The detection of high level of the neurotoxic and carcinogenic acrylamide in heat-treated foodstuffs in 2002 led to an intensive research effort. Among the parameters affecting the level of acrylamide in foods, the effect of antioxidants had not yet been elucidated satisfactorily. Several researchers have investigated the effect of various antioxidants and antioxidative extracts on acrylamide formation, but the data were discordant. Some studies claimed mitigation while others no effect or even an increase. It can be attributed to the ability of antioxidants with different structures or functional groups to react with acrylamide precursors, with intermediates of the reaction or with acrylamide itself, leading to either reducing or promoting effects. The fact that the same kind of antioxidant, or extract and its representative components behaves differently in different studies might be due to the different reaction conditions among the studies, concentrations of the antioxidant, as well as preparative methods of the extract. This review tried to give a broad overview and evident-based understanding about the role of antioxidants on acrylamide formation.
Former studies focused mainly on: (i) accurate analytical methods of acrylamide and its substrates (Biedermann, Biedermann-Brem, et al., 2002; Rosèn & Hellénäs, 2002; Zhang, Ren, Jiao, Li, & Zhang, 2011), (ii) formation mechanisms of acrylamide (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002, 2004; Zyzak et al., 2003), and (iii) the possible mitigation strategies in heat-processed foods. Existing strategies to reduce acrylamide content include three basic aspects: modification of raw materials, optimization of processing conditions and addition of exogenous additives. For raw materials, the key is to control the content of carbonyl source and asparagine. Choosing a relatively higher storage temperature (Chuda et al., 2003) can reduce the content of reducing sugars in potatoes, thus reduce the acrylamide level in the final product. For processing conditions, an appropriate heating temperature should be set and long-time processing of the foodstuffs should be avoided (Mottram et al., 2002). As for exogenous additives, many substances are reported to be effective for the mitigation of acrylamide, including some organic acids (e.g. citric acid, Cook & Taylor, 2005), some amino acids (e.g. glycine, Claeyts, De Vleeschouwer, & Hendrickx, 2005), and some mono- and divalent cations (e.g. Na⁺ or Ca²⁺, Gökmen & Senyüva, 2007). We can refer to Zhang and Zhang (2007), Friedman and Levin (2008), Zhang, Ren, and Zhang (2009) or Capuano and Fogliano (2011) for comprehensive, detailed and also up-to-date reviews.

The Confederation of the Food and Drink Industries of the EU (French: Confédération des Industries Agro-Alimentaires de l’UE; CIAA) summarized the research progress on the reduction of acrylamide and listed the parameters that influence the final level of acrylamide, creating the “CIAA Toolbox” in 2005. This toolbox was recently updated in Sep. 2011. The newly updated parameters for potato-based products included agronomical factors (sugars & asparagine), recipe factors (fermentation, dilution and piece size, pH, other minor ingredients such as amino acids and calcium salts), processing factors (asparaginase, thermal input & moisture, pre-treatment and finished product color) and final preparation factors (consumer preparation) (CIAA, 2011).

Among the parameters affecting the level of acrylamide in foods, the effect of antioxidants has not yet been elucidated satisfactorily. As a wide range of substances, the relationship between antioxidants and acrylamide formation deserves, however to be discussed.

2. Interesting results & questions asked

Previously, food researchers and technologists applied both antioxidative extracts and pure antioxidants to inhibit acrylamide generation. But both positive and negative results had been obtained. By reading and comparing the literature, interesting phenomena had drawn our attention.

