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## Acrylamide in coffee: What is known and what still needs to be explored. A review

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59 a brittle, porous texture in the bean suitable for successive grinding and brewing. The high  
60 production temperature induces extensive chemical reactions, dehydration and profound  
61 changes in the microstructure (Folmer, 2017). At the same time, roasting leads to the  
62 development of undesired compounds of concern, such as AA and furans (Schouten, Tappi, &  
63 Romani, 2020).

64 Since 2002 when AA was detected in heated foods, extensive effort has been made by public  
65 research institutions and industries to investigate ways to reduce AA formation during food  
66 processing (Summa, de la Calle, Brohee, Stadler, & Anklam, 2007). However, despite the  
67 accumulated knowledge and mitigation strategies applied so far, the reduction of AA levels in  
68 coffee is still a challenge, compared to other foods (i.e. baked or fried carbohydrate-rich  
69 foods) in which wider raw-material selection and improvements in technological processes  
70 and/or changes in the recipes are possible on an industrial level. This review presents a critical  
71 analysis of the accumulated knowledge on precursors and formation pathways of AA in coffee  
72 as well as on the mitigation strategies that have been investigated to date, with particular  
73 attention being paid to current applicability in industry and the Authors' viewpoint on topics  
74 that require further exploration.

## 75 **2. AA physico-chemical characteristics**

76 AA is an odourless white crystalline solid with the molecular formula of  $C_3H_5NO$  and a  
77 molecular weight of 71.08 g/mol. Its IUPAC name is prop-2-enamide; and its synonyms are  
78 acrylic amide and ethylene carboxamide (Figure 1). Its main physico-chemical characteristics  
79 are: melting point: 84.5 °C; vapor pressure: 0.9 Pa ( $7 \times 10^{-3}$  mm Hg) at 25 °C; solubility in water:  
80 2.155 g/L, in methanol: 1.550 g/L, in ethanol: 862 g/L, in acetone: 631 g/L at 30 °C, in benzene  
81 3.46 g/L, in chloroform: 26.6 g/L; Log Kow: -0.67; Henry's law constant at 25 °C:  $1.7 \times 10^{-9}$  atm-  
82  $m^3/mol$  (ECHA-European Chemical Agency, 2021).

83 AA is stable at room temperature, but polymerizes when heated to its melting point and even  
84 when exposed to ultraviolet radiation (WHO/IPCS, 1999). AA thermally decomposes to form  
85 ammonia and carbon monoxide, carbon dioxide and nitrogen oxides (Kitahara et al., 2012;  
86 Maan et al., 2022). AA stability is quite high in aqueous solutions, but decreases under dry  
87 conditions and can be influenced by pH and the nature of the buffer (Adams, Hamdani,  
88 Lancker, Méjri, & De Kimpe, 2010). The stability of AA and its reactivity with relevant

89 nucleophiles from various foods at elevated temperatures have been studied by Adams *et al.*  
90 in model systems. Amino acids with nucleophilic side chains decrease the amount of free AA;  
91 cysteine (Cys) is the most reactive, while other less reactive nucleophiles, such as lysine (Lys),  
92 arginine (Arg), serine (Ser) and ascorbic acid gave similar condensation products (Adams,  
93 Hamdani, Lancker, Méjri, & De Kimpe, 2010). As an unsaturated carbonyl compound with  
94 electrophilic properties, AA can react, via Michael addition with biological nucleophilic groups  
95 including amines, carboxylates, aryl and alkyl hydroxyls, imidazoles and thiols of  
96 macromolecules (e.g. Cys residues), DNA and proteins. This reactivity is the basis of its toxicity  
97 (Nehlig & Cunha, 2020).

98

### 99 **3. Mechanism of acrylamide formation**

100 Coffee beans are subject to higher temperatures than other foods during roasting (range 220–  
101 250°C). Although the Maillard reaction is predominant over others and is responsible for the  
102 AA formation, under these harsh processing conditions, it can be expected to form via  
103 pathways beyond the commonly accepted asparagine/sugar (or carbonyl) condensation  
104 (Guenther, Anklam, Wenzl, & Stadler, 2007). The additional pathways for AA formation that  
105 have been studied and proposed are reported in Figure 1.

106

#### 107 **3.1 Formation via the Maillard reaction**

108 Coffee beans mainly undergo the Maillard reaction during the roasting process and this  
109 promotes the formation of AA, which results from the combination of an amino residue of  
110 asparagine (Asn) and a carbonyl group from a reducing sugar (e.g. glucose) at temperatures  
111 above 120 °C (Anese, 2016; Bagdonaite, Derler, & Murkovic, 2008; Mottram, Wedzicha &  
112 Dodson, 2002). Stable isotope-labelled experiments have shown that the backbone of the AA  
113 molecule originates from Asn (Figure 1 A) (Pedreschi, Mariotti, & Granby, 2014).

114 In contrast to fried snacks and bakery products, green coffee apparently does not contain a  
115 source of free carbonyl compounds. However, alternative reactive carbonyls derive from  
116 linoleic acid hydroperoxide degradation or from saccharide degradation at high temperature  
117 (Belitz, Grosch, 2009). These carbonyls facilitate AA formation. Sucrose was the only sugar  
118 detected, at a concentration of approximately 8.0% in green coffee, and it tends to

119 decompose during roasting within 15 min at 220°C (Kocadagli, Göncüoğlu, Hamzalıoğlu, &  
120 Gökmen, 2012).

