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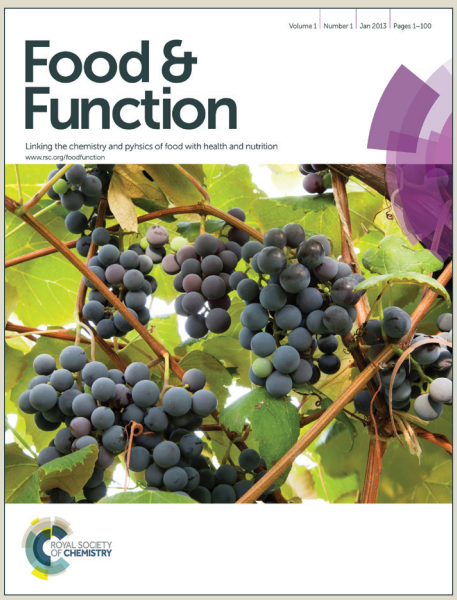

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The Colorants, Antioxidants, and Toxicants from Nonenzymatic Browning Reactions and the Impacts of Dietary Polyphenols on Their Thermal Formation

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Abstract: Nonenzymatic browning reactions proceed with the starting reactants of sugar and/or protein during thermal processing and storage of food. In addition to food color formation, the process also contributes to loss of essential nutrients, generation of beneficial antioxidants, and production of toxicants, including 5-hydroxymethylfurfural (5-HMF), reactive carbonyl species, advanced glycation endproducts (AGEs), and heterocyclic amines (HAs). Recent research has demonstrated dietary polyphenols can actively participate in nonenzymatic browning reactions, contributing to new colorants and antioxidants generation. More importantly, polyphenol addition has been realized to be an effective approach to mitigate heat-induced toxicants formation, mainly through inhibiting oxidative pathways and trapping reactive intermediates. A complex array of chemical interactions happening in the matrix of polyphenol-fortified foods, among polyphenols, traditional nutritional components, and neo-formed compounds they are thermally converted to, plays a significant role in the colorants, antioxidants as well as toxicants production. Findings support the potential of dietary polyphenols for increasing antioxidants content but reducing toxicants level when they participate into nonenzymatic browning reactions in the fortified food products.

Keywords: nonenzymatic browning reaction, dietary polyphenol, colorants, antioxidants, toxicants

1. Introduction

Browning is one of the most important chemical processes happening in food during processing and storage. It not only represents changes towards dark color as seen by naked eye but implies possible alterations in the stability, nutritional composition as well as health-related effects of food products.[1] The browning reactions can be either desirable, such as in the case of generating the golden-brown crust of bakery foods, or undesirable when the browning deteriorates the appearance and economic value of stored fruit juice. A variety of chemical species present in food may take part in the browning reactions via multiple chemical pathways. Based on whether enzymes are involved in the reaction, the group of browning reactions can be divided into enzymatic and non-enzymatic browning. The phenomenon of cut apple slice turning brown when exposed to air is a typical example of enzymatic browning facilitated by polyphenol oxidase. In comparison, nonenzymatic browning is favored by thermal treatment and includes two representative types: caramelization and Maillard reaction.

Caramelization is initiated by thermal treatment of carbohydrates. Monosaccharides (glucose and fructose), disaccharides (sucrose) or hydrolyzed polysaccharides (dextrose and glucose syrup) are usually selected for industrial caramel production. The raw carbohydrates undergo a few unselective and chemoselective reactions, and finally lead to formation of numerous polymeric and degradation products including both volatile and non-volatile products associated with food flavor and color. The key aroma compounds produced by sugar caramelization have been well characterized as

diacetyl, furans (furfural, hydroxymethylfurfural, acetylfuran, hydroxyacetylfuran), furanones (hydroxydimethylfuranone, dihydroxydimethylfuranone), 4-pyrone derivatives, maltol and hydroxymaltol. [2,3] In comparison, it remains to be a challenge to identify the diverse non-volatile caramelization products as there's lack of powerful analytical techniques. One dehydration product ($C_{12}H_{18}O_9$) and two polymers ($C_{36}H_{50}O_{25}$ and $C_{96}H_{102}O_{51}$) were firstly indicated in sucrose caramel. [4] Furfurals remaining in the non-volatile portion were examined and quantified by chromatographic analysis. [5,6] Recent advance in chemical characterization of the non-volatile caramel components has been achieved by taking advantage of combined mass spectrometric techniques. A few chemical species have been reported, such as carbohydrate oligomers (up to six sugar units), dehydration products of sugar oligomers (a maximum of losing eight water molecules, hydroxyfurfural derivatives included), hydration products of sugar oligomers, redox disproportionation products, and colored aromatic products. [2]

Maillard reaction is another typical nonenzymatic browning reaction, which is named after its first describer - a French chemist Louis Maillard. Maillard reaction is even more important than caramelization for its contribution towards food aroma, color and taste. Not only are the wide range of Maillard reaction products highly associated with sensory attributes, they are responsible for alternations in nutritional values of foods regarding digestibility and formation of antioxidants and toxicants. Starting with condensation of a reducing sugar and a compound possessing free amino group such

as protein, the reaction proceeds to encompass a whole network of reactions with great complexity and variety. Hodge [7] originally proposed the first comprehensive scheme of Maillard reaction (**Fig. 1**), which represents a milestone for understanding of the reaction and provides the basis for later interpretation and elaboration by food scientists. According to the reaction scheme, condensation of a reducing sugar and a compound with free amino group gives rise to N-substituted glycosylamine in the early stage, which rearranges to become Amadori rearrangement products (ARP). Amadori products undergo further degradation and dehydration depending on the pH of reaction system: at acidic pH, 1,2-enolization dominates and 5-hydroxymethylfurfural (5-HMF) or furfural are formed as intermediates; at alkalinic pH, ARPs are engaged in 2,3-enolization, giving rise to reductones, dehydroreductones, and a variety of fission products including acetol, diacetyl, pyruvaldehyde and so on. These reactive products in turn decarboxylate and incorporate with nitrogen compounds and form aldehydes via Strecker degradation. The sugar degradation products, ARPs, Strecker intermediates and the further derived reaction products like rearranged sugars, pyrroles, furans, carbonyls, Strecker aldehydes and pyrrazines are all important Maillard reaction-derived flavor compounds. [8] Formation of brown nitrogenous polymeric melanoidins is in the advanced stage of Maillard reaction, as a result of a couple of reactions like cyclization, dehydration, retroaldolization, rearrangement, isomerization and condensation. [9]

