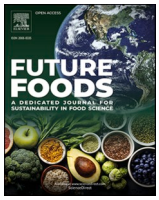
Future Foods 12 (2025) 100687

Contents lists available at ScienceDirect

Future Foods

journal homepage: www.elsevier.com/locate/fufo



An industrially applicable processing method for reducing AGEs in bread based on RSM design

Guanhua Jiang [a,b], Haili Yu [c], Liangkai Chen [a,b], Yan Zhang [c,**], Liegang Liu [a,b,*]

[a] Department of Nutrition and Food Hygiene, Hubei Key Laboratory of Food Nutrition and Safety, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China;
[b] Ministry of Education Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China.
[c] Hubei Key Laboratory of Yeast Function, Angel Yeast Co., LTD., Yichang 443000, China.

ARTICLE INFO

Keywords:
Bread
Processing conditions
Advanced glycation end-products
Response surface methodology

ABSTRACT

Intake of dietary advanced glycation end-products (AGEs) is associated with increased risk of inflammation and chronic disease. Bread, a significant source of AGEs, is an ideal model for minimizing AGEs at process scale. The study investigated the combined impact of yeast, sugar, butter contents, baking temperature, and duration on the formation of AGEs in yeast-leavened wheat bread. Protein-bound AGEs (bAGEs) predominantly localized in the crust, with negligible free form of Nε-carboxymethyllysine (CML) and Nε-carboxyethyllysine (CEL). Employing response surface methodology (RSM), we established relationships and interactions among process variables. Optimal conditions (0.6 % yeast, 23 % sucrose, 210 °C baking temperature, and 15 min baking time) minimized protein-bound CML and CEL accumulation to 24.57 ± 3.82 mg/kg and 24.74 ± 3.09 mg/kg, respectively, in the bread crust, closely aligning with predicted values derived from the RSM model.

1. Introduction

Advanced glycation end-products (AGEs) form through non-enzymatic reactions between amino groups (proteins, lipids, or nucleic acids) and reducing sugar carbonyl groups (Zhang et al., 2020). Dietary AGEs (dAGEs), ingested through food, accumulate and exacerbate oxidative stress and inflammation in the body, contributing to chronic diseases like diabetes, cardiovascular disease, and Alzheimer's (Dobi et al., 2019; Nie et al., 2022; Sergi et al., 2021; Uribarri et al., 2015). Among AGEs, Nε-carboxymethyllysine (CML) is a significant biomarker due to its considerable presence in the body (Poulsen et al., 2013). CML levels in thermally processed foods correlate with overall AGEs, making it a representative dAGEs marker (Chen, 2021; Qin et al., 2022; Yu et al., 2022). Its structural analog, Nε-carboxyethyllysine (CEL), is also widespread in foods and used in health studies (Gill et al., 2019; Jiao et al., 2019; Koska et al., 2018; Sharma et al., 2020).

AGEs formed from free amino acids are termed free AGEs (fAGEs), while produced from peptides or proteins are protein-bound AGEs (bAGEs). In protein-rich foods like meat, fish, and dairy, heating significantly increases bAGEs levels but not fAGEs(Yuan et al., 2021; Zhao et al., 2019). High-temperature, low-humidity processing promotes AGE formation, whereas additives like antioxidant phytochemicals dose-dependently inhibit it(Nie et al., 2022; Park et al., 2023; Zhang et al., 2020). Consequently, AGE mitigation strategies focus on matrix composition, processing parameters, and additives.

Bread, a globally consumed staple rich in carbohydrates, fats, and proteins, undergoes thermal processing and is a significant dAGEs source. Reported levels in commercial breads are comparatively high (CML: 2.5-94.29 mg/kg; CEL: 1.1-63.15 mg/kg) among various food categories (Cheng et al., 2021; Scheijen et al., 2016). The simplicity of ingredients and ease of preparation make bread an ideal model for studying AGEs formation. During baking, crust color is crucial for consumer appeal and indicates baking completion. Crucially, crust browning directly reflects Maillard reaction extent, with darker color signifying higher levels of harmful products like AGEs (Ahrne et al., 2007; Laroque et al., 2008). Consequently, the industry aims to minimize AGEs formation while preserving the appealing sensory qualities of bread (Jost et al., 2021).

* Corresponding author at: Department of Nutrition and Food Hygiene, Hubei Key Laboratory of Food Nutrition and Safety, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
** Corresponding author at: Hubei Key Laboratory of Yeast Function, Angel Yeast Co., LTD., Yichang 443000, China
E-mail addresses: zhangyan@angelyeast.com (Y. Zhang), lgliu@mails.tjmu.edu.cn (L. Liu).

https://doi.org/10.1016/j.fufo.2025.100687
Received 12 March 2025; Received in revised form 13 June 2025; Accepted 16 June 2025
Available online 16 June 2025

Thus, the study investigated how industrial processing parameters (yeast, sugar, butter content, baking temperature/duration) in yeast-leavened wheat bread influence CML and CEL concentrations. Parameters were varied within bakery-standard ranges to ensure acceptable products. Response surface methodology (RSM), a statistical approach efficiently resolves variables interactions via fewer runs while delivering comprehensive results, was employed combined with Box-Behnken Design (BBD) to model interactions and optimize processes for minimizing the contaminants (Nakra et al., 2025; Singh et al., 2025). This study aims to develop industrial-scale strategies for bread with reduced AGEs via BBD-RSM model, offering potential public implications for the prevention of a wide range of chronic diseases associated with AGEs in the general population.

