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1 **Acrylamide in coffee: formation and possible mitigation strategies - A**
2 **review**

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4
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24 activity is focus on aspects related to the processing and stability of foods using a
25 multidisciplinary approach.

26 **Acrylamide in coffee: formation and possible mitigation strategies - A**

27 **review**

28 It is widely known that acrylamide, present in some different heat-treated foods, is
29 an important toxic compound to humans. Coffee beverage is one of the most
30 important sources of acrylamide, because the raw bean contains the reaction
31 substrates and it is processed at very high temperature during roasting. Due to its
32 high consumption all over the world, it is necessary to find applicable solutions to
33 decrease the concentration of this undesired Maillard reaction product.

34 The present review summarizes the advance made in understanding the acrylamide
35 formation and describes the potential acrylamide reduction strategies along all
36 coffee production steps, from raw material to coffee brew preparation with a
37 dominant focus on roasting stage.

38 Currently, it is quite established that the selection of the highest quality Arabica
39 green coffee variety, high roasting thermal input and shortest brewing techniques
40 lead to low final acrylamide levels. There are also few innovative interventions
41 proposed for acrylamide control in coffee such as enzymatic treatments of raw
42 material, vacuum or steam roasting, roasted beans supercritical fluid extraction,
43 final beverage treatments like yeast fermentation and amino acids/additive
44 additions. However, for these strategies the impact on the desired sensorial and
45 nutritional coffee brew properties must be evaluated and some proposed
46 procedures are still difficult to be applied at real industrial scale. Furthermore, in-
47 depth studies are needed in order to find appropriate and practical solutions for AA
48 mitigation in coffee with a holistic risk/benefit approach.

49 Keywords: acrylamide; coffee; roasting; formation; mitigation.

50

51 **Contents**

52 **1. Introduction**

53 **2. Overview of coffee production technologies**

54 **3. Mechanisms of acrylamide formation during coffee processing**

55 **4. Fields of study on mitigation strategies of AA in coffee**

56 ***4.1. Raw materials selection and treatments***

57	<i>4.2. Roasting</i>
58	<i>4.3. Storage conditions</i>
59	<i>4.4. Brew preparation and treatments</i>

5. Conclusions

References

1. Introduction

Coffee is one of the most popular drinks in the world chosen for its sensory characteristics, stimulant effect of caffeine and beneficial impact on human health, due to the presence of bioactive components (Anese 2015; Hu et al. 2019; Seal et al. 2008).

Since its discovery in Ethiopia, coffee has played a key role in human history, developing both new processing systems and new forms of consumption (Guimarães et al. 2019).

The overall quality of a coffee cup, an apparently "simple" beverage, is the result of a careful control of a multitude of factors such as raw material selection, roasting, storage conditions and brewing extraction methods which can be differentiated according to geographical areas and consumer habits.

In particular the roasting process is the most important unit operation being responsible of the main chemical, physical and organoleptic characteristics of final coffee product, as well as of the bioactive and antioxidant compounds development (Anese 2015; Guenther et al. 2007; Soares, Alves, and Oliveira 2014). However, this thermal process induces at high temperatures the formation of undesired toxic components including acrylamide (AA), as a result of the Maillard reaction mainly between asparagine and reducing sugars, such as glucose and fructose (Anese 2015; Rannou et al. 2016; Taeymans et al. 2004; Tareke et al. 2002). AA is a common toxicant in a wide range of cooked foods that has been classified by the International Agency for Research on Cancer (IARC) as a compound "probably carcinogenic to human" (group 2A) with negative consequences on health (IARC 1994). As well as other popular carbohydrate-rich, heat-treated foods such as fried potatoes and bakery products, roasted coffee contributes to the total AA dietary intake due to its high consumption (Anese et al. 2013; Dybing et al. 2005; Guenther et al. 2007; Gökmen 2015).

Due to the high toxicological potential of AA, food industries, regulatory authorities and institutional communities are increasingly interested in applying interventions aimed at preventing and/or reducing the formation of this compound in order to obtain safer foods (European Commission 2013; Food Drink Europe 2011, 2019; Palermo et al. 2016).

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2
3 91 Recently, the Regulation (EU) 2017/2158 of 20 November 2017 established the
4
5 92 application of mitigation measures and new benchmark levels for the reduction of AA in
6
7 93 foods, including coffee. Concerning coffee products, the new AA benchmark values are:
8
9 94 400 $\mu\text{g}\cdot\text{kg}^{-1}$ for roasted ground coffee, 850 $\mu\text{g}\cdot\text{kg}^{-1}$ for soluble coffee, 500 $\mu\text{g}\cdot\text{kg}^{-1}$ and
10
11 95 4000 $\mu\text{g}\cdot\text{kg}^{-1}$ for coffee substitutes based respectively on cereal and chicory (European
12
13 96 Commission 2017). Due to the increasing restrictions of legislation and the complexity
14
15 97 of factors to be considered, the ability to control of AA in foods without affecting their
16
17 98 final quality, is becoming more and more a serious challenge for researches and
18
19 99 industries.

20
21 100 As for coffee, it is known that all processing stages (selection of raw material, roasting,
22
23 101 storage and brewing conditions) can play an important role on final AA levels in the
24
25 102 product.

26
27 103 Some efforts by the scientific community have contributed to the identification of
28
29 104 potential strategies to reduce and/or minimise AA levels in roasted coffee and
30
31 105 consequently consumer exposure. However, compared to potato and bakery products,
32
33 106 during the last 15 years only few scientific studies have been carried out to date on
34
35 107 intervention strategies for AA reduction in roasted coffee and coffee products.

36
37 108 The present paper aims to review and discuss the main possible mechanism(s) of AA
38
39 109 formation and mitigation reported in scientific literature in relation to coffee processing
40
41 110 stages, from raw material to brewing, with a main focus on roasting, being the most
42
43 111 studied processing step (Table 1). This in order to provide information on the potential
44
45 112 efficacy, applicability and potential constraints of current knowledges on AA control in
46
47 113 coffee.

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51 115 [Table 1 near here]

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54 55 117 **2. Overview of coffee production technologies**

56
57 118 Coffee drinks are produced from coffee beans after the processes of seeds separation from
58
59 119 the coffee plant, roasting and grinding (Illy and Viani 1995). The principal steps of the
60
61 120 process from the cherry to the final brew are shown in the flow sheet of Figure 1, **in which**
62
63 121 **the critical steps for the reduction of AA in coffee are highlighted.**

64
65 122 Coffee cherries must be processed strictly at the optimal level of maturation, with a
66
67 123 maximum of 5% unripe cherries present and their processing must be carried out as soon
68
69 124 as possible after harvesting to avoid qualitative deterioration (Seal et al. 2008).

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125 Depending on the climate of the country and farm tradition, after collection, ripe coffee
126 cherries are processed using a dry- or wet-method to provide a stable product for
127 exportation (Soares, Alves, and Oliveira 2014). The “dry-process” (age-old method),
128 largely used in tropical regions, corresponds to cherry drying carried out under the sun
129 (classical), in mechanical dryers (modern) or by a combination of both, and gives the
130 “natural” or “unwashed” green coffee. **With this method the drying takes different days,**
131 **depending on the used conditions and initial moisture of coffee cherries.**

132 **The “wet-process” was developed in equatorial areas characterized by constant**
133 **precipitations during the harvest stage, a condition not optimal for the dry processing.**
134 **The wet process, which gives the so-called “washed” green coffee, is based on pulp**
135 **removal (fruit skin and a part of mesocarp), fermentation of the parchment (endocarp with**
136 **a part of mesocarp mucilage) and drying under sun or in mechanical dryers. Coffee**
137 **fermentation is carried out by itself, by soaking in water or through mixed fermentation.**
138 **During mechanical drying, used for both “natural” and “washed” coffees, the temperature**
139 **of the coffee mass is gradually increased and should not exceed 40 °C to produce higher**
140 **quality coffee. This process makes it possible to reach a coffee moisture of around 11 –**
141 **12 %, ideal levels for storage.** The “wet-method” improves the characteristics of the taste
142 and aroma of coffee; it requires more time and is more expensive compared to the “dry-
143 process”. There is also an intermediate product called “semi-washed”, also known as
144 “natural depulped”, with a quality level between the washed and the natural green coffee
145 beans (Bee et al. 2005; Illy and Viani 1995; Soares, Alves, and Oliveira 2014; **Guimarães**
146 **et al. 2019**). Once dried, the beans, known collectively as “parchment” coffee or “dry
147 cherries”, are hulled to remove the outer shell (husk), sometimes polished for eliminating
148 the silver skin, graded in different classes according to their size or shape and sorted to
149 remove defective beans (i.e. black, sour, immature, broken). Subsequently, green coffee
150 beans are stored in jute or sisal bags, or big-bags with different capacity, under controlled
151 temperature and relative humidity (around 20 °C and below 70% RH) (Illy and Viani
152 1995).

