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

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

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

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

Contents

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).

Recently, the Regulation (EU) 2017/2158 of 20 November 2017 established the application of mitigation measures and new benchmark levels for the reduction of AA in foods, including coffee. Concerning coffee products, the new AA benchmark values are: 400 $\mu g \cdot kg^{-1}$ for roasted ground coffee, 850 $\mu g \cdot kg^{-1}$ for soluble coffee, 500 $\mu g \cdot kg^{-1}$ and 4000 $\mu g \cdot kg^{-1}$ for coffee substitutes based respectively on cereal and chicory (European Commission 2017). Due to the increasing restrictions of legislation and the complexity of factors to be considered, the ability to control of AA in foods without affecting their final quality, is becoming more and more a serious challenge for researches and industries.

As for coffee, it is known that all processing stages (selection of raw material, roasting, storage and brewing conditions) can play an important role on final AA levels in the product.

Some efforts by the scientific community have contributed to the identification of potential strategies to reduce and/or minimise AA levels in roasted coffee and consequently consumer exposure. However, compared to potato and bakery products, during the last 15 years only few scientific studies have been carried out to date on intervention strategies for AA reduction in roasted coffee and coffee products.

The present paper aims to review and discuss the main possible mechanism(s) of AA formation and mitigation reported in scientific literature in relation to coffee processing stages, from raw material to brewing, with a main focus on roasting, being the most studied processing step (Table 1). This in order to provide information on the potential efficacy, applicability and potential constraints of current knowledges on AA control in coffee.

[Table 1 near here]

2. Overview of coffee production technologies

Coffee drinks are produced from coffee beans after the processes of seeds separation from the coffee plant, roasting and grinding (Illy and Viani 1995). The principal steps of the process from the cherry to the final brew are shown in the flow sheet of Figure 1, in which the critical steps for the reduction of AA in coffee are highlighted.

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

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

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

Before the most important processing step (roasting), coffee can be blended or decaffeinated depending on the characteristics and the intended use of the final product. Finally, the coffee is roasted for the development of the desirable colour and flavours with different roasting techniques and time-temperature conditions. Roasting of coffee is a complex process, involving transfer of heat through the structure of the bean, transport of water vapour, generation of CO_2/volatiles, change of volume, structure, material and

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

Whole roasted coffee is grounded (using a grinder or coffee mill) to a powder characterized by different size according to the ultimate brew desired. The grinding step aims to increase the extraction surface in order to facilitate the transfer of coffee soluble constituents into the brew (Soares, Alves, and Oliveira 2014). Whole roasted coffee beans or ground coffee are usually packed prior to storage and distribution. In order to best protect the product quality, the package material should act as barrier against water and oxygen (Illy and Viani 1995).

Finally, the coffee brews can be obtained by many brewing methods according to consumer preferences, that are affected by geographical origin, cultural tradition, lifestyle, social behaviour, habit and economic aspects. The most important extraction methods can be classified into three main groups: Italian method under high pressure (i.e. Espresso, moka); infusion, by pouring hot water on ground coffee, followed by filtration (i.e. American, plunger, Neapolitan); decoction or boiling method (i.e. Turkish). Furthermore, the use of single-dose coffee, such as capsules and pods, has highly increased in recent years. The blend of roasted beans and other variables related to the different brewing methods (e.g. water temperature, grinding level of powder, coffee/water mass ratio, extraction time, volume of the final brew etc.) strongly affect the sensorial quality and the amount of extracted compounds in the coffee beverages (De Peña, Ludwing, and Cid 2019; Parenti et al. 2014; Petracco 2001).

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

It is important to underline that, since they take place in different geographical areas, the coffee processing steps are not easy to control and can be differentiated and performed according to companies and consumers demands. Consequently, any variation of the technological parameters applied in each step, for example aimed at the reduction of AA level, can significantly influence the overall quality of the final coffee brew.

[Figure 1 near here]

3. Mechanisms of acrylamide formation during coffee processing

Roasted coffee is one of the potential products, together with fried potatoes and bakery products, at risk for high AA (C_3H_5NO) levels. Green coffee contains the key precursors (e.g. free asparagine and reducing sugars) for the formation of AA during roasting at high temperatures (> 200 °C), that induces complex chemical reactions, that, according to the most recent literature, are summarized in Figure 2.

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

Different possible mechanisms and precursors of AA formation in roasted coffee have been postulated in scientific reports (Anese 2015; Cai et al. 2014; Claus et al. 2006; Friedman and Mottram 2005; Gökmen et al. 2012; Granvogl and Schieberle 2006; Khezerlou et al. 2018; Kocadağli et al. 2012; Soares, Alves, and Oliveira 2014; Stadler and Scholz 2004; Yaylayan and Stadler 2005):

- free amino acids and reducing sugars via Maillard reaction;
- decarboxylation and deamination of asparagine;
- reaction between ammonia and acrolein or acrylic acid (lipid degradation);
- 5-hydroxymethylfurfural (HMF) and free asparagine via Maillard reaction;
- pyrolytic reactions.

One of the predominant pathways of AA formation in coffee products, as in most other concerned foods, is the non-enzymatic browning group of reactions, generally referred to as Maillard reaction. This term indicates an extremely complex heat-induced set of reactions initiated by the condensation of the carbonyl group (C=O) of a reducing sugar (e.g. glucose) with an amino residue ($-NH_2$) of amino acids (e.g. asparagine) at temperatures above 120 °C (Pastoriza, Rufián-Henares, and Morales 2012; Soares, Alves, and Oliveira 2014; Stadler and Scholz 2004). The first product of this condensation is the Schiff base, that is a very unstable compound. Further reactions lead to the formation of an important intermediate known as 3-aminopropionamide (3-APA) and finally of AA (Pedreschi, Mariotti, and Granby 2014; Soares, Alves, and Oliveira 2014; Stadler and Scholz 2004; Zyzak et al. 2003). However, the Schiff base may lead, after its decomposition, directly to AA (Pedreschi, Mariotti, and Granby 2014).

Asparagine, one of the key precursors of AA by Maillard reaction, and sucrose, the main sugar found in raw coffee, are present in green coffee beans in the range of 0.30-0.90 $mg \cdot g^{-1}$ and of 35-90 $mg \cdot g^{-1}$, respectively depending on the characteristics of the raw materials (Murkovic and Derler 2006; Zhang and Zhang 2007). Sucrose is not a reducing sugar but in the initial phase of roasting his decomposition takes place, giving rise to a neo-carbonyls pool that influences the formation of AA during Maillard reaction. Moreover, neo-carbonyl compounds that can be generated from lipid oxidation during roasting of coffee, should be contemplated on AA formation by Maillard reaction (Kocadağli et al. 2012). During the Maillard reaction, not only toxic compounds are produced, but also a very large number of brown-coloured (melanoidins), volatile and non-volatile aromatic compounds are formed. These compounds are desired because they are responsible of coffee products identity and quality (Stadler and Scholz 2004). In addition, some compounds with an interesting antioxidant activity were also detected among the Maillard reaction products (Hečimović et al. 2011; Hu et al. 2019; Van der Werf et al. 2014; Vignoli et al. 2014).

