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Original article

Acrylamide: impact of precursors concentration, origin, post-harvesting process and roasting level in high-quality arabica and Robusta coffee

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Summary Origin of coffee, precursors concentration, post-harvesting processes and commercial roasting degrees were evaluated for their impact on acrylamide content in roasted coffee. Forty Seven Specialty Arabica and 7 high-quality Robusta green coffee samples were analysed to determine sugars, asparagine and A_w. Acrylamide was quantified on light, medium and dark roasted samples. In green coffee, glucose and fructose content resulted lower after wet and honey processes, no remarkable differences were found for sucrose and asparagine. In all samples, the content of asparagine was generally lower than what provided in previous works. Acrylamide concentration never exceeded the limit of 400 µg kg^{-1} in Arabica samples and it does once in Robusta, it peaked between light and medium roasting, and it was higher in Robusta. Moreover, it was lower in honey coffee than in others. Acrylamide correlated with asparagine in Robusta, while with monosaccharides and A_w in dry and honey Arabica. Coffee Origin impacted on precursors and acrylamide.

Keywords Acrylamide, coffee, origins, post-harvesting process, roasting..

Introduction

Coffee is one of the most popular beverages in the world thanks to its sensory profile, the beneficial impact on human health of some bioactive components, and the effect played by caffeine on psychophysical performances. The roasting process, which is the most important and impactful unit operation capable for the development of antioxidant and bioactive compounds, as well as many chemicals, physical and sensory characteristics of the final cup, is also the major responsible for the creation of hazardous compounds contaminating roasted coffee as furans and acrylamide (AA) (Vezzulli *et al.*, 2021). As regards AA, EFSA reported that this contaminant could increase the risk of developing cancer in all age groups (EFSA, 2015). Following the EFSA opinion, the European Commission published the Regulation (EU) 2017/2158, establishing mitigation measures and benchmark levels for the reduction in the AA presence; the benchmark level for coffee was fixed at 400 µg kg^{-1} (Commission Regulation EU, 2017). AA is mainly formed during heat processing (T > 120°C, optimal range 170–190°C) and it results prevalently by specific pathways of Maillard reactions, particularly from the reaction of reducing sugars with asparagine forming N-glycosylasparagine, an adduct that leads to more stable decarboxylated Schiff base. The Schiff base may directly decompose into AA and an imine or, after hydrolysis, it forms carbonyl compounds and 3-aminopropionamide; this last compound may also originate AA after the elimination of an ammonia group (Pedreschi *et al.*, 2014). Sucrose, the main sugar in green coffee, can also contribute to AA formation, since in the early stages of roasting, it decomposes to reducing monosaccharides. Other minor pathways of formation, starting from 5-hydroxymethylfurfural (a substance generated during roasting), acrolein and acrylic acid (deriving from fats and amino acids, respectively) together with ammonia and asparagine, can contribute to the final acrylamide content in coffee (Hamzalıoğlu & Gökmen, 2020).

Through all the coffee chain, from the different stages of selection and post-harvesting processes till the roasting, the final AA level can be decreased by specific good manufacture practices (GMP) that can provide with a reduction in precursors level. After harvest and selection

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of coffee fruits, three types of post-harvesting process (dry, wet and honey) can be alternatively applied (Vincent, 1987), with the common aim of extracting green beans from coffee fruits. Briefly, the dry process is characterised by the direct drying of the entire cherries under sunlight or in mechanical dryers. After a cleaning step to remove leaves, stones, hydraulic separation of overripe and unripe drupes from properly ripe, cleaned fruits are spread on patios or African beds (tables made of mesh), to allow irradiation by sun and air to circulate all around the fruits. After a variable period of 10–20 days, a hulling machinery will remove the dry skin and the parchment before green coffee sorting and shipping. Opposite, wet process starts, after fruits sorting by flotation, with the pulping of coffee fruits by mechanical pulper that removes skins and the majority of the mesocarp. As for natural process, unripe drupes are processed separately because of the higher energy needed to them be squeezed. After a fermentation period, the mucilage layer covering the parchment is soluble so that it can be easily washed. Lastly, green beans in parchment are dried under sunlight or in mechanical driers then hulled and sorted to be sold. As well known, the wet process reduces the concentration of reducing sugars in green beans (Knopp *et al.*, 2006). Finally, the honey (semi-dry) process is an intermediate treatment between two already mentioned: in this case, cherries pass through the pulper that leaves on seeds in parchment a variable quantity of mucilage, depending on farmer goals. This time the polysaccharidic layer is not fermented as in wet process but dried and removed in a dehuller with parchment.