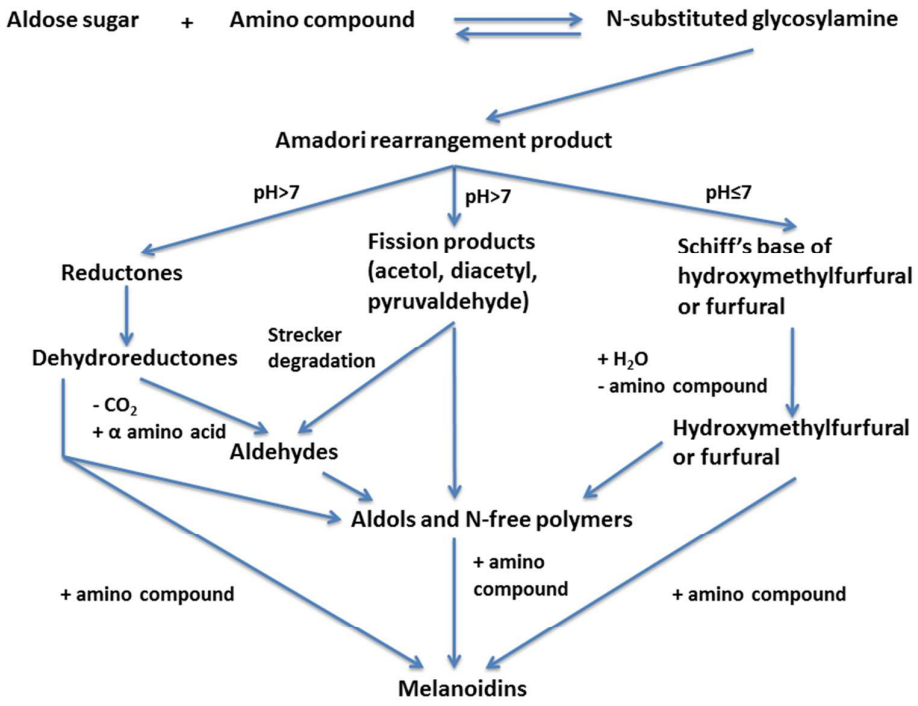

Fig. 1 Maillard reaction scheme. [7]

The formation of nonzymatic browning reaction products depend on the type and concentration of sugar/amino compounds, pH as well as the thermal preparation varieties such as heating temperature and duration. It has been a major task in food industry for long to understand nonenzymatic browning reaction pathways and the key factors affecting product composition. In latest decades, dietary polyphenols are popularly proposed as functional food ingredients, capable of affecting the formation of nonenzymatic browning reaction products and therefore improving the food appearance, taste and health effects so as to meet customers' need. Dietary polyphenols represent a large group of natural compounds synthesized as the secondary metabolites of plants for defense against ultraviolet radiation or pathogens.

[10,11] Over 8000 polyphenol structures have been identified, [12] commonly featured by multiples of phenol rings. [13] Distinction among the diverse polyphenol structures relies on the number of phenol rings and the structural elements that connect and bind to rings. [14] In addition, natural polyphenols may be in the form of association with various carbohydrates and organic acids. [11] The structural diversity is important to the various physicochemical properties of these phytochemicals [12] and this review focuses on the potential of dietary polyphenols to affect the formation of colorants, antioxidants and toxicants from nonzymatic browning reaction in chemical and food models under thermal conditions.

2. Impacts of dietary polyphenols on the thermal formation of nonenzymatic browning reaction products

2.1 Colorants

The "brownness" of food is one of the most obvious quality attributes consumers will respond to. The brown color can either increase the attractiveness or deteriorate the acceptance of food product. Caramel has been applied as a class of natural pigments for food additive for over a hundred years. [14] The use of caramel colorants accounts for more than 80% by weight in food manufacturing industry. [3] Caramel has many advantages over synthetic colorants in the aspects of safety, digestibility, dispersibility in food system and stability against heat and pressure. [3] Depending on the type or amount of reactants and manufacturing conditions, caramel colors vary in composition and provide great variety ranging from pale yellow to dark brown. [15] The

colorants effectively make food products more indicative of their taste and more appealing to consumers. For example, powdered or liquid caramel addition allows manufacturers to standardize the color of baking mixes and seasoning blends. [3] Besides, caramel colors possess useful functional properties. The caramel could be applied to enhance adhesion in rice cakes and energy bars. [16] Other functional characteristics include colloid stabilization, flavor retention, foaming promotion, dispersion facilitation and haze prevention, which are desirable in certain food products. [15]

Either a sensory panel or analytical instrument can be utilized to evaluate the color. Absorbance at 420 nm is often adopted to indicate the brown pigments formation. This spectrophotometrically measured browning value can be translated into colorants concentration if the average molar extinction coefficients of colorants formed in the nonenzymatic browning reaction system are determined. [17] Under the 3-dimensional color scale developed according to human perception, the chromatic coordinate L^* indicates the lightness of color ($L^* = 0$ yields black and $L^* = 100$ means diffuse white); a^* characterizes the position between red and green (negative values indicate green and positive values indicate red); b^* suggests the position between yellow and blue (negative values indicate blue and positive values indicate yellow).