2. Materials and Methods

2.1. Experimental materials, chemicals and instruments

Wheat flour was procured from a local supermarket in Yichang, Hubei, China. Dry yeast, butter, and sucrose frosting were sourced from Angel Yeast Co., Ltd. (Yichang, Hubei, China). The standard (CML and CEL, Purity > 97.0 %) and the isotopic internal standard (CML-d2 and CEL-d4, Purity > 97.0 %) of AGEs were purchased from Iris Biotech GmbH (Bayern, Germany). Methanol and acetonitrile were of high-pressure liquid chromatography (HPLC) grade (Purity > 99.9 %) and were purchased from Thermo Fisher Scientific, Inc. (Massachusetts, USA). Nonafluorovaleric acid (Purity > 98.0 %) was obtained from Tokyo Kasei Industry Co. (Tokyo, Japan). All other chemicals and reagents used in the study were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The ultra-high-performance liquid chromatography–tandem mass spectrometry system (UHPLC-MS/MS) comprising an Agilent 1260 UHPLC system in tandem with an Agilent 6460 triple tandem quadrupole mass spectrometer and ZORBAX Eclipse Plus C18 column (2.1 mm × 100 mm, 1.8 μm) were purchased from Agilent Technologies. (California, USA). High-speed freezing centrifuge 5810R and pipettes were purchased from Eppendorf. (Hamburg, Germany). Vortex oscillator MX-S was purchased from Scilogex. (Berlin, CT, USA). Freeze dryer ALPHA1-4 LD plus was purchased from Marin Christ (Osterode, Germany). Grinder KZ-III was purchased from Servicebio (Wuhan, China). −80 °C low temperature refrigerator was obtained from Thermo Fisher Scientific. (Waltham, USA).

2.2. Bread processing

Dry ingredients including flour, yeast, and sucrose were dissolved in water and mixed at slow speed for 4 min, followed by 1 min at high speed in a spiral kneader. Subsequently, butter was added, and the dough was mixed at low speed for 2 min and at high speed for 1 min until gluten was mature. Portions of dough, each weighing 200 g with a diameter of 5 cm and thickness of 2 cm, were placed in a fermentation tank for 120 min at 38 °C with a relative humidity of 88 %. After fermentation, the dough was baked in an airtight oven and allowed to cool to room temperature. Post-cooling, the bread was separated into crust and crumb fractions using a surgical blade (Ahrne et al., 2007). The separated crust and crumb were pre-chilled at -80°C for 1h then lyophilized at partition temperature of -10°C and 80 mTorr of vacuum pressure for 6h using a freeze-dryer and subsequently crushed into fine powder with a grinder.

2.3. Single factorial experimental design

In this experiment, the basic conditions for the bread processing included 1 % yeast, 20 % sucrose, 8 % butter, a baking temperature of 190 °C, and a baking time of 20 min, reflecting standard parameters used in Chinese bakery factories. Yeast concentration at 1 % refers to the mass of yeast relative to the mass of flour, and the same applies to butter and sucrose. The effects of varying yeast levels (0.5 %, 1.0 %, 1.5 %, 2.0 %) on CML and CEL content were investigated under conditions of 20 % sucrose, 8 % butter, baking temperature of 190 °C, and a baking time of 20 min. Additionally, the effects of varying sucrose concentrations (15 %, 20 %, 25 %, 30 %), butter concentrations (6 %, 8 %, 10 %, 12 %), baking temperature (170 °C, 190 °C, 210 °C, 230 °C), and baking times (15, 20, 25, 30 min) were explored individually while keeping the other parameters consistent.

Table 1
Bread response surface experimental factor level table.

Factor	level		
	−1	0	+1
X_1 yeast (%)	0.5	1	1.5
X_2 sucrose (%)	15	20	25
X_3 baking temperature (°C)	170	190	210
X_4 baking time (min)	15	20	25

2.4. Response surface design

Following the single factorial experiments, The BBD- RSM was used to investigate the interactions among yeast, sucrose, temperature, and baking time on the protein-bound CML and CEL contents during bread processing. The goal was to determine the conditions that minimize CML and CEL levels. The models' independent variables included yeast, sucrose, temperature, and baking time, while the response variables were protein-bound CML and CEL. Table 1 illustrates the factor levels for the 4-factor, 3-level design model.

2.5. Extraction protocol for bread samples

Freeze-dried and pulverized bread crust and crumb samples were separately suspended in deionized water to a concentration of 0.05 g/mL. After thorough vortexing, 25 μL of each suspension was transferred to a 7 mL glass tube. To each tube, 500 μL of borate buffer (pH 9.2) containing 0.1 mol/L sodium borohydride was added and allowed to react for 2 hours. Following this, 2 mL of anhydrous ethanol pre-chilled to –20 °C was added to precipitate the proteins. The mixture was centrifuged at 4000 rpm for 10 min, the supernatant was discarded, and the precipitate was retained. Subsequently, 50 μL of a mixed internal standard (CML-d2: 1 μg/ml, CEL-d4: 0.5 μg/mL) and 500 μL of 6 mol/L hydrochloric acid solution were added to the precipitate. The mixture was acid-digested for 18 hours at 110 °C with the cap tightened. After digestion, the sample was dried under nitrogen at 80 °C, then 500 μL of ultrapure water was added to redissolve the residue. The solution was vortexed thoroughly and transferred to a 1.5 mL EP tube, followed by centrifugation at 14,000 rpm for 10 min. An aliquot of 200 μL of the supernatant was transferred to a new 1.5 mL EP tube and dried under nitrogen at 80 °C. The residue was re-dissolved in 400 μL of a 5 mmol/L nonafluorovaleric acid aqueous solution. Following vortexing and centrifugation at 15,000 rpm for 10 min, approximately 200 μL of the supernatant was filtered through a 0.22 μm polytetrafluoroethylene syringe filter for analysis. All extractions were carried out in triplicate (Cheng et al., 2021).

2.6. Determination of CML and CEL by UHPLC-MS/MS

Sample analysis was performed using an ultra-high-performance liquid chromatography–tandem mass spectrometry system (UHPLC-MS/MS), comprising an Agilent 1260 UHPLC system in tandem with an Agilent 6460 triple tandem quadrupole mass spectrometer (Agilent Technologies, California, USA). Analytes were separated on a ZORBAX Eclipse Plus C18 column (2.1 mm × 100 mm, 1.8 μm) maintained at 30 °C. The mobile phase consisted of water with 5 mmol/L

Table 2
Comparison of quantitative results between SPE and protein precipitation methods (n=3).

