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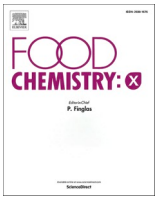

Key factors influencing the formation of α-dicarbonyls and dietary advanced glycation end products in bread and commercial bakery products: Impacts of sugar, lipid and gluten protein

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ABSTRACT

α-Dicarbonyls and dietary advanced glycation end products (dAGEs) are potentially harmful compounds formed from the Maillard reaction. Bakery products often contain high levels of α-dicarbonyls and dAGEs due to thermal processing. Additionally, the ingredients used in dough might significantly influence the formation of these compounds. Our findings indicated that adding sucrose notably increased the formation of α-dicarbonyls and methylglyoxal-derived dAGEs in bread, while the incorporation of butter and olive oil had negligible effects. In contrast, substituting high-gluten flour with medium- and low-gluten flour reduced the formation of α-dicarbonyls and dAGEs in bread. Furthermore, correlation analyses revealed a significant positive relationship between total dAGEs content and total sugar content in commercial bakery products, with no significant correlation found with total protein and fat content. This study suggests that the sugar content listed on nutrition labels could serve as a practical indicator for estimating dAGE levels in bakery products.

1. Introduction

The Maillard reaction (MR) is a non-enzymatic browning reaction that imparts distinctive flavors and colors to foods, particularly in thermally processed products. This reaction begins with the condensation of amino groups from amino acids, peptides, or proteins with the carbonyl groups of aldoses or ketoses, leading to the formation of Heyns or Amadori rearrangement products. Following enolization, these intermediates can undergo fragmentation or modification to produce α-dicarbonyl compounds, such as 3-deoxyglucosone (3-DG), glucosone, methylglyoxal (MGO), and glyoxal (GO) (Hellwig et al., 2018). These reactive carbonyl species can further interact with amino acids, especially lysine and arginine, resulting in the formation of advanced glycation end products (AGEs).

Both free and protein-bound dAGEs are present in foods. α-Dicarbonyl compounds can react with free amino acids to form free AGEs or glycate amino acid residues in proteins, generating protein-bound AGEs (Yuan et al., 2023). Emerging evidence suggests that AGEs can negatively impact human health. At the cellular level, AGEs can induce inflammatory responses and increase oxidative stress by binding to the receptor for advanced glycation end products (RAGEs) (Bastos & Gugliucci, 2015). A recent studies has demonstrated that

Abbreviations: 3-DG, 3-deoxyglucosone; 3-DGal, 3-deoxygalactosone; CEL, *N*ε-(carboxyethyl)lysine; CML, *N*ε-(carboxymethyl)lysine; DA, diacetyl; dAGEs, dietary advanced glycation end products; G-H_1, glyoxal-hydroimidazolone 1; GO, glyoxal; MG-H_1, methylglyoxal-hydroimidazolone 1; MGO, methylglyoxal; MR, Maillard reaction.

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supplementation with a heat-treated diet high in dAGEs led to a significant accumulation of free and protein-bound AGEs in plasma, liver, and kidney tissues, indicating that dAGEs are bioavailable and can enter the body's AGE pool (van Dongen et al., 2021). Meanwhile, the unabsorbed dAGEs retained in the intestinal tract can significantly affect the growth of gut microbiota. Emerging data indicate that the adverse health effects of dAGEs may be linked to gut microbiota dysbiosis caused by unabsorbed dAGEs. Supplementation with a heat-treated diet reduced both the diversity and richness of the gut microbiota in Sprague-Dawley (SD) rats, including the abundance of *Ruminococcaceae* and *Alloprevotella*, which are known producers of short-chain fatty acids (SCFAs) (Qu et al., 2017). Therefore, both absorbed dAGEs that accumulate in tissues and unabsorbed dAGEs retained in the gastrointestinal tract could have detrimental effects on health.

Given the adverse health impacts of dietary advanced glycation end products (dAGEs), their presence in food has become a critical focus in food science research. Lysine-derived and arginine-derived dAGEs are the two main types. Lysine reacts with methylglyoxal (MGO) and glyoxal (GO) to form *N*ε-(carboxyethyl)lysine (CEL) and *N*ε-(carboxymethyl)lysine (CML), respectively, which are the most common dAGEs widely present in foods. In contrast, methylglyoxal-hydroimidazolone 1 (MG-H_1), formed from the reaction between arginine and MGO, is the most prevalent arginine-derived dAGE found in various processed food products (Lin et al., 2023; Scheijen et al., 2016). Since dAGEs primarily form through the MR, their levels in both animal- and plant-based foods are closely related to heating time and temperature. For example, the levels of CML and CEL in sesame seeds increased significantly with prolonged roasting time and higher temperatures (Berk et al., 2019). Similarly, CML, CEL, and MG-H_1 levels in minced-meat hot dogs are positively correlated with deep-frying temperatures (Scheijen et al., 2016). In addition to heating conditions, food additives, condiments, and spices can also significantly influence dAGE formation. When cookie dough was mixed with glucose or fructose, the levels of CML, CEL, and MG-H_1 were notably higher than in cookies prepared with sucrose (Treibmann et al., 2017). Conversely, dAGEs content in model cookies decreased significantly when fortified with antioxidants (Chen et al., 2022).

Bakery products typically contain high levels of dAGEs due to thermal processing, making them a primary dietary source of these compounds. The estimated intake of CML from bakery products can be as high as 160 mg/day, while dairy products contribute only up to 50 mg/day (Henle, 2003). Furthermore, bread crust has been used as a high-AGE diet model to study the absorption and metabolism of dAGEs in rats (Yuan et al., 2018). Given that bread is the most widely consumed bakery product, understanding the presence of dAGEs in bread is essential for accurately estimating daily dAGE intake. Despite a recent study quantifying dAGEs during bread production (Jost et al., 2021), the specific impact of dough ingredients - such as sugar, lipids, and protein - on dAGEs formation in baked bread remains underexplored. This study aims to address this gap by investigating how commonly used dough ingredients - sucrose (the most common sugar in bakery products), better and olive oil (representing saturated and unsaturated lipids), and gluten protein (with low- and medium-gluten flours replacing high-gluten flour) - affect dAGEs formation during bread production. In addition, determining dAGEs levels in food products involves costly (e.g., LC-MS analysis) and time-intensive (e.g., 20-h acid hydrolysis) methods. Identifying potential indicators for rapidly estimating dAGEs content in bakery products is therefore critical. To explore broader implications, this study also quantified dAGEs levels in various commercial bakery products and examined correlation between their dAGEs contents and nutritional components (e.g. total sugar, lipids and protein) based on product label information. By combining controlled experiments with commercial product analyses, this study seeks to provide a comprehensive understanding of factors affecting dAGE formation in bakery products.

2. Materials and methods

2.1. Chemicals and reagents

2,3-hexanedione and MGO (40 % in aqueous solution) were purchased from Sigma (St. Louis, MO, USA). DA and GO (40 % in aqueous solution) were obtained from Alfa Aesar (Ward Hill, MA, USA). 3-DG, 3-deoxygalactosone (3-DGal), and glucosone were sourced from Cayman Chemical (Ann Arbor, MI, USA). MG-H_1, glyoxal-hydroimidazolone 1 (G-H_1), CEL, CML, and d_4-CML were purchased from Iris Biotech (Marktredwitz, Germany). All solvents used in this study were of LC-MS grade and purchased from Sigma. Packaged bakery products (pancakes, rye bread, croissants, pineapple bread, toast, bagels, and cakes), sugar, olive oil, butter, salt, baker's yeast, and wheat flour were sourced from a local supermarket (Taipei, Taiwan).

