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Formation of heterocyclic aromatic amines with the structure of aminoimidazoazarenes in food products

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ABSTRACT

Thermal food processing has many beneficial consequences, although it also produces some unintentional undesired effects, such as the formation of potentially mutagenic and carcinogenic substances. Among them, the formation of heterocyclic aromatic amines (HAAs) has been related to the declared carcinogenicity of processed meats. In spite of this importance, HAA formation pathways remain mostly unknown, which avoids the design of targeted procedures to inhibit HAA appearance. The objective of this review is to collect information recently appeared that allow advancing in the understanding of how these compounds are produced. Particularly, the possibility that aminoimidazoazarenes are produced similarly to PhIP is discussed, including their formation by cyclizations and oligomerizations of aldehydes and creatinine under usual cooking conditions. Present data suggest that HAA formation might be related to the pool of carbonyl compounds existing in foods, the food carbonylome, which can be controlled by carbonyl-trapping agents, such as amine and phenolic compounds.

Keywords: Carbonyl-amine reactions; Carbonyl-phenol reactions; Heterocyclic aromatic amines; Lipid Oxidation; Maillard reaction; Reactive carbonyls

Chemical compounds studied in this article: 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PubChem ID: 1530); 2-amino-3-methylimidazo(4,5-*f*)quinoline (PubChem ID: 53462); 2-amino-3,4-dimethylimidazo(4,5-*f*)quinoline (PubChem ID: 62274); 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (PubChem ID: 62275)

1. Introduction

Thermal food processing has many beneficial consequences: enhancing nutritional quality, improving digestibility and bioavailability of nutrients, prolonging shelf life, obtaining better sensorial and functional properties, releasing bioactive components, generating beneficial compounds, destroying anti-nutritional substances, and inactivating food-borne pathogens (Van Boekel et al., 2010). On the other hand, this kind of processing can also bring some unintentional undesired consequences. Among them, losses of certain nutrients (Halabi, Deglaire, Hamon, Bouhallab, Dupont, & Croguennec, 2020), formation of toxic compounds (Gomez-Narvaez et al., 2019), and production of compounds with negative effects on flavor perception, texture, or color (Pang et al., 2019), have been thoroughly studied. In particular, the formation of potentially mutagenic and carcinogenic substances as a consequence of processing has attracted much attention in recent years. Thus, for example, formation of acrylamide (Bedade, Sutar, & Singhal, 2019) or furan (Cepeda-Vazquez, Rega, Descharles, & Camel, 2018) in carbohydrate-rich foods, or acrolein (Ewert, Granvogl, & Schieberle, 2014) or chloropropanediols (Li, Zhou, Zhu, Wang, Nie, & Xie, 2016) in lipid-rich foods has been much studied.

Muscle foods deserve a special comment in this sense because they appear to generate the most important panel of toxicants (Meurillon & Engel, 2016). Thus, although they are rich in high-quality proteins, minerals, and vitamins (Jiménez-Colmenero, Cofrades, Herrero & Ruiz-Capillas, 2018; Sobral, Cunha, Faria, & Ferreira, 2018), consume of red and processed meat is considered dangerous because of their potential carcinogenicity. Red meat refers to unprocessed mammalian muscle meat (for example, beef, veal, pork, lamb), and processed meat refers to meat that has been transformed through salting, curing, fermentation, smoking, or other processes with the

objective of enhancing its flavor or improving preservation. To this respect, the International Agency for Research on Cancer (IARC), has considered that there are positive associations between the consumption of red meat and cancers of the colorectum, pancreas, and prostate (IARC, 2018). Therefore, IARC has concluded that consumption of red meat is probably carcinogenic to human beings (Group 2A). In relation to processed meat, IARC has considered that there is sufficient evidence in humans for the carcinogenicity of its consumption because ingesting of processed meat causes cancer of the colorectum and there are positive associations between consumption of processed meat and cancer of the stomach. Therefore, IARC has concluded that consumption of processed meat is carcinogenic to humans (Group 1).

The biological reasons for the association between red and processed meat and cancer are still unclear, although a large number of molecular mechanisms has been proposed to explain this association (Cascella et al., 2018; Chiang, & Quek, 2017; Demeyer, Mertens, De Smet, & Ulens, 2016; Jeyakumar, Dissabandara, & Gopalan, 2017). Among them, different families of compounds and chemical species have been hypothesized to contribute to these carcinogenic effects, including polycyclic aromatic hydrocarbons (PAHs), *N*-nitroso compounds, heme iron, macromolecular oxidation products, and heterocyclic aromatic amines (HAAs).

HAAs are a group of more than thirty compounds, which are usually classified into two groups (thermic and pyrolytic) depending on their formation temperature (Zamora & Hidalgo, 2015). Thus, thermic HAAs (also known as aminoimidazoazarenes because of their chemical structures) are usually produced in proteinaceous foods at temperatures typical of cooking/frying (~200 ºC), and pyrolytic HAAs are formed by pyrolysis of amino acids and proteins at temperatures higher than 250 ºC. The reason for including HAAs among the chemical compounds suspicious of contributing to meat

carcinogenicity is double: their presence in muscle foods when cooked and the identification of some of these compounds as suspicious of producing cancer in human beings. Thus, certain HAAs are considered activation-dependent carcinogens because, once activated, they can generate single-strand breaks of DNA chain, chromosomal aberrations, and DNA adducts in guanine-rich regions (Barnes, Zubair, John, Poirier, & Martin, 2018). This last consequence is the result of the attack of the activated HAA to the N^2-position of guanine (most common) or the C8-atom of guanine (less frequent) (Barnes, Zubair, John, Poirier, & Martin, 2018). Mutation frequency of produced adducts is usually reduced by polymerases. Thus, for example, Bose et al. (2016) found that, for the adduct of 2-amino-3-methylimidazo(4,5-*f*)quinoline (IQ) at N^2-position of deoxyguanosine, pol κ performed translesion synthesis (TLS) of dG-N^2-IQ in an error-free manner and pol η, pol ξ, and Rev1 cooperatively carried out the mutagenic TLS. For the adduct of IQ at position C8, Bose et al. (2015) found that pol η not only was the most efficient, but it performed TLS of dG-C8-IQ alone in an error-free manner. In contrast, pol κ and pol ξ cooperatively carried out the mutagenic TLS.