121 A range of different carbonyl compounds are involved in acrylamide formation:  
122 hydroxycarbonyls, dicarbonyls (Amrein et al., 2004; Stadler et al., 2004; Zyzak et al., 2003) and  
123 alkadienals from lipid oxidation (Gökmen, Kocadağlı, Göncüoğlu, & Mogol, 2012; Hidalgo,  
124 Delgado, & Zamora, 2009; Kocadagli et al., 2012; Zamora & Hidalgo, 2008) have been  
125 investigated so far. Model studies have demonstrated that  $\alpha$ -hydroxy carbonyls are much  
126 more effective than  $\alpha$ -dicarbonyls in converting Asn into AA as they promote the  
127 rearrangement of azomethine ylides, which are degradation products of the Schiff base  
128 (Gökmen et al., 2012; Stadler et al., 2004). The  $\beta$ -elimination reaction of the decarboxylated  
129 Amadori compound is the subsequent step and gives AA (Stadler et al., 2004; Zyzak et al.,  
130 2003). However, Hamzalıoğlu and Gökmen have, more recently, used a multi-response kinetic  
131 modelling to show that the 3-deoxyglucosone (3-DG) was the most abundant dicarbonyl to  
132 be formed from sucrose degradation and from the Maillard reaction during roasting that  
133 participates in producing AA (Hamzalıoğlu & Gökmen, 2020). 5-Hydroxymethylfurfural (HMF),  
134 which is the major sugar-decomposition product generated during roasting, can play a role in  
135 AA formation, and can generate more AA than glucose when heated together with Asn  
136 (Anese, 2016; Kocadagli et al., 2012). HMF is formed and accumulated during the early stages  
137 of roasting due to the simultaneous consumption of sucrose. It reaches its maximum content  
138 within 10 min of roasting at 220°C and then decreases (Figure 1C) (Gökmen et al., 2012). Cai  
139 et al. (Cai et al., 2014) have reported that the addition of chlorogenic acid (0.5 and 5  $\mu\text{mol/mL}$ )  
140 to the Asn/glucose-Maillard reaction system significantly promotes AA formation, mainly by  
141 increasing HMF formation, while inhibiting its elimination. A comprehensive kinetic model,  
142 including the elementary steps for acrylamide formation, was proposed by Hamzalıoğlu *et al.*,  
143 in 2020. Changes in sucrose, reducing sugars, free amino acids, Asn, AA, 3-DG, methylglyoxal,  
144 glyoxal and HMF were monitored during coffee roasting at 200, 220 and 240 °C. The results  
145 of the multi-response kinetic modelling approach indicate that sucrose degrades into glucose  
146 and a reactive fructofuranosyl cation that principally contributed to the formation of HMF,  
147 which, in turn, was found to be the most important reactive carbonyl compound in the  
148 formation of AA in coffee during roasting. Conversely, glucose mostly takes part in the  
149 formation of intermediates, glyoxal and especially 3-DG, rather than AA. Therefore, any



150 ingredient/component that promotes HMF formation also increases AA generation. By  
151 contrast, the quinone derivative of chlorogenic acid decreases AA formation via H<sub>2</sub>O<sub>2</sub>  
152 oxidation. However, this mechanism requires further investigation (Hamzalıoğlu & Gökmen,  
153 2020).

154

### 155 **3.2 Formation via triglyceride decomposition**

156 In addition to carbohydrates, lipid oxidation products also participate in AA formation. In  
157 particular, di-unsaturated hydroperoxides and their degradation products such as the  
158  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ -diunsaturated carbonyl group promote the AA formation during food heating  
159 related processes (Zamora & Hidalgo, 2008).

160 It is also well known that lipids (triglycerides) produce a large amount of acrolein when heated  
161 (Ehling, Hengel, & Shibamoto, 2005). Acrolein is oxidized to acrylic acid, which then reacts  
162 with ammonia to generate AA.  $\alpha$ -Amino acids produce ammonia via the Strecker degradation  
163 in the presence of a carbonyl compound (Figure 1B) (Stadler, Verzeegnassi, Varga, Grigorov,  
164 Studer, Riediker, Schilter, 2003; Yasuhara, Tanaka, Hengel, & Shibamoto, 2003). Aspartic acid  
165 (Asp) can also release acrylic acid without involving sugars or a carbonyl source via a  
166 concerted decarboxylation/deamination pathway. In addition to Asn, other amino acids, such  
167 as L-alanine (Ala) and L-arginine (Arg), can also release acrylic acid at temperatures above  
168 180°C (Guenther et al., 2007).

169

### 170 **3.3 Formation via pyrolysis**

171 Lactamide and AA can be generated in the presence of ammonia in pyrolytic reactions that  
172 involve Ser and Cys through conversion, via pyruvic acid, to lactic acid (Figure 1D) (Claus,  
173 Weisz, Schieber, & Carle, 2006).

174

175

## 176 **4. Factors that affect Acrylamide levels**

177 Several factors may affect AA concentrations in coffee (Figure 2A), and these are discussed in  
178 detail below.

179

#### 180 **4.1 Acrylamide precursors in green beans: pre- and post-harvesting**

181 Coffee is a perennial tropical crop unlike other acrylamide-producing agricultural crops such  
182 as potatoes and cereals, which are annual crops and need to be sown or planted annually.  
183 One advantage of annual crops is that they can be more easily manipulated to reduce AA  
184 precursor formation by changing the variety or moving the production site. This is not feasible  
185 with perennial crops, such as coffee, as soil composition, temperature, altitude and water  
186 availability determine bean quality and, thereby, the quality of the coffee product. In addition,  
187 climate change and, in particular, increases in temperature can greatly influence production.  
188 Several strategies have been proposed to manage plantations, exploit ancient species and  
189 varieties, and create new hybrids are being investigated in order to counter climatic effects.  
190 However, these projects are, in principle, oriented towards the yield and flavour and less  
191 towards the impact they may also have on AA precursors.

