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Acrylamide in Food

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CHAPTER

19

Lipid oxidation promotes acrylamide formation in lipid-rich systems

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Background

After many years of thorough investigations, it is nowadays widely accepted that acrylamide (AA) in food is mostly formed from degradation of asparagine through the Maillard reaction (MR). Despite the asparagine can theoretically form AA directly, the reaction yield is extremely low. Instead, in the presence of reducing sugars AA is produced in a substantially higher rate and yield. Formally, however, reducing sugars are not strictly necessary for the degradation of asparagine to AA. Any carbonyl compound can in principle react with asparagine and yield AA, although with different efficiency. Reducing sugars are key factors in AA generation in plant-derived food because they represent by far the most important source of carbonyl compounds (i.e., reducing sugars themselves and their degradation products, e.g., glyoxal, methylglyoxal, and deoxyosones). However, additional carbonyl compounds (e.g., flavor compounds, vitamin B_6, and products of lipid oxidation (LO)) may be present in food, albeit in a small amount. LO is one of the major chemical reactions occurring in foods during processing and cooking. It can drastically affect food sensory quality and plays an important role in the final acceptance of edible fats/oils or lipid-rich foods by the consumers. As a result of LO, LO products (LOPs) are formed which comprise a vast array of relatively unstable compounds of various molecular weight, flavor threshold, and biological significance including aldehydes, ketones, alcohol, epoxides, and hydrocarbons. Since the amount of LOPs that possess a carbonyl moiety may be significant in some cases, the hypothesis was put forward that LO can significantly contribute to AA formation in lipid-rich food. In this respect, it is important to recall that another mechanism for AA formation related to lipids has been postulated, i.e., the reaction of acrolein with ammonia. Acrolein is a typical product of lipid degradation, mainly stemming from the dehydration of glycerol. This

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pathway is usually considered minor compared with the one from MR. In any case, in this chapter, we solely speculate on the potential contribution of carbonyl compounds from LO on AA formation.

Generalities on lipid oxidation

LO (more properly referred to as lipid autoxidation to be distinguished from enzymatic oxidation) is a complex chemical phenomenon involving several consecutive steps and intermediate. Oxidation proceeds by a sequential free radical chain-reaction mechanism whose schematic representation is provided in Fig. 19.1. The first step in the reaction chain is the extraction of one hydrogen atom from the aliphatic chain of the lipid that produces a lipid radical R. This step is called the **initiation step**. The extraction is triggered by radical species that act as initiators. In polyunsaturated lipids, the generation of the lipid radical is followed by its molecular rearrangement that produces a thermodynamically favorable conjugated diene. The next step is the incorporation of an oxygen molecule with the formation of a peroxy radical ROO. This radical is able to extract a hydrogen atom from another lipid molecule producing a hydroperoxide, ROOH, and regenerating the lipid radical R. Hydroperoxides are known as **primary oxidation products**. The bulk of them will accumulate in the system but some may decompose producing RO and OH radicals that can further react with other lipid molecules. These steps globally constitute the **propagation step**. After a while, the **termination step** occurs by acollision of pair of radicals that wipes out almost all the reactive radicals.

The rate of hydroperoxide formation depends, among several other factors, on the lipid chemical structure. The energy required for the homolytic extraction of H from the lipid molecule is in the order vinylic > methylic > methylenic > allylic > diallylic which means that linolenic acid has a higher number of hydrogens more easily extractable (4 diallylic) than

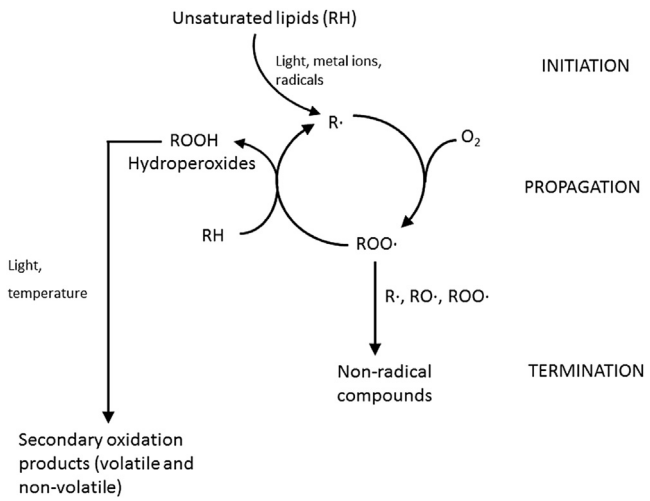

FIGURE 19.1 Schematic representation of lipid oxidation.

linoleic acid (2 diallylic) and oleic acid (no diallylic H). This explains the order in reactivity for different fatty acids and the type and relative distribution of resulting hydroperoxides reported in Table 19.1. After some time and depending on the system conditions (mainly its temperature) hydroperoxides decompose producing a vast array of **secondary oxidation products** that confer specific (and almost always undesired) taste and flavor to oxidized fats/oils. These products mostly comprise aldehydes but may also include alkanes, alkenes, esters, epoxides, ketones, and malondialdehyde (MDA). Aldehydes are mainly formed from the homolytic scission of the labile O–O bond of the hydroperoxides (catalyzed, e.g., by a radical species or metal ions) followed by β-scission of the ensuing alkyl radicals. The specific secondary oxidation products that form depend on the chemical structure of the lipid being oxidized: that is, the number and the position of the double bonds and the fatty acid chain length (Table 19.2). As an example, the formation of 2,4-decadienal from linoleic acid 9-hydroperoxide is shown in Fig. 19.2. Despite the type of fat/oil that plays a major role essentially through its level of unsaturation, numerous other factors affect the rate of LO and the

TABLE 19.1 Induction time, relative rate of oxidation at 25°C and hydroperoxides (HP) distribution for oleic, linoleic, and linolenic acid.

Fatty acid	Induction time (h)	Oxidation rate relative to stearic acid (C18:0)	HOO–group position in HP	Double bonds position in HP	Relative proportion of HP (%)
Oleic acid (C18:1)	82	100	8	9	27
			9	10	23
			10	8	23
			11	9	27
Linoleic acid (C18:2)	19	1200	8	9,12	1.5
			9	10,12	46.5
			10	8,12	0.5
			12	9,13	0.5
			13	9,11	49.5
			14	9,12	1.5
Linolenic acid (C18:3)	1.34	2500	9	10,12,15	31
			10	8,12,15	
			12	9,13,15	11
			13	9,11,15	12
			15	9,12,16	
			16	9,12,14	46

Adapted from Ref. [28].