The knowledge on the chemical nature and formation mechanism of the colorants is limited [17] while research interest is growing in consideration of these pigments'

physically and biochemically valuable functions. [18] The colorants are either unsaturated nitrogenous or nitrogen-free polymers and can be grouped into either low molecular weight pigments (two to four linked rings with extended double-bond conjugation) or high molecular weight melanoidins (molecular weight of several thousand daltons, with discrete chromophores). [19] Isolation of melanoidins has been attempted by gel/paper/capillary zone electrophoresis and reversed-phase HPLC but the diverse chemical properties of melanoidins hinder the production of satisfactory results. [18] Analytical techniques that have been applied to characterize the structure of melanoidins include infrared (IR) spectroscopy, mass spectrometry (MS) and advanced multidimensional nuclear magnetic resonance (NMR). [18, 20]

Some previous studies attempted to correlate color development with 5-hydroxymethylfurfural (5-HMF) content, but the results suggested that 5-HMF alone is not sufficient to account for color formation. [21] The sources of the colorants include Strecker aldehydes, sugar fragments, furfurals and dehydroreductones, which undergo self-condensation or condense with each other to form the brownness. [22,23] Experiments conducted in sugar-protein system supplemented that proteins can act as backbone of melanoidins and protein oligomers are cross-linked by low molecular weight colorants. [24,25] Altogether, the formation of high molecular weight melanoidins may follow two paths: 1) polymerization of low molecular weight colored/colorless reaction intermediates; 2) chromophoric low molecular weight substructures (possibly derived from carbohydrates) attachment to the backbone of colorless oligomers. [17]

The possible effect of dietary polyphenols on the colorants formation from nonenzymatic browning reactions and therefore the fortified food color development is an important issue to consider regarding the application of polyphenols as functional food ingredients. The potential alteration in color induced by natural dietary polyphenols could be both physically upon food product appearance and chemically related to the stability and compatibility of the color in a certain food category. [14]

In fructose caramelization model at 120 °C, addition of six selected polyphenols (phloretin, naringenin, quercetin, epicatechin, chlorogenic acid and rosmarinic acid) significantly increased the browning intensity of caramel. [26] The effects were especially great in phloretin and epicatechin's cases. In accordance, phloretin and epicatechin addition resulted in a lower L^* value, a higher a^* value and correspondingly a higher Chroma value and a smaller E index of caramel. pH was found to be a relevant factor regarding the impacts of polyphenols on the caramel's yellowness. Moreover, the observed difference between the browning intensity of polyphenol-treated caramel and the calculated sum of the browning of caramel control and heated polyphenol solution suggested that in addition to oxidative browning of polyphenols themselves, chemical reactions between polyphenols (or their thermal transformation products) and sugar caramelization products also contributed to the production of new brown pigments in the reaction system. [26] The shifts in chromatic

coordinates were attributable to the formation of thermal oxidative transformation products of polyphenol as well as new unknown compounds from polyphenol participation in the sugar caramelization. [26] There have been examples in Maillard reaction models as well. When glucose and lysine were mildly heated at 140 °C, addition of two apple polyphenols, phloretin and phloridzin, was reported to affect the color development, lowering lightness, yellowness but increasing redness. [27] Ferulic acid significantly inhibited the formation of brown melanoidins in model glycation mixtures systems containing fructose and soy glycinin or BSA heated at 60 °C. [28]

Further to chemical model systems, attempts to add polyphenol into real food have generated evidences on polyphenols' potential to influence food color. Grape seed extract (GSE) addition prior to bread baking induced visual colorimetric changes by reducing lightness but promoting redness and yellowness and therefore resulting in lower E index value inversely correlated to the levels of GSE addition. [29] Green tea extract (GTE) addition led to similar decrease in bread brightness. [30] 0.25% (*w/w*) quercetin, chlorogenic acid or rosmarinic acid caused decreased redness and increased yellowness of fortified cookies whereas epicatechin or naringenin elevated the cookie's redness or yellowness respectively. Comprehensively, the polyphenols increased the Chroma value but not E index. [31] On the basis of the analysis conducted in chemical models, the colorimetric changes induced by polyphenol fortification in food products are likely to be accounted by the colorants formed from thermal transformation of polyphenols together with the unknown neo-colorants produced

from polyphenol interactions with other food nutritional components. [31]

2.2 Antioxidants

In addition to aroma and color compounds, the nonenzymatic browning reactions have been shown to produce compounds exhibiting antioxidant activity. The exact chemical nature of the antioxidants formed, however, remains unclear. One of the early observations indicated that addition of 5% glucose prevented sugar cookies from oxidative rancidity. [32] Heat treatment of milk products and cereals led to improved oxidative stability, which was believed to be associated with formation of antioxidative nonenzymatic browning reaction products. These novel antioxidants may partially explain the phenomenon that roasted coffee brews had higher antioxidative activity than unroasted brews containing higher concentrations of phenolic antioxidants. [33]

When heating different sugar (glucose, fructose, ribose and xylose) solutions at 100 °C, the nonvolatile fraction of prepared sugar caramel possessed 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, reducing power and ferrous ion chelating activity. [34] The production of antioxidants seemed to be tightly associated with the formation of caramelization intermediates and brown compounds, which was dependent on the type of sugar, heating time and pH. Fructose heated at more alkalinic condition for a sufficiently long time showed the highest antioxidant activity. [34] Similar results were generated in another study trying to prepare caramelization

products from heating fructose or glucose solutions. Both sugar solutions potentially produce caramelization products with pronounced antioxidant activity at alkalinic pH for an extended time but fructose was superior to glucose in forming antioxidants. Besides, the antioxidant activity was coincidental with intermediates and browning formation. [35]