2.2. Bread preparation

The bread ingredients included flour, sugar, butter, olive oil, baker's yeast, salt, and water. To investigate the impact of sugar content on dAGEs formation in bread, 1.2, 2.4, and 3.6 g of sucrose were mixed with 22.8, 21.6, and 20.4 g of high-gluten flour, along with 0.24 g of baker's yeast, 0.24 g of salt, and 14 g of water to form a soft dough. The detailed dough recipe is provided in **Table S1**. The weight percentages of added sucrose were 5 %, 10 %, and 15 % relative to the total weight of flour and sucrose. The dough was kneaded continuously by hand for 5 min until it became smooth and elastic. Similarly, varying amounts of butter (1.2, 2.4, and 3.6 g), predominantly composed of saturated fats, or olive oil (1.2, 2.4, and 3.6 g), predominantly composed of unsaturated fats, were incorporated along with the other ingredients to evaluate the effects of different fat compositions on dAGE formation in bread. The selection of 1.2, 2.4, and 3.6 g as the addition levels for sucrose, olive oil, and butter was based on findings from preliminary studies. Specifically, we observed that when the amount of olive oil exceeded 3.6 g, it could not be fully incorporated into the dough, affecting the uniformity of the bread formulation. To ensure consistency and comparability across all factors, we set the maximum concentration of sucrose, olive oil, and butter at 3.6 g. Bread preparation for each treatment was conducted in triplicates. To assess the impact of protein content on dAGE formation, high-gluten flour was replaced with 50 % or 100 % medium- and low-gluten flour (as detailed in Table S1). For the first rise, the dough rested at 37 °C for 40 min. Subsequently, the bulk dough was shaped and rested again at 37 °C for another 30 min for the second rise. Before placing the dough in the oven, the oven was preheated to 200 °C for 5 min to ensure a consistent internal environment and minimize the risk of temperature variations. The dough was then baked at 200 °C for 15 min in an oven. After cooling to room temperature, the color characteristics - lightness (L^*), redness (a^*), and yellowness (b^*) - were measured on the bread using a colorimeter (Flu, Shenzhen, China). To minimize potential bias caused by the uneven color distribution on the bread surface, we divided each bread sample into four equal quadrants (Quadrants 1, 2, 3, and 4). The L^*, a^*, and b^* values were measured individually for each quadrant to capture the variability in color across the bread surface. Subsequently, the mean values of L^*, a^*, and b^* were calculated to provide a more reliable and representative color profile for each bread sample. After measuring the color profile, the bread was stored at −20 °C until further analysis.

2.3. dAGEs extraction from bakery products

The bread and commercial bakery products were cut into small pieces and 2 g of each sample was extracted twice with 10 mL of a dichloromethane/*n*-hexane (4:1, *v*/v) solution to remove fats. After adding 12 mL deionized water, the defatted sample was homogenized using a handheld homogenizer (IKA, Staufen, Germany) to prepare the homogenate for both dAGEs and α-dicarbonyls analysis. To prevent

excessive CML formation from the oxidative cleavage of fructoselysine, the homogenized sample was mixed with 2 mL of 1 M sodium borohydride and 1 mL of 0.2 M sodium boric acid, then allowed to stand at room temperature for 4 h. Subsequently, acid hydrolysis was performed by adding 1.5 mL of 12 M HCl, followed by heating the mixture at 110 °C for 20 h. The hydrolysate was spiked with 2 μL of d4-CML as an internal standard and then dried using a SpeedVac evaporator (Thermo Scientific, Waltham, MA, USA). The dried residue was reconstituted in a 50 % acetonitrile aqueous solution and filtered through a nylon filter before LC-MS analysis.

2.4. Quantitation of dAGEs

Determination of dAGEs was performed using a Waters UPLC system coupled with a Waters Xevo TQ-XS triple quadrupole mass spectrometer (Milford, MA, USA). Analytes were chromatographically separated using an Acquity BEH amide (2.1 × 100 m, 1.7 μm, Waters). The mobile phase consisted of (A) 5 mM ammonium formate in water:acetonitrile (98:2, *v*/v) containing 0.1 % formic acid and (B) 5 mM ammonium formate in water:acetonitrile (5:95, v/v). The gradient of the mobile phase was set as follows: 0–15 min, 100–30 %, B; 15–20 min, 30 % B; 20–21 min, 30–100 % B; 21–26 min, 100 % B. The injection volume and flow rate were set at 2 μL and 0.3 mL/min, respectively. The column oven temperature was set at 60 °C. The selected reaction monitoring (SRM) transitions, retention time, cone voltage, and collision energy of AGEs are provided in **Fig. S1** and **Table S2**. capillary voltage at 3900 V, drying gas temperature at 600 °C, drying gas flow rate at 800 L/h, and nebulizer gas pressure at 7 bar. Data analysis was conducted using Masslynx software (Waters). The limit of detection (LOD), limit of quantitation (LOQ), and linearity of dAGEs are provided in **Table S3**.

2.5. Quantitation of α-dicarbonyls

A derivatization reaction was required before analyzing α-dicarbonyls, including glucosone, 3-DG, 3-DGal, MGO, GO and DA. An aliquot (30 μL) of the homogenate prepared from dAGEs extraction was mixed with 2 μL 2,3-hexanedione as an internal standard, and 120 μL of *o*-phenylenediamine dissolved in 1.6 M perchloric acid (1 mg/mL). The mixture was allowed to stand for 20 h at room temperature, and α-dicarbonyls were derivatized to the corresponding quinoxalines. The mixture was filtered through a 0.22 μm nylon filter before LC-MS analysis. Quantitation of α-dicarbonyls was performed using a Waters UPLC system coupled with a Waters Xevo TQ-XS triple quadrupole mass spectrometer. Chromatographic separations of analytes were achieved using a Water Acquity BEH C18 column (2.1 × 100 m, 1.7 μm, Waters). The injection volume and flow rate were set at 2 μL and 0.2 mL/min, respectively. The column oven temperature was set at 30 °C. The mobile phase contained (A) 0.1 % formic acid in water and (B) 0.1 % formic acid in acetonitrile. The gradient of the mobile phase was set as follows: 0–12 min, 2–15 % B; 12–16 min, 15–100 % B; and 16–18 min, 100 % B. The selected reaction monitoring (SRM) transitions, retention time, cone voltage, and collision energy of α-dicarbonyls are provided in **Fig. S2** and **Table S2.** The global MS parameters were set as follows: capillary voltage at 3900 V, drying gas flow at 800 L/h, drying gas temperature at 600 °C, and nebulizer gas pressure at 7 bar. The LOD, LOQ, and linearity of the α-dicarbonyls are provided in **Table S3**.

2.6. Quantitation of glucose, fructose, and sucrose

Before sugar analysis, an aliquot (500 μL) of the bread homogenate was mixed with 500 μL methanol. Fructose, glucose, and sucrose were quantitated using a UPLC system (Acquity H-Class, Waters) coupled with a single-quadrupole mass spectrometer (Acquity QDa, Waters). Chromatographic separations were performed using an Acquity BEH amide (2.1 × 100 m, 1.7 μm, Waters). The mobile phase consisted of (A) water:acetonitrile (7:3, *v*/v) containing 0.1 % ammonium hydroxide and (B) water:acetonitrile (2:8, v/v) containing 0.1 % ammonium hydroxide. The gradient program was set as follows: 0–6 min, 100–50 % B; 6–8 min, 50 % B; 8–8.1 min, 50–100 % B; 8.1–13 min, 100 % B. The injection volume and flow rate were set at 2 μL and 0.2 mL/min, respectively. The column oven temperature was set at 30 °C. The bread's sugar contents were quantified using selected ion recording (SIR) mode. The negative ions for quantitation of monosaccharides (glucose and fructose) and sucrose were set at *m/z* 179.2 and *m/z* 341.3, respectively. The retention time of fructose, glucose and sucrose were 3.9, 4.4 and 5.6 min, respectively. The global MS parameter settings were as follows: cone voltage at 15 V, capillary voltage at 0.8 kV, probe temperature at 600 °C, and a target sampling rate of 10 points/*s*. Data processing was performed using Empower 2 software (Waters).

2.7. Quantitation of amino acids

For the quantitation of amino acids, 2 g of the flour sample was suspended in 12 mL of deionized water, followed by the addition of 12 mL of 12 M HCl. The mixture was hydrolyzed at 110 °C for 20 h to prepare the hydrolysate. An aliquot (20 or 200 μL) of the hydrolysate was then dried using a SpeedVac evaporator. The dried residue was reconstituted in 2 mL 50 % acetonitrile aqueous solution and filtered through a nylon filter before LC-MS (Acquity QDa) analysis. The column and mobile phase used for amino acid analysis were the same as those used for AGE analysis. The oven temperature and flow rate were set at 60 °C and 0.2 mL/min, respectively. The gradient of the mobile phase was set as follows: 0–10 min, 100–90 % B; 10–16 min, 90–80 % B; 16–20 min, 80–50 % B, 20–24 min, 50 % B. Determination of amino acids were achieved using the SIR mode. The precursor ions and retention time of amino acids are given in **Table S4**. The global MS parameter settings were as follows: cone voltage at 15 V, capillary voltage at 0.8 kV, probe temperature at 600 °C, and a target sampling rate of 6.7 points/*s*. Data processing was carried out using Empower 2 software (Waters).

