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1 **Acrylamide formation and antioxidant activity in coffee during roasting – A systematic study**

2

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26

27 **Abstract**

28 The aim of this study was to investigate the effect of the coffee roasting process on both toxic and
29 some beneficial antioxidant compounds, applying a systematic and broad approach. Arabica and
30 Robusta green coffee beans were roasted in a lab-scale roaster for different times in order to achieve
31 five roasting degrees (from light to dark) and to assess the evolution of acrylamide (AA),
32 trigonelline, nicotinic acid and caffeoylquinic acids contents (determined by HPLC) as well as
33 antioxidant activity (evaluated by Folin-Ciocalteu, FRAP, DPPH, ABTS assays).

34 The results confirmed that the AA levels and antioxidant activity reached a maximum in the first
35 coffee roasting degrees and then decreased prolonging the heating process, both in Arabica and
36 Robusta samples. Nevertheless, the thermal reduction observed was greater for AA compared to
37 antioxidant activity, which was only slightly reduced due to the balance between the degradation
38 and the neo-formation of antioxidant compounds.

39

40 **Keywords**

41 Coffee; Acrylamide; Antioxidant activity; Trigonelline; Nicotinic acid; Chlorogenic acids

42

43 **Chemical compounds studied in this article**

44 3-*O*-Caffeoylquinic acid (PubChem CID: 1794427); 5-*O*-Caffeoylquinic acid (PubChem CID:
45 5280633); 3,5-*O*-diCaffeoylquinic acid (PubChem CID: 6474310); Acrylamide (PubChem CID:
46 6579); Asparagine (PubChem CID: 6267); Caffeine (PubChem CID: 2519); Fructose (PubChem
47 CID: 2723872); Glucose (PubChem CID: 5793); Nicotinic acid (PubChem CID: 938); Sucrose
48 (PubChem CID: 5988); Trigonelline (PubChem CID: 5570).

49

50 **1. Introduction**

51 Coffee is one of the most consumed beverages in the world. During the roasting process, green
52 coffee beans undergo various changes due to different thermal reactions, most of them in the

53 context of Maillard reactions (e.g. caramelization, Strecker degradation, pyrolysis etc.) that lead to
54 the development of the desired physicochemical and organoleptic properties of roasted coffee beans
55 and derived beverages, such as flavour, aroma and colour, but also to the formation of undesired
56 compounds (Aguiar, Estevinho, & Santos, 2016).

57 One of the undesired heat-induced contaminants is acrylamide (AA), a substance formed mainly by
58 the condensation of amino group of amino acids (principally asparagine) and carbonyl group of
59 reducing sugars (e.g. glucose and fructose) during the Maillard reactions triggered at temperatures
60 above 120 °C (Schouten, Tappi, & Romani, 2020). AA has been classified by the International
61 Agency for Research on Cancer (IARC) as a substance "probably carcinogenic to humans" (group
62 2A). Following this scientific opinion, the worldwide legislation concerning the permitted AA
63 levels in a wide range of cooked foods such as fried potato, bakery and coffee products has become
64 increasingly restrictive (European Commission, 2017; Food Drink Europe, 2019). In Europe, the
65 Commission Regulation (EU) 2017/2158 defined the application of mitigation measures and
66 benchmark levels for AA in foods. Regarding roasted coffee, food business operators should apply
67 mitigation measures to ensure a minimum formation of AA below the new benchmark level of 400
68 µg/kg (European Commission, 2017). Due to legislative restrictions and the global consumption of
69 coffee beverages, a lot of researchers have been carried out to find possible solutions aimed at
70 reducing AA along the entire coffee processing (Anese, 2015; Schouten, Tappi, et al., 2020).

71 One of the strategies for the control of AA level in coffee is the selection of high-quality green
72 coffee beans. *Coffea arabica* (Arabica) is the most important coffee specie for the processing
73 industry, with about 60% of the total production, followed by *Coffea canephora* (Robusta)
74 (Schouten, Tappi, et al., 2020). At the same roasting conditions, Robusta specie presents higher AA
75 levels than Arabica, due to its higher content of asparagine, the main precursor of AA (Bagdonaite,
76 Derler, & Murkovic, 2008; Summa, de la Calle, Brohee, Stadler, & Anklam, 2007). The roasting
77 process is considered the main responsible for the formation of AA in coffee; the applied roasting
78 degree, which can range from "light" to "dark" depending on time and temperature conditions

79 adopted, seems to be a key factor (Schouten, Tappi, et al., 2020). Generally, the roasting degree is
80 determined by the habit and consumers' preferences in different countries: South European
81 consumers prefer medium-dark to dark roasted coffee, on the contrary, North European and
82 American ones prefer a lighter roasting degree (Anese, 2015). Some authors reported that in the
83 first stage of roasting (between light and medium roasting degrees) the formation rate of AA
84 reaches its maximum and decreases toward the end of the process, due to the high temperature and
85 prolonged times (Bagdonaite et al., 2008; Bertuzzi, Martinelli, Mulazzi, & Rastelli, 2020; Budryn,
86 Nebesny, & Oracz, 2015; Summa et al., 2007). However, as reported by Schouten, Tappi, et al.
87 (2020), most of the scientific researches, aimed to find solutions to reduce AA content during
88 roasting, are lacking important information concerning the roasting condition adopted, time-
89 temperature profiles during process, the number of replicates of roasting process and analysis, the
90 main physicochemical and nutritional proprieties of the final roasted coffee.

91 It is known that, despite the presence of AA, coffee is also a rich source of biologically active
92 compounds with significant antioxidant proprieties (Summa et al., 2007). The effect of roasting on
93 the antioxidant activity of coffee has been studied by several authors, but sometimes discordant
94 results have been obtained. Many studies have found an increase in the antioxidant capacity in
95 medium roasted coffee and a decrease in dark roasted one (Hečimović, Belščak-Cvitanović, Horžić,
96 & Komes, 2011; Perrone, Farah, & Donangelo, 2012; Vignoli, Bassoli, & Benassi, 2011; Vignoli,
97 Viegas, Bassoli, & Benassi, 2014); in contrast, other experimental studies have demonstrated a
98 decrease of antioxidant capacity in light roasted coffee and an increase in dark roasted one (Daglia,
99 Papetti, Gregotti, Bertè, & Gazzani, 2000; Wen et al., 2005); further researchers have found an
100 increase (Pokorná et al., 2015; Sánchez-González, Jiménez-Escrig, & Saura-Calixto, 2005) or a
101 decrease of antioxidant activity during roasting (Budryn et al., 2015; Pokorná et al., 2015; Summa
102 et al., 2007). The discrepancies between studies on the behaviours of antioxidant activity in roasted
103 coffee could be related to differences in green coffee samples, used roasting time-temperature
104 conditions, sample preparation, analytical extraction, assays methods, etc.. Caffeine, chlorogenic

105 acids, trigonelline, nicotinic acid are the characteristic coffee compounds linked to antioxidant
106 activity whose content is influenced by the roasting process (Caprioli et al., 2014; Farah &
107 Donangelo, 2006; Komes & Bušić, 2014; Vignoli et al., 2011; Zhou, Chan, & Zhou, 2012). Several
108 health benefits are attributed to these compounds and their role in the prevention of chronic diseases
109 such as cancer and cardiovascular pathologies have been the subject of a large number of scientific
110 research (Aguiar et al., 2016).

