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Review

Use of antioxidants to minimize the human health risk associated to mutagenic/carcinogenic heterocyclic amines in food

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Abstract

Heterocyclic amines (HAs) are mutagenic/carcinogenic compounds formed in meat during cooking. Several efforts have been made to minimize the risk associated to HA human exposure. Supplementation with antioxidants is considered a promising measure to reduce HA exposure because of their ability as inhibitors of HA formation or as blocking/suppressing agents on HA biotransformation/metabolism. The aim of this review is to present the current knowledge on the capability of synthetic and natural antioxidants to modulate HA-induced mutagenicity/carcinogenicity. Data show a general trend towards a reduction of HA formation both in model systems and in real foods as well as an effective modulation of biotransformation and metabolism. Phenolic compounds, particularly those from tea and olive oil, seem to be the most effective, although a great variability is observed because of the concentration-dependent pro- and antioxidant effects.
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Keywords: Reviews; Food analysis; Heterocyclic aromatic amines; Antioxidants

Contents

1. Introduction

Humans are continuously exposed to HAs, as demonstrated by detection of these compounds in cooked foods and human urine samples [1–8]. The presence of

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2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline (MeIQx)-DNA adducts found in human tissues, such as colon, rectum and kidney increased the concern about the health consequences of the intake of these compounds [9].

Although methods for estimation of HA dietary exposure in the population are still imprecise [10], in some studies epidemiological evidence suggests that consumption of high amount of HAs-containing meat products may induce colorectal, pancreatic and urothelial cancer [11–18]. On the other hand, other studies did not show such association [10,19]. Today, more than 20 mutagenic/carcinogenic HAs have been isolated and identified in cooked foods [5,17, 20–22]. Their formation is the result of complex reactions that involve creatine, free amino acids and carbohydrates through the Maillard Reaction (MR). The development of MR also occurs through a free radical mechanism which has been shown to play an important role in the formation of imidazoquinoxalines and imidazoquinolines [23–25].

The pathway of HA formation is shown in Fig. 1. Creatine forms the amino-imidazo part of the molecule by cyclization and water elimination, whereas the remaining parts of the IQ compounds arise from Strecker degradation products, such as pyridine and pyrazine [26,27].

This hypothesis was verified in model systems [26–30], and the results were later confirmed also in meat-based system [31–34].

The presence of HAs in foods depend on many factors such as cooking method, time and temperature, the presence of relative amounts of precursors, enhancers and inhibitors, lipids, antioxidants and the water content [23]. In particular, supplementation with antioxidants is considered to be an effective measure to reduce HA exposure because of the hypothetical free radical pathway leading to HA formation [8]. Single antioxidant compound and complex mixtures of antioxidants have been demonstrated to inhibit HA-induced mutagenesis or carcinogenesis. It is likely, that this effect is the final result of different actions interfering at various steps of the HA formation and of HA-toxic activity. In fact, antioxidants can act as inhibitors along the different pathways of the reaction, preventing the mutagens formation,

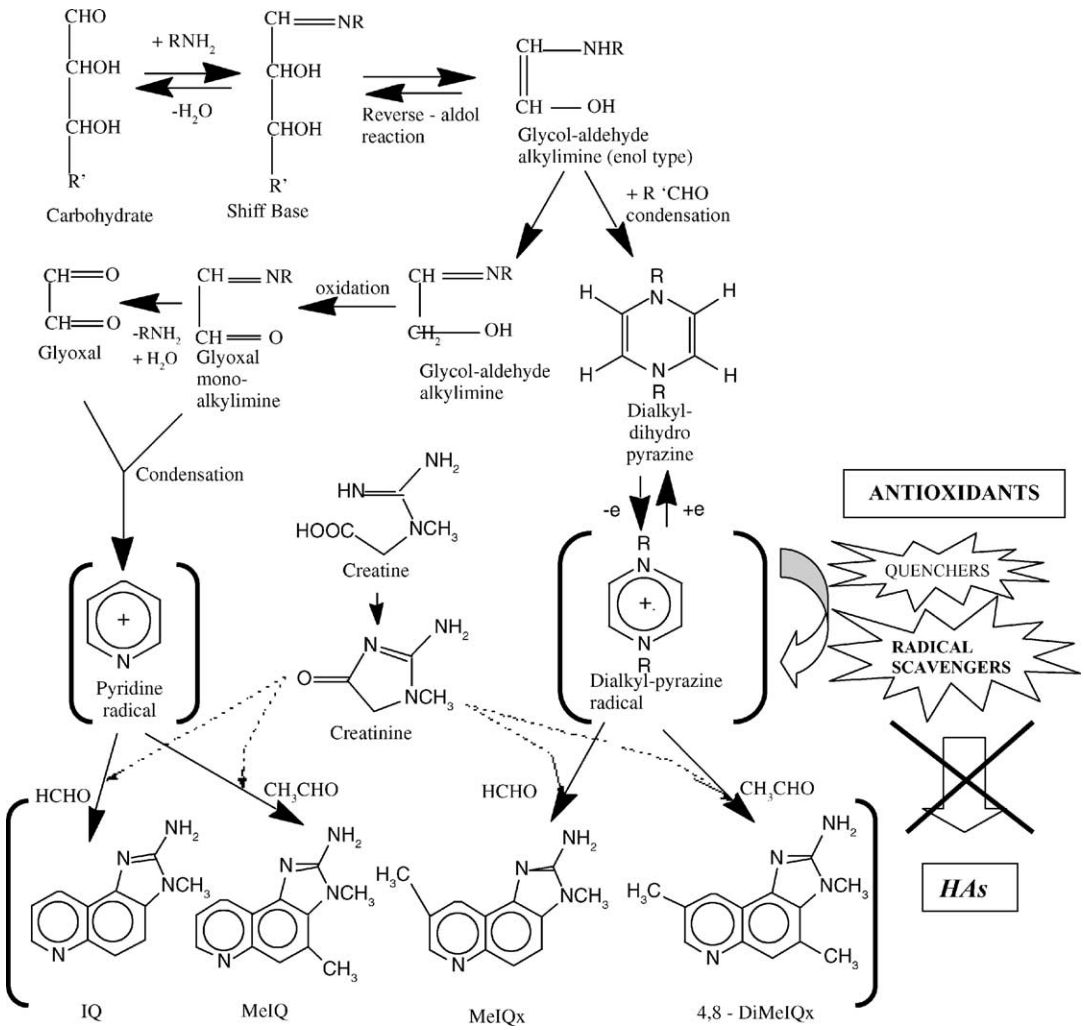


Fig. 1. Scheme of HA formation and possible sites for interference with antioxidants (modified from [25]).

