Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 88 (2004) 39–43
Food Chemistry
www.elsevier.com/locate/foodchem

Trigonelline and sucrose diversity in wild *Coffea* species

C. Campa [a,*], J.F. Ballester [b], S. Doulbeau [a], S. Dussert [a], S. Hamon [a], M. Noirot [a]

[a] *Centre IRD, B.P. 64501, Montpellier Cedex 5 34394, France*
[b] *ENSAM, 2 Place Viala, Montpellier Cedex 1 34060, France*

Received 1 August 2003; received in revised form 8 January 2004; accepted 8 January 2004

Abstract

Trigonelline and sucrose are two coffee aroma precursors. Contents of these compounds are higher in *Coffea arabica* than in *Coffea canephora* green beans and this could be the main explanation for consumers' preference for *C. arabica* coffee. This is the first evaluation of sucrose and trigonelline contents involving 14 species and six new taxa not yet botanically characterised. Trigonelline and sucrose contents varied between species from 0.39% to 1.77% dry matter basis (dmb) and from 3.8% to 10.7% dmb, respectively. *C. canephora* could be improved through both compounds by crosses with *Coffea eugenioides*.

Keywords: *Coffea*; Biochemical diversity; Sucrose; Trigonelline; HPLC

1. Introduction

Two *Coffea* species, *Coffea arabica* and *Coffea canephora*, are of worldwide importance. They are commercially known as Arabica and Robusta, respectively. *C. arabica* is mainly cultivated in tropical highlands (>600 m), as in Latin America, East Africa and India. Indeed, hot and humid climatic conditions lead to flowering problems and to a decline in cup quality. In lowlands, its cultivation is restricted to some islands (Hawaii, Réunion, Nouvelle Calédonie) where the sea moderates the temperature, and it is replaced by *C. canephora* in other tropical lowland areas. As the price of Arabica coffee is twice that of Robusta, due to its lower bitterness and better flavour, improving Robusta to achieve Arabica cup quality could boost Robusta coffee producers' incomes.

Among known aroma precursors, sucrose and trigonelline give after roasting products with appreciated flavour, such as pyrazine, alkyl-pyridines, furans, and pyrroles (De Maria, Trugo, Moreira, & Werneck, 1994; Feldman, Ryder, & Kung, 1969; Stadler, Varga, Hau, Arce Vera, & Welti, 2002; Stadler et al., 2002; Baltes and Bochmann, 1987; Baltes and Knoch, 1993). The higher sucrose and trigonelline contents in Arabica green bean could partially explain its better cup quality (Casal, Oliveira, & Ferreira, 2000; Ky et al., 2001). Increasing trigonelline and sucrose contents in the Robusta green bean could be a way to improve its cup quality.

From a breeding standpoint, this improvement depends on the available genetic diversity concerning these two important compounds in *C. canephora*. For sucrose content, there is no overlapping between these two cultivated species, thus considerably limiting the potential for intraspecific *C. canephora* breeding to improve cup quality (Ky et al., 2001). Conversely, there is substantial overlapping between the two species with respect to trigonelline content. Some *C. canephora* trigonelline contents (1.24% dmb) are higher than the mean content in *C. arabica* (1.19% dmb) (Ky et al., 2001). In this respect, the intraspecific diversity should be of interest in breeding programmes. An investigation of the trigonelline content diversity within the *Coffea* genus would nevertheless be necessary before designing a suitable breeding programme. Would it indeed be possible to find a wild species with higher trigonelline and sucrose contents than in the best *C. arabica*?

This study is the first evaluation of sucrose and trigonelline contents involving 14 wild *Coffea* species and six new taxa that have yet to be botanically characterised, with *C. arabica* and *C. canephora* serving as controls.

* Corresponding author.

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.01.020

2. Materials and methods

2.1. Plant material

The 16 species and six new taxa are field maintained at the IRD coffee breeding station (Man, Côte-d'Ivoire). Table 1 gives the geographical origin of species and taxa. Each species or taxa was represented by four trees. All samples were harvested from April to December 1999, depending on the fructification time of the species (10 weeks to 10 months).