2.8. Statistical analysis

Data are expressed as means ± standard deviations ($n = 3$). Statistical differences were detected using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test ($p < 0.05$). Pearson's correlation analysis was performed using SPSS software (IBM, Armonk, NY, USA).

3. Results and discussion

3.1. dAGEs formation in control bread sample

In this study, varying amounts of sucrose, butter, olive oil, medium-, and low-gluten flour were incorporated into the dough to investigate their effects on the formation of dAGEs in bread. To evaluate the specific impacts of sucrose, butter, and olive oil on dAGE formation, a control sample was prepared without the addition of these ingredients. After baking, the control samples contained 17.78 μg/g of CML and 2.33 μg/g of CEL, respectively (Table 1). CML and CEL are the most abundant lysine-derived dAGEs, commonly found in both animal- and plant-derived food products, such as meats and bakery goods (Hu et al., 2022; Jost et al., 2021; Lin et al., 2023). Also, the level of CML is often used as an indicator of total dAGEs in food products (Gómez-Ojeda et al., 2018). In addition to lysine-derived dAGEs, arginine-derived dAGEs, including MG-H_1 and G-H_1, were also quantified. Our results indicated that the levels of MG-H_1 and G-H_1 in the no-sucrose-added bread samples were 13.94 μg/g and 5.17 μg/g, respectively (Table 1). These results suggest that MG-H_1 and CML are the most prevalent dAGEs found in the control bread sample. Similarly, Jost et al. (2021) also demonstrated that CML and MG-H_1 were the prevalent AGEs in bread, supporting our results. Additionally, a recent comprehensive review summarized the

Table 1
Effects of sucrose, butter, olive oil, and gluten protein on dAGEs formation in bread.

	AGEs (μg/g)					Amino acids (mg/g)	
μg/g	CML	CEL	MG-H_1	G-H_1	Total dAGEs	Arginine	Lysine
Sucrose							
0 %	17.78 ± 1.87^{a}	2.33 ± 0.26^{a}	13.94 ± 1.52^{a}	5.17 ± 1.34^{a}	39.22 ± 3.38^{a}	3.54 ± 0.09^{a}	0.96 ± 0.03^{a}
5 %	18.39 ± 0.31^{a}	3.33 ± 0.49^{b}	36.52 ± 2.15^{b}	4.59 ± 0.54^{a}	62.83 ± 2.60^{b}	3.31 ± 0.08^{ab}	0.70 ± 0.02^{b}
10 %	18.17 ± 1.26^{a}	4.30 ± 0.15^{b}	32.36 ± 7.25^{b}	4.67 ± 1.10^{a}	59.51 ± 5.21^{b}	3.37 ± 0.21^{ab}	0.71 ± 0.04^{b}
15 %	19.93 ± 2.72^{a}	5.46 ± 0.50^{c}	48.22 ± 3.79^{c}	5.07 ± 0.62^{a}	78.69 ± 1.76^{c}	3.12 ± 0.14^{b}	0.65 ± 0.05^{b}
Butter							
0 %	16.89 ± 1.75^{a}	2.33 ± 0.18^{a}	13.84 ± 1.28^{a}	5.45 ± 0.76^{a}	38.51 ± 3.71^{a}	3.66 ± 0.13^{a}	0.97 ± 0.03^{a}
5 %	16.98 ± 0.17^{a}	2.07 ± 0.21^{a}	13.24 ± 3.37^{a}	4.47 ± 0.39^{a}	36.77 ± 3.44^{a}	3.77 ± 0.05^{a}	0.99 ± 0.02^{a}
10 %	16.97 ± 1.29^{a}	2.05 ± 0.20^{a}	11.32 ± 1.62^{a}	4.74 ± 0.49^{a}	35.08 ± 2.62^{a}	3.57 ± 0.21^{a}	0.93 ± 0.04^{a}
15 %	13.31 ± 0.87^{a}	2.02 ± 0.34^{a}	11.35 ± 0.64^{a}	5.44 ± 0.61^{a}	32.12 ± 1.35^{a}	3.54 ± 0.09^{a}	0.92 ± 0.01^{a}
Olive oil							
0 %	16.07 ± 2.22^{a}	2.27 ± 0.22^{a}	13.66 ± 1.34^{a}	4.11 ± 1.43^{a}	36.11 ± 2.85^{a}	3.57 ± 0.31^{a}	0.91 ± 0.11^{a}
5 %	15.17 ± 1.20^{a}	2.01 ± 0.06^{a}	13.37 ± 0.18^{a}	4.08 ± 0.15^{a}	34.62 ± 1.11^{a}	3.54 ± 0.10^{a}	0.94 ± 0.05^{a}
10 %	15.48 ± 2.18^{a}	1.97 ± 0.58^{a}	13.98 ± 3.73^{a}	3.84 ± 0.85^{a}	35.27 ± 3.78^{a}	3.51 ± 0.15^{a}	0.95 ± 0.02^{a}
15 %	16.68 ± 0.87^{a}	2.53 ± 0.43^{a}	12.91 ± 1.67^{a}	3.97 ± 0.49^{a}	36.09 ± 2.47^{a}	3.35 ± 0.19^{a}	0.89 ± 0.05^{a}
Gluten (Low/medium/high)							
0/0/100	15.64 ± 1.11^{a}	2.92 ± 0.29^{a}	13.22 ± 0.22^{a}	6.71 ± 3.07^{ab}	38.49 ± 3.35^{a}	3.67 ± 0.04^{a}	0.99 ± 0.02^{a}
0/100/0	13.52 ± 2.09^{ab}	1.92 ± 0.15^{b}	9.36 ± 0.20^{b}	8.26 ± 1.39^{a}	33.06 ± 3.60^{ab}	3.36 ± 0.31^{ab}	0.98 ± 0.09^{a}
50/50/0	15.43 ± 0.39^{a}	1.84 ± 0.17^{bc}	8.23 ± 0.24^{c}	5.52 ± 0.75^{ab}	31.02 ± 0.30^{b}	3.17 ± 0.15^{b}	0.94 ± 0.05^{a}
100/0/0	11.52 ± 1.74^{b}	1.38 ± 0.15^{c}	5.18 ± 0.37^{d}	2.83 ± 0.13^{b}	20.91 ± 2.36^{c}	2.55 ± 0.15^{c}	0.77 ± 0.06^{b}

Total contents of dAGEs are calculated as the sum of contents of CML, CEL, MG-H_1 and G-H_1. Data are expressed as means ± SDs. In the same treatment group, different alphabet letters in the same column represent significant differences ($p < 0.05$).

occurrence of dAGEs in 10 types of commonly consumed food items, highlighting that MG-H1/3 and CML were the predominant AGEs across most food types, while CEL is commonly present but at lower concentrations (Zhang et al., 2024).

3.2. α-Dicarbonyls formation in control bread sample

Since α-dicarbonyls are the primary precursors of dAGEs, 3-DG, 3-DGal, glucosone, DA, MGO, and GO in the control bread sample were also quantified. The levels of 3-DG, 3-DGal, glucosone, DA, MGO, and GO in the sugar-free bread were 7.89, 1.07, 0.61, 0.68, 7.87, and 3.54 μg/g, respectively (Table 2). Previously, a comprehensive analysis of 12 dicarbonyls during commercial bread production reported that the contents of 3-DG, glucosone, MGO, and GO in bread were 38, 29, 4.1, and 2 μg/g, respectively (Jost et al., 2021). The levels of α-dicarbonyls were also compared in bread crust-like systems prepared with different cereal flours. After baking at 200 °C for 30 min, the concentrations of MGO, GO, DA, glucosone, and 3-DG in bread crust prepared with whole

Table 2
Effects of sucrose, butter, olive oil, and gluten protein on α-dicarbonyls formation in bread.