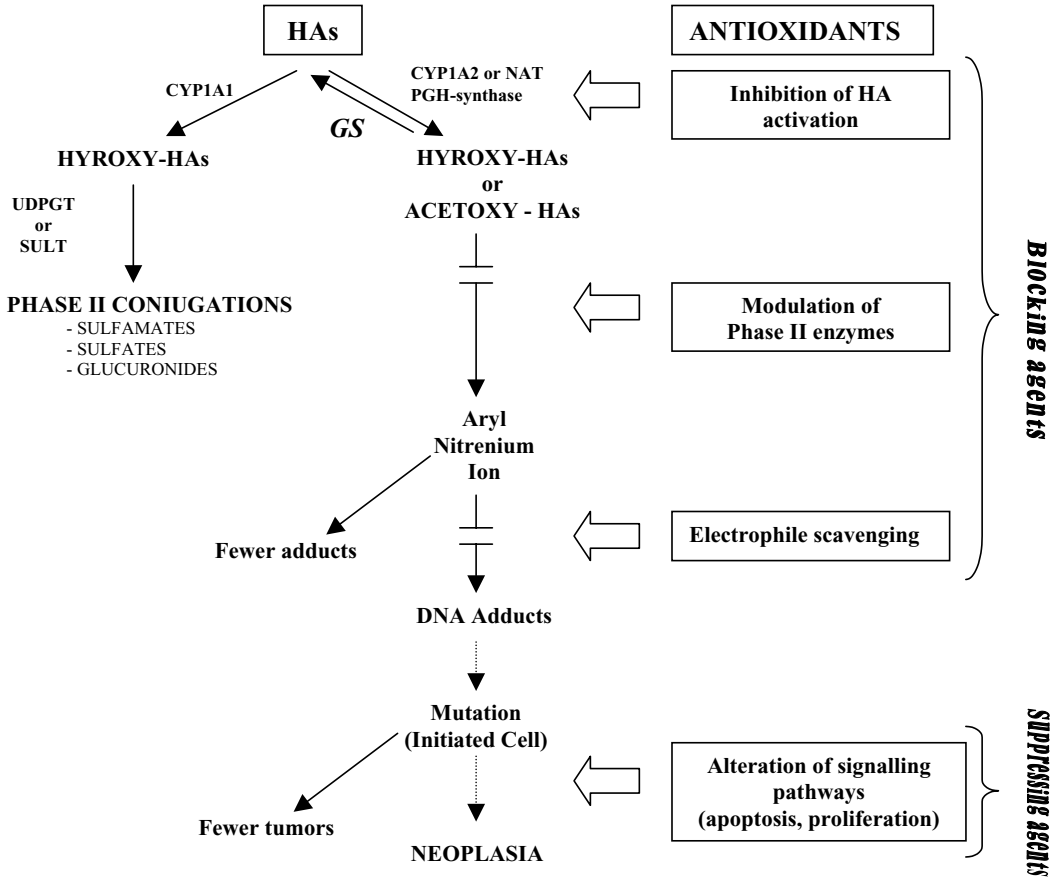


Fig. 2. Scheme of HA bioactivation and possible sites for interference with antioxidants (modified from [36]). UDP-glucuronosyl transferase (UDPGT); Sulfotransferases (SULT);Glutathione-*S*-transferase (GST); Prostaglandin H (PGH)-sinthase.

through radical quenchers and free radical scavengers activity (as shown in Fig. 1); as blocking agents, preventing the biotransformation of premutagens into reactive metabolites by inhibiting metabolic activation, by stimulating detoxification enzymes, or by scavenging reactive molecules; as suppressing agents modulating intracellular processes, which are involved in DNA repair mechanisms, tumour promotion and tumour progression [35,36] (see Fig. 2).

The aim of this review is to present the current knowledge on the ability of antioxidants to modulate HA-induced mutagenicity/carcinogenicity.

To better underline the differences of action and the potential efficacy of antioxidants to minimize the human health risk associated to cooked meat/fish consume the review was organised in two main paragraphs dealing on the effect exerted by synthetic and natural antioxidants, respectively.

2. Synthetic antioxidants

Antioxidants are known to exert both anti- and prooxidative effects depending on their concentrations. These two opposite effects make difficult the comparison between in vivo and in vitro data. This figure also occurs when antioxidants are added to a heated mixture of HA precursors or directly to foods before or during cooking [37]. In Table 1,

Table 1
Studies on the effects of synthetic antioxidants on HA formation, in chemical model system and food system, and on HA-induced mutagenesis/carcinogenesis, in Salmonella assays and in bioassays in rats

	Effects on HA formation		Effects on mutagenesis/carcinogenesis induced by HAs	
	Chemical model system	Food system	Salmonella assay	Bioassays in rats
BHA	[25,37,39,40]	[38]	[25,38,40]	
BHT	[37]	[18]		[42]
PG	[25,37,39,40]	[38]	[25,38,40]	[42]
TBHQ	[37]			[42]
HTHQ	[37]			[42]

some significant studies about the effects of synthetic antioxidants on HA formation in chemical model systems and in foods are grouped. Also studies reporting the effects of antioxidants on HA-induced mutagenesis/carcinogenesis both in Salmonella assays and on animals are listed.

Johansson and Jägerstad [37] showed that many synthetic and natural antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), α- and γ-tocopherol, increased the formation of MeIQx in heated mixture containing HA chemical precursors. The highest amount of MeIQx is formed with the addition of 100 ppm *tert*-butylhydroquinone (TBHQ) (220% of increase respect to the control).

On the other hand, opposite data where obtained by Chen [38] who reported that a concentration of 100 ppm of BHA, PG and TBHQ reduced the formation of HAs. In this study, a real food system (frying of beef) was investigated instead of a chemical model system [37].

Contrasting evidences have been reported on the effects of synthetic antioxidants on HA-induced mutagenicity and carcinogenicity. Also in this case, the comparison between the various studies is hampered by the multiplicity of markers observed, by the different experimental conditions, by the different combination of individual HA and antioxidant. Data clearly show a general trend for a decrease in the severity of HA-exposure consequence, with some relevant exception. The addition of BHT to a heated mixture of precursors showed a concentration-dependent increase in the mutagenic activity of HAs [25,38]. This effect was attributed to the alkylating action of BHT which increased the formation of the precursors for 2-amino-3,4,8-trimethylimidazo[4,5-*f*]-quinoxaline (4,8-DiMeIQx) in chemical model system [25].

BHA and PG prevent the formation of imidazoquinoxaline-mutagens in heated mixtures of creatinine, amino acids and sugars in a dose-dependent manner [39,40]. These antioxidants were also reported to decrease the mutagenic activity of IQ and MeIQx [25,38]. In particular, Kato and co-workers [40] reported that BHA at 20 and 100 mM reduced the mutagenicity, on Salmonella typhimurium TA98 strain with metabolic activation, of heated mixtures of glucose/glycine/creatinine to about 60 and 20%, respectively while epigallocatechin gallate (EGCG), tested at the same concentrations, reduced the mutagenicity to about 40 and 2%, respectively. A reduction in mutagenic activity by BHA was also demonstrated by Wang and co-workers [41]. In previous studies, BHA, TBHQ and PG had been suggested to prevent HA formation blocking the reaction with the meat mutagen precursors thank to their methoxy group that was converted into a potent free radical scavenger such as a quinone-like compound [25].