Target	Sample number	SPE		protein precipitation		significance
		quantitative value	SD	quantitative value	SD	
CML	1	7.68	0.46	7.20	0.86	$P > 0.05$
	2	25.01	1.25	23.69	1.95	$P > 0.05$
	3	45.58	1.67	42.95	4.01	$P > 0.05$
CEL	1	2.71	0.38	2.47	0.77	$P > 0.05$
	2	26.71	1.71	25.30	2.22	$P > 0.05$
	3	46.91	1.59	48.93	2.81	$P > 0.05$

Quantitative results in mg/kg; SD: standard deviation.

nonafluorovaleric acid (phase A) and acetonitrile (phase B). The gradient elution program was as follows: 96 % A for 0–1 min, 96–80 % A for 1–4 min, 80–60 % A for 4–7 min, 60–40 % A for 7–8 min, 40–20 % A for 8–9 min, 20 % A for 9–11 min, 20–96 % A for 11–11.5 min and 96 % A for 11.5–17 min, with a flow rate of 0.25 mL/min. An 8 μL aliquot of sample was injected into the UHPLC system, Positive electrospray ionization (ESI+) in multiple reaction monitoring (MRM) mode was used to detect the parent and fragment ions of the target analytes. Peak areas for CML and CEL were quantified using standard curves, with correlation coefficients of ≥0.995 for both CML and CEL across all calibration curves.

2.7. Sensory evaluation

Sensory evaluation of the basic model bread and RSM optimized bread was conducted within 30 volunteers who were refrain from eating and drinking for 2 h. Volunteers were recruited from the students of Tongji Medical College, with exclusion criterion of allergies to gluten or yeast products. The Ethics Committee of Tongji Medical College concluded that the bread tasting program of the study doesn't require ethical permission. Three slices of the two kinds of breads containing both crust and crumb portion were subjected to volunteers randomly. According to the previous study (Pešić et al., 2023), the sensory assessment comprised of appearance, texture, flavor, taste and overall acceptance was conducted. Each sensory attribute item has a 9-point scale from 1 to 9, between each item, volunteers were allowed to rinse mouth and take a while. All volunteers have signed informed consent forms for the bread tasting.

2.8. Volatile compounds

An electronic nose detector combined with chemometrics system (BosinTech, Shanghai, China) consists of 28 different metal oxide sensors was used to obtain volatiles profiles. Samples were incubated for 5 min at 60 °C and then subjected to dynamic headspace injection in a closed tube. Six independent experiments were performed for each sample, between each analysis, probes were allowed cleaning process for 120s.

2.9. Statistical analysis

Every measurement was performed in triplicate and presented as mean ± standard deviation. Statistical analyses were performed using SPSS Statistics V22.0 (IBM Corporation, NY, USA), differences between groups were compared using one-way analysis of variance (ANOVA), with significance set at $P < 0.05$. Graphs were drawn using Origin 2021

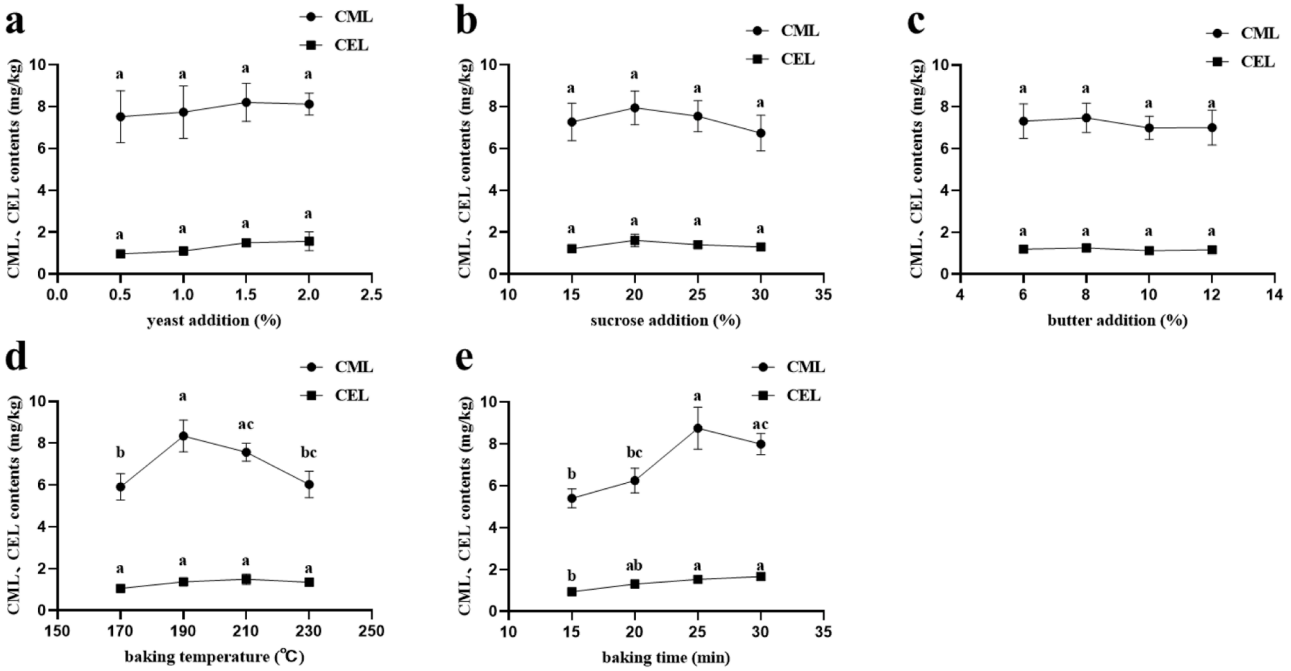


Fig. 1. Effects of yeast, sugar, butter contents, baking temperature, and duration on the contents of AGEs in bread crumbs.
(a) Effect of yeast addition on CML and CEL contents in crumbs. **(b)** Effect of sucrose addition on CML and CEL contents in crumbs. **(c)** Effect of butter addition on CML and CEL contents in crumbs. **(d)** Effect of baking temperature on CML and CEL contents in crumbs. **(e)** Effect of baking time on CML and CEL contents in crumbs. Data were expressed as mean ± SEM (n = 3). Values of points with different marked letters in the same curve are significantly different ($P < 0.05$) by Duncan's multiple range test.