TABLE 19.2 Major volatile compounds formed by autoxidation of oleic, linoleic, and linolenic acid (in descending order of abundance).[a]

Oleic acid	Linoleic acid	Linolenic acid
Nonanal	Hexanal	Propanal
Octanal	(Z)-2-Octenal	(E,Z)-2,4-Heptadienal
E-(2)-Undecenal	(E)-2-Heptenal	(Z)-3-Hexenal
Decanal	(E)-2-Octenal	2,4,7-Decatrienal
E-(2)-Decenal	(E,Z)-2,4-Decadienal	(E,E)-2,4-Heptadienal
Heptanal	(E,E)-2,4-Decadienal	(Z)-2-Pentenal

[a]*Each fatty acid in amount of 1 g was autoxidized at 20°C by uptake of 0.5 mol oxygen/mol fatty acid.*
Adapted from Ref. [28].

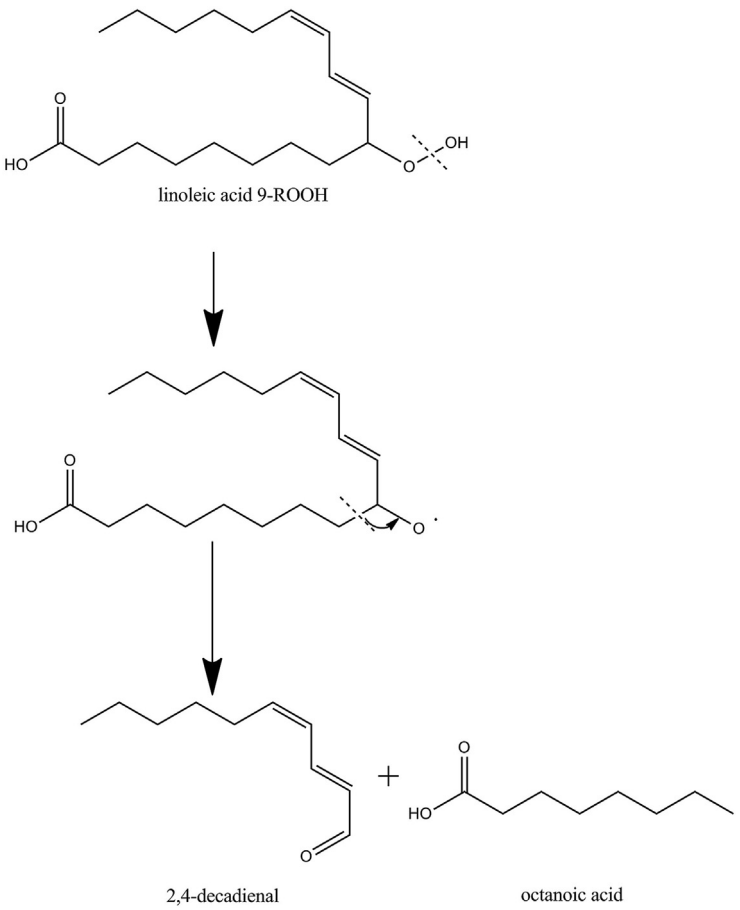

FIGURE 19.2 Formation of 2,4-decadienal from linoleic acid 9-hydroperoxide.

pattern and concentration of primary and secondary oxidation products, among which the presence and activity of pro-oxidants (e.g., cations, heme, and preformed radicals) and antioxidants (flavonoids or vitamins C and E), partial pressure of oxygen, the nature of the surface exposed to oxygen and the storage/processing conditions (e.g., temperature, light exposure, and moisture content) of fat/oil (containing food). Additionally, these lipid-derived aldehydes, such as 2-alkenals and 2,4-alkadienals, could decompose into various aldehydes due to the thermal conditions [1]. This fact also affects AA formation, since the newly formed aldehydes could be more or less reactive and involved in Maillard reaction with asparagine.

Beside autoxidation, lipids in food can also undergo what is called **lipid peroxidation** that is an enzymatic degradation phenomenon catalyzed by the enzyme lipoxygenase (linoleic acid oxygen oxidoreductase, EC 1.13.11.12), which occurs in many plants and also in erythrocytes and leukocytes. Lipoxygenase catalyzes the oxidation of some unsaturated fatty acids, preferably linoleic and linolenic acid, but not oleic acid and monounsaturated fatty acids, to their corresponding hydroperoxides, which are the same primary products of lipid autoxidation. This enzymatic reaction requires much lower temperatures to proceed at a significant rate in food. The resulting hydroperoxides can be further degraded by means of enzymatic or nonenzymatic reactions to yield secondary oxidation products that may contribute to the sensorial properties of natural food products. In addition to auto- and peroxidation, hydroperoxides can be formed from the direct addition of oxygen to the fatty acid double bond in a process known as **photo-oxidation**. Photo-oxidation requires the ground-state oxygen molecule, that is, the triplet oxygen $^3O_2$ to be activated by light (usually through the action of an intermediate photosensitizer molecule) into much more reactive singlet oxygen $^1O_2$. Singlet oxygen can add to the double bond without the prior formation of an allyl radical and, for a specific fatty acid, it produces a pattern of hydroperoxides different from that produced by autoxidation. The relative contribution of lipid peroxidation and photo-oxidation to the generation of carbonyl reactive species in food has to be considered marginal compared with the autoxidation and would therefore give a negligible contribution to AA formation in lipid-rich food.