Although the formation of AA is mainly due to the combination of free asparagine and reducing sugars, this compound can also be generated from simple asparagine in absence of sugars by direct decarboxylation and deamination reactions at high temperatures. Low amounts of free asparagine can be detected in almost all kinds of foods, for this reason most products, even when heated at high temperatures, can contain a low amount of AA (Granvogl and Schieberle 2006). The relative low concentration of asparagine and the high process temperatures lead to its rapid intramolecular cyclization, making this pathway of AA formation of limited importance (Granvogl and Schieberle 2006; Yaylayan, Wnorowski, and Perez Locas 2003).

Another pathway that does not require the presence of asparagine, is the generation of AA by acrolein and acrylic acid reacting with ammonia (NH_3) already present or formed during the thermolysis of amino acids and proteins (Anese 2015; Friedman and Mottram 2005; Khezerlou et al. 2018; Stadler and Scholz 2004; Yaylayan and Stadler 2005). Acrolein can be formed through different pathways, including the oxidative degradation of fats (i.e. triglycerides), and can generate acrylic acid by oxidation. However, acrylic acid can be also produced from the thermal decomposition of some amino acids such as aspartic acid, L-alanine and L-arginine or indirectly from serine and cysteine through the formation of pyruvic acid (Guenther et al. 2007). Acrylic acid can react with ammonia to form AA by aminodehydroxylation, which is a well-known reaction of acids leading to

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

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

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

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

[Figure 2 near here]

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

4.1. Raw materials selection and treatments

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

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

amino acids and reducing sugars). Carbohydrates are present in both species of green coffee beans, but Arabica contains higher amounts of sucrose than Robusta that, instead, contains more reducing sugars (e.g. glucose and fructose). Concerning the main free amino acids, asparagine and alanine have been found in higher concentration in Robusta compared to Arabica variety (Murkovic and Derler 2006; Pedreschi, Mariotti, and Granby 2014).

Several researches, summarized in Table 2, have studied the effect of Arabica versus Robusta beans on AA levels in roasted coffee (Alves et al. 2010; Bagdonaite, Derler, and Murkovic 2008; Bagdonaite and Murkovic 2004; Bertuzzi et al. 2020; Lachenmeier et al. 2019; Lantz et al. 2006; Summa et al. 2007). Bagdonaite and Murkovic (2004) has roasted four different types of green coffee (3 Arabica and 1 Robusta) in a small laboratory roaster at 250 °C for different times (5, 7.5, 12 and 14.5 min). The highest amount of AA (> 3500 $ng \cdot g^{-1}$) was found after 7.5 min using the variety Robusta Camerun, while the use of high-quality Arabica varieties (Santos, Columbian Excelso and Uganda) has led to lower levels (< 500 $ng \cdot g^{-1}$). In another study, Lantz et al. (2006) also found that AA formation in Robusta was on average 34% higher than in Arabica, analysing a great number of green coffee types (17 Arabica and 6 Robusta) roasted in a rotating fluidized bed roaster for 2.5 min, in order to reach a medium roasting degree. Summa et al. (2007) reported a lower concentration of AA in Arabica (> 200 $ng \cdot g^{-1}$) than in Robusta (> 250 $ng \cdot g^{-1}$) varieties, roasted with a hot air roaster at 236 °C at a medium level (370 and 430 s respectively). In three more recent studies by Bagdonaite, Derler, and Murkovic (2008), Alves et al. (2010) and Lachenmeier et al. (2019) it was confirmed the highest average levels of AA in the Robusta coffee compared to the Arabica one, roasted at different roasting conditions. In the study by Bagdonaite, Derler, and Murkovic (2008), the influence of possible precursors concentration, carbohydrates (sucrose) and amino acids (asparagine or aspartic acid), in green coffee on AA formation was also investigated. It has been concluded that asparagine is the limiting precursor because as its concentration increases the AA level increases, while the increase in the sucrose concentration lead to lower AA level. The highest levels of asparagine and lower of sucrose were found in Robusta coffee, confirming the higher AA concentration in that sample compared to the Arabica one (Table 2). Bertuzzi et al. (2020) investigated the trend of AA content during an industrial coffee roasting process with a horizontal rotating durum machine at temperature ranging from 90 °C to 215 °C for 16 min. Contrary to all previous studies, the authors found the maximum AA level was reached by Arabica samples, that contained a lower amount of

asparagine. However, according to the authors, this result is due to the higher concentration of reducing sugars in Arabica respect to Robusta coffee for the entire duration of roasting. The trend of glucose and fructose increase, due to the thermal hydrolysis of sucrose, and subsequently decrease during coffee roasting in according to AA formation, showed concentration 5-fold higher in Arabica. However, a satisfactory correlation between AA formation and asparagine degradation was found, confirming the importance of this precursor.

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

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

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

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

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

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

[Table 2 near here]

4.2. Roasting

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

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

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

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

radical scavenging capacity measured on the same coffee samples during roasting. Bagdonaite, Derler, and Murkovic (2008) found that, increasing roasting time (from 5 to 15 min) and temperature (from 220 to 260 °C), the rate of AA degradation in coffee increased too; in fact, after 15 min of roasting, for all applied temperatures, AA was no longer detected. In another study by Alves et al. (2010) a 25% decrease of AA content was measured in dark roasted coffee (210 °C for 11 min) compared to the medium one for all coffee samples investigated. Kocadağli et al. (2012) monitored the formation of AA in coffee during roasting at 220 °C for 60 min in a static oven. Also in this case, it has been clearly shown that AA increased rapidly reaching the maximum level after the first 5 min of treatment and then exponentially decreased. In order to obtain a good quality roasted coffee with low level of AA Madihah et al. (2013) used a Central Composite Design to optimize the roasting condition of different Arabica coffee samples. The lowest level of AA and the best coffee quality, tested by flavour compounds and sensory analyses, were found after 22 min of roasting at 167 °C. Another investigation of Budryn, Nebesny, and Oracz (2015), by using a specifically built pilot roaster, studied the influence of air humidity and velocity, other than time and temperature of roasting, on AA levels and polyphenols content of Robusta coffee samples. The relatively lowest levels of AA (37.6 $ng \cdot g^{-1}$) with a moderate degradation of polyphenols, were found in coffee samples roasted at 203 °C, low air velocity and humidity. Lachenmeier et al. (2019) investigated the AA levels in coffee after different roasting conditions. Six different experiments were carried out changing roasting time and temperatures in order to obtain the following coffee roast type: Scandinavian coffee (very light roasting), coffee quick drying, coffee slow drying, espresso slow dry, espresso quick drying, espresso Neapolitan (very dark roasting). The Neapolitan espresso coffee led to the lowest AA content of 130 and 250 $ng \cdot g^{-1}$ respectively for Arabica and Robusta, being roasted at the highest roasting energy. Instead, the Scandinavian coffee, roasted at the lowest roasting energy, had the highest AA content (470 and 480 $ng \cdot g^{-1}$ for Arabica and Robusta). These results confirm the inverse relationship between roasting intensity and AA content in coffee. However, the authors also found the highest content of some toxicant compounds, such as furfuryl alcohol, furan and 5-hydroxymethylfurfural (HMF), in samples containing the lowest AA levels. Furan and furfuryl alcohol are other heat-induced contaminants classified by IARC as possibly carcinogenic to humans (group 2B) (IARC 1995, 2019). For HMF, some animal studies highlighted its toxicant activity (Capuano and Fogliano 2011), but this compound has not yet been classified by IARC as carcinogenic (IARC 2019).