Ten HAAs are considered at present to be probable or possible carcinogens according to IARC (Lili, Junyan, Hongfei, Baoqing, & Bolin, 2019). They belong to the two groups of HAAs. Four of them are aminoimidazoazarenes and six of them are pyrolitic HAAs. The four aminoimidazoazarenes are: 2-amino-3-methylimidazo(4,5-*f*)quinoline (IQ) [probable carcinogen, class 2A], and 2-amino-3,4-dimethylimidazo(4,5-*f*)quinoline (MeIQ), 2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) [possibly carcinogens, class 2B]. The six pyrolytic HAAs are: 2-amino-9*H*-pyrido[2,3-*b*]indole (A-α-C), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeA-α-C), 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-1), 2-aminodipyrido[l,2-*a*:3',2'-

d]imidazole (Glu-P-2), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2)]. All of them are considered possibly carcinogens (class 2B). Chemical structures for all these compounds are given in Figure 1.

Having into account that the above described pyrolytic HAAs are produced in negligible amounts under standard cooking conditions in comparison to that of aminoimidazoazarenes (Meurillon & Engel, 2016), carcinogenicity of cooked foods due to HAAs is likely to be mostly due to the described aminoimidazoazarenes. Therefore, the knowledge of the reaction pathways conducting to the formation of these compounds would allow establishing targeted procedures to trap the reactive intermediates required for their production. The objective of this review is to collect scattered information recently appeared that allow advancing in the understanding of how these compounds are produced.

2. Proposed reaction pathways for aminoimidazoazarene formation

As observed in Figure 1, chemical structures of the different aminoimidazoazarenes are quite similar. All of them have an imidazole ring, and also either pyridine or pyrazine rings. This similarity suggests that analogous reaction pathways should be involved in the formation of all of them. Unfortunately, these formation pathways are still mostly unknown, in spite of the more than 40 years elapsed from HAA initial discover in 1977 by Prof. Sugimura's group (Sugimura, Wakabayashi, Nakagama, & Nagao, 2004).

Preliminary model studies pointed out that their formation might be produced as a consequence of Maillard reaction because HAAs were detected when mixtures of creatinine, and specific carbohydrates and amino acids were heated at high temperatures

(Skog, Johansson, & Jägerstad, 1998). Because Maillard reaction involves both free radicals and reactive carbonyls, two main general proposals were suggested: a free radical pathway and a carbonyl pathway.

Thus, some authors hypothesized that HAAs are produced as a consequence of the free radical reactions produced in foods as a consequence of processing (Pearson, Chen, Gray, & Aust, 1992). As shown in Figure 2, these authors suggested that Maillard reaction can produce pyridine and pyrazine radicals, which would then react with creatinine and one aldehyde to produce HAAs with structure of either quinoline (IQ-derivatives) or quinoxaline (IQx-derivatives). This hypothesis is based on the existence of free radicals in these reactions, as shown by ESR studies (Stoesser, Klein, Peschke, Zehl, Cammerer, & Kroh, 2007), and, also, in the inhibition of HAA formation by phenolic antioxidants (Gibis & Weiss, 2012; Vidal et al., 2020). On the other hand, this mechanism has not been demonstrated so far, has missing steps, and remains relatively controversial (Meurillon & Engel, 2016).

The second proposed pathway is based on the well-known ability of Maillard reaction to produce pyrazines, and, to a lower extent, also pyridines (Sinesio et al., 2019; Wang, Zhang, Wang, Wang, & Liu, 2019). The reaction of the appropriate pyridines or pyrazines with aldehydes and creatinine would be the origin of the different HAAs with either IQ or IQx structures (Figure 2). The main support for this hypothesis is the described synthesis of MeIQ and MeIQx by reaction of either 2-methylpyridine or 2,5-dimethylpyrazine with creatinine and acetaldehyde (Milic, Djilas, & Canadanovic-Brunet, 1993). Nevertheless, these compounds were prepared under reaction conditions far from those occurring in foods, and the proposed reaction pathway has missing steps and has not been further confirmed.

To date, a global pathway for the formation of only one HAA has been proposed: the formation of the aminoimidazoararene PhIP. Description of this mechanism was initiated almost two decades ago (Zöchling & Murkovic, 2002), but it has not been finished until very recently (Zamora, Alcon, & Hidalgo, 2014). Curiously, it does not follow any of the above described pathways. As shown in Figure 2, the heterocyclic ring is built *in situ*. Thus, the reaction is initiated by addition of creatinine to a reactive carbonyl to produce the corresponding aldol, which would be later dehydrated. Formation of the pyridine ring would be produced by ring closing of the produced adduct in the presence of ammonia and formadehyde (Zamora, Alcon, & Hidalgo, 2014). Ammonia and formaldehyde might also react between them to produce formamide (Reddy, Beatriz, Rao, & de Lima, 2019), which would be able to act as intermediate. Other aminoimidazoazarenes might also be produced similarly to PhIP. Therefore, their formation would be produced as a consequence of carbonyl chemistry, but not in the sense previously proposed. Analogously to the observed for PhIP, rings (also heterocyclic rings) present in HAAs might formed by cyclizations and oligomerizations of appropriate aldehydes under usual cooking conditions when required reactants are present. Recent studies appeared in the literature and unpublished results from this laboratory suggest that this can be an alternative mechanism to those described above. These studies will be reviewed in the next sections.