LO contribution to AA formation in model systems

The potential contribution of LO to AA formation has been thoroughly investigated in model systems and tested as early as soon after AA discovery in heated foods [2]. The potential contribution of LOPs to AA generation has been investigated in aqueous binary or ternary model systems of pure compounds [3–6]. The results of that investigation can be summarized as follows: (1) asparagine degradation to AA only occurs if the LOP possesses a carbonyl group; (2) LOP aldehydes are more reactive than LOP ketones of comparable chain length that is consistent with the higher reactivity of aldehydes in asparagine degradation to AA through carbonyl compounds; (3) short-chain carbonyl compounds (either aldehydes and ketones) are more reactive than long-chain carbonyl compounds presumably because of their higher solubility in water; and (4) α,β,γ,δ-diunsaturated carbonyl compounds have the greatest potential of generating AA when reacted with asparagine when compared

with alkenals and alkanals. This reactivity is reduced when the alkedienal is further oxidized to the corresponding epoxide. Asparagine degradation by alkadienals would likely proceed through the decarboxylation of the amino acid to 3-aminopropinamide which is then further converted to AA by deamination but decarboxylation is the key step in AA formation from asparagine/alkadienals model systems due to its higher activation energy compared with the deamination step. Lipid hydroperoxides can also produce AA when reacted with asparagine, likely as a consequence of their thermal decomposition to alkanals, alkenals, or α,β,γ,δ-diunsaturated alkadienals. A synergic effect of glucose with certain LOPs when reacted with asparagine was also reported, the most notable effect being reported with methyl linolenate but not in methyl stearate/glucose mixtures nor in the presence of antioxidants. This synergism is proposed to be a consequence of the formation of free radicals during the reaction between asparagine and glucose via MR, which would oxidize the lipid into reactive carbonyls. These results suggest that both unoxidized and oxidized lipids are able to contribute to the conversion of asparagine into AA, but unoxidized lipids need to be oxidized as a preliminary step.

The differential contribution of oil/fat unsaturation level and the amount of AA generated in the presence of asparagine was also demonstrated by reacting asparagine with different oils/fats as well as fatty acids. It has been reported, for instance, that when fats/oils are reacted with asparagine in model systems, the greatest amount of AA is generated in model systems containing sardine oil and cod oil which have a very high degree of unsaturation compared with olive oil or sunflower oil [7] (Fig. 19.3). The least amount of AA was detected in systems containing saturated fat like lard and beef. Interestingly, beef fat produced more AA than lard fat, an outcome that is explained by the fact that beef fat is not refined unlike lard. The refining step is supposed to remove preformed carbonyl LOPs or compounds that can favor LO during the processing/cooking, that is, pro-oxidants. When asparagine was heated in presence of different fatty acids/triglycerides, linoleic acid (more unsaturated

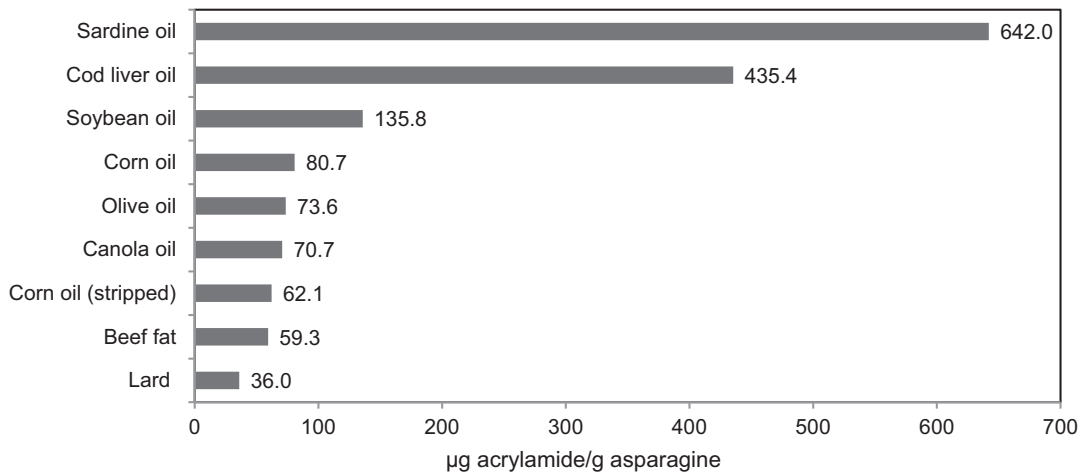

FIGURE 19.3 Formation of acrylamide from asparagine (1.76 g) and various lipids (5 g) at 180°C (30 min). Analysis performed by GC/NPD. Values are the average of two experiments. *From Ref. [7].*

and thus more prone to oxidation) is produced in much higher levels than stearic acid (saturated C18) and triolein (triglyceride with three oleic acid), but numerically less AA than oleic acid. As expected triolein produces less AA than free fatty acids which is in agreement with the fact that fatty acids are more prone to oxidation in the free form than when in the triglyceride structure. In a detailed study on the impact of LO on AA formation in model systems, Capuano et al. [8] also demonstrated that replacing palm oil (more saturated, iodine value <20) with sunflower oil (more unsaturated, iodine value ≈130) increases AA formation by 35% in solid model system formulated with water and reducing sugars. Moreover, the effect of LO was investigated upon heating of a differently formulated solid model systems at a different water activity (a_w). The systems contained sugars, lipids, and catechin or were formulated without sugars or catechin. Three sunflower oil samples with different oxidation levels were used: untreated sunflower oil with a peroxide value (PV) of 1.5 m_{eq} O_2/kg oil and a thiobarbituric acid reactive substances (TBARS) value of 0.1 mmol MDA/kg oil; sunflower oil heated for 30 min with a PV of 11.2 m_{eq} O_2/kg oil and a TBARS value of 8.2 mmol MDA/kg oil, and sunflower oil heated for 3 h with a PV of 21.5 m_{eq} O_2/kg oil and a TBARS value of 12.5 mmol MDA/kg oil. The results are summarized in Fig. 19.4. The results showed that in the complete formulations, the presence of previously oxidized lipid increases by about 25% AA formation compared with untreated oil, but further oil oxidation did not increase AA levels anymore. When lipids are the only potential source of carbonyl compounds, AA progressively increases with the oxidation level. Catechin presence reduced AA formation in systems containing untreated oil and partially oxidized oil (bars A and B), while no significant lowering effect was observed when highly oxidized oil was used (bars C). This was explained by the ability of catechin in preventing further LO, and thus further generation of reactive carbonyls from that source which would explain the lack of effect in

