, San Jose, CA, USA) equipped with an AS3000 autosampler and a P4000 quaternary pump was used. The system was controlled with Xcalibur Version 1.2 software (ThermoFisher, Austin, TX, USA). Mass spectra were acquired with an LCQ Duo ion trap mass spectrometer equipped with an ESI source (ThermoFisher, Austin, TX, USA). Collision-induced dissociation spectra were recorded at 30, 35 and 40% relative collision energy for singly charged $[M-H]^-$ ions of monomers (*m/z* 289), dimers (*m/z* 577) and trimers (*m/z* 865) respectively. A window of 1 *m/z* was set for each mass. The ESI inlet conditions were as follows: source voltage, 4.9 kV; capillary voltage, −4 V; capillary temperature, 200 °C; sheath gas pressure, 39 psi. For ESI(−)-MS/MS quantitations in cocoa beans (kaempferol used as internal standard), a relative recovery factor of 1 was applied for all compounds. Flavan-3-ol monomers were quantitated according to the calibration curves of (+)-catechin and (−)-epicatechin (0, 10, 25, 50, 100 mg L^{-1}, $R^2 = 0.99721$ and 0.99648 respectively), procyanidins B2 and B5 with that of B2 (0, 10, 25, 50, 100 mg L^{-1}, $R^2 = 0.99831$), and C1 with itself (0, 10, 25, 50, 100 mg L^{-1}, $R^2 = 0.99873$).

Enantiomeric chromatography

An ASTEC CYCLOBOND 2000 RSP column (250 mm × 4.6 mm, 5 µm) (Supelco, Bellefonte, PA, USA) was used under the conditions described above. Detection was done by ESI(−)-MS/MS (*m/z* 289). Proper identification of (epi)catechin enantiomers was performed after thermal degradation for 30 min at 90 °C of commercially available (+)-catechin and (−)-epicatechin, in order to get the four enantiomers ((+)/(−)-catechin and (+)/(−)-epicatechin) by forced epimerisation.

Statistical analyses

Multiple comparisons of means were performed with Student–Newman–Keuls tests using SAS Version 9.2 software (SAS Institute Inc., Cary, NC, USA). Values that do not share a common letter are significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Comparison of nine combinations of cocoa roasting time and temperature

To assess how the duration and strength of the roasting step might impact the flavan-3-ol content of a chocolate, a first experiment was conducted on Forastero cocoa beans from Bahia (Brazil). A temperature of 90, 120 or 150 °C was applied for 30, 60 or 90 min. After extraction of monomers, dimers and trimers, identification and quantitation were performed by RP-HPLC/ESI(−)-MS/MS.

As depicted in Fig. 1a, (−)-epicatechin, the main flavan-3-ol in cocoa, was greatly affected by roasting. At 90 °C, its concentration remained constant for the first 30 min but decreased thereafter, by about half with each additional 30 min of roasting. At higher oven temperature, a strong decrease was observed already after 30 min of roasting (by 34% at 120 °C and 72% at 150 °C), with further decreases when roasting was prolonged. Whatever the duration of the roasting period, the (−)-epicatechin concentration decreased by about half with each 30 °C increment in oven temperature. In conclusion, the only tested combination preserving (−)-epicatechin was 30 min at 90 °C. Such conditions could not be proposed to large-scale chocolate factories, where stronger Maillard reactions are required for flavour.

In these experiments, catechin (Fig. 1b) showed a very different profile. Generally, the higher the temperature, the higher was the level of catechin (except for 90 min at 150 °C, conditions under which flavan-3-ol monomers might easily be oxidised to dehydrodicatechin A and higher oligomers). This confirms the results of Cooper *et al.*,[27] who also evidenced epimerisation of (−)-epicatechin into (−)-catechin. At each tested temperature, the longer the heat treatment, the lower was the amount of recovered catechin. This could be because the balance between (−)-catechin chemical degradation and (−)-epicatechin epimerisation is in favour of the former when the epicatechin concentration is greatly diminished.

Like the monomers, the native B2 and B5 dimers and the C1 trimer also suffered greatly from roasting (Figs 1c–1e). For all three oligomers, the trend was very close to that observed with (−)-epicatechin (except at 90 min, when the results might be influenced by the nature of intermediate oligomers). The C1 trimer was more affected than the dimers and monomers (the loss was already 61% after 30 min at 90 °C and 98% after 90 min at 150 °C).

As expected, the shortest roasting at the lowest temperature (30 min at 90 °C) was the treatment most appropriate for preserving native compounds. However, as this treatment is totally insufficient for generating chocolate-like Maillard flavours, it was not kept for the next experiment.