μg/g	3-DG	3-DGal	Glucosone	DA	MGO	GO	Total
Sucrose							
0 %	7.89 ± 0.54^{a}	1.07 ± 0.08^{a}	0.61 ± 0.11^{a}	0.68 ± 0.04^{a}	7.87 ± 0.05^{a}	3.54 ± 0.53^{a}	21.66 ± 0.91^{a}
5 %	84.54 ± 4.29^{b}	2.70 ± 0.07^{b}	12.87 ± 0.52^{b}	1.04 ± 0.04^{b}	13.23 ± 0.55^{b}	5.66 ± 0.04^{b}	120.03 ± 4.03^{b}
10 %	152.87 ± 5.04^{c}	5.70 ± 0.38^{c}	24.37 ± 1.86^{c}	1.15 ± 0.05^{c}	15.84 ± 0.48^{c}	6.95 ± 0.25^{c}	206.87 ± 3.23^{c}
15 %	158.85 ± 7.41^{c}	7.98 ± 0.36^{d}	23.98 ± 1.94^{c}	1.25 ± 0.03^{d}	15.95 ± 0.73^{c}	8.25 ± 0.59^{d}	216.26 ± 6.63^{c}
Butter							
0 %	9.71 ± 1.02^{a}	1.26 ± 0.06^{a}	0.51 ± 0.02^{a}	0.68 ± 0.0^{a}	7.62 ± 0.21^{a}	3.62 ± 0.27^{a}	23.41 ± 0.92^{a}
5 %	9.62 ± 0.30^{a}	1.33 ± 0.05^{a}	0.55 ± 0.03^{a}	0.64 ± 0.09^{a}	7.16 ± 0.50^{ab}	3.44 ± 0.18^{ab}	22.75 ± 0.47^{a}
10 %	9.61 ± 0.20^{a}	1.32 ± 0.03^{a}	0.51 ± 0.06^{a}	0.64 ± 0.07^{a}	6.48 ± 0.45^{ab}	3.31 ± 0.10^{ab}	21.87 ± 0.62^{a}
15 %	10.06 ± 0.53^{a}	1.45 ± 0.13^{a}	0.55 ± 0.03^{a}	0.67 ± 0.05^{a}	6.22 ± 0.56^{b}	2.94 ± 0.39^{b}	21.89 ± 0.39^{a}
Olive oil							
0 %	9.59 ± 1.29^{a}	1.54 ± 0.07^{a}	0.54 ± 0.03^{a}	0.84 ± 0.04^{a}	8.66 ± 0.30^{a}	4.63 ± 0.58^{a}	25.80 ± 1.86^{a}
5 %	8.41 ± 0.28^{ab}	1.35 ± 0.22^{ab}	0.56 ± 0.05^{a}	0.66 ± 0.10^{b}	7.46 ± 0.87^{a}	4.09 ± 0.39^{a}	22.53 ± 1.42^{ab}
10 %	5.73 ± 0.52^{bc}	1.05 ± 0.43^{ab}	0.44 ± 0.05^{ab}	0.63 ± 0.05^{b}	8.29 ± 0.90^{a}	4.56 ± 2.86^{a}	20.71 ± 3.39^{ab}
15 %	5.64 ± 1.55^{c}	0.87 ± 0.12^{b}	0.33 ± 0.06^{b}	0.63 ± 0.01^{b}	7.74 ± 0.77^{a}	4.89 ± 0.68^{a}	20.10 ± 0.24^{b}
Gluten (Low/medium/high)							
0/0/100	7.66 ± 1.02^{a}	0.97 ± 0.16^{a}	0.62 ± 0.14^{a}	0.75 ± 0.04^{a}	8.44 ± 0.66^{a}	4.89 ± 0.55^{a}	23.34 ± 1.47^{a}
0/100/0	5.36 ± 0.29^{b}	0.85 ± 0.05^{ab}	0.62 ± 0.05^{a}	0.51 ± 0.03^{b}	7.86 ± 0.16^{a}	3.46 ± 0.29^{b}	18.67 ± 0.59^{b}
50/50/0	4.91 ± 0.31^{b}	0.76 ± 0.01^{ab}	0.45 ± 0.03^{a}	0.44 ± 0.02^{bc}	6.28 ± 0.15^{b}	2.81 ± 0.07^{b}	15.64 ± 0.37^{c}
100/0/0	4.23 ± 0.41^{b}	0.71 ± 0.03^{b}	0.54 ± 0.02^{a}	0.37 ± 0.03^{c}	5.31 ± 0.39^{b}	2.76 ± 0.15^{b}	13.93 ± 0.31^{c}

Data are expressed as means ± SDs. Different alphabet letters in the same column represent significant differences ($p < 0.05$).

wheat flour were 14.99, 6.36, 11.96, 0.50, and 6.33 μg/g, respectively (Çelik & Gökmen, 2020). Differences in α-dicarbonyl contents might result from variations in dough ingredients and the thermal processing conditions. Since formation of α-dicarbonyls is linked to the MR reaction, the levels of glucose and fructose in the control bread sample were also quantitated, showing 0.2 mg/g of glucose and 0.77 mg/g of fructose (Table 3). As no sugar was added to the control dough, glucose and fructose primarily formed from starch degradation by endogenous amylases. α-Amylase hydrolyzes starch into maltodextrins, which β-amylase further breaks down into maltose. Yeast maltase then converts maltose into glucose. Additionally, wheat flour contains small amounts of glucose, fructose, and maltose (Sahlström et al., 2004; Struyf et al., 2017). The formation mechanism of glucose in the control dough is illustrated in **Fig. S3**.

3.3. Effects of adding sugar on dAGEs and α-dicarbonyls formation in bread

Since sugar is often added to dough to enhance the sensory characteristics of bakery products, this study investigated the effects of sucrose on dAGE formation. When sucrose was added, the L^* and b^* values significantly decreased compared to bread without added sugar (**Fig. S4 and Table S5**), indicating that the bread's color became darker and less yellow. Additionally, the decreased a^* value in the sucrose-added bread indicated increased redness. In the dAGE analysis, the levels of MG-H_1 and CEL significantly increased in a concentration-dependent manner with the addition of 5 %, 10 %, and 15 % sucrose to the dough (Table 1). The MG-H_1 and CEL levels in the 0 % sucrose-added bread were 13.94 and 2.33 μg/g, respectively, while the 15 % sucrose-added bread contained 48.22 and 5.46 μg/g of MG-H_1 and CEL, respectively. In contrast, the levels of G-H_1 and CML did not significantly change with the addition of 5 %, 10 %, and 15 % sucrose. These results suggest that sucrose specifically promoted the formation of MGO-derived dAGEs (CEL and MG-H_1), while GO-derived dAGEs (CML and G-H_1) were not significantly affected. Meanwhile, the results for α-dicarbonyls showed that both MGO and GO significantly increased with the addition of 5 %, 10 %, and 15 % sucrose (Table 2). The increased levels of dAGEs observed only in CEL and MG-H_1 might be attributed to the high reactivity of MGO in protein glycation. In a bovine serum albumin (BSA)-based system, Sadowska-Bartosz et al. (2014) found that the fluorescent AGEs formed from the glycation reaction of BSA and MGO was significantly higher than those formed from the reaction of BSA and GO (Sadowska-Bartosz et al., 2014). A similar study also demonstrated that fluorescent AGE formation increased rapidly and reached a plateau when BSA was incubated with MGO at 37 °C, whereas the formation rate of fluorescent AGEs was relatively slow when BSA was incubated with GO (Li et al., 2014). These findings suggest that MGO, rather than GO, is the primary α-dicarbonyl responsible for protein glycation.

