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Acrylamide (AA) is a well-known toxic compound formed in various foods during the high thermal process. Cookies, one of the most consumed bakery goods worldwide, represent a category of food at risk of AA in the human diet. Therefore, some strategies for its control in cookies should be employed. The present review summarizes and discusses the mitigation strategies for AA reduction, reported in scientific literature, that could be carried out during the cookie's formulation and some of their effects on the final product quality. The evaluation of AA formation related to various ingredients could help the food industries and researchers to develop a more effective method to reduce this toxic compound in cookies, as well as in other bakery products.

Keywords: Acrylamide mitigation; Processing contaminant; Cookie; Formulation; Bakery ingredients.

1. Introduction

Bakery products such as bread, breakfast cereals, crackers, wafers, and cookies represent a key part of the human diet. Cookies are one of the most appreciated and consumed bakery goods worldwide, thanks to their ready-to-eat nature, availability in numerous varieties, long shelf-life, and relatively low cost. [1–3] However, cookies greatly contribute to the dietary acrylamide (AA) intake, especially in infants (<2 years of age) and children (>2 years of age) with contributions of about 27 and 56%, respectively. [4]

AA is a toxic and carcinogenic compound naturally formed during baking and other food processing/cooking methods performed at high-temperature above 120 °C. [5–7] The toxicological effects of AA on humans are neurotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity, leading to its classification as a Group 2A carcinogen by the International Agency for Research on Cancer. [8] After its absorption from the gastrointestinal tract, AA is metabolized to glycidamide, a mutagenic and genotoxic compound, following the reaction catalyzed by the cytochrome enzyme. [6, 9, 10] Glycidamide formation is considered responsible for the genotoxic effects of AA having the potential to induce mutagenic genes at the chromosomal level. [10]

The formation of AA in cookies is derived mainly from the Maillard reaction and is firstly related to the ingredient types and quantity used in the formulation, as well as their interactions during the entire preparation process which also includes the use of high temperatures during baking. The main precursors of AA are reducing sugars and free asparagine; as sugars are normally abundant in dough formulations, the concentration of free asparagine is the rate-limiting factor for AA formation in cookies. [11, 12] Extensive scientific efforts have been carried out aiming at mitigation and control of AA formation in cookies by the modification and optimization of the dough formulations. A total of 62 research papers

are present in the scientific literature since 2004, as showed in Figure 1a. No research works were published in 2005. The number of original articles has grown exponentially with a high number of new studies especially in 2012, 2019, and 2020, probably as a result of the introduction of different legal regulations, AA reference levels, and guidelines.

Considering the presence of AA in foods and its health risk, several recommendations and regulations have been established by the European Commission over the years. Among the most relevant, after several annual monitoring of AA levels in different food products and in agreement with the European Food Safety Authority (EFSA) opinion, there is the European Commission recommendation (EC 10.1.2011), that introduced the concept of AA indicative values in foods. [13] These AA indicative values were not intended as regulatory limits or safety thresholds but were set for different food categories at levels that the food industry should be able to achieve based on EFSA's 2007-2008 monitoring data. [13] Subsequently, the EC Regulation (EU 2017/2158) was introduced, establishing mitigation measures and reference levels for the reduction of the presence of AA in three food categories including coffee, potatoes, and bakery products. According to this Regulation, the food business operators are obliged to apply measures to reduce the level of AA in order to reach the lowest possible level below the reference one established in this normative act. Concerning the category "cookies and wafers", the AA benchmark value is 350 μg/kg. [14] In addition, because it was concluded that this regulation did not present sufficient available data on the presence of AA in foods, a more recent European Commission Recommendation 2019/1888/EC introduced a new list of non-exhaustive food products, including some bakery products specialties (e.g., buns, sticks, pancakes, etc.), which must be monitored to identify the AA risk and adopt new prevention and/or reduction measures against this food contaminant. [10, 15]

The selection of conventional ingredients such as flours, sugars, leavening agents, salts, oils, fats and additional ones including organic acids, amino acids, enzymes and antioxidants, can control the presence of AA precursors and reduce its formation in cookie products. The type of ingredients investigated in the reported research papers varied in percentages greatly from year to year as shown in Figure 1b and in some cases, different ingredients have been studied in the same work. The most studied ingredients for AA mitigation in cookies were sugars, flour, and leavening agents. However, it must be taken into consideration that any change aimed at improving cookie formulation can significantly influence their overall quality. [16]

The present review article aims to thoroughly describe and discuss the mitigation strategies of AA reported in the scientific literature, carried out in particular during the cookie's formulation step, and some of their effects on the final product quality. In fact, the evaluation of AA formation related to various ingredients used for cookie production could help the food industries to develop more effective methods to reduce this toxic compound in the final product as well as in other bakery products.

2. Cookie formulation

The bakery industry is in constant innovation, and bakery products are widely consumed worldwide by different consumer groups. Cookies represent a very broad category of bakery products and can be classified into different types based on their formulation: hard dough, characterized by 6-10% of fat and 10-15% of sugar; short dough with low sugar (14-20%) and low fat (12-19%); short dough with high sugar (15-30%) and high fat (18-22%); soft dough with fat and sugar around 30% and 33-60% respectively. [17, 18] Hard dough cookies are generally crispy and crunchy with an open texture (e.g., "tea cookies", "garibaldi fruit",

etc.), short dough ones are brittle and poorly plastic (e.g., shortbread, “Italian frollini”, etc.), while soft dough ones are identified by a soft texture that makes them fragile and subjected to breakage (e.g., sponge cookies, meringues, etc.). [17, 19]

The ingredients normally used in the manufacture of cookies are wheat flour, sugar, fat, water, eggs, and leavening agents in different proportions, depending on product type. [19] Moreover, other ingredients can be incorporated or replaced in the formulation to obtain different, innovative, and healthy cookie types such as pseudo-cereals (e.g., quinoa, amaranth, buckwheat) or legumes flours (e.g., chickpea, lupine, soya), fat- and sugar-mimetic ingredients (e.g., maltodextrin, lecithin, xylitol), antioxidant compounds (e.g., plant powders and extracts) and flavors (e.g., chocolate, creams, nuts). The nature and quantity of ingredients determine the sensorial and nutritional quality of the cookies. [1]

Nearly all cookies are formulated with wheat flour as the most important and basic constituent. [20] The major functions of wheat flour in cookie dough are: to form the dough during mixing, to hold all the ingredients uniformly distributed in the dough and making easy machinability; to retain gas during mixing and baking; to form the structure of the product. [21] Sugar is another important ingredient in cookies formulation that can vary from simple sugars such as glucose and fructose to more complex ones such as sucrose and maltose. The main sugars used in cookies preparation are sucrose in solid form, inverted syrup, glucose syrup, honey, and high fructose products. The principal functions of sugar in the manufacture of cookies are: to give a sweetish taste and flavor; to help water retention improving their shelf life; to participate in caramelization and Maillard's reactions necessary for the formation of aromas and color, and to give the right volume to the dough. [21] Fats and oils are present in cookies as dough ingredients, in surface sprays, cream fillings, coatings, or as a part of other ingredients such as egg yolks and chocolate. Among them, vegetable oils, butter, and

shortenings are commonly used. The main functions of fats and oils in the formula of cookies are: to give a tensor effect to the dough; to improve the machine workability of the dough; to improve the palatability of the product. [21] As cookies are generally long-life products, any used oil and fat must be stable under storage conditions. For this reason, antioxidants ingredients are often added to the formulation to prevent oxidative rancidity and unpleasant flavors. [21, 22] Water, together with other alternative liquid ingredients such as eggs and milk, also plays an important role in the cookie's formulation. Water is necessary during the mixing step, but it should be considered more correctly as a processing aid rather than an ingredient because the water added as it is or through other ingredients is largely eliminated during the baking process. Some water functions are: to help gluten formation and starch-swelling processes; to bring dough ingredients in contact; to dissolve and distribute salt, sugars, chemicals, and other water-soluble ingredients; to promote the enzyme activity; to assist temperature control of dough and to help cookie aeration by the formation of steam during baking. [21] According to some traditional recipes, in many types of cookies fresh eggs instead of water are used. In addition to their high nutritional value, eggs are added for their emulsifying, binding and yellow coloring functions particularly appreciated by the consumer. [17] However, in some cookie types, fresh eggs are replaced by powdered eggs, which are easier to use but with lower technological performances, or by fat and emulsifier substances obtained from alternative sources. [17, 21] Concerning the leavening agents, cookies are usually chemically leavened using different bicarbonates (sodium bicarbonate, $NaHCO_3$, and ammonium bicarbonate, NH_4CO_3). Chemical agents aerate the dough with the production of carbon dioxide obtained by bicarbonates decomposition thanks to the high temperatures of baking and through the chemical reaction of alkaline ingredients, such as bicarbonates, with acidic ingredients [19], that are usually cream of tartar ($KHC_4H_4O_6$) or sodium aluminum

sulfate ($NaAl(SO_4)_2$). [22, 23] The choice and the combination of different bicarbonates with different acids result in the release of leavening gases with several profiles, that expand the dough and impart specific sensorial characteristics to the final product. [19, 24]

After the selection and dosing of the ingredients, also the other cookies process steps, such as mixing, dough sheeting, dough sheet relaxation, shape forming, baking, cooling, and packaging are important in determining the overall characteristics of the final different product types. [22, 25]

3. Effect of cookies formulation on acrylamide formation

A significant number of mitigation strategies to reduce AA content in food have been proposed and tested so far. The first control step to reduce AA levels includes changes in formulation, i.e. selection of raw materials and recipe of food products. [26] The addition or removal of some conventional ingredients of bakery products, such as flours, sugars, leavening agents, salts, oils, and fats, or minor ingredients such as organic acids, amino acids, enzymes, and antioxidants could potentially increase or decrease AA levels. In fact, due to the mechanism of AA formation, the type and amount of all these ingredients can influence the presence of precursors and/or the extent of the Maillard reaction.

3.1 Conventional ingredients

3.1.1 Flours

Flour is the basic ingredient that represents the highest concentration in the composition of cookies and other bakery products. In general, each flour type, according to genetic basis, growing conditions, agronomic factors, and post-harvest processing, has a different chemical composition and physical properties. [12, 27] Depending on the origin of the flour, this is the

main source of asparagine in the cookie formulation. Hence, it is important to understand what factors influence its concentration in this ingredient and, consequently, the formation of AA in baked products. Wheat flour is the most widely used; however recently, alternative ones (i.e., not-wheat, not-cereals, pseudo-cereals, legumes) are increasingly used to improve the nutritional value of bakery products. In addition to the given main nutrients and dough technological properties, it is also necessary to determine their impact on the formation of process contaminants such as AA. [28] Several authors have studied the impact on AA formation of flour origin, mix of different flours, and their amount used in the cookie recipe, as reported in Table 1.