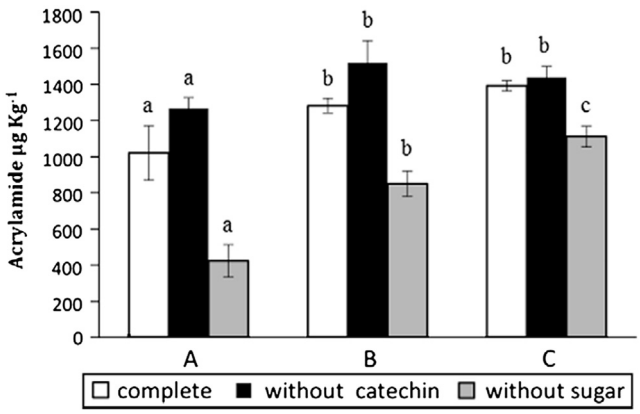

FIGURE 19.4 Acrylamide concentration formed in model systems containing oil at different oxidation level. Letter on X-axis indicates the model systems with different oxidized oils. A = model systems with unoxidized oil; B = model systems with sunflower oil heated for 30 min (mildly oxidized); C = model systems with sunflower oil heated for 3 h (severely oxidized). Within each formulation, bars with different letters are significantly different ($P < .05$). Results are expressed on a dry basis. *From Ref. [8].*

model systems formulated with severely oxidized oils. In the latter case, indeed, most of the carbonyl compounds have already formed.

Another study investigated the relation of LOPs with AA formation in preheated soybean oil, olive oil, and palm oil model systems [9]. Increasing oil heating temperature or time increased AA formation from asparagine in all oils studied. Soybean oil led to higher AA formation compared to other oils because its PV, p-anisidine value (PAV), and carbonyl group value are relatively higher than other oils. AA formation was correlated with PV of the soybean oil ($r = 0.848$) and olive oil ($r = 0.904$), while it was correlated with PAV of palm oil ($r = 0.969$). High amounts of saturated fats in oils could decompose into glycerol and fatty acids, or they could generate peroxides, aldehydes, and ketones. Therefore, the oil oxidation parameters affect AA formation due to the different compositions of the oils. Similarly, heating different oils (sesame, soy, sunflower, corn, olive, ghee, lard, and palm olein) with asparagine led to different trends in the formation of LOPs and AA [10]. The oils with high unsaturated fatty acids, such as soy oil, ended up with higher AA formation compared to other fats with high saturated fatty acids, such as lard. The AA formation was highly correlated not only with PV ($r = 0.866$) but also with the iodine value and PAV ($r = 0.922$) of the oils studied. AA formation is related to LOPs but it is greatly affected by the pH of the model system [11]. In a model system, comprising asparagine, glucose, and selected fatty acids (oleic, linoleic, linolenic acid), AA formation was generally decreased with the increase of unsaturation in nonbuffered model system (pH between 4.6 and 5.7). On the other hand, AA formation was slightly increased in the presence of unsaturated fatty acids when the model system is buffered ($pH = 6.0$). The addition of free fatty acids could affect the acidity of the nonbuffered model system and the course of Maillard reaction. Therefore, the effect of free fatty acids on the AA formation could be based on the combination of the acidity of fatty acids and lipid oxidation.

Real food systems

The potential contribution of LO to AA formation has also been investigated in real foods processed and/or cooked under real-life relevant conditions. Since the content of lipids and the physical and chemical evolution of the systems are different among different food categories it would be appropriate to discuss the state-of-art for each of the main AA contributing food categories: coffee, bakery products, and potato products.

Coffee

Currently, only one study has investigated the potential of LO during roasting on the final AA content in coffee. Green coffee (before roasting) contains on average almost equal amount of soluble carbohydrates (6%−12%) and lipid (8%−18%, higher in arabica variety). Soluble carbohydrates are mainly constituted of sucrose (8%) and nonreducing di- and trisaccharides, whereas monosaccharides are almost virtually absent (0.5%). Coffee lipids are constituted mainly of linoleic acid followed by palmitic acid. These two fatty acids are still the major fatty acids after coffee roasting. Chlorogenic acid (ester of caffeic acid and quinic

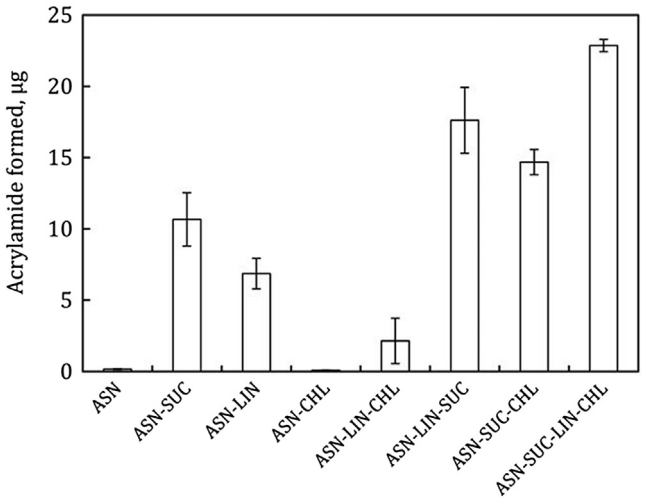

FIGURE 19.5 The amount of acrylamide formed in different model systems heated at 180°C for 10 min. *From Ref. [12].*

acid) is also very abundant in green coffee beans where it can occur at level as high as 10%. Kocadağlı and coworkers [12] identified 2-octenal, 2,4-decadienal, 2,4-heptadienal, 4-hydroxynonenal, and 4,5-epoxy-2-decenal in relatively high quantities in roasted coffee, in concentrations >1 mg/g coffee which would be about 20% of the highest content in reducing monosaccharides. This would suggest that in roasted coffee the contribution of LOPs to total asparagine degradation may not be negligible. However, in roasted coffee sucrose can also be a source of reactive carbonyls. Sucrose decomposition is favored at high temperature and yield reactive monosaccharides and/or hydroxymethylfurfural. These can react with amino compounds including asparagine through the MR. Sucrose and linoleic acid occur in green coffee at almost the same concentration, and they show almost the same reactivity toward AA in model systems with binary systems with asparagine under simulated coffee roasting conditions (Fig. 19.5). Chlorogenic acid can, however, further trigger sucrose decomposition. In the presence of chlorogenic acid, the amount of AA generated in ternary systems with sucrose and asparagine is higher than that generated in binary systems with sucrose/asparagine.