Along with MGO and GO, the levels of 3-DG, 3-DGal, glucosone, and DA in the bread also significantly increased when sucrose was added (Table 2). The formation of α-dicarbonyls primarily originates from the degradation reactions of monosaccharides during the MR and caramelization reaction (Hellwig et al., 2018). To understand why α-dicarbonyls significantly increased in sucrose-added bread, the contents of glucose, fructose, and sucrose were quantified (Table 3). The addition of 5 %, 10 %, and 15 % sucrose led to a significant increase in the concentrations of glucose, fructose, and sucrose in the bread compared to those without added sucrose. In the 15 % sucrose-added bread, the glucose, fructose, and sucrose contents were 8.73, 14.44, and 42.34 mg/g, respectively. The increased levels of glucose and fructose are likely due to the thermal degradation of sucrose during baking, which results in the formation of glucose and fructose (Richards & Shafizadeh, 1978). Additionally, invertase secreted by *S. cerevisiae* during fermentation catalyzes the hydrolysis of sucrose to produce glucose and fructose (Struyf et al., 2017). An earlier study indicated that the levels of glucose and fructose in the dough increased with prolonged fermentation time, while the sucrose content decreased (Potus et al., 1994). Interestingly, they also found that the glucose content was lower than that of fructose, which aligns with our current findings (Table 3). This is because glucose is the preferred substrate for baker's yeast during fermentation (Potus et al., 1994). Meanwhile, lysine and arginine, as primary reactive amino acids involved in the MR with MGO and GO, were also quantified in this study. As sucrose concentrations increased from 0 % to 15 %, the levels of both lysine and arginine decreased significantly (Table 3). This can be attributed to the fact that higher sucrose concentrations promoted the MR by providing reducing sugars through hydrolysis, which enhanced the formation of reactive intermediates such as MGO and GO. These intermediates readily reacted with lysine and arginine to form AGEs, resulting in a significant increase in total AGEs and a concurrent depletion of lysine and arginine. Overall, the addition of sucrose significantly increased the levels of glucose and fructose in the dough during fermentation, which further enhanced the reaction rate of the MR, leading to the generation of α-dicarbonyls and MGO-derived dAGEs during the baking process (Fig. 1).

Table 3
The levels of glucose, fructose, and sucrose in the bread with added sucrose.

mg/g	Glucose	Fructose	Sucrose	Total sugar
Sucrose				
0 %	0.20 ± 0.07^a	0.77 ± 0.07^a	0.12 ± 0.01^a	1.09 ± 0.15^a
5 %	5.43 ± 0.30^b	9.74 ± 0.23^b	4.14 ± 0.39^b	19.31 ± 0.43^b
10 %	8.45 ± 0.15^c	14.56 ± 0.50^c	19.86 ± 0.44^c	42.88 ± 0.39^c
15 %	8.73 ± 0.19^c	14.44 ± 0.25^c	42.34 ± 0.39^d	62.51 ± 0.46^d

Data are expressed as means ± SDs from triplicates. Different letters in the same column represent statistical differences among bread samples ($p < 0.05$).

3.4. Effects of adding butter and olive oil on dAGEs and α-dicarbonyls formation in bread

Animal fats and vegetable oils are commonly added during dough preparation to improve the taste and texture of bread. In this study, butter and olive oil were incorporated into the dough to investigate the effects of saturated and unsaturated fatty acids on the formation of α-dicarbonyls and dAGEs in bread. Research has shown that the addition of lipids significantly affects dAGE formation in model systems and food products (Li et al., 2023; Wang et al., 2019). However, our results indicated that the levels of α-dicarbonyls, AGEs, lysine and arginine did not significantly change with the addition of 5 %, 10 %, or 15 % butter or olive oil (Table 1 **and** Table 2). Previous studies have reported varying effects of different types of fatty acids and triacylglycerols on dAGE formation in model systems. For example, the addition of oleic acid, linoleic acid, linolenic acid, triolein, and trilinolein significantly promoted CML and CEL formation in a model system (Wang et al., 2019). Conversely, the formation of CML significantly decreased in a casein-based model system heated at 95 °C when unsaturated fatty acids were added (Lima et al., 2010). Moreover, the addition of oxidized linoleic acid inhibited CML and CEL formation in an MR model system containing glucose and lysine (Yu et al., 2016). In contrast, it promoted CML and CEL formation when lysine was replaced with myofibrillar protein. Additionally, Shen et al. (2022) found that incorporating fish oil significantly increased CML formation in silver carp surimi sausages, while coconut oil showed no significant effect (Shen et al., 2022). Although previous studies highlighted the role of specific fatty acids in promoting or inhibiting dAGE formation under controlled conditions, our findings indicate that the addition of butter or olive oil had no significant impact on dAGE formation in bread. These discrepancies could be attributed to differences in experimental models and thermal processing conditions. Most previous studies primarily focused on the effects of specific fatty acids in simplified model systems, whereas butter and olive oil are complex lipid matrices primarily composed of triacylglycerols, containing a mix of saturated and unsaturated fatty acids.

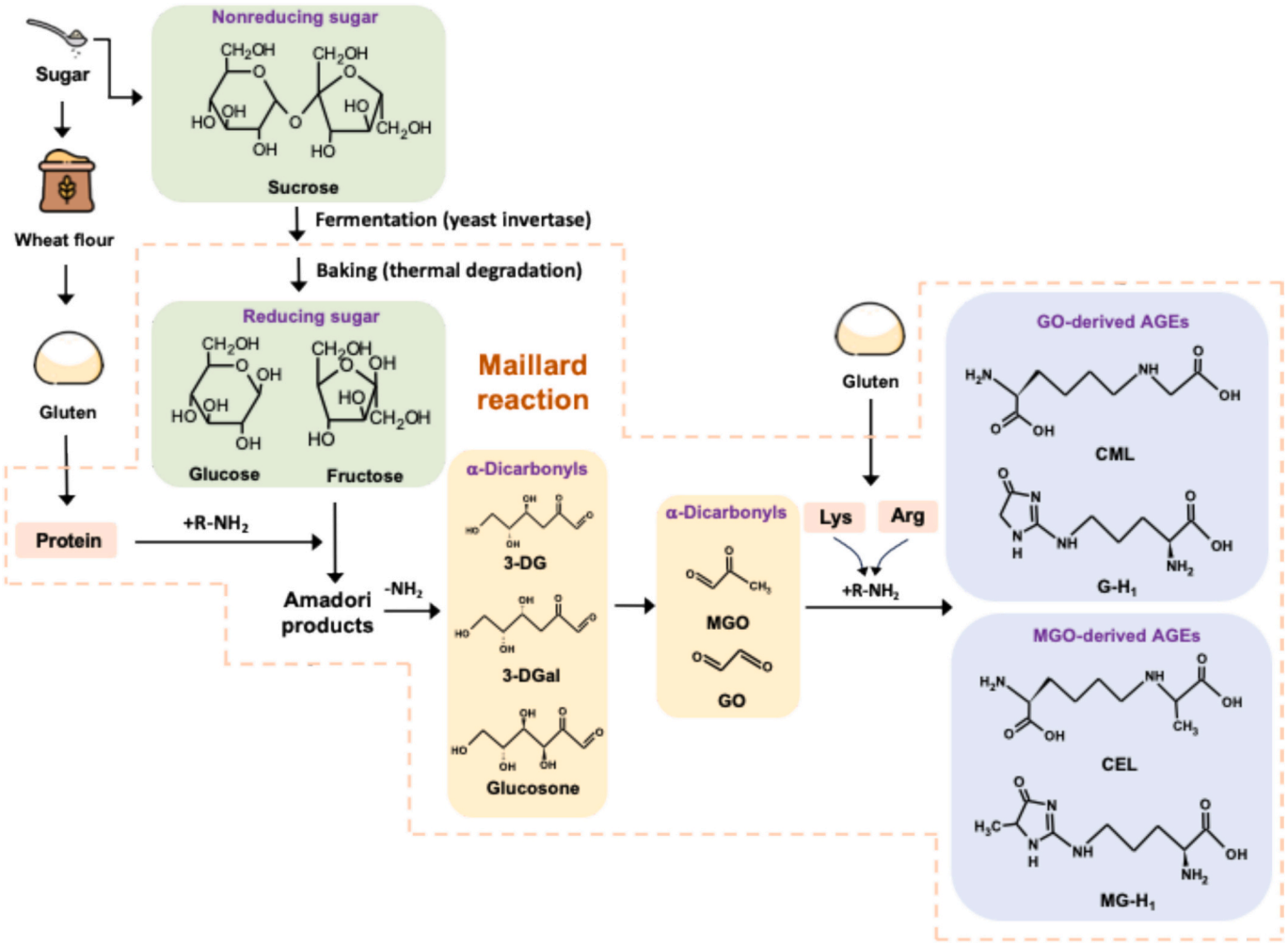

Fig. 1. Proposed mechanisms of added sugar and gluten protein on α-dicarbonyls and dAGEs formation in bread. Lys denotes lysine, and Arg denotes arginine. When sucrose is added to the flour and then kneaded into dough, it is hydrolyzed by yeast invertase during fermentation to produce glucose and fructose. Additionally, sucrose in the dough can undergo thermal degradation during baking, further generating glucose and fructose. These reducing sugars facilitate the Maillard reaction, promoting the formation of α-dicarbonyls and dAGEs. Meanwhile, high-gluten flour provides abundant amino groups, which further enhance the Maillard reaction and contribute α-dicarbonyls and dAGEs formation.