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

Regarding commercial samples, Mojska and Gielecińska (2013) did not found a significant relationship between the AA content and roasted coffee types or blend (Arabica vs Robusta vs blend). A negative correlation between AA level and colour intensity of coffee samples was found, demonstrating that the roasting degree significantly affects the AA content in the final product. Recently, Wawrzyniak and Jasiewicz (2019) analysed Arabica and Robusta commercial samples defined as "middle" and "strongly" roasted. Unlike other previous studies, the AA mean was higher in the medium roasted coffee samples compared to the strongly roasted one. The authors did not propose explanations of the results because their objective was to describe a method for AA determination.

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

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

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

Arabica and Robusta samples two different steam treatments applied prior or during roasting, using respectively an autoclave and a rotating roaster equipped with steam injection. Results showed that the steam treatments carried out on green and roasting coffee did not significantly impact the final AA content of both final roasted samples. Moreover, the steam treatments lead to a worst sensorial coffee profile that was more acid, less roasty and bitter than the conventionally roasted one. Anese et al. (2014) evaluated the possibility of reducing the formation of AA combining roasting at 200 °C with low-pressure treatments, by using a static oven equipped with a vacuum pump. Coffee samples were roasted under different roasting conditions (conventional, combined conventional-vacuum, vacuum). Under reduced pressure and in the coffee roasted at a medium degree it was possible to achieve AA levels 50% lower than the samples obtained in conventional and combined conditions. The low-pressure conditions generated inside the roaster exerted a stripping effect, preventing AA from being accumulated. In addition, all medium roasted coffee samples presented similar colour and sensorial characteristics. Nevertheless, this AA mitigation strategy could be of a limited interest since the coffee roasted at a medium degree is consumed almost only in the American and Northern European markets. Banchero, Pellegrino, and Manna (2013) suggested, for the first time, the use of supercritical CO_2 to remove AA from lab-scale roasted coffee. The efficiency of AA removal ranged from 8 to 45% when the extraction time was below 525 min and reached a maximum of 79% after 1305 min of supercritical fluid extraction treatment. The caffeine content in the coffee beans was not affected by the treatment, however the effect on the organoleptic properties of final product was not evaluated.

[Table 3 and Figure 3 near here]

4.3. Storage conditions

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

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

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

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

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

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

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

sugars, chicory, rye, barley or malt) respectively during storage at 25 °C for 12 months in their commercial closed package, thus confirming the instability of AA in coffee during storage. However, at the lowest storage temperature (4 °C) a smaller AA reduction in the coffee products was found, not statistically different at each time (6 and 12 months) compared to samples before storage. The authors concluded that the presence of -SH and $-NH_2$ groups of amino acids present in the products composition, might have a significant impact on AA reduction.

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

[Table 4 near here]

4.4. Brew preparation and treatments

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

In Table 5 the few studies concerning the impact of brewing techniques and conditions on AA in coffee beverage are summarized. Lantz et al. (2006) analysed AA in coffee 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

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

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

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

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

The use of unconventional beverage treatments could represent an interesting strategy for AA mitigation. In particular, for the removal of AA from coffee brew, Cha (2013) proposed a biochemical treatment which consisted in the use of the bacterial enzyme acrylamidase directly added into instant coffee beverages, obtained with different amount of coffee and incubated at 37 and 70 °C for 30 min. The AA in coffee beverage was partially degraded by the enzyme in relation to incubation temperatures and coffee concentrations, and completely degraded in coffee maintained at 70 °C. However, the impact of this treatment on the sensorial and safety quality of coffee brew was not evaluated. In fact, it is known that the enzyme acrylamidase hydrolyses AA to acrylic acid (a less toxic compound than AA but corrosive for skin or mucosa) and ammonia, that may affect the brew sensory profile (Anese 2015). Considering a possible industrial application of AA biochemical removal, it is necessary to evaluate the acrylamidase enzyme costs. Bedade, Sutar, and Singhal (2019) proposed to immobilize the bacterial acrylamidase on chitosan coated alginate beads. The immobilized acrylamidase was successfully used in batch and continuous packed column operation, degrading completely AA in instant coffee brews after 60 min of treatment with an initial concentration of 100-500 $mg \cdot l^{-1}$. In addition, it was demonstrated that the immobilized enzyme presented better pH, thermal and storage stability than the free one with a reusability up to four cycles application for AA removal. The results obtained with the immobilised enzyme could be very important for a possible industrial application, also if the presence of possible hydrolysis compounds have not been identified.

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

Narita and Inouye (2014) studied the effect of heat sterilization treatment (121 °C for 6 min) combined with the addition of different additives on the AA content decrease in canned coffee. The highest AA reduction in canned coffee were reached during heat treatment with the addition of cysteine and dithiothreitol (a compound with two -SH groups) and was respectively of 90 and 45%. These results emphasise the important role of thiol groups in decreasing the AA content in coffee beverages. This treatment appears promising because it can be applied in the normal canned coffee manufacturing processes. However, also in this research the impact of the used compounds on the organoleptic attributes of canned coffee was not tested, nor considered for subsequent studies.

[Table 5 near here]

5. Conclusions

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

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

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

In particular, it has been clearly showed that darker roasted coffees are characterized by a lower AA content compared to light and medium ones due to its degradation during the process. Moreover, from the reviewed literature, it seems that a certain AA loss during storage of packed roasted coffee can occur. Nevertheless, the reduction of AA in darker roasted coffee or obtained by prolonged storage, may not represent a promising option. In fact, coffee roasted at stronger degrees are appreciated by South European consumers while North European and American ones prefer lighter roasting levels; moreover, the possible formation of other toxic compounds (e.g. HMF, furan, furfuryl alcohol) which may have a negative effect on human health, has to be taken into account.

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

In addition, few innovative strategies for AA reduction/mitigation have been proposed such as enzymatic treatments of raw material, vacuum or steam roasting alternative technologies, roasted beans supercritical fluid extraction, final beverage treatments such as yeast fermentation and amino acids/additive additions. However, most of these researches are lacking important information and are not completely exhaustive; some presented AA mitigation strategies have to be evaluated based also on their impact on the highly important sensorial and nutritional properties of the final coffee brew. Sensory analysis should be included in the processing strategies used to achieve AA reduction in coffee brew, since it is of utmost importance for the applicability of each proposed methods. This challenge is even more relevant in coffee because minor changes in the standardized production processes systematically result in a significant decline of the final product's quality. Moreover, some procedures proposed by the literature are difficult to be scaled-up at industrial level in the existing manufacturing processes being less practical, technological unfeasible and costly

In conclusion, further studies are still needed in order to find appropriate and practical solutions for AA mitigation in coffee in a more holistic risk-benefit approach, which takes into account side effects such as the possible presence/formation of other toxicants in the matrix, derived from applied reduction measures or from Maillard reaction, without leading to the reduction of both beneficial compounds, such as antioxidants (Lachenmeier et al. 2019), and organoleptic properties. Only in this way and following the ALARA (as low as is reasonably achievable) concept (Food Drink Europe 2019) it will be possible to investigate other strategies for AA levels reduction in final coffee product.