The electron donating ability and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) cation radical scavenging activity of heated fructose solution was also reported to increase with increasing heating temperature and time. [36] Compared with disaccharides, heating of monosaccharides was found to produce caramel of stronger antioxidant capacity and high sugar concentration was suggested to partially overcome the negative influence of low pH on monosaccharides or high pH on disaccharides. [37] This study further pointed out that the brown pigments in caramel, instead of the colorless caramelization intermediates, likely contributed to the antioxidant activity and the increase rate of antioxidant capacity might be closely reflected by ΔpH. [37]

Protein itself is weak antioxidant but heating protein and sugar together may result in enhanced antioxidant activity, as reported by experiment conducted in a model system composed of casein and glucose/lactose. The enhancement in antioxidant capacity was indicated to be attributed by Maillard reaction products (MRPs) and was a function of initial sugar and protein concentrations. [38] A recent study incorporated

porcine plasma protein and reducing sugars (glucose, fructose and galactose) heated at 100 °C without pH control. Same as in caramelization model, the antioxidant capacity of MRPs increased with sugar concentration and coincided with intermediates and browning development. Comparison among different sugar types revealed that MRPs derived from galactose had higher DPPH radical scavenging activity and reducing power than those from glucose or fructose. [39] Amino acid – sugar model was found to more readily generate antioxidants than protein – sugar model and the antioxidant activity of MRPs may also be affected by temperature and pH. [39]

Ultrafiltration has been applied to separate MRPs into fractions based on molecular weight. [40] MRPs of different molecular weight was proposed to exhibit different antioxidant activities [41] and the high molecular weight MRPs, such as melanoidins, were indicated to be the major antioxidants formed by Maillard reaction in this study. Melanoidins extracted from vinegar exhibited DPPH/hydroxyl radical scavenging activity and reducing power. [42] Melanoidins prepared from xylose and glycine showed comparable antioxidant activity to butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) and synergistic effect was observed when melanoidin was applied in addition to tocopherol, BHA, or BHT for inhibiting linoleic acid autoxidation. [33] It's possible to draw simple positive correlation between color and antioxidant properties in foods where formation of antioxidant MRPs is a prevalent event during processing. In this case, color could be considered as an index of antioxidant properties as the formation of antioxidants and color follow the same

pathway. [1]

Traditionally, synthetic antioxidants such as BHA and BHT are used to prevent oxidative food deterioration by inhibiting free radical facilitated lipid peroxidation. The neo-antioxidants produced during nonenzymatic browning reactions may be promising safer natural alternatives to improve the oxidative stability of foods. [33] However, attention should be paid to the stability of these neo-antioxidants. Fructose caramel could retard the formation of thiobarbituric acid reactive substances (TBARS) in a comminuted saury model system during iced storage. [34] MRPs prepared by reacting casein peptides with glucose at 80 °C showed improvement on the oxidative stability of fish oil – in water emulsions, as evaluated by the length of the lag phase of oxidation. [43]

One of the most recognizable physicochemical properties of dietary polyphenols is their potential of inhibiting oxidative processes *in vitro* and free radical scavenging is the mostly widely investigated antioxidant mechanism for polyphenols. [44] On the basis of comparison on the rate constants of the reactions to the hydroxyl radical ($^{\cdot}OH$), azide radical ($N_3^{\cdot}$), superoxide anion ($O_2^{\cdot-}$), lipid peroxyl radical ($LOO^{\cdot}$) and t-butyl alkoxyl radicals ($tBuO^{\cdot}$) as well as the stability of the resulting antioxidant-derived radical, polyphenols are more effective antioxidants than vitamin C and E *in vitro* on a molar basis. [13, 45] Besides, polyphenols have also been reported of metal-chelating activity, which contributes to the prevention of free radical formation

catalyzed by transition metals, such as iron and copper. [13,44,45]

The antioxidant activity of polyphenols is derived from their ubiquitous chemical structure - a highly conjugated system sometimes with specific hydroxylation patterns. [12] The structure is ideal for donating electrons or hydrogens so as to scavenge, neutralize and stabilize the free radicals and therefore break the oxidative chain reactions. [12] The ortho 3,4-dihydroxy moiety in the B ring and meta 5,7-dihydroxy moiety in the A ring of flavonoids appear to be important structural determinants of their antioxidant capacity and hydroxylation on the carbon ortho to 4-C position may further increase antioxidant capacity. [45,46] The 2,3-double bond combined with the 3-hydroxyl and 4-keto groups in the C ring has been suggested to assist antioxidant activity as well. [45] Glycosidation, however, likely alters the hydroxyl groups pattern, or substitutes/blocks the functional groups responsible for radical scavenging or metal chelating and therefore weakens the antioxidant activity of flavonoids. [45,47] Degree of polymerization also has effects on antioxidant capacity.[48]

A recent study investigated the effects of polyphenols on the formation of heat-induced antioxidants during fructose caramelization at 120 °C and how the thermal conditions of caramelization would affect the polyphenols' antioxidant activity. Results showed that the six tested polyphenols (phloretin, naringenin, quercetin, epicatechin, chlorogenic acid and rosmarinic acid) all greatly enhanced the antioxidant activity of caramel but the increase was generally less than that of

thermally-processed polyphenols. Further instrumental analysis illustrated that in thermal caramelization system, reduction in the polyphenols' antioxidant capacity could be either due to chemical degradation or oxidation of the original polyphenol structure or chemical reaction between polyphenol and caramelization intermediates such as sugar fragments, both possibly leading to loss of the specific structural motifs carrying antioxidant activity. [26]