3. Aldehyde cyclizations and oligomerizations: an origin for the formation of heterocyclic structures in foods

According to the hypothesis suggested in the previous section, rings in HAAs might be produced as consequence of a tendency of reactive carbonyls to evolve into aromatic rings. To confirm that, the formation pathways of the most important heterocyclic rings present in HAAs is discussed in this section. As pointed out above, pyridine and

pyrazine rings are the key difference between IQ- and IQx-type HAAs. These heterocycles are known to be produced as a consequence of carbonyl-amine reactions in foods. Thus, pyrazine ring formation is a well-known consequence of the Strecker degradation produced by α-dicarbonyl compounds in the course of Maillard reaction (Scalone, Lamichhane, Cucu, De Kimpe, & De Meulenauer, 2019). Pyridines are detected less frequently in the course of Maillard reaction. However, they are produced to a significant extent as a consequence of carbonyl-amine reactions when lipid-derived reactive carbonyls are involved (Zhang et al., 2018). Formation pathways for these pyridines have been proposed very recently (Zamora, Lavado-Tena, & Hidalgo, 2020). They have been suggested to be produced by cyclizations and oligomerizations of lipid-derived short-chain reactive aldehydes produced in the presence of ammonia and ammonia-producing compounds. In fact, short-chain unsaturated aldehydes are not stable when heated in the presence of ammonia, and the formation of pyridines is rapidly observed (Zamora, Lavado-Tena, & Hidalgo, 2020).

Pyridines are always produced similarly, although the resulting products are different depending on the implicated aldehydes (Figure 3). Thus, mixtures of alkanals and 2-alkenals produce 2-alkylpyridines. In particular, formation of 2-methylpyridine occurs by addition of ammonia to crotonaldehyde (R = CH_3 in Figure 3) and, then, reaction of the produced adduct with acetaldehyde (R' = H in Figure 3). The new adduct suffers successively a cyclization reaction, a dehydration, and an aromatization to produce the corresponding pyridine. This kind of pyridines can also be produced by cyclization reaction of 2,4-alkadienals.

If the starting reactants are acrolein and alkanals, the obtained product is a 3-alkylpyridine. Thus, the formation of 3-methylpyridine is produced by reaction of acrolein (R = H in Figure 3) and propanal (R' = CH_3 in Figure 3). The reaction is

initiated again by addition of ammonia to acrolein and the formation of the corresponding imine with propanal. Cyclization, dehydration, and oxidation of this adduct produces the corresponding 3-methylpyridine.

A similar reaction pathway is also responsible for the formation of 2,5-dialkylpyridines by cyclization of 2-alkenals (R = methyl or a longer chain) and alkanals (R' = methyl or a longer chain). In this case, ammonia is added to the 2-alkenal and, then, the produced adduct reacts with the alkanal to produce the corresponding imine. The cyclization, dehydration and aromatization of the produced adduct are required for producing the 2,5-dialkylpyridine.

Analogous 2,5-dialkylpyridines are also produced by oligomerizations of 2-alkenals. Similarly to previous examples, ammonia is added to the 2-alkenal and, then, the produced adduct reacts with a new molecule of 2-alkenal to produce the corresponding imine. Cyclization and dehydration steps, but not an oxidation step, are required for producing the corresponding 2,5-dialkylpyridine. Because the oxidation step for the formation of this kind of 2,5-dialkylpyridines is not needed, their formation is produced with higher yields than other pyridines (formation yields ~30%).

All these examples show that, when appropriate aldehydes are present in the presence of ammonia and ammonia-producing compounds, aldehydes rapidly produce heterocyclic structures. However, when other compounds are present, these other compounds are also involved in the produced reactions and mixed adducts are usually produced. This happens, for example, with creatinine, which will be discussed in the next section.

4. Aldehyde cyclizations and oligomerizations in the presence of creatinine

When the pyridines produced by an aldehyde were studied comparatively in the presence of creatinine, ammonia, and other ammonia-producing compounds, the amount of pyridines obtained with creatinine was always lower than the amount of pyridines obtained with other ammonia-producing compounds (Zamora, Lavado-Tena, & Hidalgo, 2020). The reason is that creatinine is able to react with the reactive carbonyls in addition to generate the ammonia needed for producing the pyridine.

This reaction between aldehydes and creatinine was firstly hypothesized (Murkovic, Weber, Geiszler, Fröhlich, & Pfannhauser, 1999) and, then, confirmed (Zöchling & Murkovic, 2002) by Murkovic's group when investigating the origin of PhIP by initial reaction of phenylacetaldehyde and creatinine. As shown in Figure 2, the C-5 of creatinine reacts with the carbonyl group of phenylacetaldehyde in a nucleophilic addition and subsequent dehydration to form the condensation product.

This reaction is not exclusive for alkanals, and recent studies in our group have shown that analogous adducts can also be produced with other aldehydes such as 2-alkenals (unpublished results). In this case, the addition is produced in the carbon-carbon double bond, as usually occurs with this kind of aldehydes. The produced adduct has, then, a free carbonyl group that can suffer the cyclizations described in the previous section, which might finally conduct to the formation of the aromatic rings present in the HAAs. In fact, when short chain aldehydes were heated in the presence of creatinine and ammonia-producing compounds, the formation of HAAs was observed, and the HAA(s) produced depended on the specific aldehyde(s) involved in the cyclization reaction (unpublished results). Therefore, depending on the involved aldehydes, some HAAs were produced to a higher extent than others.