111 The importance of assessing the possible risks and benefits related to strategies for AA reduction in
112 coffee is therefore clear. The present work aims to develop a comprehensive study, adopting a
113 systematic approach, on the formation of both AA and antioxidant activity in Arabica and Robusta
114 coffee samples during the roasting process conducted under different time-temperature conditions.
115 This to assess how the heat treatment can be directly linked to the presence/formation of unhealthy
116 compounds, such as AA and healthy compounds, among which trigonelline, nicotinic acid and
117 caffeoylquinic acids.

118

119 **2. Materials and methods**

120

121 *2.1 Coffee samples*

122 The study was performed on two green coffee (G) samples, belonging to *Coffea arabica* L. (Brazil,
123 Santos) and *Coffea canephora* var. Robusta (India) both wet-processed, supplied by the company
124 ESSSE Caffè S.p.A. (Anzola dell'Emilia, BO, Italy).

125 Raw coffee beans batches of 250 g/run were roasted in a hot air pilot plant roaster with rotating
126 drum (mod. EXPO 500/E, STA Impianti, Crespellano, BO, Italy), pre-heated at 160 ± 2 °C. Coffee
127 samples were roasted at five different roasting degrees: light (L), light-medium (LM), medium (M),
128 medium-dark (MD), dark (D). In **Figure 1**, the adopted roasting process conditions, in terms of total
129 time and final temperature recorded for each roasting degree are reported.

130 To reach a similar degree of roasting in both Arabica and Robusta samples, preliminary trials were
131 carried out, based on the main physicochemical roasting parameters, evaluated as reported below
132 (2.3 section).

133 The air temperature inside the drum was measured, approximately every 10 seconds, by the
134 electronic control panel of the roaster in order to monitor and assess the thermal profile of each
135 cycle. Three repetitions were carried out for all roasting conditions in order to obtain a
136 representative set for each sample, producing a total of 30 roasted coffee samples (5×3 for Arabica
137 and 5×3 for Robusta), plus three replicates of both green ones. After roasting, the coffee samples
138 were left to cool at room temperature, then transferred to a sealed glass jar and stored at 4 °C until
139 analysis. A part of each sample was ground using an electric grinder (mod. M20, IKA-WERKE,
140 Staufen, Germany). Green coffee samples have been ground using small amount of material in
141 multiple cycles to avoid excessive heating of the product and to obtain a final homogeneous
142 granulometry.

143

144 *2.2 Chemicals and reagents*

145 AA (for molecular biology, ≥99% (HPLC), C₃H₅NO, molecular weight 71.08 g/mol, CAS No 79-
146 06-1), 2,3,3-*d*₃-acrylamide (AA-*d*₃) standard solution, 500 mg/mL in acetonitrile (analytical
147 standard, CAS 122775-19-3), L-asparagine (≥98% (HPLC), C₄H₈N₂O₃, molecular weight 132.12
148 g/mol, CAS No 70-47-3), D-(–)-fructose (≥99%, C₆H₁₂O₆, molecular weight 180.16 g/mol, CAS No
149 57-48-7), D-(+)-glucose (analytical standard, C₆H₁₂O₆, molecular weight 180.16 g/mol, CAS No
150 50-99-7) and sucrose (BioUltra, for molecular biology, ≥99.5% (HPLC), C₁₂H₂₂O₁₁, molecular
151 weight 342.30 g/mol, CAS No 57-50-1) were purchased from Sigma-Aldrich (St. Louis, MO,
152 USA). Analytical standards of 3-*O*-caffeoylquinic acid (3-CQA), 5-*O*-caffeoylquinic acid (5-CQA)
153 and 3,5-*O*-dicaffeoylquinic acid (3,5-diCQA), trigonelline, caffeine and nicotinic acid were also
154 purchased from Sigma-Aldrich. Individual stock solutions of AA, glucose, fructose, sucrose and
155 asparagine were prepared by dissolving the pure standard compounds in water, at a concentration of

156 1,000 mg/L. Individual stock solutions of chlorogenic acids, caffeine, nicotinic acid and trigonelline
157 were obtained by dissolving 10 mg of the analytical standard in 10 mL of HPLC-grade MeOH. All
158 stock solutions were stored in glass-stoppered bottles at $-18\text{ }^{\circ}\text{C}$. Afterwards, standard working
159 solutions at various concentrations were prepared daily by appropriate dilution of the stock solution
160 with water for sugars, AA and asparagine and with methanol for chlorogenic acids, nicotinic acid,
161 caffeine and trigonelline. For AA, an aliquot of a solution of 500 ng/mL of AA- d_3 was combined
162 with standard working solutions of native AA prepared at various concentrations. HPLC-grade
163 acetonitrile and methanol were supplied by Sigma-Aldrich (Milano, Italy). HPLC-grade formic acid
164 (99%) was obtained from Merck (Darmstadt, Germany). Bond Elut-Accucat, 200 mg 3 mL
165 cartridges for solid-phase extraction (SPE) were bought from Agilent Technology (Santa Clara, CA,
166 USA) while Oasis HLB 200 mg 6 mL cartridges were purchased from Waters (Milford, MA, USA).
167 Deionized water was further purified using a Milli-Q SP Reagent Water System (Millipore,
168 Bedford, MA, USA). Before High Performance Liquid Chromatography-tandem mass (HPLC-
169 MS/MS) analysis, all samples were filtered with PhenexTM RC 4 mm 0.2 μm syringeless filter,
170 Phenomenex (Castel Maggiore, BO, Italy) while a Captiva PTFE 13 mm 0.45 μm syringeless filter,
171 Agilent Technology (Santa Clara, CA, USA), was used before HPLC-Variable Wavelength Detector
172 (VWD) analysis.

173 Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid ($\text{C}_7\text{H}_6\text{O}_5$), TPTZ (2,4,6-tri(2-pyr-
174 idyl)-S-triazine), ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), sodium acetate ($\text{C}_2\text{H}_3\text{O}_2\text{Na}$), acetic acid
175 ($\text{C}_2\text{H}_4\text{O}_2$), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-
176 azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$),
177 disodium phosphate (Na_2HPO_4), monopotassium phosphate (KH_2PO_4), sodium acetate ($\text{C}_2\text{H}_3\text{O}_2\text{Na}$)
178 and ethanol ($\text{C}_2\text{H}_5\text{OH}$) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).
179 Hydrogen chloride (HCl), potassium chloride (KCl), acetic acid (CH_3COOH), sodium hydroxide
180 (NaOH) and glycerine ($\text{C}_3\text{H}_8\text{O}_3$) were acquired from Carlo Erba reagents (Milan, Italy). DPPH (2,2-

181 diphenyl-1-picrylhydrazyl) was obtained from Glentham Life Sciences (Corsham, UK). All
182 chemicals and reagents were of analytical grade.

