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Cafestol extraction yield from different coffee brew mechanisms

*Chen Zhang, Robert Linforth, Ian D. Fisk**

Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington,
Loughborough, Leicestershire, LE12 5RD, United Kingdom

* Corresponding Author

Division of Food Sciences, University of Nottingham, Sutton Bonington Campus, Sutton Bonington,
Loughborough, Leicestershire, LE12 5RD, United Kingdom. Tel: +44 (0)115 951 6037. Fax: +44
(0)115 951 6142. E-mail Ian.Fisk@nottingham.ac.uk

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12 Abstract: The extraction yield of cafestol from roast and ground (R&G) coffee beans was evaluated
13 using brews prepared by four brewing mechanisms (boiled, Turkish, French Press and Mocha Pot).
14 The cafestol content of the R&G coffee and the resulting brews was measured and extraction yield
15 calculated. The R&G coffee had an average cafestol content of 603 mg / 100 g R&G coffee with a
16 slight reduction at higher roast intensities. In the brews, preparation method had an impact on cafestol
17 concentration with French, Turkish and boiled preparation methods producing the highest cafestol
18 concentrations. The extraction yield of cafestol was shown to be dependent on the brew mechanism
19 and roasting time, with the lightest roast coffee prepared by French press or boiled preparations
20 having the highest cafestol extraction yield (6.5% and 5.84%) and dark roast Mocha and Turkish
21 preparations had the lowest extraction yields of 2.42% and 2.88% respectively.

22 Keywords: Coffee; Extraction; Beverage; Food

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27 **1 Introduction**

28 Coffee is a globally consumed beverage and is prepared in a wide variety of formats including
29 Scandinavian type boiled coffee, drip filtered coffee, instant or soluble coffee and espresso. Within
30 each class of brew preparation method, individual population groups consume coffee in a range of
31 formats (e.g. 37 °C - 88 °C (H.-S. Lee, Carstens, & O'Mahony, 2003), 0 % - 80 % milk (H. S. Lee &
32 O'Mahony, 2002), 0 g – 16 g of sugar, 25 mL – 880 mL in volume (Hsu & Hung, 2005), with or without
33 milk, foamed milk, cream, ice, flavourings, brew adjuncts or co-adjuncts (Fisk, Massey, & Hansen,
34 2011; Massey, Fisk, & Henson, 2011).

35 Coffee brew contains a wide range of components including medium to long chain polysaccharides,
36 melanoidins, volatile aroma compounds and lipid like compounds with a range of positive, negative
37 and neutral health benefits (Esquivel & Jiménez, 2012). Coffee also contains a number of diterpenes
38 including cafestol, which has been shown to have cholesterol raising properties (Butt & Sultan, 2011)
39 and is proposed to increase serum cholesterol by 1 mg / dL for each 2 mg of consumed cafestol,
40 although this has not necessarily been proven in all population groups (Weusten-van der Wouw,
41 Katan, Viani, Huggett, liardon, Lund-Larson, Thelle, Ahola, Aro, & Meynen, 1994).

42 The varied format and highly variable size and frequency of consumption makes prediction of risk
43 factors, such as hypertension from caffeine consumption and elevated cholesterol levels from the
44 consumption of diterpenes, challenging for health authorities and manufacturers.

45 The cafestol content of a standard cup of coffee varies depending on brew mechanism but is highest
46 in unfiltered preparation methods such as Scandinavian type boiled coffee and Turkish coffee with up
47 to 88.7 mg/L in some Turkish brews (Table 1) (Gross, Jaccaud, & Huggett, 1997; Urgert, Van Der
48 Weg, Kosmeijerschuil, Van De Bovenkamp, Hovenier, & Katan, 1995) . Filtered coffees such as drip-
49 filter and soluble coffee contain negligible levels of cafestol in the brew, as the paper filter in drip
50 filtered coffee retains the diterpenes and in soluble coffee the diterpenes are retained with the
51 grounds during production (Gross, Jaccaud, & Huggett, 1997).

