Fate of flavonoid and theobromine in cocoa processing

- Relevance of differentiated roasting and cocoa bean origin

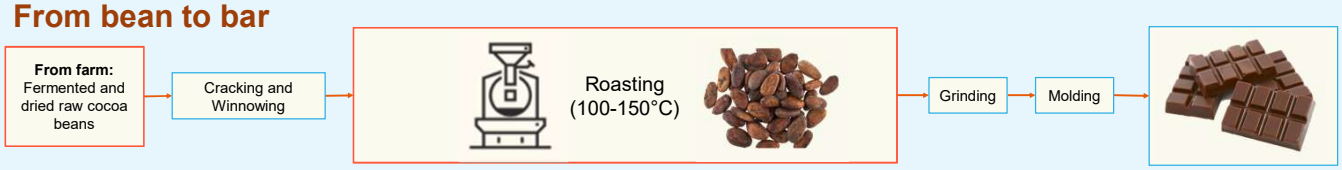
Ditte Baun Hermund, Quoc-Khanh Rose-Marie Madsen, Louise Kirstein Larsen, Sara Riegels Trangbæk, Ann-Dorit Moltke Sørensen, Jacob Kaya & Charlotte Jacobsen

dbala@food.dtu.dk

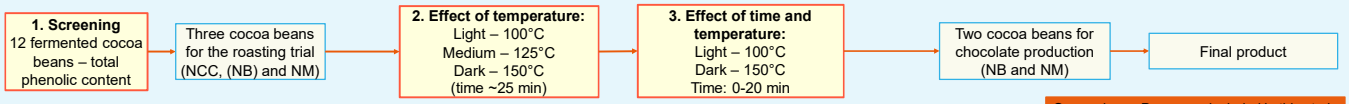
Background Phenolic compounds and theobromine from cocoa exhibit a wide range of health promoting activities, including the ability to scavenge of free radicals, inhibit generation of superoxide radicals and lower blood pressure. From bean to chocolate bar, these compounds undergo degradation, polymerization or changes in their chemical structure, which are associated with their activity. By engineering processing of cocoa into final products, the content of phenolic compounds can be optimized. Studies have previously found that the phenolic content varies with origin of cocoa beans. Moreover, during processing, fermentation and roasting changes the phenolic profile, where especially roasting temperatures have shown to play a major role in these changes.

The aim of this study was to screen 12 fermented cocoa beans and select 3 with a high phenolic content, then identify phenolic compounds in the beans and determine the fate of selected compounds due to roasting temperatures by studying the changes in the phenolic profiles during the roasting process (see Experimental design). Results were obtained by extraction of phenolics (see Extraction) for determining the total phenolic content and the concentration of selected flavonoids using UHPLC-DAD-MS.

From bean to bar



Experimental design



Abbreviations: N=Nicaragua, CC=Chuno Classico, M=Medalla, B=Barba, TPC=Total phenolic content

Orange box = Processes included in this study

Results of experiment 1, 2 and 3

1. Screening – TPC of cocoa beans depending on origin

- Cocoa beans from Nicaragua (N) had the highest TPC (up to 140 mg GAE/g dw) (data not shown) and **three beans sorts with this origin were selected for further experiments (NCC, NM and NB)**

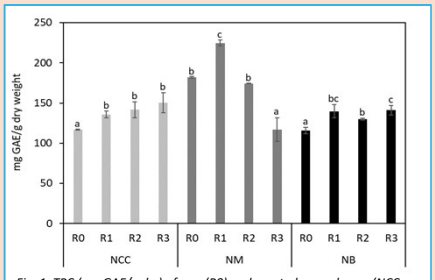


Fig. 1: TPC (mg GAE/g dw) of raw (R0) and roasted cocoa beans (NCC , NM and NB) with three roasting profiles (R1: 100°C, R2: 125°C and R3: 150°C). Mean±SD (n=3)

2. Effect of roasting temperature on TPC and phenolic profile of cocoa beans

- TPC (Fig. 1) of NCC increased (225 mg GAE/g dw) after light roasting at 100°C and afterwards decreased with increasing roasting temperatures (down to 121 mg GAE/g dw)
- Both NM and NB had a significantly higher TPC in the roasted beans compared to the raw beans
- Nine phenolic compounds were identified using UHPLC-DAD-MS – Catechin, epicatechin and procyanidin were selected and quantified including theobromine (data not shown)
- Whereas procyanidin and epicatechin followed the same pattern as TPC, theobromine was much more heat sensitive and decreased in all beans according to the increased roasting temperatures (Fig. 2). NCC and NB had similar phenolic profiles, and generally had lower content than NM. **NM and NB were selected for further studies**