References

Akıllıoglu, H. G., and V. Gökmen. 2014. "Mitigation of Acrylamide and Hydroxymethyl Furfural in Instant Coffee by Yeast Fermentation." *Food Research International* 61: 252–56. https://doi.org/10.1016/j.foodres.2013.07.057.

Alves, R. C., C. M. D. Soares, S. Casal, J. O. Fernandes, and M. B. P. P. Oliveira. 2010. "Acrylamide in Espresso Coffee: Influence of Species, Roast Degree and Brew Length." *Food Chemistry* 119 (3): 929–34. https://doi.org/10.1016/j.foodchem.2009.07.051.

Andrzejewski, D., J. A. G. Roach, M. L. Gay, and S. M. Musser. 2004. "Analysis of Coffee for the Presence of Acrylamide by LC-MS/MS." *Journal of Agricultural and Food Chemistry* 52 (7): 1996–2002. https://doi.org/10.1021/jf0349634.

Anese, M. 2015. “Acrylamide in Coffee and Coffee Substitutes.” In *Acrylamide in Food: Analysis, Content and Potential Health Effects*. https://doi.org/10.1016/B978-0-12-802832-2.00009-7.

Anese, M., L. Manzocco, S. Calligaris, and M. C. Nicoli. 2013. “Industrially Applicable Strategies for Mitigating Acrylamide, Furan, and 5-Hydroxymethylfurfural in Food.” *Journal of Agricultural and Food Chemistry* 61 (43): 10209–14. https://doi.org/10.1021/jf305085r.

Anese, M., M. C. Nicoli, G. Verardo, M. Munari, G. Mirolo, and R. Bortolomeazzi. 2014. “Effect of Vacuum Roasting on Acrylamide Formation and Reduction in Coffee Beans.” *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2013.08.047.

Bagdonaite, K., K. Derler, and M. Murkovic. 2008. “Determination of Acrylamide during Roasting of Coffee.” *Journal of Agricultural and Food Chemistry* 56 (15): 6081–86. https://doi.org/10.1021/jf073051p.

Bagdonaite, K., and M. Murkovic. 2004. “Factors Affecting the Formation of Acrylamide in Coffee.” *Czech Journal Food Science* 22: 22–24. https://doi.org/https://doi.org/10.17221/10604-CJFS.

Banchero, M., G. Pellegrino, and L. Manna. 2013. “Supercritical Fluid Extraction as a Potential Mitigation Strategy for the Reduction of Acrylamide Level in Coffee.” *Journal of Food Engineering* 115: 292–97. https://doi.org/10.1016/j.jfoodeng.2012.10.045.

Baum, M., N. Böhm, J. Görlitz, I. Lantz, K. H. Merz, R. Ternité, and G. Eisenbrand. 2008. “Fate of 14C-Acrylamide in Roasted and Ground Coffee during Storage.” *Molecular Nutrition and Food Research* 52: 600–608. https://doi.org/10.1002/mnfr.200700413.

Bedade, D. K., Y. B. Sutar, and R. S. Singhal. 2019. “Chitosan Coated Calcium Alginate Beads for Covalent Immobilization of Acrylamidase: Process Parameters and Removal of Acrylamide from Coffee.” *Food Chemistry* 275: 95–104. https://doi.org/10.1016/j.foodchem.2018.09.090.

Bee, S., C. H. J. Brando, G. Brumen, N. Carvalhaes, I. Kolling-Speer, K. Speer, F. S. Liverani, et al. 2005. “The Raw Bean.” In *Espresso Coffee: The Science of Quality*, edited by Andrea Illy and Rinantonio Viani, 2nd ed., 87–178. Elsevier Academic Press.

Bertuzzi, T., E. Martinelli, A. Mulazzi, and S. Rastelli. 2020. “Acrylamide Determination during an Industrial Roasting Process of Coffee and the Influence of

Asparagine and Low Molecular Weight Sugars." *Food Chemistry* 303: 125372. https://doi.org/10.1016/j.foodchem.2019.125372.

Bertuzzi, T., S. Rastelli, A. Mulazzi, and A. Pietri. 2017. "Survey on Acrylamide in Roasted Coffee and Barley and in Potato Crisps Sold in Italy by a LC–MS/MS Method." *Food Additives and Contaminants: Part B Surveillance* 10 (4): 292–99. https://doi.org/10.1080/19393210.2017.1351498.

Budryn, G., E. Nebesny, and J. Oracz. 2015. "Correlation between the Stability of Chlorogenic Acids, Antioxidant Activity and Acrylamide Content in Coffee Beans Roasted in Different Conditions." *International Journal of Food Properties* 18: 290–302. https://doi.org/10.1080/10942912.2013.805769.

Cai, Y., Z. Zhang, S. Jiang, M. Yu, C. Huang, R. Qiu, Y. Zou, et al. 2014. "Chlorogenic Acid Increased Acrylamide Formation through Promotion of HMF Formation and 3-Aminopropionamide Deamination." *Journal of Hazardous Materials* 268: 1–5. https://doi.org/10.1016/j.jhazmat.2013.12.067.

Capuano, E., and V. Fogliano. 2011. "Acrylamide and 5-Hydroxymethylfurfural (HMF): A Review on Metabolism, Toxicity, Occurrence in Food and Mitigation Strategies." *LWT - Food Science and Technology* 44 (4): 793–810. https://doi.org/10.1016/j.lwt.2010.11.002.

Cha, M. 2013. "Enzymatic Control of the Acrylamide Level in Coffee." *European Food Research and Technology* 236: 567–71. https://doi.org/10.1007/s00217-013-1927-8.

Claus, A., G. M. Weisz, A. Schieber, and R. Carle. 2006. "Pyrolytic Acrylamide Formation from Purified Wheat Gluten and Gluten-Supplemented Wheat Bread Rolls." *Molecular Nutrition and Food Research* 50 (1): 87–93. https://doi.org/10.1002/mnfr.200500152.

Cordoba, N., L. Pataquiva, C. Osorio, F. L. M. Moreno, and R. Y. Ruiz. 2019. "Effect of Grinding, Extraction Time and Type of Coffee on the Physicochemical and Flavour Characteristics of Cold Brew Coffee." *Scientific Reports* 9 (1): 1–12. https://doi.org/10.1038/s41598-019-44886-w.

Delatour, T., A. Périsset, T. Goldmann, S. Riediker, and R. H. Stadler. 2004. "Improved Sample Preparation to Determine Acrylamide in Difficult Matrixes Such as Chocolate Powder, Cocoa, and Coffee by Liquid Chromatography Tandem Mass Spectroscopy." *Journal of Agricultural and Food Chemistry* 52: 4625–31. https://doi.org/10.1021/jf0498362.

De Peña, M. P., I. A. Ludwing, and C. Cid. 2019. "Beverage Preparation." In *Coffee: Production, Quality and Chemistry*, edited by Adriana Farah, 1st ed., 272–88. Croydon, UK: Royal Society of Chemistry.