Hirose and co-workers [42] reported that synthetic antioxidants, such as 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ), BHT, TBHQ or PG, are all inhibitors of MeIQx-induced hepatocarcinogenesis in F344 rats, with HTHQ as the most effective. In fact each antioxidant at a concentration of 0.25% (w/w), together with MeIQx (0.03% (w/w)), inhibited development of preneoplastic glutathione-*S*-transferase placental form (GST-P) positive foci as compared with MeIQx alone, after initiation with diethylnitrosamine (DEN). 8-Hydroxydeoxyguanosine (8-OHdG), a marker for DNA damage induced by active oxygen species, malondialdehyde and 4-hydroxynonenal levels were not largely influenced by the treatment with MeIQx or antioxidants, either alone or in combination. These data suggest that free radicals may not play a major role for MeIQx-induced hepatocarcinogenesis [43].

Moreover, studies on effects of HTHQ on the in vitro metabolic activation of 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine quinoxaline (PhIP) and on PhIP-DNA adduct formation in rat colon showed that this synthetic antioxidant is a very strong chemopreventor of HA-induced carcinogenesis, but the inhibition of metabolic activation rather than antioxidant activity is responsible for this effect [42]. Medium-term bioassays using male and female F344 rats to examine the effects of HTHQ on PhIP-induced colon and mammary gland carcinogenesis, respectively, were performed by Hirose and co-workers [42]. These experiments showed that the multiplicity of colon tumours induced by 0.02% PhIP after 1,2-dimethylhydrazine (DMH) initiation was dose-dependently decreased by the combined treatment with 0.125 and 0.5% HTHQ [42].

Previous studies showed that this synthetic antioxidant inhibited colon and mammary carcinogenesis but enhanced forestomach carcinogenesis when used in post-initiation treatment (1% dietary concentration) in medium-term bioassays [44]. Thus, HTHQ was scripted as belonging to the agents that are cited as giving “stage dependent paradoxical effects” [44].

According to Hirose and co-workers [42] synthetic antioxidants are more potent than naturally occurring antioxidants, like several flavonoids, in inhibiting MeIQx-induced carcinogenesis. However, many studies addressed the use of natural antioxidants which can be considered by consumers a more friendly way to reduce HA-exposure risk.

3. Natural antioxidants

When tested as single substances, various natural compounds show marked antimutagenic activity in the Ames test and other mutation assays, but little is known about such activity when these compounds are present in food interacting with other food components, during cooking/storage and in the lumen of gastrointestinal tract.

The studies about the activity of naturally occurring antioxidants on HAs have been divided into two groups, the first reviewing papers based on use of pure compounds and the second using food extracts and whole foods. Table 2 summarizes the studies on the effects of natural antioxidants (pure compounds, extracts and whole foods) on HA formation,

Table 2
Studies on the effects of natural antioxidants on HA formation, in chemical model system and food system, and on HA-induced mutagenesis/carcinogenesis, in Salmonella assays and in bioassays in rats

	Effects on HA formation		Mutagenesis/carcinogenesis induced by HAs	
	Chemical model system	Food system	Salmonella assay	Bioassays in rats
Pure antioxidants:				
Ascorbic acid		[18,38,45]	[49,50]	
α-tocopherol		[18,38,45,46]		[47]
Flavonoids	[51]	[58]	[8,52]	[42]
EGCG	[39,40]	[40]	[8,74]	
Ellagic acid and NDGA	[8]			
Fruits and vegetables	[58]	[58,61]	[47,59,60]	[62,65,66]
Soy		[41,67]		
Tea	[39,68]	[80]	[71,72,75,79,82]	[42,81,85–87]
Olive oil	[89]			
Spices	[91]	[46]		

in chemical model systems and food systems, and on HA-induced mutagenicity/carcinogenicity, in Salmonella assays and in bioassays in rats.

3.1. Activity of pure compounds

3.1.1. Antioxidant vitamins

In a real food system, such as fried fish fibre, prepared by boiling, deboning, eviscerating, separating and pressing of snake fish meat, the addition of Vitamin C and α-tocopherol did not show any consistent effect on the formation of HAs [18]. On the contrary, previous studies showed that these antioxidants were able to inhibit mutagen formation during the frying of beef [38,45]. The addition of Vitamin E to the surface of the beef patties before frying, at the concentration of 1–10% of the fat content, produced a reduction of PhIP formation after cooking in the range of 45–75% [46]. These results can be due to the ability of tocopherols to inhibit free radical formation and/or producing compounds which may react with HA precursors and prevent formation of 4,8-DiMeIQx [25].

The capability to decrease active metabolite formation is presumably responsible of the reduction of IQ-DNA adduct-formation exerted by leaves of Kidachi aloe, which are rich in α-tocopherol, and β-carotene [47].

Anyway antimutagenic effects and possible mechanisms of action of several vitamins against genotoxic HAs have been reported by Edenharder and co-workers [48]. Results showed a lack of efficacy of ascorbic acid and α-tocopherol against HA-induced mutagenicity in the Salmonella/reversion assay.

Opposite results were obtained by Snyderwine and co-workers [49] who hypothesised that ascorbic acid could prevent the oxidative break-down of *N*-hydroxy-IQ, enabling the mutagen to remain active for a longer time in vitro with a consequent enhancement of mutagenic activity. In fact, 0.5 and 1 mM ascorbic acid, added to the plates, produced 11,000 and 11,500 revertants per plate compared to 1000 revertants per plate produced when *N*-hydroxy-IQ was tested alone. Subsequent studies using ascorbic acid and *N*-hydroxy-IQ reported no enhancement of mutagenic activity in absence of cytosolic enzymes, thus suggesting that the enhancement of mutagenicity reported by Snyderwine and co-workers (1988) was probably due to some metabolites of ascorbic acid or of *N*-hydroxy-IQ generated by cytosolic enzymes [50].

3.1.2. Phenolic antioxidants

Lee and co-workers suggested that flavones inhibited formation of IQ-type mutagens in a glycine/glucose/creatine model system reducing the formation of Maillard reaction products [51]. Edenharder and co-workers [52] some years later reported that, among the 30 flavonoids tested, all of them but two inhibited the mutagenic activity in the Salmonella assay; in presence of diosmetin and isorhamnetin a slight increase was observed. Among flavones and flavonols, the inhibitory effects increased in dependence on number and position of hydroxyl functions. This figure suggests that in poly-hydroxylated compounds the number and the position of OH groups are involved in the modulation of the activity of the enzymes responsible for IQ *n*-hydroxylation inhibition [52,53].

These in vitro evidences were not confirmed by an in vivo study performed to verify the effect of several flavonoids on hepatocarcinogenesis. Hirose and co-workers [42] reported that quercetin, rutin, curcumin, daidzin, ferulic acid and genistein were co-carcinogens for MeIQx-induced hepatocarcinogenesis, because they were able to enhance GST-P positive focus development.