153 Before the most important processing step (roasting), coffee can be blended or
154 decaffeinated depending on the characteristics and the intended use of the final product.
155 Finally, the coffee is roasted for the development of the desirable colour and flavours with
156 different roasting techniques and time-temperature conditions. Roasting of coffee is a
157 complex process, involving transfer of heat through the structure of the bean, transport of
158 water vapour, generation of CO₂/volatiles, change of volume, structure, material and

1
2
3 159 chemical composition (Seal et al. 2008). Moreover, the principal components of coffee
4
5 160 beans can react to produce a certain amount of toxic compound such as acrylamide (AA)
6
7 161 by different reaction mechanisms (which will be discussed in detail later on).

8
9 162 Whole roasted coffee is grounded (using a grinder or coffee mill) to a powder
10
11 163 characterized by different size according to the ultimate brew desired. The grinding step
12
13 164 aims to increase the extraction surface in order to facilitate the transfer of coffee soluble
14
15 165 constituents into the brew (Soares, Alves, and Oliveira 2014). Whole roasted coffee beans
16
17 166 or ground coffee are usually packed prior to storage and distribution. In order to best
18
19 167 protect the product quality, the package material should act as barrier against water and
20
21 168 oxygen (Illy and Viani 1995).

22 169 Finally, the coffee brews can be obtained by many brewing methods according to
23
24 170 consumer preferences, that are affected by geographical origin, cultural tradition,
25
26 171 lifestyle, social behaviour, habit and economic aspects. The most important extraction
27
28 172 methods can be classified into three main groups: Italian method under high pressure (i.e.
29
30 173 Espresso, moka); infusion, by pouring hot water on ground coffee, followed by filtration
31
32 174 (i.e. American, plunger, Neapolitan); decoction or boiling method (i.e. Turkish).
33
34 175 Furthermore, the use of single-dose coffee, such as capsules and pods, has highly
35
36 176 increased in recent years. The blend of roasted beans and other variables related to the
37
38 177 different brewing methods (e.g. water temperature, grinding level of powder, coffee/water
39
40 178 mass ratio, extraction time, volume of the final brew etc.) strongly affect the sensorial
41
42 179 quality and the amount of extracted compounds in the coffee beverages (De Peña,
43
44 180 Ludwing, and Cid 2019; Parenti et al. 2014; Petracco 2001).

45
46 181 While in general, the separation of green or raw beans from the fruit is carried out in the
47
48 182 country where coffee trees grow, the roasting and brewing steps are performed in the
49
50 183 countries where coffee is consumed (Soares, Alves, and Oliveira 2014).

51
52 184 It is important to underline that, since they take place in different geographical areas, the
53
54 185 coffee processing steps are not easy to control and can be differentiated and performed
55
56 186 according to companies and consumers demands. Consequently, any variation of the
57
58 187 technological parameters applied in each step, for example aimed at the reduction of AA
59
60 188 level, can significantly influence the overall quality of the final coffee brew.

189

190 [Figure 1 near here]

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192 **3. Mechanisms of acrylamide formation during coffee processing**

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3 193 Roasted coffee is one of the potential products, together with fried potatoes and bakery
4 products, at risk for high AA (C_3H_5NO) levels. Green coffee contains the key precursors
5 194 (e.g. free asparagine and reducing sugars) for the formation of AA during roasting at high
6 195 temperatures ($> 200\text{ }^\circ\text{C}$), that induces complex chemical reactions, that, according to the
7 196 most recent literature, are summarized in Figure 2.
8 197

9 198 AA is a potential toxic compound which can be formed during different food processing
10 199 methods, such as cooking, drying, frying and roasting (Khezerlou et al. 2018). Because
11 200 green coffee normally lacks AA, while the roasted one contains a significant level in the
12 201 range of 0.1-2.2 ppm; the roasting process is considered the main responsible for its
13 202 formation (Alves et al. 2010).

14 203 Different possible mechanisms and precursors of AA formation in roasted coffee have
15 204 been postulated in scientific reports (Anese 2015; Cai et al. 2014; Claus et al. 2006;
16 205 Friedman and Mottram 2005; Gökmen et al. 2012; Granvogl and Schieberle 2006;
17 206 Khezerlou et al. 2018; Kocadağlı et al. 2012; Soares, Alves, and Oliveira 2014; Stadler
18 207 and Scholz 2004; Yaylayan and Stadler 2005):

- 19 208
- 20 209 • free amino acids and reducing sugars via Maillard reaction;
 - 21 30 • decarboxylation and deamination of asparagine;
 - 22 31 • reaction between ammonia and acrolein or acrylic acid (lipid degradation);
 - 23 32 • 5-hydroxymethylfurfural (HMF) and free asparagine via Maillard reaction;
 - 24 33 • pyrolytic reactions.
- 25 34
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27 36
28 37

29 38 One of the predominant pathways of AA formation in coffee products, as in most other
30 39 concerned foods, is the non-enzymatic browning group of reactions, generally referred to
31 40 as Maillard reaction. This term indicates an extremely complex heat-induced set of
32 41 reactions initiated by the condensation of the carbonyl group ($C=O$) of a reducing sugar
33 42 (e.g. glucose) with an amino residue ($-NH_2$) of amino acids (e.g. asparagine) at
34 43 temperatures above $120\text{ }^\circ\text{C}$ (Pastoriza, Rufián-Henares, and Morales 2012; Soares, Alves,
35 44 and Oliveira 2014; Stadler and Scholz 2004). The first product of this condensation is the
36 45 Schiff base, that is a very unstable compound. Further reactions lead to the formation of
37 46 an important intermediate known as 3-aminopropionamide (3-APA) and finally of AA
38 47 (Pedreschi, Mariotti, and Granby 2014; Soares, Alves, and Oliveira 2014; Stadler and
39 48 Scholz 2004; Zyzak et al. 2003). However, the Schiff base may lead, after its
40 49 decomposition, directly to AA (Pedreschi, Mariotti, and Granby 2014).
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3 225 Asparagine, one of the key precursors of AA by Maillard reaction, and sucrose, the main
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5 226 sugar found in raw coffee, are present in green coffee beans in the range of 0.30-0.90
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7 227 mg·g⁻¹ and of 35-90 mg·g⁻¹, respectively depending on the characteristics of the raw
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9 228 materials (Murkovic and Derler 2006; Zhang and Zhang 2007). Sucrose is not a reducing
10
11 229 sugar but in the initial phase of roasting his decomposition takes place, giving rise to a
12
13 230 neo-carbonyls pool that influences the formation of AA during Maillard reaction.
14
15 231 Moreover, neo-carbonyl compounds that can be generated from lipid oxidation during
16
17 232 roasting of coffee, should be contemplated on AA formation by Maillard reaction
18
19 233 (Kocadağlı et al. 2012). During the Maillard reaction, not only toxic compounds are
20
21 234 produced, but also a very large number of brown-coloured (melanoidins), volatile and
22
23 235 non-volatile aromatic compounds are formed. These compounds are desired because they
24
25 236 are responsible of coffee products identity and quality (Stadler and Scholz 2004). In
26
27 237 addition, some compounds with an interesting antioxidant activity were also detected
28
29 238 among the Maillard reaction products (Hečimović et al. 2011; Hu et al. 2019; Van der
30
31 239 Werf et al. 2014; Vignoli et al. 2014).

32
33 240 Although the formation of AA is mainly due to the combination of free asparagine and
34
35 241 reducing sugars, this compound can also be generated from simple asparagine in absence
36
37 242 of sugars by direct decarboxylation and deamination reactions at high temperatures. Low
38
39 243 amounts of free asparagine can be detected in almost all kinds of foods, for this reason
40
41 244 most products, even when heated at high temperatures, can contain a low amount of AA
42
43 245 (Granvogl and Schieberle 2006). The relative low concentration of asparagine and the
44
45 246 high process temperatures lead to its rapid intramolecular cyclization, making this
46
47 247 pathway of AA formation of limited importance (Granvogl and Schieberle 2006;
48
49 248 Yaylayan, Wnorowski, and Perez Locas 2003).

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51 249 Another pathway that does not require the presence of asparagine, is the generation of
52
53 250 AA by acrolein and acrylic acid reacting with ammonia (NH₃) already present or formed
54
55 251 during the thermolysis of amino acids and proteins (Anese 2015; Friedman and Mottram
56
57 252 2005; Khezerlou et al. 2018; Stadler and Scholz 2004; Yaylayan and Stadler 2005).
58
59 253 Acrolein can be formed through different pathways, including the oxidative degradation
60
254 of fats (i.e. triglycerides), and can generate acrylic acid by oxidation. However, acrylic
255 acid can be also produced from the thermal decomposition of some amino acids such as
256 aspartic acid, L-alanine and L-arginine or indirectly from serine and cysteine through the
257 formation of pyruvic acid (Guenther et al. 2007). Acrylic acid can react with ammonia to
258 form AA by aminodehydroxylation, which is a well-known reaction of acids leading to

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259 amides (Yaylayan and Stadler 2005). However, the formation of AA from acrolein and
260 acrylic acid is a route of marginal importance because is limited by the availability of free
261 ammonia in food and by the necessity of relatively high temperatures for the reaction to
262 proceed efficiently (Guenther et al. 2007; Yaylayan and Stadler 2005).