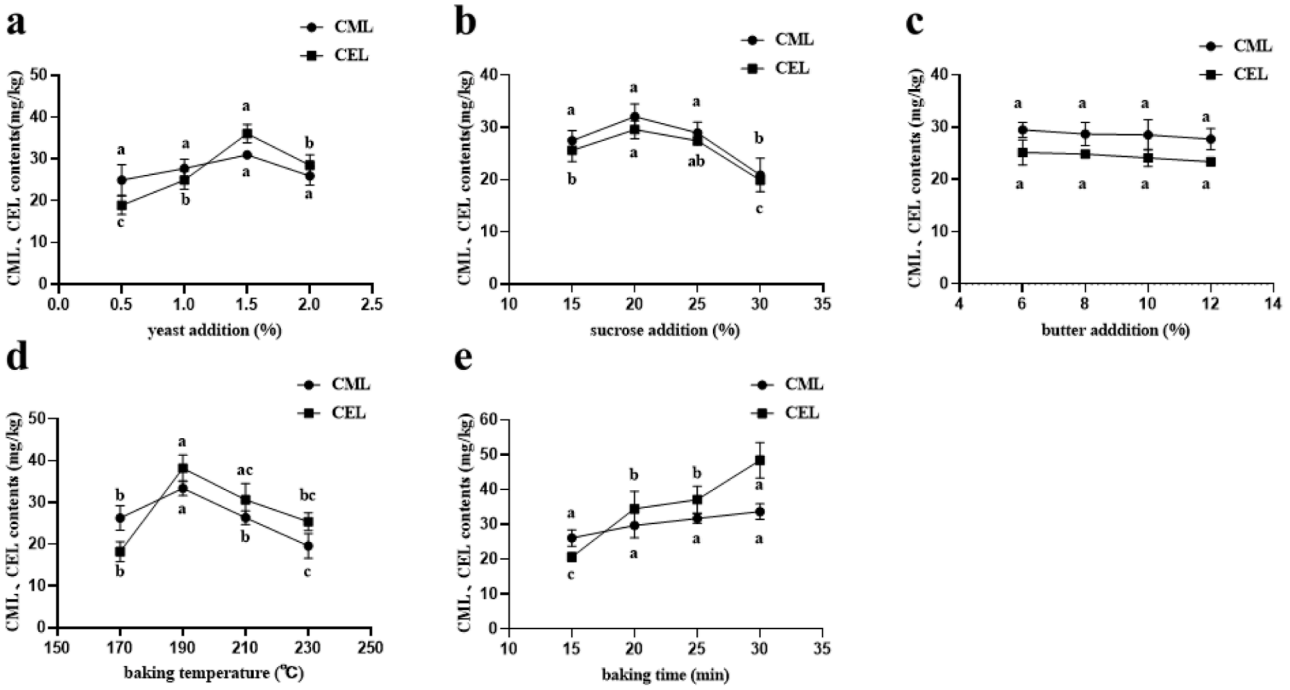


Fig. 2. Effects of yeast, sugar, butter contents, baking temperature, and duration on the contents of AGEs in bread crusts.
(a) Effect of yeast addition on CML and CEL contents in crusts. **(b)** Effect of sucrose addition on CML and CEL contents in crusts. **(c)** Effect of butter addition on CML and CEL contents in crusts. **(d)** Effect of baking temperature on CML and CEL contents in crusts. **(e)** Effect of baking time on CML and CEL contents in crusts. Data were expressed as mean ± SEM (n = 3). Values of points with different marked letters in the same curve are significantly different ($P < 0.05$) by Duncan's multiple range test.

software (Northampton, MA, USA) and GraphPad Prism 9.3.1 (GraphPad Software Inc., San Diego, CA, USA). RSM-based BBD and regression analysis were conducted using Design Expert software version 11.0 (Stat-Ease Inc., Minneapolis, USA).

3. Results and discussion

3.1. Optimization of sample pretreatment procedure for AGEs determination

Solid-Phase Extraction (SPE) is generally used for pre-treatment of protein-bound AGEs methods reported in studies (Yuan et al., 2023), however, alongside with the expense and cumbersome operation, studies tended to suggest that SPE is less effective in the purification of polar compounds (Nováková and Vlcková, 2009). Given the strong polarity of CML and CEL, we compared the effects of pretreatment of bread samples by SPE using Waters Oasis HLB columns and protein precipitation using −20 °C pre-cooled anhydrous ethanol. There was no significant difference between the quantitative results of bread samples with low, medium and high levels of AGEs processed by the two methods, presented in Table 2.Thus, prior to acid hydrolysis, protein precipitation using −20 °C pre-cooled anhydrous ethanol was used to obtain protein suspensions from bread samples.

3.2. AGEs contents in basic condition bread and levels in bread crumb

The levels of fAGEs in bread produced under basic conditions were quantified as CML (313.27 ± 6.21 μg/kg) and CEL (142.19 ± 18.02 μg/kg) using UHPLC-MS/MS system, which were significantly lower than those of bAGEs in the bread. Notably, fAGEs did not exhibit substantial variation across different processing conditions, thus not warranting further discussion. Henceforth, references to CML and CEL pertain to bAGEs unless specified otherwise. Under the standard baking conditions, comprising 1 % yeast, 20 % sucrose, 8 % butter, a temperature of 190 °C, and a baking duration of 20 min, the crust of the bread contained average levels of CML (33.69 ± 2.95 mg/kg) and CEL (30.42 ± 1.31 mg/kg). In contrast, the crumb showed average contents of CML (7.56 ± 0.83 mg/kg) and CEL (1.33 ± 0.09 mg/kg). The CML content in the crust was approximately five times higher than that in the crumb, while the CEL content was about 20 times higher. The elevated levels of AGEs in the crust can be attributed to its higher temperature and reduced moisture content compared to the crumb.

The AGEs levels in bread detected in this study were consistent with findings from other research: (1) (Scheijen et al., 2016) reported bread crust protein-bound CML (19.60 mg/kg) and CEL (14.40 mg/kg), with crust-free protein-bound CML (2.4 mg/kg) and CEL (0.50 mg/kg); (2) (Hull et al., 2012) found protein-bound CML (31.00 mg/kg) in whole wheat bread; (3) (Cheng et al., 2021) noted total CML (8.01 mg/kg) and CEL(12.48 mg/kg) in bread.

Fig. 1 shows the effects of 5 factors on the contents of AGEs in bread crumbs. No significant changes in CEL or CML content in the crumb with variations in yeast, sugar, or butter additions was observed. CML content in the crumb exhibited a trend of increasing and then decreasing with rising baking temperature, while it showed a consistent increase with longer baking times. CEL content remained relatively stable throughout the baking process.

