Discrimination and characterization of dark chocolates based on polyphenolic profiles by liquid chromatography with UV and fluorescence detection

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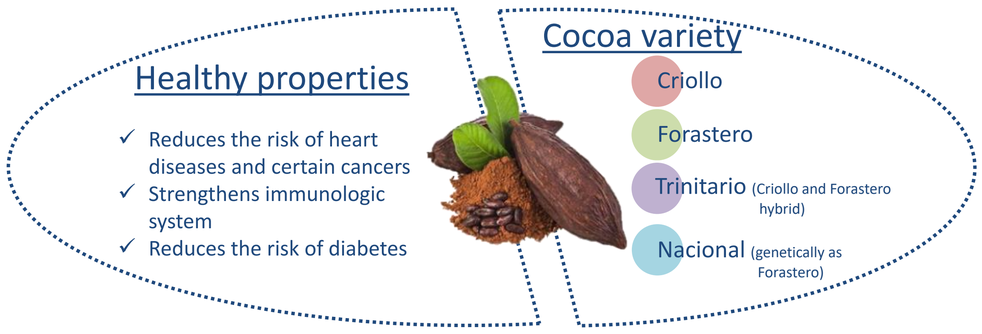
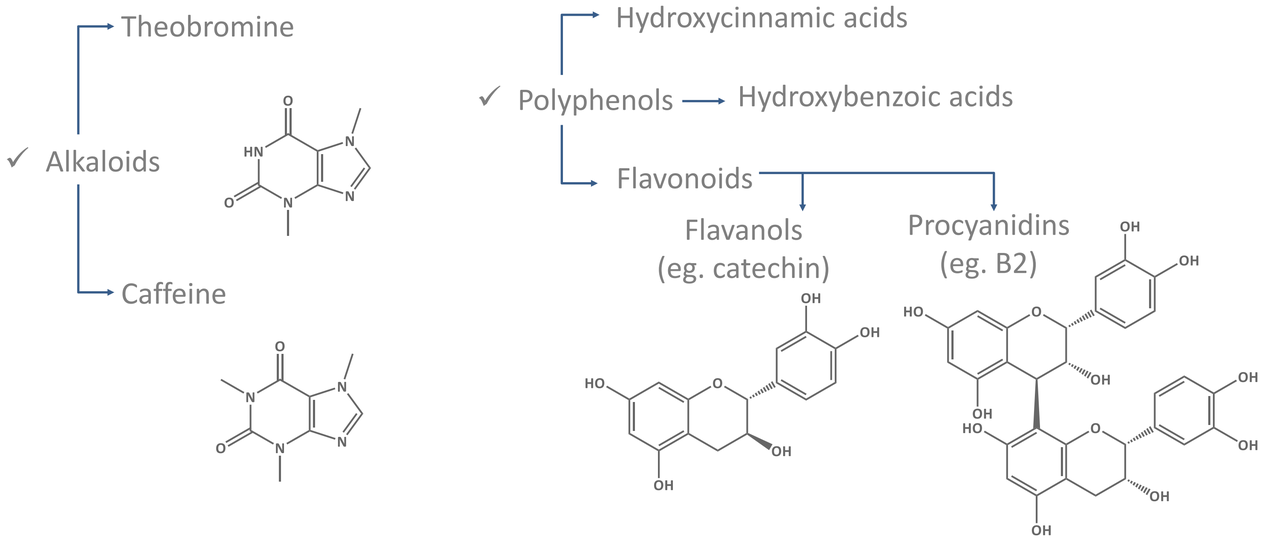
Introduction

The popularity of dark chocolate has increased greatly in recent years not only because it is considered a delicatessen, but also due to its high polyphenolic content that provides some interesting healthy properties, such as antioxidant and anti-carcinogenic activities.