2.1. Different effects of different antioxidants on acrylamide content

Rosemary, a spice with a known antioxidant property, had been proposed as one effective inhibitor of acrylamide. It was presented that acrylamide contents were lowered in fried potato slices when rosemary herb was added to either corn or olive oil (Becalski, Lau, Lewis, & Seaman, 2003). The above finding was confirmed by Hedegaard, Granby, Frandsen, Thygesen, and Skibsted (2008) who added aqueous rosemary extract, rosemary oil and dried rosemary leaves to a bread model, thus reduced the content of acrylamide by 62, 67 and 57%, respectively, compared to bread without rosemary. Zhu, Cai, Ke, and Corke (2009) applied 35 kinds of crude aqueous extracts of plants and 11 phenolic acids to mitigate acrylamide in an asparagine-glucose (ASN–GLC) model system, finding that 34 out of 35 plant extracts exerted reduction effect while 9 phenolic acids except for ferulic acid and hesperetin inhibited acrylamide formation. On the other hand, another study reported that grape seed extract had no effect on acrylamide formation in bakery products using a crust-like model (Açar & Gökmen, 2009). Tareke (2003) also found out that the addition of commonly-used antioxidants like BHT, sesamol, and Vitamin E to meat before heating enhanced the formation of acrylamide.

Cheng, Shi, Ou, Wang, and Jiang (2010) tested six fruit extracts (from apple, blueberry, mangosteen, longan and dragon fruit with white or red flesh) for their activities against acrylamide formation, and found that apple extract demonstrated potent inhibitory effect, extracts of blueberry, mangosteen and longan had not shown significant impact, whereas dragon fruit extracts enhanced acrylamide formation.

2.2. Discordant effects of the same kind of antioxidant on acrylamide formation

One obvious example was about commonly consumed antioxidant ascorbic acid and its derivatives. Biedermann, Noti, Biedermann-Brem, Mozzetti, and Grob (2002) found a relatively weak reduction of the acrylamide formation by the addition of ascorbic acid to a potato model. Similar results were obtained by Levine and Smith (2005) when using ascorbic acid and ascorbate in a cracker model. However, by investigating the effect of ascorbyl palmitate and sodium ascorbate, Rydberg et al. (2003) found no effect on acrylamide net amount in a potato model.

Another example was about the use of two kinds of phenolic acids (caffeic and gallic acids) in two independent experiments (Bassama, Brat, Boluoner, Boulanger, & Gînata, 2010; Kotsiou, Tasiouli-Margari, Capuano, & Fogliano, 2011). Results obtained from the two experiments were completely the opposite: both phenolic acids were non-effective in reducing acrylamide in the former experiment but effective in the latter one.

2.3. Discordant effects between antioxidative extract and its representative components

We (Zhang & Zhang, 2008) showed that addition of two natural antioxidants, extract of bamboo leaves and extract of green tea, and also their representative components homoorientin and galloctein gallate (EGCG) to an ASN–GLC model system both significantly reduced acrylamide formation. Contrariwise, statistics showed that applying Virgin Olive Oil (VOO) extracts to a potato model system and fresh potatoes led to an enhanced level of acrylamide, while commonly-occurring phenols in VOO were proven to be effective in inhibiting acrylamide formation (Kotsiou, Tasiouli-Margari, Kukuvou, & Ciesarova, 2010).

These experimental facts seem confusing to some extent. Meanwhile, the questions that needed to be answered are raised as follows:

1. Why do different antioxidants have different effects?
2. Why does the same kind of antioxidant either inhibit or enhance acrylamide among laboratories?
3. Why does extract and its representative component behave differently?
4. Is antioxidant capacity involved?

In the rest of the article, we tried our best to summarize useful literature and come up with a possible explanation to the questions, with discussion about acrylamide formation mechanism and possible action of antioxidants involved.

3. Current understanding about acrylamide formation

Shortly after the discovery of acrylamide in foods, it has been established that the major pathway for acrylamide formation in foods is Maillard reaction with free asparagine as main precursor (Mottram et al., 2002; Stadler et al., 2002, 2004; Zyzak et al., 2003). Asparagine was proved to provide the backbone chain of acrylamide.
by mass spectral studies demonstrating that all the three carbon atoms and the nitrogen atom are from asparagine.