The most important conclusion of this new way of explaining HAA formation is that the production of these compounds will depend on the carbonyl compounds present in the food product, which explains many previous experimental results. Thus, the HAAs that are produced with the most easily available carbonyl compounds, will be produced to a highest extent. This can be an explanation for the production of PhIP to a high extent in many food products: the required aldehyde is phenylacetaldehyde, which is produced very easily by Strecker degradation of phenylalanine. It also explains why HAAs are produced to very different extents among them and among the different food products: the produced HAAs will depend on the type and amount of carbonyl compounds present. Therefore, it is important to know the kinds and amounts of carbonyl compounds available in a food product at a certain time, because they will likely determine the kinds and amounts of HAAs formed in the food upon heating. This pool of carbonyl compounds, which is characteristic of each food and depends on processing and/or storage conditions, will be named food carbonylome. Next two sections will discuss the routes that contribute to the appearance of new carbonyls and the routes that contribute to the disappearance of these carbonyl compounds, respectively. A scheme collecting some of these routes are shown in Figure S-1 of the Supplementary Material.

5. Food carbonylome formation: the production of the reactive carbonyls required for HAA formation

The aldehydes needed to produce HAAs can have many different origins, including carbohydrates, lipids, and amino acids. Furthermore, other carbonyl compounds present in foods can also play a role in their formation. These different routes and their interactions will be briefly discussed in this section.

Carbohydrates are major contributors to food carbonylome, mainly through Maillard reaction. Carbohydrate-derived reactive carbonyls play a major role in flavor (Paravisini & Peterson, 2019a) and browning (Paravisini & Peterson, 2019b) formation in food products, and they have been related to development of disease states (Hellwig, Gensberger-Reigl, Henle, & Pischetsrieder, 2018). The most abundant carbohydrate-derived reactive dicarbonyls present in food are 3-deoxyglucosone, 3-deoxygalactosone, and glucosone, which predominate over methylglyoxal, glyoxal, and 3,4-dideoxyglucosone-3-ene (Figure 4). Common contents of 1,2-dicarbonyl compounds in food products have been reported, among others, by Degen, Hellwig, & Henle (2012).

Once produced, these reactive carbonyls suffer cyclization reactions and they are responsible for the formation of different heterocyclic structures including furanones (Erten & Cadwallader, 2017), pyranones (Andrewes, 2012), and furan derivatives (Yang et al., 2019). In addition, these reactive carbonyls contribute to the formation of other carbonyls, which convert them in indirect aroma precursors, such as in the Strecker degradation of amino acids (Chigwedere et al., 2019). This reaction is also an important source of pyrazines (Liu, Yang, Linforth, Fisk, & Yang, 2019).

Other important contributors to food carbonylome are lipids. Lipid-derived reactive carbonyls are produced as a consequence of a cascade of reactions induced by oxygen (Martínez-Yusta, Goicoechea, & Guillén, 2014). In addition to ketones, many kinds of aldehydes are produced in these reactions. The structures of produced aldehydes are very diverse and include alkanals (Wu & Wang, 2019), 2-alkenals (Bastos, de Almeida Costa, & Pereira, 2017), 2,4-alkadienals (Beltran, Ramos, Grane, Martin, & Garrigos, 2011), 4-hydroxy-2-alkenals (Csallany, Han, Shoeman, Chen, & Yuan, 2015), 4-oxo-2-alkenals (Tullberg, Vegarud, & Undeland, 2019), 4,5-epoxy-2-alkenals (Spiteller, Kern, Reiner, & Spiteller, 2001), and malondialdehyde (Bertolin, Joy, & Blanco, 2019)

294 (Figure 4). The amounts of these reactive carbonyls are very variable depending on the
295 matrix because both some of them are highly reactive and the conversion of some of
296 them into others has been described (Zamora, Navarro, Aguilar, & Hidalgo, 2015).

297 All these compounds play a major role in food flavor (Clarke, Mannion, O'Sullivan,
298 Kerry, & Kilcawley, 2019), browning (Ho, Can, Yago, Shravya, Bhandari, & Bansal,
299 2019), texture (Sun, Sun, Thavaraj, Yang, & Guo, 2017), and safety (Dehgahni,
300 Hosseini, & Regenstein, 2018). In addition, they contribute to the formation of amino
301 acid-derived carbonyl compounds by Strecker-type degradation (Hidalgo & Zamora,
302 2004; Hidalgo, Leon, & Zamora, 2016). This Strecker-like degradation also produces
303 heterocyclic structures such as pyridines and pyrroles (Hidalgo & Zamora, 2016).

304 In addition to the Strecker-type degradation produced by lipid-derived reactive
305 carbonyls, lipid-derived free radicals have also been shown to produce this degradation,
306 which constitutes an additional route to produce Strecker aldehydes (Hidalgo &
307 Zamora, 2019a) and would explain the role of free radicals in the reactions finally
308 conducting to HAA formation.

309 Other carbonyl compounds and free radicals present in foods products can also
310 produce this Strecker-type amino acid degradation. This happens, for example, with
311 phenolic compounds. Thus, under air, appropriate phenolics can be oxidized and the
312 produced quinones are able to degrade amino acids. This occurs with the easily
313 oxidizable phenolics, like *o*- and *p*-diphenols, but to a much lower extent with the less
314 oxidizable phenolics like *m*-diphenols (Delgado, Zamora, & Hidalgo, 2015). In fact, the
315 carbonyl-promoting ability of phenolics compete with their carbonyl-trapping abilities
316 that will be discussed in the next section (Delgado, Hidalgo, & Zamora, 2016).

6. Food carbonylome fate: the other reactions that compete for the carbonyl compounds needed for HAA formation

Reactive carbonyls are continuously produced as a consequence of the diverse reactions discussed in the previous section. However, once produced, these compounds are involved in many reactions, in addition to produce HAAs. All these reactions compete among them and some compounds are produced with preference to others depending on the availability of the involved reactants, reaction conditions, and activation energies, among others. Therefore, the food carbonylome is in continuous change, and conditions that favor an excess of carbonyl compounds (usually known as carbonyl stress) will likely favor the formation of HAAs.