Biscuits and bakery products

In nonsugar-added cereal-based products, asparagine is notoriously the limiting factor for AA formation. In such products, the lipid content is generally very low (about 1.5%–2.0%) and carbonyl compounds from LO are unlikely to play a significant role in asparagine degradation. However, the issue has not been properly investigated. In sweet bakery products such as biscuits or cookies, besides asparagine, reducing sugars are also correlated with AA formation during baking. In biscuits and cookies sugars, mainly sucrose or inverted sugar syrup can make up to 20% of the final formulation. Fat/oil is also added to a considerable amount,

usually accounting for up to 10% of the final formulation. The effect of LO level or fat unsaturation on the formation of AA in sweet bakery products has been addressed by Arriba-Lorenzo and coworkers [13]. They reported that AA formation dramatically increased (+59%) when cookies were formulated with oxidized sunflower oil (PVs were 33.5 m_{eq} O_2/kg and TBARS = 1.98 nmol MDA/g) compared with control cookies formulated with untreated sunflower oil (PV = 1.8 m_{eq} O_2/kg and TBARS <the detection limit). In addition, AA content was the lowest in cooked, formulated with high phenolic virgin oil compared with those formulated with olive oils with medium and low phenolic content. While there is no doubt that stressed oils can produce more AA than untreated oils, the practical significance of the reported results is questionable. The PV for the oxidized sunflower oil used in that study was much higher than the limit established by, for instance, the American Oil Chemists Society for edible oils (10 m_{eq} O_2/kg oil). The oil would have unacceptable sensory attributes and would not be used in any case.

Potato products

Potato products are the food category where AA formation poses the greatest concern because of the relatively high amount that can form therein, and the contribution of such category to the overall AA dietary intake for the general population. It is widely recognized that reducing sugars content is the limiting factor for AA formation in potato products. Comparatively, asparagine content of potato is considerably of less important. Mitigation strategies aiming at lowering the reducing sugar content in potato slices before the heat treatment such as soaking in properly formulated aqueous solutions, selection of potato varieties lower in reducing sugars, and proper tubers storage have been proposed. For fried potato products, namely French fries and potato chips, the question is whether the oxidation of frying oil during frying generates enough amount of LOP carbonyls to significantly affect the rate and the extent of AA formation. In addition to this chemical effect, LO can affect the rate and the extent of AA formation in fried foods and also through a physical effect: that is, the modification of oil thermal properties due to the accumulation of polar oxidation products, such as LOPs, glycerol, mono- and diglycerides, as well as dimers, trimers, and other polymerization products at the interface between the oil/fat and the food. The accumulation of these compounds generally not only increases oil viscosity and foaming capacity (due to their emulsifying ability), but also changes the rate of heat transfer from oil to the food being fried. On one hand, the increased viscosity decreases the heat transfer rate from oil to food. On the other hand, the polar degradation/oxidation products would change the surface tension at the interface oil/food thus allowing more heat transferred from the oil to the food in a fixed frying time.

The results from the scientific literature are conflicting. Gertz and Klostermann [14], for instance, found substantial variability in AA content in potato slices fried with different oils (from 772 to 1271 μg/kg of potato). However, the AA content did not always correlate with the oil unsaturation level. Similarly, it was reported that frying oils with a higher percentage of unsaturated fatty acids did not cause an increase in AA content in French fries [15]. While the lower amount of AA was found in potato slices fried with palm olein which is the most saturated oil used in the experiment, the highest amount was found in high-oleic sunflower oil, even higher than the amount found in sunflower oil and rapeseed oil that are

more unsaturated oils [14]. Even though the iodine value of the used oils was not reported nor was it the level of polar compounds (the fraction putatively containing reactive LOP carbonyls), it could be argued from the data that the amount of AA produced in the chips could not be explained solely on the extent of the LO level. Similarly, Mestdagh and coworkers [16] investigated the impact of oil oxidation and hydrolysis on AA formation in potato homogenates and French fries. They failed in finding a statistically significant effect of soybean oxidation level on AA content of French fries and potato homogenates heated at different temperatures. Soybean oil was selected because of the high linoleic and linolenic acid content. Potato slices were fried in progressively abused oils but the level of AA was not significantly different when the most abused soybean oil (PAV > 500) was used compared with fresh oil (PAV ≈ 1.3). Interestingly, the same authors could not find any effect of the oil hydrolysis level (measured by the content of free fatty acids and mono- and diglycerides) on AA formation either. In a companion paper, Mestdagh and coworkers [17] investigated the effect of different frying oils and fats on AA formation in French fries and potato homogenates. Fats and oils exhibited low levels of oxidation at the start of the frying step. Again the authors could not find significant differences in AA content in different frying media nor could they find a significant correlation with the oil/fat unsaturation level. The same conclusions were drawn by Matthaus and coworkers [18] and by Williams [19]. In the former investigation, the amount of AA was numerically but not significantly higher in potato slices fried with semisolid or solid deep-frying fats compared with several frying oils. Moreover, no effect could be found that the frying oil age (measured by the level of triglycerides oligomers) increases the AA concentration upon frying. On the other hand, Lim et al. [20] reported that AA content in potato slices significantly depends on the frying cycle and the type of oil used for deep frying. The AA content after the 10th frying cycle showed a threefold (coconut oil) to an eightfold (sunflower oil) increase compared with the content after the first cycle. Potato slices deep fried in palm oil showed the lowest AA content after the first cycle (298 μg/kg) and an average across the 10 cycles (1443 μg/kg) whereas potato slices fried in sunflower oil showed the highest value at first cycle (1060 μg/kg) and on average across the 10 cycles (2019 μg/kg). Coconut oil produced more AA than canola oil after the first frying, but the same average level of AA was maintained across the 10 cycles. However, no significant correlation could be found between the level of AA after the first cycle or the average level of AA across the 10 cycles and the PV, PAV, iodine value, free fatty acid concentration, and levels of polyunsaturated fatty acids of the tested oils. During frying the PV and the PAV of the frying oils significantly increased, especially PAV which were from 5 to 12 times as high as in fresh oils [19]. On the contrary, Thürer and Granvogl reported higher AA concentrations in chips fried in olive oil and linseed oil compared to chips fried in other oils (coconut, rapeseed, safflower oil, and frying fat) [21]. They stated that the highest AA formation in chips fried in linseed oil might be due to the high amount of naturally present polyunsaturated fatty acids in this frying medium.