All that considered, the objective of this work was to investigate if, besides the roasting level, the precursors concentration, specie, variety, geographical origin of green coffee and the applied post-harvesting process could influence the AA formation. To improve the consistency of sampling and samples information, together with a certified quality of green coffee, Specialty Arabica and high-quality Robusta coffees, coming from the main producer countries, were collected by a local roaster. The samples were analysed for determination of the AA precursors and, after roasting at three different levels, for AA quantification. Then, all the results were processed to evaluate whether the data on green coffee features showed correlations with AA levels in the roasted products. Then, it was also investigated whether AA mitigation in roasted coffee might be obtain by appropriate selection of green coffee lots.

Materials and methods

Sampling

A total of 54 green coffee samples, both Arabica (47 samples from Brazil, Burundi, Colombia, Costa Rica, Democratic Republic of Congo, Dominican Republic, El Salvador, Ethiopia, Guatemala, Haiti, Honduras, India, Indonesia, Kenya, Peru, Republic of Panama, Rwanda and Uganda) and Robusta (7 samples from Brazil, India, Indonesia, Uganda and Vietnam) were drawn (1 kg) from GrainPro bags of 60 kg each (S1, Supplementary Materials). All Arabica coffees were recognised as 'Specialty' or 'Premium' coffee according to the protocol established by the Specialty Coffee Association. To obtain this certification, they must have at least a cupping score of 80/100 and beans must be almost free of defects (no sour beans, foreign matter, or insect/fungus damage and only a small number of unripe or broken beans are allowed). Robusta coffee samples were selected among the coffee reaching the higher quality standard for each country (Supremo, 2021) considered in the study. Coffee samples were from 35 different varieties, representing the wide diversity of coffee on the market. Regarding the post-harvest processes, 15 Arabica and 4 Robusta samples were subjected to dry, 6 Arabica to honey and 26 Arabica and 3 Robusta to wet process.

Frozen green coffee samples (500 g) were milled using a cyclone hammer mill (1 mm sieve, Pulverisette, Fritsch GmbH, Idar-Oberstein, Germany) and homogenised. After milling and homogenisation, an aliquot (300 g) of the sample was taken and stored at −20°C until the time of analysis.

Roasting

The sample roaster IKAWA Model V2-PRO (IKAWA Ltd., UK, 2018) was used to roast the coffee samples. In this equipment, a fan draw air into a heating element and then into the roaster so that beans are agitated and roasted evenly. Chaff is removed by a cyclone system and fell into a collection jar, to separate it from the seeds. Each roasting batch was of 50 g (± 0.5 g). Three roasting profiles, namely 'light' (I roast), 'medium' (II roast) and 'dark' (III roast), were applied reaching commercial roasting levels (Vezzulli *et al.*, 2021). No adjustment was applied to roasting profile based on the differences in specie and post-harvesting processes to reduce the variability deriving from the treatment. Chamber was preheated at 174–175°C prior to coffee inlet, 'light' roasting ended at 205°C in 5.46 min, 'medium' at 210°C in 6.16 min and the 'dark' one at 215°C in 6.46 min. Moving from I roast to the II roast and finally to III roast level, there was every time an increase in 5°C and 30 s between one and the other. The temperatures were measured using the probe present in the roasting chamber. After roasting, all samples were stored at −20°C. Before the analyses, frozen seeds were ground using the Moulinex blender (Model AR110830), paying attention not to heat the beans during the milling. The I roast was

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replicated keeping the same roasting conditions, to confirm the repeatability of the process.

Moisture and A_w determination

Moisture was determined by gravimetric method after evaporation at 105°C for 24 h. Water activity (A_w) was measured using AquaLab Pre (Meter Food, Pullman, WA, USA).

