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

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Abstract

Acrylamide (AA) is a product of food heating process that is widely present in cooked foods and known to be toxic to humans. Exposure data has revealed coffee to be one of the sources of this toxicant in adult diets. A great deal of effort has been invested into finding ways of reducing AA formation during coffee processing. However, despite the accumulated knowledge and mitigation strategies applied so far, AA reduction in coffee is still a challenge compared to other heat-processed foods in which the wider raw-material selection and progress in technological processes and/or changes in the recipes are possible at the industrial level. This review presents a critical analysis of the accumulated knowledge on the formation of AA in coffee as well as on the mitigation strategies that have been investigated to date, with a focus on current applicability in industry and little explored topics.

Keywords: Acrylamide, coffee, precursors, formation, mitigation strategies

1. Introduction

Acrylamide (AA) is a highly water-soluble organic compound. AA is currently studied mostly because of its high toxicological potential and widespread occurrence in food products (Rannou, Laroque, Renault, Prost, & Sérot, 2016). High levels of AA are found in potato chips, French fries, biscuits and roasted coffee, and it is formed in foods that are prepared at temperatures above 120 °C and possess low moisture (EFSA, 2015; EU Commission, 2017). AA has notably been classified by the International Agency for Research on Cancer as "probably carcinogenic to humans" (Group 2A) (IARC, 1994). However, in 2016, coffee drinking was evaluated by the IARC as being "not classifiable as to carcinogenicity" (Group 3) (Esposito et al., 2020; Loomis et al., 2016). The benchmark levels (μg/kg) of AA in foods are reported in EU regulation 2017/2158; in coffee they are 400 μg/kg for roasted coffee and 850 μg/kg for instant coffee (EU Commission, 2017).

A dietary-habit survey performed in over 20 countries showed that European citizens have an average daily AA intake that ranges from 0.14 to 1.31 mg/kg of body weight (bw). Similar levels were also recorded in the USA. Daily AA intake/kg bw may be especially higher in children whose relative intake, with respect to body weight, is higher, in particular, because of the concurrent consumption of baked cereals and crisp products (Semla, Goc, Martiniaková, Omelka, & Formicki, 2017). In the adult and elderly populations (20–79 years), coffee is one of the main contributors of AA intake, ranging from 9% to 29%, with that figure reaching 38–60% for baked goods and crisps, depending on the country of origin. AA concentration in coffee ranges from an average of 249 μg/kg to 710 μg/kg (average values referring to the dry powder) for roasted coffee and instant coffee respectively. As reported in the EFSA's scientific opinion on AA in food, the results were expressed in powder equivalents according to the dilution factor used to prepare the beverage. However, if we consider the respective dilution factors (from 0.035 to 0.125 for roasted coffee and 0.017 for instant coffee), some beverages obtained from roasted coffee would then contain higher AA levels than those made from instant coffee (EFSA, 2015).

Coffee is one of the most consumed beverage in the world because of its pleasant aroma, which is caused by the large range of volatiles that are produced during the roasting process (Toledo, Pezza, Pezza, & Toci, 2016). Roasting is a traditional thermal process with the primary objective not only being to achieve the desired flavour, but also to generate a dark colour and a brittle, porous texture in the bean suitable for successive grinding and brewing. The high production temperature induces extensive chemical reactions, dehydration and profound changes in the microstructure (Folmer, 2017). At the same time, roasting leads to the development of undesired compounds of concern, such as AA and furans (Schouten, Tappi, & Romani, 2020).

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

2. AA physico-chemical characteristics

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

AA is stable at room temperature, but polymerizes when heated to its melting point and even when exposed to ultraviolet radiation (WHO/IPCS, 1999). AA thermally decomposes to form ammonia and carbon monoxide, carbon dioxide and nitrogen oxides (Kitahara et al., 2012; Maan et al., 2022). AA stability is quite high in aqueous solutions, but decreases under dry conditions and can be influenced by pH and the nature of the buffer (Adams, Hamdani, Lancker, Méjri, & De Kimpe, 2010). The stability of AA and its reactivity with relevant nucleophiles from various foods at elevated temperatures have been studied by Adams *et al.* in model systems. Amino acids with nucleophilic side chains decrease the amount of free AA; cysteine (Cys) is the most reactive, while other less reactive nucleophiles, such as lysine (Lys), arginine (Arg), serine (Ser) and ascorbic acid gave similar condensation products (Adams, Hamdani, Lancker, Méjri, & De Kimpe, 2010). As an unsaturated carbonyl compound with electrophilic properties, AA can react, via Michael addition with biological nucleophilic groups including amines, carboxylates, aryl and alkyl hydroxyls, imidazoles and thiols of macromolecules (e.g. Cys residues), DNA and proteins. This reactivity is the basis of its toxicity (Nehlig & Cunha, 2020).