52 Values for cafestol concentration by brew mechanism from previous studies (Table 1) are often
53 variable due to differing extraction parameters (Eulitz, Kolling-Speer, & Speer, 1999), grind sizes
54 (Buchmann, Zahm, Kolling-Speer, & Speer, 2010; Kurzrock & Speer, 2001; Sehat, Montag, & Speer,
55 1993), coffee to water ratios (Buchmann, Zahm, Kolling-Speer, & Speer, 2010), temperatures

56 (Buchmann, Zahm, Kolling-Speer, & Speer, 2010) and brewing technologies e.g. coffee pads
57 (Boekschoten, Van Cruchten, Kosmeijer-Schuil, & Katan, 2006).

58 Cafestol is not extracted by a simple dissolution kinetics, when hot water interacts with R&G coffee a
59 number of phenomena occur (T. A. Lee, Kempthorne, & Hardy, 1992; Merritt & Proctor, 1958), firstly
60 the highly soluble components dissolve in the water phase and are extracted, for example organic
61 acids (Lentner & Deatherage, 1958), secondly less soluble or physically entrapped compounds (e.g.
62 arabinogalactan) (Redgwell & Fischer, 2006) are forced out by physical mechanisms, thirdly the heat
63 leads to thermal degradation making select components more soluble and therefore more available
64 for extraction (e.g. galactomannan) and fourthly mobile water will physically lift and migrate coffee
65 fines and emulsify coffee oil into suspension (Escher, Schenker, Handschin, Frey, & Perren, 2000;
66 Eulitz, Kolling-Speer, & Speer, 1999), it is these components (coffee fines and coffee oil) that contain
67 the cafestol and deliver them to the final brew.

68 The process of extraction (coffee brew preparation), although fundamentally simple for the consumer
69 (mix then separate hot water and ground roasted coffee) is complicated to predict and requires a
70 number of technical approaches to cover each of the four brew mechanisms tested (Oosterveld,
71 Harmsen, Vorgen, & Schols, 2003; Thaler, 1978; Zaroni, Pagliarini, & Peri, 1992). In this study,
72 cafestol is the compound of interest; cafestol is a lipophilic diterpene that generally resides within the
73 oil phase of coffee and can thermally degrade to form other compounds (Kolling-Speer, Kurt, Thu, &
74 Speer, 1997). The main driving force that needs to be considered when predicting the extraction of
75 cafestol from R&G coffee to the brew is the process of oil emulsification and the removal of physical
76 barriers that would prevent the migration of the emulsified oil (e.g. cell structures or long chain
77 polysaccharide networks) into the brew. It is proposed, therefore, that both the brew mechanism and
78 the physical structure of the coffee (Bell, Wetzal, & Grand, 1996) will impact cafestol brew yield.

79 The objective of this study was therefore to determine, for the first time, the extraction yield (%) of
80 cafestol from R&G *Coffea arabica* beans at various roasting intensities (roast time) in four brew
81 mechanisms (Scandinavian boiled, Turkish, French press, mocha). This has not previously been
82 documented and will serve to be of value as a reference point for the development of future brew
83 mechanisms, the identification of technical routes to cafestol reduction, and to further explain the
84 complex interaction of brew water and R&G coffee.

85

86 **Materials and Methods**

87 *1.1 Roast and ground (R&G) coffee*

88 Green coffee beans (*Coffea arabica*) were spread evenly over roasting trays (200 g per tray) and
89 roasted at $190\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ within a Mono convection oven (Mono, BX, UK). Samples were removed at
90 10 minute intervals to produce a range of products that had been exposed to $190\text{ }^{\circ}\text{C}$ for 0 min, 10 min,
91 20 min, 30 min, 40 min and 50 min, the resulting roasted coffee beans were designated as raw, I(1),
92 I(2), I(3), I(4) and I(5) respectively to be comparable to light to medium roast intensities in small batch
93 roasting conditions.

94 Samples were moved to ambient temperature to cool for 2 hours then left to degas over two days.
95 Roasted coffee beans were stored in folded aluminium bags at $4\text{ }^{\circ}\text{C}$ until required, roasted coffee
96 beans were subsequently ground in a KG 49 grinder (Delonghi, Australia) to a uniform size and
97 sieved (Endecotts, UK) to remove fines and large particulates, R&G coffee was stored at $4\text{ }^{\circ}\text{C}$ until
98 required and samples were analysed within 5 days of roasting.