3.3. Influence of yeast, sugar, and butter dosage on CML and CEL levels in the crust

Fig. 2a shows the effect of yeast additions on CML and CEL levels in the bread crust. CEL levels increased significantly from 0.5 % to 1.5 % yeast, peaking at 36.04 ± 2.24 mg/kg at 1.5 %, before decreasing with further yeast addition to 2.0 %. In contrast, no significant trend was observed for CML content. The variation in yeast concentration impacts CML and CEL levels by altering sugar types and concentrations during dough fermentation (Heitmann et al., 2018). Three main changes occur with increasing yeast: (1) enhanced degradation of sucrose to glucose

and fructose by yeast enzymes; (2) increased consumption of reducing sugars such as glucose, fructose, and maltose during yeast glycolysis; and (3) decreased pH due to carbon dioxide accumulation, which facilitates sucrose degradation to reducing sugars. Monosaccharides typically exhibit a higher rate of Maillard reaction or AGEs formation compared to reducing disaccharides, with non-reducing disaccharides exhibiting the lowest rate (Jing and Kitts, 2002; Liang et al., 2016). Alongside the levels of reducing sugars, the levels of amino acids in the dough also manifest alterations accompanying the transamination reaction of fermentation, where amino acids are converted into aldehydes, alcohols or esters(Liu et al., 2018; Pétel et al., 2017). The observed results suggested that the increase in yeast from 0.5 % to 1.5 % enhances reducing monosaccharide formation, thereby increasing AGEs content. However, further yeast addition to 2 % may reduce AGEs content due to higher yeast metabolism and reduced availability of reducing monosaccharides.

Fig. 2b illustrates the impact of sucrose concentration on the levels of CML and CEL in the bread crust. CML content remained relatively stable as sucrose increased from 15 % to 25 %, peaking at 32.08 ± 2.40 mg/kg at 20 %, and decreased with further sucrose increase to 30 %. In contrast, CEL content initially increased and then decreased, reaching a maximum of 29.57 ± 1.73 mg/kg at 20 % sucrose.

Sucrose contributes to AGE formation through two primary mechanisms: (1) the formation of fructoselysine via the Maillard reaction with lysine, which subsequently degrades to produce AGEs; and (2) the oxidative degradation of sucrose or its Maillard reaction intermediates, leading to α-dicarbonyl compounds that react with lysine to form AGEs. The latter mechanism is primarily responsible for generating CML, CEL, and pyrraline (Fu et al., 2012). Monosaccharides, compared to non-reducing disaccharides, are more prone to AGEs formation (Li et al., 2012), implying that reducing monosaccharides such as glucose and fructose undergo a more extensive and rapid Maillard reaction process than sucrose. In our experiment, monosaccharides were primarily derived from flour and yeast during fermentation, while sucrose degradation's contribution at high-temperature was minimal (Fu et al., 2012). Consequently, higher sucrose concentrations provided more substrate for AGE formation through pathways (1) and (2). At 190 °C, pathway (2) more readily generates pyrraline, which may account for the observed decrease in CML and CEL when the sugar concentration increased to 30 %. Furthermore, Fig. 2c shows no significant changes in CML and CEL levels with butter addition ranging from 6 % to 12 %.

3.4. Influence of baking temperature and time on CML and CEL content

Fig. 2d illustrates a significant ($P < 0.05$) increase in CML content in the crust as baking temperature escalated from 170 °C to 190 °C, peaking at 33.42 ± 1.75 mg/kg before declining with further temperature increments. A similar trend was observed for CEL content, which also increased with temperature, reaching a maximum of 38.21 ± 3.17 mg/kg at 190 °C, and subsequently decreased with higher temperatures.

Fig. 2e demonstrates the impact of baking duration on the levels of CML and CEL in the crust. CEL content significantly increased during two specific baking time intervals, i.e., between 15 and 20 min and 25 and 30 min, with a maximum value of 48.43 ± 5.09 mg/kg observed at 30 min. CML content followed a similar temporal trend but did not show significant changes with varying baking times.

Baking temperature and time are fundamental parameters influencing the formation of AGEs in thermally processed foods. Generally, AGEs levels rise with increasing temperatures, although several studies report a reduction in pyrraline production once temperatures exceed 140 °C (Hull et al., 2012). The impact of temperature on CML and CEL formation can vary during food processing. For instance, under high-temperature conditions, the formation of CEL often surpasses that of CML in cakes (Srey et al., 2010). This disparity may be attributed to the enhanced reactivity of methylglyoxal (MGO, a CEL precursor) with lysine compared to glyoxal (GO, a CML precursor), under high-temperature conditions. Additionally, while extended heating time generally increases AGE levels in model systems and food processing, prolonged exposure to high temperatures might result in further reactions involving CML and CEL, potentially reducing their levels within the system (Liang et al., 2016; Nguyen et al., 2016).

Table 3
BBD matrix, experimental responses of CML and CEL in the bread crust.

Run	Yeast addition (%)	Sucrose addition (%)	Baking temperature (°C)	Baking time (min)	CML (mg/kg)	CEL (mg/kg)
1	1.5	20	190	25	31.36	42.58
2	0.5	20	190	15	28.22	31.52
3	1.5	20	170	20	31.28	38.49
4	1	20	170	15	30.09	31.18
5	1	20	190	20	31.94	41.62
6	1	20	190	20	31.16	41.13
7	1	20	210	25	29.27	37.46
8	0.5	20	190	25	31.92	36.67
9	1	25	170	20	31.74	32.30
10	1	20	190	20	32.20	41.38
11	1	15	190	15	26.63	34.36
12	1.5	15	190	20	30.27	35.78
13	1	15	210	20	28.93	33.48
14	1	25	190	25	30.68	37.57
15	1	20	170	25	26.16	36.63
16	1	20	190	20	32.09	39.52
17	1	15	170	20	28.79	31.73
18	1	25	190	15	29.18	33.22
19	0.5	25	190	20	31.22	22.72
20	1	15	190	25	28.59	43.57
21	1	20	210	15	24.05	31.04
22	0.5	15	190	20	29.90	30.67
23	0.5	20	210	20	28.52	27.29
24	1.5	25	190	20	34.27	36.50
25	1.5	20	190	15	32.74	36.74
26	1	20	190	20	30.75	39.82
27	1	25	210	20	29.47	30.16
28	0.5	20	170	20	29.93	27.12
29	1.5	20	210	20	29.26	35.85

3.5. Response surface analysis for CML and CEL

Based on preliminary single-factor experiments, the experimental range for the RSM design was established for each factor. BBD was employed to optimize processing conditions for CML and CEL, with 29 experimental runs conducted in a randomized sequence to evaluate synergistic effects between variables and responses (Table 3). All runs were performed in triplicate.