Also studies on ellagic acid and nordihydroguaiaretic acid (NDGA) gave apparently contradictory results. Ellagic acid reduced the formation of MeIQx but enhanced that of PhIP while NDGA had the opposite effect [8]. The enhancement of MeIQx formation by NDGA could be explained according to the authors by the same alkylation mechanism described by Pearson and co-workers [25] for BHT, while in the case of PhIP formation some reactive groups derived from ellagic acid were supposed to be involved [8].

Consistent evidences have been found using other phenolic antioxidants such as (−)-epigallocatechin gallate (EGCG) which has been reported to suppress the formation of imidazoquinoxaline-type HAs in heated mixtures of creatinine, amino acids and sugars [39]. Kato and co-workers [40] demonstrated that generation of the mutagens in a heated chemical model system was effectively prevented by phenolic antioxidants, sesamol, esculetin and EGCG in a dose-dependent manner (being MeIQx content reduced to 35% of control in the presence of 100 mM EGCG) and in heated-and-dried bonito meat by pre-treatment with 0.5% EGCG or 5% green tea extract. The mutagenicity of heated-and-dried bonito meat, tested on *S. typhimurium* TA98 strain with metabolic activation (by liver microsomal S9 system) after blue rayon extraction and determined as number of histidine revertant colonies, was dramatically reduced by pre-treatment with 0.5% EGCG and 5% green-tea extract resulting less than 50 and 30% respectively [40]. The authors postulated these antioxidants acted preventing the formation, through Maillard reaction [25,54–56], of the unstable pyrazine cation radical. In a subsequent study, phenolic antioxidants such as green tea catechins and their major component EGCG, two flavonoids (luteolin and quercetin), and caffeic acid were found to decrease up to 75% both MeIQx and PhIP, and to reduce the total mutagenicity of heated mixtures [8]. Also in animal experiments green-tea catechins have been shown to exert inhibitory effect against GST-P positive focus development [42].

As far as the mechanisms of action of phenolic antioxidants, beside the direct scavenging and reducing action and the modulation of enzymes involved in the detoxification system also a mechanism involving signal transduction has been described [57]. The alteration of pathways that control apoptosis or cell-proliferation could explain the protective effect observed during the initiation phase of HA-induced carcinogenesis by several phenolic antioxidants.

3.2. Activity of food-extracts and whole food

In the last years, many studies have been performed to test the effective capability and the mechanism of action of rich-antioxidant food in preventing HA-induced mutagenicity/carcinogenicity. The attention for the use of whole food or food extracts is due to the need of increase our knowledge on the behaviour of antioxidants in conditions which can resemble as much as possible the reality.

3.2.1. Fruits and vegetables

Almost all studies present in the literature consistently showed a strong antimutagenic activity, detected in liver-enzyme mediated bacterial assays, exerted by fruits and vegetables against HA action. This effect was strictly correlated to the presence in these foods of vitamins and related compounds (ascorbic acid, β-carotene, retinal, retinoic acid, α-tocopherol etc.) having antioxidant properties [48].

3.2.1.1. Carotenoid-rich foods. Vitaglione and co-workers [58] have reported that carotenoids from tomatoes inhibit the formation of imidazoquinolines both in chemical model system, containing as precursors creatine, glucose and glycine, and in meat juice model system, based on freeze-dried bovine meat juice. In particular using carotenoid extract at a concentration of 1000 ppm, inhibitions of 36 and 11% of IQx and MeIQx formation respectively in the chemical system and of 13% of MeIQx and of 5% of 4,8-DiMeIQx in the meat juice model system was observed. Quercetin, the main tomato flavonoid, tested at 10 ppm in the meat juice model system, exerted an inhibition of HA formation of 67% [58].

N-hexane extracts of some carotenoid-rich fruits and vegetables such as apricots, oranges, Brussels sprouts, carrots, yellow-red peppers, and tomatoes were directly tested on histidine-deficient strains of *Salmonella typhimurium* [59]. This study demonstrated a reduced mutagenic activity of IQ probably due to carotenoids (α-, β-carotene, lycopene), xanthophylls (β-cryptoxanthin, lutein), and also carotenoid esters (oranges) contained in the tested extracts. In particular, 100 μg of orange extract reduced the bacterial mutagenicity of IQ by 27%. Previously solvent extracts from 13 fruit and 12 vegetable residues had been demonstrated to exhibit antimutagenic activity against IQ and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) in *S. typhimurium* TA 98 assay [60].

3.2.1.2. Anthocyanin-rich foods. Tart cherry tissue has been also demonstrated to exert inhibitory effect on HA formation in ground beef patties [61].

The potential beneficial effect of fruits has been recently confirmed by Miyata and co-workers [62] who showed that grapefruit juice suppressed PhIP-induced colon DNA damage in a concentration-dependent manner. The migration of DNA and frequency of tailed colon nuclei as indicators of DNA damage were measured. In particular, a 40% reduction of DNA damage was observed in F344 rats given 60 mg/kg of PhIP by gavage after pre-treatment with grapefruit juice for 5 days. This result was independent of PhIP absorption in the intestine because serum level of PhIP was comparable between grapefruit juice—pretreated and non pretreated rats.

Furthermore, it has been reported that genotoxic activity of PhIP was strongly reduced in a dose-related manner by blueberries, blackberries, red grapes, kiwi, watermelon, parsley, and spinach and that protection by beverages, fruits, and vegetables against genotoxicity of HAs might take place by enzyme inhibition [63,64].

The administration of 5% purple corn color, an anthocyanin extracted from the seeds of corn, in rats pre-treated with 1,2-dimethylhydrazine (DMH) and given PhIP in the diet, has been evaluated to reduce development of colorectal carcinogenesis [65]. Furthermore, cacao liquor proanthocyanidins showed to inhibit in vitro mutagenicity of PhIP, as well as rat pancreatic carcinogenesis in the initiation

stage, but not mammary carcinogenesis induced by PhIP [66].

3.2.1.3. Soy. The addition of soy protein concentrates (SPC) to beef patties also have shown to inhibit the mutagen formation in pan-fried beef patties during cooking. This effect was not related to protein alone but mainly to the phenolic antioxidant component in SPC such as chlorogenic acid [41]. More recent studies have not confirmed this finding. In fact Lan and Chen [67] found that soy sauce-marinated pork, eggs, and bean cakes show an increase of HAs. Moreover, adding soybean-oil during frying of fish fibre the same effect of enhancing HA formation was exerted [18].

3.2.1.4. Tea. Polyphenolic compounds from tea are effective inhibitors of HA formation in model systems [39,68]. Several studies report that tea polyphenols attenuate mutagenic activities of HAs by various mechanisms such as: inhibition of NADPH cytochrome P450 reductase [64,69–72], inhibition of mutagenic activity of *N*-hydroxylated HAs in the absence of S9 in vitro [69,73] and electrophile-scavenging [73]. The first mechanism has been recently recapitulated using specific isoforms of human cytochromes P450, including inhibition by epigallocatechin-3-gallate (EGCG) of human CYP1A2-mediated activation of PhIP in genetically engineered Salmonella strains [74]. Data showed that the protection towards HA action depended not only from quality and concentration of antioxidants but also from their relative levels in food and from the influence of other minor food constituents on their activity.