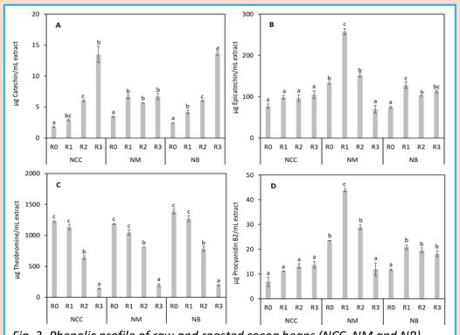


Fig. 2. Phenolic profile of raw and roasted cocoa beans (NCC, NM and NB) with three roasting profiles (light (R1: 100°C), medium (R2: 125°C) and dark (R3: 150°C)) determined by UHPLC-MS. (A) (-)-catechin, (B) (-)-epicatechin, (C) theobromine, and (D) procyanidin B2. Letters indicate significance ($p < 0.05$) between raw and the three roasted beans for specific cocoa bean sort. Mean±SD (n=2)

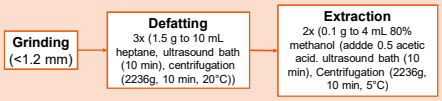
3. Effect of roasting time and temperature on phenolic profile and theobromine content (Fig. 3)

- Concentration of catechin did not change during 20 min of roasting at 100°C, but increased significantly ($p < 0.05$) after 15 min at 150°C (from 4.9 to 10.7 µg/ml extract for NCC and from 3.7 to 13.5 µg/ml extract for NM (Fig. 3A). The increase was coupled to the epimerization of epicatechin to catechin (>150°C) (Fig. 4)
- Overall the epicatechin level for NCC increased around 100 to 200 µg/ml extract by roasting at 100°C for 20 min. However, no increase or decrease was detected when roasting at 150°C event though a decrease was expected according to the catechin increase (epimerization) (Fig. 3B)
- Procyanidin levels also flocculated during roasting time without a general pattern, showing no increase or decrease in the concentration of procyanidin during roasting of NCC and NM for either roasting temperatures (Fig. 3D)
- The theobromine content in both NCC and NM did not change during roasting at 100°C. Roasting at 150°C causes a significant ($p < 0.05$) decrease in concentration after 15 min for NM and after 10 min for NCC (Fig. 3C)

Methodology

Extraction

Defatting step of the grinded cocoa beans was included in the extraction of phenolic compounds to increase the extraction yield



Analysis

- Total phenolic content (TPC). Spectrophotometric (725 nm) using Folin ciocalteu assay. mg gallic acid equivalent (GAE)/g dw
- Identification and quantification of flavonoids and theobromine using UHPLC-DAD-MS

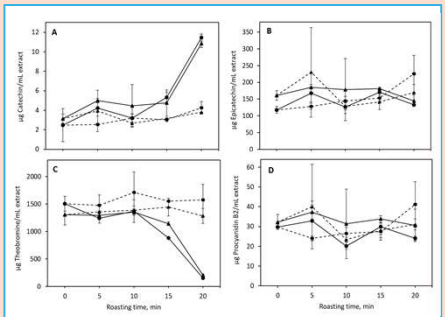
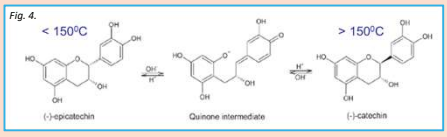


Fig. 3. Concentration (µg/mL extract) of flavonoids and theobromine during roasting (five time points; 0, 5, 10, 15 and 20 min) of NCC (dot) and NM (triangle) at two different roasting temperatures (light: 100°C (dotted line) and dark: 150°C (full line)) determined by UHPLC-MS. Mean±SD (n=2)



Conclusion It was concluded that by selecting cocoa beans (NCC and NM) with a high phenolic content and applying differentiated roasting temperatures depending on bean sort, it was possible to obtain a high phenolic content

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