Miśkiewicz et al. [29] studied the AA content in shortcrust cookies formulated with wheat Poznań flour (type 500), spelt-wheat flour (type 630), and wheat Poznań flour blended with flours from rice, chickpea, and amaranth seeds in the portions of 50, 50 and 25%, respectively. The concentration of AA resulted of 41.9 µg/kg in the cookies based on wheat flour only, while the samples prepared with a blend of wheat and chickpea flours resulted in the lowest AA formation (5.7 µg/kg). These results were attributed to the lower sucrose and reducing sugars content found in the blend of wheat and chickpea flours compared to wheat flour only. In addition, the relatively low concentration of AA in the cookies produced from this flour mixture could also result from the protective effect of chickpea proteins, which limit the reactivity of AA precursors present in the raw dough during baking as previously observed in fried potato products. [30]

Nowadays, soybeans are extensively consumed worldwide because of their several technological and health benefits; Palermo et al. [31] proposed to evaluate the effect of freeze-dried okara, a by-product of soybeans processing, on AA formation in cookies, obtained by replacing 15% of wheat flour with this product. Cookie samples enriched with okara showed

198 a more intense development of the Maillard reaction leading to a higher formation of AA
199 (+60%) than in the control. According to the authors, this phenomenon could be linked to the
200 presence of about 50% insoluble dietary fiber in okara, which reduces the water activity of
201 dough during baking, thus favoring the Maillard reaction.

202 Another study of Mesías et al. [32] investigated the effect of replacing up to 20% of
203 wheat flour with chia seeds flour in the cookie dough on the nutritional properties, antioxidant
204 content, and the formation of food toxic compounds, including AA, of the final product, with
205 the purpose to evaluate the risk/benefit of the new formulations. The incorporation of chia
206 flour into the formulation of wheat-based cookies resulted in a nutritionally enhanced product
207 with a higher amount of protein, dietary fiber, antioxidants, and mainly polyunsaturated fatty
208 acids. However, in relation to the control formulation, AA levels significantly increased by
209 around 33% with the addition of 5% of chia flour and around 700% with the addition of 10,
210 15, and 20% of chia flour. The higher formation of AA in the samples with chia could be
211 related to the levels of precursors. Chia flour showed a lower content of reducing sugars (1.6
212 g/100 g) but a higher one of free asparagine (42.8 mg/100 g) than wheat flour (respectively
213 of 5.6 g/100 g and 23.4 mg/100 g), leading to an asparagine/reducing sugars ratio of 4.2 for
214 wheat flour versus 26.8 for chia flour. In addition, chia flour presented high levels of
215 dicarbonyl compounds such as methylglyoxal and glyoxal, that were not detected in wheat
216 flour. Dicarbonyl compounds are reactive intermediates of the Maillard reaction, for this, the
217 progressive addition of chia seeds flour has promoted the AA formation during baking.

218 Similarly, the work of Manolache et al. [33] evaluated the AA content of wheat flour-
219 based cookies formulated with the addition of 25-100% of wholemeal oat flour. The amount
220 of AA formed during the baking process increased proportionally with the amount of
221 wholemeal oat flour added in the recipe, reaching around 350 µg/kg for oat flour percentages

of 75 and 100%. As it was for chia flour, these outcomes could be related to the higher protein, mineral, total fat, sugar, crude, and dietary fiber contents in cookies obtained with wholemeal oat flour than in those with wheat flour.

In a more recent study, Sazesh and Goli [6] replaced wheat flour with quinoa flour at levels of 25, 50, and 100%, also using different concentrations of sodium bicarbonate, different baking temperatures (160, 185, and 210 °C), and the same time (20 min). The authors applied the response surface methodology with the combined model design expert test (D-optimal design) to obtain cookies with desirable hardness, density, browning index, and low AA content. The two optimized formulas selected, corresponding to the replacement of wheat flour with 72 or 100% of quinoa flour, sodium bicarbonate 0.05%, and baking temperature of 160 °C, showed a drastic decrease of AA when compared to the 100% wheat flour sample. These authors did not determine the sugars content of the used flours, but, based on earlier studies of Maradini Filho et al. [34] and Navruz-Varli and Sanlier [35], the obtained results were attributed to the fact that quinoa contains about 3% of sugars represented mainly by maltose, D-galactose, and D-ribose, therefore low levels of fructose and glucose which are the most effective reducing sugars involved in the AA formation. In addition, compared to wheat flour, quinoa flour has much lower levels of asparagine, which is the most effective amino acid participating in the Maillard reaction.

A large number of flour types for the formulation of cookies were tested by Žilić et al. [12]. In detail, refined wheat flour was compared with wholemeal flours of eight genotypes of grain cereals (bread wheat i.e., *Triticum aestivum* var. *lutescens*, durum wheat, soft wheat, hard wheat, triticale, rye, hull-less barley, and hull-less oat) and four genotypes of maize (white-, yellow-, red-colored standard seeded maize, and blue-colored popping maize). The interrelationship between the initial content of proteins, free asparagine in cereal flours, and

AA in the cookies, as well as the correlation between contents of AA and free asparagine in baked cookies, were analyzed. Data indicated that hull-less oat, durum wheat, and rye flour contained the highest content of free asparagine (859.8, 603.2, and 530.3 mg/kg, respectively), hence generated the higher amount of AA in cookies baked for 13 min at 180 °C. The results confirmed once again that the use of cereal flours low in free asparagine can be an effective strategy for AA mitigation in cookies.

On the other hand, contrary to many studies in the literature, Chen et al. [36] found that, despite the higher asparagine content, rice flour when used for cookie formulation involved a lower AA formation, ranging from not detectable to 450 μg/kg, compared to the AA levels found in the cookies obtained with wheat flours, ranging from 155 to 982 μg/kg. In addition, these authors found that cookies made from finely milled rice or wheat flours had substantially lower AA levels than those from respective wholemeal flours grains. The results showed that non-wholemeal flours did not promote an increase in the reducing sugar content of the flours but increased free asparagine, especially for rice flour. Deviating from the mainstream concept, the study concluded that the AA content in cookies was apparently not dependent on the quantities of reducing sugars and free asparagine in the starting flour. To explain the results, it was hypothesized that rice flour can be rich in other amino acids, such as glycine, cysteine, and lysine, that promote competitive reactions. Moreover, a difference in AA could be related to alternative pathway formation involving oils and nitrogen-containing compounds in lipid-rich foods, such as cookies. AA can generate from acrolein, formed mainly through the oxidative degradation of fats, and ammonia (NH_3) that can be already present or formed during the thermolysis of amino acids and proteins. [37]

In literature, other authors evaluated the effect of flour extraction on the level of AA in cookies since free amino acids and sugars are not homogeneously distributed into the grain.

[16, 38–41] Flour extraction degree represents the total amount of flour obtained from 100 kg of the grain cereal. In general, wholemeal flours products are assumed to have a health-related benefit when compared with products made from white refined flours; however, it is also necessary to consider the influence of flour extraction degree on the formation of toxic compounds in the products in which they are used.

Haase et al. [38] compared the effect of wheat flour with 0.55% ash content and wholemeal wheat flour on AA level in cookies. Wholemeal flour resulted in an unchanged AA level but in a significantly higher antioxidant activity when compared with cookies formulated with 0.55% ash content flour. Hence, considering the AA/antioxidant index, wholemeal flour cookies significantly exceeded in quality those obtained with flour with low ash content.

The investigation of Mustățea et al. [39] evaluated four types of wheat flour characterized by ash contents of 0.53% (white flour), 0.44% (white flour), 2.37% (semi-white flour), and 0.88% (dietetic flour). The asparagine content in the tested flours increased with the increase of extraction degree, with the highest asparagine content found in semi-white flour (11.5 g/kg). Accordingly, the AA results showed a good correlation with the ash content, the cookies obtained from flours having the higher extraction degree had the higher amount of AA.

In another study, Negoiță et al. [16] obtained 20 different cookie formulations by combining three types of wheat flour with different extraction degrees (75-85%, 85-95%, 95-100%) and five types of fat sources. By using the same type of fat, it was noted that the lowest AA values (14.6 to 95.8 μg/kg) were obtained in cookies formulated with semi-white flour with the lowest extraction degree (75-85%). On the contrary, the highest concentrations of AA were obtained using flour with a higher extraction degree, black flour (153.3 to 608.9

294 μg/kg) and dietetic flour (166.0 to 667.7 μg/kg), this is because a greater amount of
295 asparagine is present in the outer layers of grains.

296 In another following research, Negoiță et al. [40] prepared cookies by varying three
297 types of wheat flours characterized by different ash contents (0.53, 0.44, and 2.37%). In
298 cookies obtained from the different types of flour, the AA level increased as increasing
299 baking time, when water content progressively decreased. In addition, the AA level increased
300 also with the increase in the ash content of the flours, the highest level of 1580.3 μg/kg was
301 obtained in samples with wholemeal flour with an ash content of 2.4%, followed by samples
302 with white wheat flours (ash content of 0.53 and 0.44%) that had AA levels of 387.8 μg/kg
303 and 308.4 μg/kg respectively.

304 A more recent study, with the aim of validating a methodology based on high-
305 resolution mass spectrometry for the detection and quantification of AA, evaluated three
306 types of cookies made from soft wheat flour and one type formulated from wheat bran. The
307 highest AA value was obtained in cookies made with wheat bran flour (2373 μg/kg), which
308 had higher concentrations of asparagine (691 mg/kg) compared to wheat flour type 65 (54.5
309 mg/kg). [41]

310 To the best of the author's knowledge, only the studies of Anese et al. [42] and
311 Bartkiene et al. [43] proposed using flours obtained with pre-treated grains or pre-treated
312 flaxseed and lupine to control AA formation during baking. Anese et al. [42] studied the
313 influence of a low-temperature long-time pre-treatment as a strategy to reduce AA
314 concentration in short dough cookies. In this study, the whole-wheat grains were subjected
315 to heating at 100 °C for 8 h and then milled. The low-temperature long-time pre-treatment
316 was responsible for a great decrease, up to 42%, in AA levels in the obtained cookies
317 compared to the control samples made with flour from unheated wheat. As the pre-treatment

did not cause any change in sugar and asparagine concentrations, the reduction in AA levels has been attributed to a difference in the thermal effect generated in the cookies obtained by using the unheated and pre-heated flours. In fact, as the heating pre-treatment caused a 2% moisture decrease in the flour, less time at the same temperature was required to bake cookies at a similar final moisture content.