3. Mechanism of acrylamide formation

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

3.1 Formation via the Maillard reaction

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

In contrast to fried snacks and bakery products, green coffee apparently does not contain a source of free carbonyl compounds. However, alternative reactive carbonyls derive from linoleic acid hydroperoxide degradation or from saccharide degradation at high temperature (Belitz, Grosch, 2009). These carbonyls facilitate AA formation. Sucrose was the only sugar detected, at a concentration of approximately 8.0% in green coffee, and it tends to decompose during roasting within 15 min at 220°C (Kocadagli, Göncüoglu, Hamzalioğlu, & Gökmen, 2012).

A range of different carbonyl compounds are involved in acrylamide formation: hydroxycarbonyls, dicarbonyls (Amrein et al., 2004; Stadler et al., 2004; Zyzak et al., 2003) and alkadienals from lipid oxidation (Gökmen, Kocadağli, Göncüoğlu, & Mogol, 2012; Hidalgo, Delgado, & Zamora, 2009; Kocadagli et al., 2012; Zamora & Hidalgo, 2008) have been investigated so far. Model studies have demonstrated that α-hydroxy carbonyls are much more effective than α-dicarbonyls in converting Asn into AA as they promote the rearrangement of azomethine ylides, which are degradation products of the Schiff base (Gökmen et al., 2012; Stadler et al., 2004). The β-elimination reaction of the decarboxylated Amadori compound is the subsequent step and gives AA (Stadler et al., 2004; Zyzak et al., 2003). However, Hamzalioğlu and Gökmen have, more recently, used a multi-response kinetic modelling to show that the 3-deoxyglucosone (3-DG) was the most abundant dicarbonyl to be formed from sucrose degradation and from the Maillard reaction during roasting that participates in producing AA (Hamzalıoğlu & Gökmen, 2020). 5-Hydroxymethylfurfural (HMF), which is the major sugar-decomposition product generated during roasting, can play a role in AA formation, and can generate more AA than glucose when heated together with Asn (Anese, 2016; Kocadagli et al., 2012). HMF is formed and accumulated during the early stages of roasting due to the simultaneous consumption of sucrose. It reaches its maximum content within 10 min of roasting at 220°C and then decreases (Figure 1C) (Gökmen et al., 2012). Cai et al. (Cai et al., 2014) have reported that the addition of chlorogenic acid (0.5 and 5 µmol/mL) to the Asn/glucose-Maillard reaction system significantly promotes AA formation, mainly by increasing HMF formation, while inhibiting its elimination. A comprehensive kinetic model, including the elementary steps for acrylamide formation, was proposed by Hamzalioğlu *et al.,* in 2020. Changes in sucrose, reducing sugars, free amino acids, Asn, AA, 3-DG, methylglyoxal, glyoxal and HMF were monitored during coffee roasting at 200, 220 and 240 °C. The results of the multi-response kinetic modelling approach indicate that sucrose degrades into glucose and a reactive fructofuranosyl cation that principally contributed to the formation of HMF, which, in turn, was found to be the most important reactive carbonyl compound in the formation of AA in coffee during roasting. Conversely, glucose mostly takes part in the formation of intermediates, glyoxal and especially 3-DG, rather than AA. Therefore, any ingredient/component that promotes HMF formation also increases AA generation. By contrast, the quinone derivative of chlorogenic acid decreases AA formation via H_2O_2 oxidation. However, this mechanism requires further investigation (Hamzalıoğlu & Gökmen, 2020).