Reagents and standards

The solvents and chemicals used for the extraction and clean-up were ACS grade or equivalent (Carlo Erba, Milan, Italy); deionised water was purified through a Milli-Q treatment system (Millipore, Bedford, MA, USA). Solvents and formic acid used for LC–MS/MS analysis were HPLC grade (Merck, Darmstadt, Germany). Deuterium labelled d_3-acrylamide (AA-d_3; internal standard) standard solution, AA, fructose, glucose, sucrose and asparagine were obtained from Sigma-Aldrich (St. Louis, MO, USA). AA stock and working standard solutions were prepared as reported in our previous work (Bertuzzi *et al.*, 2017). Sugars standard solutions were prepared at concentration from 0.2 to 4 mg l^{-1}; asparagine standard solutions from 8 to 160 μg kg^{-1}. All the solutions were stored at −20°C when not in use.

Low molecular sugar determination

Sugars were extracted from 0.5 g of ground green coffee into a centrifuge tube with 50 mL of 80% (v/v) ethanol for 10 min in an ultrasonic bath at 80°C, according to Bertuzzi *et al.* (2020); after centrifugation (3500 *g*, 5 min), the extract was diluted (0.5 + 9.5 v/v) using H_2O:CH_3CN = 25 + 75 (v/v). Quantification was performed by LC–MS/MS. The LC–MS/MS system consisted of a LC 1.4 Surveyor pump (Thermo Fisher Scientific, San Jose, CA, USA), a PAL 1.3.1 sampling system (CTC Analytics AG, Zwingen, Switzerland) and a Quantum Discovery Max triple quadrupole mass spectrometer; the system was controlled by an Excalibur 1.4 software (Thermo Fisher Scientific). Chromatographic separation was obtained using an Xbridge BEH Amide column (2.5 μm particle size, 100 × 2.1 mm i.d., Waters Corporation, MA, USA) and a gradient elution 8 mM ammonium formate—CH_3CN. The linear gradient was from 25% to 55% ammonium formate within 5 min, isocratic for 6 min and conditioning of the column for 7 min. The flow rate was 0.2 mL min^{-1}. For fructose and glucose, the ionisation was performed in negative mode (ESI interface), considering the $[M + HCOO]^-$ ion (225 *m/z*), while for sucrose in positive mode, considering the $[M + Na]^+$ ion (365 *m/z*). The fragment ions were 90, 113 and 179 *m/z* for fructose, 90, 119 and 179 *m/z* for glucose, 185 and 203 *m/z* for sucrose. For all sugars, the limit of detection (LOD) and of quantification (LOQ) were 100 and 300 mg kg^{-1}, respectively.

Free asparagine determination

Asparagine was quantified by LC–MS/MS (Thermo Fisher Scientific) as reported by Bertuzzi *et al.* (2018); briefly, after extraction from 2 g of green coffee using 50 mL 0.01 M formic acid for 40 min and dilution (1 + 9 v/v) with H_2O:CH_3CN = 90:10 (v/v), asparagine was separated using a X-Select HSS T3 column (2.5 μm particle size,150 × 2.1 mm i.d., Waters Corporation) and a gradient elution H_2O-CH_3CN (both acidified with 0.2% formic acid; pH = 2.6). The gradient program was 100% acidified H_2O for 2.5 min.; linear gradient to 15% acidified CH_3CN within 0.5 min, then isocratic for 1 min; conditioning of the column 7 min. The flow rate was 0.2 mL min^{-1}. The ionisation was performed in positive mode (ESI interface) and the fragment ions were 116, 87 and 74 *m/z*. The LOD and the LOQ were 0.5 and 1.5 mg kg^{-1}, respectively.

Acrylamide determination

Acrylamide quantification was performed following the method by Bertuzzi *et al.* (2017). Briefly, an aliquot of sample (2 g) was weighed into a centrifuge vial, 20 mL of Milli-Q water, 5 mL of hexane, 1 mL of Carrez I and 1 mL of Carrez II solutions were added and the mixture was agitated using a rotary-shaking stirrer for 45 min. After centrifugation (4500 *g* for 10 min), 10 g of $MgSO_4$, 1 g of NaCl and 10 mL of CH_3CN were added to 5 mL of aqueous phase. The vial was shaken for 5 min, centrifuged again at 4500 *g* for 10 min and 3 mL of the organic phase were transferred in a vial together with 150 mg of basic Al_2O_3, hand shaken and centrifuged for 3 min. Finally, an aliquot of the organic phase (1 mL) was purified on the column OASIS HLB column (60 mg, Waters Corporation, Bedford, MA, USA) previously conditioned with 3 mL of H_2O and 3 mL of CH_3CN; AA was collected into a vial, adding 1 mL of CH_3CN to complete the elution. The liquid was almost completely evaporated using a gentle stream of nitrogen and the residue was immediately re-dissolved in 1 mL of CH_3CN:formic acid 0.2% (v/v) aqueous solution 10:90 (v/v). An aliquot of 100 μL of a deuterium-labelled AA (AA-d_3) internal standard solution (1 mg l^{-1}) was added to 900 μL of the extract; then, 20 μL were injected into the LC–MS/MS (Thermo-Fisher Scientific) in positive mode. Chromatographic analysis was performed using a X-Select HSS T3 column (2.5 μm particle size, 150 × 2.1 mm i.d., Waters Corporation) and a