One way for carbonyl compound disappearance is their involvement in carbonyl-amine reactions. Thus, carbohydrate-derived reactive carbonyls react with amines, amino acids, aminophospholipids, and proteins to produce advanced Maillard reaction products, also known as advanced glycation-end products (AGEs) (Wei, Liu, & Sun, 2018). These AGEs are common components of processed foods, mainly as a result of their heating (ALjahdali & Carbonero, 2019). There are many types of AGEs, and the most commonly studied are *N*(6)-carboxymethyllysine (CML) as example of non-crosslinking AGEs, and pyrraline and pentosidine as examples of crosslinking AGEs. They are widely used as indicators of the nutritional quality of foodstuffs. Thus, for example, *N*(6)-carboxymethyllysine has been shown to increase as a consequence of the changes produced during the reheating of dairy products (Dong, Wu, Wang, Wu, Zhang, & Wang, 2019) or to be present to a high extent in infant formulas containing high contents of whey protein (Prosser, Carpenter, & Hodgkinson, 2019). In addition, pyrraline has been suggested as a reliable index of heat load in puffed wheat kernels

(Cattaneo, Hidalgo, Masotti, Stuknyte, Brandolini, & De Noni, 2015), and pentosidine has been regarded as a biomarker for AGEs (Li & Yu, 2018).

Analogously to the above described for carbohydrate-derived reactive carbonyls, carbonyl-amine reactions are also a common way of disappearance for lipid-derived reactive carbonyls (Hidalgo & Zamora, 2000). In fact, lipid- and carbohydrate-derived carbonyls compete among them for the amine compounds existing in the nearby and both reactions are so interrelated that lipid oxidation and Maillard reaction should be considered simultaneously to understand the products of the Maillard reaction in the presence of lipids and vice versa (Zamora & Hidalgo, 2005). This reaction is responsible for the formation of advanced lipoxidation-end products (ALEs), among which the formation of pyrrole-derivatives during the storage of fat-rich food products has been described (Hidalgo & Zamora, 1993; Lu, Bruheim, Haugsgjerd, & Jacobsen, 2014). Some of these pyrrole derivatives are shown in Figure 5. Among them, *N*(6)-pyrrolylnorleucine has been shown to be a common component in many foods (Zamora, Alaiz, & Hidalgo, 1999). In addition to ALE formation, lipid-derived carbonyl-amine reactions have been related, for example, to acrylamide formation during coffee roasting (Kocadagli, Goncuoglu, Hamzalioglu, & Gokmen, 2012; Zamora & Hidalgo, 2008), or to the formation of PhIP (Zamora, Alcon, & Hidalgo, 2012).

Another important way of disapperance for reactive carbonyls is phenolic compounds (Zamora & Hidalgo, 2016). The carbonyl scavenging ability of phenolics has been long known (Totlani & Peterson, 2005), but its role as inhibitor of AGEs or ALEs formation is more recent (Elrod, Greenspan, & Hofmeister, 2017; Hidalgo, Delgado, & Zamora, 2017; Yuan et al., 2019; Zamora, Navarro, & Hidalgo, 2018). The reactive carbonyls mostly studied in this sense have been glyoxal and methylglyoxal (Yu, Xu, & Yu, 2017; Zhu, Poojary, Andersen, & Lund, 2019). The structures of the

carbonyl-phenol adducts produced with these reactive carbonyls are shown in Figure 6. In addition, different studies have described the ability of phenolics to scavenge a wide range of lipid-derived reactive carbonyls, including alkanals (Hidalgo, Aguilar, & Zamora, 2017), 2-alkenals (Hidalgo & Zamora, 2014), 2,4-alkadienals (Hidalgo & Zamora, 2018), 4-hydroxy-2-alkenals (Hidalgo & Zamora, 2019b), 4-oxo-2-alkenals (Hidalgo, Aguilar, & Zamora, 2018), and 4,5-epoxy-2-alkenals (Zamora, Aguilar, & Hidalgo, 2017). Typical structures for the carbonyl-phenol adducts produced are also collected in Figure 6. These carbonyl-trapping reactions have been shown to occur in foods under common cooking conditions (Zamora, Aguilar, Granvogl, & Hidalgo, 2016).

This inhibitory ability of phenolic compounds has been shown to be effective for inhibiting HAA production (Viegas, Amaro, Ferreira, & Pinho, 2012) and to be mainly produced by specific phenolics (Hidalgo, Navarro, & Zamora, 2018; Salazar, Arambula-Villa, Hidalgo, & Zamora, 2014). Thus, certain phenolics are able to trap the reactive carbonyls needed for HAA formation. The phenolics that exhibit the highest carbonyl-trapping abilities are those having hydroxyl groups at *meta* positions, because these derivatives concentrate a high electronic density at certain carbons. This high electronic density is needed so that phenolic compounds can be added to the reactive carbonyls and produce the corresponding adducts (Zamora & Hidalgo, 2018). On the other hand, phenolics with a substitution pattern that favors electronic delocalization will have lower abilities for carbonyl trapping but higher abilities for free-radical scavenging. This explains the wide variety of inhibiting properties for HAA formation observed for the different phenolics, which is not well correlated with the antioxidant/free radical-scavenging capacity of phenolic compounds and spice extracts (Cheng, Chen, & Wang, 2007). Furthermore, some of these phenolic compounds and spice extracts promoted the

391 formation of HAAs (Damasius, Venskutonis, Ferracane, & Fogliano, 2011), most likely
392 because of the above discussed possibility of appropriate phenolics to be converted into
393 quinones that act as reactive carbonyls and can promote the formation of the carbonyls
394 required for HAA formation.