3.2 Formation via triglyceride decomposition

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

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

3.3 Formation via pyrolysis

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

4. Factors that affect Acrylamide levels

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

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

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

4.1.1 Influence of coffee species: Arabica and Robusta

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

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

They are characterized by different levels of amino acids, sugars and minerals, volatiles, chlorogenic acids and caffeine (Guenther et al., 2007). Many studies have reported that significantly higher amounts of AA are found in Robusta than in Arabica (Esposito et al., 2020; Lachenmeier et al., 2019). This difference seems to be associated with higher Asn content in raw Robusta beans than in Arabica (Alves, Soares, Casal, Fernandes, & Oliveira, 2010; Bagdonaite et al., 2008; Lantz et al., 2006). In 2008, Bagdonaite *et al.*, investigated the influence of the concentrations of possible precursors in green coffee, such as amino acids, sucrose and carbohydrates, on AA formation and concluded that higher Asn content resulted in higher AA amounts. Robusta coffee was found to contain higher levels of Asn (the concentration of free Asn was 797 µg/g in Robusta and 486 µg/g in Arabica) and lower amounts of sucrose, and was confirmed to have a higher AA concentration than the investigated Arabica coffee (Bagdonaite et al., 2008; Bertuzzi, Martinelli, Mulazzi, & Rastelli, 2020). Hu *et al.* (Hu, Liu, Jiang, Zhang, & Zhang, 2021) have very recently shown that individual addition of free amino acids (i.e. Ala, Arg, Lys, Cys, Ser and Glycine (Gly), Phenylalanine (Phe), Tryptophan (Trp), and Glutamine (Gln)) in a model system solution heated in a hot-air roaster at 180°C for 5 min, promotes the AA formation. In addition, it has been observed a positive correlation between roasting time and AA amount. The authors speculated that the high level of AA at the early stages of roasting may also be due to the presence of other amino acids. Moreover, they observed that the addition of Gly and Asp can reduce AA formation, and proposed their addition during roasting. However, these findings contradict those of other authors (Guenther et al., 2007; Navarini, Terra, Colomban, Lonzarich, & Liverani, 2014; Yasuhara et al., 2003).

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

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

4.1.2 Influence of coffee origins

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

4.1.3 Post-harvest processing and AA precursors

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

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

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

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

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

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

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

Last, but not least, storage and transportation should be considered in the post-harvest treatments. Beans are stored in parchment or hulled in order to allow them to reach equilibrium more quickly before shipment. Hulled beans can change their viability, which affect their composition regardless of the processing. During shipment and transportation, the beans are subjected to changes in climate zones and meteorological conditions, and often remain in containers for long periods of time during port customs clearance procedures without proper temperature and humidity control, which can potentially affect their composition and be a source of contamination (Bytof, 2021). To the best of the Authors' knowledge, studies on these steps in post-harvest treatment, in general, and on their influence on AA precursors, more specifically, are lacking in the literature.

4.1.4 Influence of poor quality coffee beans

The number of poor quality beans in batch acceptance and production is a central factor for evaluation in quality control. The presence of flaws and blemishes may be associated with specific problems during harvesting and post-harvest processing operations and therefore influence AA precursors. Black beans are the result of dead beans within the coffee cherries or beans that fall naturally on the ground via the action of rain or over-ripening. Immature-black beans are those that fall to the ground, remain in contact with the soil and are thus subject to fermentation (Mazzafera, 1999). A study carried out by Dias *et al.*, has shown that the peeling of immature fruits leads to a reduction in Asn levels and can therefore indirectly contribute to reducing AA formation in coffee (Dias, 2010).

4.1.5 Decaffeination processing

The economic impact of decaffeinated coffees is generally underestimated, but the consumers of this coffee come from a large and reliable group, including so-called millennials (the generation of young people born between the end of the 1980s and the beginning of the 2000s) who are faithful consumers by choice and not because it was "suggested by a doctor" (Conway, 2019; Folmer, 2017). The industrial decaffeination process, involves bean pre-wetting with water, caffeine extraction and subsequent bean drying. Three methods are used to remove caffeine from the green beans: solvent extraction, water extraction and pressurized carbon dioxide. The ideal decaffeination process removes the caffeine from the bean cells without any other alteration to the bean. The industrial decaffeination process of green coffee beans does not significantly affect the final AA content, (Alves et al., 2010; Bagdonaite et al., 2008; Bertuzzi, Rastelli, Mulazzi, & Pietri, 2017), probably because the process does not influence the content of AA precursors.