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gradient elution H_2O-CH_3CN (both acidified with 0.2% formic acid). The gradient program was 100% acidified H_2O for 3 min.; linear gradient to 15% acidified CH_3CN within 5 min, then isocratic for 2 min; conditioning of the column for 7 min. The fragment ions were: 55 and 44 m/z for AA, 58 m/z for AA-d_3. The LOD and the LOQ were 5 and 15 μg kg^{-1}, respectively. All the results were corrected for the recovery (83.2%). The trueness of the method was evaluated by AA analysis in a FAPAS coffee reference material with reference value of 638 μg kg^{-1} (expanded uncertainty U = 109 μg kg^{-1}). The average concentration of three replicates, corrected for the recovery, was 658 μg kg^{-1} with a standard deviation of 6.3%. To confirm these findings, 30% of samples for each roasting level was analysed in duplicate.

Statistical analysis

Statistical analysis of AA precursors and AA concentration data was carried out using package IBM SPSS statistics (ver. 27, Inc., Chicago, IL, USA). Homogeneity of variance was checked. T-test was conducted to evaluate whether significant differences was present between wet and dry Robusta coffee and discrimination between set of data from Ethiopian and Southern American coffees. One-way ANOVA and discriminant analysis were applied to evaluate significant differences between the three Arabica subgroups (dry, honey and wet) and Robusta. The Waller Duncan post hoc test was applied in homoscedastic sets (AA concentration in Arabica Coffee) while Games-Howell in heteroscedastic (AA precursors in Arabica Coffee). The relationship between AA precursors in green coffee and AA was evaluated with Excel linear regression model.

Results and discussion

AA precursors in green coffee

In Supplementary materials (S2 and S3) moisture, A_w and the AA precursors (Sucrose, Fructose Glucose and Asparagine) concentrations in green coffee are shown. A_w and moisture showed no significant differences depending on the origin, species and post harvesting process. On the contrary and as expected from what provided by several works (Illy & Viani, 2005), the concentration of sugars and asparagine in Specialty Arabica and high-quality commercial Robusta coffees revealed some important differences.

As regards Arabica coffee, glucose and fructose levels are strongly affected by the post-harvest processing, confirming and substantiating the findings of Knopp *et al.* (2006), Kleinwächter & Selmar (2009) and Amalia *et al.* (2021). Although the initial level of the two monosaccharides in coffee fruits was not known, Waller Duncan's test provided with significant differences between their concentration in dry and the two other processes (Table 1). In detail, glucose and fructose content in dry processed beans (15 samples) is higher ($P < 0.01$) than honey (6 samples) and wet one (26 samples). These findings also support what provided by (Tarzia *et al.*, 2010) in the respect of the amount of extractable saccharides in coffee obtained by different post-harvesting processes: natural process is characterised by an higher time of contact between coffee seeds and whole pulps—in comparison with honey and wet process—and by the absence of fermentation that result in the scenario mentioned above. Even if a larger number of data are required to provide with consistent results, with a preliminary analysis no differences was observed between dry ($n = 4$) and wet ($n = 3$) Robusta coffee samples.