The potential application of fermented lupine and flaxseed by pure culture of *Lactobacillus sakei*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, and solid-state (SSF) or submerged (SMF) fermentation to produce safe and high nutritional value cookies with reduced AA was demonstrated by Bartkiene et al. [43]. The obtained flours led to cookies with lower AA compared to samples obtained from not pre-fermented ingredients; the fermentation process decreased asparagine content on average of 67.6 and 80.6% and reduced saccharides content of 18 and 79.4% in flaxseed and lupine, respectively. The most effective AA reduction of 78 and 85% was reached in cookies obtained with flaxseed (SMF) and lupine (SSF) flours pre-fermented by *P. acidilactici*. Significant effects of lupine or flaxseed addition, fermentation method, type of microorganisms, and interaction of these factors on AA concentration in wheat cookies were found.

All these results indicate that the source of the flour and its composition also related to the extraction degree after the milling process, play a primary role in determining the AA content in cookies. However, it is important to consider that the nature and quantity of flours alternative to wheat can alter the processability of the dough and impact some important characteristics of bakery products, such as taste, color, texture, density, related to consumers acceptability. [6, 28, 44, 45]

3.1.2 Sugars

Other than flours, sugars are one of the key ingredients of cookies that influence their main desired sensorial quality. [46] The type, quantity, granulation of sugar used contributes to texture, flavor, sweetness, and color of sweet bakery products. [46, 47] In addition, besides asparagine, the type and quantity of sugars chosen in the formulation of cookies may also play an important role in AA development. [44] For this reason, their presence in the cookie dough as ingredients alone or as a component of other ingredients must be carefully evaluated. For an overview, the studies that investigated the effect of sugars on AA levels in cookie products have been summarized in Table 2.

Many studies have suggested that the replacement of reducing sugars with sucrose (non-reducing sugar) is an effective way to significantly reduce the AA content in cookie products. Amrein et al. [48] firstly studied this issue in cookies evaluating the reduction of AA content in gingerbread by replacing the ingredients rich in reducing sugars, such as honey, inverted sugar syrup, and caramel coloring, with sucrose in an amount corresponding to the sum of glucose and fructose present in the previous ingredients. Results showed a 95% decrease in AA content in these cookie samples compared to the control ones due to the reduction of reactive carbonyls for the Maillard reaction.

Similarly, Graf et al. [49] reported an AA content reduction of 70% in industrially produced cookies formulated with sucrose solution instead of inverted sugar syrup (46 vs 170 μg/kg AA).

The research of Summa et al. [50] investigated the kinetics of AA formation and sugars decrease in cookies formulated with sucrose or fructose during baking at 180 °C up to 20 min. The use of fructose has led to a greater formation of AA in cookies due to its reaction with asparagine in the first baking period (up to 10 min) following a linear rate kinetic. In contrast, sucrose promoted an exponential kinetic reaction showing that a prolonged heating

time is required to break the bond between the glucose and fructose monosaccharides before reacting with the amino acid. The authors also studied the impact of the amount of sucrose added in the cookie recipe. Contrary to expectations, a low AA concentration was found in the samples to which sucrose was added in the highest amount (28%) reaching a similar result of the samples in which sugars were not added. The authors explained that these higher levels of AA could be due to the higher relative concentration of protein and, in particular, the amino acid asparagine, which is considered the limiting factor for AA formation in bakery products. Indeed, an asparagine concentration resulted in higher amounts of AA in the final products. On the contrary, increasing the content of sugar in the formulation is equivalent to a dilution of the flour and thus of the concentration of asparagine. However, this was not the case for the fructose formulation, probably due to its high reactivity in the Maillard reaction.

Also, Gökmen et al. [51] prepared different cookie doughs by varying the concentration of sucrose and glucose. Because replacing entirely sucrose with glucose adversely affected the cookie structure, a fixed amount of sucrose (7% of the dough) was necessarily included in the recipe. The progressive replacement of sucrose with glucose turned into a drastic enhancement in the AA level up to 50% or more. Under the applied baking conditions (205 °C for 11 min), it was considered that the hydrolysis of sucrose can be very limited. Similar results were observed also by Ramadan [46] and Nguyen et al. [5].

Another study evaluated the effects of cookie formulation in terms of the presence of different sugars (glucose or sucrose) and leavening agents on some risk/benefit indexes based on the concomitant formation of AA and compounds with antioxidant activity. For the same leavening agent, cookie recipes with sucrose showed a higher risk/benefit index compared to samples with glucose indicating that the formation of antioxidant activity compounds does not compensate for that of AA. Glucose compared to sucrose have a higher reactivity in both

Maillard and caramelization reactions which enhance both the formation of AA and antioxidant activity. This study, therefore, highlighted the importance of also considering the effect of individual sugar types on the formation of beneficial compounds. [52]

The use of blackstrap molasses as an alternative to glucose and sucrose was also evaluated in cookies formulated with different leavening agents [46]. Compared to glucose and sucrose, samples formulated with blackstrap molasses showed a higher AA content ranging from 511 to 740 μg/kg and from 1260 to 2390 μg/kg in cookies formulated with 3 and 2 g of leavening agent, respectively. Besides the high reducing sugars level, these results were attributed to the low pH value in the dough of cookies prepared with glucose or black molasses compared to other samples prepared with sucrose. [46]

Indeed, the findings of Sung and Chen [53], analyzing a very simple cookie dough model, made from wheat flour, sugar, and water, showed that fructose reacted significantly faster with amino acids of flour during the first 10 min of baking inducing AA formation compared to glucose and sucrose. Nevertheless, the authors did not find differences in AA levels after 20 min of baking. This indicates that the sucrose had already been converted to glucose and fructose due to the thermal process before this time.

The high reactivity of fructose for AA formation was also confirmed by Miśkiewicz et al. [54], who evaluated the effect of different reducing sugars in low humidity carbohydrate-asparagine model systems comparable to a cookie product. The replacement of fructose with glucose or sucrose caused a decrease in the resulting AA content by 29.8 and 44.1%, respectively. These results were attributed to the low melting point temperature of the different sugars that have an impact on the degree of asparagine-to-AA conversion. In detail, sucrose, due to its high melting point temperature, equal to 184 °C, is the least reactive among the analyzed carbohydrates leading to a lower formation of AA. On the other hand, fructose,

having the lowest melting point temperature (between 119 and 122 °C), is the most reactive leading to the highest formation of AA.

The recent research of Aarabi and Ardebili [55] investigated different combinations of inverted sugar syrup and sucrose to study the formation of AA in rotary mould cookies produced on an industrial scale in a three-zones oven, for the following baking temperatures for zone-1, zone-2, and zone-3, respectively, and baking times: 250-320-350 °C for 7 min and 45 s (condition I); 240, 350 and 380 °C for 7 min and 20 s (condition II); and 230, 380 and 410 °C for 7 min (condition III). In detail, three recipes with different combinations of sucrose and invert sugar syrup have been studied during three different time-temperature industrial baking conditions. The results confirmed that either type or level of sugars has a strong influence on AA formation, so that decreasing inverted syrup from 9 to 5% and simultaneously increasing sucrose from 13 to 15%, promoted a reduction of AA formation during the baking process carried out at the tested temperature-time conditions.

Another sugar widely used in the special cookies and bakery products formulations is brown sugar. Some bakeries prefer using this ingredient because it is considered to be healthier and gives a unique appearance and flavor. The study by Passos et al. [56] concluded that using sucrose and brown sugar allowed to obtain cookies with AA values (139-188 µg/kg) lower than the ones obtained using only fructose (256-388 µg/kg).

However, brown sugar may contain traces of AA itself due to its production process, for this reason, Shyu et al. [57] studied AA formation in cookies prepared with dark brown sugars with high and low AA contents (908 and 140 µg/kg, respectively) instead of sucrose. As could be expected, the higher the initial AA content in dark brown sugar, the higher the amount of AA in the final baked product. The addition of dark brown sugar, as a replacer of sucrose, significantly increased the AA levels, both because the content of reducing sugar is

higher in brown sugar than in sucrose, and because brown sugar already contains a certain amount of AA.

Other authors have evaluated, as a strategy for AA reduction, the use of alternative high-intensity sweeteners and polyols, increasingly used in place of sugar in bakery products to maintain the glycaemic index low. The research of Garcia-Serna et al. [58] aimed at evaluating new cookie formulations with sucrose, maltitol, and stevia as sweeteners to obtain high-quality diet products, also determining the AA contents. The use of maltitol and stevia alone allowed to obtain a significant AA content mitigation of 26.4 and 25% respectively compared to the cookie sample with sucrose. However, under the formulation and baking conditions applied, AA levels were very low even for samples with sucrose.

The research of Singh and Kumar [59] focused on optimizing the formulation of gluten-free cookies using sugar and fat substitutes, such as acesulfame-k and maltodextrin, thus adding a new and healthy choice to the range of commercially available bakery products for celiac, obese, and diabetic people. The replacement of sucrose and fat content with binary (fat and sugar) substitutes also promoted a strong reduction of AA in the final product (from 500 to 320 µg/kg).

In addition, Suman et al. [60] aimed at investigating how AA concentration may be influenced by bakery-making parameters, including dextrose percentage, within a parallel strategy of mycotoxin mitigation related to wholegrain and cocoa cookie production. The increase of dextrose content contributed to the overall AA increase. When a high dextrose level and a high thermal input were employed (200 °C for 8 min) an AA increase up to 120% was observed (data not shown). On the other hand, a combination of lower dextrose content and moderate thermal input (180 °C for 8 min) may lead to an AA reduction up to 77% (data not shown).

Therefore, from the study of the literature, it can be concluded that a reduction in sugar content and a careful choice of sugar types could reduce AA levels in cookies. However, changing the type and amount of sugar is a challenge for the bakery industry because of the many functions that this ingredient has in the process and its effect on the main sensory properties of the baked product especially in terms of color and texture. [46, 48, 49, 56–59]

3.1.3 Leavening agents

Leavening, raising, or baking agents, are key ingredients used in sweet bakery dough that cause a foaming action which lightens and softens the finished baked product. [19] Small sweet products such as cookies that bake quickly need a fast-acting leavener that releases the gas before the structure sets. Therefore, to provide the desired uniform pore structure and improved eating quality, chemical leavening agents are normally used in the production of cookies. Furthermore, since cookies are characterized by a high amount of sugars, biological yeasts are usually not recommended as the sugars would inhibit their activity and development. [21]

The two major chemical leavening agents used in the manufacture of cookies are sodium bicarbonate ($NaHCO_3$), called also baking soda, and ammonium bicarbonate (NH_4HCO_3), both systems being decomposed into carbon dioxide gas when exposed to heat during baking. [44] Although NH_4HCO_3 is the most widely used leavening agent, it leads to an indirect increase in the formation of AA in cookies probably because it provides more reactive carbonyls originating from the reaction of ammonia with glucose and fructose present in the dough. Glyoxal, methylglyoxal, and many other formed R-dicarbonyls have been shown to react more rapidly with amino acids than glucose or fructose. [48] For this

484 reason, several studies have been conducted in the literature on its replacement by other
485 leavening agents, as summarized in Table 3.