Dias, E. C., F. M. Borém, R. G. F. A. Pereira, and M. C. Guerreiro. 2012. "Amino Acid Profiles in Unripe Arabica Coffee Fruits Processed Using Wet and Dry Methods." *European Food Research and Technology* 234 (1): 25–32. https://doi.org/10.1007/s00217-011-1607-5.

Dria, G. J., D. V. Zyzak, R. W. Gutwein, F. V. Villagran, H. T. Young, P. R. Bunke, P. Y. T. Lin, J. K. Howie, and R. G. Schafermeyer. 2007. Method for reduction of acrylamide in roasted coffee beans, roasted coffee beans having reduced levels of acrylamide, and article of commerce. US 7,220,440 B2, issued 2007.

Dybing, E., P. B. Farmer, M. Andersen, T. R. Fennell, S. P. D. Lalljie, D. J. G. Müller, S. Olin, et al. 2005. "Human Exposure and Internal Dose Assessments of Acrylamide in Food." *Food and Chemical Toxicology* 43 (3): 365–410. https://doi.org/10.1016/j.fct.2004.11.004.

European Commission. 2013. "Commission Recommendation 2013/647/EU on Investigations into the Levels of Acrylamide in Food." Official Journal of the European Union. 2013. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32013H0647.

European Commission. 2017. "Commission Regulation (EU) 2017/2158 Establishing Mitigation Measures and Benchmark Levels for the Reduction of the Resence of Acrylamide in Food." Official Journal of the European Union. 2017. https://doi.org/http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf.

Food Drink Europe. 2011. "Acrylamide Toolbox 2011." 2011. https://www.fooddrinkeurope.eu/uploads/publications_documents/Toolboxfinal260911.pdf.

Food Drink Europe. 2019. "Acrylamide Toolbox 2019." 2019. https://www.fooddrinkeurope.eu/uploads/publications_documents/FoodDrinkEurope_Acrylamide_Toolbox_2019.pdf.

Friedman, M., and D. Mottram. 2005. *Chemistry and Safety of Acrylamide in Food*. Boston, MA: Springer. https://doi.org/10.1007/B106417.

Gökmen, V. 2015. *Acrylamide in Food*. Edited by Vural Gökmen. 1st ed. Academic Press. https://doi.org/https://doi.org/10.1016/C2014-0-02160-0.

Gökmen, V., T. Kocadağli, N. Göncüoğlu, and B. A. Mogol. 2012. "Model Studies on the Role of 5-Hydroxymethyl-2-Furfural in Acrylamide Formation from Asparagine." *Food Chemistry* 132 (1): 168–74. https://doi.org/10.1016/j.foodchem.2011.10.048.

Granvogl, M., and P. Schieberle. 2006. "Thermally Generated 3-Aminopropionamide as a Transient Intermediate in the Formation of Acrylamide." *Journal of Agricultural and Food Chemistry* 54 (16): 5933–38. https://doi.org/10.1021/jf061150h.

Guenther, H., E. Anklam, T. Wenzl, and R. H. Stadler. 2007. "Acrylamide in Coffee: Review of Progress in Analysis, Formation and Level Reduction." In *Food Additives and Contaminants*, 24:60–70. https://doi.org/10.1080/02652030701243119.

Guimarães, R. J., F. M. Borém, J. Shuler, A. Farah, and J. C. P. Romero. 2019. "Coffee Growing and Post-Harvest Processing." In *Coffee: Production, Quality and Chemistry*, edited by Adriana Farah, 1st ed., 26–83. Croydon, UK: Royal Society of Chemistry.

Hečimović, I., A. Belščak-Cvitanović, D. Horžić, and D. Komes. 2011. "Comparative Study of Polyphenols and Caffeine in Different Coffee Varieties Affected by the Degree of Roasting." *Food Chemistry* 129: 991–1000. https://doi.org/10.1016/j.foodchem.2011.05.059.

Hendriksen, H. V. 2013. "Asparaginase for Acrylamide Mitigation in Food." *Aspects of Applied Biology* 116: 41–50.

Hoenicke, K., and R. Gatermann. 2005. "Studies on the Stability of Acrylamide in Food during Storage." *Journal of AOAC International* 88 (1): 268–73.

Hu, G. L., X. Wang, L. Zhang, and M. H. Qiu. 2019. "The Sources and Mechanisms of Bioactive Ingredients in Coffee." *Food and Function* 10 (6): 3113–26. https://doi.org/10.1039/c9fo00288j.

IARC. 1994. *Acrylamide. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. Some Industrial Chemicals* 60: 389–434.

IARC. 1995. *Furan. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, 63: 393–407.

IARC. 2019. *Furfuryl Alcohol. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans*, 119: 83–113.

Illy, A., and R. Viani. 1995. *Espresso Coffee: The Chemistry of Quality*. Edited by

Andrea Illy and Rinantonio Viani. *Academic Press*. 1st ed.

Khezerlou, A., M. Alizadeh-Sani, N. Z. Firouzsalari, and A. Ehsani. 2018. "Formation, Properties, and Reduction Methods of Acrylamide in Foods: A Review Study." *Journal of Nutrition Fasting and Health* 6 (1): 52–59. https://doi.org/10.22038/JNFH.2018.34179.1133.

Knopp, S., G. Bytof, and D. Selmar. 2006. "Influence of Processing on the Content of Sugars in Green Arabica Coffee Beans." *European Food Research and Technology* 223 (2): 195–201. https://doi.org/10.1007/s00217-005-0172-1.

Kocadağli, T., N. Göncüoğlu, A. Hamzalioğlu, and V. Gökmen. 2012. "In Depth Study of Acrylamide Formation in Coffee during Roasting: Role of Sucrose Decomposition and Lipid Oxidation." *Food and Function* 3: 970–75. https://doi.org/10.1039/c2fo30038a.

Lachenmeier, D. W., S. Schwarz, J. Teipel, M. Hegmanns, T. Kuballa, S. G. Walch, and C. M. Breitling-Utzmann. 2019. "Potential Antagonistic Effects of Acrylamide Mitigation during Coffee Roasting on Furfuryl Alcohol, Furan and 5-Hydroxymethylfurfural." *Toxics* 7 (1): 1–13. https://doi.org/10.3390/toxics7010001.

Lantz, I., R. Ternité, J. Wilkens, K. Hoenicke, H. Guenther, and G. H. D. Van Der Stegen. 2006. "Studies on Acrylamide Levels in Roasting, Storage and Brewing of Coffee." *Molecular Nutrition and Food Research* 50: 1039–46. https://doi.org/10.1002/mnfr.200600069.

Lynglev, G. B., and S. Schoesler. 2016. Method for producing roasted coffee beans. US 2016/0295877 A1, issued 2016. https://doi.org/10.1037/t24245-000.

Madihah, K. Y. K., S. Norashikin, A. H. Zaibunnisa, J. Misnawi, and O. Rozita. 2013. "Optimization of Roasting Conditions for High-Quality Robusta Coffee." *International Food Research Journal* 20 (4): 1623–27.

Mazzafera, P. 1999. "Chemical Composition of Defective Coffee Beans." *Food Chemistry* 64 (4): 547–54. https://doi.org/https://doi.org/10.1016/S0308-8146(98)00167-8.