99 *1.2 Coffee brew preparation*

100 Turkish coffee was prepared using a traditional Turkish coffee pot (Grunweg, Sheffield, UK) prepared
101 with 40 g R&G coffee and 300 ml distilled water (Pur1te select, ONDEO, UK). The brew was heated
102 until it had foamed twice, allowed to settle (5 min) then decanted for analysis. Individual cup size was
103 60 mL.

104 Scandinavian type boiled coffee was prepared by adding R&G coffee (40 g) to boiling distilled water
105 (300 ml), allowed to settle (10 min) then decanted for analysis. Individual cup size was 160 mL.

106 French press coffee was prepared by pouring boiling water (300 mL) on to R&G coffee (40 g) in a
107 glass French press pot (Fisherbrand, US), allowed to stand for 5 minutes and the plunger depressed
108 to separate the brew from the grounds. Individual cup size was 160 mL.

109 Mocha style brewed coffee was prepared with 40 g R&G coffee and 300 ml distilled water in an
110 aluminium Mocha-maker (Oroley, Spain). Individual cup size was 60 mL.

111 All coffee brews were prepared at sea level in an air conditioned room at 21°C. Brews once prepared
112 were frozen for 24 h at -18 °C then placed in a Edwards Freeze Dryer Super Modulyo Pirani 1001
113 (Edwards, Crawley, UK) at -40 °C for 72 hours or until a constant weight was achieved (Fisk,
114 Gkatzionis, Lad, Dodd, & Gray, 2009).

115 1.3 *Colour*

116 The colour of the R&G coffee was measured, as per (Morales & Jiménez-Pérez, 2001) with slight
117 modifications, in the CIE Lab scale (McLaren & Rigg, 1976) (L^* , a^* , b^*) using a tristimulus colorimeter
118 ColourQuest XE (HunterLab, US) after equilibration and calibration (8° standard angle). L^* denotes
119 black to white component, luminosity, a^* denotes +red to - green component, b^* denotes +yellow to -
120 blue component (Hunter, 1942) (Standard illumination: D65, colorimetric normal observer angle: 10°,
121 ASTM E308 RSIN Mode, LAV, 1.00 Port, UV Nominal). Samples were placed in transparent square
122 containers and reported as the mean of five determinations at 21°C.

123 1.4 *Tap density and bulk density*

124 Tap density and bulk density were measured by the ratio of sample weight to tap volume and bulk
125 volume respectively. R&G coffee was poured into a 20 ml cylinder and tapped three times. The
126 volume and weight was measured before and after tapping of the cylinder on the table three times.
127 Bulk density and Tap density were then calculated.

128 The physical structure of the R&G coffee was affected by varying roast intensities. There was no
129 change in the tap density (after compaction), but there was a significant change in the bulk density
130 (measured after free flow with no shaking or settling) (Table 2). Coffee that had been roasted to a L(5)
131 roast intensity was less dense than coffee roasted to a L(2) roast intensity. Therefore all subsequent
132 experimentation was conducted on a weight basis, to exclude any volume effects on extraction
133 efficiency.

134 1.5 *Cafestol extraction*

135 2 mL of 2.5 M KOH (AnalaR, BDH Laboratory Supplies, UK) in 96 % ethanol (Fisher Scientific, UK)
136 was added to R&G coffee (200 mg) or freeze dried coffee brews (200 mg) and saponified at 80 °C for
137 1 h (GC 8000 series, FISONs instrument, Germany). After saponification, distilled water (2 mL) was

138 added and the water phase extracted three times with diethyl ether (4 mL, laboratory reagent grade,
139 Fisher Scientific, UK). Samples were shaken for 10 min at 250 oscillations / min (Denley Spiramix,
140 Thermo Electron Corporation, US) and centrifuged for 5 min at 3000 RPM (CR3i Multifunction,
141 JOUAN, US). Organic phases were pooled then evaporated (15 min, 70 °C, HC502, Bibby Scientific,
142 UK), residues were dissolved with methanol (HPLC grade, Fisher Scientific, UK) to 25 ml and stored
143 at -40°C in brown glass bottles with Teflon lids.