263 HMF, a furanic compound which can be generated during roasting from sugar
264 decomposition, has been recently suggested as an alternative precursor for AA formation.
265 Owing to its carbonyl group, HMF may rapidly react with asparagine in the Maillard
266 reaction. There is limited research on this possible pathway for AA formation, but it
267 seems that HMF can acts as a more efficient precursor than sugars (Anese 2015; Cai et
268 al. 2014; Gökmen et al. 2012; Kocadağlı et al. 2012).

269 Additional possible mechanisms of AA formation could occur during coffee roasting,
270 caused by pyrolytic reactions as previously observed in bakery products at high
271 temperature (Anese 2015; Claus et al. 2006).

272 It is easy to understand that each of these AA formation routes in coffee is influenced by
273 several factors like raw material (e.g. coffee species, origin and post-harvest treatments)
274 and process interventions (e.g. roasting, storage and brewing conditions) (Anese 2015).

275

276 [Figure 2 near here]

277

278 **4. Fields of study on mitigation strategies of AA in coffee**

279 ***4.1. Raw materials selection and treatments***

280 The correct choice of the raw materials is an important factor that can influence the final
281 content of AA in foods, and it becomes even more important for roasted coffee and coffee
282 brew obtained from a single ingredient, whose purity and quality are fundamental
283 attributes.

284 The two main green coffee species of processing industry interest in the genus *Coffea*,
285 are *C. arabica* L. (around 65% of the world's production) and *C. canephora* (around 35%
286 of the world's production) conventionally called Arabica and Robusta respectively
287 (Soares, Alves, and Oliveira 2014). The two botanical species are characterized by a
288 distinct chemical composition and consequently different sensorial properties; they
289 normally are used alone (e.g. 100% Arabica) or in blend (e.g. 70% Arabica and 30%
290 Robusta) according to the company's decisions and consumers preferences (Guenther et
291 al. 2007; Rubayiza and Meurens 2005). Different chemical composition means also that
292 the two species are characterized by different concentration of AA precursors (i.e. free

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3 293 amino acids and reducing sugars). Carbohydrates are present in both species of green
4
5 294 coffee beans, but Arabica contains higher amounts of sucrose than Robusta that, instead,
6
7 295 contains more reducing sugars (e.g. glucose and fructose). Concerning the main free
8
9 296 amino acids, asparagine and alanine have been found in higher concentration in Robusta
10
11 297 compared to Arabica variety (Murkovic and Derler 2006; Pedreschi, Mariotti, and Granby
12
13 298 2014).

14 299 Several researches, summarized in Table 2, have studied the effect of Arabica versus
15
16 300 Robusta beans on AA levels in roasted coffee (Alves et al. 2010; Bagdonaite, Derler, and
17
18 301 Murkovic 2008; Bagdonaite and Murkovic 2004; Bertuzzi et al. 2020; Lachenmeier et al.
19
20 302 2019; Lantz et al. 2006; Summa et al. 2007). Bagdonaite and Murkovic (2004) has roasted
21
22 303 four different types of green coffee (3 Arabica and 1 Robusta) in a small laboratory roaster
23
24 304 at 250 °C for different times (5, 7.5, 12 and 14.5 min). The highest amount of AA (> 3500
25
26 305 ng·g⁻¹) was found after 7.5 min using the variety Robusta Camerun, while the use of high-
27
28 306 quality Arabica varieties (Santos, Columbian Excelso and Uganda) has led to lower levels
29
30 307 (< 500 ng·g⁻¹). In another study, Lantz et al. (2006) also found that AA formation in
31
32 308 Robusta was on average 34% higher than in Arabica, analysing a great number of green
33
34 309 coffee types (17 Arabica and 6 Robusta) roasted in a rotating fluidized bed roaster for 2.5
35
36 310 min, in order to reach a medium roasting degree. Summa et al. (2007) reported a lower
37
38 311 concentration of AA in Arabica (> 200 ng·g⁻¹) than in Robusta (> 250 ng·g⁻¹) varieties,
39
40 312 roasted with a hot air roaster at 236 °C at a medium level (370 and 430 s respectively). In
41
42 313 three more recent studies by Bagdonaite, Derler, and Murkovic (2008), Alves et al. (2010)
43
44 314 and Lachenmeier et al. (2019) it was confirmed the highest average levels of AA in the
45
46 315 Robusta coffee compared to the Arabica one, roasted at different roasting conditions. In
47
48 316 the study by Bagdonaite, Derler, and Murkovic (2008), the influence of possible
49
50 317 precursors concentration, carbohydrates (sucrose) and amino acids (asparagine or aspartic
51
52 318 acid), in green coffee on AA formation was also investigated. It has been concluded that
53
54 319 asparagine is the limiting precursor because as its concentration increases the AA level
55
56 320 increases, while the increase in the sucrose concentration lead to lower AA level. The
57
58 321 highest levels of asparagine and lower of sucrose were found in Robusta coffee,
59
60 322 confirming the higher AA concentration in that sample compared to the Arabica one
323 (Table 2). Bertuzzi et al. (2020) investigated the trend of AA content during an industrial
324 coffee roasting process with a horizontal rotating durum machine at temperature ranging
325 from 90 °C to 215 °C for 16 min. Contrary to all previous studies, the authors found the
326 maximum AA level was reached by Arabica samples, that contained a lower amount of

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3 327 asparagine. However, according to the authors, this result is due to the higher
4
5 328 concentration of reducing sugars in Arabica respect to Robusta coffee for the entire
6
7 329 duration of roasting. The trend of glucose and fructose increase, due to the thermal
8
9 330 hydrolysis of sucrose, and subsequently decrease during coffee roasting in according to
10
11 331 AA formation, showed concentration 5-fold higher in Arabica. However, a satisfactory
12
13 332 correlation between AA formation and asparagine degradation was found, confirming the
14
15 333 importance of this precursor.

16
17 334 Post-harvest green coffee beans processing (dry- or wet-process), important to provide a
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19 335 stable product for exportation, also may have an impact on the AA precursors profile and
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21 336 concentration in the raw material (Illy and Viani 1995; Knopp, Bytof, and Selmar 2006;
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23 337 Soares, Alves, and Oliveira 2014). The amount of main reducing sugars, such as glucose
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25 338 and fructose, was less than 80% in wet-processed green coffee compared to dry-processed
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27 339 one (Knopp, Bytof, and Selmar 2006). This difference on soluble solids can be explained
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29 340 by osmotic phenomena (Illy and Viani 1995) or anaerobic fermentation (Knopp, Bytof,
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31 341 and Selmar 2006) that occurs during wet post-harvest processing. Despite these, Lantz et
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33 342 al. (2006) and Alves et al. (2010) did not found significant differences on AA
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35 343 concentration between dry- and wet-processed coffee bean samples. However, Lantz et
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37 344 al. (2006) did not show related data and Alves et al. (2010) compared AA results obtained
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39 345 only from 1 Arabica dry-processed sample versus 7 Arabica wet-processed ones.

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41 346 A factor that could led to a relatively higher AA content in roasted coffee is the presence
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43 347 in the blend of immature beans with lower quality (called defective beans). In fact, the
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45 348 unripe beans contain significantly higher concentration of free asparagine compared to
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47 349 ripe ones, therefore a careful removal of these beans is recommended for AA formation
48
49 350 control (Mazzafera 1999). In most cases the green beans are classified and separated
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51 351 according to its maturation level, however, the presence of a portion of unripe fruit in the
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53 352 processing is unavoidable. A recent work of Dias et al. (2012) demonstrated that post-
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55 353 harvest coffee processing also influences the amino acids profile of unripe coffee fruits.
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57 354 The asparagine levels were significantly lower when the immature coffee beans were
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59 355 processed by the wet-process then by dry-process one. It has been hypothesized that these
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356 differences were caused by the presence of more active metabolism in coffee beans that
357 are processed using the wet method.