Fig. 1 shows the main pathways from asparagine to acrylamide, including the up-to-date understanding of acrylamide formation so far. When asparagine is heated alone, a very limited amount of acrylamide is formed (Granvogl, Jezussek, Koehler, & Schieberle, 2004). So it needs a carbonyl to accelerate its conversion to acrylamide. In a classic pathway (marked as “A”) in which asparagine and an α-hydroxycabonyl compound (e.g. the reducing sugars) are heated together, an N-glycosyl conjugate (structure not shown) and its corresponding Schiff base are formed as a key intermediate after dehydration. The Schiff base would mainly rearrange to the Amadori compound, which leads to 1- and 3-deoxyosones formation, and further decomposes to generate color and flavor (Ledl & Schleicher, 1990). Amadori compound is not an efficient precursor to acrylamide, which is in accordance with the relatively low transformation yield of asparagine to acrylamide (Blank, 2005). Alternatively, the Schiff base may decarboxylate, either directly via the Schiff betaine or indirectly via oxazolidine-5-one intermediate, to form an azomethine ylide (also called decarboxylated Schiff base), which further furnishes the decarboxylated Amadori compound after tautomerization (Yaylayan, Wnorowski, & Locas, 2003). Afterward, acrylamide may be formed directly from azomethine ylide, through β-elimination of the decarboxylated Amadori compound, or through deamination of 3-aminopropionamide (3-APA), which is proved to be a key direct precursor of acrylamide (Granvogl & Schieberle, 2006).

In recent years, continuous efforts have been made to explore the formation pathway of arylamide, and finally scientists found out that carbonyls other than α-hydroxycabonyl can also react with asparagine to yield acrylamide. The typical pathway is shown as pathway B in Fig. 1. Asparagine and the carbonyl compound form the corresponding Schiff base and the Schiff base decarboxylates later on to its decarboxylated form and yield acrylamide either via or not via 3-APA.

Research also showed that not any carbonyl compound can be involved in the reactions of pathway B (Zamora & Hidalgo, 2008). Sugar fragments such as glyoxal, hydroxyethanal and glyceraldehydes (Amrein, Andres, Manzardo, & Amadô, 2006), as well as specific lipid oxidized products like alkeniadienes and their analogous ketodienes and hydroperoxides (Hidalgo, Delgado, Navarro, & Zamora, 2010) seem preferable in this pathway. It seems that whether the carbonyl is available in both A & B pathways depends on the functional group in the β-position to the nitrogen atom. The presence of a hydroxyl group favors the rearrangement from azomethine ylide to the decarboxylated Amadori product to afford acrylamide (pathway A). α,β,γ,δ-diunsaturated carbonyl group and α-dicarbonyl groups may play a key role in pathway B. As for the carbonyl group, aldehydes are more reactive than ketones.

Also, a pathway named acrolein pathway (pathway C) was put up by Yasuhara, Tanaka, Hengel, and Shibamoto (2003), stating that acrolein and asparagine, as well as acrylic acid (oxidized acrolein) and ammonia produced significant amount of acrylamide. But this pathway might be marginal, since the nitrogen atom of acrylamide comes from asparagine, and other carbonyl compounds such as reducing sugars are way more reactive with asparagine that it’s hard to spare nitrogen atom to acrolein in foodstuffs.

4. Possible reactions in acrylamide formation that antioxidants are involved

Asparagine needs a carbonyl compound to convert to acrylamide. The carbonyl can come from multiple sources. Due to the diversity of antioxidants in structure and properties and the complexity of the reactions, different antioxidants are involved in different reactions during the process of Maillard reaction, thus caused different effects towards acrylamide formation.

4.1. Reaction with asparagine to form acrylamide

It was discussed above that carbonyl compounds, especially those that bear α,β,γ,δ-diunsaturated carbonyl group can react with asparagines to yield acrylamide. Owing to its carbonyl group (which is
α,β,γ,δ-diusaturated), curcumin is expected to produce acrylamide after a reaction with asparagine. A relevant study for confirmation was performed by Hamzalioğlu, Mogol, Lumaga, Fogliano, and Gökmen (2012) in which curcumin was heated in binary and ternary model systems of asparagine–curcumin and asparagine–curcumin–fructose. High resolution mass spectrometry was used to identify the structure of the reaction intermediates and products, thus presenting the exact mechanism. The results showed that curcumin significantly increased acrylamide formation, though to a lesser extent compared with fructose, and acrylamide formation was also enhanced in the ternary model system by binary system of Asn–Fru. Curcumin can contribute to acrylamide formation under long-term heating as long as asparagine is present, since fructose reacts more rapidly with asparagine than curcumin. Also, a reasonable reaction pathway was settled up according to the mass spectrometry results (already described as pathway B above).

