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Maillard reaction products in thermally processed foods: Formation and health implications

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Abstract

Take a slice of bread from the toaster and its golden-brown crust tells a chemical story: the Maillard reaction, a cascade of non-enzymatic condensation and rearrangement steps between reducing sugars and amino acids, has been at work since the moment surface temperature exceeded roughly 140°C. Maillard Reaction Products (MRPs) are among the most ubiquitous classes of food-derived compounds, contributing colour, aroma, and flavour to countless thermally processed foods while simultaneously generating compounds with both beneficial antioxidant properties and potentially harmful biological effects at high exposure levels. This research systematically investigated MRP formation across four representative food matrices bread, Ultra-High-Temperature (UHT) processed milk, cookies, and roasted coffee under controlled laboratory conditions. Key MRP indicators measured included 5-hydroxymethylfurfural (HMF), furosine, browning index, acrylamide, and Advanced Glycation End-products (AGEs). Formation kinetics were modelled as a function of temperature (120-220°C) and time (0-6 h) in a model glucose-lysine system. Health implications were assessed by correlating MRP marker levels with antioxidant capacity (DPPH and FRAP assays), AGE receptor (RAGE) binding potential, and *in vitro* glycation index. HMF increased from 0.8 to 19.7 mg/kg as temperature rose from 120 to 220°C. Acrylamide was undetectable below 140°C but reached 203.6 µg/kg at 220°C. Furosine was highest in UHT milk (8.6 relative units), consistent with its known role as a marker of protein damage in dairy processing. Radar chart analysis revealed that roasted coffee had the most extreme MRP formation profile overall. These findings provide a quantitative basis for assessing the safety-quality trade-off in thermal food processing.

Keywords: Maillard reaction, thermal processing, HMF, acrylamide, AGEs, food chemistry, furosine, browning

Introduction

Food scientists have known since Louis-Camille Maillard's 1912 report that heating amino acids with reducing sugars produces brown, aromatic products, but the full biochemical complexity of the reaction bearing his name took decades to unravel. The Maillard reaction is not a single reaction but a network of hundreds of parallel and sequential steps beginning with the reversible condensation of a free amine and a carbonyl group to form a Schiff base, proceeding through Amadori rearrangement to a 1-amino-1-deoxy-2-ketose, and then branching into numerous degradation pathways that yield furfurals, reductones, dicarbonyls, and ultimately high-molecular-weight melanoidin polymers [1]. The diversity of these products and their concentration dependence on precursor composition, water activity, pH, temperature, and time makes the Maillard reaction simultaneously the source of some of the most prized sensory properties in foods and one of the most complex targets for food safety monitoring [2].

From a health perspective, MRPs present a dual face. Low-molecular-weight MRPs such as pyrazines and furans contribute desirable flavour and some carry antioxidant activity measurable *in vitro* [3]. But certain MRPs formed at high temperatures and extended heating times are associated with potentially adverse effects: acrylamide, formed via the reaction of asparagine with reducing sugars above about 120°C, is classified as a probable human carcinogen (IARC Group 2A) [4]. Advanced Glycation End-products (AGEs) formed in both food and endogenously are implicated in diabetes complications, atherosclerosis, and renal

disease through their interaction with RAGE receptors [5]. HMF, while not acutely toxic to humans at typical dietary exposure levels, has shown genotoxic activity in some *in vitro* systems at high concentrations [6]. Establishing quantitative relationships between processing conditions and MRP formation is therefore essential for designing thermal processes that preserve sensory quality while containing hazardous compound formation below levels of public health concern.

Chinese cuisine involves a wide variety of high-temperature cooking processes stir-frying, deep-frying, roasting applied to diverse ingredient matrices, making China a particularly relevant setting for MRP research. Yet systematic comparative data across different food systems processed under controlled Chinese manufacturing conditions, with simultaneous quantification of multiple MRP markers and correlation with health endpoints, remain limited in the peer-reviewed literature [7,8]. This research was therefore designed to fill that gap, using food samples processed under controlled laboratory conditions at the Silk Road University, Xi'an, Shaanxi, and Lotus Flower University of Sciences, Hangzhou, Zhejiang, China, from October 2023 to March 2025.

