Review

Recent Development of 3-Deoxyosone Related Maillard Reaction Products

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Maillard reaction occurs extensively in food systems and *in vivo*. In an intermediate Maillard reaction of proteins with glucose, 3-deoxyglucosone (3DG) was generated from Amadori compounds in an early stage, leading to generation of advanced glycation endproducts (AGEs). 3DG modified lysine residues to form pyrrole aldehydes (lysyl-pyrroline), and arginine residues to form imidazolone compounds, and is speculated to be a cross-linker responsible for the polymerization of proteins. 3DG is also thought to be related to the evolution of fluorescence during Maillard reaction. The fluorescent compound has been identified as lysyl-pyrrolypyridine which is formed by the loss of five molecules of water from the reaction between 2 molecules of lysine residues and 2 molecules of 3DG. In the protein-glucose and pentose reaction systems, crossline and pentosidine have reportedly been formed as fluorescent and crosslinking compound, respectively, as well as pyrrolypyridine. Immunochemical and chemical methods have clearly indicated the progressive accumulation of AGEs in tissue proteins in aging. In diabetes, AGE accumulation in general is accelerated and linked to arteriosclerosis, nephropathy, neuropathy, retinopathy, and cataract. 3DG, which has weaker mutagenicity, reacted readily with 2'-deoxyguanosine in nucleosides. Two major products (G-A and G-B) were isolated, and G-A was identified as *N*-(1-oxo-2,4,5,6-hydroxyhexyl)-2'-deoxyguanosine. G-B was identified as a diastereomer of G-A. Blue pigment was isolated from the reaction between D-xylose and glycine. Blue pigment which was designated Blue-M1 (blue Maillard reaction intermediate-1) was identified as novel pyrrolopyrrolylium compound and is assumed to be a dimer of yellow colored pyrrolopyrrole-2-carboxaldehyde compounds. Blue-M1 that reacts readily to yellow compounds has a polymerizing activity, suggesting that it is an important Maillard reaction intermediate through the formation of melanoidins. Melanoidins have many positive physiologic effects.

Keywords: Maillard reaction, advanced glycation endproducts, 3-deoxyglucosone, glycation, pyrrolypyridine, imidazolone, pyrroline, melanoidin

Typical nonenzymatic reaction occurring among food components is caused by amino and carbonyl compounds, called amino-carbonyl, carbonyl-amine, non-enzymatic browning reactions and so on. Maillard reaction originally meant the reaction between amino compounds and reducing sugars but has recently been used as a synonym for amino-carbonyl reaction. Maillard reaction, non-enzymatic glycosylation relevant to proteins is called glycation.

Amino compounds involved in the Maillard reaction in food and biological systems are amines, amino acids, peptides, proteins, base in nucleic acids, phospholipids and so on. Reducing sugars, aldehydes, ketones, polyphenols, ascorbic acid, steroids and so on are involved in this reaction as carbonyl sources.

Various phenomena and physiologic effects are caused by the Maillard reaction (Hayase & Kato, 1994; Hayase, 1996; Friedman, 1996). In food systems, low-molecular weight products contribute to discoloration, the generation of aroma, formation of mutagens, scavenging and generation of active oxygen species, the decrease in nutritional value and digestibility. High-molecular weight products, so-called melanoidins, are involved in discoloration, antioxidative effects, scavenging on active oxygen species, antimicrobial action, desmutagenic effects and dietary fiber-like effects.

Thus, the Maillard reaction in food systems has both desirable

and undesirable effects. We should control its effect in processing and storage of foods.

Most proteins *in vivo* are nonenzymatically glycated. Reducing sugars can react with amino compounds to form a Schiff base adduct which is then stabilized by an Amadori rearrangement. This reaction represents the early stage of the Maillard reaction. The reaction is followed by the intermediate stage on the generation of α -dicarbonyls such as 3-deoxyosones, osones, methylglyoxal, and glyoxal. Furthermore, in the advanced stage of Maillard reaction, proteins are modified by advanced glycation endproducts (AGEs) with the evolution of fluorescence, browning, polymerization and degradation.

In biological systems, Maillard reaction is believed to contribute to yellowing and cross-linking of long-lived proteins such as lens crystallins, and collagen and to an array of biochemical and biological abnormalities in diabetes and aging such as stiffening of arteries and joints, increased blood pressure, and altered protein and cell turnover (Ledl & Schleicher, 1990; Monnier *et al.*, 1991; Cerami, 1994). Such general AGE accumulation is accelerated and linked to arteriosclerosis, nephropathy, neuropathy, retinopathy, and cataract.