183

184 *2.3 Physicochemical analysis*

185 In order to assess the uniformity of the roasting conditions adopted, the following parameters were
186 evaluated on each green and differently roasted coffee sample:

- 187 - weight loss (%) was determined as the percentage weight variation between whole coffee
188 beans before and after each roasting run;
- 189 - density (g/mL) of whole coffee beans was evaluated by volume displacement in a
190 pycnometer, using glycerine ($\rho = 1.26$ g/mL);
- 191 - water activity (a_w) was determined on ground samples using a dew point hygrometer
192 AQUALAB (Meter 4TE, Pullman, USA);
- 193 - moisture (%) was determined on ground coffee by gravimetric method, after heating in a
194 stove (mod. UF110, Memmert, Schwabach, Germany) at 105 °C up to constant weight;
- 195 - colour of whole coffee beans was measured by using a tristimulus spectrophotometer
196 HunterLab (mod. ColorFlex EZ, s/n: CFEZ 1206, Virginia, USA) with geometry 45°/0°,
197 illuminant D65 (6500 K) and equipped with a glass sample cup (64 mm diameter) and a
198 19.1 mm diameter measuring head. The instrument was calibrated with a white tile and
199 black glass before the measurements. Colour was expressed in standard CIE L*a*b* scale;
200 a* and b* parameters were converted into hue angle ($h^\circ = \tan^{-1}(b^*/a^*)$).

201

202 *2.4 Analyte extraction and sample clean-up*

203 For all analytes, water has been chosen as extraction solvent with a sample/solvent ratio of 1:10
204 since all monitored compounds were sufficiently polar for migrating and dissolving in water as
205 reported in other works (Andrzejewski, Roach, Gay, & Musser, 2004; Nielsen, Granby, Hedegaard,
206 & Skibsted, 2006; Schouten, Genovese, et al., 2020). For AA extraction and sample purification, a

207 previous procedure was followed (Andrzejewski et al., 2004) with some adjustments. 1 g of coffee
208 powder was spiked with 0.4 mL (500 ng/mL) of AA-d₃ internal standard and the sample was diluted
209 with 9.6 mL of water. The extraction of monitored molecules was performed at controlled
210 temperature (80 °C) for 30 min, under magnetic stirring. Then, the sample was centrifuged at 5,000
211 rpm (3,661 g) for 10 min and the supernatant was collected and stored at 4 °C until use.

212 For AA analysis, the supernatant was filtered by 0.45 µm syringeless filter and purified by SPE,
213 following a previous procedure (Andrzejewski et al., 2004). Briefly, Oasis HLB columns were first
214 conditioned with 3.5 mL of MeOH and then with 3.5 mL of water. 1.5 mL of filtered supernatant
215 was loaded onto cartridge and the sample was allowed to pass completely through the sorbent
216 material and was followed with 0.5 mL of water. For AA elution, 1.5 mL of water was added onto
217 the cartridge and the eluent was collected in an 8 mL glass vial. Before conditioning the second SPE
218 column, a mark was placed on the outside of the cartridge at a height equivalent to 1 mL of liquid
219 above the sorbent bed. The Bond Elut-Accucat column was conditioned with 2.5 mL of methanol
220 followed by 2.5 mL of water. The solvents used for conditioning were discarded. The eluent
221 collected from the first cartridge was added to the Bond Elut-Accucat cartridge. The sample was
222 allowed to eluate from the column up to the mark previously placed on the outside; the eluent was
223 then collected to a 6 mL glass vial. Before injection into HPLC-MS/MS system, it has been filtered
224 by 0.2 µm syringeless filter. The purpose of discarding the first 0.5 mL of the sample was to avoid
225 collecting residual water used to wash the column, which could dilute any AA collected.

226 For the analysis of glucose, fructose, and asparagine an aliquot of supernatant was centrifuged at
227 5,000 rpm for 10 min and diluted 1:20 with mobile phase while for sucrose analysis the dilution
228 was 1:100. Then, before HPLC-MS/MS injection the diluted samples were filtered by 0.2 µm
229 syringeless filter.

230

231 *2.5 Sugars, asparagine and acrylamide analysis by HPLC-MS/MS system*

232 The analysis of the three sugars, asparagine and AA has been developed taking the cue from
233 previous procedure (Schouten, Genovese, et al., 2020). The present method has been implemented
234 by using isotopically labelled internal standard (ILIS) and adding the analysis of sucrose. All AA
235 precursors such as asparagine, sucrose, glucose, and fructose were monitored in green coffee while
236 AA in green and roasted beans. HPLC-MS/MS studies were performed using an Agilent 1290
237 Infinity series and a Triple Quadrupole 6420 from Agilent Technology (Santa Clara, CA, USA)
238 equipped with an electrospray ionization (ESI) source operating in positive ionization mode. The
239 HPLC-MS/MS parameters of sucrose and AA-d₃ were optimized in flow injection analysis (FIA) (1
240 μ L of a 10 mg/L individual standard solution) by using optimizer software (Agilent) while the other
241 HPLC-MS/MS parameters were applied according to a previous method (Schouten, Genovese, et
242 al., 2020). The separation of target compounds was achieved on a Kinetex Hilic analytical column
243 (100 mm \times 4.6 mm i.d., particle size 2.6 μ m) from Phenomenex (Torrance, CA, USA) preceded by
244 a KrudKatcher ULTRA HPLC In-Line Filter (2.0 μ m Depth Filter \times 0.004 in I.D.). The mobile
245 phase for HPLC-MS/MS analysis was composed of 15% water (A) and 85% HPLC-grade
246 acetonitrile (B), both with 0.1% formic acid. The separation was obtained by flowing at 0.8 mL/min
247 with this gradient elution: isocratic condition until 2.5 min (85% B), 3.5 min (70% B), 5.5 min
248 (70% B), 6.5 min (85% B) and then constant until the end of the run (15 min). The injection volume
249 was 2 μ L. The temperature of the column was 25 $^{\circ}$ C and the temperature of the drying gas in the
250 ionization source was 350 $^{\circ}$ C. The gas flow was 12 L/min, the nebulizer pressure was 45 psi and the
251 capillary voltage was 4,000 V. Detection was performed in the multiple reaction monitoring
252 (MRM) mode. The MRM peak areas were integrated for quantification and the most abundant
253 transition was used for quantitation, and the rest of the product ions were used for qualitative
254 confirmation. For AA quantification the response factor was measured by calculating the ratio
255 between the area of AA and AA-d₃. The selected ion transitions and the mass spectrometer
256 parameters are reported in **Table S1** (in **Supplementary materials**). As an example, **Figure S1** (in
257 **Supplementary materials**) reports (a) the HPLC-MS/MS chromatogram of a standard mixture of

258 sugars, AA and asparagine plotted as overlapped multiple reaction monitoring (MRM) transitions of
259 each analyte and (b) the HPLC-MS/MS chromatograms of AA and AA-d₃ of a coffee sample
260 (Robusta light-medium roasted). The limit of quantification (LOQ) and of detection (LOD) have
261 been calculated as 10:1 and 3:1 signal-to-noise ratio (SNR), respectively; the LOQ was 5 µg/L,
262 while the LOD was 1 µg/L. The recovery and matrix effects have been calculated even if a previous
263 validated procedure was followed (Andrzejewski et al., 2004). The obtained values were
264 satisfactory since the recovery was 90 ± 5% and matrix effects were 110 ± 5%.