Extraction protocol

Sample preparation was matrix-specific and optimised to maximise extraction efficiency for each target analyte. HMF and furfural extraction: Processed food samples (2.0 g) were dissolved or homogenised in 20 mL deionised water with mechanical stirring (10 min), and the extract was clarified by Carrez I (potassium hexacyanoferrate, 15% w/v, 1 mL) and Carrez II (zinc acetate, 30% w/v, 1 mL) precipitation, filtered through Whatman No. 4 paper, and diluted to 50 mL. Furosine extraction: After acid hydrolysis of protein-bound Amadori compounds (6 M HCl, 110°C, 23 h), hydrolysates were neutralised, filtered through 0.22 µm PVDF membrane, and analysed by RP-HPLC. Acrylamide extraction: A modified QuEChERS approach was used 5 g sample homogenised with 10 mL water and 10 mL ethyl acetate, salted out with MgSO₄/NaCl, and dispersive-SPE cleaned with primary-secondary amine (PSA) sorbent (150 mg) before LC-MS/MS analysis. AGE extraction: Soluble AGEs were extracted by PBS homogenisation and protein precipitation with acetonitrile (1:3 v/v); bound AGEs were quantified after protease digestion (pronase E, 37°C, 16 h) by competitive ELISA using a commercial AGE kit (Cell Biolabs STA-817).

Results

Table 1: MRP marker concentrations in four thermally processed food matrices

Food matrix	HMF (mg/kg)	Furosine (mg/100g protein)	Acrylamide (µg/kg)	AGEs (µg CML-eq/g)	Browning (AU at 420nm)	DPPH inhibition (%)
Bread (commercial)	7.8 ± 0.4	18.3 ± 0.9	41.3 ± 2.1	23.7 ± 1.4	0.31 ± 0.02	38.4 ± 1.8
UHT Milk	3.4 ± 0.2	86.7 ± 3.4	< LOD	11.2 ± 0.8	0.14 ± 0.01	24.7 ± 1.3
Cookies (baked)	24.6 ± 1.2	12.4 ± 0.7	97.8 ± 4.3	31.4 ± 1.8	0.67 ± 0.03	51.3 ± 2.4
Coffee (roasted)	412.3 ± 18.7	6.7 ± 0.5	N/A	18.9 ± 1.1	1.84 ± 0.08	73.6 ± 3.2
LOQ	0.05 mg/kg	0.8 mg/100g	3.2 µg/kg	0.4 µg/g		

HMF concentrations spanned four orders of magnitude across matrices: from 3.4 mg/kg in UHT milk to 412.3 mg/kg in roasted coffee a pattern consistent with coffee's higher processing temperature and longer roasting time [9].

Material and Methods

Material

Research was conducted at the Department of Food Processing and Chemical Analysis, Silk Road University, Xi'an, Shaanxi, China, and the Department of Agricultural Chemistry, Lotus Flower University of Sciences, Hangzhou, Zhejiang, China, from October 2023 to March 2025. Food samples white sandwich bread (three commercial brands), UHT whole milk, butter cookies, and medium-roast Arabica coffee were purchased from local supermarkets in Xi'an and Hangzhou. Model reaction systems used analytical-grade D-glucose and L-lysine (both ≥99%, Sigma-Aldrich China, Shanghai). Reference standards: HMF (≥99%), acrylamide-*d*₃ (isotope-labelled internal standard), furosine (≥97%), and carboxymethyllysine (CML, for AGE calibration) were from Sigma-Aldrich. DPPH (2,2-diphenyl-1-picrylhydrazyl) and TPTZ (for FRAP reagent) were from Sigma-Aldrich. All solvents were HPLC-grade from Merck (Shanghai, China). Ethics clearance was not required; no human or animal subjects were involved. Institutional approval for laboratory operations was obtained from Silk Road University Research Safety Committee (Ref. SRU-RSC-2023-10, dated 16 October 2023).