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359 358 Raw coffee beans can be also subjected to pre-treatments aimed to reduce the level of AA
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361 359 precursors. Being free asparagine a limiting factor for the AA formation in coffee, some
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363 360 authors studied the possibility to reduce this component in green coffee and the

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3 361 consequent AA formation during roasting by using the enzyme asparaginase (Dria et al.
4 362 2003; Hendriksen 2013; Lynglev and Schoesler 2014; Navarini et al. 2014). This enzyme
5 363 efficiently catalyses the hydrolysis of free asparagine amino acid into aspartic acid and
6 364 ammonia (Anese 2015). Hendriksen (2013) evaluated the effect of commercial
7 365 asparaginase (produced by *Aspergillus oryzae*) on asparagine reduction in green coffee.
8 366 The results indicated that treating green coffee beans with low dosage of asparaginase
9 367 achieved a decrease by 70-80% of asparagine and by 55-74% of AA after roasting.
10 368 Moreover, there are various patents that suggest the use of asparaginase combined with
11 369 various techniques aimed at improving the contact between enzyme and the coffee
12 370 substrate, overcoming the incomplete hydrolysis of asparagine. The green coffee bean is
13 371 a very dense, hardly permeable raw material; for this reason, it was suggested to pre-treat
14 372 it by drying, hydrating, grinding, steaming and soaking in bath water, eventually in
15 373 combination with reduced or increased pressure (Dria et al. 2003; Lynglev and Schoesler
16 374 2014; Navarini et al. 2014). These pre-processes would create a driving force for the
17 375 enzyme solution to migrate into the whole beans.

18 376 **However, in most of the reported research works and patents, the effects of the applied**
19 377 **treatments on the organoleptic characteristics of the final brews have not been verified.** It
20 378 is also important to evaluate the application of some studied pre-treatments at industrial
21 379 level; for example, grinding is a strategy that cannot be realistically applied because
22 380 coffee beans have to be roasted whole (Anese 2015); **in addition hydrating or soaking**
23 381 **would include in the process a further dehydration step of green beans.**

24 382 For what concern the decaffeination pre-treatment of green coffee beans, sometimes used
25 383 at the industrial level, Alves et al. (2010) and Bertuzzi et al. (2017) found that this
26 384 treatment has no significantly effect on the final AA content, probably because the
27 385 process does not influence the content of AA precursors in the raw material.

28 386

29 387 [Table 2 near here]

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31 389 **4.2. Roasting**

32 390 Roasting is an intense thermal treatment during which coffee beans are heated at
33 391 temperatures higher than 200 °C for different times, depending on the desired colour,
34 392 aroma and taste of the final product (Anese 2015; Bagdonaite, Derler, and Murkovic
35 393 2008; Soares, Alves, and Oliveira 2014). Temperature and time are important process
36 394 parameters affecting the Maillard reaction behaviour, and thus possible AA formation in

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395 foods (Rannou et al. 2016). For this reason, the final AA concentration in roasted coffee
396 strictly depends on the roasting degree achieved (light, medium or dark), that influences
397 also the sensorial characteristics of final product.

398 Roasting degree is normally determined by the consumers preferences in different
399 countries. In particular, the South European countries' consumers have a general
400 preference for a medium-dark roasting degree, on the contrary the North European and
401 American ones prefer a lighter roasting degree (Anese 2015; Seal et al. 2008; Soares,
402 Alves, and Oliveira 2014). In the scientific literature there are some studies, summarised
403 in Table 3, on the impact of different applied roasting conditions on the final AA levels
404 in roasted coffee (Alves et al. 2010; Bagdonaite, Derler, and Murkovic 2008; Bagdonaite
405 and Murkovic 2004; Bertuzzi et al. 2020; Budryn, Nebesny, and Oracz 2015; Kocadağlı
406 et al. 2012; Lachenmeier et al. 2019; Lantz et al. 2006; Madihah et al. 2013; Şenyuva and
407 Gökmen 2005; Summa et al. 2007).

408 Bagdonaite and Murkovic (2004) studied the formation of AA in Robusta coffee beans
409 roasted in a static oven at different temperatures (220, 240, 260 °C) and times (5, 10, 15
410 min). The lowest amount of AA was reached at the highest temperatures (260 °C) and at
411 the longest time (15 min) for all tested roasting temperatures. Şenyuva and Gökmen
412 (2005) studied the effect of the heating process on AA contents and colour (CIEL*a*b*)
413 of coffee samples. Green ground coffee samples were placed in sealed vials and heated
414 in an oven at different temperatures (150, 200, 225 °C) from 5 to 30 min. The amount of
415 AA increased reaching a peak after 10 and 5 min of roasting, respectively at 200 and 225
416 °C. After reaching the highest peak, AA contents exponentially decreased. Moreover, the
417 results showed a good correlation between AA and chromatic parameter a* ($r^2= 0.929$)
418 data of roasted coffee samples. Lantz et al. (2006) studied the AA formation kinetics of
419 coffee samples during 1.5 to 16 min roasting in three roasters at different roasting degree
420 (unspecified temperature). The authors concluded that the maximum level of AA is
421 formed early during the heating process and decrease increasing roasting time and degree.
422 Furthermore, Lantz et al. (2006) comparing AA results obtained in coffee samples roasted
423 with three types of equipment (fluidizer bed, rotating fluidized bed and drum roaster),
424 reported that the type of roaster used did not affect the kinetic of formation and
425 degradation of AA. Summa et al. (2007) found that prolonging roasting at 236 °C over
426 around 4 min caused a reduction of AA both in Arabica and Robusta coffee samples. In
427 agreement with Lantz et al. (2006), Summa and co-workers found the higher AA levels
428 in light roasted coffees. At the same time the authors found a proportional reduction of

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3 429 radical scavenging capacity measured on the same coffee samples during roasting.
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5 430 Bagdonaite, Derler, and Murkovic (2008) found that, increasing roasting time (from 5 to
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7 431 15 min) and temperature (from 220 to 260 °C), the rate of AA degradation in coffee
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9 432 increased too; in fact, after 15 min of roasting, for all applied temperatures, AA was no
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11 433 longer detected. In another study by Alves et al. (2010) a 25% decrease of AA content
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13 434 was measured in dark roasted coffee (210 °C for 11 min) compared to the medium one
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15 435 for all coffee samples investigated. Kocadağlı et al. (2012) monitored the formation of
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17 436 AA in coffee during roasting at 220 °C for 60 min in a static oven. Also in this case, it
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19 437 has been clearly shown that AA increased rapidly reaching the maximum level after the
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21 438 first 5 min of treatment and then exponentially decreased. In order to obtain a good quality
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23 439 roasted coffee with low level of AA Madihah et al. (2013) used a Central Composite
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25 440 Design to optimize the roasting condition of different Arabica coffee samples. The lowest
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27 441 level of AA and the best coffee quality, tested by flavour compounds and sensory
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29 442 analyses, were found after 22 min of roasting at 167 °C. Another investigation of Budryn,
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31 443 Nebesny, and Oracz (2015), by using a specifically built pilot roaster, studied the
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33 444 influence of air humidity and velocity, other than time and temperature of roasting, on
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35 445 AA levels and polyphenols content of Robusta coffee samples. The relatively lowest
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37 446 levels of AA (37.6 ng·g⁻¹) with a moderate degradation of polyphenols, were found in
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39 447 coffee samples roasted at 203 °C, low air velocity and humidity. Lachenmeier et al. (2019)
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41 448 investigated the AA levels in coffee after different roasting conditions. Six different
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43 449 experiments were carried out changing roasting time and temperatures in order to obtain
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45 450 the following coffee roast type: Scandinavian coffee (very light roasting), coffee quick
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47 451 drying, coffee slow drying, espresso slow dry, espresso quick drying, espresso Neapolitan
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49 452 (very dark roasting). The Neapolitan espresso coffee led to the lowest AA content of 130
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51 453 and 250 ng·g⁻¹ respectively for Arabica and Robusta, being roasted at the highest roasting
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53 454 energy. Instead, the Scandinavian coffee, roasted at the lowest roasting energy, had the
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55 455 highest AA content (470 and 480 ng·g⁻¹ for Arabica and Robusta). These results confirm
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57 456 the inverse relationship between roasting intensity and AA content in coffee. However,
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59 457 the authors also found the highest content of some toxicant compounds, such as furfuryl
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458 alcohol, furan and 5-hydroxymethylfurfural (HMF), in samples containing the lowest AA
459 levels. Furan and furfuryl alcohol are other heat-induced contaminants classified by IARC
460 as possibly carcinogenic to humans (group 2B) (IARC 1995, 2019). For HMF, some
461 animal studies highlighted its toxicant activity (Capuano and Fogliano 2011), but this
462 compound has not yet been classified by IARC as carcinogenic (IARC 2019).

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3 463 In a more recent study Bertuzzi et al. (2020) determined AA concentration during an
4 464 industrial coffee roasting process. The results, both for Arabica and Robusta samples,
5 465 confirmed that AA formation is dominant at the first 10 min of roasting and decreases
6 466 toward the end of the process, as reported in the small-scale roasting studies.

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9 467 Regarding commercial samples, Mojska and Gielecińska (2013) did not found a
10 468 significant relationship between the AA content and roasted coffee types or blend
11 469 (Arabica vs Robusta vs blend). A negative correlation between AA level and colour
12 470 intensity of coffee samples was found, demonstrating that the roasting degree
13 471 significantly affects the AA content in the final product. Recently, Wawrzyniak and
14 472 Jasiewicz (2019) analysed Arabica and Robusta commercial samples defined as “middle”
15 473 and “strongly” roasted. Unlike other previous studies, the AA mean was higher in the
16 474 medium roasted coffee samples compared to the strongly roasted one. The authors did
17 475 not propose explanations of the results because their objective was to describe a method
18 476 for AA determination.