It was reported that applying VOO extracts to a potato model system and fresh potatoes led to an enhanced level of acrylamide, while applying oregano extracts to the same systems led to a reduced level (Kotsiou et al., 2010). Now that the reaction conditions were the same, the authors attributed it to the different compositions of phenolic compounds in VOO and oregano extracts. As shown in Fig. 2, I–IV are major phenolics occurring in VOO, V–VIII are major phenolics occurring in oregano. It was interpreted that phenolic compounds occurring in VOO differ from oregano phenols as they contain more aldehyde groups in the side chain, which may undergo reactions with asparagine to form acrylamide.

Fig. 2. Structure of major polar phenolics occurring in virgin olive oil and oregano. Virgin olive oil secoiridoids: R=OH: (I) oleuropein, (II) aldehydic form of oleuropein aglycone (OFAO) (III) dialdehydic form of decarboxymethyl oleuropein aglycone (DAFOA), and (IV) hydroxytyrosol, R=H: (I) ligstroside, (II) aldehydic form of ligstroside aglycone (AFOA) (III) dialdehydic form of decarboxymethyl ligstroside aglycone, and (IV) tyrosol. Oregano phenolics: (V) rosmarinic acid, (VI) gallic acid, (VII) protocatechuic acid and (VIII) querectin. Adapted from Kotsiou et al. (2010).

In addition to a direct reaction with asparagine, it is known that several types of phenolic antioxidants like tannins can precipitate amino acids through complexation (Shahidi & Ho, 2005). So an observed reduction effect of proanthocyanidins (condensed tannins) on acrylamide was assumed to this reason (Zhu et al., 2009).

4.2. Reaction with sucrose to trigger its decomposition

5-Hydroxymethylfurfural (i.e. 5-(hydroxymethyl)furan-2-carbaldheyde, HMF) is a furanic compound which forms as an intermediate in the Maillard reaction (Ames, 1992) or from direct dehydration of sugars under acidic conditions (caramelization) during thermal treatments applied to foods (Kroh, 1994). Fig. 3 depicts the main pathways to HMF formation in foods, which includes key intermediate 3-deoxyosone formation via 1,2 enolization and dehydration of glucose or fructose, and further dehydration and cyclization of 3-deoxyosone to yield HMF. Under dry and pyrolytic conditions an alternative pathway to HMF formation from sucrose and sucrose has been proposed. It involves the formation of a highly reactive fructofuranosyl cation which can be effectively and directly converted to HMF (Perez-Locas & Yaylayan, 2008).

Recently, it was shown that HMF, which contains an α,β,γ,δ-diunsaturated carbonyl group, can convert free asparagine to acrylamide very efficiently during heating. The ASN–HMF model system generated more acrylamide than the ASN–GLC model system, though the reaction rate was higher in the ASN–GLC system. High resolution mass spectrometry analysis confirmed its role as a potent carbonyl accelerating acrylamide formation (Gökmen, Kocadagli, Gönçüoğlu, & Mogol, 2012).

A corresponding research conducted by Kocadagli, Gönçüoğlu, Hamzalioğlu, and Gökmen (2012) claimed an observation of chlorogenic acid (CGA), a main phenolic constituent in green coffee, promoting sucrose decomposition to form HMF. Statistics obtained showed that the amount of acrylamide formed in the ASN–SUC–CGA model system was found 1.38 times higher than that formed in the ASN–SUC model system. Mass spectrometry confirm the presence of 3,4-dideoxyosone and HMF as sucrose decomposition product, and conversion of CGA into caffeic acid and quinic acid during roasting of coffee. The authors thought that CGA can trigger SUC decomposition to form HMF and 3,4-dideoxyosone, which both react with asparagine to yield acrylamide.

4.3. Trapping of Maillard reaction intermediates

As stated above, reactive carbonyls heated with asparagine form a series of intermediates, and finally lead to yielding of acrylamide. During the process, several antioxidants, especially some specific flavonoid, were found to be able to trap Maillard reaction intermediates by C6 or C8 position of the A-ring.