486 Amrein et al. [48] investigated the influence of NH_4HCO_3 added in gingerbread dough
487 in different amounts. The results showed that AA formation in cookies was proportional to
488 its content; when the leavening agent was not used almost no AA was formed, on the other
489 hand, gingerbread, prepared according to the traditional recipe with a leavening agent
490 concentration of 0.8%, contained 501 µg/kg of AA. When 0.4% of NH_4HCO_3 was added, the
491 AA content decreased by 60% (170 µg/kg), whereas 1.6% led to a strong increase in AA
492 content (880 µg/kg). The same authors also evaluated the influence of $NaHCO_3$ added in two
493 concentrations (0.83 and 1.67%) as an alternative baking agent to NH_4HCO_3. Its application
494 reduced the AA content to one-third compared to NH_4HCO_3 both for the concentration of
495 0.83 and 1.67%. However, the pH values of the doughs (from 8.2 to 8.8) were significantly
496 higher in the samples with $NaHCO_3$, when compared to those obtained with 0.8% NH_4HCO_3
497 (pH 6.9). These results showed that $NaHCO_3$ allows the preparation of cookies with a
498 substantially lower AA concentration and that a more alkaline pH does not necessarily imply
499 a higher AA content in gingerbread.

500 Following these previous findings, Graf et al. [49] and Sadd et al. [61] tested various
501 combinations of baking agents such as NH_4HCO_3, $NaHCO_3$, and tartaric acid ($C_4H_6O_6$), an
502 organic acid often added to baking powders to enhance leavening in sweet bakery products.
503 The amount of each individual compound in each combination was chosen to obtain the same
504 volume of gas released from the standard baking agent composed of 127 g of NH_4HCO_3, 273
505 g of $NaHCO_3$, and 195 g of $C_4H_6O_6$ per 100 kg of dough. The complete replacement of
506 NH_4HCO_3 by $NaHCO_3$ promoted a reduction of over 70% of AA content. The authors stated
507 that part of this effect on the AA content might also be ascribed to a lower pH related to the

presence of more tartaric acid when NH_4HCO_3 was fully replaced by $NaHCO_3$. [49] The pH-dependence of the Maillard reaction exhibits a maximum of AA formation at pH values around 8, on the other hand, lower pH induces a reduction of AA formation [60, 62]; the effect of the presence of organic acid is discussed below in the section "3.2.1 Organic acids".

Various following studies confirmed that generally, any leavening agent increased AA and that ammonium-based agents gave the highest levels while the replacement with $NaHCO_3$ as the only baking agent could be a strategy to decrease the AA in shortbread. Moreover, the presence of tartaric acid allows to reduce AA formation inducing the dough pH reduction. [38, 46, 53, 61, 63, 64]

Contrary to previous findings, Courel et al. [65] observed that AA in cookies appeared to be not affected by the presence or absence of NH_4HCO_3 (0 or 0.33% of dough). According to the authors, this observation may be partly due to the limited number of AA analyses in this study, leading to an insufficient data set for discrimination purposes.

A further study tested the effects of recipe composition in terms of leavening agents and sugars contents on a risk/benefit index considering the formation of AA and antioxidant compounds in cookies. [52] Cookies prepared with sucrose and $NaHCO_3$ showed a significantly higher index for AA/antioxidants than those prepared with NH_4HCO_3. These results highlighted that NH_4HCO_3 was not efficient for enhancing the formation of substances with antioxidant activity. The lack of antioxidant formation was not observed in the recipe with glucose and NH_4HCO_3; in fact, the presence of glucose probably increased the formation of compounds with higher antioxidant activity compared with sucrose recipes as discussed in the section "3.1.2. Sugars". [52]

Kukurová et al. [64] also observed that using sodium pyrophosphate ($Na_4P_2O_7$) as a leavening agent in cookie formula allowed to obtain a final AA concentration similar to the

control sample obtained without leavening agents. However, the author did not provide a possible explanation for this result. Also, in this study, the highest AA levels were found in cookies obtained with NH_4HCO_3.

In the study of Suman et al. [60] a predictive model was developed, suggesting a significant role of low pH values of cookie dough, related to the presence of $NaHCO_3$ as a leavening agent, on the reduction of AA formation in the final product. However, the obtained AA levels were not reported.

A more recent study of Sazesh and Goli [6] aimed to optimize cookie formulation considering three levels of $NaHCO_3$ (0.05, 0.10, and 0.15% based on the final dough weight), five wheat, and quinoa flour blends, and different baking conditions. The authors concluded that the amount of AA in cookies was mainly affected by the amount of the leavening agent when wheat flour was used. In particular, at the baking temperature of 185 °C, the AA amount increased with increasing $NaHCO_3$ and wheat flour in cookie formulation, while the lowest amount of AA was observed with increasing levels of quinoa flour at all levels of $NaHCO_3$ highlighting that the formation of AA is more likely to be influenced by flour rather than the leavening agent. Since at least 0.05% $NaHCO_3$ raised the pH level to more than nine and quinoa flour compared to wheat flour has a low concentration of asparagine and reducing sugars as AA-producing agents, the AA-producing Maillard reaction occurred to a lesser extent.

The choice of type and amount of leavening agents can be a strategy to reduce AA levels in cookies, however, it must be considered that different leavening powders in the formulation can significantly influence the final quality of cookies mainly in terms of textural, physical, and organoleptic characteristics. [19] For example, according to Graf et al. [49], cookies prepared with a leavening agent without NH_4HCO_3 showed a lesser leavening

as compared to the standard product formulated with a traditional baking agent composed of a mixture of NH_4HCO_3 and $NaHCO_3$. However, in this study, the difference in leavening capacity was not problematic as the cookies were used as a semi-finished ingredient for other bakery products, and its suitability for further use was not negatively affected. Sensorial analysis carried out on cookie samples indicated that the addition of $NaHCO_3$ at 1% did not affect the main sensorial proprieties compared to the other formulas prepared with NH_4HCO_3. [46] However, Kukurová et al. [64] and Canali et al. [19] reported that in general the addition of high doses of $NaHCO_3$ provides an alkaline taste, a yellowish crumb, and surface coloration and an unpleasant taste, known as "soda bite". On the other hand, the use of $NaHCO_3$ must be handled with care, as it increases the sodium content of the formulation, with organoleptic changes and nutritional consequences. Further results revealed that NH_4HCO_3 and $NaHCO_3$ leavening agents led to a shape expansion and a crispy texture of the cookies when desired, whereas cookies with $Na_4P_2O_7$ were paler, smaller, and harder. [64]

3.1.4 Oils and fats

Fats and oils are added to the formulations of many bakery products to improve sensory and rheological characteristics; moreover, the presence of fat influences the dough processability and the shelf-life of products. [66] However, as reported by several studies summarized in Table 4, the type and amount of fats used in the cookie's formulation can also influence the AA content of the final product.

Cookie doughs enriched with three types of virgin olive oils, classified according to their content in phenolic compounds in high, intermediate, and low oleic oils were evaluated in terms of AA formation during baking at 190 °C up to 16 min by Arribas-Lorenzo et al. [67]. No significant differences in AA levels were found among the different cookies for the

shortest baking times of 8, 10, 12, and 14 min. However, after baking for 16 min, the use of low oleic oil resulted in the highest level of AA (805 μg/kg) while high oleic oil resulted in an AA value reduced by 20% (637 μg/kg). The same authors also evaluated the impact of the oxidation degree of the oil, using in cookie formulation sunflower oil previously heated at 180 °C for 17 h in a laboratory oven compared to the control one. Both samples of cookies showed a significantly different AA content when baked for 8 min and a significantly similar one when baked for 14 min. After 16 min of baking, AA rapidly increased in cookies formulated with oxidized oil reaching levels about 59% higher compared with control one. It can be concluded that the use of oxidized oil in the cookie formulation led to a huge increase in AA formation during cooking, thus the presence of antioxidant compounds is a possible strategy to control AA formation.

Anese et al. [68] tested the effect of different amounts of margarine (0, 8, and 15%) and alternative fats such as palm oil and monoglyceride-palm-oil-water gel (hydrogel) added in the cookie recipe. Concerning margarine and palm oil, the highest AA concentration was found in the free-fat cookies, while both fats addition significantly reduced (from 41 to 28%) the formation of AA. These data seem to indicate that during baking the presence of melted margarine (transition phase at 62 °C) could hamper the interaction between the precursors in the aqueous phase, leading to lower amounts of AA. However, even if the two fats had different chemical compositions and physical properties, no significant differences in AA formation were found between margarine and palm oil-containing cookies. On the contrary, the substitution of fat with the hydrogel caused a significant increase of AA content, leading to levels comparable to those obtained for the fat-free formulation. This result indicates that the incorporation of palm oil in the form of the hydrogel may modify the “hampering effect” of fat towards AA formation.

603 Another study of Haase et al. [38] assessed the AA formation in relation to the fat
604 content used in the cookie formulation to alter volume during baking. It was concluded that
605 a reduction of shortening content by around 40% improved the final volume of cookies.
606 Nevertheless, AA content dropped down non-significantly. However, in this study, the
607 authors did not specify any possible assumptions related to the obtained results.

608 A further in-depth study of Negoiță et al. [16] focused on the influence of five types of
609 fat, such as sunflower oil, palm oil, margarine, lard, and butter, on the AA content of cookies
610 also formulated with different flours. Although the processing conditions were the same, for
611 each type of flour, the use of the same amount of fat in the formulation with different lipid
612 content, ranged from 60 to 100%, led to an increase in the level of AA with the following
613 trend: margarine < butter < lard < sunflower oil < palm oil. Fats with a high lipid content
614 (100%) like lard, sunflower, and palm oils, provided a higher level of AA compared to the
615 types of fat with less lipid content of 60 and 65%, respectively.

616 For cookies formulated with black wheat flour (85-95% ash content) the use of
617 different amounts of fat with the same lipid content (60%) was evaluated. [16] In general, using
618 a smaller amount of fat resulted in a decrease in AA content of 11-15% compared to cookies
619 where the same amount of fat with a different lipid content (60-100%) was used. Thus, the
620 AA content was higher when the fat contained a higher level of triglycerides, mainly with
621 unsaturated fatty acids. Triglycerides are responsible for the formation of AA, probably
622 because they lead to increased formation of acrolein through oxidation. It is known that the
623 formation of AA from the reaction of acrolein and ammonia (NH_3) is a possible secondary
624 route to be considered in fat-rich products. [69–72]

625 Sung and Chen [53] evaluated the effect on AA formation of adding or not shortening,
626 a very common fat in the formulation of bakery products, in model cookies consisting of

flour and water. From 10 to 20 min of baking model cookies with shortening in formulation had a lower level of AA compared to those without shortening. The mitigating effect on AA formation given by the addition of shortening in formulation could be due to their partial hydrogenation that prevents the reaction between the amino acids and the reducing sugars.