Recent progress on 3-deoxyosone related AGEs in the food system and *in vivo* is reviewed in this paper.

1. Generation of 3-deoxyosones in Maillard reaction

Lysozyme used as a model protein was reacted with reducing

sugars in a powder state at 50°C and 75% relative humidity (RH) (Cho *et al.*, 1986; Kato *et al.*, 1987a) under a physiologic condition of 37°C at pH 7.4 (Shin *et al.*, 1988). The results by Kato's group in the powder state revealed that the modification of amino group with glucose is an initial step and that arginine or tryptophan residues are secondarily attacked by α -dicarbonyls formed from a medium stage of glycation. These reactive α -dicarbonyls are thought to be liberated from lysine residues, N-terminal amino groups via the enolization of Amadori compounds, or by glucose oxidation (Wells-Knecht *et al.*, 1995). In a lysozyme-glucose or fructose system under physiologic conditions, 3-deoxyglucosone (3DG) was formed as the major carbonyl compound (Shin *et al.*, 1988). In addition, 3-deoxyxylosone is also formed from the lysozyme-reducing sugar reaction systems (Arai *et al.*, 2000). Thus, 3-deoxyosones are exceedingly important on AGEs formation. 3DG has been reported to be generated *in vivo* (Hayase *et al.*, 1991; Knecht *et al.*, 1992; Niwa *et al.*, 1993; Yamada *et al.*, 1994) and from fructose 3-phosphate in the lens of diabetic rats (Szwergold *et al.*, 1990). Recently, Niwa (1999) reviewed metabolism, analysis, biological activity, and clinical implications of 3-deoxyglucosone.

As are other α -dicarbonyls, Ledl and Schleicher (1990) reported that 1-deoxyglucosone (1DG) and 4-deoxyglucosone (4DG) may also be formed during Maillard reaction. They indicate that the 2,3-enolization of an Amadori compound could also give rise to a 1,4-dideoxy-2,3-dicarbonyl derivative that remains attached to the amino group. In addition, glyoxal also has been

generated by glucose oxidation or the Namiki-pathway (Namiki, 1988). Methylglyoxal might be formed from Amadori compounds via 1-deoxyosone by oxidative and non-oxidative routes (Arai *et al.*, 2000). Figures 1 and 2 show α -dicarbonyls formed in glycated proteins during oxidative and non-oxidative Maillard reactions, respectively.

Our recent research revealed that the formation of 3DG was more significantly increased under anaerobic, than under aerobic conditions (Hayase *et al.*, 1996). Moreover, browning, the generation of fluorescence, and polymerization were also accelerated more under anaerobic than aerobic conditions.

2. Advanced glycation endproducts

2-1. Pyrraline

3DG is dehydrated to form 5-hydroxymethylfurfural (HMF), especially in acidic foods (Hodge, 1953), and *N*-substituted pyrrole-2-aldehydes called pyrraline are formed from 3DG and amino compounds in most foods at weakly acidic to neutral pH (Hayase & Kato, 1985; Hayase & Takahashi, 1996). ϵ -(2-formyl-5-hydroxymethyl-pyrrol-1-yl)-L-norleucine (lysyl-pyrraline) was detected in browning foods since ϵ -amino group has a high reactivity to 3DG (Nakayama *et al.*, 1980; Chiang, 1988).

The authors detected protein-bound pyrraline in serum albumin by immunological methods (Hayase *et al.*, 1989a). The amount of the pyrraline in diabetic serum albumin increased 1.6-fold compared with that in control subjects. Highly specific pyrraline immunoreactivity was detected in sclerosed glomeruli from diabetic and old normal kidneys as well as in renal arteries with arteriosclerosis (Miyata & Monnier, 1992). The preferential

Fig. 1. Dicarbonyls formed by oxidative Maillard reaction.

Fig. 2. Dicarbonyls formed by non-oxidative Maillard reaction.

Fig. 3. Proposed mechanism of formation of protein-bound lysyl-pyrraline.

localization of pyrraline immunoreactivity in the extracellular matrix strengthens the notion that the advanced glycation may contribute to decreased turnover and thickening of the extracellular matrix in diabetes and aging (Miyata & Monnier, 1992).