144 1.6 Cafestol quantification

145 Cafestol extracts were analysed by HPLC-UV composed of an automatic injector (AS-2055 Plus
146 intelligent sampler, JASCO, Japan), solvent pump (PU-980 intelligent HPLC pump, JASCO, Japan),
147 variable-wavelength UV detector (RI-2031 Plus intelligent RI Detector, JASCO, Japan) and a C₁₈
148 reverse-phase column (250 mm x 4.6 mm, 5 µm). The mobile phase (85 : 15) was methanol (HPLC
149 grade, Fisher Scientific, UK) and water with an isocratic flow rate of 0.7 ml / min and a detection
150 wavelength of 230 nm (Benassi, Dias, Campanha, Vieira, Ferreira, Pot, & Marraccini, 2010). The
151 mobile phase was prepared and degassed for 30 min in an ultrasonic bath (F5300b, Decon, UK).
152 Cafestol was quantified by retention time and peak area of authentic standards (ChromaDex, Irvine,
153 USA) using a six point calibration curve. All samples were within the calibration curve range and
154 repeatability was acceptable at $R^2 > 0.99$. All results are presented on a wet weight basis (mg/L) or
155 (mg/cup).

156 All samples were prepared in triplicate and analysed in duplicate. Statistical differences were
157 evaluated by ANOVA-LSD post hoc test (XLSTAT 2011, addinsoft, UK) at a significance level of $p \leq$
158 0.05.

159

160 **2 Results**

161 Coffee brews were prepared by four brewing mechanisms to investigate the extraction efficiency of
162 cafestol in each process, the absolute concentration of cafestol within a brew is detailed in Table 3 on
163 a mg/L basis for each brew mechanism, this is then further detailed in Table 4 on a mg/cup basis, to
164 illustrate parity and to enable comparisons with previous literature. The extraction yield of cafestol
165 from R&G coffee is subsequently shown in Figure 1 for each roast colour and brew preparation.

166 *2.1 Impact of brew mechanism and roast time on cafestol brew concentration*

167 The concentration of cafestol within the R&G coffee significantly reduced with higher roast intensities,
168 this is detailed in Table 3. There was a significant reduction from raw green beans to the lightest roast
169 intensity, I(1) and further roasting at levels I(4) and I(5) gave further reductions in the concentration of
170 cafestol.

171 The concentration of cafestol in the coffee brews was dependent on both the roast colour and the
172 brewing method. The cafestol concentration of the brew ranged from 19.2 mg/L to 74.4 mg/L with the
173 highest brew concentration found in the raw coffee sample for all brew preparation methods, further
174 roasting reduced the cafestol brew concentration. French press, boiled and Turkish preparation
175 methods produced the highest cafestol brew concentration and the lowest concentration was found in
176 the Mocha preparation method at all roast colours.

177 The relative differences in cafestol concentrations were further highlighted on a cup basis (Table 4) as
178 the two highest cafestol brew concentration samples (French and Boiled) also had the highest cup
179 volume. On a mg/cup basis French press and boiled coffee preparations had the highest cafestol
180 level per cup and mocha had the lowest cafestol per single cup serving.

181 *2.2 Impact of brew mechanism and roast time on cafestol extraction yield*

182 When directly comparing the brew extraction yields between different brew preparation mechanisms
183 (French press, Turkish, Mocha, boiled coffee), a marked and significant difference in extraction yield
184 was identified.

185 Cafestol extraction yield was in the order French>boiled>Turkish>Mocha for both Raw and L(1) coffee,
186 boiled=French>Turkish>Mocha for L(2), and boiled>Turkish>French=Mocha for L(3),

187 boiled=Turkish=French>Mocha for L(4) and for L(5) French>boiled=Turkish>Mocha as calculated by
188 ANOVA-LSD ($P>0.05$). There was also a strong correlation of roast intensity with cafestol extraction
189 yield (Figure 1), with green coffee and the lightest roasts having significantly greater cafestol
190 extraction yields than the brews prepared with darker roast coffee (Figure 1). Of the roasted samples
191 L(1) French press and boiled preparations had the highest cafestol extraction yield (6.5% and 5.84%)
192 and L(5) Mocha and Turkish preparations had the lowest extraction yields (2.42% and 2.88%).