Michalak, J., E. Gujska, M. Czarnowska, J. Klepacka, and F. Nowak. 2016. "Effect of Storage on Acrylamide and 5-Hydroxymethylfurfural Contents in Selected Processed Plant Products with Long Shelf-Life." *Plant Foods for Human Nutrition* 71: 115–22. https://doi.org/10.1007/s11130-015-0523-4.

Mojska, H., and I. Gielecińska. 2013. "Studies of Acrylamide Level in Coffee and

Coffee Substitutes: Influence of Raw Material and Manufacturing Conditions." *Roczniki Państwowego Zakładu Higieny* 64 (3): 173–81.

Murkovic, M., and K. Derler. 2006. "Analysis of Amino Acids and Carbohydrates in Green Coffee." *Journal of Biochemical and Biophysical Methods* 69 (1–2): 25–32. https://doi.org/10.1016/j.jbbm.2006.02.001.

Narita, Y., and K. Inouye. 2014. "Decrease in the Acrylamide Content in Canned Coffee by Heat Treatment with the Addition of Cysteine." *Journal of Agricultural and Food Chemistry* 62: 12218–22. https://doi.org/10.1021/jf5035288.

Navarini, L., L. Del Terra, S. Colomban, V. Lonzarich, and F. S. Liverani. 2014. Method for reducing the content of acrylamide in a roasted coffee. US 2014/0193541 A1, issued 2014. https://doi.org/10.1037/t24245-000.

Palermo, M., V. Gökmen, B. De Meulenaer, Z. Ciesarová, Y. Zhang, F. Pedreschi, and V. Fogliano. 2016. "Acrylamide Mitigation Strategies: Critical Appraisal of the FoodDrinkEurope Toolbox." *Food and Function* 7 (6): 2516–25. https://doi.org/10.1039/c5fo00655d.

Parenti, A., L. Guerrini, P. Masella, S. Spinelli, L. Calamai, and P. Spugnoli. 2014. "Comparison of Espresso Coffee Brewing Techniques." *Journal of Food Engineering* 121 (1): 112–17. https://doi.org/10.1016/j.jfoodeng.2013.08.031.

Pastoriza, S., J. Á. Rufián-Henares, and F. J. Morales. 2012. "Reactivity of Acrylamide with Coffee Melanoidins in Model Systems." *LWT - Food Science and Technology*. https://doi.org/10.1016/j.lwt.2011.08.004.

Pedreschi, F., M. S. Mariotti, and K. Granby. 2014. "Current Issues in Dietary Acrylamide: Formation, Mitigation and Risk Assessment." *Journal of the Science of Food and Agriculture* 94 (1): 9–20. https://doi.org/10.1002/jsfa.6349.

Petracco, M. 2001. "Technology IV: Beverage Preparation: Brewing Trends for the New Millennium." In *Coffee: Recent Developments*, edited by R. J. Clarke and O. G. Vitzthum, 1st ed., 140–64. Oxford: Blackwell Science. https://doi.org/10.1002/9780470690499.ch7.

Rannou, C., D. Laroque, E. Renault, C. Prost, and T. Sérot. 2016. "Mitigation Strategies of Acrylamide, Furans, Heterocyclic Amines and Browning during the Maillard Reaction in Foods." *Food Research International* 90: 154–76. https://doi.org/10.1016/j.foodres.2016.10.037.

Rubayiza, A. B., and M. Meurens. 2005. "Chemical Discrimination of Arabica and Robusta Coffees by Fourier Transform Raman Spectroscopy." *Journal of*

Agricultural and Food Chemistry 53 (12): 4654–59. https://doi.org/10.1021/jf0478657.

Seal, C. J., A. de Mul, G. Eisenbrand, A. J. Haverkort, K. Franke, S. P. D. Lalljie, H. Mykkänen, et al. 2008. "Risk-Benefit Considerations of Mitigation Measures on Acrylamide Content of Foods - A Case Study on Potatoes, Cereals and Coffee." *British Journal of Nutrition* 99: S1–46. https://doi.org/10.1017/S0007114508965314.

Şenyuva, H. Z., and V. Gökmen. 2005. "Study of Acrylamide in Coffee Using an Improved Liquid Chromatography Mass Spectrometry Method: Investigation of Colour Changes and Acrylamide Formation in Coffee during Roasting." *Food Additives and Contaminants* 22 (3): 214–20. https://doi.org/10.1080/02652030500109834.

Soares, C. M. D., S. Cunha, and J. Fernandes. 2006. "Determination of Acrylamide in Coffee and Coffee Products by GC-MS Using an Improved SPE Clean-Up." *Food Additives and Contaminants* 23 (12): 1276–82. https://doi.org/10.1080/02652030600889608.

Soares, C. M. D., Rita C. Alves, and M. B. P. P. Oliveira. 2014. "Factors Affecting Acrylamide Levels in Coffee Beverages." In *Coffee in Health and Disease Prevention*, 217–24. https://doi.org/10.1016/B978-0-12-409517-5.00024-3.

Stadler, R. H., and G. Scholz. 2004. "Acrylamide: An Update on Current Knowledge in Analysis, Levels in Food, Mechanisms of Formation, and Potential Strategies of Control." *Nutrition Reviews* 62 (12): 449–67. https://doi.org/10.1301/nr.2004.janr.449-467.

Summa, C. A., B. de la Calle, M. Brohee, R. H. Stadler, and E. Anklam. 2007. "Impact of the Roasting Degree of Coffee on the in Vitro Radical Scavenging Capacity and Content of Acrylamide." *LWT - Food Science and Technology* 40: 1849–54. https://doi.org/10.1016/j.lwt.2006.11.016.

Taeymans, D., A. Andersson, P. Ashby, I. Blank, P. Gondé, P. Van Eijck, V. Faivre, et al. 2005. "Acrylamide: Update on Selected Research Activities Conducted by the European Food and Drink Industry." *Journal of AOAC International* 88 (1): 234–41.

Taeymans, D., J. Wood, P. Ashby, I. Blank, A. Studer, R. H. Stadler, P. Gondé, et al. 2004. "A Review of Acrylamide: An Industry Perspective on Research, Analysis, Formation, and Control." *Critical Reviews in Food Science and Nutrition*.

https://doi.org/10.1080/10408690490478082.

Tareke, E., P. Rydberg, P. Karlsson, S. Eriksson, and M. Törnqvist. 2002. "Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs." *Journal of Agricultural and Food Chemistry* 50 (17): 4998–5006. https://doi.org/10.1021/jf020302f.

Theurillat, V., V. Leloup, R. Liardon, R. Heijmans, and P. Bussmann. 2006. "Impact of Roasting Conditions on the Formation of Aroma Compounds in Coffee Beans." In *Journal of Food Science*, 67:590–95. https://doi.org/10.1111/j.1365-2621.2002.tb11359.x.

Van der Werf, R., C. Marcic, A. Khalil, S. Sigrist, and E. Marchioni. 2014. "ABTS Radical Scavenging Capacity in Green and Roasted Coffee Extracts." *LWT - Food Science and Technology* 58 (1): 77–85. https://doi.org/10.1016/j.lwt.2014.02.053.

Vignoli, J. A., M. C. Viegas, D. G. Bassoli, and M. de Toledo Benassi. 2014. "Roasting Process Affects Differently the Bioactive Compounds and the Antioxidant Activity of Arabica and Robusta Coffees." *Food Research International* 61: 279–85. https://doi.org/10.1016/j.foodres.2013.06.006.