192

##### 193 **4.1.1 Influence of coffee species: Arabica and Robusta**

194 According to a research group from the Royal Botanical Gardens in Kew (Davis et al., 2019),  
195 *Coffea arabica* is a vulnerable species and at risk of extinction due to deforestation and  
196 climate change. To ensure its survival, experts suggest moving crops to higher and colder  
197 areas or upgrading irrigation systems. Unfortunately, these recommendations cannot be  
198 adopted everywhere, their application depending on origin, farm size and the nature of the  
199 land. *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) are the two leading species in  
200 the coffee market. *C. arabica* grows in a narrower range of regions, compared to *C.*  
201 *canephora*, as it can be cultivated in mountainous rainforests with average annual  
202 temperatures of between 18 and 21°C and rainfall of between 1100 and 2000 mm.

203 *C. canephora* mainly grows in soils that are flat or gently sloping and are well-drained.

204 They are characterized by different levels of amino acids, sugars and minerals, volatiles,  
205 chlorogenic acids and caffeine (Guenther et al., 2007). Many studies have reported that  
206 significantly higher amounts of AA are found in Robusta than in Arabica (Esposito et al., 2020;  
207 Lachenmeier et al., 2019). This difference seems to be associated with higher Asn content in  
208 raw Robusta beans than in Arabica (Alves, Soares, Casal, Fernandes, & Oliveira, 2010;  
209 Bagdonaite et al., 2008; Lantz et al., 2006). In 2008, Bagdonaite *et al.*, investigated the

210 influence of the concentrations of possible precursors in green coffee, such as amino acids,  
211 sucrose and carbohydrates, on AA formation and concluded that higher Asn content resulted  
212 in higher AA amounts. Robusta coffee was found to contain higher levels of Asn (the  
213 concentration of free Asn was 797  $\mu\text{g/g}$  in Robusta and 486  $\mu\text{g/g}$  in Arabica) and lower  
214 amounts of sucrose, and was confirmed to have a higher AA concentration than the  
215 investigated Arabica coffee (Bagdonaite et al., 2008; Bertuzzi, Martinelli, Mulazzi, & Rastelli,  
216 2020). Hu *et al.* (Hu, Liu, Jiang, Zhang, & Zhang, 2021) have very recently shown that individual  
217 addition of free amino acids (i.e. Ala, Arg, Lys, Cys, Ser and Glycine (Gly), Phenylalanine (Phe),  
218 Tryptophan (Trp), and Glutamine (Gln)) in a model system solution heated in a hot-air roaster  
219 at 180°C for 5 min, promotes the AA formation. In addition, it has been observed a positive  
220 correlation between roasting time and AA amount. The authors speculated that the high level  
221 of AA at the early stages of roasting may also be due to the presence of other amino acids.  
222 Moreover, they observed that the addition of Gly and Asp can reduce AA formation, and  
223 proposed their addition during roasting. However, these findings contradict those of other  
224 authors (Guenther et al., 2007; Navarini, Terra, Colombari, Lonzarich, & Liverani, 2014;  
225 Yasuhara et al., 2003).

226 An investigation of the sugar fraction by Bagdonaite *et al.* (Bagdonaite et al., 2008), using a  
227 laboratory scale roaster, indicated that higher sucrose amounts lead to lower AA formation,  
228 while Stadler *et al.* (Stadler & Theurillat, 2012) reported no correlation between AA formation  
229 and reducing sugars during industrial scale roasting. Total sugars were significantly higher in  
230 the Arabica green coffee beans than in Robusta (sucrose: 7.5% in Arabica and 4.5% in Robusta  
231 (Stadler & Theurillat, 2012). Recently, Bertuzzi et al. 2020 quantified the reducing sugars  
232 during an industrial roasting process of Arabica and Robusta. The increase in reducing  
233 monosaccharides due to thermally induced hydrolysis of sucrose (i.e.  $936 \pm 78$  mg/kg and  $424$   
234  $\pm 69$  mg/kg for fructose and glucose in Arabica,  $338 \pm 41$  and  $138 \pm 19$  mg/kg for fructose and  
235 glucose in Robusta, respectively) could explain the higher AA content in their Arabica coffees  
236 (Bertuzzi et al., 2020).

237 Factors, such as cultivation conditions, coffee origin and processing, can influence the content  
238 of amino acids and free reducing sugars and, thereby, the formation of AA (Bertuzzi et al.,  
239 2020; Schouten et al., 2020).

240

#### 241 4.1.2 Influence of coffee origins

242 Coffee production is restricted to the humid tropical regions of Asia and Oceania, South  
243 America, Africa, some regions of Mexico and Central America and their respective islands (in  
244 order of productive yield). Despite the widespread of production sites, only a few studies have  
245 correlated geographical origin to AA and its precursors, while several research works have  
246 connected the different coffee species to the presence of AA, on the basis of their different  
247 chemical compositions (Alves, Soares, Casal, Fernandes, & Oliveira, 2010; Bagdonaite et al.,  
248 2008; Guenther et al., 2007; Lantz et al., 2006; Summa et al., 2007). Bagdonaite *et al.*,  
249 (Bagdonaite & Murkovic, 2004) have reported differences in AA levels in some wet-processed  
250 Arabica varieties (Columbian Excelso, Uganda Organico Biocoffee, Santos Brazil) and Robusta  
251 (Cameroon) after roasting under identical conditions. The latter was shown to contain the  
252 highest amount of AA. Among the Arabica, high quality beans (Columbian Excelso, and  
253 Uganda Organico Biocoffee) contained lower AA amounts than low quality beans (Santos  
254 Brasil). The potential effects of origin, within the same treatment and species groups (i.e. wet  
255 processed for Arabica samples and dry-processed for Robusta), can be inferred from the  
256 results of Alves *et al.*, (Alves et al., 2010). Table 1S shows the concentration of AA in the final  
257 espresso coffee product. For Robusta, higher amounts of AA were observed in coffee from  
258 African regions than from Asian samples, and there was a certain variability within the same  
259 geographical macro-area. A similar trend, although to a different extent, can be observed in  
260 Arabica samples. In 2015, Pugajeva *et al.* (Pugajeva, Jaunbergs, & Bartkevics, 2015) measured  
261 the AA content in 22 samples of roasted commercial coffee of different varieties, available in  
262 local supermarkets and labelled as monovarietal, and a variation from 166 to 503  $\mu\text{g}/\text{kg}$  was  
263 found (table 1S). However, the variability in their results does not allow any conclusions to be  
264 drawn as the pre-processing methods applied to the green beans were not known and their  
265 influence on the AA precursors can therefore not be evaluated. Origin and fertilization  
266 practices can influence AA precursors. In general, the effect of fertilization, climate and soil  
267 can be monitored via the state of the leaves, the growth rate of the trees, the development  
268 of the beans and the production yield, rather than in the chemical composition of the beans  
269 (Seal et al., 2008). To the best of the authors' knowledge, no data on the impact of agricultural  
270 practices on the amount of AA precursors in beans are available. This is currently an  
271 underexplored field.