193 **3 Discussion**

194 For all roast intensities, Mocha produced the lowest cafestol concentration, this confirms work by
195 Gross (1997) who showed that Mocha has the lowest brew concentration when comparing boiled,
196 Turkish and Mocha preparations (Table 1), but is contrary to findings by Urgert (1995) who showed
197 that on a concentration basis, Boiled coffee and French press had concentrations of 13 mg/L and 10-
198 14mg/L respectively and that Mocha had an intermediate cafestol brew concentration of 18 ± 2 mg/L
199 when compared to Turkish preparation method (17-33 mg/L). The low concentration of cafestol found
200 in the Mocha preparation is presumed to be due to the fact that the coffee fines and coffee oil
201 (containing the diterpenes) are not significantly transferred to the final brew and are retained in the
202 water tank. The geometry and fill volume will therefore impact transfer rate and may explain Urgert's
203 results.

204 On both a cup and concentration basis Boiled and French press prepared brews had the highest
205 cafestol concentration, this is due to the elevated levels of physical and thermal stresses imposed on
206 the coffee grounds by these methods and subsequent release of oil and diterpenes into the brew.
207 Turkish style prepared brews contain an intermediate level of cafestol due to the decanting procedure
208 during preparation, but exceeded that of French press at intermediate roast intensities. Both Urgert
209 (1995) and Gross (1997) showed that French press, boiled and Turkish extraction preparation method
210 can produce high cafestol brew concentrations (Boiled, Turkish, Mocha and French were studied);
211 Gross did not study French press, and found Turkish to be the highest whereas Urgert found
212 French press and Turkish to have the highest concentration. It should be noted that all the data in
213 Table 1 are not truly comparable due to differences in brew geometry, brew volumes and roast colour,
214 but do serve to highlight trends that support the general findings shown in Table 3.

215 There is a small but statistically significant reduction in cafestol in the R&G coffee, with I(5) containing
216 96% the cafestol of the I(1) coffee, this is presumed to be due to thermal degradation of the cafestol
217 with heating. When considering the coffee brews prepared from I(1) and I(5) roast intensities, the I(5)
218 contains, on average, only 58% of the cafestol that brews prepared from I(1) contain. Given that the
219 original coffee only has a slight reduction in cafestol levels due to thermal damage, there must be a
220 significant impact of roast intensity on the physical release mechanisms occurring during extraction to
221 drive this difference. Kurzrock (2001) and Ugert (1995) have previously shown only small or no
222 changes in cafestol concentrations with roast intensity, which supports this finding, but do not elude to
223 the impact of roast intensity on the extraction efficiency of cafestol during brewing.

224 The range of brew extraction yields is shown in Figure 1, the reason for the significant difference in
225 extraction yield with roast intensity is proposed to be due to changes in the physical structure of the
226 R&G coffee, making it entropically less favourable for the thermal and physical processes to release
227 and emulsify the entrapped oil. As this is driven by the roast intensity, there must therefore be a
228 causal link between heating time and the physical availability of the internal oil reserves of the R&G
229 coffee.

230 Previously Kurzrock (2001) and Speer (2000) summarised work by Sehat (1993) and suggested that
231 in a Scandinavian type brew up to 23 % of the total diterpene esters are extracted from the coffee into
232 the beverage, whereas, for espresso and filtered coffee an extraction yield of 0.3 % and 2.5 % was
233 found.

234 Sehat (1993) demonstrated that for Scandinavian style brews there was an impact of grind size on
235 extraction yield, with very fine ground coffee having a greater extraction yield when compared to
236 coffee prepared with coarse grind size, which serves to support the conclusion that the physical
237 availability of the cafestol within the R&G coffee has a significant impact on the cafestol extraction
238 yield. Specific numerical comparisons cannot be carried out due to difference in choice of preparation
239 method but the literature results do serve to indicate that the results shown (extraction yield of 2.5 % -
240 9.0 %) are similar to those previously published (0.3 % - 23 %).

241 Although this study robustly evaluates the extraction yield of cafestol from within a defined number of
242 samples, it does not address all technologies employed by the coffee industry to create R&G coffee.
243 Future studies should therefore include a more comprehensive investigation into coffee brew

244 extraction kinetics to allow a full understand of the extraction physics which can then be applied to
245 new brewing technologies (e.g. on demand home brew machines, self-service coffee machines) to
246 control the extraction of cafestol to the brew and minimise consumption by the consumer.