19 477 AA formation and reductions kinetics during coffee roasting, selected from some studies
20 478 reported above, are shown in Figure 3. Most of the researchers found an initial increase
21 479 and a subsequent decrease of AA during roasting, also if the maximum level reached and
22 480 the kinetics of formation and degradation are different, being related to the process
23 481 parameter and type of coffee used. In all cases the maximum AA levels have been found
24 482 between light and medium roasting degrees.

25 483 To our knowledge, no research work reported a possible mechanism of AA evaporation
26 484 or degradation during extended coffee roasting. Only Pastoriza, Rufián-Henares, and
27 485 Morales (2012) proposed a possible pathway studied on a low-moisture model system
28 486 composed of AA and coffee melanoidins at different real concentrations during heating
29 487 at 180 °C from 2 to 12 minutes. The authors concluded that the decrease of AA during
30 488 roasting is due to its chemical interaction with coffee melanoidins, whose concentration
31 489 have a direct effect. In order to explain these results, the authors hypothesized that
32 490 nucleophilic amino groups of amino acids from the proteinaceous backbone of
33 491 melanoidins react via the Michael addition reaction with AA. **However, further researches
34 492 are necessary to identify the specific mechanisms of this reaction and to clarify if the
35 493 degradation of AA leads to possible formation of other toxic compounds, which may have
36 494 a negative effect on human health.**

37 495 Some authors studied possible alternative strategies in order to minimise or reduce the
38 496 AA levels in coffee during or after heat treatment. Theurillat et al. (2006) evaluated in

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3 497 Arabica and Robusta samples two different steam treatments applied prior or during
4 498 roasting, using respectively an autoclave and a rotating roaster equipped with steam
5 499 injection. Results showed that the steam treatments carried out on green and roasting
6 500 coffee did not significantly impact the final AA content of both final roasted samples.
7 501 Moreover, the steam treatments lead to a worst sensorial coffee profile that was more
8 502 acid, less roasty and bitter than the conventionally roasted one. Anese et al. (2014)
9 503 evaluated the possibility of reducing the formation of AA combining roasting at 200 °C
10 504 with low-pressure treatments, by using a static oven equipped with a vacuum pump.
11 505 Coffee samples were roasted under different roasting conditions (conventional, combined
12 506 conventional-vacuum, vacuum). Under reduced pressure and in the coffee roasted at a
13 507 medium degree it was possible to achieve AA levels 50% lower than the samples obtained
14 508 in conventional and combined conditions. The low-pressure conditions generated inside
15 509 the roaster exerted a stripping effect, preventing AA from being accumulated. In addition,
16 510 all medium roasted coffee samples presented similar colour and sensorial characteristics.
17 511 Nevertheless, this AA mitigation strategy could be of a limited interest since the coffee
18 512 roasted at a medium degree is consumed almost only in the American and Northern
19 513 European markets. Banchemo, Pellegrino, and Manna (2013) suggested, for the first time,
20 514 the use of supercritical CO₂ to remove AA from lab-scale roasted coffee. The efficiency
21 515 of AA removal ranged from 8 to 45% when the extraction time was below 525 min and
22 516 reached a maximum of 79% after 1305 min of supercritical fluid extraction treatment.
23 517 The caffeine content in the coffee beans was not affected by the treatment, however the
24 518 effect on the organoleptic properties of final product was not evaluated.

25 519

26 520 [Table 3 and Figure 3 near here]

27 521

28 522 **4.3. Storage conditions**

29 523 Numerous scientific researches demonstrate that AA is not stable during storage of
30 524 packed roasted coffee (Andrzejewski et al. 2004; Delatour et al. 2004; Hoenicke and
31 525 Gatermann 2005; Lantz et al. 2006; Michalak et al. 2016; Taeymans et al. 2005). Results
32 526 of these experiments indicate that the AA loss extent during coffee storage was affected
33 527 by ambient conditions such as time, temperature and atmosphere inside the package. The
34 528 studies regarding AA reduction during storage of coffee are summarized in Table 4.

35 529 In particular, Andrzejewski et al. (2004) observed important losses of AA (40-65%) in
36 530 roasted ground coffees after 6 months of secondary shelf-life in the original opened

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531 package at room temperature. At a lower temperature of -40 °C the content of AA did not
532 change in the same coffee sample after 8 months of secondary shelf-life. These results
533 suggest that AA loss occurs during prolonged storage at room temperature after the
534 container is opened. Delatour et al. (2004) reported 28 and 66% AA losses respectively
535 in roasted ground and soluble coffee samples from different manufacturers stored at room
536 temperature respectively for 7 and 12 months in original package tightly closed. However,
537 in both previous studies the authors did not hypothesize the possible causes of the
538 measured AA reduction during coffee storage, probably because the main aim of both
539 researches was to improve the analytical techniques for AA determination.

540 In a research of Hoenicke and Gatermann (2005) more focused on the study of AA
541 stability during storage of different types of foods, an AA decrease of around 30% was
542 found in ground and beans coffee samples after 3 months storage at 10-12 °C in their
543 closed original package. At the same storage conditions, AA loss was not found in soluble
544 coffee samples (spray-dried extracts). Analysing other products (cookies, cornflakes,
545 crispbread, raw sugar, potato chips and peanuts), the same authors observed that the
546 decrease of AA during storage takes place with more evidence for coffee and cacao
547 powder than for the other examined foods. It was hypothesized that AA losses probably
548 are due to its reactions with several roasted coffee constituents that are not present in
549 soluble coffee extracts and other products. In detail, it was assumed that AA reacts with
550 -SH groups containing substances during coffee and cocoa storage, **but the possible new
551 compounds resulted have not been reported, nor identified.**

552 Taeymans et al. (2005) evaluated the reduction of AA in roasted coffee beans samples
553 stored in a closed and in an opened jar at 60 °C for 34 h. A reduction of 30% AA was
554 found in the coffee beans stored in closed jar, whereas the content of AA in the opened
555 one remained unchanged. However, also if in some cases AA reduction was achieved, the
556 applied storage conditions inevitably affected the sensory characteristics of coffee.

557 An evident and proportional temperature dependence of AA loss was observed by Lantz
558 et al. (2006) in vacuum packed ground coffee samples stored for 12 months at
559 temperatures ranging from -18 to 37 °C. The highest AA reduction (90%) and rate were
560 registered in the samples stored at the highest temperature (37 °C), following a second
561 order reaction kinetic. Moreover, the authors calculated the activation energy of AA
562 reduction in coffee that was 73 kJ·mol⁻¹.

563 In a more recent study, Michalak et al. (2016) reported a significant AA reduction of 33
564 and 28% in instant coffee and coffee substitutes (coffee with different proportions of

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3 565 sugars, chicory, rye, barley or malt) respectively during storage at 25 °C for 12 months in
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5 566 their commercial closed package, thus confirming the instability of AA in coffee during
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7 567 storage. However, at the lowest storage temperature (4 °C) a smaller AA reduction in the
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9 568 coffee products was found, not statistically different at each time (6 and 12 months)
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11 569 compared to samples before storage. The authors concluded that the presence of -SH and
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13 570 -NH₂ groups of amino acids present in the products composition, might have a significant
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15 571 impact on AA reduction.

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17 572 At present there is no clear knowledge on the mechanism(s) of AA reduction during
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19 573 storage, although some hypotheses have been put forward. Baum et al. (2008) evaluated
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21 574 the possible fate of AA in stored roasted and ground coffees, using a ¹⁴C-AA radiotracer.
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23 575 Coffee samples were spiked with the radiotracer and stored for 48 weeks at room
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25 576 temperature and at 37 °C. Total radiolabelled contents and derived products were
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27 577 measured in coffee brew obtained by stored coffee samples, related filter residues and
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29 578 volatiles. The authors found a ¹⁴C-AA decrease in the obtained brews that resulted higher
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31 579 in the coffee samples stored for 16 weeks at 37 °C. It was concluded that the reduction of
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33 580 AA observed during coffee storage may be associated mainly to its covalent binding to
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35 581 insoluble matrix constituents of coffee. However, the chemistry and mechanisms of AA
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37 582 loss during coffee storage deserve further elucidation (Baum et al. 2008).

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39 584 [Table 4 near here]

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41 586 ***4.4. Brew preparation and treatments***

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43 587 Roasted coffee, after grinding, is used to prepare brews with various hot water brewing
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45 588 methods. AA is a compound soluble in water, hence during brewing process may be
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47 589 extracted to the coffee infusion depending on the extraction conditions (Andrzejewski et
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49 590 al. 2004). There are different techniques of brewing, in according to geographic, local
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51 591 traditions and social context that differ for many aspects: coffee grinding degree, coffee-
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53 592 to-water ratio, pressure, extraction temperature and time. All these parameters may
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55 593 significantly affect the AA level in the final coffee beverage (Cordoba et al. 2019; Soares,
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57 594 Alves, and Oliveira 2014).