The previously mentioned authors who studied the influence of oils and fats in the cookie formulation on the formation of AA did not consider, at the same time, their influence on the final quality characteristics of the product, particularly on the overall sensory properties. Thereby, further studies are needed to simultaneously evaluate the influence of fat/oil on both desired final cookie characteristics and AA formation.

3.1.5 Salts

Salts have traditionally been used during the manufacture of bakery products as they cause several important changes in rheological, technological, and sensory parameters. [44] Sodium chloride (NaCl) is the main salt added as a flavor enhancer and is also used in low quantities in sweet bakery products, including cookies. Monovalent and bivalent ions such as NaCl can influence the development of Maillard reaction through the dehydration of various key intermediate compounds. [73] Due to the common use of salt in cookie formulation, many researchers, reported in Table 5, have studied its effect on the AA formation in the product.

Based on previous studies showing that the addition of polyvalent cations such as Ca^{2+} prevents the formation of AA in bakery products [74–76], Fiore et al. [73] evaluated the incorporation of microencapsulated NaCl into cookie recipes in the increasing percentages of 0, 0.32, 0.65 and 1%. It was found that the formation of AA was not significantly modified by the presence of salt. Cookies with 0.65% of NaCl showed an average AA concentration of 278 μg/kg, whereas the control without NaCl had the highest concentration equal to 313

650 µg/kg. These data showed that there was not a direct relationship between NaCl concentration
651 and AA levels in cookies.

652 Van Der Fels-Klerx et al. [77] prepared cookies formulated without and with 0.65%
653 NaCl, baked at different temperatures of 180, 190, and 200 °C for 15 min. The results
654 revealed a significant reduction in AA of approximately 16 and 30% in samples prepared
655 with the presence of salt, when baked at the lower temperatures of 180 and 190 °C,
656 respectively. This was attributed to the inhibition of the formation of Schiff's base, which is
657 formed in the condensation reaction between asparagine and reducing sugars, by the release
658 of the two monovalent ions (Na^+ and Cl^-) during baking at 180 and 190 °C. In addition, AA
659 concentration reached the maximum when cookies from both recipes were baked at 200 °C,
660 with no significant differences between them, demonstrating that the effect of salt
661 concentration does not occur at high baking temperatures.

662 In agreement with these results, Sung and Chen [53] found a positive action of salt in
663 reducing AA levels when added to 1% in model cookies made from flour and water and
664 baked at 205 °C for 15 min. Specifically, at this baking time, an AA content of 103.3 µg/kg
665 was found in cookies with NaCl and 790.1 µg/kg in cookies without salt. However, after 20
666 min of baking at the same temperature, no significant differences were found between
667 cookies made with (739.8 ±118.0 µg/kg) and without (953.4 ± 26.8 µg/kg) NaCl.

668 The use of NaCl in the formulation as a strategy to reduce AA in cookies requires
669 further investigation; furthermore, its impact on the organoleptic and health characteristics
670 of the products must also be taken into account, so the choice of the amount used must be
671 undertaken with some degree of care. [45] Some authors in the literature have tried to assess
672 the replacement of NaCl with other salts as another useful strategy to reduce the presence of
673 AA in cookies, without increasing sodium intake beyond the amounts recommended by the

World Health Organization (WHO). It has been suggested that adding divalent metal ions could promote the stability of the interaction between asparagine and the food matrix at high temperatures (stable polymer network); thereby rendering this amino acid unavailable for reaction with carbonyl precursors to produce AA. [78] To test this effect, Sadd et al. [61] incorporated calcium in cookie dough as chloride ($CaCl_2$), carbonate ($CaCO_3$), or propionate ($C_6H_{10}CaO_4$) in the concentration of 2, 1, and 0.7 or 0.35%, respectively. When incorporated into cookie dough, calcium in the form of chloride and carbonate reduced AA by 60 and 15%, respectively. On the contrary, calcium propionate, already added to bakery products in the UK as a preservative (up to 0.2%), incremented AA levels of about 15 and 20%, respectively. The reasons for this behavior were not clear for authors because the addition of propionic acid alone had little effect on AA levels, it allowed only a slight reduction of about 2% compared to the control cookie. Calcium supplementation seems promising for AA control, but interactions with other ingredients (especially propionate) need further investigation.

The study conducted by Quarta and Anese [79] found no changes in AA formation in cookies formulated with 0.25% of $CaCl_2$ or $MgCl_2$ compared to the control sample without salt. However, a 60% reduction was achieved when these salts were added in combination (1:1, w/w), suggesting a synergic effect of the cations Ca^{2+} and Mg^{2+} on the AA reduction in cookies. On the other hand, the results showed that the potassium acetate (CH_3COOK) was responsible for the greatest increase in AA of 116% in the cookies compared to the sample with no salt.

The effect of calcium derivatives on AA levels in cookies was also evaluated by Açar et al. [80] adding in cookie recipe 0.04, 0.2, and 0.4% of $CaCl_2$ and calcium salts of lactic acid such as Puracal Act 100 (PA100) and Puracal Act 200 (PA200), characterized by a ratio of

calcium to lactate of 23 to 35% and 20 to 44%, respectively. Compared to the control sample without salt, each calcium derivative contributed to a decrease in AA formation in cookies directly related to the amount of calcium added. At the amounts of 0.2%, PA100 and $CaCl_2$ were found more effective to mitigate AA formation in cookies compared to PA200, leading to a reduction in AA of 72.4 and 66.3%, respectively. These results were explained by the presence of less calcium and more lactate in PA200 than PA100, the organic acid may have facilitated the formation of AA by promoting the hydrolysis of sucrose. Moreover, in this study, experimental cookies with and without calcium addition were prepared to determine the effect of calcium on the rate of AA formation during baking. The reaction rates based on the slopes of AA formation indicated that the presence of 0.4% of $CaCl_2$ significantly reduced the AA formation in cookies. The AA inhibition ratios were found to be 63.7, 74.1, and 73.7% at 150, 200, and 250 °C of baking, respectively.

Chang et al. [81] compared the effects on AA of adding different quantities of various calcium salts such as calcium lactate ($C_6H_{10}CaO_6$), calcium citrate ($Ca_3(C_6H_5O_7)_2$), calcium acetate ($C_4H_6CaO_4$), and calcium carbonate ($CaCO_3$) plus NaCl in cookies and alone in model cookies prepared with only flour, water, and sucrose. All calcium salts addition has been shown to reduce AA in all samples. The AA concentration of the model and control cookies mainly decreased when fortified with $CaCO_3$; the addition of a concentration of 0.06% (% w/w of dough) of this salt resulted in a reduction in AA of 30 and 13% compared for model and control cookies, respectively. On the other hand, the AA content of calcium lactate-added cookies was significantly lower than that of the corresponding control cookies. The reducing sugar content in the model cookies with calcium lactate was higher than that of those fortified with other calcium salts, confirming the enhance of hydrolysis of sucrose to reducing sugar in presence of organic acid as reported previously by Açar et al. [80].

722 The effect of replacing NaCl with different salts such as $CaCl_2$, potassium chloride
723 (KCl), and two different salt replacements consisting of 13.8 g Na/100 g plus 20.0 g K/100
724 g (SR-I) and 14.3 g Na/100 g plus 17.1 g K/100 g (SR-II) in cookies baked at 190 °C for 20
725 min was studied by Mesías et al. [82]. The AA levels detected ranged from 153.4 µg/kg to
726 380.8 µg/kg, with the highest values in cookies containing NaCl, KCl, and the salt substitute
727 SR-I. The AA concentration decreased by up to 17% when NaCl was replaced by SR-II,
728 while reductions of 35 and 40% were observed in cookies formulated with a mixture of NaCl
729 and SR-I or SR-II, respectively. This could be explained by a possible synergistic effect
730 between the salts when they are mixed in the same cookie recipe, in agreement with the
731 observations of Quarta and Anese [79].

732 Contrary to the previous finding, in the more recent study of Shyu et al. [57], the
733 presence of 1% of calcium ions was not associated with either a reduction in AA formation
734 or an increase in the amount of reducing sugars in cookies formulated with dark brown sugars
735 with high and low AA contents (908 and 140 µg/kg, respectively). Gökmen et al. [51] reported
736 that the Schiff base formation was mitigated and changed to another pathway, with the
737 dehydration of glucose generating hydroxymethylfurfural and furfural. The reaction
738 proceeded in this way when calcium ions were increased. However, the results of Shyu et al.
739 [57] agree with the mechanistic model based on an asparagine-related pathway proposed by
740 Nguyen et al. [83]. Both authors claimed that fructose reacted with asparagine to form a Schiff
741 base without any Amadori rearrangement product or sugar fragmentation before
742 decarboxylation to produce AA.

743 In light of these results, the addition of NaCl or other salts is a possible intervention
744 to minimize AA formation in cookies. However, it must be pointed out that types and/or
745 quantities of some usable salts may be responsible for undesired effects, such as failure in

746 the development of desired sensory properties. [61, 80, 81] For example, calcium chloride, when
747 used in certain concentrations, hindered the growth of sweet cookies and the products had an
748 unpleasant taste. [61] In addition, the calcium derivatives affected the cookie's surface colors
749 by increasing surface lightness (L*) and decreasing the redness (a*) parameter. [80] On the
750 other hand, Chang et al. [81] showed that the overall acceptability of the fortified cookies is
751 significantly improved by the addition of calcium carbonate, while other calcium salts had a
752 significant negative effect on the appearance and spread ratio of the product.

753 ***3.2 Additional ingredients***

754 *3.2.1 Organic acids*

755 Other minor ingredients such as organic acids, commonly added to bakery products to
756 regulate acidity and improve flavor or leavening, have been tested in numerous studies in the
757 literature for the control of AA formation in cookies. In Table 6, the studies that evaluated
758 the effect of this and other feasible additional ingredients used in cookie formulations for the
759 mitigation of AA formation are reported. It is widely established that pH values can influence
760 the formation of AA. Lowering the pH of a food matrix prevents the nucleophilic addition of
761 asparagine with a carbonyl compound and the formation of the corresponding Schiff base, a
762 key intermediate in the Maillard reaction and thus AA formation. [72, 84]

763 Amrein et al. [48] carried out various experiments checking the ability of citric acid to
764 reduce AA content in gingerbread cookies. The addition of 0.5 and 1.0% of dough weight
765 resulted in drops of pH to 5.6 and 5.0, and in a reduction of the AA concentration by factors
766 of 4 and 40, respectively.