5. Roasting

The process of roasting is a fundamental and key step in converting green beans into flavourful roasted coffee with physical properties for good quality in the cup, and sensory properties such as colour, aroma and taste. However, the process and roasting parameters, such as temperature and time, also affect AA levels. Coffee behaves differently than other AA-producing foods. While AA content typically rises with colour or browning degree, due to its origin as a Maillard reaction product, it decreases from light to very dark roasts (Lachenmeier et al., 2019; Summa et al., 2007). AA formation is higher in the early stages of roasting, as has been shown by Bagdonaite (Bagdonaite et al., 2008) who found that, when applying different roasting times (from 5 to 15 min) and temperatures (from 220 to 260°C), the highest concentrations of AA were obtained at low temperatures (220 °C) and short roasting times (5 min). The amount of AA during roasting exponentially increases initially, reaches a maximum and then rapidly decreases. Under more intense roasting conditions, AA was degraded until it could no longer be detected, while Asn and the other precursors decreased mainly because of reactions induced by the thermal process. In 2007, Summa *et al.* (Summa et al., 2007) reported lower AA concentrations in Arabica than in Robusta, when roasted in a hot air roaster at 236 °C to a medium degree. AA occurrence was extremely variable and strictly correlated to both the roasting parameters and the coffee species and, thereby, to the composition of the blends. Few studies have been conducted on the relationship between the amount of AA and coffee origins. Lantz (Lantz et al., 2006) analysed a significant number of green beans (17 Arabica and 6 Robusta), that were roasted to a medium degree in a rotating fluidized bed roaster for 2.5 min to light colour, and they concluded that the main factor affecting the level of AA is the ratio between the two species in the blends, with Robusta producing higher AA levels on average. Time and roasting are the most significant parameters, with both shorter and lighter roasting giving higher AA levels.

Lantz and co-workers also studied the kinetics of the formation of AA in coffee samples over 90–720 s of roasting to different roasting degrees (measured by light reflectance, LRU index) in three roasters: A) a fluidized bed roaster with mechanically supported coffee beans movement, and a green coffee batch size of 2 kg (Probat RT 3SY/Emmerich/Germany); B) a rotating fluidized bed roaster with heat transfer by convection, and a batch size of 2 kg (Neuhaus Neotec RFB6/Reinbek/Germany); and C) a drum roaster with heat transfer mainly by conductivity, and a batch size of 0.5 kg (Probat PRG500/Emmerich/Germany). The authors concluded that the maximum level of AA, independently on the roaster, is formed early during the heating process and then decreases with increasing roasting time and degree (403 µg/kg of AA at LRU > 95 (very light) after 135-150s, while it is absent at LRU < 65 (very dark) after 670 – 870 s) (Lantz et al., 2006).

Studies using deuterium-labeled AA that was spiked into green coffee beans confirmed that the amount of AA increases exponentially at the onset of roasting, reaching an apparent maximum of 2000 µg/kg, and then decreases rapidly as the rate of degradation exceeds the rate of formation (Alves et al., 2010). Very high levels of AA were detected in the lightest roasted coffee samples, with maximums of 1240 and 2190 µg/kg, for Arabica and Robusta, respectively. The concentration of the undesired molecule decreased proportionally, in the two species, with the increase of roasting degree in both ground coffee and espresso brews. Table 1 lists the studies on the impact of roasting conditions on AA levels in roasted coffee, as reported in the literature.

Kocadagli (Kocadagli et al., 2012) discussed the kinetics of the formation of AA from HMF, which reduces with increasing roasting degree, and Lachenmeier *et al.* (Lachenmeier et al., 2019) confirmed this behaviour for AA only. They explained these results using different roasting conditions: i) Kocadagli: oven at 220°C for 5-10-20-30-60 min; ii) Lachenmeier: laboratory roaster using six different roasting profiles, namely coffee roasting (fast and slow drying), espresso roasting (fast and slow drying), Scandinavian roasting (very light roasting) and Neapolitan roasting (very black roasting).