In respect of sucrose, even if it is not a direct AA precursor being a no reducing sugar, many studies demonstrated it has a role in the AA formation. In particular, Oosterveld *et al.* (2003) evaluated the carbohydrate composition of extracts obtained from roasted coffee beans and proved that most of the sucrose was converted into sugar degradation products, even at mild roasting conditions. Stadler *et al.* (2002) found that AA could be formed by the pyrolysis of asparagine and other amino acids with an equimolar amount of fructose, galactose, lactose or sucrose, giving comparable yields in terms of AA. As reported in previous works, sucrose in Arabica coffee is not affected by the post-harvesting processing (Joët *et al.*, 2009); (Kleinwächter & Selmar, 2009); (Knopp *et al.*, 2006). Our data on Arabica coffee confirmed these findings; the slightly lower average content found for dry processed beans is probably attributable to sample variability rather than post-harvesting process, together with level of cherry ripening, required to be higher for fruits to be submitted to natural process, that is directly related with the degree of sugar depolymerisation. As already known, sucrose was markedly lower in Robusta coffee.

Asparagine levels was not affected by the post-harvest processing in Arabica and Robusta coffee. Robusta showed higher asparagine levels, confirming what already provided in several works (Bagdonaite *et al.*, 2008); (Lantz *et al.*, 2006); (Murkovic & Derler, 2006). From our data, the level of asparagine in the green coffee samples was always below 400 and 500 μg kg^{-1} in Arabica and Robusta, respectively, and the mean content was often lower than those provided by other studies. Several authors proved that unripe and defective beans had normally a higher asparagine content (Dias *et al.*, 2011; Schouten, Tappi, & Romani, 2020), then the low level of asparagine detected in our samples can be a confirm of the high-

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Table 1 Average sucrose (g kg^{-1}), glucose (mg kg^{-1}), fructose (mg kg^{-1}) and asparagine (mg kg^{-1}) content in dry, honey, wet Arabica and Robusta green coffee

	Dry Arabica	Honey Arabica	Wet Arabica	Robusta
Sucrose (g kg^{-1})	65.1 ± 4.6^{A}	71.6 ± 4.3^{B}	70.8 ± 7.7^{B}	32.6 ± 2.0
Glucose (mg kg^{-1})	1760.8 ± 636.2^{B}	707.9 ± 563.7^{A}	515.9 ± 270.6^{A}	941 ± 291.1
Fructose (mg kg^{-1})	3472.2 ± 1437.4^{B}	814.6 ± 755.6^{A}	560.9 ± 408.8^{A}	1093 ± 305.0
Asparagine (mg kg^{-1})	231.0 ± 35.0^{a}	242.0 ± 55.5^{a}	246.4 ± 61.9^{a}	365.6 ± 99.1

^{A,B}Letters in superscript provides with significant differences from Games-Howell (uppercase) and Waller Duncan's (lowercase) test among Arabica coffees on the same line.

quality of Arabica 'Specialty' and high-quality Robusta coffees. Indeed, the certification is released only whether defective beans are not present or at a very low level (<10 beans/300 g green coffee).

Finally, discriminant analysis was conducted considering all AA precursors concentrations: besides a discrimination between Robusta and Arabica samples, these last were subclustered in dry group and wet and honey one (Fig. 1).

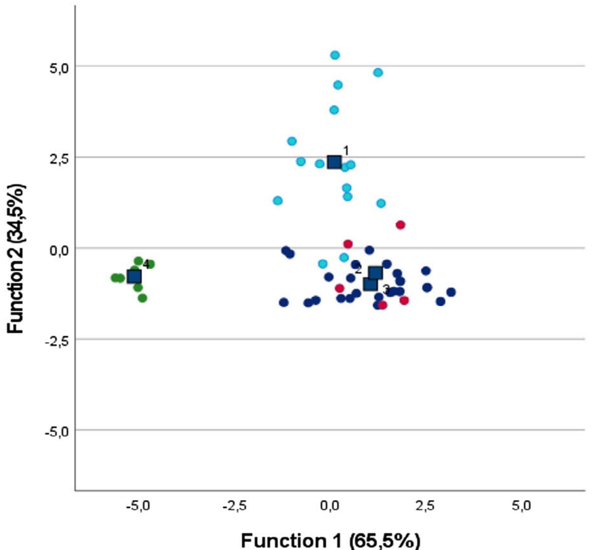
AA content and correlations with its precursors

The concentration of AA was analysed after coffee was submitted to three commercial roasting profiles (I, II and III roast). AA levels of I roast and its replicate were similar; then, no replicates were performed for II and III roast. Regarding AA analysis, no remarkable difference was found between the replicates. AA concentration levels for each sample at different roasting degree are reported in Supplementary materials S4 and S5.