2.2. Sample preparation

Coffee cherries were harvested at full maturity and depulped using the wet processing method. After desiccation on silicagel, 50 green beans per tree were frozen in liquid nitrogen before crushing in a ball mill (Dangoumill) for 2 min. The fine powder was split into six samples, three to estimate the water content and three for extraction and analysis.

2.3. Extraction and purification

Trigonelline was extracted using the caffeine extraction methods of Trugo, Macrae, and Dick (1983) and Barre et al. (1998), but slightly modified. Each 50 mg powder sample was placed in a 50-ml capped tube (Sarstedt) with 50 mg of magnesium oxide (Merck) and 25 ml of distilled water. Tubes were heated for 20 min at 105 °C in an autoclave. Extracts were filtered (0.2 μm pore size filter) and directly analysed by HPLC.

Table 1
Geographical origin of species and taxa

Species and taxa	Geographical origin
C. arabica	Ethiopia
C. brevipes	Cameroon
C. canephora	Côte-d'Ivoire
C. congensis	Congo Democratic Republic
C. eugenioides	Kenya
C. heterocalyx	Cameroon
C. humblotiana	Comores
C. humilis	Côte-d'Ivoire
C. kapakata	Angola
C. liberica dewevrei	Central African Republic
C. liberica Koto	Cameroon
C. liberica liberica	Côte-d'Ivoire
C. pseudozanguebariae	Kenya
C. racemosa	Tanzania
C. salvatrix	Tanzania
C. pocsii	Tanzania
C. stenophylla	Côte-d'Ivoire
C. sp. Bakossi	Cameroon
C. sp. Congo	Congo Democratic Republic
C. sp. Ngongo 2	Congo Democratic Republic
C. sp. Moloundou	Congo Democratic Republic
C. sp. Koumbala	Cameroon

Sucrose was extracted using the method described in Ky et al. (2000a).

2.4. Analytical HPLC

For trigonelline analysis, chromatography was carried out in a system consisting of: (i) two Waters Associates Model 510 pumping units; (ii) an automated sample injector (Waters 717 plus autosampler); (iii) a photodiode-array detector (Waters 996); (iv) a C_{18} pre-column and (v) a 250 mm × 4 mm Merck LiChrospher 100 RP-18 column (5 μm particle size). The elution system (1 ml/min) consisted of two solvents filtered (0.2 μm pore size filter), degassed and sonicated (Ney, 300 ultrasonik): solvent A (10 mM acetic acid/triethylamine (1000/1), pH 5.3) and solvent B (methanol). The gradient was : 0–15 min, 65% solvent A, isocratic; 15–20 min, 100% solvent B, linear; 20–26 min, 100% solvent B, isocratic; 26–30 min, 65% solvent A, linear. Identification and quantification were performed at room temperature by means of reference standard (Sigma Chemical Co.) at 263.3 nm. The calibration curve was plotted using three replicate points for a trigonelline solution at 10, 20, 30 and 40 mg/l.

Sucrose was analysed using anion-exchange chromatography coupled to pulsed amperometric detection (Ky et al., 2000a). The solvent system was degassed with helium and delivered by a GP 40 Gradient Pump (Dionex Chromatography Co, Jouy-en-Josas, France) at 1 ml min^{-1}. It consisted of distilled filtered water (0.2 μm pore size filter) in 150 mM NaOH (50% sodium hydroxide from Mallinckrodt Baker, Deventer, Netherlands) (25/75). An automated sampler (Dionex Chromatography Co.) was used with a fixed 50 μl loop. A Carbo-Pac PA 1 (4 × 250 mm) column was used in conjunction with a Carbo-Pac PA 1 Guard pre-column (10 × 32 mm). A pulsed amperometric detector II (Dionex Chromatography Co.), equipped with a gold working electrode, was used at the following settings: measurement potential, $E_1 = 50$ mV; measurement time, $t_1 = 300$ ms; $E_2 = 60$ mV; $t_2 = 300$ ms; $E_3 = -800$ mV; $t_3 = 480$ ms. Solid sucrose standard from Sigma (Ref. S-9378, HPLC grade, Saint-Quentin-Fallavier, France) was dissolved in water and filtered through 0.2 μm pore size filters.