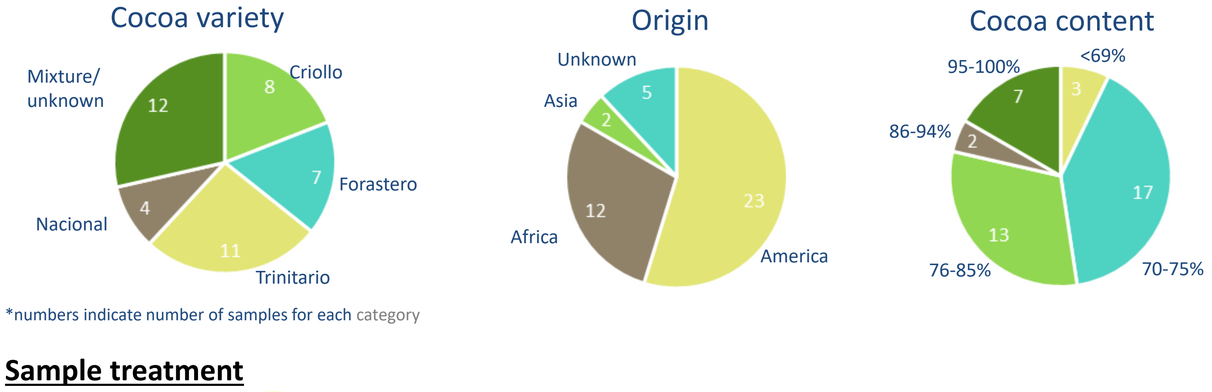
Cocoa content



This work aims at characterizing and classifying dark chocolate samples based on their geographical origin, cocoa variety and cocoa content using alkaloid and polyphenolic composition as the data.

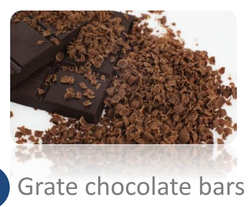
Samples and methods

Samples analyzed (42 dark chocolate samples commercially available)



*numbers indicate number of samples for each category

Sample treatment



1 Grate chocolate bars

2 Defatting process

Fat was extracted with cyclohexane (5 mL/g sample). The mixture was gently mixed and sonicated during 15 min. Supernatant was separated by centrifugation (5 min, 4500 rpm). This procedure was repeated three times. Finally, defatted cocoa was left to dry at room temperature.

3 Extraction

4 mL of MeOH/1% formic acid in water (60:40) were added to 0.2 g of defatted cocoa. The mixture was stirred and sonicated for 15 min. Supernatant was separated by centrifugation (5 min, 4500 rpm) and filtered with 0.2 µm nylon filter before LC analysis

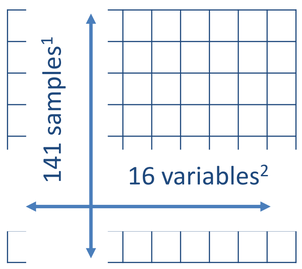
HPLC-UV-FLD

Column: Kinetex® C18 2.6 µm (150 x 4.6 mm) (Phenomenex)
Mobile phase: Channel A: 0.1% formic acid in water;
Channel B: acetonitrile
Flow rate: 0.7 mL·min^{-1}
UV detection at λ 280, 325, and 370 nm
Fluorescence detection at λ_{ex} 280 and λ_{em} 330 nm

t (min)	% B	Remarks
0	18	Separation range
20	48	
22	90	Cleaning step
24	90	
24.2	18	Stabilization time
29	18	

Data analysis

Data matrix

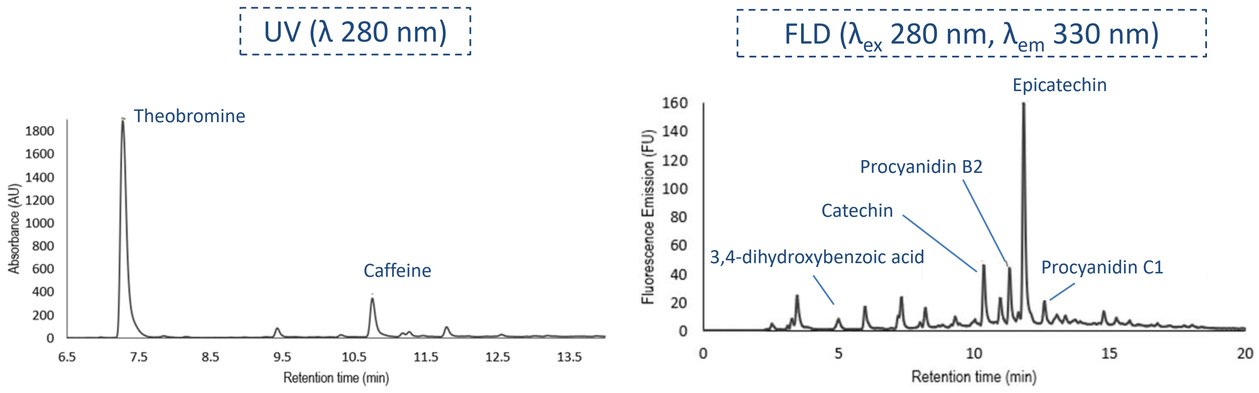


[1] 42 samples extracted by triplicate + QC sample injected 15 times (every 10 samples)
[2] chromatographic area of 14 peaks (identified and non identified compounds) selected from LC-UV (280 and 370 nm) and LC-FLD chromatogram + total peak area of the LC-UV (280 nm) and LC-FLD chromatograms