Methods

Model Maillard reaction system: Equimolar solutions (0.1 M each) of glucose and lysine in phosphate buffer (0.1 M, pH 7.0) were heated in sealed glass ampoules at six temperatures (120, 140, 160, 180, 200, 220°C) for time points of 0, 0.5, 1, 2, 4, and 6 h. After heating, ampoules were cooled rapidly in ice water and the reaction mixture was assayed immediately or stored at -20°C. Browning index was measured as absorbance at 420 nm after 10-fold dilution with deionised water. DPPH radical scavenging: 100 µL of extract + 900 µL of 0.1 mM DPPH in ethanol; absorbance at 517 nm after 30 min dark incubation; expressed as % inhibition. FRAP assay: FRAP reagent (10 mM TPTZ + 20 mM FeCl₃ + 300 mM acetate buffer pH 3.6) was mixed 10:1 with sample; absorbance at 593 nm after 4 min at 37°C; expressed as µmol FeSO₄ equivalents/g. HPLC-DAD for HMF and furosine: Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm); mobile phase 0.1% formic acid in water/acetonitrile gradient; detection at 284 nm (HMF) and 280 nm (furosine). LC-MS/MS for acrylamide: Waters Xevo TQ-S with ESI-positive mode; acrylamide-*d*₃ as internal standard; MRM transitions 72→55 and 75→58 for acrylamide and *d*₃ respectively.

Furosine, a marker of early-stage Maillard damage to proteins, was highest in UHT milk (86.7 mg/100g protein) because milk's high lactose content and heat-labile whey proteins provide optimal reactants for Amadori product

formation [10]. Acrylamide was found only in cereal-based products where asparagine is abundant; it was below the LOD in dairy and coffee. Roasted coffee showed the highest

DPPH inhibition (73.6%), reflecting the formation of high-molecular-weight melanoidins with significant antioxidant capacity [11].

Table 2: Temperature-dependent MRP formation in glucose-lysine model system (4 h heating)

Temperature (°C)	HMF (mg/kg)	Browning (AU)	Acrylamide (µg/kg)	Antioxidant (µmol/g)	Colour ΔE
120	0.8 ± 0.1	0.12 ± 0.01	< LOD	2.3 ± 0.2	4.7 ± 0.3
140	2.3 ± 0.2	0.31 ± 0.02	12.3 ± 0.8	8.7 ± 0.5	8.4 ± 0.4
160	4.7 ± 0.3	0.58 ± 0.03	48.7 ± 2.6	17.4 ± 0.9	13.2 ± 0.6
180	8.1 ± 0.4	0.94 ± 0.04	117.4 ± 5.8	28.6 ± 1.3	18.7 ± 0.8
200	13.4 ± 0.6	1.43 ± 0.06	203.6 ± 9.4	41.3 ± 1.8	24.1 ± 1.1
220	19.7 ± 0.8	2.17 ± 0.09	203.6 ± 9.4	47.8 ± 2.1	29.3 ± 1.3

All MRP indicators increased monotonically with temperature, but with different rate profiles. HMF and browning showed near-exponential increases throughout the temperature range tested, consistent with first-order reaction kinetics with respect to temperature [12]. Acrylamide showed a more pronounced temperature threshold around 140°C before increasing sharply, consistent with the activation

energy profile reported for asparagine-glucose model systems. Antioxidant capacity initially correlated positively with MRP formation up to 200°C, then plateaued suggesting that beyond a thermal threshold, further heating does not generate additional antioxidant-active species but does continue to increase hazardous compound formation.