This correlation has been demonstrated in green- and black-tea extracts whose antimutagenic activity reduction corresponded with reduction in antioxidants and in particular with a decrease in concentration of three catechins (catechin, epigallocatechin gallate and epigallocatechin) [75]. Furthermore, an artificial tea produced by mixing nine of the major constituents found in green tea (including high level of EGCG and several other polyphenols) exhibited a smaller antimutagenic potency in Salmonella assays compared with the complete tea [76].

The bioavailability of potential antimutagenic compounds is another key-factor that has to be taken into account. Krul and co-workers [75] evaluated the influence of the food matrix on the bioavailability of tea antioxidants using an in vitro gastrointestinal model, simulating the conditions in the human digestive tract. In particular, they investigated whether black tea and green tea preserved their antimutagenic properties against HAs, alone or together with milk at different fat content, after passage through the in vitro model. The antimutagenic activity of the filtrates obtained introducing in the gastrointestinal model tea extracts alone, together with MeIQx and with whole or semi-skimmed or skimmed milk, was determined in the Ames test. The maximum inhibition of mutagenicity in Salmonella assay was observed with black tea filtrates, while adding together with the tea extract whole, semi-skimmed or skimmed milk, a reduction of the antimutagenic activity of 22, 42 and 78%, respectively, was observed. The authors hypothesized that these findings were due to the formation of milk protein-tea polyphenol complexes, resistant to gastric hydrolysis, with consequent reduction of absorbed antimutagenic compounds [75,77]. Furthermore, antimutagens of green-tea, such as catechins (90% of dry weight) and quercetin, bind stronger to milk proteins than the antimutagens of black-tea, thus explaining why the effect of green-tea was more affected by milk than that of black tea [75]. When tea and MeIQx were added together into a digestion model system, MeIQx mutagenicity was efficiently inhibited, green tea had a slightly stronger antimutagenic activity than black tea. Moreover, purified polyphenols from black tea extracts are more potent inhibitors of mutagenicity caused by PhIP in the *S. typhymurium* TA98 assay than the polyphenols from green tea extract [78]. Application of polyphenols extracted from both tea varieties to the surfaces of ground beef before cooking is able to inhibit the formation of mutagens in a dose-related fashion [79]. Furthermore, both theaflavins and theafulvins from black tea brews are able to prevent, at a dose-range 0.1–0.5 mg/ml, in a concentration-dependent manner, the DNA damage caused by heterocyclic amine 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2) [80].

The chemical mechanisms implicated in the inhibition by tea extracts of both HA-formation and HA-induced mutagenicity/carcinogenicity, have been amply studied.

Stavric and co-workers [72] reported that extracts of various teas (green, oolong, orange pekoe, decaffeinated orange pekoe) and herbal teas (chamomile, orange spice, linden flowers), were able to inhibit the Salmonella assay mutagenic activity of eight different HAs. The effect was strongly dependent from the concentration of tea extracts and it decreased at high concentrations. In particular, studies on the antimutagenic actions towards IQ of tea extracts (green, pouching, oolong and black tea) using a Salmonella/microsome assay showed that they were due to a combination of two distinctive mechanisms: inhibition of the cytochrome P450-mediated metabolism of IQ to its mutagenic metabolite form and interaction with IQ promutagens and metabolites in such a way to reduce its mutagenic potential [70].

Tea polyphenols could have also additional mechanisms of protection against HAs, such as the induction of UDP-glucuronosyl transferase (UDPGT), another enzyme involved in phase II metabolism of HAs [57,82,83]. This is the prevalent mechanism associated to the inhibitory activities of white, green, and black teas against IQ and PhIP-induced colonic aberrant crypt foci (ACF) and DNA adducts in the rat [84,85]. In fact rats fed with white or green teas extracts (1.5 or 2% w/v), in place of drinking water, before treatment with PhIP or IQ had reduced parent compounds and sulfamate in the urine. Accordingly, the concentration of ring glucuronides increased [84–86].

White tea is also able to inhibit PhIP-induced colonic ACF inducing glutathione-*S*-transferase (GST) [85] thus

assessing glutathione-dependent reduction of *N*-acetoxy-heterocyclic amines back to the parent compound [87].

On the contrary, liver S9 or microsomes from rats given aqueous extracts of green tea enhanced the mutagenic activity of 2-amino-6-methyl-pyrido[1,2-a:3′,2′-*d*]imidazole (Glu-P-1) and IQ in the Salmonella assay [71] through the induction of CYP1A and consequent enhanced formation of *N*-hydroxylated HAs, which were substrates for further activation by bacterial *O*-acetyltransferase and by enzyme S9 [36].

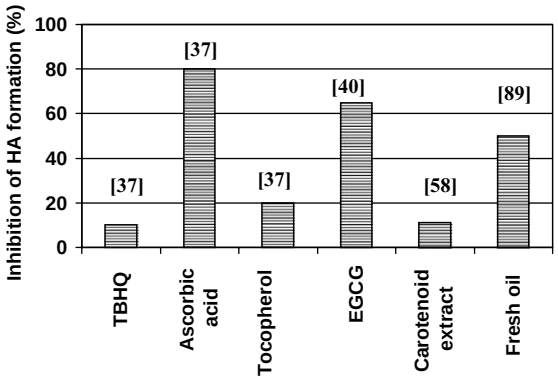
3.2.1.5. Olive oil. Monti and co-workers [88] reported that olive oil inhibited the formation of different IQ-type HA, in a chemical model system. The fresh made oil was more effective than the same oil after 1 year of storage thanks to the presence in the first of largest amount of dihydroxyphenylethanol derivatives. Their data supported the hypothesis that HA formation partially involved free-radical reactions, thus a reaction between phenolic compounds and key intermediates of HA formation could also contribute to reduce the levels of HAs.

Previously Johansson and co-workers [89] reported that using frying fats with an initial high antioxidant level lower amounts of MeIQx and DiMeIQx in the beef burgers and in the pan residues after frying were obtained.

3.2.1.6. Spices. Thyme, marjoram, rosemary and Monascus red extracts added, each at three different concentrations (4, 10, and 50 mg that is about 0.0005, 0.0025 and 0.00625%), in chemical model system, caused an increase of PhIP formation stronger for thyme, marjoram and Monascus red in comparison to rosemary flavour [90]. No correlation could be found between the antioxidative properties of the tested food additives, measured by the Rancimat system, and the formation of PhIP. In fact PhIP content increased independently to pro- or anti-oxidative properties of flavours. The extracts of thyme and Monascus red showed an antioxidative potential measured toward rapeseed oil oxidation while marjoram and rosemary showed pro-oxidative properties. On the contrary, some years before Murkovic and co-workers [91] found that the addition of spices including rosemary, thyme and sage to the ground beef, reduced the concentrations of HAs in fried beef. Balogh and co-workers [46] also showed that rosemary oleoresin added directly to the ground beef patties or to the surface of the patties before frying, at the concentration of 1–10% of the fat content, produced a reduction of PhIP formation after cooking of 44%.