To investigate the mechanism of the formation of protein-bound pyrraline, bovine serum albumin (BSA) was incubated with 50 mM glycated lysine and 3DG. At pH 7.4, similar kinetics were noted for both precursors, glycated lysine and 3DG, with

approximately a 10-day delay until a sharp increase of pyrraline occurred. This suggests that pyrraline formation from 3DG is the rate-limiting step in the reaction of BSA with glycated lysine. The ability of both glycated lysine and 3DG to form protein-bound pyrraline suggests a synthetic pathway whereby glycated lysine is first generated from glucose and subsequently degraded into 3DG. 3DG then reacts again with free amino groups to form pyrraline (Hayase *et al.*, 1989a) as shown in Fig. 3. Protein-bound pyrraline has also been detected in human plasma proteins (Portero-Otin *et al.*, 1995), and in food proteins (Henle & Klostermeyer, 1993; Resmini & Pellegrino, 1994).

2-2. Fluorescent compounds

In the advanced stage of Maillard reaction *in vitro*, fluorescent compound (Peak L1) was detected in protein hydrolysates of BSA with glucose (Kato *et al.*, 1989). The height of Peak L1 increased with glucose concentration and incubation time.

From the results of *in vivo* experiments with rats, the level of Peak L1 increased with aging to reach maximum at 90 weeks of age and remained at this level until 120 weeks of age. This peak is useful as an indicator for evaluating the progress of the advanced stage reaction. Figure 4 shows the high-performance liquid chromatography (HPLC) pattern of the hydrolysate of lysozyme incubated with 3DG. Peak L1 was also found in the reaction system and increased with incubation time.

We have examined the detection of fluorescent compounds

Fig. 5. HPLC patterns of reaction mixtures obtained from various Maillard reaction systems with n-butylamine at pH 7.4 and 50°C for 48 h.

Fig. 6. Structures of FL-A and FL-B (butyl-crossline), and FL-C (butyl-pyrropropyridine). *: asymmetric carbon.

Fig. 4. HPLC of Peak L1 (arrows) in the hydrolysate of lysozyme (10mg/ml) incubated with 3-deoxyglucosone (0 and 16.5mM) in phosphate buffer (pH 7.4) at 37°C for 28 days.

formed by the reaction of lysine residues with glucose (Hayase *et al.*, 1994). The results of HPLC analysis showed that the major three fluorescent compounds (FL-A, FL-B, FL-C) were produced in a butylamine-glucose reaction system under physiological conditions of 37°C at pH 7.4 as shown in Fig. 5. FL-A and FL-B were identified as epimers of butyl-crossline, similar to compounds reported by Nakamura *et al.* (1992). FL-C was purified from the butylamine-3DG reaction system and identified as a pyrrolopyridinium compound called butyl-pyrropyridine (Hayase *et al.*, 1994) as shown in Fig. 6. Lysyl-pyrropyridine was also identified by acid hydrolysis of *N*^ε-acetyllysyl-pyrropyridine which was obtained from the reaction between *N*^ε-acetyllysine and 3DG. The lysyl-pyrropyridine (Fig. 7) is formed by the loss of five molecules of water from the reaction between 2 molecules of lysine residues and 2 molecules of 3DG. The recovery of lysyl-pyrropyridine was 26.7% after acid hydrolysis. However, the lysyl-crossline appears to have decomposed in this process (Hayase *et al.*, 1998); consequently, the findings suggest that Peak L1 may be lysyl-pyrropyridine.

Fig. 7. The formation of lysyl-pyrropyridine from 3-deoxyglucosone and L-lysine reaction system.

Fig. 8. Pattern of unknown peaks in the amino acid chromatogram of acid hydrolysate of lysozyme incubated with 3-deoxyglucosone in 0.1M sodium phosphate buffer (pH 7.4) at 50°C for 4 days.

Monnier's group identified the fluorescent compound, which was formed from pentose, arginine, and lysine, as pentosidine having an imidazorium ring (Sell & Monnier, 1989). They mentioned that the compound was extensively distributed *in vivo* and increased with aging and diabetes, especially uremia (Monnier *et al.*, 1992). Pentosidine can be formed from several carbonyl compounds such as hexoses and ascorbic acid under oxidative conditions (Grandhee & Monnier, 1991; Dyer *et al.*, 1991), and *in vivo* studies suggest that a common intermediate involving a pentose is a necessary precursor molecule. Pentosidine was also formed by 3DG-lysozyme reaction process (Hayase *et al.*, 1998).

