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

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Abstract

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

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

Keywords

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

Chemical compounds studied in this article

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

1. Introduction

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

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

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

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

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

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

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

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

2.2 Chemicals and reagents

AA (for molecular biology, ≥99% (HPLC), C_3H_5NO, molecular weight 71.08 g/mol, CAS No 79-06-1), 2,3,3-d_3-acrylamide (AA-d_3) standard solution, 500 mg/mL in acetonitrile (analytical standard, CAS 122775-19-3), L-asparagine (≥98% (HPLC), $C_4H_8N_2O_3$, molecular weight 132.12 g/mol, CAS No 70-47-3), D-(–)-fructose (≥99%, $C_6H_{12}O_6$, molecular weight 180.16 g/mol, CAS No 57-48-7), D-(+)-glucose (analytical standard, $C_6H_{12}O_6$, molecular weight 180.16 g/mol, CAS No 50-99-7) and sucrose (BioUltra, for molecular biology, ≥99.5% (HPLC), $C_{12}H_{22}O_{11}$, molecular weight 342.30 g/mol, CAS No 57-50-1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical standards of 3-*O*-caffeoylquinic acid (3-CQA), 5-*O*-caffeoylquinic acid (5-CQA) and 3,5-*O*-dicaffeoylquinic acid (3,5-diCQA), trigonelline, caffeine and nicotinic acid were also purchased from Sigma-Aldrich. Individual stock solutions of AA, glucose, fructose, sucrose and asparagine were prepared by dissolving the pure standard compounds in water, at a concentration of

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

Folin-Ciocâlteu reagent, sodium carbonate (Na_2CO_3), gallic acid ($C_7H_6O_5$), TPTZ (2,4,6-tri(2-pyridyl)-S-triazine), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), sodium acetate ($C_2H_3O_2Na$), acetic acid ($C_2H_4O_2$), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), potassium persulfate ($K_2S_2O_8$), disodium phosphate (Na_2HPO_4), monopotassium phosphate (KH_2PO_4), sodium acetate ($C_2H_3O_2Na$) and ethanol (C_2H_5OH) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Hydrogen chloride (HCl), potassium chloride (KCl), acetic acid (CH_3COOH), sodium hydroxide (NaOH) and glycerine ($C_3H_8O_3$) were acquired from Carlo Erba reagents (Milan, Italy). DPPH (2,2-

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

2.3 Physicochemical analysis

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

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

2.4 Analyte extraction and sample clean-up

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

previous procedure was followed (Andrzejewski et al., 2004) with some adjustments. 1 g of coffee powder was spiked with 0.4 mL (500 ng/mL) of AA-d_3 internal standard and the sample was diluted with 9.6 mL of water. The extraction of monitored molecules was performed at controlled temperature (80 °C) for 30 min, under magnetic stirring. Then, the sample was centrifuged at 5,000 rpm (3,661 g) for 10 min and the supernatant was collected and stored at 4 °C until use.

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

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

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

The analysis of the three sugars, asparagine and AA has been developed taking the cue from previous procedure (Schouten, Genovese, et al., 2020). The present method has been implemented by using isotopically labelled internal standard (ILIS) and adding the analysis of sucrose. All AA precursors such as asparagine, sucrose, glucose, and fructose were monitored in green coffee while AA in green and roasted beans. HPLC-MS/MS studies were performed using an Agilent 1290 Infinity series and a Triple Quadrupole 6420 from Agilent Technology (Santa Clara, CA, USA) equipped with an electrospray ionization (ESI) source operating in positive ionization mode. The HPLC-MS/MS parameters of sucrose and AA-d_3 were optimized in flow injection analysis (FIA) (1 µL of a 10 mg/L individual standard solution) by using optimizer software (Agilent) while the other HPLC-MS/MS parameters were applied according to a previous method (Schouten, Genovese, et al., 2020). The separation of target compounds was achieved on a Kinetex Hilic analytical column (100 mm × 4.6 mm i.d., particle size 2.6 µm) from Phenomenex (Torrance, CA, USA) preceded by a KrudKatcher ULTRA HPLC In-Line Filter (2.0 µm Depth Filter × 0.004 in I.D.). The mobile phase for HPLC-MS/MS analysis was composed of 15% water (A) and 85% HPLC-grade acetonitrile (B), both with 0.1% formic acid. The separation was obtained by flowing at 0.8 mL/min with this gradient elution: isocratic condition until 2.5 min (85% B), 3.5 min (70% B), 5.5 min (70% B), 6.5 min (85% B) and then constant until the end of the run (15 min). The injection volume was 2 µL. The temperature of the column was 25 °C and the temperature of the drying gas in the ionization source was 350 °C. The gas flow was 12 L/min, the nebulizer pressure was 45 psi and the capillary voltage was 4,000 V. Detection was performed in the multiple reaction monitoring (MRM) mode. The MRM peak areas were integrated for quantification and the most abundant transition was used for quantitation, and the rest of the product ions were used for qualitative confirmation. For AA quantification the response factor was measured by calculating the ratio between the area of AA and AA-d_3. The selected ion transitions and the mass spectrometer parameters are reported in **Table S1** (in **Supplementary materials**). As an example, **Figure S1** (in **Supplementary materials**) reports (**a**) the HPLC-MS/MS chromatogram of a standard mixture of

sugars, AA and asparagine plotted as overlapped multiple reaction monitoring (MRM) transitions of each analyte and (**b**) the HPLC-MS/MS chromatograms of AA and AA-d_3 of a coffee sample (Robusta light-medium roasted). The limit of quantification (LOQ) and of detection (LOD) have been calculated as 10:1 and 3:1 signal-to-noise ratio (SNR), respectively; the LOQ was 5 μg/L, while the LOD was 1 μg/L. The recovery and matrix effects have been calculated even if a previous validated procedure was followed (Andrzejewski et al., 2004). The obtained values were satisfactory since the recovery was 90 ± 5% and matrix effects were 110 ± 5%.