The formation of dAGEs in bread may depend on the structural features of lipids, such as their degree of saturation and solubility under specific baking conditions. Consistent with our findings, a recent study demonstrated that lipid peroxidation only significantly influenced malondialdehyde-derived protein modifications in wafers, with minimal influence on the formation of GO- and MGO-derived dAGEs (Eggen et al., 2022). Malondialdehyde-derived protein modifications significantly change due to lipid peroxidation because malondialdehyde is a direct product of polyunsaturated fatty acid oxidation and reacts readily with lysine and arginine residues to form advanced lipoxidation end products. In contrast, GO and MGO are primarily generated from carbohydrate degradation and glycolytic pathways, with minimal contribution from lipid peroxidation, resulting in no significant changes under lipid oxidation conditions.

3.5. *Effects of gluten protein on dAGEs and α-dicarbonyls formation in bread*

Since the MR initiates from the reaction between reducing sugars and amino groups, making the protein content of flour a critical factor influencing dAGE formation in bread. Additionally, the protein content can significantly affect the sensory attributes of bread. High-gluten flour is typically used in bread preparation. To evaluate the effects of protein content on dAGE formation, high-gluten flour was partially or entirely substibuted with medium- or low-gluten flour during bread making. Our analysis showed that replacing high-gluten flour with medium- or low-gluten flour significantly reduced the levels of both MGO- and GO-derived dAGEs (Table 1). The total dAGE content in bread prepared with high-gluten flour was 38.49 μg/g, whereas bread made with low-gluten flour contained only 20.91 μg/g of dAGEs. Similarly, the concentrations of all α-dicarbonyls significantly decreased when high-gluten flour was substituted with medium- or low-gluten flour (Table 2). For instance, bread made with high-gluten flour had a total α-dicarbonyl content of 23.34 μg/g, compared to 13.93 μg/g in bread made with low-gluten flour.

Color browning, a reliable indicator of the MR, also supported these findings. Substitution of high-gluten flour with medium- or low-gluten flour produced lighter-color bread, as reflected by increased L^* values (**Fig. S4 and Table S5**). The lower a^* and b^* values observed in bread made with medium- or low-gluten flour indicated that the bread became less yellow and red compared to bread made with high-gluten flour. These results suggest that the MR occurred more extensively in bread made with high-gluten flour than in bread made with medium- or low-gluten flour.

The primary difference between low-, medium-, and high-gluten flour lies in their protein content. According to the nutritional labels,

low-, medium-, and high-gluten flours contained 7.5, 12.0, and 13.3 g of protein per 100 g, respectively (**Table S6**). Amino acid analysis showed that all amino acids were present at higher levels in high-gluten flour, with total amino acid concentration of 99.2 mg/g in high-gluten flour, compared to 77.36 mg/g and 58.77 mg/g in medium- and low-gluten flours (Table 4). These higher amino acid levels in high-gluten flour likely provided more amino groups to facilitate the MR, resulting in greater α-dicarbonyl production. Notably, lysine and arginine levels in high-gluten flour were significantly higher in high-gluten flour than in medium- or low-gluten flour (Table 4). Consequently, bread made with high-gluten flour contained higher levels of lysine and arginine compared to bread made with low-gluten flour (Table 1). As key reactants with MGO and GO, lysine and arginine play critical roles in dAGE formation. Therefore, the higher dAGE content observed in bread made with high-gluten flour can be primarily attributed to the higher concentrations of lysine and arginine in high-gluten flour. Supporting this, Shen et al. (2019) demonstrated that the formation of melanoidins in white pan bread increased significantly with higher amino acid levels in the flour (Shen et al., 2019). Similarly, Ma et al. (2012) showed that the production of Maillard-type volatiles was positively correlated lysine levels in an aqueous model system (Ma et al., 2012). Collectively, the higher amino acid concentrations in high-gluten flour enhanced the MR, leading to increased production of α-dicarbonyls, which subsequently reacted with lysine and arginine to produce dAGEs (Fig. 1).

3.6. Contents of dAGEs and α-dicarbonyls in commercial bakery products

Since this study found that sugar and protein contents in the dough significantly affected dAGE formation in bread, we aimed to identify potential indicators of dAGEs from the nutritional fact labels of various commercial bakery products, with a particular focus on their sugar, lipid, and protein contents. To establish reliable indicators of dAGEs, both fermented and non-fermented bakery products made with low-, medium-, and high-gluten flour, and containing varying amounts of added sugar and fats, were included. A total of nine groups of commercial bakery products were selected for α-dicarbonyl and dAGE quantifications, including pancakes, rye bread, croissants, pineapple bread, toast, bagels, and cakes. Pancakes and cakes are typically made with low- and medium-gluten flour, while rye bread, croissants, pineapple bread, toast, and bagels are usually made with medium- and high-gluten flour. Additionally, the products were categorized based on their fat and sugar content. Croissants, pineapple bread, and cakes typically contain high levels of fat, while bagels and toast have relatively low fat content. Similarly, pineapple bread and cakes are rich in sugar, whereas rye bread and toast tend to have lower sugar content (Table S7). Since this study focused on packaged bakery products with nutritional fact labels, the availability of certain products was limited, with only three samples of pancakes and rye bread were obtainable from the market. In the analysis of dAGEs, both lysine- and arginine-derived dAGEs, including CML, CEL, MG-H_1, and G-H_1, were detected in all commercial bakery products. MG-H_1 and CML were the most abundant dAGEs found in these products, followed by CEL and G-H_1. The highest levels of CML, CEL, MG-H_1, and G-H_1 were found in cake samples (54.94, 41.67, 76.40, and 8.95 μg/g, respectively), while the lowest levels of CML, CEL, MG-H_1, and G-H_1 were observed in bagels, toast, pineapple bread, and toast, respectively (3.73, 2.58, 9.89, and 3.91 μg/g)(Table 5). The total dAGEs contents, calculated as CML + CEL + MG-H_1 + G-H_1, in different types of bakery products are provided in Fig. 2. The highest amount of total dAGEs was found in the cake (172.31 μg/g), while the toast sample contained the lowest level of total dAGEs (24.23 μg/g). The mean total dAGE content in cake samples was 90.51 μg/g, which was significantly higher than that in pancakes, rye bread, pineapple bread, toast, and bagels. Previously, Jost et al. (2021) analyzed dAGE levels in different types of bread and found that bread products contained 2.1–8.1, 13–27, and 4.5–10.4 mg/kg of CEL, MG-H_1, and CML, respectively (Jost et al., 2021). Similarly, Zhou et al. (2015) reported that white bread, roughage bread, and whole-meal bread contained 4.69, 6.01, and 5.20 mg/kg of CEL, respectively (Zhou et al., 2015). These previous findings are consistent with our current results for rye bread samples. Notably, a wide range of total dAGE content was observed in the cake samples. The lowest and highest dAGE contents in the cake samples were 45.48 and 172.31 μg/g, respectively (Fig. 2). A previous study also showed that the levels of CML in the cake samples ranged from 16.1 to 171 μg/g (Zhou et al., 2015). This wide variation in dAGE content in cake samples is possibly due to differences in the types and amounts of added sugar (Srey et al., 2010).

In the analysis of α-dicarbonyls, glucosone, 3-DG, 3-DGal, GO, MGO, and DA were detected in all bakery products. Among them, 3-DG was the most abundant α-dicarbonyl found, followed by glucosone, 3-DGal, and MGO (Table 5). The highest levels of 3-DG were observed in pancakes and rye bread (315.31 and 272.14 μg/g, respectively), while the lowest levels were found in toast and rye bread (14.44 and 16.52 μg/g, respectively). In contrast to 3-DG, the highest levels of MGO and GO were found in cakes and croissants, respectively. The total α-dicarbonyl content, calculated as the sum of glucosone, 3-DG, 3-DGal, MGO, GO, and DA, in different types of bakery products is also presented in Fig. 2. The highest mean values of total α-dicarbonyls were found in croissants (268.8 μg/g) and bagels (256.0 μg/g), while the lowest levels were found in cakes (157.71 μg/g) and pancakes (168.37 μg/g). However, no statistically significant differences in total α-dicarbonyls were detected among the different bakery products, likely due to the large variations within the same type of product. Previously, Maasen et al. (2021) analyzed the contents of α-dicarbonyls in commonly consumed foods and drinks (Maasen et al., 2021). Their results indicated that 3-DG was the most abundant α-dicarbonyl in various food products. In addition, their study also showed that white and rye bread contained 2.6–8.0, 1.5–11, and 2.4–72 mg/kg MGO, GO and 3-DG, respectively. Degen et al. (2010) also found that bread products contained n.d – 28, 13–619 and n.d – 47 mg/kg MGO, 3-DG, and 3-DGal, respectively (Degen et al., 2012). The wide range of α-dicarbonyls found in the same type of bread products may be attributed to differences in processing conditions, such as baking temperature and the use of various condiments.