767 These results are in line with those reported by Graf et al. [49], who added tartaric acid
768 in different proportions to the dough of semi-finished cookies, leading to a decrease of AA

formation at all additional levels. The use of 0.24% tartaric acid by weight of the dough decreased the AA content by one-third, while an even higher acid addition of 0.29% had a slightly greater effect on the reduction of AA content by 44%, which was not significantly lower than in the previous experiment.

Also, Gökmen et al. [51] evaluated the addition of citric acid in three different percentages in cookies prepared with sucrose and with glucose plus sucrose, corresponding to doughs pH of 7.40, 4.37, and 3.28 in both recipes. Lowering the pH by adding citric acid to the dough with glucose and sucrose, resulted in a 67% of reduction in the AA content of cookies. However, the addition of citric acid to dough formula with sucrose alone increased the AA formation in cookies, probably due to the excessive hydrolysis of sucrose, which increased the concentration of reactive sugars.

The work of Mogol and Gökmen [85] showed that the addition of formic acid in cookie dough did not significantly affect the formation of AA at all baking temperatures studied.

Another more recent study by Passos et al. [56], tried to modulate the AA formation in cookies by adding polymeric acid compounds such as galacturonic acid (monomer) other than pectin (polymer) or partially hydrolyzed pectin (oligomer) as substitutes to tartaric acid. When using the monomer galacturonic acid, which consists of both a reducing sugar and an acidic part, the formation of AA and its mitigation were simultaneously achieved. Consequently, when 1% of this monomer was added to the cookie dough, the AA formation increased by 95% compared to the control sample without pectic polysaccharides. When using 1 or 5% of partially hydrolyzed pectin, the amount of AA in cookies was significantly lower than galacturonic acid-based cookies but comparable to the amount of AA observed in the control cookies. The partially hydrolyzed pectin although presenting 13.8% of reducing sugars, which had a net contribution to the formation of AA, had also an AA mitigation effect

due to the pH-lowering outcome of the repeating units of galacturonic acid residues in its oligomeric structure. Using 1% of pectin, which contains a lower amount of reducing sugar compared to galacturonic acid, the AA level was significantly lower than that of cookies with galacturonic acid but still comparable to the amount of AA observed in the control and partially hydrolyzed pectin cookies. Nevertheless, although having the same galacturonic acid content, 1% pectin samples contained half of methyl-esterified residues, which did not contribute to the same extent of pH-lowering effect of galacturonic acid and partially hydrolyzed pectin samples. In conclusion, the authors of this experiment suggested that the lowest amount of reducing sugars of cookie samples with pectin is the cause of the lowest formation of AA when compared with the other samples. Furthermore, this hypothesis is also corroborated by the experiment with the addition of pectin by 5%, where the AA formation was significantly reduced by 67% compared to the control without pectin addition. Similarly, the addition of only 1% of tartaric acid, which contributes to the acidifying effect without adding reducing sugars towards AA formation, promoted a 52% decrease in AA content compared with the corresponding control. In addition, a 5% of tartaric acid addition to the dough resulted in an 81% AA content reduction.

In summary, the addition of some organic acids is a possible way to control the AA level in cookies. However, the amount of acid must be carefully assessed evaluating its effects on sensorial and physic-chemical properties of the cookies. [48, 51, 56] The addition of citric acid in gingerbread led to cookies with a clearly acidic taste, not homogeneous color surface, and insufficient volume which limited their acceptability. [48, 51] Cookies prepared with tartaric acid and examined by an untrained sensory panel showed a difference in hardness, crispness, and firmness to the touch compared to those prepared without any organic acid; a harder, brittle, and sandy texture on the tongue and a sour taste. [56]

817 *3.2.2 Amino acids*

818 Another strategy to control the formation of AA in bakery products is the use of different
819 amino acids that can compete with asparagine in the Maillard reaction or that can react with
820 the nucleophilic amino group of AA formed through Michael addition reaction, promoting
821 its elimination. [44, 86] In the literature, different types of amino acids and certain protein
822 isolates have been tested in cookies formulations to control AA (Table 6).

823 The first study of Amrein et al. [48] did not found a reduction in AA contents in
824 gingerbread cookies with the addition of the amino acids L-glutamine, L-lysine, or glycine
825 at a 0.2% of dough weight. For glycine, a concentration of 1% was also evaluated which
826 reduced the AA content in cookies to one-third. Another tested amino acid, L-cysteine,
827 showed a tendency to reduce the dough pH and AA content at the concentration of 0.05 and
828 0.2%.

829 Salazar et al. [87] in an attempt to investigate additional beneficial properties of the
830 underexploited plant, investigated the AA mitigating effect of amaranth proteins isolate used
831 in cookies formulation. The addition of amaranth protein isolates significantly decreased the
832 AA formation in the cookies upon baking. In particular, AA mitigation was reduced from
833 89% (using a baking time of 7 min, which was the optimum baking time for the assayed
834 cookies) to 26% (with a baking time of 9 min). This result is due to the amino acid
835 composition of amaranth proteins that are rich in lysine (4.8-6.4 g/100 g of protein) and sulfur
836 amino acids (3.7-5.5 g/100 g of protein).

837 Another study tested the effects of cysteine plus glycine amino acids or in
838 combination also with $CaCl_2$ on the reduction of AA formation in cookies. All the added
839 compound mixtures reduced the contents of AA, showing a synergistic effect when amino
840 acids were combined with each other or with $CaCl_2$. The optimal formulations were 0.36%

of cysteine plus 0.2% glycine plus 0.06% $CaCl_2$ and 0.29% cysteine plus 0.2% glycine which led to a drastic AA reduction of 97.8 and 98%, respectively. [88]

As regards protein-based ingredients commonly added in the formulation of cookies such as milk and egg, Suman et al. [60] that tested different recipes obtained from an experimental design, based on the overall statistical evaluation, found no differences in mitigation effect on wholegrain and cocoa cookies baked at pilot-plant processing conditions (data not shown). This result was attributed to the fact that the ranges of variation studied for milk and egg content in the recipe formulation were very close, 5-8 and 4-7% respectively.

It has been demonstrated that the addition of certain amino acids, in supplement of asparagine, to the formulation of cookies can reduce the AA content. However, even for these ingredients, it is necessary to evaluate possible modifications on the sensory proprieties of the final products. For example, the ready reaction between glycine and reducing sugars strongly increases the browning of the cookies' surface, as more melanoidins result, while the amino acid L-cysteine has an unpleasant taste and odor presumably caused by S-containing decomposition products. [48] Nevertheless, to minimize these adverse effects, the amount of the amino acids added can be reduced and these compounds can be used in combination, or natural protein ingredients, such as amaranth protein, can be employed. [87, 88] However, a much more detailed sensory evaluation of cookies obtained with these ingredients is needed.

3.2.3 Enzymes

Among the most effective mitigation strategies tested for reducing AA in the most at-risk foods, including cookies, there is the use of the enzyme asparaginase (Table 6). This enzyme can hydrolyze asparagine into aspartic acid; hence it represents a good way to remove this

AA precursor from the primary ingredients before thermal processing. The effectiveness of asparaginase depends on its concentration, time, and temperature of its incubation, water activity of the food matrix that affects its mobility, and the pH at which the asparagine conversion reaction takes place. [45]

From its use in the cookies formulation, excellent results were achieved leading to an AA reduction of up to 55% in gingerbread baked at 180 °C for 3 min plus 190 °C for 7 min. [48] Analysis of the fresh dough treated with asparaginase revealed that it still contained 22 mg/kg of free asparagine and that 75% of the total free asparagine had been degraded, which explains why AA formation was not fully inhibited. Its incomplete hydrolysis was probably due to the limited mobility of both the enzyme and the substrate within the cookie dough.

Hendriksen et al. [89] used asparaginase from the fungus *Aspergillus oryzae* in semisweet and ginger cookies formulations. A clear reduction of AA levels was observed in the semisweet cookies, especially with increasing the amount of the enzyme. Treatment with 525 ASNU/kg of flour (one ASNU is defined as the amount of enzyme that produces 1 μmol of ammonia per minute at 37 °C and pH 7.0) and a dough resting time of 15 min resulted in AA reduction of 65% when compared to the control sample, while cookie treated with twice the enzyme amount and the same resting time had a reduction in AA content of 84%. For all asparaginase levels, the AA reduction effect increased when resting time was longer than 30 min, illustrating that the system operated within the dynamic response range of both enzyme dosages and resting times.

A clear enzyme dose-dependent reduction in AA levels was confirmed also by Huang et al. [90] evaluating the effect of asparaginase produced from *Rhizomucor miehei* in cookies. Approximately 15 and over 80% of AA reduction was reached when the concentration of this enzyme was 0.5 and 100 U/g flour, respectively.

The importance of dough water content on the asparaginase activity and asparagine mobility was further investigated by Hendriksen et al. [89] in ginger cookies prepared with different water contents and a constant enzyme dose of 1000 ASNU/kg of flour. AA levels measured in the control cookies without the addition of asparaginase were rather constant, despite the differences in dough water contents. For the other samples, a clear correlation between cookies' AA level and dough moisture content was observed. Increasing water content from 11 to 19% with the same concentration of asparaginase, allowed an increase of AA reduction from 34 to 90%. This result was attributed to a limited enzyme-substrate contact in the low-water doughs because of limited diffusion, confirming the previous hypothesis of Amrein et al. [48].

A further study of Anese et al. [68] studied the influence of the matrix composition and structure on the capacity of asparaginase to reduce AA formation in short dough cookies. In detail, formulations differed for water (10 to 20% on total weight) and fat (0 to 15% on total weight) contents, moreover fat type (margarine, palm oil) and lipid phase distribution were considered. The results showed that high water contents, by favoring reactants mobility, promoted AA formation as well as, the enzyme capability, when added to the formulation, of reducing asparagine levels in the final product. Thus, when present, the asparaginase enzyme was responsible for a 58% AA reduction in the sample with the highest water amount of 20%. On the contrary, the presence of fat significantly reduced both enzyme activity and AA development compared with the fat-free cookie formulation, suggesting that fat would make more difficult the contact between reactants. In fact, the highest AA concentration was found in the fat-free cookies and the percentages of AA reduction caused by the addition of the enzyme to the doughs decreased progressively as the fat concentration increased (69, 62, and 58% AA reductions corresponding to 0, 8 and 15% of fat). In addition, the asparaginase

capability to lower AA formation seemed to be influenced also by the different structures of systems due to the presence of a different type of fat such as margarine, palm oil, and hydrogel. The AA reduction in the hydrogel-containing cookies (66%) was significantly higher compared to margarine (58%) and palm oil-containing (58%) formulations. Being water-soluble, asparaginase would be confined in the aqueous domain of the hydrogel together with AA reactants. Therefore, probably due to the higher proximity between the enzyme and substrate in the hydrogel system, asparaginase efficiently mitigated AA formation like in the fat-free system. However, the AA level in the hydrogel containing cookie treated with asparaginase was still higher than those found in the margarine and palm oil systems due to the reasons explained above in section "3.1.4 Oils and fats".