265

266 *2.6 Caffeine, chlorogenic acids, trigonelline and nicotinic acid analysis*

267 The quantification of caffeine, trigonelline, nicotinic acid and chlorogenic acids was performed by
268 following two developed procedures (Caprioli et al., 2014, 2013). Briefly, the supernatant collected
269 after water extraction was diluted 1:20 in mobile phase, filtered with 0.45 µm syringeless filter and
270 then injected into high-performance liquid chromatography-variable wavelength detector (HPLC-
271 VWD) system. All analytes were monitored in green and roasted coffee except caffeine which was
272 quantified only in green beans. The system used for the analysis was a Hewlett Packard (Palo Alto,
273 CA, USA) HP-1090 Series II, made of an autosampler and a binary solvent pump. The separation of
274 caffeine, trigonelline and nicotinic acid was achieved on a Gemini C18 110 Å analytical column
275 (250 × 3 mm I.D., 5 µm) from Phenomenex (Cheshire, UK) using a mobile phase composed of water
276 (A) containing 0.3% of formic acid and methanol (B), at a flow rate of 0.4 mL/min. The gradient
277 program was: 0 min, 25% B; 0–10 min, 60% B; 10–15 min, 60% B; 15–20 min, 25% B; held at
278 25% until the end of the run at 25 min. The acquisition was performed at two different wavelengths
279 in the same run: 265 nm for trigonelline and nicotinic acid and 270 nm for caffeine. The separation
280 of chlorogenic acids was performed on a Polar-RP 80 Å analytical column (150 × 4.6 mm I.D., 4
281 µm) from Phenomenex (Cheshire, UK) with a mobile phase constituted by water (A) and methanol
282 (B) both with 0.1% of formic acid, at a flow rate of 1 mL/min. The elution was carried out in
283 gradient mode: 0–5.5 min, 25% B; 5.5–8 min, 50% B; 8–13.5 min, 50% B; 13.5–18 min, 25% B.

284 The acquisition was performed by monitoring a wavelength of 325 nm for all three chlorogenic
285 acids.

286

287 *2.7 Antioxidant activity determination*

288 Antioxidant activity analysis was determined in coffee extracts prepared according to the procedure
289 described by Herawati et al., 2019. Around 2.5 g of ground coffee (green and roasted) was brewed
290 with 50 mL of hot water at 95 °C, stirred for 1 min using a magnetic stirrer, cooled in an ice bath
291 for 2 min and filtered with a filter paper (1300/80 125 mm, FILTER-LAB, Spain). The coffee
292 extracts have been stored at –80 °C until the determinations.

293 To adequately represent the antioxidant activity of coffee samples, different *in vitro* methods were
294 used. Folin-Ciocalteu (FC), which is often used as a determination of total phenolic content, is also
295 a good indicator of the total reducing capacity (Vignoli et al., 2011). FRAP (Ferric Reducing
296 Antioxidant Power) was used to evaluate the ability of reducing iron, while ABTS and DPPH
297 assays represented the radical scavenging ability.

298 FC method was applied according to the procedure reported by Vignoli et al. (2011). An aliquot of
299 coffee extract (100 µL) was added to 300 µL of FC reagent (0.9 mol/L) and 1 mL of Na₂CO₃
300 solution (20% w/w); distilled water was then added until 10 mL was reached. The solution obtained
301 was kept in the dark and at room temperature for 60 min. The total reducing capacity of the coffee
302 samples was determined by measuring the absorbance at a wavelength of 765 nm with a UV-Vis
303 spectrophotometer (mod. UV-1601, SHIMADZU EUROPA GmbH, Duisburg, Germany). Standard
304 aqueous solutions of gallic acid at known concentrations were used for calibration. The results were
305 expressed in mg equivalent of gallic acid/100 g of ground coffee.

306 FRAP method was used according to the procedure described by Sánchez-González, Jiménez-
307 Escrig, & Saura-Calixto (2005). The FRAP reagent was obtained by combining 2.5 mL of TPTZ
308 solution (10 mM) in HCl (40 mM), 2.5 mL of FeCl₃·6H₂O (20 mM) and 25 mL of acetate buffer
309 (0.3 mM) at pH 3.6. The mixture obtained was warmed at 37 °C for 20 min in a stove (mod. UF110,

310 Memmert, Schwabach, Germany). Subsequently, 900 μL of FRAP reagent, 90 μL of distilled water
311 and 10 μL of diluted coffee extract were mixed. After 20 min at 37 $^{\circ}\text{C}$ the absorbance was
312 measured at a wavelength of 595 nm with a UV-Vis spectrophotometer (mod. UV-1601,
313 SHIMADZU EUROPA GmbH, Duisburg, Germany). Standard Trolox water solutions at known
314 concentrations were used for calibration. The results were expressed in mg equivalent of Trolox/100
315 g of ground coffee.

316 ABTS method was applied according to Sánchez-González et al. (2005). The ABTS radical cation
317 solution was obtained by reaction of a stock solution of ABTS (7 mM) with potassium persulphate
318 (2.45 mM), left to rest in the dark and at room temperature for 12–16 h. The ABTS radical cation
319 solution was diluted with an alkaline phosphate buffer (pH 7.5, 5 mM) to reach an absorbance of
320 0.700 ± 0.020 at 734 nm. In 4 mL of obtained solution and 10 μL of coffee extract were added, then
321 the absorbance was measured at 734 nm after 6 min using a UV-Vis spectrophotometer (mod. UV-
322 1601, SHIMADZU EUROPA GmbH, Duisburg, Germany). Standard Trolox water solutions at
323 known concentrations were used for calibration. The results were expressed in mg equivalent of
324 Trolox/100 g of ground coffee.

325 DPPH method was used following the protocol of Vignoli et al. (2011). Different concentration (50,
326 25, 20, 15 and 10 mg/mL) for each coffee sample were prepared. A solution was prepared by
327 mixing 0.5 mL of ethanolic DPPH solution (250 M), 1 mL of acetate buffer (100 mM; pH 5.5), 1
328 mL of ethanol and 10 μL of the sample at the different concentrations. After resting the solution for
329 10 min in the dark at room temperature, the absorbance was read at 517 nm using a UV-Vis
330 spectrophotometer (mod. UV-1601, SHIMADZU EUROPA GmbH, Duisburg, Germany). The
331 results were expressed in IC₅₀ (coffee concentration able to reduce the radical DPPH by 50%)
332 calculating the percentage of inhibition of absorbance (IA%) for each coffee concentration.

333

334 *2.8 Statistical analysis*

335 All physicochemical and analytical determinations were conducted in triplicate for each green and
336 roasted coffee sub-sample. The results were reported as the mean value \pm standard deviation.
337 Significant differences between results were calculated by unidirectional analysis of variance
338 (ANOVA) followed by Tukey's post-hoc comparison test, with a significance level of $p < 0.05$. The
339 Pearson correlation coefficient (r), with a level of significance $p < 0.05$, was calculated to evaluate
340 the relationship between the average values of AA, antioxidant activity (determined with FC,
341 FRAP, ABTS, DPPH methods), total chlorogenic acids, trigonelline and nicotinic acid measured in
342 all coffee samples.