The effects of yeast, sucrose, baking temperature, and time on CML and CEL formation in bread crust are summarized in Table 3. Generally, CML and CEL concentrations ranged from 24.05 to 34.27 mg/kg and 22.72 to 43.57 mg/kg, respectively. The quadratic regression models derived from the RSM data are as follows:

$$CML=31.62+0.7885\ X_1+1.12\ X_2-0.7077\ X_3+0.5897\ X_4+0.6688\ X_1X_2-0.1502\ X_1X_3-1.27\ X_1X_4-0.6022\ X_2X_3-0.1157\ X_2X_4+2.29\ X_3X_4+0.6138\ X_1^2-0.5382\ X_2^2-2.06\ X_3^2-1.89\ X_4^2$$

$$CEL=40.69+4.16\ X_1-1.43\ X_2-0.18\ X_3+3.03\ X_4+2.17\ X_1X_2-0.7033\ X_1X_3+0.1734\ X_1X_4-0.9716\ X_2X_3-1.21\ X_2X_4+0.2421\ X_3X_4-4.05\times1^2-4.03\times2^2-5.20\times3^2-0.2204\times4^2$$

Here, CML represents the concentration of CML in mg/kg, CEL denotes the concentration of CEL in mg/kg, X_1, X_2, X_3, and X_4 correspond to yeast addition (%), sugar addition (%), heating temperature (°C), and heating time (minute), respectively.

The ANOVA results for the BBD-based RSM, as presented in Table 4, highlighted the significant impact of the regression models on CML and CEL formation ($P < 0.0001$ for both). The linear relationships between the response variables and the parameters were also highly significant. The Lack-of-Fit values for CML (P=0.6164) and CEL (P=0.1056) exceeded the significance threshold of 0.05, indicating that the models

Table 4
ANOVA Results from BBD for CML and CEL model parameters.

Source	CML Coefficient	F-value	*P*-value	CEL Coefficient	F-value	*P*-value
model	31.62	10.48	<0.0001	40.69	17.74	<0.0001
X1	0.7885	9.12	0.0092	4.16	76.41	<0.0001
X2	1.12	18.45	0.0007	-1.43	8.96	0.0097
X3	-0.7077	7.34	0.0169	-0.1800	0.1429	0.7111
X4	0.5897	5.10	0.0404	3.03	40.63	<0.0001
X1×2	0.6688	2.19	0.1614	2.17	6.91	0.0199
X1×3	-0.1502	0.1102	0.7448	-0.7033	0.7274	0.4081
X1×4	-1.27	7.90	0.0139	0.1734	0.0442	0.8365
X2×3	-0.6022	1.77	0.2044	-0.9716	1.39	0.2584
X2×4	-0.1157	0.0654	0.8019	-1.21	2.17	0.1629
X3×4	2.29	25.53	0.0002	0.2421	0.0862	0.7733
$X1^2$	0.6138	2.09	0.1701	-4.05	39.06	<0.0001
$X2^2$	-0.5382	3.23	0.0940	-4.03	38.79	<0.0001
$X3^2$	-2.06	36.97	<0.0001	-5.20	64.41	<0.0001
$X4^2$	-1.89	31.27	<0.0001	-0.2204	0.1158	0.7387
Lack-of-Fit		0.8607	0.6164		3.79	0.1056
R^2	0.9129			0.9466		
Adj R^2	0.8259			0.8933		

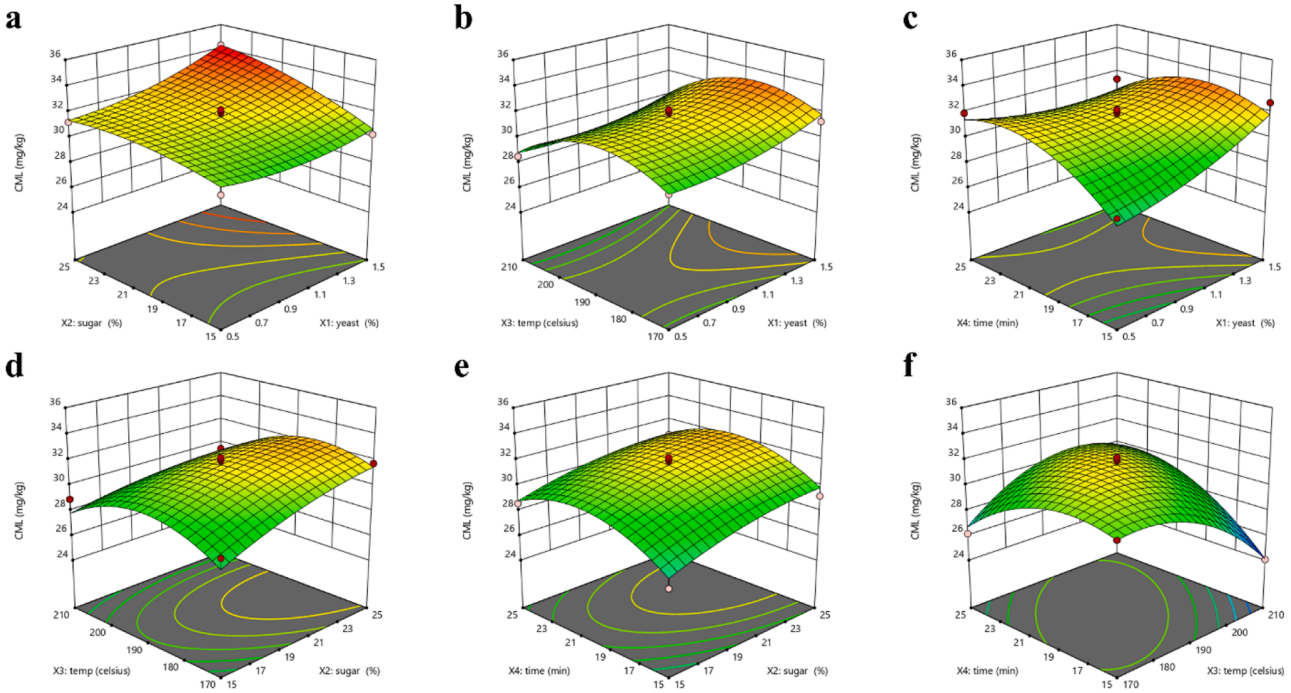


Fig. 3. 3D response surface curve and corresponding contour plot showing effect of independent variables on the formation of CML in the bread crust. **(a)** Surface and contour plot between sugar and yeast addition. **(b)** Surface and contour plot between baking temperature and yeast addition. **(c)** Surface and contour plot between baking time and yeast addition. **(d)** Surface and contour plot between baking temperature and sugar addition. **(e)** Surface and contour plot between baking time and sugar addition. **(f)** Surface and contour plot between baking time and temperature.