Behaviours of beans of different genetic groups

The above-described results obtained with Forastero beans from Bahia (Fig. 1) were compared with those obtained with Trinitario

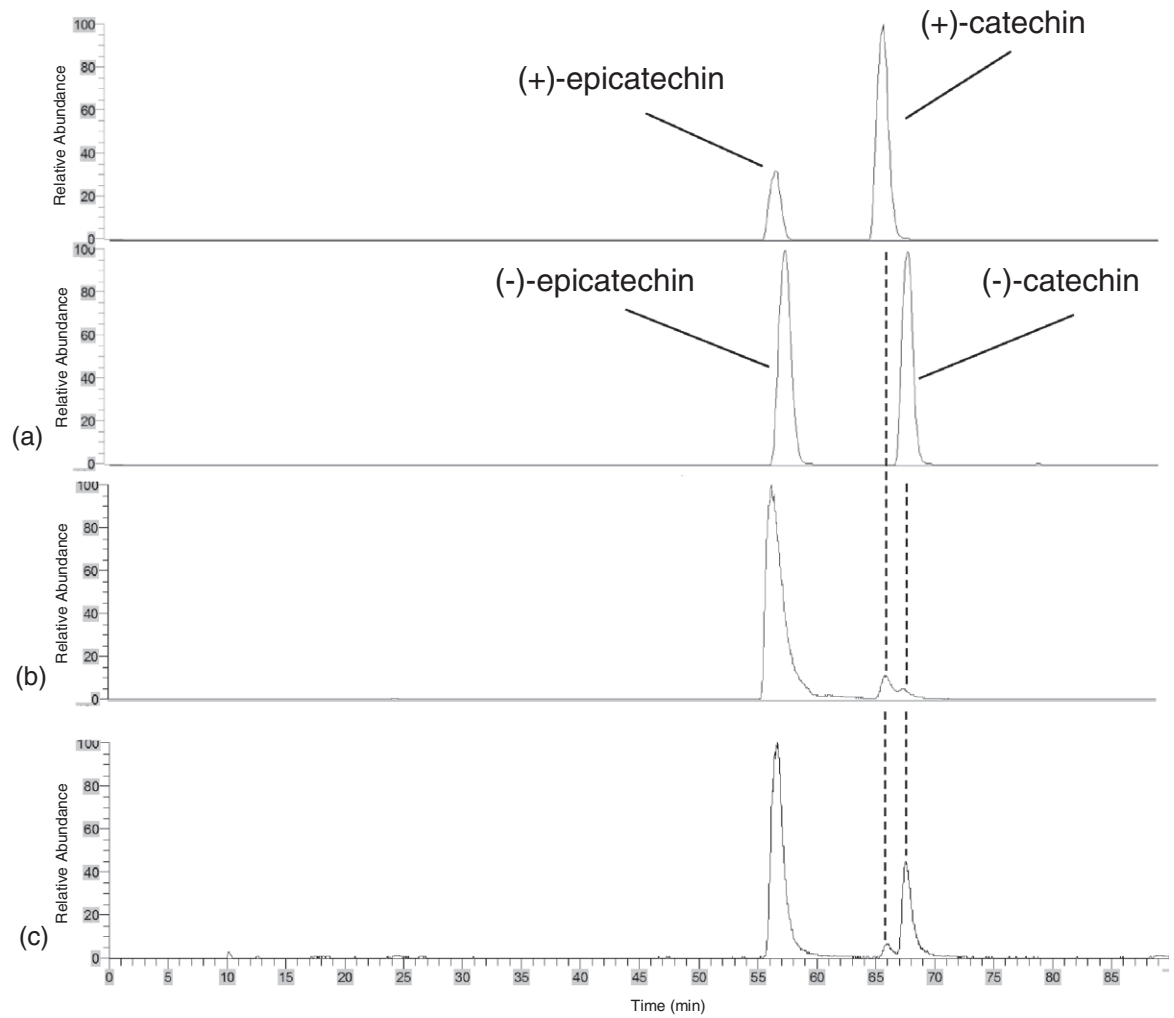

Figure 6. Enantiomeric HPLC/ESI(−)-MS/MS chromatograms (*m/z* 289) of (a) a mixture of catechin and epicatechin obtained by epimerisation (30 min at 150 °C) of pure (+)-catechin (above) or (−)-epicatechin (below) and (b, c) the flavan-3-ol extract issued from Malagasy Criollo beans (b) before and (c) after roasting for 30 min at 150 °C.

UF654 (Fig. 2) and Criollo C411 (Fig. 3) beans subjected to roasting for 60 min at 90 °C, for 30 min at 120 °C and for 30 min at 150 °C.