2.6 Caffeine, chlorogenic acids, trigonelline and nicotinic acid analysis

The quantification of caffeine, trigonelline, nicotinic acid and chlorogenic acids was performed by following two developed procedures (Caprioli et al., 2014, 2013). Briefly, the supernatant collected after water extraction was diluted 1:20 in mobile phase, filtered with 0.45 μm syringeless filter and then injected into high-performance liquid chromatography-variable wavelength detector (HPLC-VWD) system. All analytes were monitored in green and roasted coffee except caffeine which was quantified only in green beans. The system used for the analysis was a Hewlett Packard (Palo Alto, CA, USA) HP-1090 Series II, made of an autosampler and a binary solvent pump. The separation of caffeine, trigonelline and nicotinic acid was achieved on a Gemini C18 110 Å analytical column (250 × 3 mm I.D., 5 μm) from Phenomenex (Chesire, UK) using a mobile phase composed of water (A) containing 0.3% of formic acid and methanol (B), at a flow rate of 0.4 mL/min. The gradient program was: 0 min, 25% B; 0–10 min, 60% B; 10–15 min, 60% B; 15–20 min, 25% B; held at 25% until the end of the run at 25 min. The acquisition was performed at two different wavelengths in the same run: 265 nm for trigonelline and nicotinic acid and 270 nm for caffeine. The separation of chlorogenic acids was performed on a Polar-RP 80 Å analytical column (150 × 4.6 mm I.D., 4 μm) from Phenomenex (Chesire, UK) with a mobile phase constituted by water (A) and methanol (B) both with 0.1% of formic acid, at a flow rate of 1 mL/min. The elution was carried out in 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-Ciocâlteu (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 Na_2CO_3
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 $FeCl_3 \cdot 6H_2O$ (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 °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*

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

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

3. Results and discussion

3.1 Time-temperature roasting profiles and physicochemical characterization

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

In **Table 1** results of the physicochemical characteristic of all green and roasted coffee samples are 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*

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

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

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

3.3 Influence of coffee roasting degree on antioxidant properties

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

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

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

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

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

As reported in **Table 3**, no significant differences in reducing and radical scavenging activity values were found between Robusta and Arabica green samples. However, after roasting Robusta samples showed significantly higher antioxidant activity values compared to Arabica ones at each roasting degree. According to Vignoli et al. (2014), the higher antioxidant activity of roasted Robusta coffee is ascribable to its higher caffeine (alkaloid with antioxidant proprieties) content whose levels are 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 ± 30 mg/kg) than in
491 Arabica samples (23,740 ± 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 Yeretzian, 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

3.4 Coffee acrylamide content and antioxidant activity correlation

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

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

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

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

4. Conclusions

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

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

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

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Declaration of Competing Interest

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

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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 (μg/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.

asting ree	**3-CQA (mg/kg)**	**5-CQA (mg/kg)**	**3,5-diCQA (mg/kg)**	**Trigonelline (mg/kg)**	**Nicotinic acid (mg/kg)**
abica					
	4200 ± 20^{d}	24940 ± 110^{a}	2320 ± 10^{cd}	13540 ± 200^{a}	240 ± 30^{bcd}
	6610 ± 650^{a}	20890 ± 2230^{b}	1810 ± 90^{ef}	12900 ± 360^{a}	120 ± 0^{ef}
	5690 ± 490^{ab}	13360 ± 1020^{c}	1400 ± 140^{fg}	11440 ± 840^{b}	110 ± 0^{f}
	4460 ± 260^{cd}	9350 ± 710de	1090 ± 90^{gh}	10750 ± 70^{b}	190 ± 10^{def}
)	2850 ± 310^{e}	5210 ± 670^{fg}	960 ± 0^{h}	7820 ± 220^{cd}	170 ± 20^{cde}
	1980 ± 80^{e}	2940 ± 150^{g}	700 ± 10^{i}	4800 ± 380^{e}	270 ± 0^{bc}
obusta					
	2840 ± 0^{e}	21020 ± 30^{b}	3000 ± 110^{ab}	7090 ± 90^{d}	260 ± 20^{bcd}
	6310 ± 380^{a}	22480 ± 1340^{ab}	3280 ± 250^{a}	8870 ± 380^{c}	130 ± 20^{ef}
	6600 ± 190^{a}	16590 ± 680^{c}	2680 ± 100^{bc}	8660 ± 190^{c}	130 ± 10^{ef}
	5370 ± 520^{bc}	11750 ± 1360^{cd}	2020 ± 210de	8840 ± 550^{c}	250 ± 30^{bcd}
)	3810 ± 170^{d}	7610 ± 370^{ef}	1700 ± 40^{ef}	7160 ± 180^{d}	300 ± 10^{b}
	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 ± 660de	6100 ± 200de	23 ± 1^{bc}
M	4470 ± 260^{d}	10170 ± 290de	6770 ± 340^{cd}	22 ± 1^{cd}
MD	4280 ± 520^{d}	9800 ± 280de	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 ± 2de
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}

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

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

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

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

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

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

Author statement

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Highlights

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