Addition of polyphenol into food has been reported to result in health-beneficial increase in food's antioxidant potential. Grape seed extract fortification at levels of 0.06-0.2% (*w/w*), for example, obviously elevated the antioxidant activity of the corresponding bread sample. [29] Naringenin, quercetin, epicatechin, chlorogenic acid and rosmarinic acid at a fortification level of 0.25% (*w/w*) led to significantly-increased antioxidant capacity of fortified cookie extract prepared by extraction with solvents of different polarity. [31] The two studies shared a similar observation that the thermal processing lowered the antioxidant capacity of the originally-added polyphenols, especially in the cases of polyphenols with poor thermal stability. The influence of the usual thermal food cooking conditions on the overall antioxidant activity of foods is a result of different events happening consecutively or simultaneously. [49] It's expected that thermal processing may cause structural degradation of polyphenols. For example, when boiling and steaming of black beans, significant decrease in phenolic quantity was associated with reduced antioxidant power. [50] Under the low-moisture and high temperature (200 °C) cookie baking

environment, it took as short as 10 mins for naringenin to lose over 30% in its primary compound and around 88% dramatic loss was recorded for quercetin. [31] For quercetin, besides degradation, heat-stimulated oxidation is an alternative way to deteriorate the polyphenol structure for expressing antioxidant activity. [31]

In some other cases, including the processes of pressure steaming yellow beans, cooking tomato, and high pressure processing of tomato and carrot purees, thermal processing may have positive effects on the phenolic contents and antioxidant activity. [50-52] The partially oxidized polyphenols are possible to exhibit similar antioxidant activity as non-oxidized ones. [1] Meantime, the thermal reactions between polyphenols and other food nutritional components may lead to improvement on the antioxidant capacity of originally occurring compounds and also formation of neo-antioxidants. [29,31,49] Therefore, it seems to be necessary to consider the polyphenols' thermal stability and reactivity in the food matrix when the food product is fortified with polyphenols in purpose of increasing the level of health-beneficial antioxidants.

2.3 Toxicants

It's in latest decades that the toxicological activities of Maillard reaction products (MRPs) have attracted much attention as certain types of MRPs have been reported to contribute to disease and cancer development. Four major categories of toxic compounds originated from nonenzymatic browning reactions are 5-hydroxymethylfurfural (5-HMF), dicarbonyls, advanced glycation endproducts

(AGEs) and heterocyclic amines (HAs).

2.3.1 5-Hydroxymethylfurfural (5-HMF)

The 5-hydroxymethylfurfural (5-HMF) was a common type of furfural intermediates of nonenzymatic browning reactions and it's regarded as the most important heat-induced contaminant in bakery products. [53] Measurement of 5-HMF content in foods was traditionally performed by colorimetric absorbance reading at 443 nm, which reflects the amount of the complex formed from 5-HMF with 2-thiobarbituric acid. The method, however, suffers from low specificity to 5-HMF because 2-thiobarbituric acid generally reacts with all aldehydes. Therefore, reverse-phase HPLC technique is currently widely adopted for much more accurate quantification of 5-HMF. [54] Given that sugar is the source of precursor to 5-HMF formation, foods rich in sugars or ingredients such as sugar caramel or honey tend to contain higher quantity of 5-HMF. Besides, the amount of 5-HMF is directly related to the heat load during processing. Dietary intake of 5-HMF is mainly through bread and coffee and the daily exposure is estimated to be several orders of magnitude higher than that of other heat-induced food toxicants such as acrylamide and furan. [53]

As shown in **Fig. 2** , formation of 5-HMF starts from 1,2-enolization and dehydration of sugar to produce the key intermediate – 3-deoxyosone, which further dehydrates and cyclizes to yield 5-HMF. The rate of formation would increase with the increase in temperature, time and was favored in acidic conditions. [53,55] The type and

concentration of sugar, water activity, the concentration of divalent cations, and other nutritional components like fat in the reaction media/food system have also been reported to affect 5-HMF formation. [53,56] Alternative mechanism of 5-HMF formation under high-temperature dry pyrolytic conditions was proposed, which emphasized the formation of a very reactive fructofuranosyl cation that could be effectively converted into 5-HMF. [57]

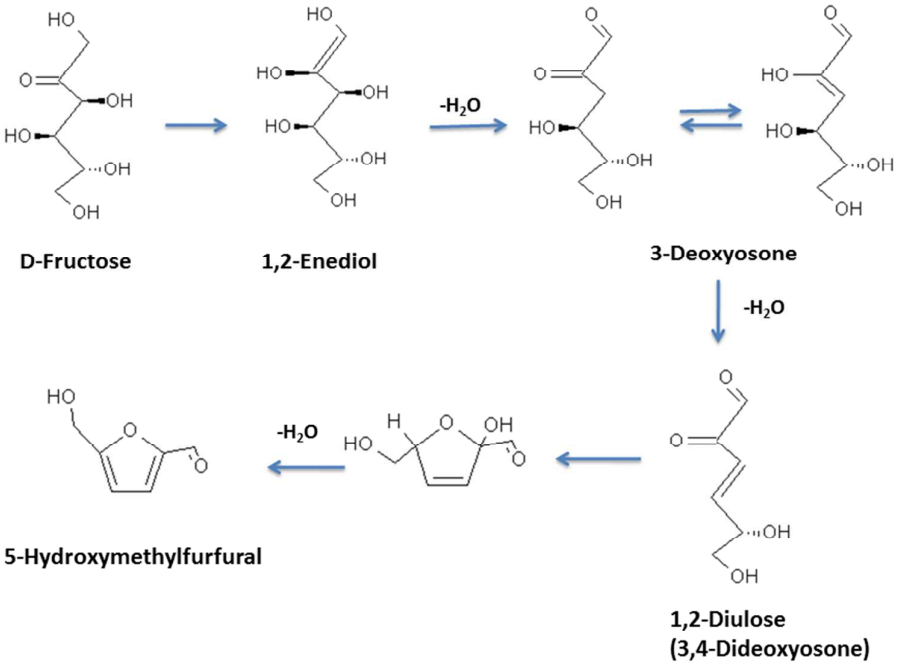

Fig. 2 Formation pathway of 5-HMF from D-fructose. [53]