The processing order was fully randomized for both dosages. Every 10 extracts, a control was used to verify the measurement consistency. The content unit was the percentage dry matter basis (% dmb).

2.5. Statistical analysis

All results were analysed using the Statistica software package (5.1 version, 1997 for Microsoft Windows).

No data transformation was required to apply ANOVA. Between species differences were tested using a one-way ANOVA.

3. Results and discussion

3.1. Trigonelline content

Trigonelline content seems to be highly dependent on the variety, but also on the extraction and dosage methods implemented. In *C. arabica*, trigonelline content reportedly ranges from 0.83% to 1.01% dmb (Smola & Sontag, 1983), 0.79% to 1.06% (Stennert & Maier, 1994), 0.88% to 1.77% dmb (Ky et al., 2001), 1% to 1.94% dmb (Martin, Pablos, & Gonzales, 1998) and 1.52% to 2.9% dmb (Mazzafera, 1991), whereas in *C. canephora* it ranges from 0.66% to 0.68% dmb (Stennert & Maier, 1994), 0.75% to 1.24% dmb (Ky et al., 2001) and 0.91% to 1.94% (Martin et al., 1998). The only single value given by Mazzafera (1991) is clearly higher (3.08% dmb). Concerning species other than *C. arabica* and *C. canephora*, data are scarce and equally heterogeneous. For *Coffea stenophylla*, contents reported are 1.89% dmb (Mazzafera, 1991) and 0.51% (Stennert & Maier, 1994). Consequently, two conditions are required when comparing species: (1) the estimation must concern more than one tree, with each tree represented by several extractions and (2) all species have to be compared using the same methodology. These conditions are fulfilled in the present study.

Here, trigonelline ranged from 0.39% dmb in *C. liberica* Koto to 1.77% dmb in *C. kapakata*. Between species differences were highly significant ($F_{21,64} = 23.7$; $p < 0.001$), representing 82% of the total variance. The distribution was homogeneous over the entire range (Fig. 1). The interspecific Max/Min ratio was 4.54. This is much higher than within *C. arabica* (2.01) and *C. canephora* (1.65) (Ky et al., 2001).

Trigonelline inheritance has been studied using an interspecific cross between *Coffea pseudozanguebariae* and *Coffea liberica* spp. *dewevrei* (Ky, Guyot, Louarn, Hamon, & Noirot, 2000b). As confirmed in the present study, parental species have very different trigonelline contents. Trigonelline content inheritance would be nucleo-cytoplasmic. Indeed, similar levels of trigonelline content characterize all hybrids having the same maternal cytoplasm and a nuclear QTL is located on the G linkage group (Ky et al., 2000b). Lastly, there is no year effect or (genotype × year) interaction and the genotypic value can be easily estimated from the phenotypic value (high heritability *sensu largo*).

All of these data explain the high trigonelline level in *C. arabica* relative to *C. canephora*. Indeed, *C. arabica* is amphiploid, as initially suggested by chromosome pairing behaviour in *C. arabica* × *C. canephora* hybrids (Grassias & Kammacher, 1975). Two species – *C. canephora* and *C. eugenioides* – are proposed to constitute its genome, with *C. eugenioides* being the original female parent (Berthou, Trouslot, Hamon, Vedel, & Quetier, 1980; Lashermes, Combes, Trouslot, & Charrier, 1997; Lashermes et al., 1999). From a cup quality viewpoint, this clearly shows that genes from *C. eugenioides* can improve *C. canephora*. Here, there was no significant difference between *C. eugenioides* and *C. arabica* (Fig. 1), thus confirming the role of the maternal cytoplasm on the mean content.

