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Review

Mitigation strategies to reduce the impact of heterocyclic aromatic amines in proteinaceous foods

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ABSTRACT

Background: Meat and fish are currently the main sources of proteins necessary for a healthy diet. Cooking proteinaceous food helps reduce biological risks and produce odor-active compounds, but it also generates heat-induced toxicants, of which **heterocyclic aromatic amines** (HAAs) are probably the most problematic as they are strongly mutagenic and carcinogenic.

Scope and approach: This review highlights the most promising strategies for mitigating the impacts of HAAs on human health. These strategies revolve around reducing HAA formation by impacting HAA **precursors**, **controlling the process,** adjusting **formulations** or adapting diets to limit HAA assimilation and **metabolism**.

Key findings and conclusions: Identifying the different mechanisms of HAA formation and metabolism has made it possible to propose **mitigation** strategies to limit the risks related to HAA consumption. Various kinds of levers exist. While cooking methods for industrial processed foods can be regulated, it is far more difficult to influence household practices. Mitigation strategies involving other food ingredients are probably more promising from a consumer point of view if pushed by health education campaigns. Efforts to reduce the health risk from HAA consumption should now turn to ingredients like carvacrol that present different concomitant modes of action.

1. Heat-induced food toxicants

Since the discovery of fire, humankind heats its foods to increase palatability and reduce bacteriological risks. Unfortunately, cooking also forms toxicants that present potential risks for human health (Stadler & Lineback, 2009). The big neurotoxicant-producing food categories are carbohydrate-rich foods (formation of acrylamide and hydroxymethylfurfural), meat and fish products (formation of heterocyclic aromatic amines, *N*-nitrosamines and polyaromatic hydrocarbons), and oils (formation of monochloropropane diols and derivatives). Meat and fish products appear to generate the most important panel of heat-induced toxicants and should thus be monitored to assess the real risk tied to their consumption. The IARC has recently published an article in the Lancet Oncology where red meat and processed meat have been classified as probably carcinogenic (group 2B) and carcinogenic (group 1) (Bouvard et al., 2015), respectively. This is the position adopted by the health authorities (ANSES, 2011; National Toxicology Program – Department of Health and Human Services, 2011) which have found that several categories of food and beverages are potential risk factors for cancer. They have also found convincing evidence that higher risk for cancer (especially colorectal cancer) is linked to the consumption of red and processed meat, which should thus be reduced. Their studies concluded that the risk was not linked to a particular family of heat-induced toxicants but to *N*-nitrosamines, heme iron or HAAs (ANSES, 2011). Polycyclic aromatic hydrocarbons could also be listed but they are considered less a threat as only barbecueing presents a real risk of inducing their formation by pyrolysis of organic materials, direct contact of fat with a flame, or incomplete charcoal combustion (Alomirah et al., 2011). After its latest total diet study, the ANSES rejected the population risk from exposure to polycyclic aromatic hydrocarbons but proned keeping a close watch on their occurrence in food. *N*-nitroso compounds are a potential factor in colorectal cancer etiology in relation with heme iron. However, while the precursors are found in relatively high quantity in cured meat (addition of nitrites or nitrates for bacteriological, nutritional, sensory reasons), there are very little of them in red meat and their impact is negligible compared to the amount contributed by vegetables and water and knowing that saliva

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produces about 93% of the total ingested nitrite (Sindelar & Milkowski, 2012). Furthermore, although *N*-nitroso compounds are often formed endogenously in healthy human stomach (via acid catalysis in presence of nitrite, at 37 °C), the conditions under which they are preformed in cured and processed meat are very specific as they require the presence of secondary amines with nitrite available to react, a near-neutral pH, and a temperature above 130 °C (Honikel, 2008). The potential risk related to *N*-nitroso compounds should also be balanced against the importance of their precursors for human health. The nitrite involved in *N*-nitroso compound formation in cured and processed meats is also a significant source for endogenous production of nitrite oxide, which a recent report claims may play a crucial role in the prevention of cardiovascular disorders and immune response (Sindelar & Milkowski, 2012). Conversely, several studies suggest that consumption of well-done or grilled meat is associated with increased cancer risk in humans, and this close relationship is often associated with the increase of HAA formation in well-done meat. Eight of the known HAAs are classified as probably (2A) or potentially (2B) carcinogenic to humans (IARC, 1993), and as they are potently genotoxic, mutagenic and/or carcinogenic in animal testing, they are likely also involved in human cancer etiology (IARC, 1993; National Toxicology Program - Department of Health and Human Services, 2011). To clarify the issue, several epidemiological studies have been led to determine the impact of HAA exposure on human health, but more reliable data are still needed (Sinha, 2002), since while there is evidence of links between the consumption of well-done meat and the onset of colon, prostate or female mammary gland cancers, the contribution of HAAs is not as clear (Ollberding, Wilkens, Henderson, Kolonel, & Le Marchand, 2012) and is a subject of debate as the health risks may be related not only to HAAs but also to other heat-induced food toxicants and their cocktails in processed foods (HAAs, PAHs, *N*-nitroso compounds, lipid peroxides) (Jamin et al., 2013). Nevertheless, health authorities recommend minimizing their occurrence as they possibly induce mutations and can therefore, if combined with other food mutagens, increase sensitivity to tumor promoters (Sanz Alaejos, Pino, & Afonso, 2008). The metabolism of HAAs is individual-dependent, and the associated cancer risk is mainly linked to genetic predisposition (Sanz Alaejos et al., 2008). Given their mutagenicity and carcinogenicity for animals, it is not unreasonable to believe that their consumption could cause human cancers. Further studies are needed to fill in the gaps in our knowledge of this family of compounds. Questions over the toxicological impact and methods of detection of HAAs in food have already been addressed in several full reviews (Cheng, Chen, & Wang, 2006; Pais & Knize, 2000; Sanz Alaejos et al., 2008) but knowledge of their mechanisms of formation and metabolism could be mobilized to develop efficient mitigation strategies to reduce the related health risk. To achieve this goal, there are three big strategy options: (i) reduce the formation of HAAs by acting directly on their precursors or on the process via different cooking conditions and adapted formulations (Alaejos & Afonso, 2011); (ii) modulate the bioaccessibility of HAAs by adapting formulations or diets; (iii) modulate the metabolism of HAAs in the human body by using appropriate formulations or diets (Schwab et al., 2000). This papers discusses the options for mobilizing these three levers. The impact of thermal treatments on HAA formation is not discussed in depth here as it is widely described elsewhere. The main focus will be on the other new perspectives for reducing the health impact of HAAs.