3.7. Correlations among dAGEs, α-dicarbonyls, and nutrients in commercial bakery products

The nutrient information of commercial bakery products, including protein, fat, saturated fat, carbohydrate, sugar, and sodium contents, is provided in **Table S7**. To explore the correlations among dAGEs, α-dicarbonyls, and nutrients in bakery products, Pearson's correlations were performed, and a corresponding heatmap was generated based on

Table 4
The levels of amino acids in low-, medium-, and high-gluten flour.

mg/g	Low	Medium	High
Alanine	1.42 ± 0.04^a	1.73 ± 0.06^b	2.19 ± 0.11^c
Arginine	2.08 ± 0.36^a	2.81 ± 0.09^b	3.79 ± 0.21^c
Aspartic acid	2.07 ± 0.31^a	2.37 ± 0.19^a	3.17 ± 0.30^b
Glutamic acid	22.32 ± 0.62^a	29.55 ± 0.56^b	37.53 ± 2.37^c
Glycine	2.24 ± 0.19^a	2.96 ± 0.09^b	3.65 ± 0.30^c
Histidine	1.15 ± 0.24^a	1.54 ± 0.19^a	2.09 ± 0.12^b
Leucine	3.02 ± 0.26^a	4.34 ± 0.38^b	5.42 ± 0.17^c
Isoleucine	1.85 ± 0.13^a	2.42 ± 0.21^b	3.00 ± 0.13^c
Lysine	1.34 ± 0.03^a	1.51 ± 0.03 ^{b}	2.64 ± 0.56 ^{c}
Methionine	1.24 ± 0.19^a	1.66 ± 0.08^a	2.15 ± 0.19^b
Phenylalanine	2.88 ± 0.37^a	4.67 ± 0.49^b	6.24 ± 0.25^c
Proline	9.46 ± 0.21^a	11.91 ± 0.33^b	14.54 ± 0.76^c
Serine	4.22 ± 0.14^a	5.69 ± 0.29^b	7.54 ± 0.76^c
Threonine	1.42 ± 0.08^a	1.68 ± 0.07^a	2.05 ± 0.15^b
Tyrosine	0.76 ± 0.08^a	1.05 ± 0.06^b	1.39 ± 0.09^c
Valine	1.29 ± 0.16^a	1.47 ± 0.11^a	1.80 ± 0.08^b
Total amino acids	58.77 ± 1.94^a	77.36 ± 2.16^b	99.20 ± 4.62^c

Data are expressed as means ± SDs from triplicates. Different letters in the same row represent statistical differences among flour samples ($p < 0.05$).

Table 5
Contents of dAGEs and α-dicarbonyls in commercial bakery products.

	Pancake	Rye bread	Croissant	Pineapple bread	Toast	Bagel	Cake
Sample number	3	3	5	5	12	9	9
dAGEs[a]							
CML	4.50–18.15	5.50–8.90	5.98–12.17	6.71–32.21	3.81–14.84	3.73–12.13	10.54–54.94
CEL	3.32–8.36	3.92–6.65	6.60–12.87	2.90–4.84	2.58–10.42	2.82–6.09	4.64–41.67
MG-H_1	13.86–22.41	14.46–29.28	24.58–52.23	9.89–13.35	10.68–39.34	11.14–24.86	14.71–76.40
G-H_1	4.83–7.05	6.31–6.70	4.10–6.01	5.12–6.49	3.91–7.23	4.45–6.86	3.97–8.95
α-dicarbonyls[a]							
Glucosone	3.20–36.80	3.02–58.12	18.67–38.39	15.74–42.72	4.74–139.03	36.49–88.57	7.86–36.05
3-DG	29.82–315.31	16.52–272.14	186.36–225.83	84.13–166.02	14.44–194.51	102.25–192.33	60.03–172.81
3-DGal	7.58–12.02	1.50–11.43	6.81–14.43	10.28–28.73	2.78–12.89	5.74–13.84	11.73–23.82
GO	4.30–5.94	2.71–7.04	5.59–11.60	4.77–9.00	3.27–11.04	4.98–11.32	0.74–9.70
MGO	3.37–12.60	4.37–15.78	13.89–23.14	6.70–15.21	6.33–19.67	9.42–18.99	5.03–32.70
DA	1.25–2.58	1.42–5.28	2.42–4.44	0.99–2.08	1.25–6.89	2.18–4.07	1.28–4.65
Amino acids[b]							
Arginine	2.12–2.96	2.74–3.00	1.73–2.61	1.39–2.86	1.62–3.01	2.26–3.58	1.21–3.36
Lysine	0.76–1.53	0.46–0.66	0.15–0.70	0.35–1.21	0.32–0.83	0.42–0.94	0.38–1.86

[a] dAGE and α-dicarbonyl concentrations are given as μg/g. [b]Amino acid concentrations are given as mg/g.

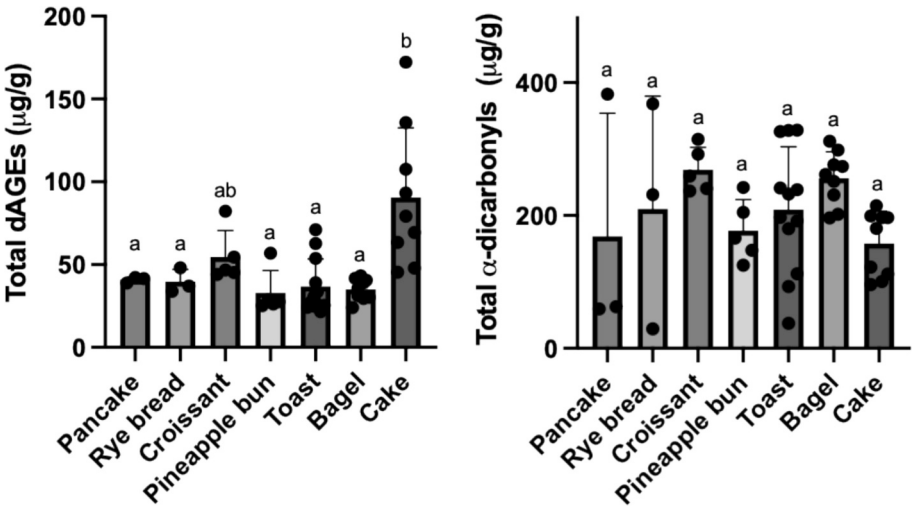

Fig. 2. Total contents of (a) dAGEs and (b) α-dicarbonyls in various types of bakery products. Data are expressed as means ± SDs. Different alphabet letters represent significant differences among different types of bakery products ($p < 0.05$).

the *p*-value matrix (Fig. 3). Total dAGE content in bakery products showed a positive relationship with carbohydrate and sugar content, while exhibiting a negative relationship with sodium content. The positive relationship between total dAGEs and sugar content is consistent with our findings, where adding sugar significantly increased dAGE formation in bread (Table 1). A recent study investigated the impact of baking factors on dAGE formation in butter cookies (Hu et al., 2022). The protein-bound CML and CEL levels in butter cookies significantly increased with the addition of 10, 15, and 20 g of sucrose. Furthermore, reducing sugars appear to have a greater impact on dAGE formation in bakery products compared to non-reducing sugars. For instance, the CEL level in sponge cake was only 5.93 mg/kg when refined sucrose was added, but it significantly increased to 86.2 mg/kg when fructose was added (Srey et al., 2010). On nutritional fact labels, it should be noted that total sugar content is calculated as the sum of glucose, fructose, galactose, maltose, lactose, and sucrose. Besides sugar content, our results also showed a positive correlation between total dAGEs and carbohydrate content. To assess this relationship further, the sugar content was subtracted from the total carbohydrate content. A negative correlation was found between total dAGEs and the adjusted carbohydrate content (**Fig. S5**). These findings confirm that the positive correlation between total dAGE content and carbohydrate content in commercial bakery products is primarily attributed to their sugar content.