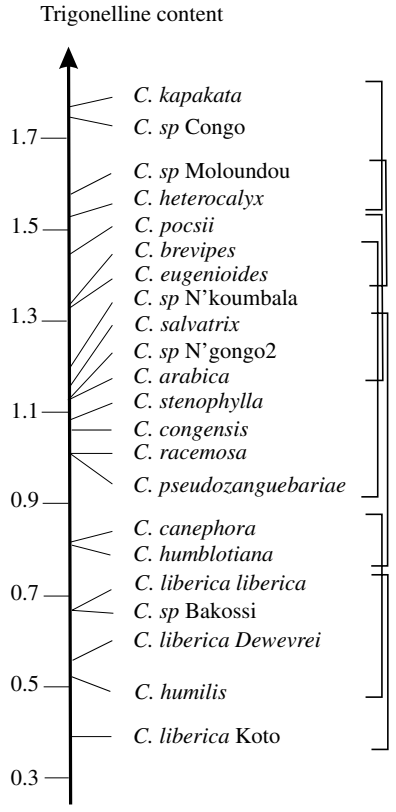

Fig. 1. Results of the multiple mean comparison (Newmann and Keuls test) for trigonelline content, expressed in percent dry matter basis (% dmb). Species within bracket were not significantly different.

From a breeding standpoint, eight species, *C.* sp. Koumbala, *C. eugenioides*, *C. brevipes*, *C. pocsii*, *C. heterocalyx*, *C.* sp. Moloundou, *C.* sp. Congo and *C. kapakata* showed levels higher than 1.24% dmb, which means that they constitute genetic resources for potential trigonelline content improvement in *C. canephora* (0.8% dmb). Trigonelline contents in *C. kapakata* and *C.* sp. Congo, that belong to the same phylogenetic clade (Lashermes et al., 1997), were similar (Fig. 1). These species could have the same maternal cytoplasm and be used as female parent in interspecific crosses with *C. canephora*. Within *C. kapakata* and *C. canephora*, it is possible to choose the best genotypes as parents according to their phenotypes.

3.2. Sucrose content

Sucrose content ranged from 3.8% dmb in *C.* sp. Bakossi to 10.7% dmb in *C. pocsii* (Fig. 2). Sucrose

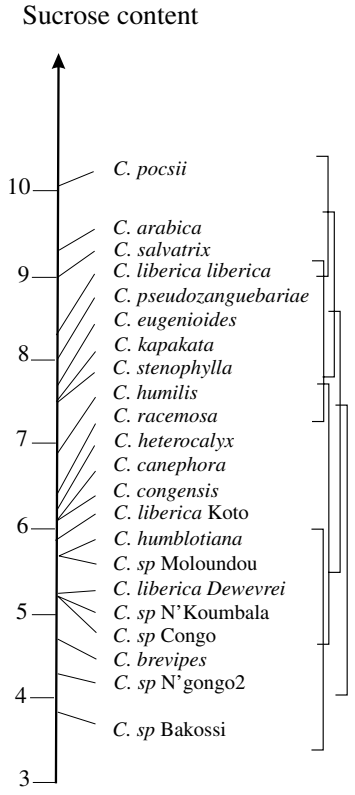

Fig. 2. Results of the multiple mean comparison (Newmann and Keuls test) for sucrose content, expressed in percent dry matter basis (% dmb). Species within bracket were not significantly different.

content differed significantly between species ($F_{21,61} = 11.9$; $p < 0.001$) and these interspecific variations represented 74% of the total variance (Table 2). These results are consistent with those of Chabrillange, Dussert, Engelmann, Doulbeau, and Hamon (2000), who assessed nine species that were also tested in this study. Pearson correlation and rank correlation between both evaluations are $r = 0.85$ and $r = 0.95$, respectively. Note that, in the Chabrillange et al. study (2000) *C. liberica* actually corresponds to the *C. liberica dewevrei* subspecies (pers. comm.).

The sucrose content distribution was quasi-homogeneous over the entire range (Fig. 2). The two controls, *C. arabica* and *C. canephora*, were significantly different, as expected. *C. arabica* had one of the highest sucrose contents (9.3% dmb), close to that of *C. pocsii*. The interspecific Max/Min ratio was lower than for trigonelline (2.82). Nonetheless, this was substantially higher than within *C. arabica* (1.74) and *C. canephora* (1.50) (Ky et al., 2001).