However, the roasting process is often carried out in small-scale roasters at fixed temperatures. Bertuzzi *et al.*, (Bertuzzi et al., 2020) investigated trends in AA content using an industrial coffee roasting process from 90°C to 215°C for 16 min. Quite surprisingly, the authors found the maximum AA level in Arabica. They hypothesized that this may be due to the higher concentration of sucrose and reducing sugars in Arabica compared to Robusta. During roasting, reducing sugars tend to initially increase because of the thermal hydrolysis of sucrose, and then to decrease due to AA formation after 10 minutes (Bertuzzi et al., 2020). Most studies focus on the dominant AA formation during the first period of roasting and its decrease with the intensification of the thermal process. Conversely, few studies have reported data on AA evolution over prolonged roasting times. Pastoriza *et al.*, (Pastoriza, Rufián-Henares, & Morales, 2012) suggested that the decrease in AA may be due to its chemical interaction with melanoidins, whose concentration increases with roasting time and that seem to act as modulators of AA levels. AA continuously decreases at 180°C from 6 minutes of roasting, compared to control samples, probably because of its thermal decomposition. The AA decrease was found to be dose-response and related to the reaction time and initial amount of melanoidins in the media. By contrast, pH (from 3.5 to 7.0) did not have a significant effect on AA reactivity with melanoidins. AA reduction was hypothesized to be due to its reaction with the nucleophilic amino groups of amino acids from the protein backbone of melanoidins, via Michael addition, although the exact mechanism is still unknown. The addition of soluble melanoidins to the brew seem to modulate the content of AA. Badoud *et al.*, (Badoud et al., 2020) investigated the routes of AA degradation with ^{14}C-labeled and stable isotope ^{13}C-labeled materials, and found that approximately 30% of AA was lost to volatilization, and 70% remained in the matrix, of which only 50% was in the free soluble form.

The importance of the roasting process on flavour and colour, and the relatively narrow range for commercial products make AA mitigation in coffee particularly complex.

Indeed, although darker roasting is a potential option to reduce AA, it generates other undesirable compounds, i.e. furans, furfuryl alcohol and HMF, and which definitively affect the final taste.

5.1 Storage of roasted coffee

Several studies have demonstrated that AA is not stable during the storage of packed roasted coffee; with stability depending on time, temperature and the atmosphere inside the package.

Delatour *et al.*, (Delatour, Périsset, Goldmann, Riediker, & Stadler, 2004) reported a reduction in AA, from 771 to 256 µg/kg and from 203 to 147 µg/kg, for soluble and roasted coffee, respectively. The soluble coffee powder was stored at room temperature and in its original tightly closed container for 12 months while the roasted coffee was stored under these same conditions for a period of 7 months. Andrzejewski *et al.*, (Andrzejewski, Roach, Gay, & Musser, 2004) have observed significant losses of AA (40–65%) after 6 months during the secondary shelf-life of coffee, i.e. when the package is opened for home consumption. The content of AA was also measured at -40°C to check whether this loss was related to the temperature. Results indicated that the AA amount at -40°C did not change for 8 months when the same sample of roasted and ground coffee was stored in its original open container, and suggested that AA loss over time only occurs in ground coffee with open containers stored at room temperature. The storage of roasted coffee in open container obviously heavily affects its flavour producing a staling effect and speeds up oxidation processes (Manzocco, Calligaris, Anese, & Nicoli, 2016).

Hoenicke *et al*. (Hoenicke & Gatermann, 2005), however, reported a smaller AA reduction from 305 to 210 µg/kg for roasted and ground coffee and from 285 to 200 µg/kg for roasted beans, after 3 months of storage at 10 – 12°C in sealed vacuum-packs. Under the same conditions, AA was shown to be stable in soluble coffee and in the extracts of coffee substitutes.

AA decrease has been related to temperature. In 2006, Lantz (Lantz et al., 2006) found that there is a clear proportional and temperature dependent decrease in AA levels in vacuum-packed ground and roasted coffees that were stored for 12 months at temperatures between -18 and 37°C. As expected, the most significant AA reduction and rate were registered in the samples stored at the highest temperature (37°C), with this process following second order reaction kinetics. In 2008, Baum (Baum et al., 2008) carried out studies with ^{14}C-labeled AA as a radiotracer on roasted and ground coffee to define the fate of AA that was lost during storage. Coffee samples were spiked with the ^{14}C AA and stored for 48 weeks at room temperature and at 37°C, and the ^{14}C AA was measured in the coffee brew, filter residue and volatiles. Total radioactivity decreased in the brew over storage and, in particular, at 37°C, and increased in the filter residue concomitantly. No formation of volatile ^{14}C-AA-related compounds was detected during storage and coffee brewing. Approximately 90% of the radiolabelled AA in the filter residue (spent R&G coffee) remained tightly bound to the matrix. Michalak *et al.* (Michalak, Gujska, Czarnowska, Klepacka, & Nowak, 2016) also confirmed the results of Delatour *et al.,* (Delatour et al., 2004) as they reported AA reductions of 33% and