395 **7. Conclusions**

396 Above discussed results seem to confirm that the tendency of reactive carbonyls to
397 suffer cyclizations and oligomerizations may be related to the appearance of HAAs in
398 foods. Therefore, the understanding of how the food carbonylome changes (the changes
399 produced in the pool of carbonyl compounds existing in each food at each time) can be
400 essential to control HAA formation. Thus, short-chain reactive carbonyls are produced
401 in foods from many sources and the same carbonyls can have different formation routes.
402 This food carbonylome is in constant change because reactive carbonyls are
403 continuously produced and the most reactive compounds rapidly disappear. In fact,
404 these compounds react with different carbonyl scavengers present in foods to produce
405 carbonyl-amino and carbonyl-phenol adducts, among others. When they are not
406 scavenged, reactive carbonyls suffer cyclizations and oligomerizations in the presence
407 of ammonia to produce heterocyclic rings. When creatinine is present, creatinine also
408 participates in these cyclizations and oligomerizations, which is suggested to be the
409 origin of HAAs. Because of their carbonyl-trapping abilities, phenolics are able to block
410 HAA formation, as observed in many studies. However, free radical-scavenging
411 phenolics can also originate quinones that act as reactive carbonyls and can contribute
412 to the formation of the aldehydes required for HAAs production, as also observed in
413 other studies. The specific carbonyl compounds responsible for the formation of each
414 HAA remain to be identified. This identification is needed to design specific targeted
415 procedures that can either block their formation or trap them once formed.

Conflict of interest

The authors declare no conflicts of interest.

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735

736 **Figure legends**

737 **Fig. 1.** Chemical structures of the HAAs classified, at present, as probable (class 2A) or
738 possible (class 2B) carcinogens by IARC (International Agency for Research on
739 Cancer).

740 **Fig. 2**. Proposed pathways for HAA formation.

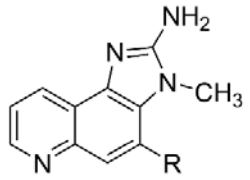
741 **Fig. 3**. Pyridine ring formation by aldehyde cyclization and oligomerization in the
742 presence of ammonia.

743 **Fig. 4**. Reactive carbonyls commonly found in foods and components of the food
744 carbonylome.

745 **Fig. 5**. Chemical structures of common carbonyl-amine adducts.

746 **Fig. 6**. Chemical structures of common carbonyl-phenol adducts (resorcinol is shown as
747 a model *m*-diphenol, but analogous adducts with other *m*-diphenols are also produced).

748



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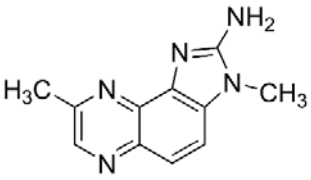
MeIQ: R = CH_3



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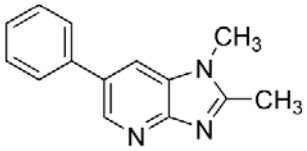

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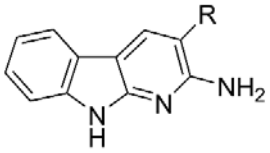
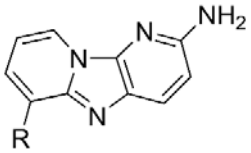
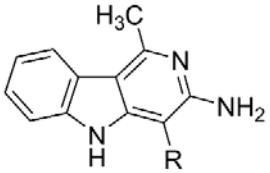