Results confirmed that AA, during roasting, reached a maximum and then degraded (Table 2), as already demonstrated by many authors (Schouten, Tappi, Angeloni, *et al.*, 2020). In our work, at light roasting degree, about half of dry and wet Arabica and all Robusta samples reached the maximum contamination. The other half of dry and wet Arabica showed higher levels at the medium roasting degree, as most of the honey Arabica samples did. The III roast (dark) caused a decrease in AA concentration for all the samples, independently from Origin and processes, confirming the pathway of AA decomposition increasing the roasting level (Bagdonaite *et al.*, 2008; Schouten, Tappi, & Romani, 2020).

Usually, to a light roasting degree corresponds a higher AA level because the process ends when the pathway of formation is favoured in the respect to degradation one. However, this is not a general trend, because roasting curve parameters are not standardised, and coffee behave is different due to the species, the chemical composition and the post-harvest processing.

Independently from the roasting degree, honey Arabica always presented the lowest mean AA levels: after I and III roast, AA level of honey Arabica was



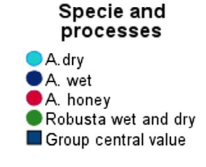


Figure 1 Discriminant analysis based on species and post-harvesting processes.

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Table 2 Average acrylamide content (μg kg^{-1}) in dry, honey, wet Arabica and Robusta coffee at three different roasting levels

	Dry Arabica	Honey Arabica	Wet Arabica	Robusta
AA I roast (μg kg^{-1})	212.1 ± 70.6^{b}	148.2 ± 48.0^{a}	193.3 ± 56.6$^{a.b}$	300.4 ± 97.4
AA I roast-replicate (μg kg^{-1})	212.8 ± 87.6^{b}	130.3 ± 53.5^{a}	196.0 ± 81.5$^{a.b}$	309.5 ± 188.8
AA II roast (μg kg^{-1})	197.9 ± 45.6$^{.b}$	159.0 ± 34.8^{a}	199.2 ± 38.2$^{.b}$	229.9 ± 68.4
AA III roast (μg kg^{-1})	150.7 ± 32.8^{b}	104.2 ± 28.3^{a}	134.3 ± 40.4$^{a.b}$	213.5 ± 78.9

$^{a.b}$Letters in superscript provides with significant differences from Waller Duncan's test among Arabica coffees on the same line.

significantly ($P < 0.05$) lower than that of dry Arabica and after II roast it was lower ($P < 0.05$) than both other two processes. This fact can be explained considering the processing flow that characterises honey coffee: together with the removal of exocarp also the outer layers of the mesocarp are mechanically removed before drying (Illy & Viani, 2005), resulting in a lower reducing sugar concentration in green beans once compared with dry process. Additionally, to get depulped coffee with a consistent amount of mucilage left on the parchment, drupes must be all homogeneously ripe, that results in a very low probability to have underripe seeds rich in Asparagine (Dias *et al.*, 2011;)– more suitable for wet one. Even if the statistics was not strict due to high difference in samples abundance (47 *vs* 7), Robusta was always the most contaminated, confirming previous data from the literature (Table 2).

In general, AA concentration found in our samples never exceeded the benchmark level of 400 μg kg^{-1} fixed by Commission Regulation (2017) in Arabica samples and it does once in Robusta (average value 471.5 μg kg^{-1}). AA levels were lower than those presented by the EFSA Scientific opinion on AA in food of 2015 (the mean level, independent from the roasting degree, is 185 μg kg^{-1} *vs* 249 μg kg^{-1}). Additionally, more recent scientific works (Bertuzzi *et al.*, 2017; Schouten, Tappi, Angeloni, *et al.*, 2020; Várady *et al.*, 2021) provided with AA higher than ours, except for the study by (Lachenmeier *et al.*, 2018) which reported comparable data. However, the number of samples involved in this study was less representative (only 2 samples).