Wawrzyniak, R., and B. Jasiewicz. 2019. "Straightforward and Rapid Determination of Acrylamide in Coffee Beans by Means of HS-SPME/GC-MS." *Food Chemistry* 301: 125264. https://doi.org/10.1016/j.foodchem.2019.125264.

Yaylayan, V. A., and R. H. Stadler. 2005. "Acrylamide Formation in Food: A Mechanistic Perspective." *Journal of AOAC International* 88 (1): 262–67.

Yaylayan, V. A., A. Wnorowski, and C. P. Locas. 2003. "Why Asparagine Needs Carbohydrates to Generate Acrylamide." *Journal of Agricultural and Food Chemistry* 51 (6): 1753–57. https://doi.org/10.1021/jf0261506.

Zhang, Y., and Y. Zhang. 2007. "Formation and Reduction of Acrylamide in Maillard Reaction: A Review Based on the Current State of Knowledge." *Critical Reviews in Food Science and Nutrition* 47: 521–42. https://doi.org/10.1080/10408390600920070.

Zyzak, D. V., R. A. Sanders, M. Stojanovic, D. H. Tallmadge, B. L. Eberhart, D. K. Ewald, D. C. Gruber, et al. 2003. "Acrylamide Formation Mechanism in Heated Foods." *Journal of Agricultural and Food Chemistry* 51 (16): 4782–87. https://doi.org/10.1021/jf034180i.

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

Figure 2. Simplified scheme of possible routes of acrylamide (C_3H_5NO) formation during roasting in coffee.

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).

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) Lachenmeier et al. (2019) Bertuzzi et al. (2020)
	Post-harvest processing	Illy and Viani (1995) Knopp, Bytof, and Selmar (2006) Soares, Alves, and Oliveira (2014)
	Quality of green coffee beans	Mazzafera (1999) Dias et al. (2012)
	Asparaginase pre-treatment	Dria et al. (2003) Hendriksen (2013) Lynglev and Schoesler (2014) Navarini et al. (2014)
	Decaffeination	Alves et al. (2010) Bertuzzi et al. (2017)
Roasting	Time and temperature optimization	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) Kocadağli et al. (2012) Madihah 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	Theurillat et al. (2006)
	Vacuum roasting	Anese et al. (2014)
	Supercritical fluid extraction	Banchero, Pellegrino, and Manna (2013)
Storage	Time and temperature optimization Secondary shelf-life	Andrzejewski et al. (2004) Delatour et al. (2004) Hoenicke and Gatermann (2005) Taeymans et al. (2005) Lantz et al. (2006) Michalak et al. (2016)
Brewing	Selection of brewing methods	Lantz et al. (2006) Soares, Cunha, and Fernandes (2006) Alves et al. (2010)
	Enzymatic treatment	Cha (2013)

		Bedade, Sutar, and Singhal (2019)
	Yeast fermentation	Akıllıoglu and Gökmen (2014)
	Additive addition	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 ($ng \cdot g^{-1}$)**	**Reference**
Arabica Robusta	Laboratory roaster	T= 250 °C t= 7.5 min	< 500 > 3500	Bagdonaite and Murkovic (2004)
Arabica Robusta	Fluidized bed convection roaster	T= n. r. t= 2.5 min	251 ± 45 378 ± 32	Lantz et al. (2006)
Arabica Robusta	Bench-scale roaster	T= 236 °C t= 370 s T= 236 °C t= 430 s	> 200 > 250	Summa et al. (2007)
Arabica Robusta	Small-scale convection roaster	T= 240 °C t= 7.5 min	374 ± 86 708 ± 77	Bagdonaite, Derler, and Murkovic (2008)
Arabica Robusta	Probat L12 roaster	T= 210 °C t= 10 min	178* 355*	Alves et al. (2010)
Arabica Robusta	Laboratory roaster	Six different roasting profiles	from 130 to 470 from 250 to 480	Lachenmeier et al. (2019)
Arabica Robusta	Horizontal rotating drum industrial-scale roaster	T= 136-138 °C t= 10 min	1045 ± 56 795 ± 45	Bertuzzi et al. (2020)

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 Rotating fluidized roaster Drum roaster	T= n. r. t= 1.5-16 min	Lantz et al. (2006)
Arabica Robusta	Laboratory roaster	T= 236 °C t= 235-560 s T= 236 °C t= 260-620 s	Summa et al. (2007)
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	Kocadağli 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 $m \cdot s^{-1}$	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 T= -40 °C T= 8 months	40-65% 0%	Andrzejewski et al. (2004)
Ground Soluble	Opened original package	T= room t= 7 months T= room t= 12 months	28% 66%	Delatour et al. (2004)
Beans Ground Soluble	Closed original package Closed vacuum package Closed original package	T= 10-12 °C t= 3 months	30% 31% 0%	Hoenicke and Gatermann (2005)
Beans	Closed jar Opened jar	T= 60 °C t= 34 hours	30% 0%	Taeymans et al. (2005)
Ground	Closed vacuum package	T= -18 °C t= 12 months T= 4 °C t= 12 months T= room t= 12 months T= 37 °C t= 12 months	14% 25% 80% 88%	Lantz et al. (2006)
Soluble Coffee substitutes	Closed package	T= 4* and 25** °C t= 6/12 months	12/18% (*) 20/33% (**) 7/12% (*) 7/28% (**)	Michalak et al. (2016)

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 $g \cdot l^{-1}$	104%	Lantz et al. (2006)
	Household drip-filter (1 l capacity)	C/W= 49 $g \cdot l^{-1}$	102%	
	Plunger pot	C/W= 49 $g \cdot l^{-1}$	99%	
	Fresh brew filter coffee (vending machine)	C/W= 62 $g \cdot l^{-1}$	102%	
	Espresso (manual equipment)	C/W= 146 $g \cdot l^{-1}$	75%	
Commercial roasted	Espresso	C/W= 200-230 $g \cdot l^{-1}$ t= 30 ±5 s p= 9 ±2 bar	21 $\mu g \cdot l^{-1}$	Soares, Cunha, and Fernandes (2006)
Instant	Water solution	C/W= 200 $g \cdot l^{-1}$	72.4 $\mu g \cdot l^{-1}$	
Arabica	Espresso	C/W= from 93 to 325 $g \cdot l^{-1}$	from 62 to 99%	Alves et al. (2010)
Robusta			from 59 to 98%	