Another study found a positive and strong correlation between AA and free fatty acids ($r = 0.750$), PAV ($r = 0.777$) over 160 frying cycles of French fries fried in palm oil, soybean, canola, and sunflower oils [22]. Lee Kuek and coworkers tested the effect of intermittent frying (80 cycles) on the formation of AA in French fries fried in various oils (palm olein, sunflower, red palm olein, and soybean oil) [23]. There was no common trend in AA formation in French fries fried in various oils upon the 80th frying cycle. For example, there is a

significant decrease after the 32nd cycle when sunflower oil was used, while there was no significant difference ($P > .05$) in AA formation in French fries fried in palm olein up to the 80th cycle. The PV values of all oils exhibited increment throughout 48 frying cycles before showing subsequent decline afterward. The changes in PAV over frying were not linear; however, free fatty acids of all oils studied showed a linear increase across the 80 cycles of frying. Zhang and coworkers reported no significant ($P > .05$) change in the AA content of French fries fried in various oils with the increased number of frying cycles (up to 600 cycles) [15].

In lipid-rich, nonfried potato-based products the effect of oil type and oxidation level is likely to be very limited or negligible. In potato dough containing 10% oil, potato powder, flour, and water, shaped into thin discs and baked at 180°C for different heating times neither the type of oil used (olive, sunflower, or soybean) nor the oil oxidation level (low oxidation: 5.51 m_{eq} O_2/kg oil, MDA content: not detected; medium oxidation: 7.18 m_{eq} O_2/kg oil, MDA content: 23.11 μmol/kg; high oxidation: 8.84 m_{eq} O_2/kg oil, MDA content: 51.64 μmol/kg) significantly affected AA formation [24].

The role of antioxidant compounds in preventing AA formation

Since there is convincing evidence that carbonyl LOPs may contribute to AA formation, **antioxidants** have been proposed as mitigation strategy for AA formation in lipid-rich food. Antioxidant compounds are defined as compounds that are able to inhibit or delay the oxidation of other molecules. A number of antioxidant compounds can be naturally found in foods or formulated into food as additives (vitamins C and E, phenolic acids, and polyphenols, carotenoids, butylated hydroxyanisol, butylated hydroxytoluene, and so on) or can be formed during heat treatment of carbohydrate-rich food through MR, for example, melanoidins (brown anionic nitrogenous polymers) in coffee, bread, and bakery products. The addition of antioxidant compounds or natural extracts rich in antioxidants provides conflicting effects with respect to AA mitigation. A comprehensive review on the subject can be found in Jin et al. [25]. Some (mixture of) antioxidants are effective in reducing AA formation in several real food formulations, whereas other compounds or combinations are ineffective or even enhance AA formation. This contradictory results stem from the fact that antioxidant compounds are a class of chemically heterogeneous compounds that may modulate AA formation in different steps and with diverse mechanisms and from the fact that model systems compositions and/or reaction conditions may be very different across scientific studies. Specific antioxidants can trigger ingredients decomposition (e.g., sucrose), which can generate reactive carbonyls, trap specific MR products or intermediates, prevent lipids from oxidation, react with asparagine directly to form AA, cause precipitation of asparagine, directly react with AA. In the perspective of what has been discussed in this chapter, antioxidant compounds may mitigate AA formation by limiting formation of LOPs through their mere antioxidant capacity, that is, by reducing or delaying LO and thus accumulation of additional carbonyls (i.e., LOPs). However, this does not result necessarily in the mitigation of AA formation because of the multiple ways antioxidants can interact with other food components. A notable example is provided by chlorogenic acid in coffee. Chlorogenic acid reduces LOPs formation from linoleic acid less than it triggers the formation of reactive carbonyls from

sucrose degradation, the net effect being an increase in AA formation when chlorogenic acid is present (see Fig. 19.5).

Conclusions

As proven by several studies in model systems there is convincing evidence that secondary oxidation carbonyl compounds generated during advanced LO can react with asparagine and contribute to its degradation to AA. Secondary oxidation carbonyl compounds can not only react directly with asparagine but also react with other amino acids generating reactive intermediates that can contribute even further to asparagine degradation. What is not clear at the present is the practical significance of this additional source of reactive carbonyls to AA formation under industrial processing or domestic cooking real-life conditions. The fraction of secondary oxidation carbonyl compounds to reducing sugars is usually minor in most food products, unless severely oxidized lipids are used in food formulations or severe processing/cooking conditions are applied which would be the exception rather than the norm in almost all the practical cases. All in all, AA formation from LO is considered minor in most of the scientific literature and the evidence for a significant effect in real food can be considered at best conflicting, if not very thin. At the present, no mitigation strategy or suggested practice referring to, or based on, formation of AA from LO is mentioned in the most updated version of the CIAA acrylamide toolbox [26] or in the Codex Alimentarius Code of Practice for the reduction of AA in foods [27]. Overall, the evidence available points to the fact that LO may contribute to AA formation in lipid-rich food but its actual contribution is likely negligible or minor compared with other sources of AA for most of its potential dietary sources, the only notable exception potentially being roasted coffee.

Synopsis of analytical techniques

Lipid oxidation

LO level can be measured in different ways: by measuring (1) the amount of oxygen taken up by the oil/fat, (2) the reactants change, (3) the amount of primary oxidation products, (4) the amount of secondary oxidation products, (5) free radicals, and (6) by other methods. The amount of oxygen taken up by the lipid sample can be assessed by measuring the weight gain of the sample or the consumption of headspace oxygen. The measurement of reactant change involves mainly measuring the change in specific fatty acid concentration. Fatty acids can be easily measured after trans-esterification to corresponding methyl esters by gas chromatography with flame ionization or mass spectrometry detector. Primary oxidation products can be expressed by the PV which represents the total hydroperoxides content of the test fat. PV can be measured by iodometric titration, ferric ion complex measurement spectrophotometry, and Fourier Transform Infrared spectroscopy. Detailed information on the structure and the amount of specific hydroperoxides can be obtained by direct or reverse-phase HPLC. As an alternative, dienes, and trienes (produced during the formation of hydroperoxides from unsaturated fatty acids) can be assayed by measuring