A-α-C: R = H
MeA-α-C: R = CH_3



Glu-P-1: R = CH_3
Glu-P-2: R = H

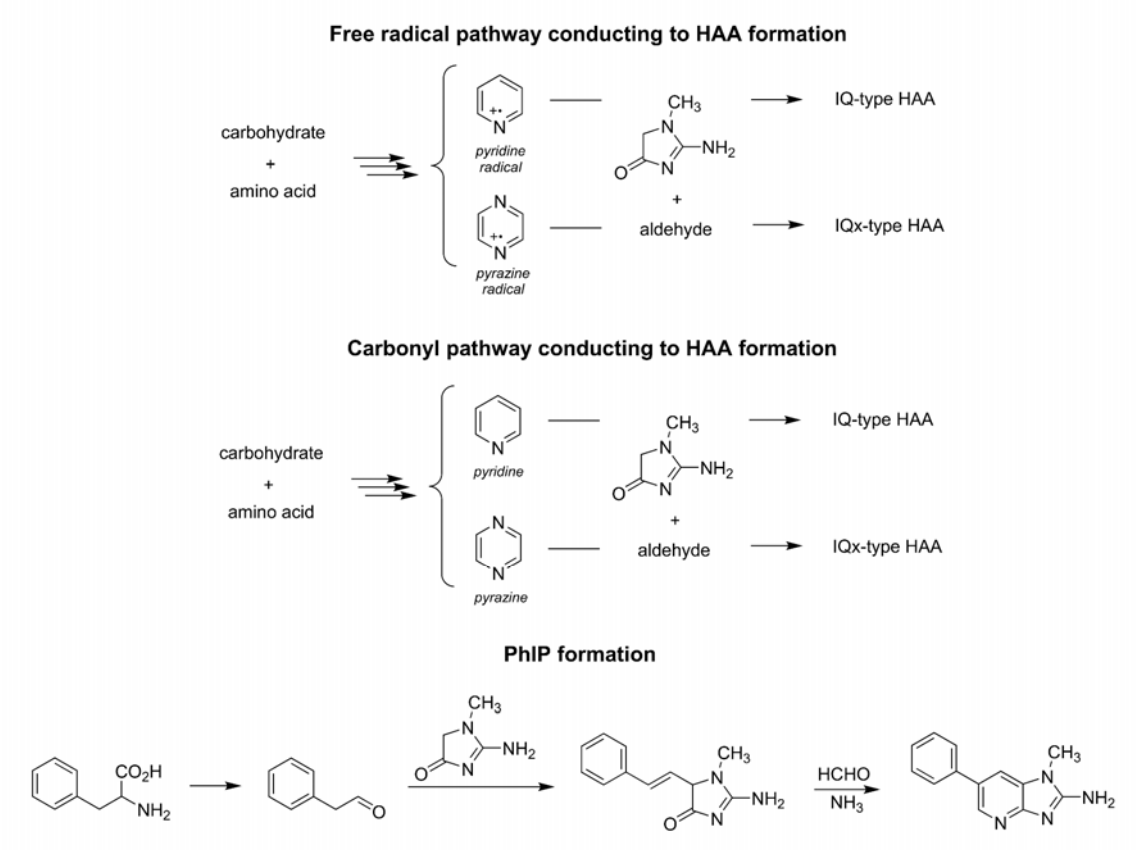


Trp-P-1: R = CH_3
Trp-P-2: R = H

749

750 **Figure 1**

751



752

753 **Figure 2**

754

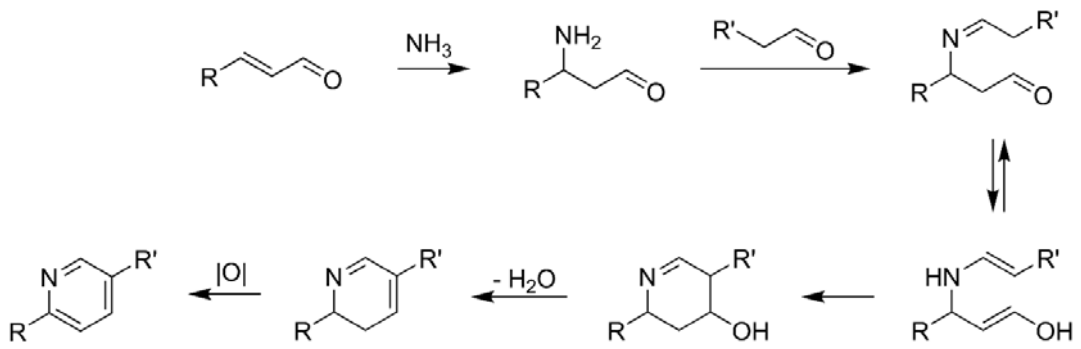
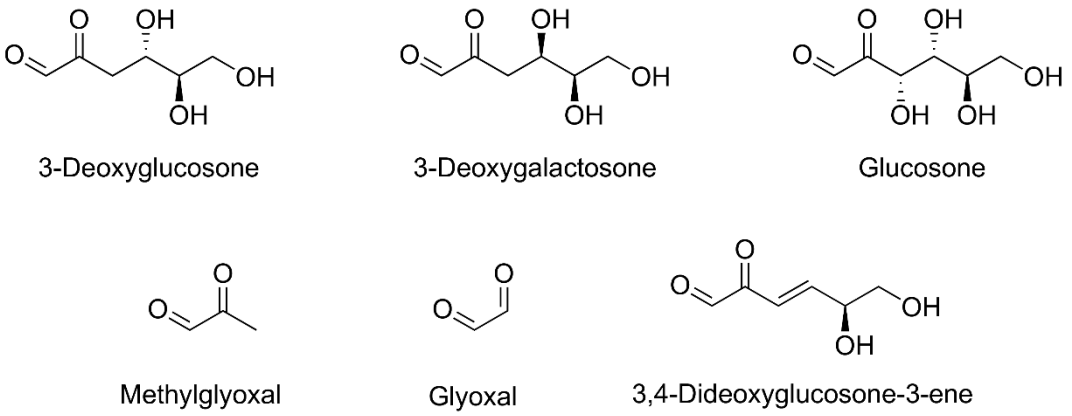
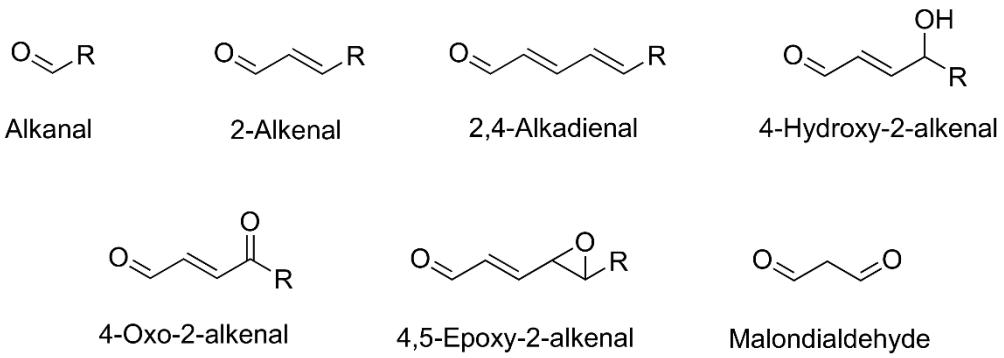


Figure 3

Carbohydrate-derived reactive dicarbonyls



Lipid-derived reactive carbonyls



758

759 **Figure 4**

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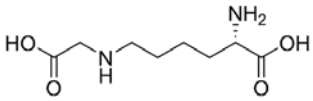
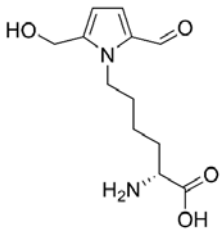
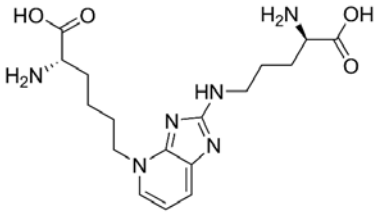
Carbohydrate-derived carbonyl-amine adducts