343 The statistical package STATISTICA 8.0 software (Statsoft Inc., Tulsa, UK) was used.

344

345 **3. Results and discussion**

346

347 *3.1 Time-temperature roasting profiles and physicochemical characterization*

348 The obtained temperature profiles for the roasting process are shown in **Figure 1A** and **1B** for
349 Arabica and Robusta coffee samples, respectively. Each thermal profile represents the average
350 value of triplicate roasting cycles for each coffee sample. At the beginning of each roasting cycle, a
351 rapid drop in the air temperature inside the roaster (set at 160 °C) of about 70 °C was observed, as a
352 consequence of green beans insertion. After 1 min the temperature started to rise, reaching the final
353 values reported in the **Figure 1** for each roasting degree in both Arabica and Robusta samples. The
354 Robusta sample took a longer time than the Arabica to reach the final temperature set for each
355 roasting degree. It is well known that these two coffee types do not reach an analogous degree of
356 roasting at the same time, due to their differences in composition, volume and bean shape (Romani,
357 Cevoli, Fabbri, Alessandrini, & Dalla Rosa, 2012). The overlapping of thermal profiles confirms a
358 very high reproducibility of the roasting cycles carried out.

359 In **Table 1** results of the physicochemical characteristic of all green and roasted coffee samples are
360 reported. Coffee beans showed a significant and progressive weight loss, that at the longest roasting

361 time (dark degree) reached around 17% and 18% in Arabica and Robusta samples, respectively. The
362 weight loss in the first roasting degrees (L, LM, M) can be attributed to water loss, while from the
363 medium-dark degree it is mainly related to thermal degradation of organic matter into gas and
364 volatile compounds (Fernandes, 2019; Schenker & Rothgeb, 2017). In fact, the decrease in moisture
365 (%) and water activity (a_w) was faster in the early stages of the roasting process and then (from MD
366 and D degrees) became slower and similar in both Arabica and Robusta samples. The moisture
367 content in the Robusta sample until light-medium degree, was higher than that of Arabica, probably
368 due to its higher initial moisture content. Therefore, this higher moisture content in the Robusta
369 samples has led to greater weight loss.

370 During roasting, a significant change in the colour of the coffee beans occurred. As expected, the
371 variations of lightness (L^*) and hue angle (h°) parameters showed that the colour of both samples
372 becomes progressively more brownish and more uniform at the highest roasting degree. In terms of
373 h° , the colour of coffee beans changed from greenish-grey-blue, typical of green coffee, to
374 gradually yellow, orange, brown and brown-black in the dark roasting degree, as a result mainly of
375 brown polymers melanoidins formation with the progress of Maillard reactions (Fernandes, 2019).

376 Another important roasting parameter is the density of coffee beans that decreased during roasting
377 due to the simultaneous decrease in weight and increase in volume, associated with loss of water
378 and generation of volatile compounds (Schenker & Rothgeb, 2017). For both Arabica and Robusta
379 samples, the dark roasted coffee showed density values halved compared to the corresponding green
380 samples.

381 The values of the roasting parameters measured in the coffee samples are within the typical ranges
382 for the defined roasting degree. The medium roasted coffee samples showed characteristics suitable
383 for the preparation of an American-style drip coffee brew, while the dark roasted coffee samples for
384 the preparation of an Italian-style espresso coffee brew (Romani, Pinnavaia, & Dalla Rosa, 2003).

385

386 *3.2 Influence of coffee roasting degree on acrylamide content*

387 **Figure 2** shows the behaviour of AA development in Arabica and Robusta coffee samples at the
388 different roasting degrees. In green coffee samples AA levels were always below the limit of
389 quantification (LOQ). At the applied roasting conditions, the highest AA levels were reached in
390 both Arabica and Robusta samples at the light-medium roasting degree, with a value of 730 ± 30
391 $\mu\text{g}/\text{kg}$ for Arabica and $1,130 \pm 10 \mu\text{g}/\text{kg}$ for Robusta. Increasing the roasting degree, the AA content
392 decreased rapidly by 85% and 88% respectively for Arabica and Robusta dark roasted samples,
393 starting from the highest value (LM degree), reaching a similar final content. M, MD and D samples
394 showed AA contents below the benchmark level of $400 \mu\text{g}/\text{kg}$, reported in the EU Regulation
395 2017/2158 (European Commission, 2017).

396 The general trend obtained in both samples during roasting confirmed, as reported in numerous
397 studies, that AA formation is dominant during the first period of roasting and decreases toward the
398 intensification of the thermal process (Bagdonaite et al., 2008; Bertuzzi et al., 2020; Esposito et al.,
399 2020; Hamzalıoğlu & Gökmen, 2020; Summa et al., 2007). However, until now, very few research
400 works attempted to identify a potential mechanism of AA evaporation or degradation during
401 prolonged roasting. Pastoriza et al. (2012) suggested that the decrease of AA during roasting could
402 be due to its chemical interaction with coffee melanoidins, whose concentration has a direct effect.
403 The authors hypothesized that nucleophilic amino groups of amino acids from the proteinaceous
404 backbone of coffee melanoidins react via the Michael addition reaction with AA. Recently, Badoud
405 et al. (2020) investigated the fate of AA during roasting and brew preparation using ^{14}C - and ^{13}C -
406 labeled AA. The results highlighted the complexity of the reactions involved in coffee roasting and
407 indicated that while about 25% of AA was lost by volatilization, the remaining 75% was detectable
408 in the final products, but only 50% was in free soluble form. However, further researches are still
409 required to determine the entire mechanisms of this reaction and to clarify if the degradation of AA
410 contributes to the possible development of other toxic compounds, which may have a negative
411 impact on human health.

412 In this study, Robusta coffee showed a significantly higher AA content, especially at the lowest
413 roasting degrees. This is probably attributed to the different content of AA precursors in green
414 coffee samples. The sum of total sugars was significantly higher in the Arabica green coffee beans
415 than in Robusta (sucrose: $55,630 \pm 3,600$ mg/kg and $48,010 \pm 480$ mg/kg respectively in Arabica
416 and Robusta; reducing sugars: $12,850 \pm 150$ mg/kg and $8,000 \pm 110$ mg/kg respectively in Arabica
417 and Robusta), on the other side the levels of asparagine were 540 ± 40 mg/kg in Arabica and $800 \pm$
418 50 mg/kg in Robusta. These results confirm that the amino acid asparagine is the limiting factor for
419 the formation of AA in coffee, as already reported in other studies (Bagdonaite et al., 2008;
420 Bertuzzi et al., 2020). The difference between Arabica and Robusta coffee in terms of AA content
421 found in this study is in agreement with previous findings (Bagdonaite et al., 2008; Esposito et al.,
422 2020; Summa et al., 2007).

423

424 *3.3 Influence of coffee roasting degree on antioxidant properties*

425 In order to evaluate whether the applied roasting process affected the concentration and type of
426 antioxidant compounds in the studied coffee species, the content of caffeoylquinic acids,
427 trigonelline, nicotinic acid and the antioxidant activity by reducing and radical scavenging ability
428 were determined.