Sucrose inheritance has been studied using the same interspecific cross as that used for the trigonelline content inheritance (Ky et al., 2000a). As confirmed in the present study, *C. pseudozanguebariae* and *C. liberica* spp. *dewevrei* show significantly different sucrose contents. Inheritance was found to be quantitative, i.e. polygenic, and additive. In contrast with trigonelline, no QTLs were highlighted.

Table 2
Trigonelline and sucrose content variations between and within species (the within-species range is in brackets)

Species and taxa	Trigonelline content		Sucrose content	
	Mean	Range	Mean	Range
C. arabica	1.13	(0.95–1.29)	9.32	(7.99–11.0)
C. brevipes	1.35	(1.26–1.43)	4.68	(4.12–5.14)
C. canephora	0.82	(0.52–1.06)	6.10	(5.94–6.47)
C. congensis	1.06	(0.91–1.12)	6.06	(5.22–7.25)
C. eugenioides	1.33	(1.13–1.42)	7.70	(5.83–8.87)
C. heterocalyx	1.53	(1.50–1.57)	6.22	(5.59–6.66)
C. humblotiana	0.81	(0.71–0.88)	5.73	(5.00–7.20)
C. humilis	0.52	(0.36–0.73)	6.89	(6.11–8.02)
C. kapakata	1.77	(1.55–1.99)	7.51	(7.12–7.83)
C. liberica dewevrei	0.56	(0.45–0.66)	5.33	(3.76–6.24)
C. liberica Koto	0.39	–	5.86	–
C. liberica liberica	0.67	(0.52–0.87)	8.28	(7.30–9.13)
C. pseudozanguebariae	1.02	(0.87–1.16)	7.95	5.94–9.25
C. racemosa	1.02	(0.75–1.23)	6.44	(5.26–7.04)
C. salvatrix	1.16	(1.08–1.22)	8.96	(7.21–10.64)
C. pocsii	1.45	(1.38–1.48)	10.1	(8.95–10.87)
C. stenophylla	1.09	(0.98–1.26)	7.50	(6.41–8.56)
C. sp. Bakossi	0.66	(0.50–0.88)	3.81	(3.08–4.61)
C. sp Congo	1.75	(1.35–1.92)	5.16	(4.11–6.08)
C. sp. Ngongo 2	1.13	(0.97–1.19)	4.34	(3.87–4.87)
C. sp. Moloundou	1.58	(1.39–1.87)	5.68	(5.47–6.08)
C. sp. Koumbala	1.20	(1.09–1.34)	5.22	(4.72–5.54)

Contents are expressed in percent dry matter basis (% dmb).

Means and ranges are based on four trees, except for *C. liberica* Koto (only one tree). For each tree, the individual value was the average of three extraction-analysis processings.

This type of inheritance does not clarify the high levels of sucrose in *C. arabica* relative to *C. canephora*. Indeed, according to the additivity hypothesis, sucrose content in *C. eugenioides* should be about 12.5% dmb, instead the 7.7% observed. This suggests complementarity between genes arising from the two parental species within the *C. arabica* genome. From a breeding standpoint, this clearly shows that *C. canephora* could be improved through crosses with *C. eugenioides*. Other species, phylogenetically close to *C. eugenioides*, such as *C. heterocalyx* and *C.* sp Moloundou, could also be tested as parents.

References

Barre, P., Akaffou, S., Louarn, J., Charrier, A., Hamon, S., & Noirot, M. (1998). Inheritance of caffeine and heteroside contents in an interspecific cross between a cultivated coffee species *Coffea liberica* var "*dewevrei*" and a wild species caffeine free *C. pseudozanguebariae*. *Theoretical and Applied Genetics, 96*, 306–311.

Berthou, F., Trouslot, P., Hamon, S., Vedel, F., & Quetier, F. (1980). Analyse en électrophorèse du polymorphisme biochimique des caféiers: variation enzymatique dans dix-huit populations sauvages/variations de l'ADN mitochondrial dans les espèces: *C. canephora*, *C. eugenioides* and *C. arabica*. *Café Cacao Thé, 24*, 313–326.