272

#### 273 4.1.3 Post-harvest processing and AA precursors

274 Post-harvest treatments have a decisive influence on the final coffee quality and content of  
275 AA. Harvesting should take place when most of the cherries are ripe, as unripe cherries have  
276 a higher Asn content (Dias, Borém, Pereira, & Guerreiro, 2012). Cherries are harvested either  
277 by hand or mechanically (i.e. by stripping or using a vibrating ring applied to the trunk of the  
278 coffee plant) depending on the size and shape of the plantation. In general, hand picking  
279 provides harvest with riper cherries than stripping or mechanical harvesting because it makes  
280 it possible to better select the fruit, but this practice is discontinuous and costly. Cherry  
281 metabolism varies with the degree of ripeness, producing biochemical and chemical  
282 conversions that also affect the final composition of amino acids, sugars and other  
283 metabolites in the green beans, and conditioning the AA precursors (Dias, 2010; Dias et al.,  
284 2012).

285 After harvesting, coffee cherries must be separated from the skin and pulp, mucilage, and the  
286 parchment and then dried (Folmer, 2017). These processes allow the fruit to dry to a safe  
287 moisture content in order to inhibit the activity of bacteria and moulds.

288 Three main processes are possible: the dry method, the wet-process and the 'semi-washed'  
289 process. The first method, commonly named *the natural process*, consists of drying the whole  
290 fruits under the sun on raised beds or on the floor. The mucilage and skin are removed once  
291 dried. This process is mainly used for Robusta coffee.

292 The wet-process, also called the *washed process*, involves the fresh mature cherries being de-  
293 pulped, fermented and washed before drying. This process is mainly used for Arabica coffee.

294 The *semi-washed process*, also known as the honey process, involves fruit depulping and  
295 drying, and the removal of the mucilage and parchment after drying. The chemical  
296 composition changes depending on the process and provide coffees with different flavour  
297 qualities in the cup, and also have an impact on the AA precursors.

298 The coffee processing method does not significantly affect sucrose, the major disaccharide in  
299 green coffee beans (Kleinwächter & Selmar, 2010; Knopp, Bytof, & Selmar, 2006); the sucrose  
300 concentration, unlike that of glucose and fructose, is more significantly determined by pre-  
301 harvest, rather than post-harvest, factors. Only small amounts of glucose and fructose were

302 detected after wet-processing, whereas their contents were significantly higher after dry-  
303 processing.

304 Fermentation during wet-processing results in the specific consumption (and decrease) of  
305 free sugars (glucose, fructose, arabinose, galactose and mannose). During fermentation in  
306 wet-processing, the oxygen concentration in the tank drops, due to microbial action, creating  
307 anaerobic conditions that lead to alcoholic or lactic fermentation. Conversely, in dry-  
308 processing, the coffee remains in a well-aerated environment throughout treatment, during  
309 which aerobic metabolic conditions can be maintained until the reduction of the water  
310 content deactivates the metabolic activity. The anaerobic fermentation in the fruits in wet-  
311 processed coffee leads to a greater consumption of hexoses for the generation of the same  
312 number of ATP-molecules with a major decrease of glucose and fructose (Knopp et al., 2006).  
313 Changes in amino acids occur during processing, with glutamic acid (Glu) and Asp mainly being  
314 present in the untreated beans followed by Ala and Asn in order of concentration. The wet-  
315 process led to a decrease in the concentrations of Asp, Ala and Asn, while the concentration  
316 of Glu increased. In the dry-process, the concentrations of most amino acids were either  
317 similar to those in the unfermented beans or lower. Galactose also diminishes in the dry-  
318 process (Kleinwächter & Selmar, 2010; Knopp et al., 2006). Several diverse metabolic  
319 processes occur inside coffee beans during post-harvest processing, and these can alter the  
320 chemical composition of the green beans. Drying at 40°C considerably reduces the  
321 concentrations of Asn and the other amino acids, while the steam treatment of the beans  
322 influences the free and total amino acids, and accounts for a 10% decrease in protein-bound  
323 amino acids, and a 50% loss in free amino acids (Seal et al., 2008). In 2014, Navarini (Navarini  
324 et al., 2014), demonstrated that Asp, which is present in green beans in non-negligible  
325 concentrations compared to Asn, also plays a role of similar significance to that of Asn in the  
326 formation of AA during roasting. However, Asn levels, as well as those of other amino acids,  
327 were significantly lower when beans were processed using the wet method (Dias et al., 2012).  
328 The high water content in green coffee beans renders them metabolically active. The level of  
329 moisture in green beans has been investigated by Lantz *et al.*, (Lantz et al., 2006) who  
330 reported that changing their moisture, from 14 to 7%, did not affect AA formation in further  
331 processing steps. However, the article did not report data on the relationship between  
332 moisture content in green coffee beans and AA formation, and other studies on this topic are  
333 not available in the literature.