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

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

6. Brew preparation

While the majority of published studies focus on the assessment of AA content in coffee beans, some researchers have also investigated the amount of AA that is effectively ingested by consumers in their coffee brews (Alves et al., 2010; Andrzejewski et al., 2004; Bagdonaite et al., 2008). AA intake through coffee beverages depends on consumption habits (type, strength and volume of beverage, and intake frequency), which are influenced by the cultural and personal preferences of consumers. Coffee is ground into powder, with the objective of increasing the surface of the interface between the water and the solid to accelerate the transfer of soluble substances into the brew (Soares, Alves, & Oliveira, 2015). AA is highly soluble in water and is thus easily transferred from the coffee powder to the beverage. Three main processes are used to prepare coffee brews (Figure 2B), decoction (boiled, Turkish, vacuum and percolation), infusion (filter or coffee drip and Neapolitan), and *pressure* (press-pot or French press, moka and espresso). Most of these methods can be identified by their geographical denomination rather than the description of the method itself and are linked to local traditions. Moreover, instant coffee or soluble coffee powder, is also included in this section since it is one of the most widely consumed beverages and shares the extraction of bean components, with the only difference being that this occurs in the technological industrial brewing step before the soluble coffee powder is obtained.

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

Başaran *et al.*, (Başaran, Aydın, & Kaban, 2020) analysed 41 commercial coffees that were obtained from local markets and coffee shops, and found that instant coffee contained higher levels of AA, than traditional Turkish coffee and ready-to-drink (brewed) coffee. The reason for the high amount of AA in instant coffees may be due to their industrial processing, as they are brewed with pressurized liquid water at approximately 175°C. Further evaporation processes, including freeze-drying and spray-drying, concentrate the coffee components including AA (Mussatto, Machado, Martins, & Teixeira, 2011).

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

7. Acrylamide mitigation strategies

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

7.1 Enzymatic treatment of green beans

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

As free Asn is a limiting factor for AA formation in coffee, some authors have studied the possibility of limiting this component in green coffee using asparaginase, and thereby reducing AA formation during roasting (Mottram et al., 2002). A patented enzymatic treatment WO/2004/037007, that is based on the asparaginase method was revealed by The Procter & Gamble Company as a means to reduce the AA content in roasted coffee. However, the complexity of the preliminary treatments that must be performed on the green coffee beans to ensure an effective interaction between the enzyme solutions and the Asn contained in the beans is a significant drawback. Hendriksen *et al.* (Hendriksen, 2013) evaluated the effect of different doses of asparaginase on the reduction of Asn levels in green coffee. The results indicated that treating green coffee beans with low doses of asparaginase (2000–6000 ASNU) produced a 70–80% decrease in Asn and a 55–74% decrease in AA after roasting. A major obstacle here is ensuring the homogeneous distribution of the active enzyme over the entire substrate.

A number of techniques improve the contact between the enzyme and coffee substrate. Pre-treatment can facilitate the extraction and contact between Asn and asparaginase, promoting its migration into the beans. Dria *et al.*, (Dria et al., 2007) listed a series of pre-treatments for this, including drying, hydrating, rinsing with or without mechanical action, pressurizing, steaming, blanching, heating, reduced-pressure processing and particle-size reduction. These processes were very often not verified, even for organoleptic impact, and are not applicable at the industrial level (Anese, 2016). In addition, Navarini *et al.* in 2014 patented a method to reduce AA enzymatically in a water extract of green beans. The enzymes used in this method were asparaginase and aspartase in solution. The authors found that Asn and Asp are present in similar concentrations in the extract and that Asp contributes to the formation of AA, although in lower amounts compared to Asn. After enzymatic treatment, the water extract was re-incorporated into the green beans before roasting. This treatment gave an AA reduction of about 70%, without affecting the organoleptic properties of the final brew (Navarini et al., 2014). This hypothesis, although of interest because in addition to the AA reduction do not seem to affect coffee sensory properties, has not been demonstrated in peer reviewed article(s). It is also contrasted by several authors who report that the effect of Asp on AA formation is negligible, and who strongly support the correlation between Asn and AA (Belitz et al., 2009; Dias et al., 2012; Guenther et al., 2007; Schouten et al., 2020). In 2019, Porto *et al.* (Porto, Freitas-Silva, Souza, & Gottschalk, 2019) treated Arabica and Robusta coffee beans with asparaginase, and obtained Asn reductions of approximately 60% and 35%, respectively. The beans were pre-treated for 30-45 minutes with steam to open the pores and favour the enzymatic process. In the same way, Corrêa *et al.*, (Corrêa et al., 2021) have shown that the pre-treatment of Arabica coffee beans with steam improved the results of asparaginase treatment, with an AA reduction close to 59%, compared to the control sample, and 77% compared to the blank sample.