Cheng et al. (2009) tested the effect of a citrus flavonoid, naringenin, on the formation of acrylamide in chemical model systems under mild heating conditions. Results showed that naringenin significantly and dose dependently inhibited the formation of acrylamide by 20–50% relative to the control. Structural information obtained by LC-MS/MS and NMR showed that naringenin reacted with Maillard intermediates, forming two new derivatives 8-C-(E-propenamide)-naringenin and 6-C-(E-propenamide)-naringenin [Fig. 4]. In other words, naringenin scavenged asparagine-derived intermediates (also amide source of the reaction) at positions 6 and 8 of the A-ring, diverted them from the pathways leading to acrylamide formation, thus reduced the acrylamide content.

Totlani and Peterson (2005) reported that epicatechin (EC) in aqueous glucose–glucose model systems functioned as a carbonyl trapping agent of C2, C3, and C4 sugar fragments or key transient precursors of the Maillard reaction (i.e., glyoxal, methylglyoxal, acetal, ethyrole, etc.). The authors suggested that EC underwent electrophilic aromatic
substitution reactions with these carbonyl compounds. This mechanism was further confirmed by NMR analysis of a EC-methylglyoxal adduct product, which indicated that the C6 or C8 position of the flavanol (A-ring) and the carbonyl-bearing carbon of the aldehyde compound were the bonding sites (Totlani & Peterson, 2006). EC exerts a trapping effect on sugar fragments or 3-deoxy-2-hexosulose intermediates and consequently inhibits Maillard products (including acrylamide) formation.

4.4. Destructive & protective reactions with acrylamide

Once formed, the acrylamide content is not always constant. After it has reached the highest concentration, the acrylamide content can decrease again due to exhaustion of one of the reactants and/or by elimination of acrylamide. It’s obvious that acrylamide possesses two functional groups, an amino group and an electron-deficient vinylic double bond, making it available for a wide range of reactions, including nucleophilic and Diels–Alder addition and radical reactions (Capuano & Fogliano, 2011).

Acrylamide may undergo Michael addition type reactions to the vinylic double bond with nucleophiles, including amino and thiol groups of amino acids and proteins. On the other hand, the amide group can undergo many reactions including hydrolysis, dehydration, alcoholysis and condensation with aldehydes (Friedman, 2003).

For example, Zamora, Delgado, and Hidalgo (2010) proved that acrylamide can react with amino compounds by means of a Michael addition to form corresponding 3-(alkylamino)propionamides, which may produce acrylamide again by heating.

For further understanding, Hidalgo, Delgado, and Zamora (2011) tested glutamic acid (Glu, with an amino group), N-acetylcysteine (AcCys, with a sulfhydryl group), and glutamic acid/N-acetylcysteine mixture, glutathione (GSH, with both amino and sulfhydryl groups), cysteine (Cys, with both amino and sulfhydryl groups) for the combined action of amino and sulfhydryl groups on acrylamide reduction. Results showed that the acrylamide disappearance decreased in the following order: Cys > GSH > AcCys > Glu. It can be inferred that the sulfhydryl group can suppress acrylamide, and the presence of amino group in the same molecule can encourage the effect of the sulfhydryl group. Also, the closer the position of the two groups, the...
stronger the positive interaction between them. When the two groups are present in different molecules, the positive interaction can only be observed when small concentrations are employed. Yuan, Shu, Zhou, Qi, and Xiang (2011) also reported an inhibitory effect of allicin, a natural sulfhydryl-containing antioxidant, towards acrylamide.

A hypothesis explaining why different antioxidants act differently was raised by Ou et al. (2010) who related it to possible radical induced elimination of acrylamide. Six kinds of antioxidants, three of which are relatively stable (not easily oxidized) and the rest of which are unstable were tested in both the acrylamide model system and ASN–GLC model system. Results showed that stable antioxidants (BHA, BHT and TBHQ) had no effect or even promoted acrylamide formation, while unstable antioxidants (ferulic acid, EGCG and Vitamin C) slightly inhibited acrylamide. And when H_2O_2 was added to the acrylamide model system, or oxidized antioxidants were used in the ASN–GLC system, acrylamide formation was significantly reduced. The authors assumed that acrylamide suffers from radical induced elimination, so antioxidants can help protect acrylamide against radicals by scavenging them. But if the antioxidant is unstable or can be easily oxidized, it may turn into its oxidized form to attack the acrylamide, resulting in reduced acrylamide content.