In another study, Anese et al. [91] also evaluated the effect of asparaginase in AA reduction in shortbread cookies by preparing, according to a three-factor, three-level cube central composite design, 15 recipes different in asparaginase concentration and incubation temperature and time, from 100 to 900 U/kg of flour, 20 to 54 °C, and 10 to 30 min, respectively. In agreement with the results reported previously, the variable that showed the biggest effect in reducing AA was the concentration of asparaginase, followed by the incubation temperature, while the incubation time of the asparaginase infused dough seemed to be the least effective variable. Within the ranges considered in this study, the intermediate asparaginase concentration of 500 U/kg combined with the lowest temperature and 20 min of incubation resulted in the lowest AA formation of 90 µg/kg in short dough cookies.

The results of Haase et al. [38] demonstrated once again that AA in cookies was significantly reduced when asparaginase was added to the dough and the thermal input was the most relevant criterion. Samples produced without enzymatic treatment showed an exponential increase in AA with increasing baking temperatures. On the other hand, the

temperature-related increase in AA in asparaginase-treated samples was on a linear basis indicating that the benefit of the enzymatic activity was especially pronounced at higher thermal input.

As already mentioned, a further aspect to consider in order to achieve maximum efficiency of asparaginase application is the pH of the dough which may be modified also by the type of chemical leavening agent used in the cookie formulation. By changing the asparaginase incubation time and pH of the cookie dough by varying the type of leavening agent, Kukurová et al. [64] observed that a deviation of the pH out of the optimal range (about pH 7) for the action of the enzyme leads to a strong limitation in its activity. The highest AA reduction efficiencies of 66 and 75% in the cookie baked at 205 °C for 11 and 15 min respectively were achieved with a 60 min enzymatic pre-treatment at pH 6.78 which is close to the optimum pH value for asparaginase activity. While shifting of pH to 8.10 diminished the asparaginase efficiency to about 50% of AA reduction after 60 min of incubation. To assess whether the required results can be obtained, it is necessary to test prolonging the enzyme incubation or increasing the enzyme dosage.

A clear advantage of using the asparaginase enzyme, compared to the other strategies for the AA formation control in cookies, is the low impact it has on the sensory characteristics of the final product. In evidence, some authors found that taste and color of cookies prepared with asparaginase were almost identical to those of the standard product. [48, 68, 91]

3.2.4 Antioxidants

Other minor ingredients studied for the AA mitigation in cookies, as reported in the literature, are represented by antioxidant compounds. Antioxidant compounds can react with AA precursors or intermediates, which may inhibit the overall rate of the Maillard reaction. In

particular, they could control AA formation in three ways: by trapping of carbonyls, reduction of sugar degradation through Maillard reaction processes, and radical scavenging activity. [92, 93] Some antioxidants ingredients and/or certain plant powder or extracts were able to reduce AA formation in cookies, while others showed no effect or even an enhancing effect (Table 6).

Significant AA reductions in cookies have been demonstrated by Zhu et al. [94] adding raw powders and crude aqueous extracts of many different common dietary plants such as cinnamon, clove, coriander, cumin, turmeric, red onion, and some phenolic compounds including cinnamaldehyde, curcumin, and eugenol. Among all plant-based raw materials tested, clove bud powders at various addition concentrations (0.25-4%) showed the highest AA reduction rates in the range of 21.6-41%. For all powders, the inhibitory effects at higher concentrations (from 2 to 4%) were not as marked as those at lower concentrations (from 0.25 to 1%). The aqueous extracts from the selected plant materials had slightly better efficiency in reducing AA, with an average decrease of 30.2% compared to the crude powders tested (26.7%). As well as with raw powders, plant extracts decreased AA content in cookies to varying degrees in a nonlinear dose-dependent manner, and the most effective in controlling AA was clove bud extract with reduction rates from 25.1 to 50.9% at different levels of 0.25-4% addition. Data for the three pure phenolic compounds (cinnamaldehyde, curcumin, and eugenol) showed slightly greater efficacy in reducing AA compared to crude plant powders (26.7%) with an average decrease of 29.8%, but comparable levels to aqueous extracts (30.2%). Eugenol promoted less reduction in AA (31.6%) than aqueous extract of clove buds (40.7%), suggesting that other factors than the major additional phenolic constituents in plant materials might be influential in AA formation. For example, crude aqueous extracts usually had strong hygroscopicity and might alter water activity levels in

cookies during heating thus affecting AA content. Crude aqueous extracts also contain other food ingredients such as protein and peptides, non-reducing saccharides, and low levels of monovalent/divalent cations, which might also play a role in the AA formation, as explained in the previous sections of this review.

Similarly, Li et al. [95], evaluating different antioxidant concentrations from 0 to 0.1% of bamboo leaves added in cookie dough, found that the highest AA inhibitory rate of 63.9% was achieved by the antioxidant concentration of 0.02%. This result indicated that after a first positive effect, a threshold value was reached, and increasing the concentration of bamboo leaves antioxidants beyond this value, a negative effect was found. This is the so-called "antioxidant paradox" since polyphenol-rich bamboo leaves can reduce free radicals and reactive free electrons that cause a rapid conversion of asparagine to AA, but on the other hand, a high concentration of antioxidants did not suppress AA formation as it did with a lower dose. Based on these considerations, the same authors evaluated the potential effectiveness of other antioxidants such as sodium erythorbate, tea polyphenols, vitamin E, and tert-butyl hydroquinone added in 0.01 or 0.02%. Results showed that the addition of these antioxidants significantly mitigated the formation of AA in cookies up to 43.0, 71.2, 54.1, and 49.6%, respectively. The difference in the inhibitory effect of these antioxidants was attributed to their antioxidation and polarity diversity.

Ten other pure and partially pure plant polyphenolic compounds (caffeic acid, chlorogenic acid, European cranberry bush juice (ECJ), ellagic acid, epicatechin, oleuropein, olive mill wastewater (OMWWE), pomegranate peel (PPE), punicalagin, and tyrosol) were tested on AA formation in cookies by Oral et al. [96]. All of them slightly decreased the AA formation in cookies at levels between 10.3 and 19.2% in comparison with the control sample obtained without antioxidants addition.

Passos et al. [97] investigated the impact of four instant coffee fractions differently obtained, by simple centrifugation (WSn), ethanol precipitation (fraction ethanol-soluble EtSn and fraction precipitated EtPp), or ultrafiltration (fraction HWSn) as ingredients for antioxidant-rich cookies. The impact of 0.5, 2.3, and 4.6% w/w relative to the dough (corresponding to 1, 5, and 10% w/w flour) of coffee melanoidin-rich fractions supplementation on the cookies was evaluated also in terms of AA formation. The content of AA in the coffee fractions was negligible and did not account for the final level of AA in the baked cookies. The highest content of AA in cookies was observed for the addition of 2.3% of EtSn fraction (274 µg/kg). This result is supported by the highest percentage of monosaccharides (18.9%) precursors in AA formation. On the other hand, the addition of 2.3% of EtPp resulted in a significant AA decrease (31%) when compared to control. This effect is supported by the lower percentage of monosaccharides (4.8%) of EtPp fraction. No differences to control in AA contents were observed for any fraction at 4.6% supplementation. The differences observed between 2.3 and 4.6% addition may result from a complex balance between AA formation and mitigation opposite effects. AA mitigation, on the other hand, maybe explained by the scavenger ability of the antioxidants towards the radical fragments of hydrocarbons that are formed during baking, preventing the formation of carbonyl groups by lipid peroxidation.

Miśkiewicz et al. [98] evaluated the positive effects of dough supplementation with different amounts of freeze-dried aqueous rosemary extract on AA content in shortcrust cookies, baked at 170 °C in dry or humid (90% RH) air. The addition of rosemary extract to the cookie dough resulted in an AA reduction proportional to its concentration in both baking methods. Compared with products without the antioxidant extract, the greatest decrease was

observed in cookies containing 0.5% of the extract, with a reduction of 18.4 and 15.8% for dry and moist air baking conditions, respectively.

Again, evaluating two different baking conditions, in conventional and microwave ovens, AL-Ansi et al. [93] proposed the addition of fine fennel and black cumin seeds as a promising strategy for AA reduction in cookies. The addition of black cumin seeds in the formulation gradually decreased the AA content by 17-53% in conventionally baked cookies and by 23-68% in microwave-baked ones. Meanwhile, the addition of fennel seeds significantly decreased AA to the minimum limit of the quantitation in microwave-baked cookies and up to 78% in conventionally baked cookies. These results were attributed to the high antioxidant activity of the seeds, highlighting a potential plant antioxidant source and mitigation strategy for AA reduction.

Two recent studies have evaluated the addition of ginger [99] and of green and spent roasted coffee samples [100] on AA formation in cookies. Ground freeze-dried ginger added in different amounts of 1, 3, 5, and 7% (w/w of dough) in cookie formulation was able to significantly reduce the AA content. The AA inhibition rate was 6.2 at the 1% ground ginger level, and 15.6, 19.1, and 23.7% at the 3, 5, and 7% ginger levels, indicating a dose-dependent relation. This result was attributed to the phenol hydroxyl group of gingerol that plays a more important role in the reaction of AA formation than the side chain. In addition, ginger contains bioactive constituents that alleviate protein glycation by trapping glucose thermal decomposition product called methylglyoxal, which might affect the inhibition of AA formation. However, more studies should be carried out on the mechanism of this ingredient in AA reduction. [99]

Cookies obtained with flour fortified with different percentages (3-12% w/w) of spent roasted coffee (RGCS) and spent unroasted green coffee (UGCS) evaluated by Desai et al.

[100] recorded low AA in the range of 32.6-37.8 µg/kg and 23.4-29.7 µg/kg, respectively. This low AA content was associated with the phenolic antioxidant compounds present in spent coffee. However, the authors did not determine the content of AA in control cookies obtained without the addition of both RGCS and UGCS ingredients.