It's regarded as a difficult and challenging task to specifically mitigate 5-HMF formation during thermal food processing, because its formation pathways tightly correlate with the production of color and flavor compounds. None of the previously proposed strategies, including lowering heating temperature, shortening heating time

or using less reactive carbohydrates, is capable of lowering 5-HMF formation without negatively affecting browning development. A recent study found that in fructose caramelization model at 120 °C, rosmarinic acid addition showed over 40% inhibition of 5-HMF formation at both neutral and alkaline pH whereas the inhibitory activity of quercetin and epicatechin was only shown at neutral pH. A concurrent reduction in furfural level was also observed for rosmarinic acid addition. [26] Given that the approach of rosmarinic acid addition caused no destruction on the browning intensity of caramel, rosmarinic acid might be a promising natural HMF inhibitor with practicality in caramel-type food preparation.

2.3.2 Reactive carbonyl species

The α-dicarbonyl compounds are another group of important nonenzymatic browning reaction intermediates, including glyoxal (GO), methylglyoxal (MGO) and glucosones. During food processing, they are either directly derived from sugar fragmentation, thermal transformation of Schiff bases and Amadori rearrangement products, or lipid degradation. [58,59] As shown in **Fig. 3**, glucosone is produced by sugar autoxidation; GO can be generated from glucose by retro-aldol condensation or through oxidation of early Maillard reaction products; deprotonation and dehydration of glucose lead to formation of 1,2-enol or 2,3-enol and thereby 1-deoxyglucosone (1-DG) or 3-deoxyglucosone (3-DG), fragmentation of which forms MGO. [59,60] Sugar fragmentation is favored by alkaline and oxidative conditions. [60] Monosaccharides are more capable of forming dicarbonyls and glucose tends to yield more dicarbonyls

than fructose. [59] Besides, the dicarbonyls formation is influenced by phosphate buffer and trace metal ions, which may catalyzes the deprotonation and autoxidation of sugar.[60]

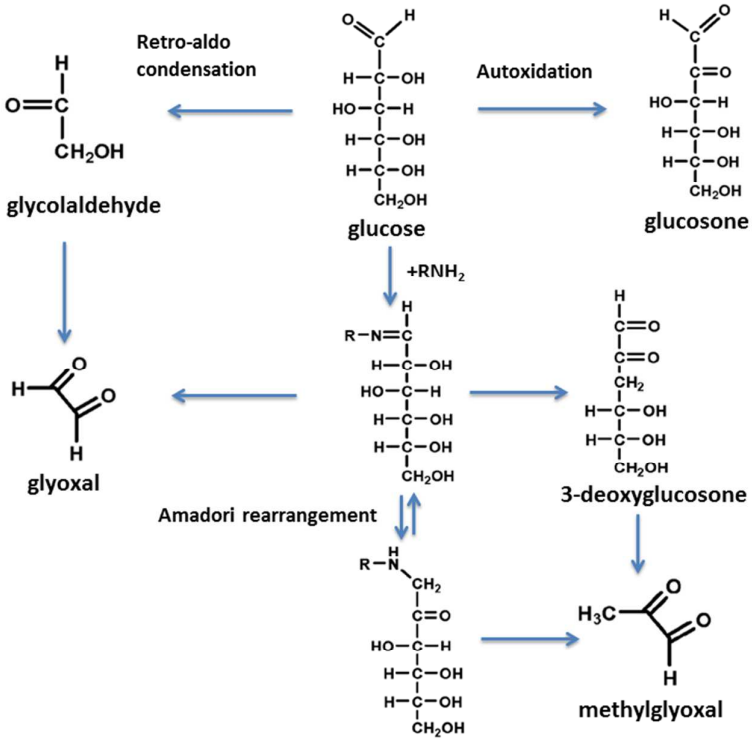

Fig. 3 Formation of GO and MGO from glucose and Maillard reaction. [59,60]

Compared to sugar, dicarbonyls possess a much higher browning activity and their formation during food processing is essential to the production of heterocyclic aroma and color compounds. [33,58] Dicarbonyls have been detected in a range of food product including cookie, honey, coffee, and other beverages [58] but the data are still limited. The high reactivity and ease of volatilization and polymerization make it difficult to specifically quantify the amount of dicarbonyls, such as GO and MGO, in food products and biological samples. [61] The quantification is facilitated by a necessary

derivatization process with derivatization agents before HPLC or GC analysis and the derivatives are feasible to be detected by UV, MS, ECD (electron-capture detector), NPD (nitrogen phosphorus detector), and FPD (flame photometric detector). [60]

In aqueous thermal Maillard reaction model systems at 125 °C, epicatechin (EC) was found to directly trap MGO in the way that the C6 or C8 of EC's A ring could covalently link to the C1 of MGO, presumably by hydroxyalkylation and aromatic substitution reactions. [62] In another thermal glucose-casein glycation model at 120 °C, chlorogenic acid was elucidated to be a GO and MGO inhibitor and the inhibition was likely to be achieved by lowering the amount of dicarbonyls' precursors, including Schiff bases and fructosamine. [63] The same research group also attempted to investigate the carbonyl inhibitory activity of dietary polyphenols in the cookie model with a baking temperature of 200 °C and it was found that the five tested polyphenols (naringenin, quercetin, epicatechin, chlorogenic acid and rosmarinic acid) were all GO but not MGO inhibitors. On the basis of the correlative analysis between inhibition rate and polyphenols' free radical scavenging capability, the GO inhibition mechanism was hypothesized to be through blocking the peroxidation of the unsaturated lipids contained in cookie recipe. [31] Therefore, it seems that under thermal conditions, multiple mechanisms may exist for polyphenols' inhibition against reactive dicarbonyls formation, depending on the type of chemical components in the reaction model and condition parameters.