4. Conclusions and future needs

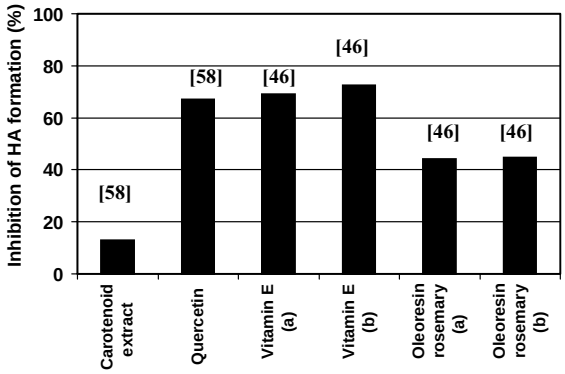
Data above reviewed show that various antioxidants are able to inhibit HA formation in chemical model systems and often to reduce the HA-induced mutagenicity/carcinogenicity in vitro and in vivo biological systems. Some of the most impressive results obtained in chemical systems and in cooked meat foods are summarised in Figs. 3 and 4, respectively. This reduction can be partially responsible for the reduction of the risk associated to HA-induced mutagenicity/carcinogenicity with a consequent beneficial



Antioxidant	Antioxidant amount	Chemical model system
TBHQ	1000 ppm	0,9 mmol creatinine, 0,9 mmol glycine, 0,45 mmol glucose and (10 g) corn oil in water (2,5 mL) 180˚Cx10 min
Ascorbic acid	1000 ppm	
Tocopherol	100 ppm (f.c.)*	
EGCG	100 mM	0,4 M creatinine, 0,4 M glycine, 0,2 M glucose in diethylenglycol-water (8:2 v/v) 120˚Cx2h
Carotenoid extract	1000 ppm	creatinine 0,4 5mmol, glycine 0,45 mmol, glucose 0,225 mmol in water-diethylenglycol (2:1 v/v) 180˚Cx30 min
Fresh oil	500 mg	creatinine 0,9 mmol, glycine 0,9 mmol, glucose 0,45 mmol in water-methanol (2:1 v/v) 180˚Cx30 min

* (f.c.) is for (fat content)

Fig. 3. Selection of data on the inhibition of HA formation in chemical model system. The number on the top of each bar represents the reference while the conditions of each experiment are summarized in the table below the histogram.



Antioxidant	Antioxidant amount	Meat system
Carotenoid extract	1000 ppm	freeze-dried bovine meat-juice:water (1:2 w/w) 180˚Cx30 min
Quercetin	10 ppm	
Vitamin E (a)	1% (f.c.)*	Antioxidants+1 mL corn oil added to the ground beef patties 2h before frying (10 min/side at 225˚C)
Vitamin E (b)	10% (f.c.)*	
Oleoresin rosemary (a)	1% (f.c.)*	
Oleoresin rosemary (b)	10% (f.c.)*	

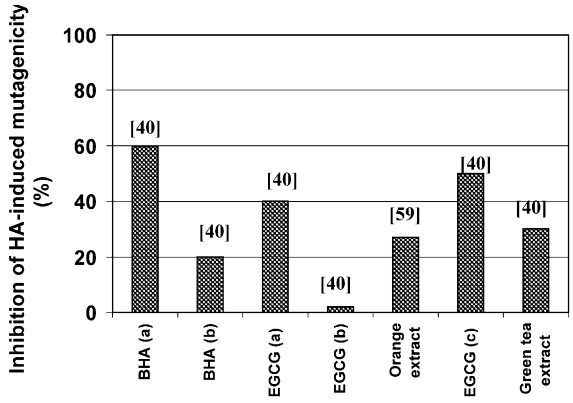
* (f.c.) is for (fat content)

Fig. 4. Selection of data on the inhibition of HA formation in meat-system. The number on the top of each bar represents the reference while the conditions of each experiment are summarised in the legend below the histogram.

effect on human health. In Fig. 5, some data about this effect have been resumed. Many findings summarised in this review should be carefully considered as they have been obtained in model systems. Further studies should prove if the observed results can be transferred to living organisms which are more complex than chemical or biological systems. Only the complete elucidation of the in vivo working mechanisms would suggest the most appropriate strategies to reduce HA-related risk for humans.

Several strategies for the prevention or reduction of HAs, and consequently exposure to these compounds, are simple and are a matter of changing cooking and eating habits, whereas in other cases more research is needed [24].

The experimental works carried out using food-extracts or whole-food as source of antioxidants provide easy-to-use tools to reduce the HA dietary intake. Pre-treatment with phenolic antioxidants derived from tea and olive oil results in a marked reduction of HA formation. Cooking meat and fish together with foodstuffs containing phenolic antioxidants may be useful to lessen the levels of carcinogenic HAs produced [8]. The same effect may be obtained cooking meat with tomatoes, carrots or other vegetables rich of



Antioxidant	Antioxidant amount	Experimental model
BHA (a)	20 mM	creatinine 2 mmol, glycine 2 mmol, glucose 1 mmol and antioxidants in water 200°Cx30 min
BHA (b)	100 mM	
EGCG (a)	20 mM	
EGCG (b)	100 mM	
Orange extract	100 μg	directly added to the plate in Ames Test
EGCG (c)	0,5%	bonito-meat and antioxidants boiled and then heated-and-dried at 100°Cx24/48h
Green tea extract	5%	

Fig. 5. Selection of data on the inhibition of HA-induced mutagenicity in Salmonella assay. The number on the top of each bar represents the reference, while the experimental model adopted to prepare the compound to test in Salmonella assay (Ames Test) are summarised in the table below the histogram.

carotenoids and antioxidant vitamins able to exert inhibition of HA formation [92,93]. In particular, Mediterranean way of cooking can be regarded as particularly suitable, as it is based on the use of sauces and spices rich in antioxidants.

The formation of HAs should also to be taken into account when optimising the conditions for various food processes and food processing equipment, in order to increase both quality and safety. Food industry have to handle carefully the current knowledge about HAs considering the large amount of meat-based products offered on the market. Meat processing industry could evaluate the effective possibility to use spices, not only as flavouring or food preservative but also as protective agents against HA formation during technological processes, and domestic cooking. This approach could be well accepted by consumers that are against synthetic food additives and which usually respond favourably to the concept of food regarded as natural.