In our whole sample set, no correlation was found between AA and its precursors in green coffee. However, a linear correlation was found for dry Arabica coffees between AA level at I roast and the sum of glucose and fructose by A_w value in green coffee ($r = 0.7579$; $n = 15$, Fig. 2), following the equation Acrylamide = 0.0434[(fructose+glucose)*A_w] + 82.27. Just mentioned correlation was also verified including honey Arabica coffees in the model ($r = 0.7812$, $n = 21$) and, despite the small number of samples available (6), it persists in honey subcluster processed at II roast ($r = 0.9027$; $n = 6$, Fig. 3) thanks to the AA increase from I to II roast. Even never reported before in coffee, A_w value improved the significance of our correlation; its impact on AA formation can be justified by its role as an enhancer of

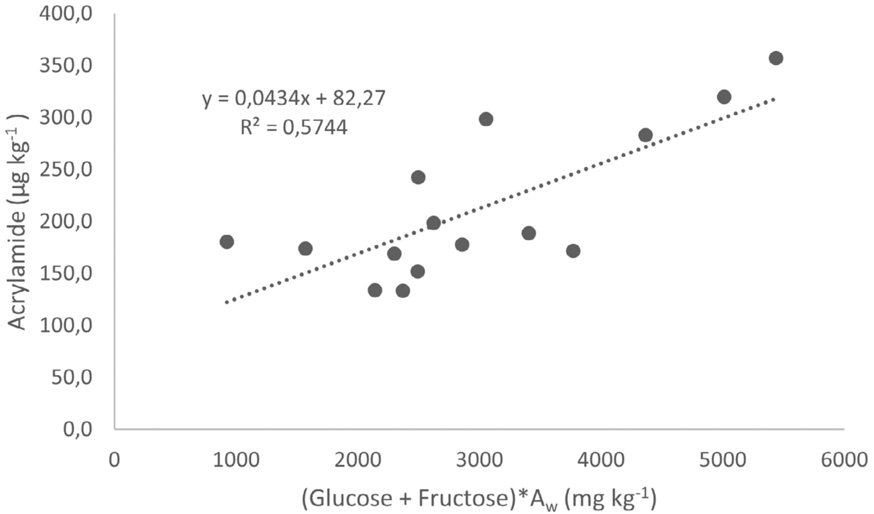

Figure 2 Relationship between (Glucose + Fructose)*Aw (mg kg^{-1}) in Arabica dry green coffees and AA level of I roast (μg kg^{-1}).

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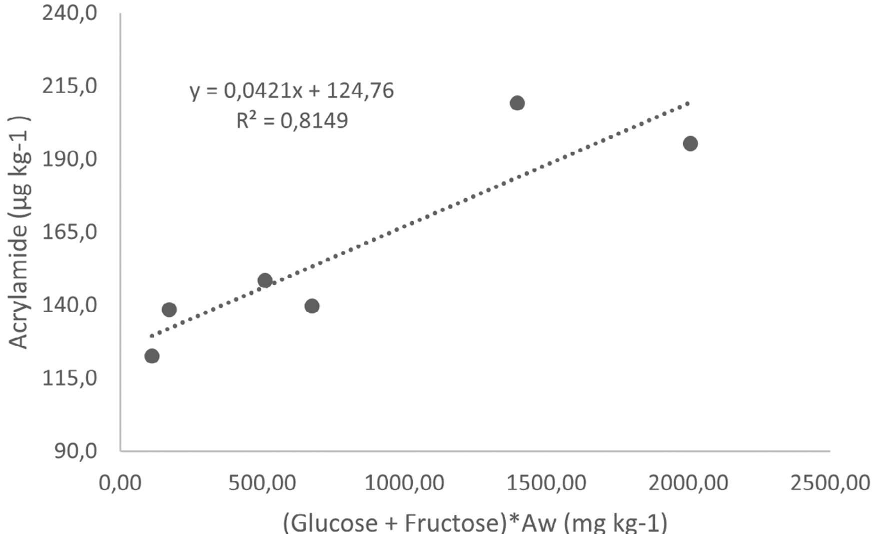

Figure 3 Relationship between (Glucose + Fructose)*Aw (mg kg^{-1}) in Arabica honey green coffees and AA level of II roast (μg kg^{-1}).

Maillard reaction rate when ranging values between 0.6 and 0.8 (de Vleeschouwer *et al.*, 2007). No significant improvement in correlation (r = 0.730;) was found including asparagine concentration in our equation, proving that low levels of this amino acid did not affect AA formation.

On the contrary, in Robusta coffees asparagine correlated with AA values at light roast (r = 0.8269), while no correlation was found with sugars concentration.