2.3.3 Advanced glycation endproducts (AGEs)

As indicated by the name, advanced glycation endproducts (AGEs) are produced in the advanced stage of Maillard reaction. The group of intermediate compounds produced during Amadori rearrangements, including the reactive carbonyl species (glyoxal, methylglyoxal and 3-deoxyglucosone) have been proposed to be the immediate precursors of AGEs. [28,61,64-67] The reaction between these precursors and amino groups of proteins could happen either oxidatively or non-oxidatively, giving rise to different types of AGEs. [68] Carboxymethyllysine (CML) and pentosidine are two of the best characterized AGEs detectable in a wide variety of foodstuffs, such as meat, milk products and bakery products. [69]

CML is a type of nonfluorescent and non-crosslinking AGEs. A couple of instrumental methods have been utilized to quantify CML in foods, including GC-MS, [70] RP-HPLC, [71] (UP)LC-MS/MS [72,73] and ELISA (enzyme-linked immunosorbent assay). [69,74] In certain study, CML was relied on as base marker to reflect general AGE level in foods. [74] It's found that foods belonging to the meat and meat substitutes category are richer in CML than those divided into carbohydrates group. Thermal processing elevated CML amount from lower than a thousand in raw meat and fish to several thousand kilounits in processed state. [74] The extent of new CML formation was suggested to be largely dependent on the cooking styles differentiating from each other on the temperature, duration and moisture content. Broiling and frying led to more CML formation than roasting and boiling and microwaving tend to be the

practice resulting in least CML amount. [69]

Pentosidine, in contrast, is fluorescent and cross-linked. It is present at relatively low levels in foods and can be quantified by ion exchange chromatography with fluorescence detection and subsequent ninhydrin derivatization. [75] Roasted coffee was found to contain the highest amount of pentosidine up to 40 mg/kg protein, followed by bakery goods from non-detectable to over 20 mg/kg protein. [75] 148-672 μg pentosidine was detected per 100 g sauced meat or fish sample cooked in three different ways (boiling, frying, baking). [76]

The formation of both CML and pentosidine in food is an oxidative process. In addition to heat and dryness, the glycoxidation process is tightly associated with the nutritional composition of food products. High lipid content, especially polyunsaturated fatty acids, has been reported to promote AGEs formation possibly attributable to the free radicals released during lipoxidation. [69,73,76] Niquet-Leridon and Tessier [77] supplemented that the more the protein, the less the proportion of lysine residues converted to CML. Reducing sugars were considered to be the primary precursor of CML in drink mixes and the sugar contained in sauce exerted synergistic effects with heat during pentosidine formation in sauced meat and fish. [73,76,77] It is generally believed that both Maillard Reaction and lipid peroxidation play roles in dietary AGEs formation and their relative importance depends. [78] Typically, the AGEs formation in food is a comprehensive result of amino group blockage by the oxidative

degradation products of sugar, lipid or ascorbic acid. Metal ions, such as iron, were noted for catalyzing dietary AGEs formation by activating sugar and ascorbate oxidation. [71,72,79]

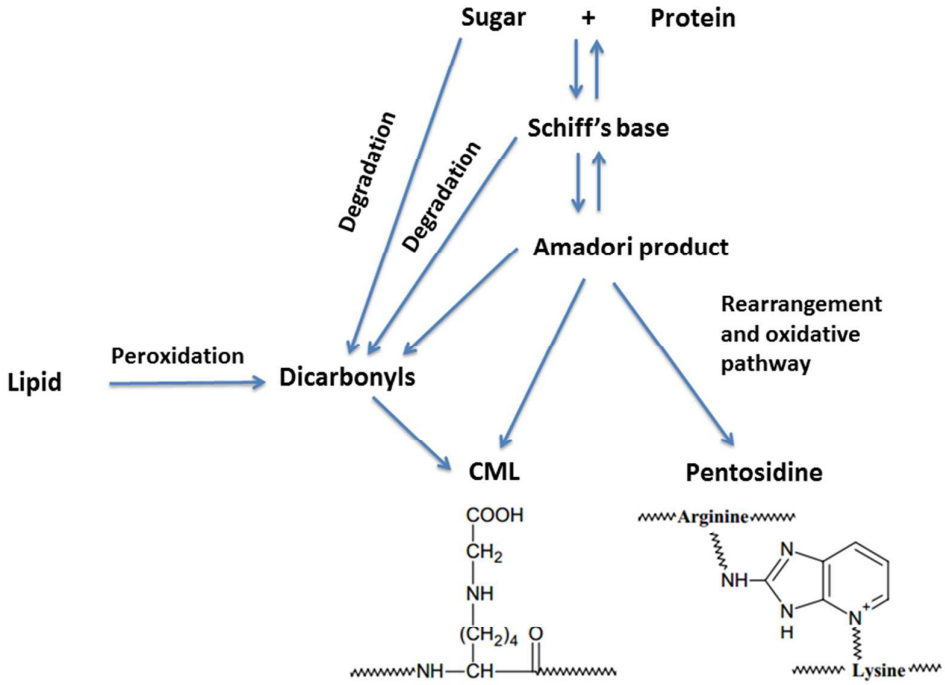

Fig. 4 Formation pathways of CML and pentosidine in food. [68]