References

[1] R.H. Adamson, U.P. Thorgeirsson, E.G. Snyderwine, S.S. Thorgeirsson, J. Reeves, D.W. Dalgard, S. Takayama, T. Sugimura, Jpn. J. Cancer Res. 81 (1990) 10.
[2] H. Ohgaki, S. Takayama, T. Sugimura, Mutat. Res. 259 (1991) 399.
[3] H. Ushiyama, K. Wakabayashi, M. Hirose, H. Hitoh, T. Sugimura, M. Nagao, Carcinogenesis 12 (1991) 1417.
[4] T. Sugimura, Science 258 (1992) 603.
[5] K. Wakabayashi, M. Nagao, H. Esumi, T. Sugimura, Cancer Res. 52 (Suppl.) (1992) 2092.
[6] B. Stavric, Food Chem. Toxicol. 32 (1994) 977.
[7] D.W. Layton, K.T. Bogen, M.G. Knize, F.T. Hatch, V.M. Johnson, J.S. Felton, Carcinogenesis 16 (1995) 39.
[8] A. Oguri, M. Suda, Y. Totsuka, T. Sugimura, K. Wakabayashi, Mutat. Res. 402 (1998) 237.
[9] Y. Totsuka, K. Fukutome, M. Takahashi, S. Takahashi, A. Tada, T. Sugimura, K. Wakabayashi, Carcinogenesis 17 (1996) 1029.
[10] K. Skog, Food Chem. Toxicol. 40 (2002) 1197.
[11] M.H. Schiffman, J.S. Felton, Am. J. Epidemiol. 131 (1990) 376.
[12] G. Steineck, U. Hagman, M. Gerhardsson, S.E. Norell, Int. J. Cancer 45 (1990) 1006.
[13] W. C. Willett, M. J. Stampfer, G. A. Colditz, B. A. Rosner, F. E. Speizer, New England J. Med., (1990) 1664.
[14] M. Gerhardsson de Verdier, U. Hagman, R.K. Peters, G. Steineck, E. Overvik, Int. J. Cancer 49 (1991) 520.
[15] M. Gerhardsson de Verdier, in: H. A. Adamson, J. Gustafsson, N. Ito, M. Nagao, T. Sugimura, K. Wakabayashi, Y. Yamazoe (Eds.), Heterocyclic Amines in Cooked Foods: Possible Human Carcinogens, Princeton Scientific Publishing, Princeton, NJ, 1995, p. 92.
[16] S.E. Norell, A. Ahlbom, R. Erwald, G. Jacobson, I. Lindberg-Navier, R. Olin, B. Tornberg, K.L. Wiekel, Am. J. Epidemiol. 124 (1986) 894.
[17] T. Sugimura, Mutat. Res. 376 (1997) 211.
[18] C.Y. Tai, K.H. Lee, B.H. Chen, Food Chem. 75 (2001) 309.
[19] K. Augustsson, G. Steineck, in: M. Nagao, T. Sugimura (Eds.) Food Borne Carcinogens: Heterocyclic Amines, Wiley, Chichester, West Sussex, 2000, p. 332.
[20] M. Nagao, Mutat. Res. 431 (1999) 3.
[21] T. Sugimura, Carcinogenesis 21 (2000) 387.
[22] J. S. Felton, M. Jägerstad, M. G. Knize, K. Skog, K. Wakabayashi, in: M. Nagao, T. Sugimura (Eds.), Food Borne Carcinogens: Heterocyclic Amines, Wiley, Chichester, West Sussex, 2000, p. 31.
[23] M. Jägerstad, K. Skog, P. Arvidsson, A. Solyakov, Z. Lebensm, Unters. Forsch. 207 (1998) 419.
[24] K. Skog, M.A. Johansson, M. Jägerstad, Food Chem. Toxicol. 36 (1998) 879.
[25] A.M. Pearson, C. Chen, J.I. Gray, S.D. Aust, Free Radical Biol. Med. 13 (1992) 161.
[26] M. Jägerstad, K. Olsson, S. Grivas, C. Negishi, K. Wakabayashi, M. Tsuda, S. Sato, T. Sugimura, Mutat. Res. 126 (1984) 239.
[27] M. Jägerstad, K. Skog, S. Grivas, K. Olsson, Mutat. Res. 259 (1991) 219.
[28] M. Jägerstad, A. Laser-Reutersward, R. Öste, A. Dahlqvist, K. Olsson, S. Grivas, T. Nyhammar, in: G. Waller, M. Feather (Eds.), The Maillard reaction in Foods and Nutrition, American Chemical Society, Washington, DC, 1983, p. 507.
[29] C. Negishi, K. Wakabayashi, M. Tsuda, S. Sato, T. Sugimura, H. Saito, M. Maeda, M. Jägerstad, Mutat. Res. 140 (1984) 55.
[30] M. Shioya, K. Wakabayashi, S. Sato, M. Nagao, T. Sugimura, Mutat. Res. 191 (1987) 133.
[31] J.S. Felton, M.G. Knize, Mutat. Res. 259 (1991) 205.
[32] J. S. Felton, M. G. Knize, in: H. Hayatsu (Ed.), Mutagens in Food: Detection and Prevention, CRC Press, Boston, 1991, p. 57.
[33] J.S. Felton, M.A. Malfatti, M.G. Knize, C.P. Salmon, E.C. Hopmans, R. Wu, Mutat. Res. 376 (1997) 37.
[34] P. Arvidsson, M.A.J.S. van Boekel, K. Skog, M. Jägerstad, J. Food Sci. 62 (1997) 911.
[35] G. Bailey, D. Williams, Food Technol. 47 (1993) 105.
[36] R.H. Dashwood, Mutat. Res. 511 (2002) 89.
[37] M. Johansson, M. Jägerstad, Food Chem. 56 (1996) 69.
[38] C. Chen, Ph.D. Thesis, Michigan State University, East Lansing, Michigan, 1988.
[39] J.H. Weisburger, M. Nagao, K. Wakabayashi, A. Oguri, Cancer Lett. 83 (1994) 143.
[40] T. Kato, T. Harashima, N. Moriya, K. Kikugawa, K. Hiramoto, Carcinogenesis 17 (1996) 2469.
[41] Y.Y. Wang, L.L. Vuolo, N.E. Spingarn, J.H. Weisburger, Cancer Lett. 16 (1982) 179.
[42] M. Hirose, S. Takahashi, K. Ogawa, M. Futakuchi, T. Shirai, Food Chem. Toxicol. 37 (1999) 985.
[43] M. Hirose, T. Ito, S. Takahashi, M. Ozaki, T. Ogiso, Y. Nihro, T. Miki, T. Shirai, Eur. J. Cancer Prev. 7 (1998) 233.
[44] N. Ito, M. Hirose, M. Futakuchi, S. Tamano, T. Shirai, in: A.Y. Conney, N. Ito, T. Sugimura, M. Terada, K. Wakabayashi, I.B. Weinstein (Eds.). Fundamentals of Cancer Prevention, Princess Takamatsu Cancer Research Fund, Tokyo, Japan, 1997, p. 48.
[45] K. Kikugawa, K. Hiramoto, T. Kato, Biofactors 12 (2000) 123.
[46] Z. Balogh, J.I. Gray, E. Gomaa, A.M. Booren, Food Chem. Toxicol. 38 (2000) 395.
[47] N. Uehara, Y. Iwahori, M. Asamoto, H. Baba-Toriyama, M. Iigo, M. Ochiai, M. Nagao, M. Nakayama, M. Degawa, K. Matsumoto, I. Hirono, H. Beppu, K. Fujita, H. Tsuda, Jpn. J. Cancer Res. 87 (1996) 342.
[48] R. Edenharder, A. Worf-Wandelburg, M. Decker, K.L. Platt, Mutat. Res. 444 (1999) 235.
[49] E.G. Snyderwine, P.J. Wirth, P. Roller, R.H. Adamson, S. Sato, S.S. Thorgeirsson, Carcinogenesis 9 (1988) 411.
[50] C. Liew, H.A.J. Schut, S.F. Chin, M.W. Pariza, R.H. Dashwood, Carcinogenesis 16 (1995) 3037.
[51] H. Lee, J. Chyr-Yir, S.-J. Tsai, Food Chem. 45 (1992) 235.
[52] R. Edenharder, R. Rauscher, K.L. Platt, Mutat. Res. 379 (1997) 21.
[53] F.T. Hatch, F.C. Lightstone, M.E. Colvin, Environ. Mol. Mutagen 35 (2000) 279.
[54] B.L. Milic, S.M. Djilas, J.M. Canadanovic-Brunet, Food Chem. 46 (1993) 273.

