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Toward the biochemical and sensory characterization of *C. stenophylla* in Côte d'Ivoire.

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Introduction

Coffea stenophylla, a rare and indigenous coffee species from West Africa, is gaining renewed interest in Côte d'Ivoire due to its natural heat tolerance and exceptional sensory qualities (flowers and fructification are shown in **Figure 1**). This study aims to characterize its biochemical composition and flavor attributes in order to assess its potential for varietal diversification and the revitalization of the Ivorian coffee sector.

Fig. 1. Flowers and fructification of *C. stenophylla*

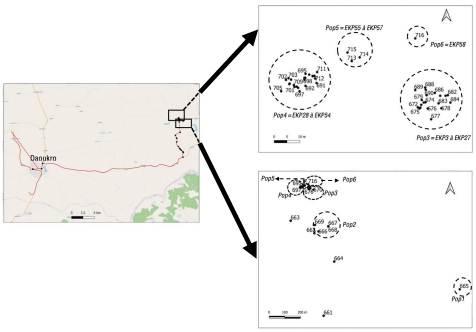

Fig. 2. Discovery of a native *C. stenophylla* forest in eastern Côte d'Ivoire and sampling of individuals from local populations.

Experimental

Mature beans were collected from forest zones in the Côte d'Ivoire (see **Figure 2**) and post-harvest processed to get green coffee beans. Two different samples were prepared and named sample 1 (130 g) and sample 2 (40 g). Green coffee beans were ground in a ball mill (Retsch MM400) to fine powder and the samples were analyzed using standard analytical protocols to determine alkaloids, chlorogenic acids, carbohydrates and diterpenes content. In particular quantification of trigonelline, caffeine and theobromine was performed on aqueous green coffee solution by Agilent 1290 Infinity UHPLC operating at 254 nm for trigonelline and 273 nm for caffeine and theobromine, Zorbax SB-C18 column (Agilent) and gradient elution: acetonitrile and aqueous formic acid. For chlorogenic acids analyses an aqueous methanolic extraction following a previously published method [1] was used and quantification performed by comparison to an authentic standard of 5-O-caffeoylquinic acid. Gradient elution: acetonitrile and aqueous formic acid, Acquity BEH C18 column (Waters) and 305, 324 nm wavelength UV detector as previously reported [1]. Carbohydrates were analyzed by HPLC with refraction index detector (RID) on an Agilent 1260 series system (Agilent Technologies, USA) equipped with a ZORBAX-Carbohydrate analytical column (5 µm, 4.6 mm x 250 mm, Agilent Technologies, USA). Aqueous green coffee solutions were analyzed by using ACN:H2O 60:40 w/w, as the mobile phase at a flow rate of 1.0 mL/min, the injection volume was 5 µL and the column temperature 30 °C. For the determination of diterpenes (cafestol, kahweol, 16OMC and 16OMK), coffee samples were saponified and analyzed in triplicate. Ground powders (0.5 g) were saponified with 5 mL of ethanolic potassium hydroxide (prepared dissolving 10 g of potassium hydroxide in 10 mL of water and making up to 100 mL with ethanol) at thermostatic dry bath G-Block (Fratelli Galli, Italy) at 90 °C for 2 hrs. After adding 10 mL of NaCl 2%, the unsaponifiable fraction was extracted with 10 mL of diethyl ether three times, and the organic phase was washed with 10 mL of NaCl 2%. The organic solvent was removed by using a rotary evaporator (CVC 2000, Vacuubrand, DE). After complete dryness, the organic phase was resuspended in 2 mL of mobile phase (H2O:ACN 40:60 w/w), filtered using a nylon membrane filtration (0.45 µm Millipore), diluted and injected into the 1290 UHPLC system (Agilent Technologies, USA) for the determination of cafestol and kahweol, not diluted and injected into 1290 UHPLC (Agilent Technologies, USA) interfaced to a SCIEX Triple QuadTM 4500 (Sciex, USA) for the determination of 16OMC and 16OMK. Sensory analysis was performed according to illycaffè spa internal protocol on *ad hoc* roasted & ground samples (Medium roast degree).

Results

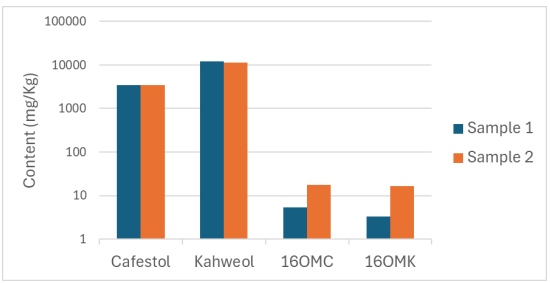

Fig. 3. Diterpenes profile of *C. stenophylla* samples

The results revealed a relatively high level of **chlorogenic acids (8.0–8.2%)**, along with a **caffeine content (1.6-1.7%)** slightly higher than the average value for *C. arabica* and lower than that generally reported in the literature, making it a promising option for caffeine-sensitive consumers. **Trigonelline content (1.0–1.1%)** in line with the scarce reported data and **theobromine (0.1%)** were also determined. **Sucrose (5.5 – 6.0%)** and **trace amount of raffinose and stachyose**, together with **myo-inositol (0.1-0.2%) and traces of galactinol** defined the carbohydrate profile of the analyzed samples. As reported in **Figure 3**, diterpenes profile is characterized by a relatively high content of **kahweol (11-12 g/kg) and by cafestol (3.4 - 3.5 g/kg), 16-O-methylcafestol (5 -18 mg/kg) and 16-O-methylkahweol (3 -16 mg/kg)**. The latter could be considered a sort of a chemo-taxonomic marker for Stenophylla in view of previous studies [2]. A sensory evaluation was carried out by a trained panel to assess flavor profile, acidity, bitterness, and body, using two preparation methods: V60 and espresso. Overall, the findings highlight a **complex and highly appreciated aromatic profile, with fruity, caramel and chocolate notes and a naturally smooth, low-bitter taste**. While some defects were noted with the V60 preparation, the espresso method produced a flavor profile comparable to that of Central American Arabica, with a low incidence of perceivable defects.

Conclusion

The obtained experimental results underscore the agronomic and commercial potential of this overlooked species in the context of climate change and sustainable coffee production. Its rediscovery opens new opportunities for coffee cultivation in Côte d'Ivoire. However, it is crucial to protect and conserve the forests where *C. stenophylla* naturally grows, as they are increasingly threatened by human activities.

REFERENCES: [1] S. Colomban, M. de Rosso, R. Flamini, L. Navarini *J. Mass Spectrom.* (2023), 9999: e4970. 10.1002/jms.4970. [2] B. de Roos, G. van der Weg, R. Urgert, P. van de Bovenkamp, A. Charrier, M.B. Katan, *J. Agric. Food Chem.* (1997), 45, 3065-3069.