There have been limited investigations of the antiglycation activity of dietary polyphenols under thermal conditions but the available results suggested the polyphenols' potential effectiveness. For example, ferulic acid inhibited the formation of fluorescent AGEs and carboxymethyllysine (CML) by nearly 90% and 85% respectively, in a thermal fructose/soy glycinin model heated at 60 °C. [28] In a glucose-casein glycation model at 120 °C, five tested polyphenols (phloretin, naringenin, epicatechin, chlorogenic acid and rosmarinic acid) inhibited the formation of total

fluorescent AGEs by over 50% at pH=7; the inhibition rates for phloretin and naringenin were as high as over 70%. For CML, weaker inhibitory activity ranging from 13% to 45% was recorded at pH=7. Besides, the antiglycation activity at pH=10 of polyphenols followed similar order but was generally lower. [63] Under baking condition at 190 ℃, significant reduction in CML level of sponge cake was observed as a result of ferulic acid addition. [80] Dose-dependent CML inhibition during bread baking was reported for grape seed extract (GSE) rich in phenolic compounds. [29] When fortified at same mass proportion into model cookie baked at 200 °C, quercetin was the most potent fluorescent AGEs inhibitor (over 80%), followed by naringenin, rosmarinic acid and epicatechin. [31]

Free radical scavenging and reactive carbonyl trapping are two most recognizable antiglycation mechanisms for polyphenols proposed by researchers. These two mechanisms, however, seemed to be insufficient to account for the polyphenols' antiglycation activity under thermal conditions. The thermal treatment, including heating temperature and time, would influence polyphenols' structural integrity and therefore their reactivity effectiveness in inhibiting protein glycation process. Moreover, the food context represents a much more complicated reaction system than sugar-protein chemical model that extra nutritional components or chemical additives might influence the process either positively or negatively. More studies, therefore, need to be performed in thermal and food models regarding the antiglycation activity and mechanisms of dietary polyphenols, in purpose of shedding light on the

mitigation strategies of AGEs during food processing and storage.

2.3.4 Heterocyclic amines

Heterocyclic amines (HAs) are a class of amino compounds produced in proteinaceous foods of both animal and plant origin. [81] They can be divided into different groups based on their formation temperature or polarity. Amino acids or proteins are the main precursors for HAs formation above 300 ^{o}C; HAs formation under mild thermal conditions lower than 300 ^{o}C, however, is a result of Maillard reaction and Strecker degradation involving sugars, amino acids, peptides and creatinine. [82] The molecule of HAs formed at lower temperature is composed of two parts, either from creatinine cyclization and dehydration or aldol condensation of Strecker degradation products. [81] Generally, the formation pathways have been suggested to be mediated by free radicals and reactive fragments. [82] Besides, the yield would be influenced by a variety of factors, such as the heating parameters (temperature and duration), type and concentration of principle precursors, as well as the type and content of fats. Therefore, it is expected that the types and levels of HAs that predominate in a certain food product are dependent on the food's nutritional composition and thermal processing conditions. [83] The quantitative analysis of HAs in food usually starts from liquid-liquid or solid phase extraction followed by liquid or gas chromatography coupled to mass spectrometry. [84] The amounts are higher in cooked pure meat than mixed meat products or fish and crust contains more HAs than inner parts. PhIP is the most abundant HA detected in cooked food, with the level up

to several hundred ng per g food. [85]

Polyphenols and the plant extracts rich in polyphenols have been realized to be promising HAs inhibitor during food processing. Rosemary, thyme, sage, garlic, pycnogenol, and tea extracts have all been reported to either suppress the thermal formation of HAs or alleviate HAs-induced toxicity. [86-89] Recently, experiments performed using fried beef patties (200 ^{o}C**)** revealed the efficacy of apple or grape seed extract in reducing total and three individual HAs (MeIQx, 4,8-DiMeIQx and PhIP) and phloridzin or proanthocyanidins was pointed out to be the key inhibitor in either apple or grape seed extract. [90] The research group further explored the HAs inhibitory potential of twelve polyphenols in both chemical models (composed of glucose, phenylalanine and creatinine, heated at 125 ^{o}C) and fried beef patties and the results supported the effectiveness of theaflavin 3, 3'-digallate, epicatechin gallate, rosmarinic acid, and especially naringenin. [91]

Addition of phenolic antioxidants did not always lead to lowered HAs amount. [92] Meantime, the inhibition rate was found to show no significant correlation with polyphenols' antioxidant activity. [91] These phenomena suggest the occurrence of alternative HAs inhibitory mechanisms other than antioxidation. Breakthroughs in mechanistic study elucidated that in chemical models, naringenin, a weak phenolic antioxidant, could scavenge the reactive carbonyl species by forming stable adducts, therefore diverting these Maillard reaction intermediates from HAs formation

pathways. [93] The two adducts were identified to be 8-C-(E-phenylethenyl)naringenin and 6-C-(E-phenylethenyl)naringenin, which were electrophilic substitution products from naringenin and phenylacetaldehyde. This phenylaetaldehyde scavenging mechanism was later proven on EGCG or in real food system and suggested to be the dominant mechanism for weak or non-antioxidants' inhibition of HAs. [94]

3. Summary and concluding remarks

Nonenzymatic browning reactions are particularly important chemical reactions during thermal food processing. The active participation of dietary polyphenols during thermal proceeding of nonenzymatic browning reactions, with regards to the production of color, antioxidative and toxic compounds, has been documented in both chemical and food models. The thermal stability and transformation of polyphenols would affect their antioxidant activity and inhibitory efficacy of toxicants formation. The mechanisms for toxicants inhibition have not been fully understood, but polyphenols' free radical scavenging and reactive carbonyl trapping activities are suggested to take important roles. It's a general conclusion that dietary polyphenol are promising agents for developing colorful and healthy food products containing more antioxidant but less toxicant than traditional ones, but more efforts need to be focused on the structural conversions and reaction mechanisms behind the phenomena. It may be useful to develop cautious strategies to efficiently retain the primary active structural motifs of polyphenols during thermal treatment. Meantime, the complex array of chemical transformations taking place in the polyphenol-fortified food matrix

is waiting for clearer understanding and the neo-formed compounds' structure, physicochemical properties and bioactivity need to be better characterized.

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