[55] M. Namiki, T. Hayashi, in: C. Eriksson (Ed.), Progress in Food and Nutrition Science, Pergamon Press, Oxford, 1981, p. 31.
[56] M. Namiki, T. Hayashi, in: G.R. Waller, M.S. Feather (Eds.), Proceedings of the American Chemical Society Symposium on The Maillard Reaction in Foods and Nutrition, American Chemical Society, Washington, DC, 1983, p. 21.
[57] G.D. Stoner, M.A. Morse, G.J. Keloff, Environ. Health Perspect. 105 (1997) 945.
[58] P. Vitaglione, S.M. Monti, P. Ambrosino, K. Skog, V. Fogliano, Eur. Food Res. Technol. 215 (2002) 108.
[59] R. Rauscher, R. Edenharder, K.L. Platt, Mutat. Res. 413 (1998) 129.
[60] R. Edenharder, C. Leopold, M. Kries, Mutat. Res. 341 (1995) 303.
[61] C. Britt, E.A. Gomaa, J.I. Gray, A.M. Booren, J. Agric. Food Chem. 46 (1998) 4891.
[62] M. Miyata, H. Takano, K. Takahashi, Y.F. Sasaki, Y. Yamazoe, Cancer Lett. 183 (2002) 17.
[63] R. Edenharder, J.W. Sager, H. Glatt, E. Muckel, K.L. Platt, Mutat. Res. 521 (2002) 57.
[64] N. Hasaniya, K. Youn, M. Xu, J. Hernaez, R. Dashwood, Cancer Res. 88 (1997) 553.
[65] A. Hagiwara, K. Miyashita, T. Nakanishi, M. Sano, S. Tamano, T. Kadota, T. Koda, M. Nakamura, K. Imaida, N. Ito, T. Shirai, Cancer Lett. 171 (2001) 17.
[66] M. Yamagishi, M. Natsume, N. Osakabe, H. Nakamura, F. Furukawa, T. Imazawa, A. Nishikawa, M. Hirose, Cancer Lett. 185 (2002) 123.
[67] C.M. Lan, B.H. Chen, Food Chem. Toxicol. 40 (2002) 989.
[68] G.C. Yen, H.Y. Chen, J. Agric. Food Chem. 43 (1995) 27.
[69] H. Hayatsu, N. Inada, T. Kakutani, S. arimoto, T. Negishi, K. Mori, T. Okuda, I. Sakata, Prev. Med. 21 (1992) 370.
[70] A. Bu-Abbas, M.N. Clifford, R. Walker, C. Ioannides, Mutagenesis 9 (1994) 325.
[71] A. Bu-Abbas, M.N. Clifford, R. Walker, C. Ioannides, Carcinogenesis 15 (1994) 2575.
[72] B. Stavric, T.I. Matula, R. Klassen, R.H. Downie, Food Chem. Toxicol. 34 (1996) 515.
[73] M. Hernaez, M. Xu, R.H. Dashwood, Environ. Mol. Mutagen. 30 (1997) 468.
[74] S. Muto, K. Fujita, Y. Yamazaki, T. Tamataki, Mutat. Res. 479 (2001) 197.
[75] C. Krul, A. Luiten-Schuite, A. Tenfelde, B. van Ommen, H. Verhagen, R. Havenaar, Mutat. Res. 474 (2001) 71.
[76] G. Santana-Rios, G.A. Orner, A. Amantana, C. Provost, S.-Y. Wu, R.H. Dashwood, Mutat. Res. 495 (2001) 61.
[77] P.J. Brown, W.B. Wright, J. Chromatogr. 11 (1963) 504.
[78] Z. Apostolides, D.A. Balentine, M.E. Harbowy, J.H. Weisburger, Mutat. Res. 359 (1996) 159.
[79] J.H. Weisburger, E. Veliath, E. Larios, B. Pittman, E. Zang, Y. Hara, Mutat. Res. 516 (2002) 19.
[80] A. Dhawan, D. Anderson, S. de Pascual-Teresa, C. Santos-Buelga, M.N. Clifford, C. Ioannides, Mutat. Res. 515 (2002) 39.
[81] H.-Y. Chen, G.-C. Yen, Mutat. Res. 393 (1997) 115.
[82] A. Bu-Abbas, M.N. Clifford, C. Ioannides, R. Walker, Food Chem. Toxicol. 33 (1995) 27.
[83] C.W. Embola, O.S. Sohn, E.S. Fiala, J.H. Weisburger, Food Chem. Toxicol. 40 (2002) 841.
[84] M. Xu, A.C. Bailey, J.F. Hernaez, C.R. Taoka, H.A. Schut, R.H. Dashwood, Carcinogenesis 17 (1996) 1429.
[85] G. Santana-Rios, G.A. Orner, M. Xu, M. Izquierdo-Pulido, R.H. Dashwood, Nutr. Cancer 41 (2001) 98.
[86] C.W. Embola, M.C. Weisburger, J.H. Weisburger, Food Chem. Toxicol. 39 (2001) 629.
[87] T.W. Kensler, Environ. Health Perspect. 105 (1997) 965.
[88] S.M. Monti, P. Ambrosino, K. Skog, V. Fogliano, J. Agric. Food Chem. 49 (2001) 3969.
[89] M. Johansson, L. Fredholm, I. Bierne, M. Jägerstad, Food Chem. Toxicol. 33 (1995) 993.
[90] S. Zöchling, M. Murkovic, Food Chem. 79 (2002) 125.
[91] M. Murkovic, D. Steinberger, W. Pfannhauser, Food Res. Technol. 207 (1998) 477.
[92] J.P. Brown, Mutat. Res. 75 (1980) 243.
[93] E. Wollenweber, V.H. Dietz, Phytochemistry 20 (1981) 869.