429 The content of caffeoylquinic acids (3-CQA, 5-CQA, 3,5-diCQA), trigonelline and nicotinic acid is
430 reported in **Table 2**. Chlorogenic acids (CGAs) are the main phenolic antioxidant compounds in
431 coffee and are formed by the esterification of quinic and hydroxycinnamic acids (Komes & Bušić,
432 2014). The major class of CGAs in coffee are caffeoylquinic acids (CQAs) and dicaffeoylquinic
433 acids (diCQAs) with their main isomers 3-*O*-caffeoylquinic acid (3-CQA), 5-*O*-caffeoylquinic acid
434 (5-CQA) and 3,5-*O*-di-caffeoylquinic acid (3,5-diCQA) (Farah & Donangelo, 2006; Komes &
435 Bušić, 2014). The most abundant CGAs in the analysed coffee samples were 5-CQA (about 80%),
436 followed by 3-CQA and 3,5-diCQA. The total content of the analysed CGAs was higher in green
437 and light roasted samples for Arabica ($31,460 \pm 130$ mg/kg) and Robusta ($32,080 \pm 1,970$ mg/kg),

438 respectively. The increased value in the light sample compared to the green one in Robusta coffee
439 can probably be explained by the loss of other compounds more sensitive to heat, as a consequence
440 this caused a relative, but fictitious increase in the levels of the remaining ones. Moreover, an
441 increase of 3-CQA from green to light roasting degree and a decrease or similar level of 5-CQA
442 have been noticed as a possible result of the isomerization phenomenon of chlorogenic acids, that
443 takes place at the beginning of the roasting process, as reported by Farah, De Paulis, Trugo, &
444 Martin (2005). In both species, the total amount gradually decreased as roasting time increased. In
445 the dark roasted samples, a reduction of about 90%, 70% and 70% were observed respectively for
446 5-CQA, 3-CQA and 3,5-diCQA, starting from their highest values. Due to their instability at high
447 roasting temperatures, these phenolic substances are partially degraded during roasting and can be
448 found in the pigment fraction as free quinic acid and as low molecular weight phenolic compounds
449 (Vignoli et al., 2014). Moreover, at the beginning of the roasting process, part of the chlorogenic
450 acids are incorporated into large molecular weight molecules generated through Maillard reactions
451 forming several derivative compounds (i.e. melanoidins) also characterized by antioxidant
452 properties (Hečimović et al., 2011; Komes & Bušić, 2014). However, increasing roasting time leads
453 to a degradation of melanoidins (Vignoli et al., 2014).

454 Trigonelline is one of the major components of green coffee beans (Komes & Bušić, 2014). It is an
455 alkaloid known to contribute to the formation of desired volatile and non-volatile compounds,
456 important precursors of coffee flavour and aroma, but also of products of nutritional importance
457 (Farah, Ferreira, & Vieira, 2019). Moreover, trigonelline seems to possess some beneficial effects
458 on diabetes and its complications, and on central nervous system which are related to its antioxidant
459 activity as well (Zhou et al., 2012). Nonetheless, the contribution of trigonelline and its derivatives to
460 global coffee flavour and health is mostly unclear and requires further in-depth investigation (Farah
461 et al., 2019). As reported in **Table 2**, the trigonelline content of green coffee samples in both
462 species was in good agreement with the ranges reported in the literature, with higher values in
463 Arabica coffee (Farah et al., 2019), and gradually decreased during roasting. However, while in

464 Arabica coffee a significant reduction was already observed in the light-medium samples, in
465 Robusta one a first increase was observed in the light sample compared to the green one, probably
466 due to an easier extraction, followed by a significant reduction observed in the medium-dark and
467 dark samples. In dark roasted samples a reduction of about 60% for Arabica and 40% for Robusta
468 was reached. Although the initial difference in trigonelline content between the two species, in both
469 medium-dark and dark roasted samples values were significantly similar, probably due to
470 differences in cell wall resistance during the roasting process. Despite the reduction during roasting,
471 according to Farah et al. (2019), the trigonelline content in the ranges found in this study can still be
472 considered relevant with regard to the potential health benefits.

473 Nicotinic acid is the main compound obtained from the thermal conversion of trigonelline during
474 roasting. However, its content, at the dark roasting degree compared to green samples, has increased
475 only by 10% for Arabica coffee and 40% for Robusta coffee. These percentages confirming that
476 trigonelline degradation leads also to the generation of other nitrogenous compounds such as
477 nicotinamide, N-methylpyridinium, 1,2-, 1,3-, 1,4-dimethylpyridinium (non-volatile compounds),
478 pyridine and pyrrole derivatives (volatile compounds) (Ashihara et al., 2015; Komes & Bušić, 2014).

479 To provide a better insight of the health characteristics of coffee samples, antioxidant activity was
480 measured. FC, FRAP, ABTS and DPPH assays have been reported as effective methods to evaluate
481 the antioxidant capacity of coffee and coffee-based products (Sánchez-González et al., 2005).
482 However, every assay tests a different mechanism for antioxidant activity, hence, with the aim of
483 better represent this property, a variety of in-vitro determinations was used.

484 As reported in **Table 3**, no significant differences in reducing and radical scavenging activity values
485 were found between Robusta and Arabica green samples. However, after roasting Robusta samples
486 showed significantly higher antioxidant activity values compared to Arabica ones at each roasting
487 degree. According to Vignoli et al. (2014), the higher antioxidant activity of roasted Robusta coffee
488 is ascribable to its higher caffeine (alkaloid with antioxidant properties) content whose levels are
489 not significantly altered during roasting (Vignoli et al., 2011). In this study, the caffeine content

490 analysed in green coffee beans was significantly higher in Robusta ($26,520 \pm 30$ mg/kg) than in
491 Arabica samples ($23,740 \pm 90$ mg/kg), while total CGAs and trigonelline contents were higher in
492 Arabica. Hence, it can be assumed that a different combination of all singular components has led to
493 the similar measured antioxidant activity in the two green coffee species.

494 Both reducing (FC and FRAP assays) and radical scavenging (ABTS and DPPH assays) capacities
495 of coffee samples, underwent a rapid increase compared to green ones during the first roasting
496 minutes (from L to LM and M degrees) in the range of 40-60% and 50-70% for Arabica and
497 Robusta samples respectively. After a plateau observed generally for all samples, at prolonged
498 roasting times (MD and D degrees) a slight decrease, although not always significant, in reducing
499 and radical scavenging activities was observed for both species. These outcomes are in agreement
500 with earlier studies in which an increase in coffee antioxidant capacity at light and medium roasting
501 degrees and a subsequent decrease with the increasing of roasting time was observed (Bobková et
502 al., 2020; Hečimović et al., 2011). The variation of antioxidant activity is related to a balance
503 between the degradation and the neo-formation of antioxidant compounds. The highest antioxidant
504 activity of light and/or medium roasted coffee can be attributed to the release of low molecular
505 weight phenols from the green coffee constituents and to the formation of compounds by Maillard
506 reactions during the roasting process (Komes & Bušić, 2014; Vignoli et al., 2014). In specific,
507 several antioxidant mechanisms have been attributed to melanoidins, such as chain breakage, metal
508 chelation, radical scavenging and reducing abilities (Delgado-Andrade, Rufián-Henares, & Morales,
509 2005). The majority of melanoidins are already formed in the early stage of the roasting process and
510 their relative contribution to the total antioxidant activity increases towards darker roasting degree,
511 mainly due to the degradation of CGAs during the thermal process (Smrke, Opitz, Vovk, &
512 Yeretizian, 2013). The overall decrease in antioxidant activity observed in this study in the last part
513 of the roasting might indicates that the degradation of antioxidant compounds is not fully
514 compensated by the generation of new ones.