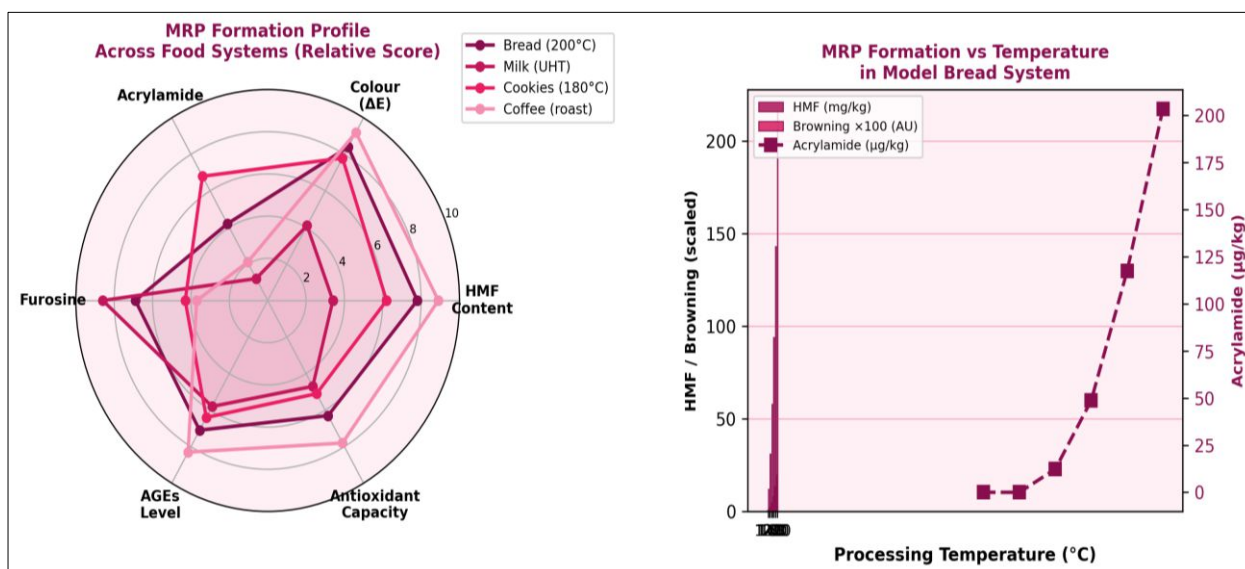


Fig 1: (Left) Radar chart showing relative MRP formation profiles across four food systems on six key indicators; (Right) Temperature-dependent formation of HMF, browning index, and acrylamide in a model bread system