489 28% in instant coffee and coffee substitutes respectively, in storage at 25°C after 12 months,  
490 and a less significant decrease at 4°C.

491 Hoenicke *et al.* suggested, in 2005, that AA losses over time probably occur because of  
492 reactions with other components in coffee beans and powders. Reactions with compounds  
493 containing SH groups may have a significant impact on AA reduction during storage. In  
494 general, the high reactivity of AA with nucleophilic components, such as the sulfhydryl, amino  
495 and hydroxyl groups of peptides, proteins and melanoidins, might be responsible for its  
496 reduction in stored coffee. AA is therefore rather stable in foods such as cereal-based  
497 products because they do not contain sulfur derivatives (Hoenicke & Gatermann, 2005;  
498 Michalak et al., 2016). However, experiments with <sup>14</sup>C-labeled AA as a radiotracer have shown  
499 that furanthiol, which is an abundant aroma component in roasted coffee, was not involved  
500 in the formation of covalent AA adducts and thus does not substantially contribute to  
501 decreases in AA during storage. Table 2 lists the studies that are available in the literature on  
502 AA decreases in roasted coffees and coffee products during storage under different  
503 conditions.

504

## 505 **6. Brew preparation**

506 While the majority of published studies focus on the assessment of AA content in coffee  
507 beans, some researchers have also investigated the amount of AA that is effectively ingested  
508 by consumers in their coffee brews (Alves et al., 2010; Andrzejewski et al., 2004; Bagdonaite  
509 et al., 2008). AA intake through coffee beverages depends on consumption habits (type,  
510 strength and volume of beverage, and intake frequency), which are influenced by the cultural  
511 and personal preferences of consumers. Coffee is ground into powder, with the objective of  
512 increasing the surface of the interface between the water and the solid to accelerate the  
513 transfer of soluble substances into the brew (Soares, Alves, & Oliveira, 2015). AA is highly  
514 soluble in water and is thus easily transferred from the coffee powder to the beverage. Three  
515 main processes are used to prepare coffee brews (Figure 2B), decoction (boiled, Turkish,  
516 vacuum and percolation), infusion (filter or coffee drip and Neapolitan), and *pressure* (press-  
517 pot or French press, moka and espresso). Most of these methods can be identified by their  
518 geographical denomination rather than the description of the method itself and are linked to

519 local traditions. Moreover, instant coffee or soluble coffee powder, is also included in this  
520 section since it is one of the most widely consumed beverages and shares the extraction of  
521 bean components, with the only difference being that this occurs in the technological  
522 industrial brewing step before the soluble coffee powder is obtained.

523 During brew preparation, AA extraction can be affected by factors such as the temperature  
524 of the water, the time that the water is in contact with the ground coffee and the applied  
525 pressure. In any case, AA is almost completely extracted, in proportions of around 92 to 99%  
526 because of its high polarity and water solubility (Alves et al., 2010; Andrzejewski et al., 2004;  
527 Bertuzzi et al., 2017). AA levels between 6 and 16 µg/L were found in brewed coffee prepared  
528 using an electric drip coffee maker by measuring the variation when the brew was heated in  
529 the coffeepot over time. The results showed that AA is quite stable in brewed coffee, since  
530 no significant decreases in its levels were observed after 5 hours of heating (Andrzejewski et  
531 al., 2004). Similar results were also found by Alves (1.7 to 75 µg/L), Sirot (37 µg/L), and Mesías  
532 (7.7 to 40 µg/L) (Alves et al., 2010; Mesías & Morales, 2016; Sirot, Hommet, Tard, & Leblanc,  
533 2012). In 2006, Lantz *et al.* reported that espresso-coffee brewing only partially extracts AA  
534 from ground coffee due to the short contact time with water, unlike with other coffee brewing  
535 methods, such as the plunger pot and filtered coffee (Lantz et al., 2006). Alves *et al.*, (Alves et  
536 al., 2010) found that the AA extraction rate using the percolation method was very similar for  
537 both Arabica and Robusta, and that the increase in the water volume that percolates through  
538 the coffee cake is responsible for higher AA extraction, ranging from 59 to 98% for Robusta,  
539 and from 62% to 99% for Arabica, with volumes of extract ranging from 20 (Italian “ristretto”  
540 coffee) to 70 mL (Italian “lungo” coffee). Although the short contact time results in incomplete  
541 AA extraction during espresso coffee preparation, the high coffee/water ratio leads to higher  
542 AA concentration than in other coffee brews (Mesías & Morales, 2016; Soares et al., 2015).  
543 The ever-increasing success of coffee capsules has brought attention to AA contents in the  
544 resulting brews. Alves, however, did not find significant differences between espresso caps  
545 and conventional espresso (33.4–55.3 µg/L). Furthermore, they found similar AA contents in  
546 decaffeinated coffee (24.8–49.5 µg/L), confirming that the decaffeination process does not  
547 influence acrylamide precursors (Alves et al., 2010).

548 Başaran *et al.*, (Başaran, Aydın, & Kaban, 2020) analysed 41 commercial coffees that were  
549 obtained from local markets and coffee shops, and found that instant coffee contained higher

550 levels of AA, than traditional Turkish coffee and ready-to-drink (brewed) coffee. The reason  
551 for the high amount of AA in instant coffees may be due to their industrial processing, as they  
552 are brewed with pressurized liquid water at approximately 175°C. Further evaporation  
553 processes, including freeze-drying and spray-drying, concentrate the coffee components  
554 including AA (Mussatto, Machado, Martins, & Teixeira, 2011).