515

516 *3.4 Coffee acrylamide content and antioxidant activity correlation*

517 In **Table 4** the results of Pearson's correlation matrix analysis carried out between the values of AA,
518 chlorogenic acids, trigonelline, nicotinic acid, reducing and radical scavenging capacities found in
519 Arabica and Robusta coffee samples are reported.

520 A strong correlation was found between the reducing capacity, measured by FC and FRAP
521 methods, and the radical scavenging ability determined by ABTS and DPPH assays. The correlation
522 of all used methods with DPPH ones was negative because for this assay the antioxidant activity
523 was expressed in IC50, low IC50 values correspond to higher antioxidant activity values and vice
524 versa.

525 The results of antioxidant activity determined by FC, FRAP and DPPH methods were also
526 significantly correlated with the AA content results. Both AA and antioxidant activity increased
527 remarkably during the early roasting degrees indicating a strong relationship between Maillard
528 reactions and the formation of antioxidant compounds. The following decrease is observed for both
529 AA and antioxidant activity but to a different extent. Indeed, AA levels decreased by more than
530 80% while for antioxidant activities the decrease was lower. This is highlighted by the fact that the
531 AA content was not correlated with the antioxidant activity determined with the ABTS assay,
532 probably because ABTS results decreased during roasting in both coffee samples slower than data
533 measured by the other methods. In detail, the reduction percentage of reducing and radical
534 scavenging capacities in coffee samples, calculated between the reached maximum value (light or
535 light-medium degree) and the dark roasted degree were 50% and 30%, 20% and 30%, 30% and
536 50%, 5% and 10% respectively for Arabica and Robusta samples determined by the FC, FRAP,
537 DPPH and ABTS assays. The different percentages of reduction of the antioxidant activity outlined
538 that both coffee composition and analytical method used for the determination influenced the trend
539 of these coffee health properties.

540 Finally, the trigonelline content in both species was positively correlated to CQAs, indicating a
541 progressive degradation of both classes of components during roasting, and negatively correlated
542 with nicotinic acid, indicating an inverse relationship between them (**Table 4**).

543

544 **4. Conclusions**

545 The results obtained in this systematic study confirmed that the increase in coffee roasting degree
546 promotes a decrease both in AA and in antioxidant content; however, the observed thermal
547 reduction in the medium, medium-dark and dark roasted Arabica and Robusta samples was greater
548 for AA (always below the Commission Regulation (EU) 2017/2158 reference value) compared to
549 antioxidant activity that was only slightly reduced.

550 The present study underlines the importance of considering the impact of heat treatments on both
551 toxic (AA) and beneficial (CGAs, trigonelline, nicotinic acid) compounds, applying a holistic risk-
552 benefit research approach. Indeed, any change in the selection of coffee species and roasting
553 conditions with the intention to reduce AA in the product could also lead to some reduction in the
554 final content of beneficial compounds, such as antioxidant and biologically active ones.

555 Moreover, the overall obtained results, such as those from other scientific comprehensive studies,
556 can be important and useful both for the food industry and international authorities to identify and
557 evaluate potential intervention helpful to reduce AA formation in the most at-risk food products
558 widely consumed.

559

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563

564 **Declaration of Competing Interest**

565 The authors declare that they have no known competing financial interests or personal relationships
566 that could have appeared to influence the work reported in this paper.

567

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725

726 **Figure Captions**

727 **Figure 1.** Total times, final temperatures and corresponding profiles recorded during the roasting of
 728 Arabica (A) and Robusta (B) coffee at different degrees (L = light; LM = light-medium; M =
 729 medium; MD = medium-dark; D = dark).

730

731 **Figure 2.** Acrylamide contents ($\mu\text{g}/\text{kg}$) in Arabica and Robusta coffee samples roasted at different
 732 degrees. Different letters indicate significant differences among samples at $p < 0.05$ level.

733 **Table 1.** Roasting parameters of green (G) and differently roasted (L = light; LM = light-medium;
 734 M = medium, MD = medium-dark; D = dark) Arabica and Robusta coffee samples.

Roasting degree	Weight loss (%) *	Moisture (%) **	Water activity (a_w) **	Lightness (L^*) *	Hue angle (h°) *	Density (g/mL) *
Arabica						
G	-	9.36 ± 0.13^b	0.53 ± 0.00^b	46.80 ± 1.10^a	82.83 ± 1.34^a	1.13 ± 0.01^a
L	7.10 ± 0.06^l	4.15 ± 0.21^d	0.32 ± 0.02^c	44.41 ± 1.44^{ab}	67.11 ± 0.95^d	0.81 ± 0.03^b
LM	9.56 ± 0.08^h	2.77 ± 0.21^f	0.20 ± 0.01^d	34.35 ± 2.51^e	62.24 ± 1.92^f	0.76 ± 0.01^c
M	12.56 ± 0.15^f	1.70 ± 0.26^{gh}	0.12 ± 0.02^e	28.20 ± 1.03^f	58.73 ± 1.11^g	0.66 ± 0.01^d
MD	15.08 ± 0.11^d	1.10 ± 0.27^h	0.08 ± 0.03^f	23.34 ± 1.54^g	54.18 ± 1.65^h	0.59 ± 0.00^{ef}
D	16.83 ± 0.06^b	1.20 ± 0.16^h	0.07 ± 0.01^f	20.60 ± 0.80^h	50.98 ± 1.44^i	0.54 ± 0.00^f
Robusta						
G	-	11.59 ± 0.08^a	0.61 ± 0.01^a	41.11 ± 1.33^c	78.01 ± 1.05^b	1.16 ± 0.01^a
L	8.15 ± 0.15^i	5.08 ± 0.43^c	0.33 ± 0.01^c	43.31 ± 1.52^{bc}	69.68 ± 0.67^c	0.79 ± 0.01^b
LM	11.03 ± 0.34^g	3.44 ± 0.46^e	0.20 ± 0.02^d	36.05 ± 1.02^d	65.37 ± 1.22^e	0.76 ± 0.01^c

M	13.98 ± 0.33 ^e	2.07 ± 0.21 ^g	0.11 ± 0.01 ^e	29.02 ± 1.23 ^f	60.70 ± 1.20 ^g	0.69 ± 0.01 ^d
MD	15.88 ± 0.18 ^c	1.51 ± 0.15 ^h	0.08 ± 0.01 ^f	24.12 ± 1.01 ^g	56.53 ± 1.44 ^h	0.64 ± 0.01 ^e
D	18.01 ± 0.09 ^a	1.45 ± 0.17 ^h	0.07 ± 0.01 ^f	21.21 ± 1.12 ^h	53.10 ± 2.03 ⁱ	0.59 ± 0.01 ^f

735 Values in the same column followed by different letters differ significantly at a $p < 0.05$ level.

736 *Whole bean coffee samples. **Ground coffee samples.