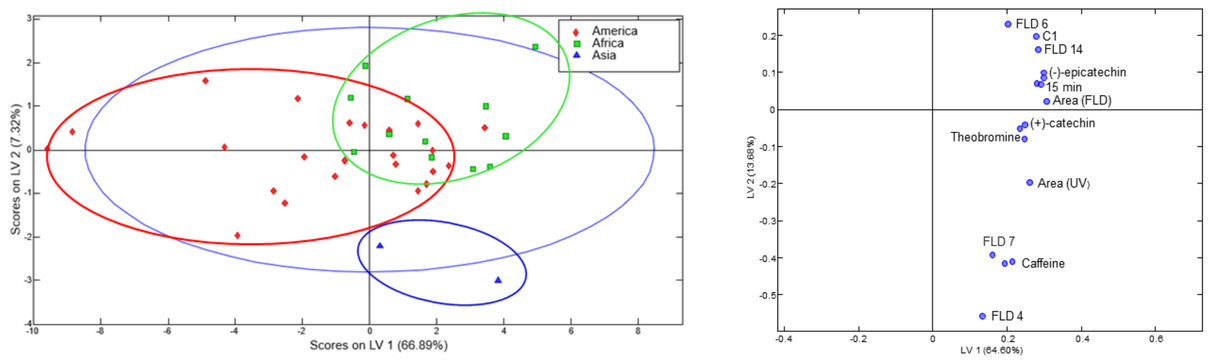
Principal component analysis (PCA) → Exploratory
Partial least squares – discriminant analysis (PLS-DA) → Classification

Results

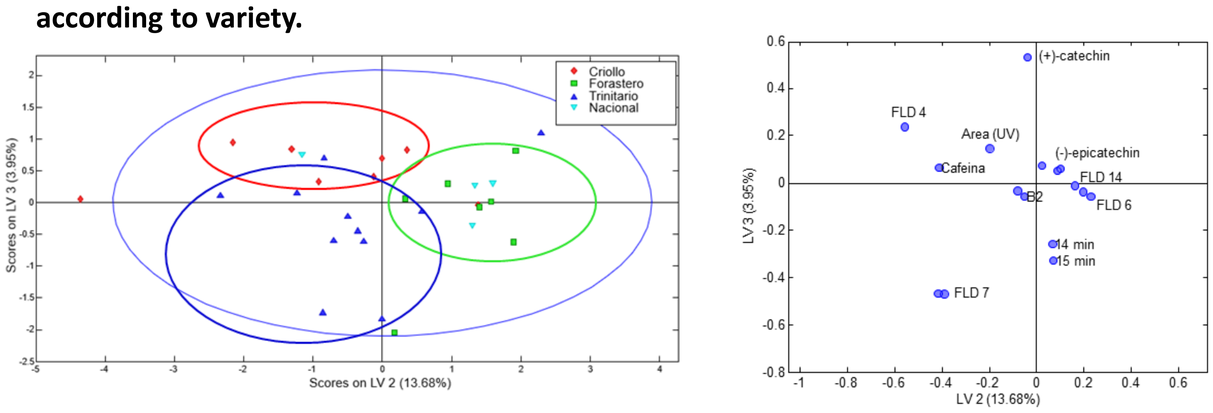
Chromatograms of a QC sample (prepared by mixing 70 µL of the extract obtained from each chocolate sample)



Characterization of samples by PLS-DA using the X-matrix as the data. Scatter plot of scores of LV1 vs LV2 (left); Scatter plot of loadings of LV1 vs LV2 (right). Classification according to origin.



Characterization of samples by PLS-DA using the X-matrix as the data. Scatter plot of scores of LV2 vs LV3 (left); Scatter plot of loadings of LV2 vs LV3 (right). Classification according to variety.



Conclusions

- ✓ Classification and discrimination of the chocolates were achieved based on their variety and origin.
- ✓ In general, PLS-DA provides better separation of the samples than PCA.
- ✓ Geographical origin can be discriminated using LV1 and LV2, whereas LV2 and LV3 classify samples according its variety.
- ✓ African samples are characterized by the abundance of flavanols while American samples are richer in alkaloids.
- ✓ (-)-epicatechin, (+)-catechin and procyanidins B2 and C1 are more abundant in Forastero and Nacional derived chocolates, caffeine and theobromine in Criollo, and Trinitario is characterized by the high levels of flavonols and poor (+)-catechin content.

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