555 Kang *et al.*, (Kang, Lee, Davaatseren, & Chung, 2020) investigated the presence of AA in cold  
556 and hot brews; cold brews were prepared at 5°C and 20°C for 12 h using steeping and  
557 dripping, whereas hot brews were obtained at 80°C and 95°C for 5 min using the pour-over  
558 method. Cold brews showed higher levels of AA than hot brews, probably because of the  
559 relatively longer contact time with water. The brewing time and, thereby, the water/coffee  
560 ratio, the blend composition and roasting degree all significantly influence the level of AA in  
561 the final beverage. AA intake through coffee brews therefore essentially depends on  
562 consumption habits (type, strength and volume of beverage, together with intake frequency),  
563 which vary with cultural and consumer preferences. Table 3 reports literature studies on the  
564 impact of brewing techniques and conditions on AA levels in final coffee beverages.

565

## 566 **7. Acrylamide mitigation strategies**

567 To reduce AA intake, the food industry has tried to change processes and/or product  
568 parameters without compromising taste, texture and appearance of their products (Food and  
569 Drink Europe, 2019; Pedreschi *et al.*, 2014; Schouten *et al.*, 2021, 2020). Many mitigation  
570 techniques can be adopted, at different steps during coffee processing (Figure 3).

571

### 572 **7.1 Enzymatic treatment of green beans**

573 The formation of AA in coffee can be limited by two enzymatic treatments: i) with  
574 asparaginase, which catalyses the hydrolysis of Asn into Asp and ammonia via the hydrolysis  
575 of the Asn side-chain amide group (Corrêa *et al.*, 2021); and ii) with acrylamidase, which can  
576 convert AA into acrylic acid (Cha, 2013).

577 As free Asn is a limiting factor for AA formation in coffee, some authors have studied the  
578 possibility of limiting this component in green coffee using asparaginase, and thereby  
579 reducing AA formation during roasting (Mottram *et al.*, 2002). A patented enzymatic

580 treatment WO/2004/037007, that is based on the asparaginase method was revealed by The  
581 Procter & Gamble Company as a means to reduce the AA content in roasted coffee. However,  
582 the complexity of the preliminary treatments that must be performed on the green coffee  
583 beans to ensure an effective interaction between the enzyme solutions and the Asn contained  
584 in the beans is a significant drawback. Hendriksen *et al.* (Hendriksen, 2013) evaluated the  
585 effect of different doses of asparaginase on the reduction of Asn levels in green coffee. The  
586 results indicated that treating green coffee beans with low doses of asparaginase (2000–6000  
587 ASNU) produced a 70–80% decrease in Asn and a 55–74% decrease in AA after roasting. A  
588 major obstacle here is ensuring the homogeneous distribution of the active enzyme over the  
589 entire substrate.

590 A number of techniques improve the contact between the enzyme and coffee substrate. Pre-  
591 treatment can facilitate the extraction and contact between Asn and asparaginase, promoting  
592 its migration into the beans. Dria *et al.*, (Dria et al., 2007) listed a series of pre-treatments for  
593 this, including drying, hydrating, rinsing with or without mechanical action, pressurizing,  
594 steaming, blanching, heating, reduced-pressure processing and particle-size reduction. These  
595 processes were very often not verified, even for organoleptic impact, and are not applicable  
596 at the industrial level (Anese, 2016). In addition, Navarini *et al.* in 2014 patented a method to  
597 reduce AA enzymatically in a water extract of green beans. The enzymes used in this method  
598 were asparaginase and aspartase in solution. The authors found that Asn and Asp are present  
599 in similar concentrations in the extract and that Asp contributes to the formation of AA,  
600 although in lower amounts compared to Asn. After enzymatic treatment, the water extract  
601 was re-incorporated into the green beans before roasting. This treatment gave an AA  
602 reduction of about 70%, without affecting the organoleptic properties of the final brew  
603 (Navarini et al., 2014). This hypothesis, although of interest because in addition to the AA  
604 reduction do not seem to affect coffee sensory properties, has not been demonstrated in peer  
605 reviewed article(s). It is also contrasted by several authors who report that the effect of Asp  
606 on AA formation is negligible, and who strongly support the correlation between Asn and AA  
607 (Belitz et al., 2009; Dias et al., 2012; Guenther et al., 2007; Schouten et al., 2020). In 2019,  
608 Porto *et al.* (Porto, Freitas-Silva, Souza, & Gottschalk, 2019) treated Arabica and Robusta  
609 coffee beans with asparaginase, and obtained Asn reductions of approximately 60% and 35%,  
610 respectively. The beans were pre-treated for 30-45 minutes with steam to open the pores and

611 favour the enzymatic process. In the same way, Corrêa *et al.*, (Corrêa et al., 2021) have shown  
612 that the pre-treatment of Arabica coffee beans with steam improved the results of  
613 asparaginase treatment, with an AA reduction close to 59%, compared to the control sample,  
614 and 77% compared to the blank sample.

615

## 616 **7.2 Mitigation using roasting strategies**

617 Some authors have proposed optimizing the roasting process to mitigate AA content, with  
618 the aim of finding the best conditions to obtain both the desired roasting degree and lower  
619 AA concentrations. Madihah *et al.* (Madihah, 2013) optimized the roasting time and  
620 temperature conditions for Arabica coffee beans, and found the optimal conditions to be  
621 168°C for 22 min. Under these roasting conditions, a low amount of AA was formed (1110  
622 µg/kg) with a score of the overall sensory evaluation of the brews of 7.5 out of 10 points.