Troise et al. [101] demonstrated that cookies enriched with polyphenol powders from virgin-olive oil mill wastewater (OMWP), rich in secoiridoids, showed a lower concentration of AA in comparison to control cookies. Specifically, the addition of 0.05 and 0.1% OMWP resulted in a reduction of AA to 47 and 55%, respectively. However, for 0.2% OMWP samples there were no significant differences in AA concentration when compared to the control cookies. [101] The authors did not give a specific explanation for the effect of OMWP concentration, but based on other studies, given their chemical nature, the use of secoiridoids for the reduction of AA and other highly reactive amides is controversial. [102–104]

The inhibitory effects of glutathione (GSH), a tripeptide with antioxidant properties, consisting of cysteine and glycine, on AA in cookie model systems were investigated by Zhu et al. [105]. The presence of GSH in cookies inhibited the AA formation but without a proportional relationship between the GSH level and the AA inhibition ratio. No significant inhibition on AA formation was observed when the GSH amounts were 0.0002-0.001% of weight dough. However, for other tested GSH levels ranged from 0.002 to 0.01%, the AA decreasing ratios were in the range of 21-48% compared to the control samples (no GSH added). The addition of 0.002% GSH showed the best inhibitory effect and decreased the AA concentration by 48%. Additionally, monitoring GSH and asparagine concentrations it turned out that after baking, only 6-17% of the initial amount of GSH remained in the cookies and meanwhile, the residual asparagine levels in the cookies gradually increased with the increase

of the GSH adding amount. This result indicated that GSH participated in the Maillard reaction and competitively react with glucose against asparagine.

Many of the antioxidant ingredients studied in the literature are plants or spices, so in some works, their impact on the sensory characteristics of cookies has been evaluated. [93, 95, 99, 101, 105] For example, a sensorial panel evaluation results showed that color, texture, and flavor of cookies processed with either bamboo leaves (0.2 g/kg) or vitamin E (0.1 g/kg) did not differ significantly from control cookies. Nevertheless, other ingredients such as polyphenols from OMWP are characterized by bitterness and astringency, especially when added at high concentrations. [101] Therefore, in parallel with the reduction of AA, it is necessary to make a careful organoleptic evaluation of final products according to the type and quantity of ingredients added and kind of cookie.

3.2.5 Other ingredients

The industry of bakery products is constantly evolving to offer healthier and environmentally friendly alternative products that provide consumers an improved nutritive quality. Therefore, some by-products and fermented ingredients were used to enrich and diversify the cookies formulations, and the formation of toxic compounds was also evaluated (Table 6).

For example, coffee silverskin can be used in the preparation of functional bakery products. This by-product of roasting coffee is natural coloring and rich in dietary fiber, which makes it a good candidate for improving the overall quality of cookies. [106] Garcia-Serna et al. [58] aimed to evaluate the usefulness of Arabica coffee silverskin finding that the addition of this ingredient did not inhibit AA formation. Moreover, cookies with silver coffee skin extract, made by boiling in water and drying, had an AA content of 205.9 μg/kg dry weight which was significantly higher than that found in the control cookies. This is probably

because coffee silverskin extract contained 11.4 μg/L of AA, although this level is approximately 10 times lower than that reported in coffee beverages (175-263 μg/L) by Food and Drug Administration.

Another coffee by-product is the spent coffee grounds (SCG) obtained after beverage extraction, including those obtained from instant coffee. Martinez-Saez et al. [107] evaluated the use of SCG from instant coffee as an ingredient in cookie formulation also analyzing AA levels. Results showed that SCG presented a low concentration of residual AA (37.2 μg/kg) and was a natural source of antioxidant insoluble fiber, essential amino acids, low glycaemic sugars, resistant to thermal food processing and digestion process, and totally safe. However, this coffee by-product did not affect AA levels in cookies formulated also with stevia and oligofructose; AA values were 166 μg/kg and 169 μg/kg when SCG was added or not added, respectively. Therefore, the results seem to indicate that SCG does not influence the formation of AA during baking.

Troise et al. [108] studied the impact of rapeseed press-cake (RPC), a by-product of rapeseed oil production, rich in proteins and fiber on the formation of AA in cookies. RPC was added in different forms to cookie model systems, as cold-pressed RPC, RPC fiber isolate, and RPC alkaline extract. The addition of cold-pressed RPC led to a significant increase of AA up to 66.9% in the cookies that was attributed to its high content of AA precursors, such as glucose and crude protein that could actively contribute to the final concentration of AA. In addition, considering that also the fatty acid composition affected AA levels, as cold-pressed RPC is rich in monounsaturated fatty acids and polyunsaturated fatty acids it is likely that more AA is formed due to the formation of AA via two pathways: the Strecker degradation of N-(1-deoxy-D-fructos-1-yl)-L-asparagine and the reaction of asparagine with lipid oxidation products from fatty acids. On the contrary, AA concentration

was reduced to 39.6% in presence of the alkaline extract and down to 4.4% in the presence of 5.2% of fiber extract. The reduction of AA in cookies containing the alkaline extract can be ascribed to the direct elimination of AA through Michael's addition of nucleophilic amino acids, particularly the thiol group of the cysteine side chain. In addition, AA precursors may react with polyphenols present in the protein extract.

An additional study aimed to investigate the effect of the addition of the vegetable Jerusalem artichoke (JA) fermented with different lactobacilli (LAB; *Lactobacillus sakei* KTU05-6, *Pediococcus acidilactici* KTU05-7, and *Pediococcus pentosaceus* KTU05-9) by solid-state fermentation (SSF) or by submerged fermentation (SMF) on AA content in cookies. The fermentation technologies were able to reduce AA levels in cookies to different extents. In particular, the addition of LAB fermented by SMF promotes higher AA reduction due to lower acidity and higher protease and alpha-amylase activities compared to the application of SSF. Therefore, fermentation of JA with selected LAB could be the method of choice to minimize the AA content in cookies without adversely affecting the nutritional quality, safety, and sensory attributes, including color and flavor, while maintaining consumer acceptance. [109]

Another very promising minor ingredient is the hydrocolloid chitosan, a popular natural food preservative due to its antibacterial and antifungal activities. It may be used in products subjected to thermal processing as an AA mitigation strategy, due to the availability of its amino groups to compete with the amino group of asparagine. [53, 57, 85] Mogol and Gökmen [85] investigated the effect of chitosan and formic acid solutions on the formation of AA in cookies, however, they did not significantly affect the AA formation at all considered baking temperatures. Nevertheless, it was clear the necessity to also consider the pH-

lowering effect of the acidic solutions in which chitosan is solubilized when determining the AA mitigation mechanism.

Accordingly, the 1% of chitosan addition was not effective on AA reduction in brown sugar cookies. [57] In contrast to these findings, Sung and Chen [53] found significant mitigation of AA in cookies enriched with chitosan after 15 min baking time.

A methodological approach for the incorporation of other food hydrocolloids such as gum Arabic (GA), pectin, and carboxymethylcellulose (CMC) in the cookie dough to investigate the formation of AA in ammonia cookies was applied by Mousa [110]. Results revealed that the use of 0.03% GA in the dough reduced significantly AA content up to 58.6% compared to the control cookies baked at 180 and 200 °C. The reasons for this behavior could be due to the gelling or thickening effect of GA on the texture modification of cookies which consequently could interfere with the molecular interactions between fructose and asparagine as precursors of AA formation. Moreover, the acidic pH value of GA solution (pH = 4.9) could be another factor to facilitate the reduction of AA formation in cookies. Contrary to GA, the use of pectin and CMC at all tested concentration levels did not significantly affect the AA formation at all temperatures compared to the control cookies.

A recent study investigated the incorporation of passion fruit epicarp flour (PFEF) up to 9% as a source of high nutritional value into cookies by also assessing the AA content. As PFEF was added the AA content of cookies considerably increased, attributed to the content of reducing sugars in PFEF. The highest AA content of 228.4 μg/kg was reached in the cookies prepared with 9% of PFEF, however, this AA value is lower than the European standard (350 μg/kg). [111]

4. Conclusion and future directions

The presence of AA in widely consumed foods including cookies and other bakery products is currently a challenging issue due to its carcinogenic, mutagenic, and reproductive toxicological effect on humans. In addition, global regulatory authorities and institutional communities are becoming increasingly restrictive on the levels of AA allowed in the final products and its control throughout the food production processes.

Several strategies to control the level of AA in cookies have been extensively evaluated in the actual literature. Given the wide variety of traditional, innovative, and usually complex formulations of cookies, it is necessary to evaluate the effect of each type of major or minor ingredient on the formation of AA during baking. Recipe optimization is a crucial factor for the control of AA levels in cookies, as the reduction of its formation can be achieved mainly by:

- selecting ingredients with low asparagine and reducing sugar content, such as refined cereal flours, pseudo-cereal flours (e.g., quinoa), pre-fermented cereal flour, white sucrose, and alternative sweeteners (e.g., stevia);
- adopting the lowest amount of leavening agent, preferring $NaHCO_3$ instead of NH_4HCO_3 and combination of leavening agents for example $NaHCO_3$ plus NH_4HCO_3 or $NaHCO_3$ plus tartaric acid;
- adding an adequate amount of fat, choosing oils with a high polyphenol content, low oxidating degree and not exposed to heat, using fats with low lipid content such as margarine and butter;
- using monovalent or polyvalent cations by $CaCl_2$, $CaCO_3$, NaCl addition and a right combination of ions such as NaCl + mix of Na and K or $CaCl_2$+$MgCl_2$;

- employing some additional ingredients (different acids to control the pH, amino acids that compete with asparagine, antioxidant compounds, asparaginase, etc.).

Many of the most successful reviewed intervention strategies could also be applied to other sweet and non-sweet bakery products, and the critical summary of applied studies on cookies can be useful for the industry and other research in this specific production area.

Moreover, it is important to take into account that some of the AA mitigation strategies related to cookies formulation changes may have an impact on the organoleptic and nutritional properties of the final product (e.g., excessive or insufficient browning, generation of off-flavors, inadequate rising, excess sodium intake, etc.) and thus on the final quality and consumers' acceptance. The studies reported in the literature have not all thoroughly assessed the industrial feasibility point of view and not all evaluated in detail the effect of the AA mitigation strategies on the overall quality of the final product, making further research on the most promising reduction solutions necessary.

CRediT authorship contribution statement

Maria Alessia Schouten: Writing - Original Draft, Investigation, Visualization. **Silvia Tappi:** Review & Editing. **Pietro Rocculi:** Review & Editing. **Santina Romani:** Conceptualization, Writing - Review & Editing, Supervision.

Conflicts of Interest

The authors declare no conflicts of interest.

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Figure 1. Number (a) of articles published per year, from 2004 to the present, on the effect of cookies' formulation on acrylamide formation with the related cumulative trend and percentage proportion (b) of each ingredient studied per year.