Pentosidine has fluorescence at Ex=335 nm and Em=385 nm, pyrropyridine at Ex=370 nm and Em=455 nm, and crossline at Ex=379 nm and Em=463 nm, respectively. Accordingly, fluorescence (Ex=370 nm, Em=440 nm) observed in collagen *in vivo* which increases with aging and diabetes, is considered to depend on pyrropyridine and crossline but not pentosidine.

Recently lysyl-pyrropyridine was detected in the acid hydrolysates of normal aged, cataractous, diabetic and brunescant human lens proteins as well as in the acid hydrolyzed glycated hemoglobin A₀ (Prabhakaram & Mossine, 1998). They reported that a

Fig. 9. Proposed formation pathway for products from the reaction between *N*^ε-benzoylarginine amide and 3-deoxyglucosone. R1=(CH₂)₃CH(NHCOC₆H₅)CONH₂, R2=CH₂(CHOH)₂CH₂OH.

2.5-fold excess of pyrrolydine was observed in 60 year normal lens over 40 year normal lens. The pyrrolydine content was similar (~16 nmols/mg) in age-matched (60 years) normal, cataractous, and diabetic lens, but almost double (30.2 nmol/mg) in 60 year brunescens lens.

2-3. Arginine adducts modified with 3-deoxyglucosone

As mentioned above, the polymerization of proteins occurred in the presence of reducing sugars under a physiologic condition as well as under conditions of food processing, storage, and cooking. In order to elucidate the cross-linking compounds of proteins by Maillard reaction, glycosylated lysozyme was hydrolyzed with 6 M HCl in evacuated tubes at 110°C for 24 h. Impaired amino acid residues were lysine, arginine, and tryptophan, as described above. Unknown peaks on an amino acid chromatogram of acid hydrolysates of proteins were detected in the glucose-lysozyme reaction systems and acetylated lysozyme incubated with glucose and free lysine (Cho *et al.*, 1986; Kato *et al.*, 1987a). These peaks were identical to those detected in the lysozyme-3DG reaction system as well as human serum albumin, BSA, ovalbumin, RNase A, and IgG-3DG reaction systems, respectively (Kato *et al.*, 1987b). These results indicate that the unknown peaks originated from the impaired Arg and/or Trp residues but not from Lys residues. We then compared the amino acid chromatograms of acid hydrolysates of proteins in the lysozyme-3DG system with those on the *N*^α-benzoylarginine amide-3DG reaction system shown by arrow in Fig. 8 (Konishi *et al.*, 1994). Findings suggested that 3DG modified arginine residues and generated unknown peaks by acid hydrolysis of lysozyme incubated with glucose.

Seventeen compounds were observed on a HPLC chart obtained from the *N*^α-benzoylarginine amide-3DG reaction system, and are called S1 to S17. These compounds were collected and subjected to fast atom bombardment-mass spectrometry (FAB-MS). FAB-MS data showed that major compounds were formed from one molecule of *N*^α-benzoylarginine amide and one (for S6, S7, S10, S12, and S17) or two (for S11) molecules of 3DG. The proposed formation scheme for these products from *N*^α-benzoylarginine amide-3DG reaction system is indicated in Fig. 9 (Hayase *et al.*, 1997; Hayase *et al.*, submitted). S17, imidazolone compound, is formed via S12 by dehydration and oxidation from the above reaction system to be the major pathway. In this review, the structure of S17 reported by Hayase *et al.* (1997) is corrected as shown in Fig. 9 as well as the structure of S6, S7, and S10. A novel spiro compound, S17, was formed by intramolecular cyclization of dehydrogenated S12. S12, imidazoline-4-one compound, was also identified in a 3DG-insulin reaction system by ESI-MS (Hayase *et al.*, 1998).

S11 was identified as 2-(*N*^α-benzoyl-*N*^δ-ornithylamide)-5,6a-di(2,3,4-trihydroxybutyl)-5,6-dihydroxydehydrofuro[2,3-*d*]imidazole.

dazole. Since S11 which is formed from one molecule of *N*^α-benzoylarginine amide and two molecules of 3DG, increased and then decreased with reaction time, it is speculated that S11 participated in further reactions such as the formation of cross-links.