4.5. Role of antioxidant capacity in reaction towards lipid oxidation

It has been found that in lipid-rich systems, lipid oxidation products are able to convert asparagine into acrylamide. This conversion is favored by oxidized lipids having an α,β,γ,δ-diunsaturated carbonyl group, which comes from oxidation of unsaturated fatty acids and their corresponding esters. For example, lipid oxidation products were prepared via oxidation of methyl linoleate, the structures of which were shown in Fig. 5 and numbered as compounds 2–9. Benzaldehyde (compound 10) was also included for comparison purposes. Heating experiments showed that compounds 2, 4, 7 and 9 were reactive to asparagine, and 2,4-decadienal (compound 7) showed the highest reactivity. While heating together with asparagine and glucose, compound 1 promoted the reaction by a synergism factor of 1.6, which may be due to the oxidation of compound 1 to exert compound 2 and other primary and secondary oxidized products (Zamora & Hidalgo, 2008). Capuano, Oliviero, Açar, Gökmén, and Fogliano (2010) confirmed that lipid oxidation positively influenced the formation of acrylamide, especially in sugar-free systems where lipids became the main sources of carbonyls. Under this frame, antioxidants can inhibit acrylamide formation by preventing lipid oxidation, thus limit the accumulation of carbonyls. This theory was proved by data from Kocadağlı et al. (2012), which showed that chlorogenic acid lowered both lipid oxidation product accumulation as well as acrylamide formation, comparing data from an asparagus–linoleic acid system and the system in which chlorogenic acid was added.

However, as for antioxidant capacity (AOC) of the system, Serpen and Gökmén (2009) found a close linear correlation (r = 0.8322, n = 36) between acrylamide and total AOC in a potato matrix. It can be concluded that antioxidative compounds and acrylamide were formed in the similar stages of the Maillard reaction, and at similar rates as well, suggesting no direct effect of AOC on acrylamide formation.

Bassama et al. (2010) in their article stated that the AOC of the model system was not positively correlated to the mitigation of acrylamide formation. After adding caffeic acid at different levels, the AOC changed but no significant effect on the kinetics of acrylamide formation/elimination was observed. Lorenzo, Fogliano, and Morales (2009) used three kinds of oil which were of phenolic compounds at high, intermediate and low levels individually. These different oil samples were used to make cookies, and the acrylamide content and AOC were determined. Results showed that as time passed, the AOC was always not significantly different among the oils, but the acrylamide content gradually showed significant difference.

Actually, the antioxidant capacity of the system is influenced by several factors, that is, the residue of the antioxidants added (if not all reacted), and the antioxidants formed through Maillard reaction, mainly melanoidins. Melanoidins are brown anionic nitrogenous polymers formed during the final stage of the Maillard reaction (Borreli, Visconti, Mennella, Anese, & Fogliano, 2002). It was known to us, long before the discovery of acrylamide as a product formed during the Maillard reaction, that melanoidins have strong antioxidant properties comparable to those of commonly used food antioxidants (Lingnert & Wailer, 1983). Results likely show that the antioxidant capacity of the system, which is measured by means of ferric reducing/antioxidant power (FRAP), 2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonicacid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) or oxygen radical absorbance capacity (ORAC), mainly depends on the melanoidins formed rather than antioxidants added, because melanoidins are produced in such a large amount, and that antioxidants added are
soon consumed thus can’t be fully measured. So, antioxidant capacity might not be a quality assay to express acrylamide inhibitory ability.

5. Other factors influencing the effect of antioxidants towards acrylamide

By discussing the antioxidants with various functional groups to react with different compounds during Maillard reaction, we basically can explain the reason why different antioxidants showed different effects on acrylamide formation. Other questions why the same kind of antioxidant can either inhibit or enhance acrylamide among studies, and why extract and its representative component behaves differently are tried to be answered in this session, by discussion of reaction conditions, concentration, polarity and comparative methods.