fit the data well and are reliable. The CML model demonstrates a high correlation coefficient ($R^2 = 0.9129$) with an adjusted R^2 of 0.8259, rendering it robust for analyzing and predicting conditions that minimize CML contents. Similarly, the CEL model has an R^2 of 0.9466 and an adjusted R^2 of 0.8933, demonstrating its effectiveness for prediction.

At a significant level of $P < 0.05$, the linear parameters X_1, X_2, X_3, X_4, the interaction parameters X_1X_4, X_3X_4, and the quadratic parameters X_3^2, X_4^2 significantly impact CML content. For CEL, significant effects are observed with linear terms X_1, X_2, and X_4, the interaction term X_1X_2, and quadratic terms $X1^2$, $X2^2$, and $X3^2$. Each factor exhibited a quadratic relationship with CML and CEL content. The influence of parameters on the CML content is ranked as follows: X_2 (sucrose addition) > X_1 (yeast addition) > X_3 (baking temperature) > X_4 (baking time). For CEL, the ranking is X_1 (yeast addition) > X_4 (baking time) > X_2 (sucrose addition) > X_3 (baking temperature).

Three-dimensional response surfaces illustrating interactions among yeast, sucrose, baking temperature, and time on AGEs are shown in Figs. 3 and 4. For CML, a lower sucrose level was preferred to minimize formation, with the lowest CML content achieved at 15 % sucrose, beyond which levels may increase (Fig. 3a, d, e). The effect of baking temperature on CML, depicted in Fig. 3b, d, f, shows an initial increase and followed by a decrease with rising temperature. Furthermore, significant interactions between baking temperature and time affected CML content, supporting the notion that high temperatures and prolonged heating may enhance CML formation (Nguyen et al., 2016). For CEL, the content significantly increased with prolonged baking time, peaking at 25 min (Fig. 4c, e, f), with shorter baking times resulting in lower CEL levels. CEL content also showed a tendency to increase and then

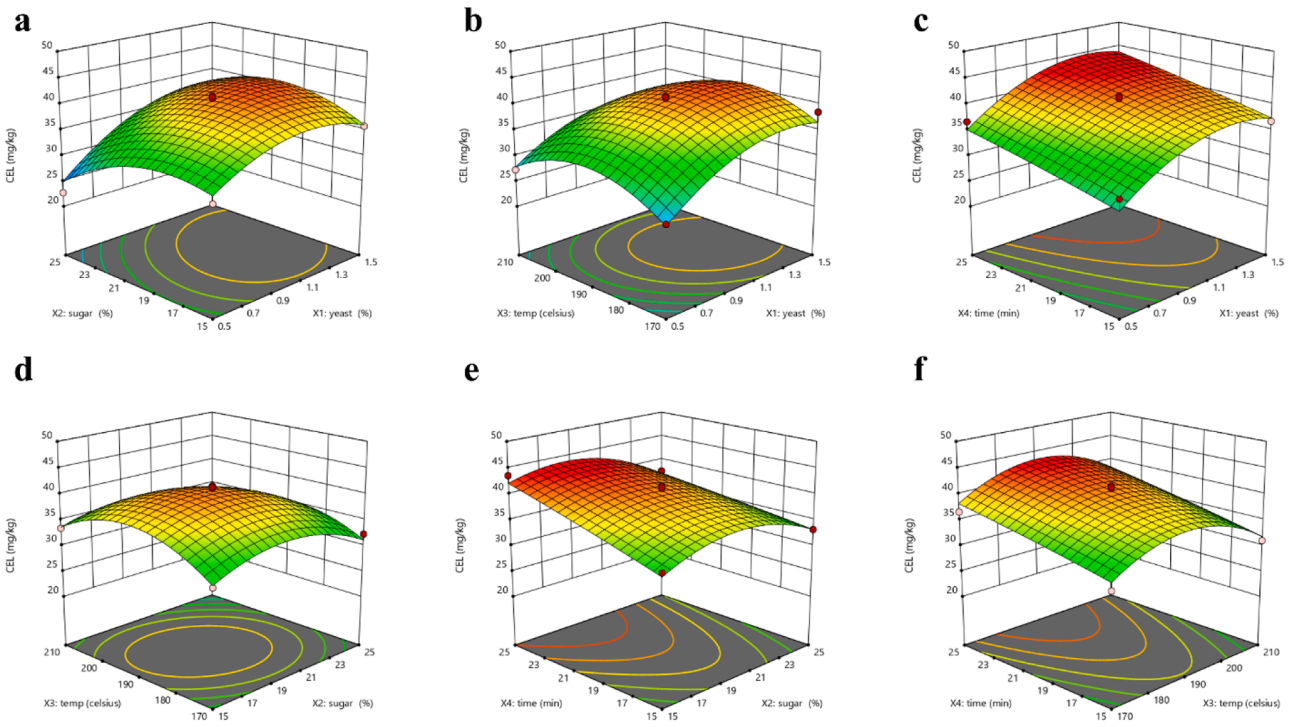


Fig. 4. 3D response surface curve and corresponding contour plot showing effect of independent variables on the formation of CEL in the bread crust. **(a)** Surface and contour plot between sugar and yeast addition. **(b)** Surface and contour plot between baking temperature and yeast addition. **(c)** Surface and contour plot between baking time and yeast addition. **(d)** Surface and contour plot between baking temperature and sugar addition. **(e)** Surface and contour plot between baking time and sugar addition. **(f)** Surface and contour plot between baking time and temperature.

decrease with yeast dosage, sugar dosage, and heating temperature (Fig. 4a~ f). Temperature and time are two crucial parameters in AGEs formation; generally, AGEs levels increase with temperature. However, the levels of CML and CEL increased 1-fold and 10-fold, respectively, in raw meat after high-temperature treatment, suggesting that high temperatures are more likely to promote CEL production (Sun et al., 2015).