2. HAA formation in proteinaceous food

Unlike other processed food toxicants such as acrylamide, the mechanisms of HAA formation are only partially understood, and while some factors seem to play a role in the reduction of HAA formation levels, the way they act is far from clear. Here we first present the different classes of HAAs with the known data on their mechanisms of formation.

HAAs were discovered about 40 years ago by Professor Sugimura who studied the charred part and smoke generated by broiled meat and fish (Sugimura et al., 1977). The major source of human exposure to HAAs is through cooked meat and fish (Alaejos & Afonso, 2011) and depends on the kind of meat/fish and cooking preferences (Skog, Solyakov, & Jägerstad, 2000). So far, about 25 HAAs have been identified in proteinaceous foods (Skog, Johansson, & Jägerstad, 1998). They are classified into two distinct families, the carbolines or pyrolytic HAAs, and the aminoimidazoazaarenes.

2.1. Formation of pyrolytic HAAs

The pyrolytic HAAs, as their name suggests, are mainly formed at high temperature (generally more than 250 °C) (Skog, Solyakov, Arvidsson, & Jägerstad, 1998). As depicted in Fig. 1, depending on their structures, pyrolytic HAAs belong to one of 5 different groups: pyridoindoles (harman, norharman, AαC, MeAαC, Trp-P-1, Trp-P-2), pyridoimidazoles (Glu-P-1, Glu-P-2), phenylpyridines (Phe-P-1), tetraazofluoranthenes (Orn-P-1) or benzimidazoles (Cre-P-1). As shown in Table 1, they are formed from amino acid precursors such as tryptophan, phenylalanine, glutamic acid or ornithine, but also from the pyrolysis of casein and soy bean globulin. At high temperature, reactive radicals are formed as well as deaminated and decarboxylated products (Supplementary data 1). These different compounds react together to generate the pyrolytic HAAs (Murkovic, 2004). Table 1 also charts their occurrence in proteinaceous food according to various cooking conditions. To illustrate, the pyrolysis of tryptophan induces the formation of Trp-P-1, Trp-P-2, AαC, MeAαC, harman and norharman. Unfortunately, only the first steps of their formation are generally understood. As they were long believed to be only formed at temperatures above 250 °C, research mainly focused on pyrolytic HAAs formed in substantial quantity in products with an active pyrolysis step, such as cigarette smoke which produces large amounts of MeAαC and AαC or harman and norharman (Totsuka et al., 1999). Snook & Chortyk found 12 μg/g of norharman and 4 μg/g of harman in cigarette smoke condensate (Snook & Chortyk, 1982) whereas the amounts found in chicken pan-fried at 220 °C for 12 min are more than a thousand times less (3.5 ng/g and 5.7 ng/g, respectively (Pfau & Skog, 2004)). Totsuka et al. showed that pan-frying bacon for 15 min at only 176 °C produced a non-negligible 40.2 ng/g of norharman and 5.5 ng/g of harman, and stressed the differences in amount of HAA precursors in various kinds of meat (Totsuka et al., 1999). Various studies have measured the amount of pyrolytic HAAs in proteinaceous foods cooked by standard methods. Skog et al. in 2004 (Pfau & Skog, 2004) and more recently Alaejos & Afonso (Alaejos & Afonso, 2011) summarized the different results. It first appears that harman and norharman are by far the most abundant pyrolytic HAAs formed in food. Their formation is mainly favored by high temperature methods such as flame-broiling, oven-broiling or pan-frying. For flame broiling, the high amount of pyrolytic HAAs can be explained by direct contact with the flame inducing a very high temperature at the meat surface. Food contains much higher concentrations of norharman and harman than other HAAs (pyrolytic and aminoimidazoazaarenes), suggesting that human exposure to these two HAAs is far more important (Totsuka et al., 1999). Nevertheless, based on the results obtained with *Salmonella typhimurium* strains, these two HAAs, even if they are ubiquitous in proteinaceous food, are not mutagenic *per se* but only become mutagenic in presence of aromatic amines such as aniline or *o*-toluidine. This co-mutagenic effect is attributed to the endogenous