the increase of absorbance of the test sample at 234 nm (dienes) and 268 nm (trienes). To avoid interferences from substances absorbing at the same wavelength (e.g., carotenoids), hydroperoxides can be converted to conjugated chromophores followed by measurement of absorbance at 268 and 301 nm (tetraenes). Secondary oxidation products are expressed as TBARS assay. With this assay, secondary oxidation products (mainly MDA) are conjugated to thiobarbituric acid and the concentration of the conjugates is measured spectrophotometrically at 530−535 nm. As an alternative, PAV can be used. PAV measures the content of aldehydes (principally 2-alkenals and 2,4-alkadienals) generated during the decomposition of hydroperoxides. It is based on the color reaction of p-methoxyaniline (anisidine) and the aldehydic compounds under acidic conditions that produces a derivative that absorbs at 350 nm. The total content in carbonyl compounds (aldehydes and ketones) can be quantified by reacting the carbonyls with 2,4-dinitrophenylhydrazine followed by the reaction of the resulting hydrazones with alkali. The resulting derivative is measured spectrophotometrically at a given wavelength. Volatile secondary oxidation products can also be measured by gas chromatography/mass spectrometry (GC−MS) or by the oxidative stability instrument or Rancimat. The latter instrument quantifies the volatile compounds by monitoring the change in electrical conductivity when effluent from oxidizing oils is passed through water. Another approach to measuring lipid oxidation level is represented by the measurement of free radicals. This is possible by electron spin resonance, also referred to as electron paramagnetic resonance spectroscopy which relies on the paramagnetic properties of the unpaired electrons in radicals. Finally, the evolution of LO can be followed by differential scanning calorimetry which monitors the thermally induced transitions occurring during oxidation such as the transfer of oxygen molecules to unsaturated fatty acids or by 1H nuclear magnetic resonance spectroscopy. Theoretically, the extent of LO can also be quantified by means of the sensory evaluation of the fat sample by expert and trained sensory panels. The sensory evaluation consists in the descriptive analysis of the test samples which includes detection and description of the qualitative and quantitative sensory aspects of the product.

Lipid unsaturation level

Lipid unsaturation level can be conveniently measured by the iodine value that gives a measure of the average degree of unsaturation of a lipid: the higher the iodine value, the greater the number of CC double bonds. By definition, the iodine value is expressed as the grams of iodine absorbed per 100 g of lipid. One of the most commonly used methods for determining the iodine value of lipids is the "Wijs method" which consists in adding an excess of iodine chloride that reacts with CC double bonds and titrating the excess of iodine chloride (which did not react with double bonds) with sodium thiosulfate after quantitative liberation of iodine from iodine chloride after addition of excess potassium iodine. Lipid unsaturation level can also be determined by profiling fatty acid composition that consists of the quantitative saponification of triglycerides and simultaneous trans-esterification of fatty acid in the corresponding fatty acid methyl ester (FAME) followed by FAME determination by GC-FID or GC-MS.

Key facts

- Carbonyl compounds generated through lipid oxidation can contribute to asparagine degradation to AA.
- The amount of carbonyl generated through lipid oxidation depends on the lipid properties (fatty acids composition, level of unsaturation, content in antioxidants, and prooxidants) as well as storage and processing conditions (such as temperature, time, and level of oxygen).
- A significant contribution to AA formation from lipid oxidation has been unambiguously demonstrated in model systems.
- In potato and cereal-based products the contribution to AA formation from lipid oxidation may be of limited significance whereas it may be significant in coffee.
- Antioxidant compounds can theoretically reduce AA formation by limiting the rate and the extent of lipid oxidation but their actual effect is difficult to predict because of the multiple ways antioxidants can interact with other precursors/intermediate of AA formation and with AA itself.

Mini dictionary

Lipid oxidation (LO): It is the degradation of fat/oil through the incorporation of oxygen. In the final stage, LO produces volatile compounds that are detrimental to the oil/fat sensory quality.

Radical: Chemical species (atom, molecule, or ion) that has unpaired valence electrons or an open electron shell.

Primary oxidation products: Products of incorporation of oxygen in the fat/oil molecule. They are hydroperoxides.

Secondary oxidation products: Products of chemical decomposition of primary oxidation products. They are responsible for the rancidity of oxidized oils/fats and may contribute to AA formation.

Homolysis: It is the dissociation of a chemical bond of a neutral molecule in two free radicals.

Lipid unsaturation: It is the number of double bonds in the aliphatic chain of fatty acids in an oil/fat.

Iodine value: It is the grams of iodine incorporated by 100 g of the test substance. It is an indicator of oil/fat unsaturation level.

Peroxide value: It is the amount of peroxide oxygen per 1 kg of fat or oil. It is expressed in milliequivalent of oxygen. It is an indicator of the content of primary oxidation products (hydroperoxides) in fat/oil.

TBARS: Thiobarbituric acid reactive substances are a by-product of lipid peroxidation, which can be detected by the TBARS assay which uses thiobarbituric acid as a reagent. It is essentially, if not exclusively, malondialdehyde (MDA).

Decarboxylation: It is a reaction where a carboxyl group is removed from a molecule and released as carbon dioxide.

Deamination: It is a reaction where an amino group is removed from a molecule and released as ammonia.

Antioxidant compounds: These are substances that inhibit or delay the oxidation of other molecules.

Oxidation: It is a chemical reaction where electrons or hydrogen are transferred from a substance (which is oxidized in the process) to an oxidizing agent (which is reduced in the process).

Catechin: It is a plant secondary metabolite belonging to the family of flavan-3-ols, part of the chemical family of flavonoids. It possesses antioxidant activity.

Summary points

Lipid oxidation can contribute to AA formation in lipid-rich model systems. The contribution depends on the generation of reactive carbonyl oxidation products. The level of unsaturation and oxidative status of lipid affect AA formation. The level and nature of pro-oxidants and antioxidants occurring in lipid also can affect AA formation. The significance of lipid oxidation contribution in real food is questionable because typical level of lipid oxidation products is much lower than reducing sugars and their carbonyl degradation products. In coffee lipid oxidation during roasting may significantly contribute to AA formation.