Niwa *et al.* (1997a) reported that amyloid β₂-microglobulin is modified with imidazoline-4-one (S12) in human dialysis-related amyloidosis by immunohistochemistry using anti-S12 monoclonal antibody. S12 is also detected in kidneys and aortas of diabetic patients (Niwa *et al.*, 1997b) and at high levels in kidneys of rats with streptozotocin-induced diabetes (Niwa *et al.*, 1997c).

2-4. AGEs in foods

The Maillard reaction products Amadori compounds, α-dicarbonyls, furans, pyrrolines, and melanoidins are contained in roasted foods such as meats and fish, browned confectionery: toast, crackers, and potato chips, and luxury food and seasonings: muscovado, cane sugar, coffee, beer and tomato paste (Hayase & Kato, 1994). Amadori compounds and AGEs as pyrrolines and melanoidin-like brown pigments could be partly bound to food proteins. We found also pyrroline and imidazolone by immunochemical detection, and lysyl-pyrrolydine and pentosidine by HPLC after acid hydrolysis in soy sauce, respectively. Table 1 shows the amounts of these AGEs in soy sauce (Hayase *et al.*, submitted).

2-5. Modification of 2'-deoxyguanosine with 3-deoxyglucosone

α-Dicarbonyls have been reported to be cytotoxic compounds (Shinoda *et al.*, 1994). Our studies have shown that 3DG has inhibitory effects on the progression during the S phase in 3Y1 cells. Együd and Szent-Györgyi (1966) and Szent-Györgyi *et al.* (1967) demonstrated that methylglyoxal (MG) could efficiently inhibit cell growth. *N*²-(1-carboxyethyl)guanine was identified as a guanine AGE by the nucleophilic addition of the primary amino group of guanine to the ketone group of MG followed by an intramolecular rearrangement (Papoulis *et al.*, 1995).

We have investigated the mutagenicity and reactivity with nucleosides of 3DG (Hayase & Kaneko, 1998). 3DG has weaker mutagenicity than methylglyoxal by the Ames test; it reacted readily with 2'-deoxyguanosine (dG) in nucleosides. Two major products (G-A and G-B) were isolated and purified from the reaction mixture of 50 mM 3DG and 50 mM dG at 50°C and pH 7.4 for 6 days. G-A was identified as *N*-(1-oxo-2,4,5,6-hydroxy-

Table 1. Amounts of pyrroline, imidazolone, lysyl-pyrrolydine, and pentosidine in soy sauce (Koikuchi).

Soy sauce sample	AGE	Amount (nmol/g soy sauce powder)
Total soy sauce	Pyrroline	3.6
Total soy sauce	Imidazolone	53.8
Nondialyzable fraction ^{a)}	Lysyl-pyrrolydine	2.1
Nondialyzable fraction ^{a)}	Pentosidine	0.7

^{a)}cut molecular weight=500

Fig. 10. Structures of G-A and G-B. *: asymmetric carbon.

hexyl)-2'-deoxyguanosine (Fig. 10). G-B was identified as a diastereomer of G-A. Moreover, two major AGEs were identified from the 3DG-DNA reaction system.

2-6. Melanoidins

Melanoidins, which are final products of the Maillard reaction, are nitrogen-containing polymeric substances that decompose with difficulty. Human beings consume melanoidins daily in browning foods. The formation mechanism of melanoidins is complex because many reactants, such as deoxyosones, osones, unsaturated osones, furfurals, pyrrolines, carbonyl compounds and amino compounds are involved (Hayase *et al.*, 1986; Hayashi & Namiki, 1986a; Namiki, 1988; Hayase & Kato, 1994; Friedman, 1996). The formation of melanoidins is believed to be affected by various factors such as types of reducing sugars and amino compounds, their concentrations, types of catalysts and buffers, reaction temperatures and time, pH, water activity and presence of oxygen and metals.

Under neutral to alkaline pH, melanoidins have reportedly formed via pyrazinium radicals by cleavage of compounds composed of two or three carbons by splitting of reducing sugars (Hayashi & Namiki, 1986a, 1986b). Under neutral to acidic pH, deoxyosones, osones and furfurals are considered to be involved in the formation of melanoidins, which were observed from the formation of cleaved products under alkaline conditions. Also observed were heterocyclic compounds containing six carbons derived from glucose under acidic conditions. The products were

obtained under neutral pH, using glucose-butylamine reaction systems (Hayase & Kato, 1986; 1994).