247 **4 Conclusion**

248 Roasting time and choice of brew mechanism impacts in-cup delivery of cafestol with French press,
249 boiled coffee and Turkish preparation methods producing higher cafestol concentrations than the
250 Mocha preparation method. Higher roasting times led to a 42% reduction in cafestol concentration on
251 a concentration basis within the brews.

252 The extraction yield of cafestol from R&G coffee is dependent both on the choice of brew mechanism
253 and roasting time, with lighter roast coffee brews having a greater cafestol extraction yield and darker
254 roast coffee brews having a lower cafestol extraction yield.

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326 **Table 1 : Literature values for cafestol concentration in different brew mechanisms, all literature values**
 327 **use different cup sizes therefore values are all converted to mg/L to facilitate comparison, cup volume is**
 328 **provided in parenthesis.**

329

	Cafestol mg/L	Light Roast ^f mg/L	Dark Roast ^g mg/L
Instant	1.9±0.05 ^a (150)		330 331
Drip Filter	0.12±0.02 ^a (150) 3.3 ^b (150)		332
Boiled	48.3±3.8 ^a (150) 13 ^b (150)	43.9±1.36 (160)	25.9±3.54 (160) 333
Turkish	88.7±4.0 ^a (60) 17-33 ^b (60)	39.1±0.04 (60)	22.8±0.12 (60) 334
Mocha	37.5±1.3 ^a (60) 18±2 ^b (60)	26.2±0.60 (60)	19.2±0.37 (60) 335
French Press	20-27 ^b (150) 10-14 ^c (70-180)	43.6±0.98 (160)	29.0±0.53 (160) 336 337
Espresso	16.7-17.3 ^a (60) 40-80 ^b (25) 22-30 ^c (40-90) 12 ^d (50) 26 ^e (50)		338 339 340

341 ^a (Gross, Jaccaud, & Huggett, 1997); ^b (Urgert, Van Der Weg, Kosmeijerschuil, Van De Bovenkamp,
 342 Hovenier, & Katan, 1995); ^c (Buchmann, Zahm, Kolling-Speer, & Speer, 2010); ^d (Kurzrock & Speer,
 343 2001); ^e (Speer, Hruschka, Kurzrock, & Kolling-Speer, 2000); ^f 1(2) roast colour; ^g 1(5) roast colour

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346 **Table 2: Colour parameters (Lightness (L*), a*, b* value) and density (tap density and bulk density) of**
347 **roast coffee by roast intensity.**

Roasting intensity	Tap density (kg/m ³)	Bulk density (kg/m ³)	L*	a*	b*
Raw	493 ^a ±0.01	415 ^a ±1.27	67.3 ^c ±1.04	0.66 ^a ±0.06	14.5 ^e ±0.42
I(1)	514 ^a ±0.03	404 ^a ±7.21	63.8 ^d ±0.48	8.03 ^e ±0.14	22.1 ^f ±0.41
I(2)	504 ^a ±0.01	374 ^b ±7.65	46.6 ^c ±0.54	7.92 ^e ±0.17	10.8 ^d ±0.28
I(3)	497 ^a ±0.01	354 ^b ±14.7	44.2 ^b ±0.11	7.17 ^d ±0.15	8.82 ^c ±0.31
I(4)	490 ^a ±0.01	349 ^{bc} ±18.4	41.7 ^a ±0.43	6.26 ^b ±0.10	6.86 ^a ±0.12
I(5)	483 ^a ±0.02	322 ^c ±0.18	42.0 ^a ±0.31	6.59 ^c ±0.16	7.63 ^b ±0.30

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349 *Mean ± standard deviation of values in five replicates. Different letters indicate a difference*
350 *within a column (p≤0.05).*

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357 **Table 3: Cafestol concentration of roast and ground coffee (mg/100g) and coffee brews (mg/L) by roast**
358 **intensity and brew mechanism.**