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59 595 In Table 5 the few studies concerning the impact of brewing techniques and conditions
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596 on AA in coffee beverage are summarized. Lantz et al. (2006) analysed AA in coffee
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598 beverages obtained by different brewing systems (horeca pour-over system, household
drip-filter, plunger pot, fresh-brew filter of vending machine and espresso). The authors

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599 reported that the use of espresso method leads to the lower AA extraction (75%) in
600 comparison with the other tested brewing systems. The minor extraction efficacy in coffee
601 espresso brew was attributed to the highest coffee-to-water ratio ($146 \text{ g}\cdot\text{l}^{-1}$) and to the
602 shortest extraction time (not reported). In this work the only information reported, related
603 to the different brewing methods studied, was the coffee-to-water ratio. Another study of
604 Soares, Cunha, and Fernandes (2006) reported a higher AA mean level in soluble coffee
605 brew ($72.4 \mu\text{g}\cdot\text{l}^{-1}$) compared to espresso one ($21 \mu\text{g}\cdot\text{l}^{-1}$), both obtained from commercial
606 roasted products. Also, in this case the authors generally attributed these results to the
607 different extraction methods, without studying them in detail being the main purpose of
608 this study the development of a new method for AA determination in different coffee and
609 coffee products brew.

610 Alves et al. (2010) compared the AA concentration in espresso brews obtained at the same
611 brewing conditions (coffee-to-water ratio) from commercial caffeinated, decaffeinated
612 and serving coffee blends. The differences between AA average levels found in the
613 obtained espresso samples were not statistically significant and highly variable, probably
614 due to different coffee species ratio in the blends and roasting degree. Moreover, the AA
615 variability levels found in 30 ml espresso coffee by Alves et al. (2010) are in agreement
616 with those reported by Soares, Cunha, and Fernandes (2006), ranging from around 20 to
617 $50 \mu\text{g}\cdot\text{l}^{-1}$. In the same work Alves et al. (2010) studied the influence of espresso coffee
618 volume on the AA levels (Table 5), demonstrating that a long espresso (70 ml) leads to
619 the highest AA extractability of 98-99% than shorter espresso coffees (50, 30 and 20 ml),
620 due to the longest contact time between coffee and water. However, considering a
621 standard espresso coffee, with a volume ranging from 20 to maximum 30 ml, the AA
622 extracted was almost half of that initially present in coffee cake.

623 Some authors studied the fate of AA in the brewed coffee heated over time. Andrzejewski
624 et al. (2004) analysed the AA level in drip coffee brew for 5 h of heating (temperature not
625 reported). No change was found in AA level during time. The abovementioned study of
626 Lantz et al. (2006) also demonstrated that the AA extracted in the household drip-filter
627 coffee brew remained stable in a thermos-jar for 1.5 h, that is the usual time for
628 consumption. Pastoriza, Rufián-Henares, and Morales (2012) evaluated the stability of
629 AA in a brewed coffee obtained adding hot water to a coffee model system (previously
630 described in the paragraph 4.2) during a cooling time of 1 h at room temperature. The
631 authors reported a slight decrease in AA only during the first cooling minutes, concluding

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3 632 that the effect of soluble coffee melanoidins in modulating AA content in the coffee brew
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5 633 should be considered.

6 634 The use of unconventional beverage treatments could represent an interesting strategy for
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8 635 AA mitigation. In particular, for the removal of AA from coffee brew, Cha (2013)
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10 636 proposed a biochemical treatment which consisted in the use of the bacterial enzyme
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12 637 acrylamidase directly added into instant coffee beverages, obtained with different amount
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14 638 of coffee and incubated at 37 and 70 °C for 30 min. The AA in coffee beverage was
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16 639 partially degraded by the enzyme in relation to incubation temperatures and coffee
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18 640 concentrations, and completely degraded in coffee maintained at 70 °C. However, the
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20 641 impact of this treatment on the sensorial and safety quality of coffee brew was not
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22 642 evaluated. In fact, it is known that the enzyme acrylamidase hydrolyses AA to acrylic
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24 643 acid (a less toxic compound than AA but corrosive for skin or mucosa) and ammonia, that
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26 644 may affect the brew sensory profile (Anese 2015). Considering a possible industrial
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28 645 application of AA biochemical removal, it is necessary to evaluate the acrylamidase
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30 646 enzyme costs. Bedade, Sutar, and Singhal (2019) proposed to immobilize the bacterial
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32 647 acrylamidase on chitosan coated alginate beads. The immobilized acrylamidase was
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34 648 successfully used in batch and continuous packed column operation, degrading
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36 649 completely AA in instant coffee brews after 60 min of treatment with an initial
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38 650 concentration of 100-500 mg·l⁻¹. In addition, it was demonstrated that the immobilized
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40 651 enzyme presented better pH, thermal and storage stability than the free one with a
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42 652 reusability up to four cycles application for AA removal. The results obtained with the
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44 653 immobilised enzyme could be very important for a possible industrial application, also if
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46 654 the presence of possible hydrolysis compounds have not been identified.

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48 655 Another biochemical treatment proposed by Akıllıoğlu and Gökmen (2014) for the
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50 656 mitigation of AA in coffee beverage was baker's yeast (*Saccharomyces cerevisiae*)
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52 657 fermentation. In this study, instant coffee beverages were added with sucrose (1-10% w/v)
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54 658 and baker's yeast (1-2% w/v) and were kept at 30 °C in an orbital shaker (100 rpm). AA
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56 659 reductions of about 46 and 62% were achieved after 24 and 72 h of fermentation
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58 660 respectively, by using 1% of yeast and 10% of sugar. These results revealed that sugar
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60 661 and yeast concentration affect the AA reduction during coffee brew fermentation.
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663 However, the effect of the applied biochemical treatment on sensorial characteristics of
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665 coffee brew, and the presence of some metabolic degradation product have not been
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667 investigated.

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3 665 Narita and Inouye (2014) studied the effect of heat sterilization treatment (121 °C for 6
4 666 min) combined with the addition of different additives on the AA content decrease in
5 667 canned coffee. The highest AA reduction in canned coffee were reached during heat
6 668 treatment with the addition of cysteine and dithiothreitol (a compound with two -SH
7 669 groups) and was respectively of 90 and 45%. These results emphasise the important role
8 670 of thiol groups in decreasing the AA content in coffee beverages. This treatment appears
9 671 promising because it can be applied in the normal canned coffee manufacturing processes.
10 672 However, also in this research the impact of the used compounds on the organoleptic
11 673 attributes of canned coffee was not tested, nor considered for subsequent studies.
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675 [Table 5 near here]

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677 **5. Conclusions**

678 The intake of AA in coffee and coffee products is reported extensively in literature,
679 however less studies and feasible solutions are available on possible AA
680 reduction/mitigation strategies.

681 As for the intake of AA in coffee through various stages of production it is quite
682 established that, in order to reach low levels in the final product, it is necessary:

- 683 • to select good quality green coffee, removing defective beans;
- 684 • to prefer Arabica than Robusta specie, or higher Arabica content in the blends;
- 685 • to roast at the highest thermal input (“very dark” degree);
- 686 • to prefer shorter brewed coffees than longer ones.

687 In particular, it has been clearly showed that darker roasted coffees are characterized by
688 a lower AA content compared to light and medium ones due to its degradation during the
689 process. Moreover, from the reviewed literature, it seems that a certain AA loss during
690 storage of packed roasted coffee can occur. Nevertheless, the reduction of AA in darker
691 roasted coffee or obtained by prolonged storage, may not represent a promising option.

692 **In fact, coffee roasted at stronger degrees are appreciated by South European consumers**
693 **while North European and American ones prefer lighter roasting levels; moreover, the**
694 **possible formation of other toxic compounds (e.g. HMF, furan, furfuryl alcohol) which**
695 **may have a negative effect on human health, has to be taken into account.**

696 **As for the prolonged storage of roasted coffee** it can be directly linked to a simultaneous
697 reduction of desired organoleptic properties of the final beverage.