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

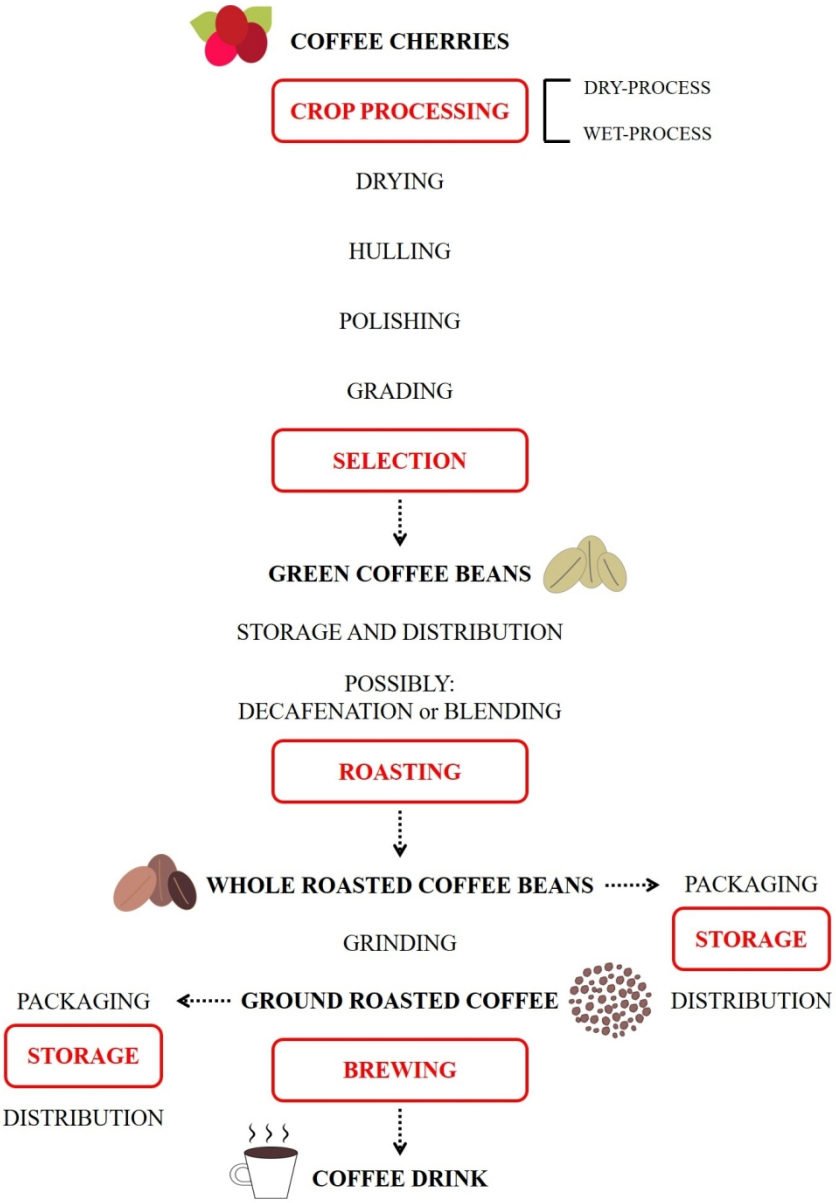

Figure 1. Scheme of coffee processing, from the cherry to the brew. The critical steps for the reduction of acrylamide in coffee are highlighted.

240x331mm (150 x 150 DPI)

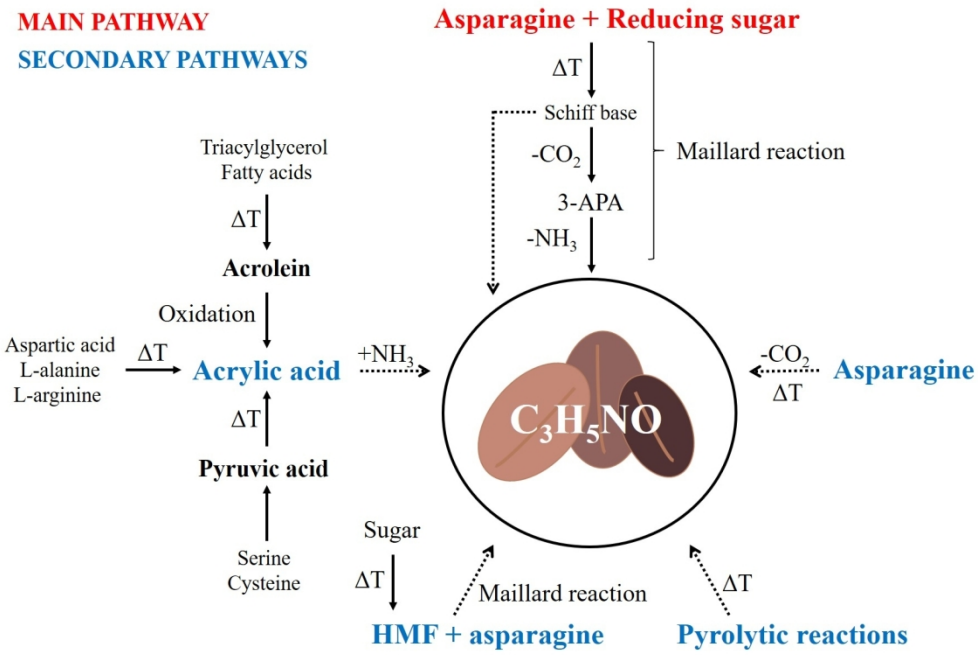

Figure 2. Simplified scheme of possible routes of acrylamide (C3H5NO) formation during roasting in coffee.

304x205mm (150 x 150 DPI)

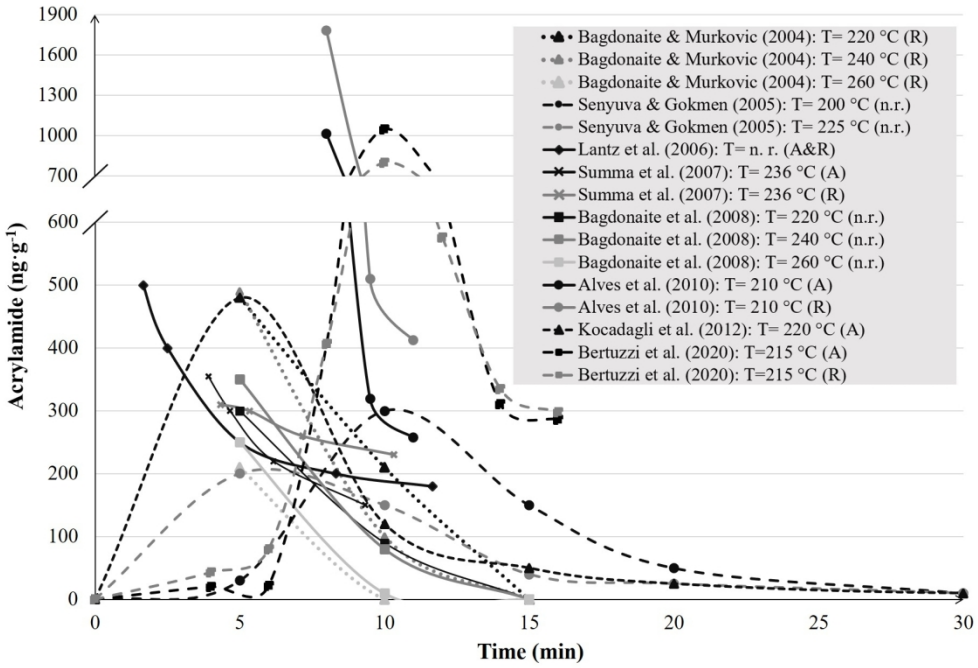

Figure 3. Selected typical profiles of acrylamide formation during coffee roasting processes as reported in some literature studies (A: Arabica, R: Robusta, n. r.: not reported).

314x213mm (150 x 150 DPI)