737

738 **Table 2.** Content of chlorogenic acids (3-CQA, 5-CQA, 3,5-diCQA), trigonelline and nicotinic acid
739 content in green (G) and differently roasted (L = light; LM = light-medium; M = medium; MD =
740 medium-dark; D = dark) Arabica and Robusta coffee samples.

Roasting Coffee	3-CQA (mg/kg)	5-CQA (mg/kg)	3,5-diCQA (mg/kg)	Trigonelline (mg/kg)	Nicotinic acid (mg/kg)
Arabica					
G	4200 ± 20 ^d	24940 ± 110 ^a	2320 ± 10 ^{cd}	13540 ± 200 ^a	240 ± 30 ^{bcd}
L	6610 ± 650 ^a	20890 ± 2230 ^b	1810 ± 90 ^{ef}	12900 ± 360 ^a	120 ± 0 ^{ef}
LM	5690 ± 490 ^{ab}	13360 ± 1020 ^c	1400 ± 140 ^{fg}	11440 ± 840 ^b	110 ± 0 ^f
M	4460 ± 260 ^{cd}	9350 ± 710 ^{de}	1090 ± 90 ^{gh}	10750 ± 70 ^b	190 ± 10 ^{def}
MD	2850 ± 310 ^e	5210 ± 670 ^{fg}	960 ± 0 ^h	7820 ± 220 ^{cd}	170 ± 20 ^{cde}
D	1980 ± 80 ^e	2940 ± 150 ^g	700 ± 10 ⁱ	4800 ± 380 ^e	270 ± 0 ^{bc}
Robusta					
G	2840 ± 0 ^e	21020 ± 30 ^b	3000 ± 110 ^{ab}	7090 ± 90 ^d	260 ± 20 ^{bcd}
L	6310 ± 380 ^a	22480 ± 1340 ^{ab}	3280 ± 250 ^a	8870 ± 380 ^c	130 ± 20 ^{ef}
LM	6600 ± 190 ^a	16590 ± 680 ^c	2680 ± 100 ^{bc}	8660 ± 190 ^c	130 ± 10 ^{ef}
M	5370 ± 520 ^{bc}	11750 ± 1360 ^{cd}	2020 ± 210 ^{de}	8840 ± 550 ^c	250 ± 30 ^{bcd}
MD	3810 ± 170 ^d	7610 ± 370 ^{ef}	1700 ± 40 ^{ef}	7160 ± 180 ^d	300 ± 10 ^b
D	2170 ± 180 ^e	3370 ± 380 ^g	1070 ± 180 ^{gh}	4410 ± 400 ^e	480 ± 10 ^a

741 Values in the same column followed by different letters differ significantly at $p < 0.05$ level.

742

743 **Table 3.** Reducing capacity (FC, FRAP) and radical scavenging activity (ABTS, DPPH) of green
744 (G) and differently roasted (L = light; LM = light-medium; M = medium; MD = medium-dark; D =
745 dark) Arabica and Robusta coffee samples.

Roasting degree	FC (mg gallic acid/100 g)	FRAP (mg trolox/100 g)	ABTS (mg trolox/100 g)	DPPH IC ₅₀ (mg/mL) *
Arabica				
G	2400 ± 80 ^e	5030 ± 270 ^f	3690 ± 230 ^f	40 ± 3 ^a
L	5890 ± 860 ^b	8490 ± 880 ^e	5500 ± 250 ^e	24 ± 1 ^{bc}
LM	5770 ± 510 ^b	10170 ± 660 ^{de}	6100 ± 200 ^{de}	23 ± 1 ^{bc}
M	4470 ± 260 ^d	10170 ± 290 ^{de}	6770 ± 340 ^{cd}	22 ± 1 ^{cd}
MD	4280 ± 520 ^d	9800 ± 280 ^{de}	7010 ± 280 ^c	21 ± 1 ^{cd}
D	3100 ± 400 ^e	8530 ± 320 ^e	6680 ± 80 ^{cd}	27 ± 2 ^b
Robusta				
G	2390 ± 190 ^e	4250 ± 420 ^f	4400 ± 410 ^f	40 ± 3 ^a
L	6950 ± 900 ^a	13040 ± 1870 ^{ab}	8150 ± 910 ^b	14 ± 4 ^f
LM	6940 ± 1050 ^a	14440 ± 1210 ^a	9080 ± 320 ^a	17 ± 3 ^{def}
M	6740 ± 1340 ^a	13270 ± 620 ^{ab}	9080 ± 260 ^a	17 ± 2 ^{de}
MD	5300 ± 490 ^{bc}	12150 ± 690 ^{bc}	8910 ± 120 ^a	17 ± 2 ^e
D	4580 ± 290 ^{cd}	10710 ± 1450 ^{cd}	8000 ± 690 ^b	22 ± 1 ^c

746 Values in the same column followed by different letters differ significantly at $p < 0.05$ level.

747 * IC₅₀ represents the concentration of coffee able to inhibit 50% of the radical solution, low values
748 correspond to a high antioxidant activity and vice versa.

749

750 **Table 4.** Correlation matrix of acrylamide, reducing capacity (FC, FRAP), radical scavenging
751 activity (ABTS, DPPH), chlorogenic acids (CQAs tot), trigonelline and nicotinic acid values of
752 Arabica and Robusta coffee samples roasted at all considered roasting degrees.

	Acrylamide	FC	FRAP	ABTS	DPPH	CQAs tot	Trigonelline
FC	<u>0.834</u>	-	-	-	-	-	-
FRAP	<u>0.646</u>	<u>0.883</u>	-	-	-	-	-
ABTS	0.421	<u>0.737</u>	<u>0.946</u>	-	-	-	-
DPPH	<u>-0.618</u>	<u>-0.869</u>	<u>-0.944</u>	<u>-0.889</u>	-	-	-

CQAs tot	0.482	0.205	-0.154	-0.347	0.213	-	-
Trigonelline	0.258	0.123	-0.202	-0.464	0.198	<u>0.688</u>	-
Nicotinic acid	<u>-0.648</u>	-0.394	-0.111	0.147	0.153	<u>-0.601</u>	<u>-0.659</u>

753 Pearson correlation coefficient (r): $0.6 \leq r \leq 1$ = positive linear correlation, $-1 \leq r \leq -0.6$ = negative
754 linear correlation and $-0.6 < r < 0.6$ = no correlation.

755

756 **Author statement**

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764

765 **Highlights**

- 766 • The risks/benefits induced by roasting thermal process in coffee were evaluated.
- 767 • Acrylamide and antioxidant activity decreased during prolonged roasting process.
- 768 • Robusta samples showed the highest acrylamide and antioxidant compounds content.
- 769 • Antioxidant levels are related to degradation/formation of beneficial compounds.

770