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3 698 In addition, few innovative strategies for AA reduction/mitigation have been proposed
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5 699 such as enzymatic treatments of raw material, vacuum or steam roasting alternative
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7 700 technologies, roasted beans supercritical fluid extraction, final beverage treatments such
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9 701 as yeast fermentation and amino acids/additive additions. However, most of these
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11 702 researches are lacking important information and are not completely exhaustive; some
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13 703 presented AA mitigation strategies have to be evaluated based also on their impact on the
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15 704 highly important sensorial and nutritional properties of the final coffee brew. **Sensory**
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17 705 **analysis should be included in the processing strategies used to achieve AA reduction in**
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19 706 **coffee brew, since it is of utmost importance for the applicability of each proposed**
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21 707 **methods. This challenge is even more relevant in coffee because minor changes in the**
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23 708 **standardized production processes systematically result in a significant decline of the**
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25 709 **final product's quality.** Moreover, some procedures proposed by the literature are difficult
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27 710 to be scaled-up at industrial level in the existing manufacturing processes being less
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29 711 practical, technological unfeasible and costly
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31 712 In conclusion, further studies are still needed in order to find appropriate and practical
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33 713 solutions for AA mitigation in coffee in a more holistic risk-benefit approach, which takes
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35 714 into account side effects such as the possible presence/formation of other toxicants in the
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37 715 matrix, derived from applied reduction measures or from Maillard reaction, without
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39 716 leading to the reduction of both beneficial compounds, such as antioxidants (Lachenmeier
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41 717 et al. 2019), and organoleptic properties. Only in this way and following the ALARA (as
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43 718 low as is reasonably achievable) concept (Food Drink Europe 2019) it will be possible to
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45 719 investigate other strategies for AA levels reduction in final coffee product.
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- 1002 Figure 1. Scheme of coffee processing, from the cherry to the brew. **The critical steps**
1003 **for the reduction of acrylamide in coffee are highlighted.**
- 1004 Figure 2. Simplified scheme of possible routes of acrylamide (C_3H_5NO) formation
1005 during roasting in coffee.
- 1006 Figure 3. Selected typical profiles of acrylamide formation during coffee roasting
1007 processes as reported in some literature studies (A: Arabica, R: Robusta, n. r.: not
1008 reported).

Table 1. Intervention strategies studied in literature for the reduction of acrylamide level in coffee throughout the main processing steps.

Process step	Intervention strategies	Reference
Raw material	Selection of variety	Bagdonaite and Murkovic (2004)
		Lantz et al. (2006)
		Summa et al. (2007)
		Bagdonaite, Derler, and Murkovic (2008)
		Alves et al. (2010)
	Post-harvest processing	Lachenmeier et al. (2019)
		Bertuzzi et al. (2020)
		Illy and Viani (1995)
	Quality of green coffee beans	Knopp, Bytof, and Selmar (2006)
		Soares, Alves, and Oliveira (2014)
Asparaginase pre-treatment	Mazzafera (1999)	
	Dias et al. (2012)	
	Dria et al. (2003)	
Decaffeination	Hendriksen (2013)	
	Lynglev and Schoesler (2014)	
	Navarini et al. (2014)	
Roasting	Time and temperature optimization	Alves et al. (2010)
		Bertuzzi et al. (2017)
		Bagdonaite and Murkovic (2004)
		Şenyuva and Gökmen (2005)
		Lantz et al. (2006)
		Summa et al. (2007)
		Bagdonaite, Derler, and Murkovic (2008)
		Alves et al. (2010)
		Kocadağlı et al. (2012)
		Madiah et al. (2013)
Mojska and Gielecińska (2013)		
Budryn, Nebesny, and Oracz (2015)		
Lachenmeier et al. (2019)		
Wawrzyniak and Jasiewicz (2019)		
Bertuzzi et al. (2020)		
Steam treatments	Vacuum roasting	Theurillat et al. (2006)
		Anese et al. (2014)
		Supercritical fluid extraction
Storage	Time and temperature optimization Secondary shelf-life	Banchero, Pellegrino, and Manna (2013)
		Andrzejewski et al. (2004)
		Delatour et al. (2004)
		Hoenicke and Gatermann (2005)
		Taeymans et al. (2005)
Brewing	Selection of brewing methods	Lantz et al. (2006)
		Soares, Cunha, and Fernandes (2006)
		Alves et al. (2010)
Enzymatic treatment	Enzymatic treatment	Cha (2013)

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		Bedade, Sutar, and Singhal (2019)
	Yeast fermentation	<hr/>
		Akıllıoğlu and Gökmen (2014)
	Additive addition	<hr/>
		Narita and Inouye (2014)

Table 2. Studies in the literature concerning the impact of raw green coffee varieties on acrylamide levels in roasted products.

Green coffee variety	Roasting equipment	Roasting parameters	Max acrylamide level found during roasting (ng·g⁻¹)	Reference
Arabica	Laboratory roaster	T= 250 °C t= 7.5 min	< 500	Bagdonaite and Murkovic (2004)
Robusta			> 3500	
Arabica	Fluidized bed convection roaster	T= n. r. t= 2.5 min	251 ± 45	Lantz et al. (2006)
Robusta			378 ± 32	
Arabica	Bench-scale roaster	T= 236 °C t= 370 s	> 200	Summa et al. (2007)
Robusta		T= 236 °C t= 430 s	> 250	
Arabica	Small-scale convection roaster	T= 240 °C t= 7.5 min	374 ± 86	Bagdonaite, Derler, and Murkovic (2008)
Robusta			708 ± 77	
Arabica	Probat L12 roaster	T= 210 °C	178*	Alves et al. (2010)
Robusta		t= 10 min	355*	
Arabica	Laboratory roaster	Six different roasting profiles	from 130 to 470	Lachenmeier et al. (2019)
Robusta			from 250 to 480	
Arabica	Horizontal rotating drum	T= 136-138 °C	1045 ± 56	Bertuzzi et al. (2020)
Robusta	industrial-scale roaster	t= 10 min	795 ± 45	

T: temperature, t: time, n. r.: not reported.

*Data calculated from acrylamide levels measured by authors in espresso brews obtained from Arabica and Robusta coffee.

Table 3. Studies in the literature concerning the impact of roasting conditions on acrylamide levels in roasted coffee.

Green coffee variety	Roasting equipment	Roasting parameters	Reference
Robusta	Static oven	T= 220, 240, 260 °C t= 5-10-15 min	Bagdonaite and Murkovic (2004)
n. r.	Static oven	T= 200 and 225 °C t= 5-30 min	Şenyuva and Gökmen (2005)
Arabica and Robusta	Fluidized bed roaster		Lantz et al. (2006)
	Rotating fluidized roaster	T= n. r. t= 1.5-16 min	
	Drum roaster		
Arabica	Laboratory roaster	T= 236 °C t= 235-560 s	Summa et al. (2007)
Robusta		T= 236 °C t= 260-620 s	
Arabica and Robusta	Static drying oven	T= 220, 240, 260 °C t= 5-15 min	Bagdonaite, Derler, and Murkovic (2008)
Arabica and Robusta	Probat roaster	T= 210 °C t= 8-11 min	Alves et al. (2010)
Arabica	Static oven	T= 220 °C t= 5-60 min	Kocadağlı et al. (2012)
Arabica	Probat roaster	T= 155-185 °C t= 15-30 min	Madihah et al. (2013)
Robusta	Pilot roaster	T= 190, 203, 216 °C t= 20-35 min dry or humid air air velocity= 0.5-1 m·s ⁻¹	Budryn, Nebesny, and Oracz (2015)
Arabica and Robusta	Laboratory roaster	Six different roasting profiles	Lachenmeier et al. (2019)
Arabica and Robusta	Industrial roaster	T=90-215°C t= 4-16 min	Bertuzzi et al. (2020)

T: temperature, t: time, n. r.: not reported.

Table 4. Studies in the literature concerning the decrease of acrylamide in roasted coffee and coffee products during storage at different conditions.

Roasted coffee type	Packaging	Storage conditions	Acrylamide loss	References
Ground	Opened original package	T= room t= 6 months	40-65%	Andrzejewski et al. (2004)
		T= -40 °C T= 8 months	0%	
Ground	Opened original package	T= room t= 7 months	28%	Delatour et al. (2004)
Soluble		T= room t= 12 months	66%	
Beans	Closed original package		30%	Hoenicke and Gatermann (2005)
Ground	Closed vacuum package	T= 10-12 °C t= 3 months	31%	
Soluble	Closed original package		0%	
Beans	Closed jar	T= 60 °C t= 34 hours	30%	Taeymans et al. (2005)
	Opened jar		0%	
Ground	Closed vacuum package	T= -18 °C t= 12 months	14%	Lantz et al. (2006)
		T= 4 °C t= 12 months	25%	
		T= room t= 12 months	80%	
		T= 37 °C t= 12 months	88%	
Soluble	Closed package		12/18% (*)	Michalak et al. (2016)
Coffee substitutes		T= 4* and 25** °C t= 6/12 months	20/33% (**)	
			7/12% (*) 7/28% (**)	

T: temperature, t: time.

Table 5. Studies in the literature concerning the impact of brewing techniques and conditions on acrylamide levels in final coffee beverage.