623 Esposito *et al.* optimized roasting conditions on an industrial scale for Arabica and Robusta in  
624 order to fulfil the requirements of taste and aroma, as well as to reduce the concurrent AA  
625 formation. They found that, with the proper set up of roasting conditions, AA concentration  
626 can be reduced in Robusta samples by between 20% and 90%. However, the roasting degree  
627 was measured colorimetrically without any sensory evaluation of the final products (Esposito  
628 et al., 2020).

629 Alternative roasting technologies have been attempted. Theurillat *et al.* (Theurillat, Leloup,  
630 Liardon, Heijmans, & Bussmann, 2006) evaluated the use of a steam/pressure roasting pilot  
631 plant unit. Results showed that the steam treatment carried out on green and roasted coffee  
632 did not significantly influence the final AA content in either of the final roasted samples.

633 Anese *et al.*, ( Anese et al., 2014) subjected coffee beans to a medium-roasting process under  
634 reduced pressure conditions leading to a reduction in AA levels of 50% compared to  
635 conventionally roasted coffee, and a minimal impact on sensory properties. The low-pressure  
636 conditions generated inside the roaster, which exerted a stripping effect, preventing AA from  
637 being accumulated. Nevertheless, this AA-mitigation strategy is probably not of general  
638 interest as coffee roasted to a medium degree is almost only consumed in the American and  
639 Northern European markets.



640 Budryn *et al.*, (Budryn, Nebesny, & Oracz, 2015) studied AA formation upon the roasting of  
641 Robusta samples with air at different speeds, humidity, time and temperature. These roasting  
642 conditions resulted in lower AA formation when air velocity was decreased at temperature in  
643 the range of 190–216 °C and air humidity was increased at higher temperatures (e.g., at 216  
644 °C). A relatively low AA level (0.0376 µg/g) was found in coffee samples roasted at 203°C,  
645 although polyphenols underwent moderate deterioration.

646 Guenther *et al.* found that saturated steam roasting can reduce AA content by up to 10%.  
647 However, the process had a negative impact on the taste and aroma of the coffee (Guenther  
648 *et al.*, 2007).

649 Rattanarat *et al.*, (Rattanarat, Chindapan, & Devahastin, 2021) studied the effect of  
650 superheated steam (SHS) roasting on the formation and reduction of AA, and, interestingly,  
651 found that SHS roasting resulted in lower AA content in medium- (~16%) and dark-roasted  
652 (~25%) beans at 250°C. Nevertheless SHS, used as an alternative to roasting, impacted upon  
653 the flavour of (Robusta) coffee, producing brews with higher sweetness and citrus-like acidity  
654 (Chindapan, Soydok, & Devahastin, 2019).

655

### 656 **7.3 AA removal from roasted coffee beans and brews**

657 All methods and proposed technologies should, of course, also be tested for their impact on  
658 the sensory quality of the final product and feasibility from an industrial point of view before  
659 being adopted. Banchemo *et al.*, (Banchemo, Pellegrino, & Manna, 2013) proposed the use of  
660 supercritical CO<sub>2</sub> to remove AA from roasted coffee. The efficiency of AA removal ranged from  
661 8% to 45% at an extraction time of 525 min, and increased to 79% after 22 hours. Changes in  
662 pressure did not affect the results, but temperature was the variable that drove the extraction  
663 process. Furthermore, the addition of ethanol (up to 9.5% w/w) changed the polarity of the  
664 supercritical solvent mixture, resulting in an increase in extraction performance. The most  
665 effective operative conditions were found to be 100°C, 200 bar and 9.5% w/w ethanol.

666 Cha (Cha, 2013) reported a technique that can remove AA from brews using bacterial enzymes  
667 at relatively high temperatures. Extracts from *Ralstonia eutropha* AUM-01 and a thermophilic  
668 strain, *Geobacillus thermoglucosidasius* AUT-01, were used to remove 50% of AA from coffee  
669 brews. In 2016, Anese *et al.* (Anese, 2016), proposed using acrylamidase to hydrolyse AA to

670 acrylic acid, which is less toxic than AA, but corrosive for the skin and mucosa, and ammonia.  
671 However, this process may affect the brew's sensory profile. Akillioglu *et al.* (Akillioglu &  
672 Gökmen, 2014) proposed a mitigation method for AA in instant coffee that is based on baker's  
673 yeast (*Saccharomyces cerevisiae*, 1–2%, w/v) mixed with sucrose (0–10, w/v). The mixture  
674 was fermented at 30°C for 48h with an AA concentration decrease of about 70%. The results  
675 revealed that both the sucrose and yeast concentrations affected the AA mitigation during  
676 fermentation and that its reduction was due to the effect of metabolic conversion via yeast  
677 metabolism.

678 Using immobilized enzymes is a further possibility for minimizing AA content. Bedade *et al.*,  
679 (Bedade, Sutar, & Singhal, 2019) have proposed immobilizing bacterial acrylamidase from  
680 *Cupriavidus oxalaticus* ICTDB921 on chitosan-coated alginate beads. The immobilized  
681 acrylamidase has an optimal pH/temperature of 8.5/65 °C, showed improved pH/thermal and  
682 shelf stability and retained 80% activity after four cycles. They applied it to instant coffee with  
683 complete AA degradation after 60 min of treatment, starting from an initial concentration of  
684 100–500 µg/L. The authors successfully tested the immobilized acrylamidase in both batch  
685 and continuous operations on a packed column for the effective AA removal from a roasted  
686 instant coffee solution, although some limitations in continuous operation, which were linked  
687 to column performance, were found.