Of the clones tested in our laboratory, Trinitario UF654 emerged as the richest in native flavan-3-ols, and notably much richer than Forastero (up to 2200 mg kg^{-1} epicatechin, 180 mg kg^{-1} catechin, 650 mg kg^{-1} B2, 140 mg kg^{-1} B5 and 890 mg kg^{-1} C1). It also displayed much better resistance to heat treatment. Even the harshest treatment tested (30 min at 150 °C) caused no more than 45% loss of epicatechin (Fig. 2a) compared with 71% for Forastero. This apparent resistance of (−)-epicatechin to heat could be due to depolymerisation of oligomers, also present here in higher amounts, and to the high induced antioxidant activity of the beans. Trinitario UF654 also showed better recovery of dimers B2 and B5 (Figs 2c and 2d) and trimer C1 (Fig. 2e) after roasting than Forastero (−69 *vs* −83% for B2, −62 *vs* −74% for B5 and −91 *vs* −92% for C1 after 30 min at 150 °C). However, as observed for Forastero, trimers were more affected than monomers or dimers, since fewer were regenerated from higher oligomers. Yet catechin (Fig. 2b) was less preserved in UF654 than in Bahia beans after roasting under usual conditions (−44 *vs* −25%).

Compared with our Forastero and Trinitario samples, Criollo beans exhibited a very different behaviour upon roasting (Fig. 3). The initial amount of (−)-epicatechin, close to that measured in Forastero beans, remained stable through roasting, whatever the heat treatment applied (Fig. 3a). Even more surprising was the level of catechin found after roasting for 30 min at 150 °C, about three times the expected level (Fig. 3b), in the absence of any drop in (−)-epicatechin. Two hypotheses could explain our results: either the additional amount was native (+)-catechin or it was generated through (−)-epicatechin epimerisation. As classical RP-HPLC columns cannot separate the two catechin enantiomers, an appropriate chiral column was used to distinguish them (see below). As in the case of Forastero and Trinitario beans, major losses of all three oligomers were observed at 150 °C (−55, −42 and −76% for B2, B5 and C1 respectively after 30 min at 150 °C) (Figs 3c–3e). Trimer C1 was again the most affected flavan-3-ol. Yet B2, B5 and C1 were completely preserved after 60 min at 90 °C. This suggests either a particular resistance of flavan-3-ols in Criollo (devoid of anthocyanins) or the presence of higher amounts of oligomers able to refill the pools depleted of native compounds through degradation. In all three beans investigated, procyanidin B5 proved more resistant to heat treatment than procyanidin B2, whatever the time and temperature. This resistance might be due to different interflavane bonds in the two dimers (C4—C6 in B5 and C4—C8 in B2).

Comparison of three Criollo samples during roasting for 30 min at 150 °C

To see if white beans other than from C411 clone would show the same behaviour as above, white beans from Mexico (Carmelo) and Madagascar were roasted for 30 min at 150 °C (Figs 4 and 5). When unroasted, the Criollo beans of all three origins showed similar levels of epicatechin and B2, B5 and C1 oligomers (generally lower than in the Trinitario beans from Cuba; Figs 4a–4d, left). After roasting for 30 min at 150 °C, lower losses were recorded for Criollo beans, as indicated by lower unroasted/roasted (UR/R) concentration ratios (Figs 4a–4d, right). In beans of the same genetic type, the apparent degradation ratio increased from monomer to trimer.

Like the Cuban white-seeded beans, the Mexican and Malagasy Criollo beans showed substantially more catechin than Forastero and Trinitario after roasting for 30 min at 150 °C (Figure 5, higher roasted/unroasted (R/UR) catechin ratios). This trend thus appears characteristic of the Criollo genetic origin.

The ratio of catechin enantiomers was determined by enantiomeric chromatrography. As depicted in Fig. 6a, the four (epi)catechin enantiomers eluted in the order suggested by Cooper *et al.*,[27] namely (+)-epicatechin, (−)-epicatechin, (+)-catechin and (−)-catechin. Under our elution conditions optimised for catechin enantiomers (i.e. the enantiomers we wanted to distinguish), (+)-epicatechin and (−)-epicatechin co-eluted.

In unroasted Criollo cocoa beans from Madagascar, (+)-catechin was as expected the major epimer (Fig. 6b). Traces of (−)-catechin were also detected, probably because of native (+)-catechin epimerisation during drying in the sun. As shown in Fig. 6c, the additional amount of catechin detected after roasting at 150 °C was mainly (−)-catechin.

CONCLUSION

We proved that the apparent degradation rate of cocoa flavan-3-ols through roasting was higher for trimer and dimers than for monomers. We also showed that this degradation was higher in cocoa beans containing anthocyani(di)ns. The evidence of (−)-catechin by chiral chromatography confirmed the atypical behaviour of white beans when roasted at 150 °C. Complementary analyses are now needed to determine which anthocyanin-derived products[30] are most involved in the disappearance of flavan-3-ols in Forastero and Trinitario.

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