Roasted coffee type and variety	Brewing techniques	Brewing conditions	Acrylamide extraction and level	Reference
Arabica and Robusta	Horeca pour-over system (5 l capacity)	C/W= 46 g·l ⁻¹	104%	Lantz et al. (2006)
	Household drip-filter (1 l capacity)	C/W= 49 g·l ⁻¹	102%	
	Plunger pot	C/W= 49 g·l ⁻¹	99%	
	Fresh brew filter coffee (vending machine)	C/W= 62 g·l ⁻¹	102%	
	Espresso (manual equipment)	C/W= 146 g·l ⁻¹	75%	
Commercial roasted	Espresso	C/W= 200-230 g·l ⁻¹ t= 30 ±5 s p= 9 ±2 bar	21 µg·l ⁻¹	Soares, Cunha, and Fernandes (2006)
Instant	Water solution	C/W= 200 g·l ⁻¹	72.4 µg·l ⁻¹	
Arabica Robusta	Espresso	C/W= from 93 to 325 g·l ⁻¹	from 62 to 99% from 59 to 98%	Alves et al. (2010)

C/W: coffee/water ratio, t: time, p: pressure.

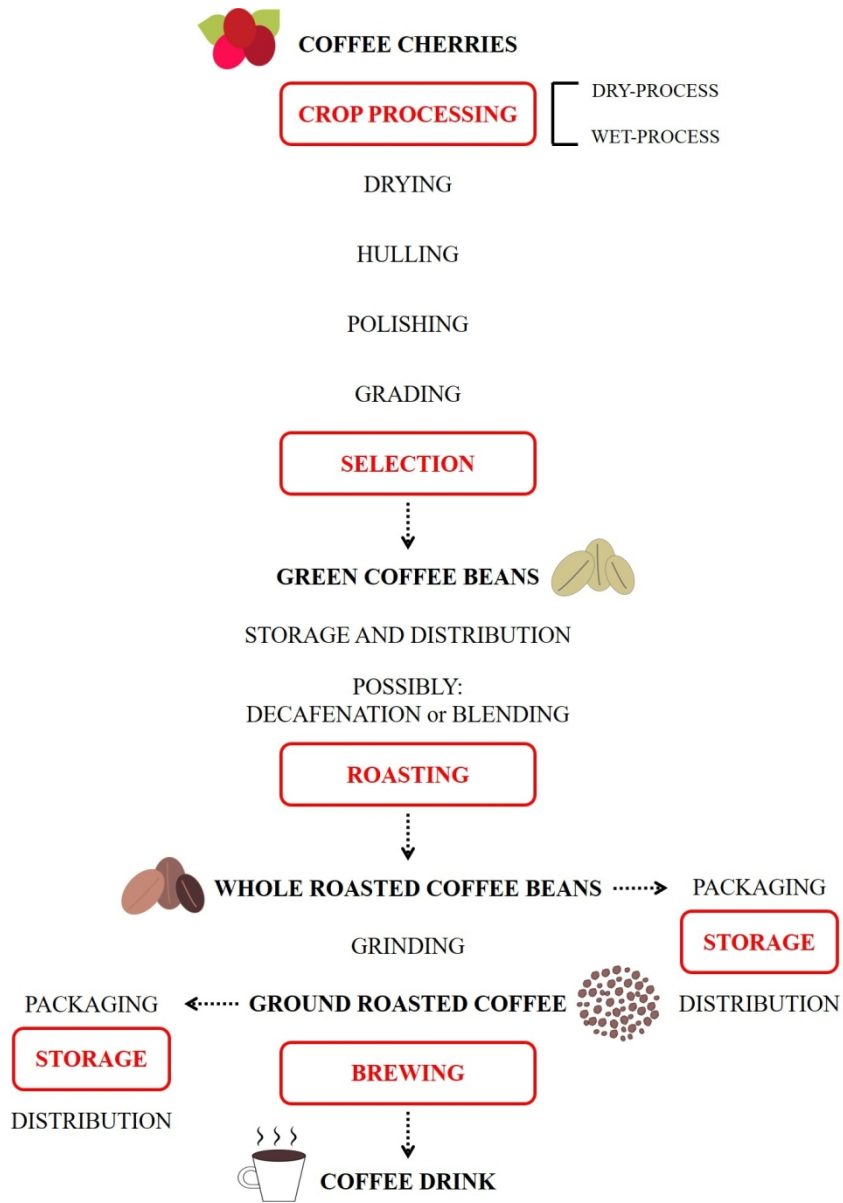


Figure 1. Scheme of coffee processing, from the cherry to the brew. The critical steps for the reduction of acrylamide in coffee are highlighted.

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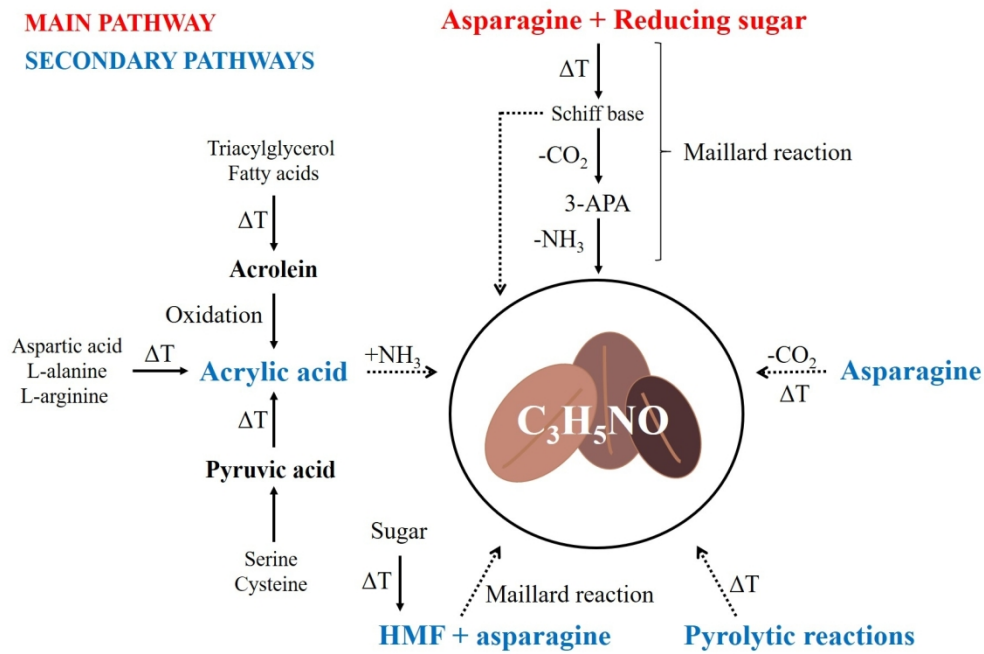


Figure 2. Simplified scheme of possible routes of acrylamide ($\text{C}_3\text{H}_5\text{NO}$) formation during roasting in coffee.

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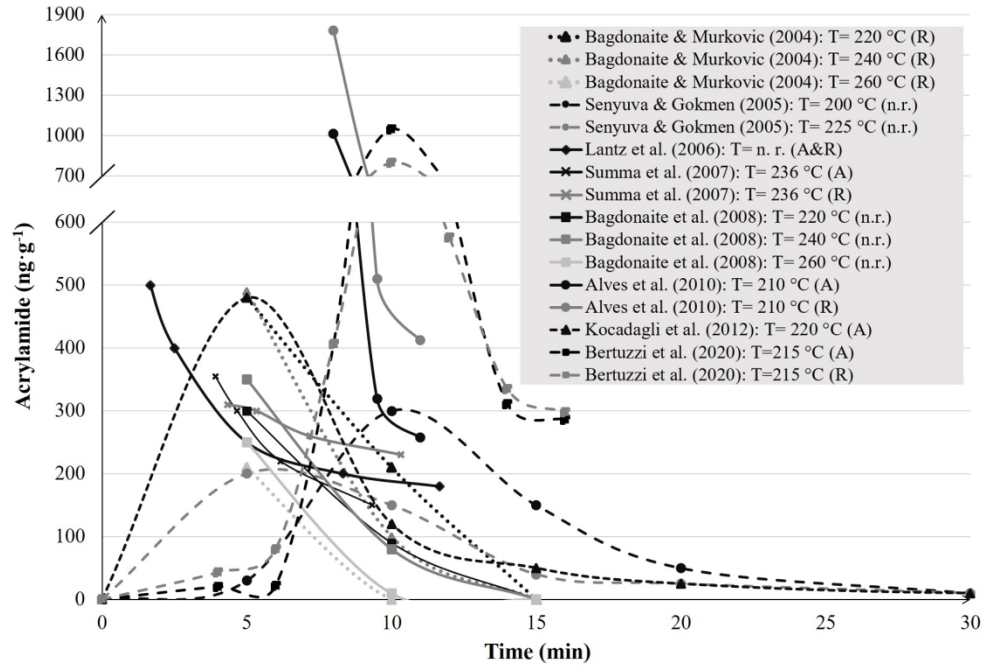


Figure 3. Selected typical profiles of acrylamide formation during coffee roasting processes as reported in some literature studies (A: Arabica, R: Robusta, n. r.: not reported).

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