References

[1] R. Zamora, J.L. Navarro, I. Aguilar, F.J. Hidalgo, Lipid-derived aldehyde degradation under thermal conditions, Food Chem. 174 (2015) 89–96.
[2] A. Becalski, B.P.Y. Lau, D. Lewis, S.W. Seaman, Acrylamide in foods: occurrence, sources, and modeling, J. Agric. Food Chem. 51 (2003) 802–808.
[3] F.J. Hidalgo, R.M. Delgado, R. Zamora, Degradation of asparagine to acrylamide by carbonyl-amine reactions initiated by alkadienals, Food Chem. 116 (2009) 779–784.
[4] F.J. Hidalgo, R.M. Delgado, J.L. Navarro, R. Zamora, Asparagine decarboxylation by lipid oxidation products in model systems, J. Agric. Food Chem. 58 (2010) 10512–10517.
[5] R. Zamora, F.J. Hidalgo, Contribution of lipid oxidation products to acrylamide formation in model systems, J. Agric. Food Chem. 56 (2008) 6075–6080.
[6] R. Zamora, R.M. Delgado, F.J. Hidalgo, Conversion of 3-aminopropionamide and 3-alkylpropionamide into acrylamide in model systems, Mol. Nutr. Food Res. 53 (2009) 1512–1520.
[7] S. Ehling, M. Hengel, T. Shibamoto, Formation of acrylamide from lipids, in: M. Friedman, D. Mottram (Eds.), Chemistry and Safety of Acrylamide in Food, Springer, New York, US, 2006.
[8] E. Capuano, T. Oliviero, Ö.Ç. Açar, V. Gökmen, V. Fogliano, Lipid oxidation promotes acrylamide formation in fat-rich model systems, Food Res. Int. 43 (2010) 1021–1026.
[9] X. Nan, Q. Wu, S. Nan, X. Zeng, Y. Dai, L. Kang, Effect of oil oxidation on acrylamide formation in oil-rich model systems without the participation of reducing sugars, J. Food Prot. (2020) 342–349.
[10] G. Daniali, S. Jinap, P. Hajeb, M. Sanny, C.P. Tan, Acrylamide formation in vegetable oils and animal fats during heat treatment, Food Chem. (2016) 212 244–249.
[11] Y. Wang, H. Hu, D.J. McClements, S. Nie, M. Shen, C. Li, Y. Huang, Y. Zhong, J. Chen, M. Zeng, M. Xie, pH and lipid unsaturation impact the formation of acrylamide and 5-hydroxymethylfurfural in model system at frying temperature, Food Res. Int. 123 (2019) 403–413.
[12] T. Kocadağlı, N. Göncüoğlu, A. Hamzalioğlu, V. Gökmen, In depth study of acrylamide formation in coffee during roasting: role of sucrose decomposition and lipid oxidation, Food Funct. 3 (2012) 970–975.

[13] G. Arribas-Lorenzo, V. Fogliano, F.J. Morales, Acrylamide formation in a cookie system as influenced by the oil phenol profile and degree of oxidation, Eur. Food Res. Technol. 229 (2009) 63–72.
[14] C. Gertz, S. Klostermann, Analysis of acrylamide and mechanisms of its formation in deep-fried products, Eur. J. Lipid Sci. Technol. 104 (2002) 762–771.
[15] H. Zhang, H. Zhang, L. Cheng, L. Wang, H. Qian, Influence of deep-frying using various commercial oils on acrylamide formation in French fries, Food Addit. Contam. Part A 32 (7) (2015) 1083–1088.
[16] F. Mestdagh, B. De Meulenaer, V. Van Peteghem, Influence of oil degradation on acrylamide formation in a model system and in French fries, Food Chem. 100 (2007) 1153–1159.
[17] F.J. Mestdagh, B. De Meulenaer, C. Van Poucke, C. Detavernier, C. Cromphout, C. Van Peteghem, Influence of oil type on the amounts of acrylamide generated in a model system and in French fries, J. Agric. Food Chem. 53 (2005) 6170–6174.
[18] B. Matthäus, N.U. Haase, K. Vosmann, Factors affecting the concentration of acrylamide during deep-fat frying of potatoes, Eur. J. Lipid Sci. Technol. 106 (2004) 793–801.
[19] J.S.E. Williams, Influence of variety and processing conditions on acrylamide levels in fried potato crisps, Food Chem. 90 (2005) 875–891.
[20] P.K. Lim, S. Jinap, M. Sanny, C.P. Tan, A. Khatib, The influence of deep frying using various vegetable oils on acrylamide formation in sweet potato (*Ipomoea batatas* L. Lam) chips, J. Food Sci. 79 (2013) 115–121.
[21] A. Thürer, M. Granvogl, Generation of desired aroma-active as well as undesired toxicologically relevant compounds during deep-frying of potatoes with different edible vegetable fats and oils, J. Agric. Food Chem. 64 (47) (2016) 9107–9115.
[22] R.A. Abd Razak, A.H. Ahmad Tarmizi, A. Kuntom, M. Sanny, I.S. Ismail, Intermittent frying effect on French fries in palm olein, sunflower, soybean and canola oils on quality indices, 3-monochloropropane-1,2-diol esters (3-MCPDE), glycidyl esters (GE) and acrylamide contents, Food Control 124 (2021) 107887.
[23] S. Lee Kuek, A.H. Ahmad Tarmizi, R.A. Abd Razak, S. Jinap, S. Norliza, M. Sanny, Contribution of lipid towards acrylamide formation during intermittent frying of French fries, Food Control 118 (2020) 107430.
[24] K. Kotsiou, M. Tasioula-Margari, E. Capuano, V. Fogliano, Effect of standard phenolic compounds and olive oil phenolic extracts on acrylamide formation in an emulsion system, Food Chem. 124 (2010) 242–247.
[25] C. Jin, X. Wu, Y. Zhang, Relationship between antioxidants and acrylamide formation: a review, Food Res. Int. 51 (2013) 611–620.
[26] CIAA, Acrylamide toolbox. http://www.fooddrinkeurope.eu/uploads/publications_documents/Toolbox final260911.pdf.
[27] Codex Alimentarius, Code of Practice for the Reduction of Acrylamide in Foods. Available at: http://www.codexalimentarius.net/input/download/standards/11258/CXP_067e.pdf.
[28] H.D. Belizt, W. Grosch, P. Schieberle, Lipids, in: Food Chemistry, 4th Revised and Extended Edition, Springer Verlag, Berlin Heidelberg, 2009.