Although this study found that the type of wheat flour added to the dough significantly affected dAGE formation in bread, the total dAGE content in bakery products did not show a significant correlation with their total protein content. These results may be influenced by the diverse protein sources present in commercial bakery products. When discussing the impact of wheat flour type on dAGE formation in bread, gluten is the primary protein source. However, in commercial bakery products, protein sources can originate not only from gluten but also from milk and eggs. The ingredient lists on the packaging showed that several bakery products, including pancakes, croissants, pineapple bread, toast, and cakes, contained eggs and/or milk (data not shown). The impact of adding milk and eggs on dAGE formation in bakery products may differ from that of gluten protein. In contrast to the positive correlation between total dAGEs and sugar content, sodium content was negatively correlated with total dAGE content ($p < 0.01$). The primary sources of sodium in bakery products are salt and baking powder. How these sodium-containing ingredients affect AGE formation is not yet fully understood. A recent study demonstrated that adding sodium chloride significantly reduced CML and CEL formation in a myofibrillar

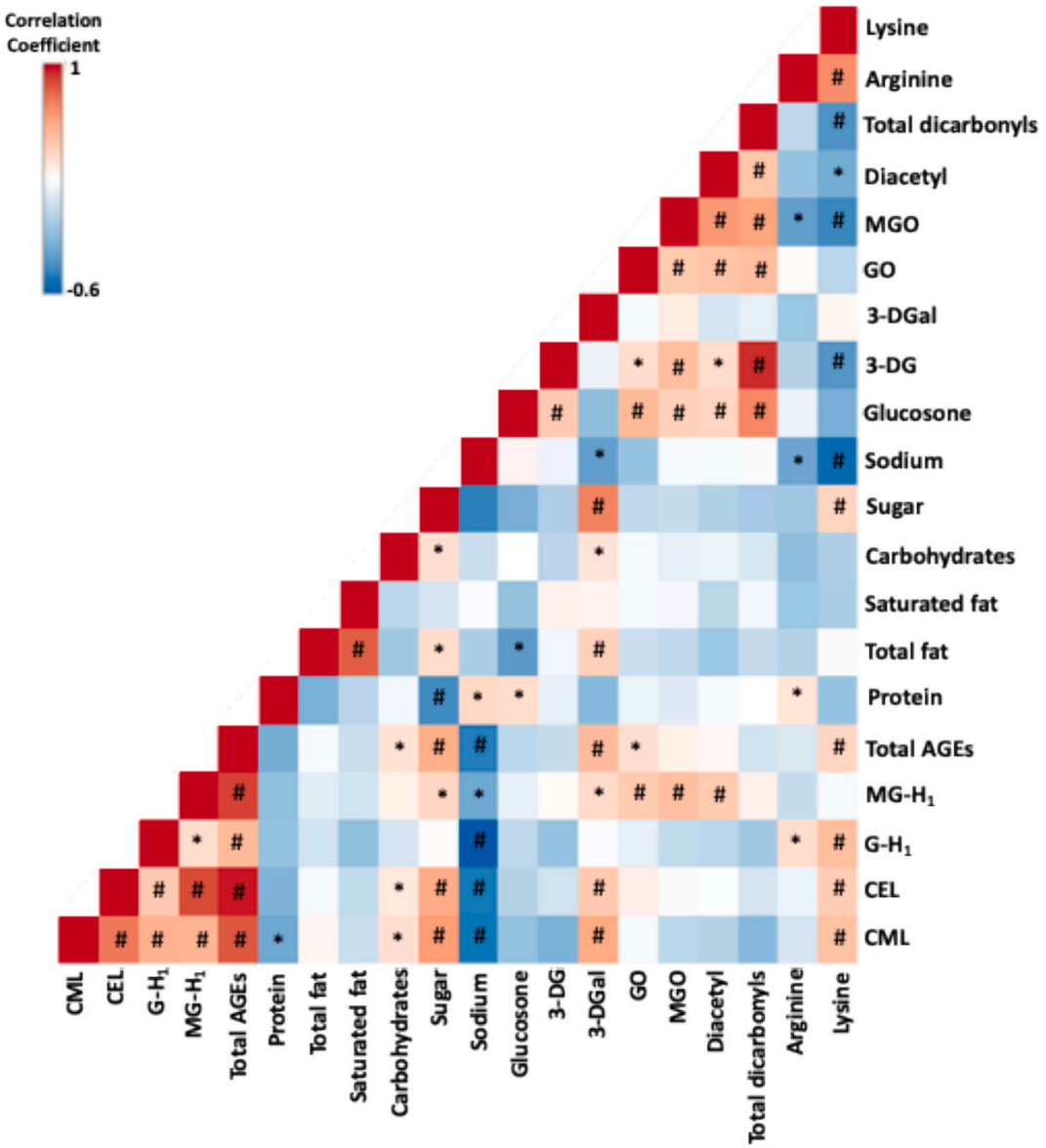

Fig. 3. Heatmap of Pearson's correlations among dAGEs, α-dicarbonyls, and nutrients. $*p < 0.05$, $\#p < 0.01$.

protein-reducing sugar-oleic acid model system (Niu et al., 2024). Interestingly, sodium chloride addition significantly promoted CML and CEL formation in commercially sterilized ground pork, while their formation significantly decreased with the addition of sodium nitrite (Niu et al., 2018). Therefore, further research is needed to investigate the influence of different sodium sources on dAGE formation in bakery products.

Although α-dicarbonyls are the primary precursors of dAGEs, the correlation between total α-dicarbonyls and total dAGEs was not significant (Fig. 3). Total dAGEs were only positively correlated with 3-DGal and GO, while no significant correlations were observed between total dAGEs and 3-DG, glucosone, or MGO, despite these being the most abundant α-dicarbonyls in bakery products. This could be due to the fact that dAGE formation in bakery products does not always exhibit a positive relationship with α-dicarbonyl formation under different thermal processing conditions. For example, the formation of protein-bound CEL and CML in butter cookies baked at 175 °C was higher than that at 180 °C, whereas the formation of 3-DG and MGO consistently increased with rising baking temperatures (130–180 °C)(Hu et al., 2022). Additionally, butter cookies made with 12 g of egg liquid contained 6.38 and 69.19 mg/kg of protein-bound CML and CEL, respectively, which were significantly higher than those made with 3, 6, or 9 g of egg liquid. However, the concentrations of 3-DG and MGO in the butter cookies reached their maximum levels when 6 g of egg liquid was added.

4. Conclusion

This study investigated the effects of sugar, butter, olive oil, and gluten protein on the formation of α-dicarbonyls and dAGEs in bread. Our findings indicated that adding sucrose specifically promoted the formation of MGO-derived dAGEs (CEL and MG-H_1), while the formation of GO-derived dAGEs (CML and G-H_1) was not significantly affected. Additionally, although previous studies have shown that fatty acids significantly impact dAGE formation during thermal processing, the present study found that the levels of α-dicarbonyls and dAGEs in bread were not significantly altered by the addition of butter or olive oil. Additionally, bread made with low- and/or medium-gluten flour significantly reduced the formation of α-dicarbonyls and dAGEs compared to bread made with high-gluten flour. Importantly, we further investigated whether sugar and protein content could serve as potential indicators of total dAGE content in commercial bakery products. Correlation analysis confirmed a significant positive correlation between

total dAGE content and total sugar content in these products, while no significant correlations were found with total protein and fat content. Taken together, our findings suggest that sugar content listed on the nutrition facts label, rather than lipid or protein content, may be a useful indicator for estimating the total dAGE content in commercial bakery products.

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CRediT authorship contribution statement

Cheng-Yi Tsai: Writing – original draft, Software, Methodology, Investigation, Formal analysis. **Kai-Wei Liao:** Writing – review & editing, Supervision. **Shih-Min Hsia:** Writing – review & editing, Supervision. **Yin-Chieh Tsai:** Investigation, Formal analysis. **Keng-Jui Lin:** Investigation, Formal analysis. **Chi-Tang Ho:** Writing – review & editing, Supervision, Conceptualization. **Wei-Lun Hung:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102286.

Data availability

Data will be made available on request.

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