5.1. Reaction conditions

The reaction condition of Maillard reaction is a multiple concept which includes the reaction matrix, the pH value, moisture and the overall heat treatment. All these factors have been proven to be important in acrylamide formation, thus are able to affect the relationship between antioxidant and acrylamide.

The influence of temperature and heating time on acrylamide formation has been repeatedly demonstrated and results indicated that the acrylamide amount increased with processing temperatures (Rydberg et al., 2003). But acrylamide levels were reduced under high temperature combined with prolonged heating time, due to the elimination process of acrylamide (Corradini & Peleg, 2006). It was also clarified that acrylamide elimination did not occur at low temperatures, even after prolonged heating times (Bråthen & Knutsen, 2005). The heat treatment may also affect the status of antioxidants since some antioxidants with a poor heat-stability might have decomposed under high temperature, thus being unable to protect lipids from oxidation, or take part in the Maillard reaction.

Researches also indicated that the initial pH value and the moisture content of the reaction system may also remarkably influence acrylamide formation. For example, Weisshaar (2004) reported that in an ASN–GLC model system, the acrylamide content significantly increased when the pH was beyond 7.0, and the highest acrylamide content was found when the pH was at 8.0. Mestdagh et al. (2008) found that acrylamide content reached the highest point between pH 7.0 and 7.5 in homogeneous potato powder mixture systems with different initial pH values. Similar phenomenon was also observed by Yuan et al. (2011). When the initial pH values of the aqueous model ASN–FRU and ASN–GLC systems varied from 5.0 to 7.5, the acrylamide content increased, and when the pH passed 7.5, the acrylamide content decreased with the increase of the pH values from 7.5 to 9.0. It can be concluded that the pH values between 7.0 and 8.0 are the most suitable ones for acrylamide to form. As a result, we can modify and maintain the pH value in this range to obtain higher acrylamide response when we are setting up model systems, and we can adjust the pH to a lower value to reduce the formation of acrylamide in industrial applications.

As for moisture content, Capuano et al. (2010) found that there was a large difference in acrylamide contents in two different food systems with 4% and 16% water, respectively. They attributed it to the different time-temperature profiles of the heating procedure caused by the different water contents. In the model system with 4% of water, both on the surface and in the core, the temperature reached the oven temperature more rapidly than that with 16% of water. As a consequence, the more the initial water content, the more the surface and the core take time to reach the optimum acrylamide formation condition. Also, water content may affect the distribution and the status of the antioxidant applied, as well as its combination and reaction with acrylamide or its intermediates.

In order to study the scientific problems under a simplified condition, scientists have set up different model systems, e.g. dry systems (microwave or oven conditions), oily systems (frying conditions), semi-dry conditions, etc.
systems (baking conditions), emulsion systems (multiphase system) and aqueous systems, etc. Mainly, matrix, moisture content and heating condition have become the key factors to distinguish different systems. Also, there are reaction systems made of food stuffs, such as potato, cookies, bread and crust. As far as we are concerned, model systems might be more suitable for mechanism analyzing since food systems are much more complicated, while food systems are better for application experiments. Choosing either the model system or the food system may lead to different results. Kotsiou et al. (2010) treated potato model system with different concentrations of VOO phenolic extracts, and found out that the lowest concentrations resulted in a reduction of 15% (p<0.05), while at intermediate concentrations an increase up to 21% (p<0.05) on acrylamide formation was observed. Finally, a remarkable increase of 49% was induced by the highest concentration.

Also, similar phenomenon occurs in ascorbic acid cases where at low concentrations (0.2%, 0.5%, 0.8% and 1.2%), ascorbic acid inhibited acrylamide formation, with a highest inhibitory rate of 58%, but the increase of ascorbic acid concentration to 1.5% promoted acrylamide formation by more than 90% in an ASN–GLC model system (Yuan et al., 2011). Such findings suggest that the effect of antioxidant towards acrylamide is not only determined by the type but also the concentration of the antioxidants, which makes the case more complicated.