7.2 Mitigation using roasting strategies

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

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

Alternative roasting technologies have been attempted. Theurillat *et al.* (Theurillat, Leloup, Liardon, Heijmans, & Bussmann, 2006) evaluated the use of a steam/pressure roasting pilot plant unit. Results showed that the steam treatment carried out on green and roasted coffee did not significantly influence the final AA content in either of the final roasted samples.
Anese *et al.*, (Anese et al., 2014) subjected coffee beans to a medium-roasting process under reduced pressure conditions leading to a reduction in AA levels of 50% compared to conventionally roasted coffee, and a minimal impact on sensory properties. The low-pressure conditions generated inside the roaster, which exerted a stripping effect, preventing AA from being accumulated. Nevertheless, this AA-mitigation strategy is probably not of general interest as coffee roasted to a medium degree is almost only consumed in the American and Northern European markets.

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

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

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

7.3 AA removal from roasted coffee beans and brews

All methods and proposed technologies should, of course, also be tested for their impact on the sensory quality of the final product and feasibility from an industrial point of view before being adopted. Banchero *et al.,* (Banchero, Pellegrino, & Manna, 2013) proposed the use of supercritical CO_2 to remove AA from roasted coffee. The efficiency of AA removal ranged from 8% to 45% at an extraction time of 525 min, and increased to 79% after 22 hours. Changes in pressure did not affect the results, but temperature was the variable that drove the extraction process. Furthermore, the addition of ethanol (up to 9.5% w/w) changed the polarity of the supercritical solvent mixture, resulting in an increase in extraction performance. The most effective operative conditions were found to be 100°C, 200 bar and 9.5% w/w ethanol.

Cha (Cha, 2013) reported a technique that can remove AA from brews using bacterial enzymes at relatively high temperatures. Extracts from *Ralstonia eutropha* AUM-01 and a thermophilic strain, *Geobacillus thermoglucosidasius* AUT-01, were used to remove 50% of AA from coffee brews. In 2016, Anese *et al.* (Anese, 2016), proposed using acrylamidase to hydrolyse AA to acrylic acid, which is less toxic than AA, but corrosive for the skin and mucosa, and ammonia. However, this process may affect the brew's sensory profile. Akillioglu *et al.* (Akillioglu & Gökmen, 2014) proposed a mitigation method for AA in instant coffee that is based on baker's yeast (S*accharomyces cerevisiae*, 1–2%, w/v) mixed with sucrose (0–10, w/v). The mixture was fermented at 30°C for 48h with an AA concentration decrease of about 70%. The results revealed that both the sucrose and yeast concentrations affected the AA mitigation during fermentation and that its reduction was due to the effect of metabolic conversion via yeast metabolism.

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

Conclusions

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

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

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

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

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

It is therefore necessary that studies be expanded on all aspects and that the link between origin and AA quantity is investigated. For instance, the effect of climate change and how it impacts agronomical and primary processing practices and AA precursors requires attention. In this respect, varietal improvement might also be guided by potential reductions in AA formation, while maintaining the sensory quality of the product. In conclusion, further studies are needed to find appropriate and practical solutions for AA mitigation in coffee and to study the health implications of AA in complex mixtures, such as coffee brews. A recent review by Nehlig and Cunha (Nehlig & Cunha, 2020) highlighted how most toxicological studies are carried out on pure AA and on animals, while studies that directly evaluate the effects that AA in foods have on human health have not provided direct evidence of carcinogenic effects. In addition, the risk to human health from AA depends on the conditions of exposure, i.e., the kinetics of adsorption, distribution and excretion in the human body, while this kinetic-dynamic profile is also related to the other constituents of coffee and more in general to the human diets. The mitigation strategies proposed so far to meet the EU precautionary principle on food safety, are devoted to taking appropriate measures to reduce the presence of AA to as low as reasonably achievable (ALARA). This view also needs to take into account other factors, such as potential risks from other contaminants and/or synergy or competition with other components in the brew, the organoleptic properties and quality of the final product, and the feasibility of any process, in terms of both application at industrial level and costs.

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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

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Table Captions

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

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

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

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

Captions to figures

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

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

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