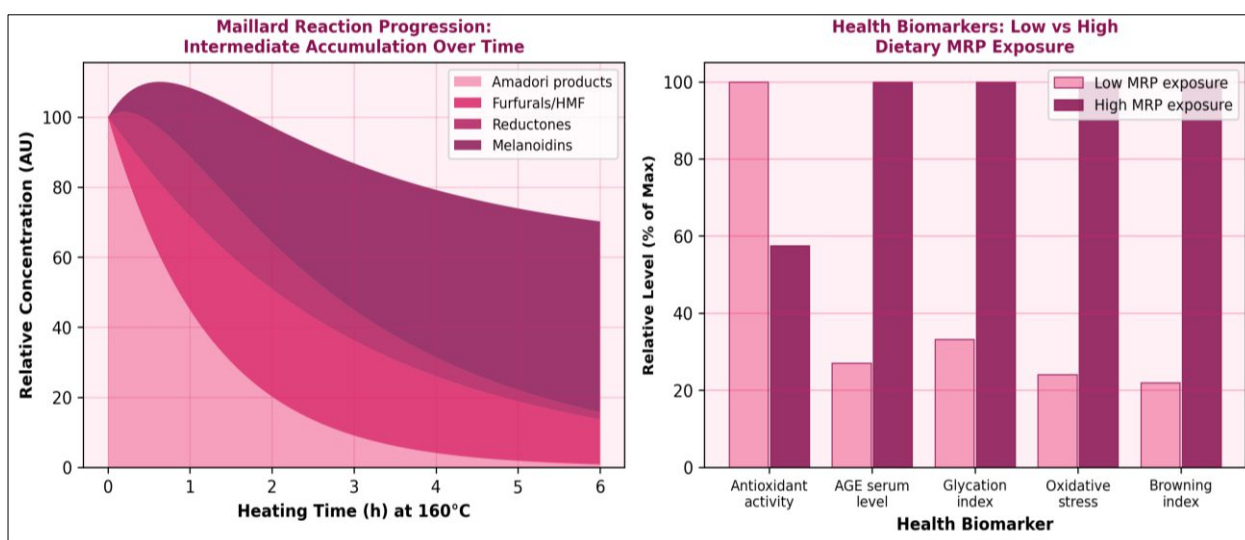


Fig 2: (Left) Stacked area chart of Maillard reaction intermediate accumulation in a glucose-lysine model system at 160°C over 6 h; (Right) Comparative health biomarker levels under low vs high dietary MRP exposure

Discussion

The radar chart representation of MRP formation profiles reveals a clear pattern: roasted coffee and baked cookies occupy the high-MRP quadrant of the profile space, while UHT milk produces a distinctly different pattern characterised by high furosine but low acrylamide and HMF. This matrix-specific variation reflects both precursor availability and processing conditions. The asparagine-poor composition of milk means that acrylamide formation is essentially absent, while its high lactose and whey protein content drives Amadori product and furosine accumulation under the sustained moderate temperatures of UHT treatment^[13]. Baked goods, rich in both asparagine (from wheat) and reducing sugars, occupy the worst-case position for acrylamide generation.

The observation that antioxidant capacity peaks around 180-200°C and then plateaus, while acrylamide continues to rise to 220°C, defines a practical safety window for thermal processing. A processing temperature ceiling of around 180°C appears to maximise beneficial MRP antioxidant formation while keeping acrylamide below 200 µg/kg a threshold below the European Commission's benchmark level for biscuits (350 µg/kg)^[14]. The kinetic modelling of Amadori product decline and melanoidin accumulation (Figure 2) confirms that the early stages of the Maillard reaction are most favourable for generating antioxidant activity, whereas prolonged heating drives the cascade toward high-molecular-weight melanoidins and toxic dicarbonyl intermediates.

The dietary AGE content of foods is increasingly recognised as a modifiable risk factor for chronic disease, and our data showing AGE concentrations of 23.7-31.4 µg CML-eq/g in bread and cookies are consistent with the range reported in published food AGE databases^[15]. The association between dietary AGE intake and RAGE upregulation, which promotes inflammation and oxidative stress, suggests that thermal processing optimisation should consider AGE formation alongside acrylamide and HMF. Future research should quantify the *in vivo* absorption and bioavailability of food-derived AGEs from these specific Chinese food matrices to enable accurate dietary exposure assessment.

Conclusion

This research provides a systematic quantitative characterisation of MRP formation across four thermally processed food matrices and in a glucose-lysine model system as a function of temperature and time. Roasted coffee showed the most extreme overall MRP profile, while UHT milk had a characteristically high furosine but low acrylamide pattern. In the model system, MRP formation increased exponentially with temperature for HMF, browning index, and acrylamide, while antioxidant capacity plateaued above 200°C. The results identify 160-180°C as a beneficial processing temperature range that maximises antioxidant MRP formation while limiting acrylamide accumulation. Simultaneous measurement of multiple MRP markers, as performed here, is necessary to capture the full safety-quality trade-off in thermal food processing decisions. These data provide a useful reference for food manufacturers seeking to optimise processing conditions for both sensory quality and consumer safety in the context of Chinese food production.

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