688

## 689 **Conclusions**

690 The intake of AA from coffee and coffee products has been widely discussed in the literature.  
691 However, not many studies on possible AA mitigation are available. A number of strategies  
692 and approaches (Figure 3) that the coffee industry may use to mitigate AA levels in their final  
693 products are currently available (Food and Drink Europe, 2019), they include:

- 694 • selecting good quality green coffee and removing poor quality beans
- 695 • favouring Arabica over Robusta coffees
- 696 • roasting at the highest thermal input (dark degree)
- 697 • storing roasted coffee for a long time
- 698 • favouring shorter coffees brews over longer ones.

699 In particular, several articles have reported that darker roasted coffees are characterized by  
700 lower AA contents than light and medium ones, due to AA degradation during processing.  
701 Nevertheless, the reduction of AA in darker roasted coffee may not be a generally applicable  
702 solution as this type of coffee is mainly appreciated from the Southern European consumers,  
703 in contrast to Northern European and American consumers who prefer lighter roasted  
704 products (Schouten et al., 2020). In addition, stronger roasting can increase the formation of  
705 other toxic substances (i.e. furans).

706 The applicability of the asparaginase enzyme in the treatment of green coffee is limited due  
707 to the poor permeability of the green beans and the additional processing steps required  
708 (steam treatment and soaking in a water bath) for enzyme effectiveness. Moreover, this  
709 treatment influences the sensory properties of coffee and therefore cannot be expected to  
710 become a generalized AA mitigation process in coffee production. However, this technique  
711 should be evaluated on a case-by-case basis according to the origin of the green coffee, the  
712 amount of enzyme to be used and the desired quality of the final product.

713 Although some innovative strategies for AA reduction have been proposed and may be of  
714 interest, including roasting in modified environments, vacuum or superheated vapor, and the  
715 use of bacterial enzymes to remove AA from brews, they still need to be tested at an industrial  
716 level. Moreover, can lead to changes in aroma composition, not only affecting the quality of  
717 the product, but also its acceptance by consumers. However, most research on AA mitigation  
718 fails to report completely exhaustive information and some of them are also contradictory  
719 (i.e. Bertuzzi et al., 2020). In particular, there is a significant lack of knowledge on the effect  
720 of agricultural practices and geographical origins on AA precursors. Finally, most processes  
721 are studied in laboratory/pilot plants and the scaling-up conditions and sustainability of these  
722 processes are still to be investigated.

723 It is therefore necessary that studies be expanded on all aspects and that the link between  
724 origin and AA quantity is investigated. For instance, the effect of climate change and how it  
725 impacts agronomical and primary processing practices and AA precursors requires attention.  
726 In this respect, varietal improvement might also be guided by potential reductions in AA  
727 formation, while maintaining the sensory quality of the product. In conclusion, further studies  
728 are needed to find appropriate and practical solutions for AA mitigation in coffee and to study  
729 the health implications of AA in complex mixtures, such as coffee brews. A recent review by  
730 Nehlig and Cunha (Nehlig & Cunha, 2020) highlighted how most toxicological studies are

731 carried out on pure AA and on animals, while studies that directly evaluate the effects that  
732 AA in foods have on human health have not provided direct evidence of carcinogenic effects.  
733 In addition, the risk to human health from AA depends on the conditions of exposure, i.e., the  
734 kinetics of adsorption, distribution and excretion in the human body, while this kinetic-  
735 dynamic profile is also related to the other constituents of coffee and more in general to the  
736 human diets. The mitigation strategies proposed so far to meet the EU precautionary principle  
737 on food safety, are devoted to taking appropriate measures to reduce the presence of AA to  
738 as low as reasonably achievable (ALARA). This view also needs to take into account other  
739 factors, such as potential risks from other contaminants and/or synergy or competition with  
740 other components in the brew, the organoleptic properties and quality of the final product,  
741 and the feasibility of any process, in terms of both application at industrial level and costs.

742

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747

748

749

750 List of acronyms

AA	Acrylamide
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
Cys	Cysteine
3-DG	3-Deoxyglucosone
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
HMF	5-Hydroxymethylfurfural
LRU	Light reflectance units
Lys	Lysine
Phe	Phenylalanine
R&G coffee	Roast and Ground coffee
Ser	Serine
SHS	Saturated steam
Trp	Tryptophan

751

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1027 **Table Captions**

1028 Table 1 Studies on the impact of roasting conditions on acrylamide levels in roasted coffee

1029 Table 2 Studies on the decrease of acrylamide in roasted coffee and coffee products during  
1030 storage under different conditions

1031 Table 3 Impact of brewing techniques and conditions on acrylamide levels in final coffee  
1032 beverage

1033 Table 1S Monovarietal commercial roasted samples of different origin available in local  
1034 supermarkets adapted from (Alves et al., 2010; Pugajeva et al., 2015).

1035 **Captions to figures**

1036 Figure 1 Formation pathways of AA: a) Maillard reaction pathway in yellow; b) via triglyceride  
1037 decomposition in green; c) from 5-hydroxymethylfurfural (HMF) in violet; d) formation from  
1038 pyrolysis in light blue.

1039 Figure 2 A. Main factors affecting acrylamide levels in coffee: coffee species (Robusta coffee  
1040 contains higher levels of acrylamide than Arabica; roasting conditions (acrylamide is formed  
1041 in the early stages of roasting and its content decreases with increasing temperature and  
1042 roasting time); storage (acrylamide is not stable in commercial coffee stored in its original  
1043 container); beverage preparation (acrylamide is extracted differently into the beverages);  
1044 defective coffee beans (in particular immature ones that contain higher amounts of free  
1045 asparagine). B. Examples of coffee brewing techniques and their respective grinding grades.  
1046 Coffee brews are prepared using a certain volume of water (boiled, under pressure...) and a  
1047 defined amount of coffee powder. The optimal grinding degree varies with coffee brewing  
1048 preparation.

1049 Figure 3. Options for reducing acrylamide amount in final coffee beverages.