N(6)-Carboxymethyllysine

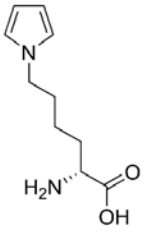


Pyrraline

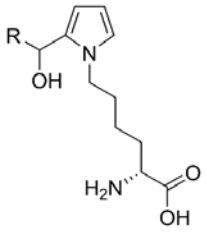


Pentosidine

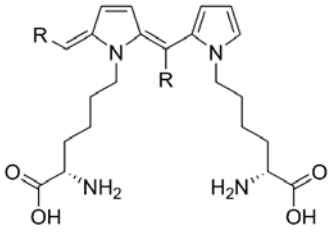
Lipid-derived carbonyl-amine adducts



N(6)-Pyrrolylnorleucine



2-Amino-6-(2-(1-hydroxyalkyl)-1*H*-pyrrol-1-yl)hexanoic acid

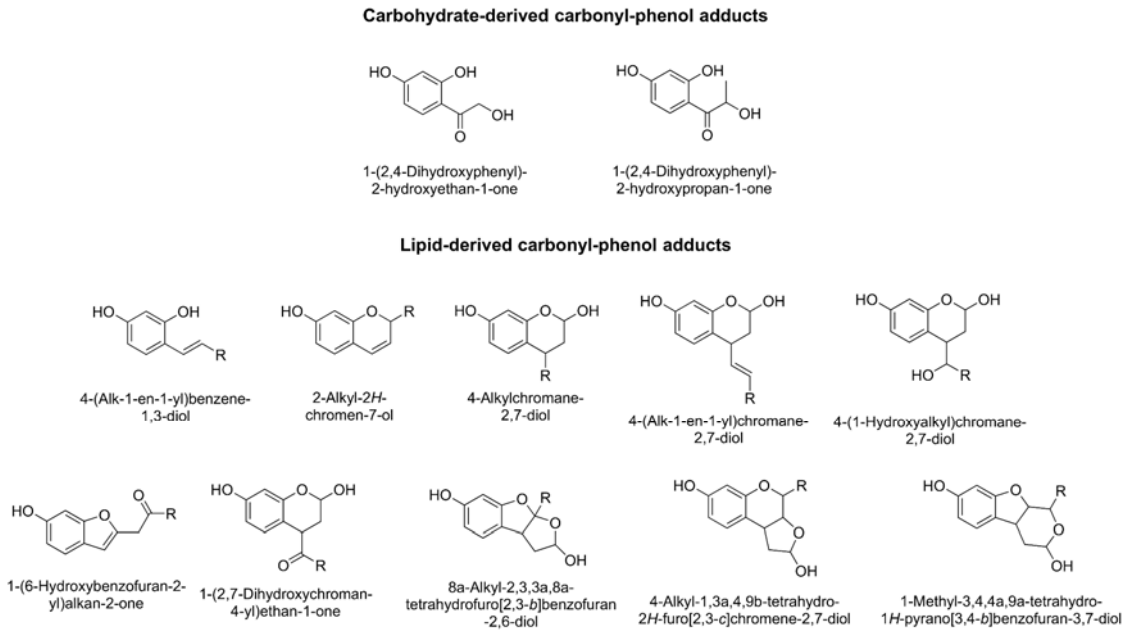


(2-Amino-6-(2-(1-(1-(5-amino-5-carboxypentyl)-1*H*-pyrrol-2-yl)alkylidene)-5-alkylidene-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoic acid

761

762 **Figure 5**

763



764

765 **Figure 6**

766

Supporting Information

Formation of heterocyclic aromatic amines with the structure of aminoimidazoazarenes in food products

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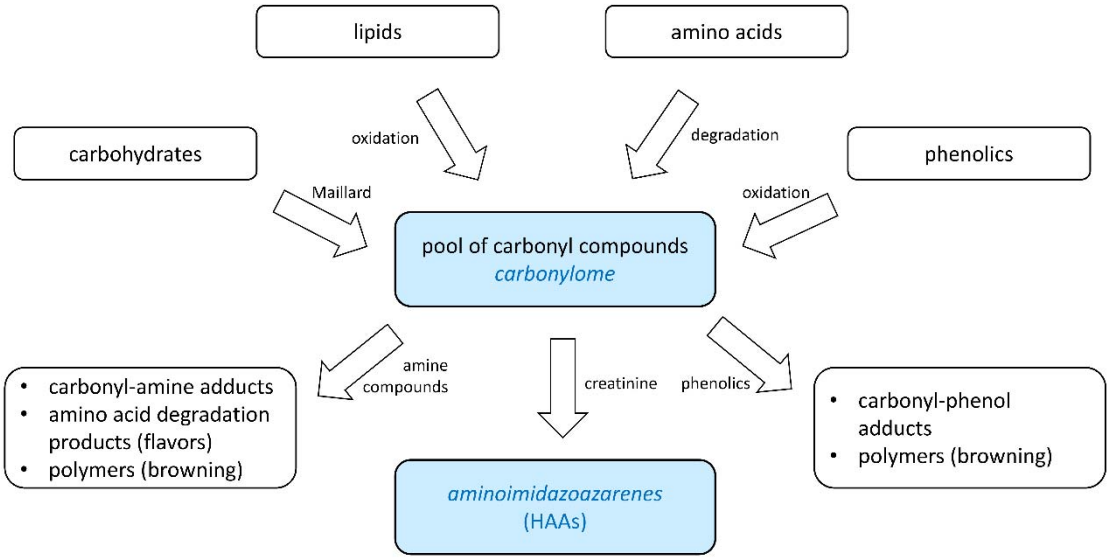

Fig. S-1. Selected routes for the formation and fate of the pool of carbonyl compounds existing in foods (the food carbonylome). Although it has not indicated in the figure, some routes of disappearance act as feedback of the pool of new carbonyl compounds, such as the Strecker degradation of the amino acids produced by either carbohydrate- or lipid-derived reactive carbonyls.