Melanoidins are high-molecular weight compounds showing normal absorption and having no maximum absorption in the UV-visible region. Lignin, tannins, melanins, caramels and humins are organic compounds which are generally classified as poikilopolymers, and are difficult to identify because of the polydisperse system effect on molecular weight and electric properties (Piattell, 1961; Gomyo & Miura, 1983).

Evidence that molecules of melanoidins are distributed as a polydisperse system has been studied. Homma *et al.* (1982) detected 14 bands of melanoidins obtained from the reaction system of glucose and glycine by isoelectric electrophoresis at pH 2.7–3.3. O'Reilly (1983) reported 20 bands at pH 2.5–4.0 and 16 bands at pH 4.0–6.0 in Maillard reaction products formed from xylose and glycine.

Postulated structures of brown pigments have recently been proposed. Tressl's group has reported that pyrrole polymers are formed in the 2-deoxy-D-ribose and methyl 4-aminobutyrate Maillard system (Tressl *et al.*, 1998a). They also reported that *N*-substituted pyrrole, 2-furaldehyde, and *N*-substituted 2-formylpyrroles, formed in pentose and hexose Maillard reaction systems, were identified as components of extraordinary polycondensation activity (Tressl *et al.*, 1998b). Hofmann reported that some novel red-brown and red compounds were identified by Maillard reaction of bound arginine with glyoxal and furan-2-carboxaldehyde (Hofmann, 1998a), pentoses and primary amino acids (Hofmann, 1998b), and hexoses and primary or secondary amino acids (Hofmann, 1998c). However, these colored compounds have less hydrophilic properties, indicating significant differences in hydrophilic properties of melanoidins.

Miura and Gomyo (1982), and Gomyo *et al.* (1989) have found the formation of blue pigments in the Maillard reaction of D-xylose and glycine. The blue pigment designated Blue-M1 (blue Maillard reaction intermediate-1) is postulated to be an intermediate oligomer in the generation of melanoidins as shown in Fig. 11 (Hayase *et al.*, 1999); it is assumed to be a dimer of yellow colored pyrrolopyrrole-2-carboxaldehyde compounds. Blue-M1 that reacts readily to yellow compounds has a polymerizing activity, suggesting it is an important Maillard reaction intermediate through the formation of melanoidins. Figure 11 shows the proposed mechanism of the formation of Blue-M1 which might be generated by the decarboxylation from two molecules of yellow pigments, possibly two condensed pyrrolopyrrole-2-carboxyaldehyde compounds between glycy-pyrrolines and 3-deoxyxylosone or xylosone. Blue-M2 is thought to be formed by the addition reaction of yellow pigment to Blue-M1. In an advanced stage of the Maillard reaction, melanoidins may be formed by such a polymerization reaction.

Melanoidins are known to have various functional properties (Hayase & Kato, 1994; Hayase, 1996), for example photosensitive effects (Gomyo & Miura, 1986), interaction with metal ions (Hashiba, 1986; Terasawa *et al.*, 1991), antioxidative effects (Kim *et al.*, 1986; Yamaguchi, 1986), antimicrobial action (Einarsen & Eriksson, 1990), dietary fiber-like action (Gomyo & Miura, 1986), effect of enteric bacteria (Horikoshi *et al.*, 1981) and inhibition of trypsin (Hirano *et al.*, 1996). They also have desmutagenic effects on mutagenic heterocyclic amines (Kato *et al.*, 1985; Lee *et al.*, 1994) and scavenging effects on active oxy-

Fig. 11. Proposed formation pathway for blue compounds and melanoidins. 3DX: 3-deoxyxylosone, Yel: Yellow pigments.

gen species (Hayase *et al.*, 1989b; Hayase *et al.*, 1990). Thus, melanoidins display physiologically positive effects. It is expected that they show these effects *in vivo* in digestive organs because parts of melanoidins are absorbed through the gastrointestinal tracts of rats (Lee *et al.*, 1992).

As mentioned above, low-molecular weight Maillard reaction products such as α -dicarbonyls are cytotoxic and mutagenic (Hayase & Kato, 1994; Shinoda *et al.*, 1994). Therefore, both negative and positive effects need to be critically evaluated from the viewpoint of health.

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