But, this may partly explain the different behaviors of the same antioxidant among studies, since the applied concentrations were mostly different.

5.4. Antioxidant preparative methods

Research results showed that the standard compounds of commonly-occurring phenols in VOO (tyrosol, oleuropein and 4-hydroxyphenyl acetic acid) were effective in inhibiting acrylamide formation, in contrast to VOO phenolic extracts’ behavior which enhanced acrylamide formation. The authors ascribed it to the preparation procedure of the phenolic extract. Oleuropein is hydrolyzed during VOO extraction to form dialdehydic or aldehydic derivatives, which makes VOO phenols contain aldehydic groups. As mentioned before in this article, aldehydic groups may increase the formation of acrylamide as reactive carbyls, thus preparative methods of the antioxidant may influence acrylamide formation by changing the composition of the antioxidative extract during reaction.

6. Conclusions and outlooks

In recent years, there have been significant improvements in acrylamide field including its formation mechanism and mitigation strategies. Several researchers have investigated the effect of various antioxidants and antioxidative extracts on acrylamide formation using different model systems, as well as actual food matrices.

However, there are confusing results in the literature, where some studies claim mitigation while others no effect or even an increase. This can be attributed to the ability of antioxidants with different structures or functional groups to react with acrylamide precursors, with intermediates of the reaction or with acrylamide itself, leading to either reducing or promoting effects.

Fig. 6 summarizes the possible reactions antioxidants may take part in. As shown in the figure and discussed above, a reactive carbonyl compound is needed to accelerate the conversion of asparagine to acrylamide. The carbonyl compound can come from ingredients of the food matrix, from sucrose decomposition, from Maillard reaction, from lipid oxidation and from the antioxidant added, as shown in the figure. Among these pathways to form the so-called “reactive carbonyl pool”, specific antioxidants can trigger sucrose decomposition [marked as (1)] to promote acrylamide formation, trap specific Maillard reaction products [marked as (2)] to inhibit acrylamide formation, prevent lipids from oxidation [marked as (3)] to lower acrylamide content or even react with asparagine directly to form acrylamide. Also, some antioxidants can cause precipitation of asparagine, which clearly mitigates acrylamide formation [marked as (4)]. As the Maillard reaction proceeds, antioxidants of some specific structures can react with the intermediates, thus alter the reaction pathway to reduce acrylamide formation [marked as (5)]. Acrylamide may undergo Michael addition type reactions with antioxidants bearing nucleophilic groups to be eliminated [marked as (6)]. Either protective
or destructive reactions can be expected from antioxidants or their oxidized forms against acrylamide [marked as (7)].

Since the reaction condition can dramatically influence the final concentration of acrylamide, the confusing fact that the same kind of antioxidant acts differently may be attributed to the variety of reaction conditions among studies. The fact that several antioxidants show different effects under different concentrations may also help explain this phenomenon, for the concentrations applied in researches are mostly different. But the mechanism still remains unclear. We think it may be due to the change of dominating reaction the antioxidant undergoes when the concentration varies, since antioxidants can get involved in several reactions.

The preparative procedure, which often includes heat-assisted extraction or concentration steps, may change the composition of the extract, thus making it different from the raw material or the representative component, especially for heat unstable antioxidants. Also, since the extract is relatively complicated compared to pure compounds, the effect of co-extracts and interaction between representative components and the co-extracts should be taken into consideration.

Antioxidants can prevent lipid oxidation to limit carbonyl accumulation, by its antioxidant capacity. But since the antioxidant capacity measured may be dramatically influenced by melanoidins generated during the Maillard reaction, it might not be an adequate assay to evaluate the antioxidant’s ability to influence acrylamide formation.

The application of high resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) has made it possible to identify the structure of reaction intermediates and assume the pathway by identification of the results. Further investigations should focus on: finding other reactive structure patterns or functional groups in acrylamide formation and reduction, ensuring the mechanism of acrylamide elimination, exploring the possible mechanism of concentration-activity relationship, and foundation and optimization of standard reacting systems to compare results among laboratories. For mitigation researches, the interactions among reduction strategies and risk–risk analysis to evaluate its side effects should be investigated or applied as well.

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