Casal, S., Oliveira, M. B., & Ferreira, M. A. (2000). HPLC/diode-array to the thermal degradation of trigonelline, nicotinic acid and caffeine in coffee. *Food Chemistry, 68*, 481–485.

Chabrillange, N., Dussert, S., Engelmann, F., Doulbeau, S., & Hamon, S. (2000). Desiccation tolerance in relation to soluble sugar contents in seeds of ten coffee (*Coffea* L.) species. *Seed Science Research, 10*, 393–396.

De Maria, C. A. B., Trugo, L. C., Moreira, R. F. A., & Werneck, C. C. (1994). Composition of green coffee fractions and their contribution to the volatile profile formed during roasting. *Food Chemistry, 50*, 141–145.

Feldman, J. R., Ryder, W. S., & Kung, J. T. (1969). Importance of nonvolatile compounds to the flavour of coffee. *Journal of Agriculture and Food Chemistry, 17*, 733–739.

Grassias, M., & Kammacher, P. (1975). Observations sur la conjugaison chromosomique de *Coffea arabica* L. *Café Cacao Thé, 19*, 177–190.

Ky, C.-L., Doulbeau, S., Guyot, B., Charrier, A., Hamon, S., Louarn, J., & Noirot, M. (2000a). Inheritance of sucrose content in the interspecific cross: *Coffea pseudozanguebariae* × *Coffea liberica* '*dewevrei*'. *Plant Breeding, 119*, 165–168.

Ky, C.-L., Guyot, B., Louarn, J., Hamon, S., & Noirot, M. (2000b). Trigonelline inheritance in the interspecific cross between *Coffea pseudozanguebariae* × *C. liberica* var. *dewevrei*. *Theoretical and Applied Genetics, 102*, 630–634.

Ky, C. L., Louarn, J., Dussert, S., Guyot, B., Hamon, S., & Noirot, M. (2001). Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L. and *C. canephora* P. accessions. *Food chemistry, 75*, 223–230.

Lashermes, P., Combes, M. C., Trouslot, P., & Charrier, A. (1997). Phylogenetic relationships of coffee-tree species (*Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. *Theoretical and Applied Genetics, 95*, 947–955.

Lashermes, P., Combes, M.-C., Robert, J., Trouslot, P., D'Hont, A., Anthony, F., & Charrier, A. (1999). Molecular characterisation and origin of the *Coffea arabica* L. genome. *Molecular and General Genetics, 261*, 259–266.

Martin, M. J., Pablos, F., & Gonzales, A. G. (1998). Discrimination between arabica and robusta green coffee varieties according to their chemical composition. *Talanta, 46*, 1259–1264.

Mazzafera, P. (1991). Trigonelline in coffee. *Phytochemistry, 30*, 2309–2310.

Smola, U., & Sontag, G. (1983). Polargraphische Bestimmung von Trigonellin im Kaffee. *Zeitschrift für Lebensmitteluntersuchung und -forschung, 177*, 114–116.

Stadler, R. H., Varga, N., Hau, J., Arce Vera, F., & Welti, D. H. (2002). Alkylpyridiniums. 1. Formation in model systems via thermal degradation of trigonelline. *Journal of Agriculture and Food Chemistry, 50*, 1192–1199.

Stadler, R. H., Varga, N., Milo, C., Schilter, B., Arce Vera, F., & Welti, D. H. (2002). Alkylpyridiniums. 2. Isolation and quantification in roasted and ground coffees. *Journal of Agriculture and Food Chemistry, 50*, 1200–1206.

Stennert, A., & Maier, H. G. (1994). Trigonelline in coffee II. Content of green, roasted and instant coffee. *Zeitschrift für Lebensmitteluntersuchung und-forschung, 199*, 198–200.

Trugo, L. C., Macrae, R., & Dick, J. (1983). Determination of purine alkaloids and trigonelline in instant coffee and other beverages using high performance liquid chromatography. *Journal of Science and Food Agriculture, 34*, 300–306.