As expected, no correlations were identified for III roast, confirming the hypothesis of Lantz *et al.* (2006) which reported that correlations between green coffee precursors and AA formed in the early stages of roasting process are obscured by its reduction during the following stages. The same authors reported a correlation between asparagine and AA (R^2 = 0.5602) in 20 commercial coffee samples (15 Arabica and 5 Robusta), while glucose levels in the green coffees did not show correlation with AA. Similarly, Bagdonaite *et al.* (2008) reported that an increased content of asparagine resulted in a higher AA level.

All that considered, it is possible to conclude that the method of production of Specialty Arabica and high-quality Robusta coffees, including selective picking and appropriate sorting of the drupes, permitting to process only ripe and not defective drupes, resulted a global low AA level after roasting.

Impact of origin on AA formation and its precursors

From our data (Table 3), significant higher levels of fructose ($P < 0.05$), glucose and sucrose ($P < 0.1$) are verifiable in samples of dry coffee from Ethiopia when compared with dry samples from Southern America (Brazil and Colombia). In particular, coffees from Ethiopia, very likely due to the high level of selection of the green beans and the higher average altitude of their plantation, are characterised by high levels of low molecular sugars, according to Worku *et al.* (2018), about 1.5 times higher than Brazilian and Colombian ones. Consequently, the AA concentration reached by Ethiopian dry coffees is significantly ($P < 0.05$) higher than the level quantified in southern Americans, both in light and medium roasted samples.

Table 3 Sucrose (g kg^{-1}), glucose (mg kg^{-1}), fructose (mg kg^{-1}), asparagine (mg kg^{-1}) and acrylamide content (μg kg^{-1}) in Ethiopian and South American dry Arabica green and roasted coffee

	Ethiopian dry	South American dry
Sucrose (g kg^{-1})*	68.0 ± 3.7	61.0 ± 3.8
Glucose (mg kg^{-1})*	2413.8 ± 417.2	1663.1 ± 863.4
Fructose (mg kg^{-1})**	5583.0 ± 357.1	3346.2 ± 1082.2
Asparagine (mg kg^{-1})	223.6 ± 16.4	228.2 ± 45.7
AA I roast average (μg kg^{-1})**	325.0 ± 37.0	180.4 ± 27.7
AA II roast (μg kg^{-1})**	262.2 ± 33.3	170.6 ± 23.5
AA III roast (μg kg^{-1})	160.6 ± 30.6	163.6 ± 23.3

*T-test significance $p < 0.10$.
**T-test significance $p < 0.05$.

Conclusions

Outcomes from this work suggest that the certification as Specialty Arabica coffees allows at getting not only a coffee with a superior sensorial quality, but also a

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safer product giving rise to less AA. On a total of 47 Arabica coffee roasted at three different levels, AA never exceeded the benchmark limit of 400 μg kg^{-1} fixed by EFSA; only 1 Robusta coffee of high quality showed a slightly higher concentration (at I roast). As regards the post-harvest process, it was confirmed that wet and honey treatments reduced the content of glucose and fructose, AA precursors; the coffee samples subjected to honey process showed a slightly lower average AA content after each roasting level. During the roasting, maximum AA level was reached at I or II roast, depending on the species, the chemical composition and the post-harvest processing. Considering the species Arabica and Robusta, higher AA levels were found in the samples belonging to Robusta, due to a higher asparagine content in green beans. Different correlations were found among AA precursors and AA content, depending on the post-harvesting process. Finally, coffee origin can also impact on AA content.

Through our findings, AA mitigation in roasted coffee might be obtain by an appropriate selection of green coffee lots, depending on easily available information.

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Author contributions

Fosca Vezzulli: Conceptualization (lead); data curation (equal); formal analysis (equal); investigation (equal); resources (lead); software (equal); validation (lead); writing – original draft (equal). **Sara Triachini:** Formal analysis (equal); methodology (equal). **Annalisa Mulazzi:** Formal analysis (equal); methodology (equal). **Milena Lambri:** Project administration (equal); resources (equal); supervision (lead). **Terenzio Bertuzzi:** Data curation (lead); formal analysis (equal); methodology (equal); visualization (equal); writing – original draft (equal).

Conflict of interest

No potential conflict of interest was reported by the authors.

Ethical statement

Ethics approval was not required for this research.

Peer review

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Data availability statement

Data available on request from the authors and in article Supplementary Materials

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supporting information.

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