Roasting intensity	R&G (mg/100g)	Boiled (mg/L)	Turkish (mg/L)	French (mg/L)	Mocha (mg/L)
Raw	642 ^a ±10.7	63.9 ^a ±1.94	45.9 ^a ±0.04	74.4 ^a ±0.42	40.0 ^a ±1.41
I(1)	619 ^b ±0.9	48.2 ^b ±3.47	41.3 ^b ±0.12	53.3 ^b ±0.68	32.7 ^b ±0.73
I(2)	608 ^{bc} ±0.32	43.9 ^c ±1.36	39.1 ^c ±0.04	43.6 ^c ±0.98	26.2 ^c ±0.60
I(3)	593 ^{bc} ±5.68	42.5 ^c ±1.84	34.7 ^d ±0.12	25.8 ^d ±0.14	24.1 ^d ±0.52
I(4)	600 ^c ±12.4	35.0 ^d ±1.64	34.4 ^d ±1.67	29.0 ^d ±6.51	22.3 ^e ±0.27
I(5)	595 ^c ±5.56	25.9 ^e ±3.54	22.8 ^e ±0.12	29.0 ^d ±0.53	19.2 ^f ±0.37

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360 *Mean ± standard deviation of values in five replicates. Different letters indicate a difference*
361 *within a column (p≤0.05), R&G is roasted and ground coffee*

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367 **Table 4: Cafestol concentration (mg/cup) by roast intensity and brew mechanism.**

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Roasting intensity	Boiled (mg/cup)	Turkish (mg/cup)	French (mg/cup)	Mocha (mg/cup)
Raw	10.2 ^a ±0.31	2.8 ^a ±0.00	11.9 ^a ±0.07	2.4 ^a ±0.08
I(1)	7.7 ^b ±0.56	2.5 ^b ±0.01	8.5 ^b ±0.01	2.0 ^b ±0.04
I(2)	7.0 ^c ±0.22	2.3 ^c ±0.00	7.0 ^c ±0.16	1.6 ^c ±0.04
I(3)	6.8 ^c ±0.29	2.1 ^d ±0.01	4.1 ^d ±0.00	1.4 ^d ±0.03
I(4)	5.6 ^d ±0.26	2.1 ^d ±0.10	4.6 ^d ±1.04	1.3 ^e ±0.02
I(5)	4.1 ^e ±0.56	1.4 ^e ±0.01	4.6 ^d ±0.08	1.1 ^f ±0.02

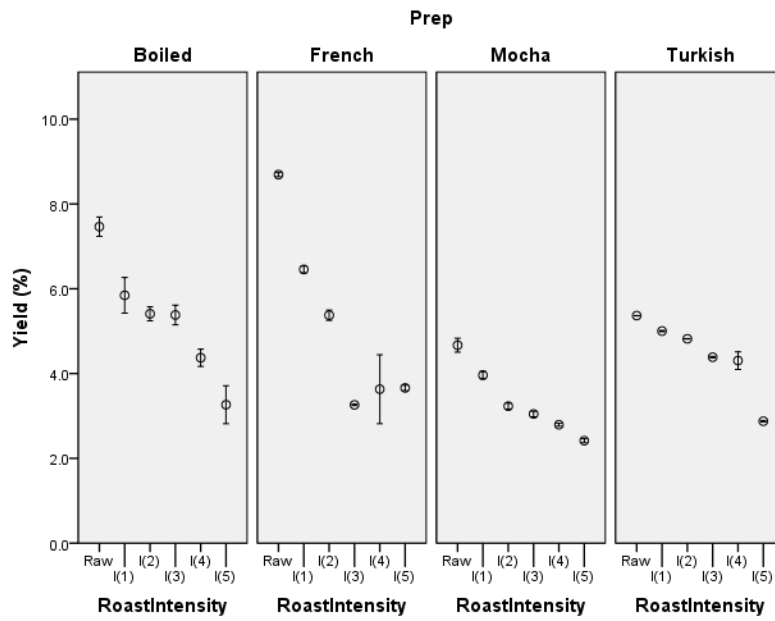
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375 *Mean ± standard deviation of values in five replicates. Different letters indicate a difference within a*
376 *column (p≤0.05) on a cup basis, cup size for each preparation: Boiled (160mL), Turkish (60mL),*
377 *French (160mL), Mocha (60mL).*

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382 **Figure 1: Cafestol extraction yield by roast intensity and brewing mechanism +/- 1 Standard Deviation.**

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384 $Yield = [Brew\ cafestol\ concentration\ (mg/L) \times total\ brew\ volume\ (L)] / [R\&G\ cafestol\ concentration$
385 $(mg/kg) \times total\ R\&G\ (kg)] \times 100$, where R&G is roasted and ground coffee.