3.6. Optimization of bread processing conditions

Given the high correlation coefficients ($R^2 = 0.9129$ for CML and $R^2 = 0.9466$ for CEL), the RSM analysis suggested that optimal processing conditions can effectively minimize AGE formation during industrial bread processing. The parameters for achieving the minimum CML and CEL content were identified in the model as follows: yeast addition at 0.53 %, sucrose addition at 23.22 %, baking temperature at 208.71 °C, and baking time at 15.26 min. Under the optimal parameters, the predicted value of CML output from the model was: 23.47 mg/kg and the predicted value of CEL: 22.13 mg/kg. To ensure practical applicability and accuracy in industrial settings, these conditions were modified to the following integer values: yeast addition at 0.6 %, sugar addition at 23 %, baking temperature at 210 °C, and baking time at 15 min. Under these modified conditions, the AGEs content of 6 parallel-baked bread samples was measured as 24.57 ± 3.82 mg/kg for CML and 24.74 ± 3.09 mg/kg for CEL, corresponding to 104.5 % and 111.8 % of predicted values, respectively. The deviation between observed and predicted values was acceptable. Compared to the bread processed under standardly used parameters, these adjustments resulted in a reduction of the CML and CEL levels by 27.07 % and 18.67 %, respectively. Our findings suggested that optimized processing conditions can effectively control the generation of AGEs during industrial bread processing, thereby reducing population exposure to AGEs from bread.

Table 5
Sensory evaluation scores of basic bread and optimized bread.

Breads	Appearance	Texture	Flavor	Taste	Overall acceptance
Basic bread	7.43 ± 1.04	7.03 ± 1.24	6.70 ± 1.05	6.93 ± 0.98	7.10 ± 0.88
Optimized bread	7.23 ± 0.97	6.73 ± 0.91	7.27 ± 0.87*	7.63 ± 0.85*	7.13 ± 0.87

The data are presented as mean ± SD (n=3). Values with * in the same column are significantly different ($P < 0.05$).

3.7. Sensory evaluation and volatile profiles

An integral part of the study was to reduce the amount of CML and CEL formation in bread while preserving acceptable sensory limits of the bread. The sensory evaluation scores for the basic model bread and RSM optimized bread by volunteers, as presented in Table 5, demonstrated no significant difference between basic bread and the optimized in terms of overall acceptability, while the scores varied for each attribute parameter. The optimized breads exhibited a deeper crust color, a harder texture, and reduced crumb elasticity. However, no significant differences were observed in the appearance or textural characteristics scores between the two bread types. The optimized breads received significantly higher taste and flavor scores than the basic breads. Volunteers described them as tasting sweeter and more pleasant, with a notably more appealing aroma.

With a further order to render certain the flavor properties manifest little alterations accompanying the thermal process optimized, this study evaluate the volatile files of the basic and optimized bread by employing an electronic nose detector combined chemometrics system. Those volatile compounds, as a source for the hedonic odor attribute of bread, are generated through various chemical reactions during bread processing. The volatile compounds in the crumb are deemed to be

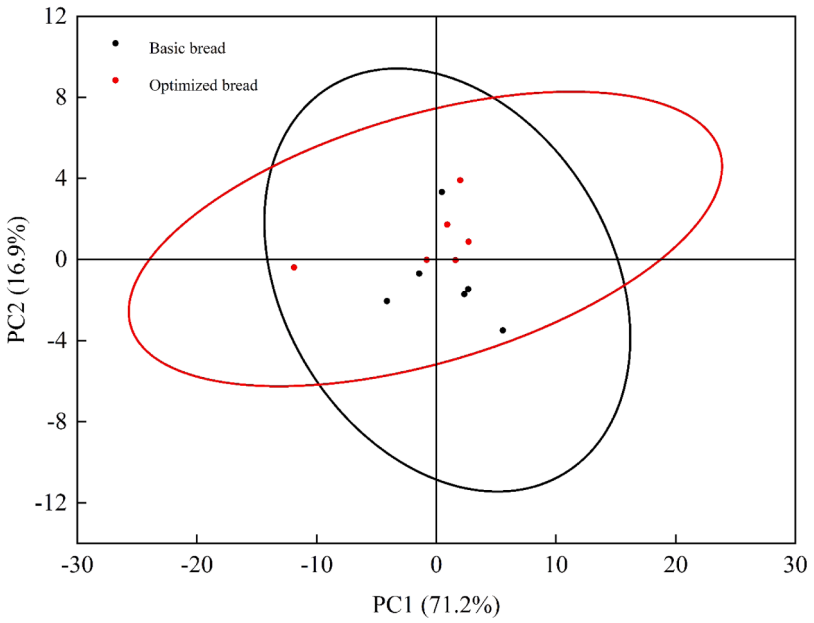


Fig. 5. Principal Component Analysis of the volatiles for the Basic model bread and optimized bread (n=6).

primarily generated by yeast fermentation, lipid oxidation and enzymatic reaction, while the volatiles give off from the crust are found to be associated with non-enzymatic reactions, particularly the Maillard reaction (Ma et al., 2021; Pico et al., 2015). In our results of the electronic nose test, the volatiles of bread mainly include 8 categories, alcohols, esters, aldehydes, ketones, acids, aliphatic hydrocarbons, aromatic compounds and nitrogen-containing compounds. The response values for some certain alcohols and esters of the optimized appeared to exhibit an uptrend than those of the basic bread, though, there was no statistical difference. To further recognize the differences of volatile compounds between basic model and RSM optimized bread, Principal Component Analysis (PCA) was employed. Fig. 5 shows an PCA scores plot reflecting clustering of the two groups. The sum of PC1 and PC2 described 88.1 % (71.2 % for PC1 and 16.9 % for PC2, respectively). The confidence ellipse plots of the optimized and basic bread overlapped for the most part, indicating that few alterations of volatiles in bread were rendered during the optimized process compared to the basic one.

4. Conclusion

The present study indicated that the formation of CML in yeast-leavened wheat bread is influenced by yeast and sucrose additions, as well as baking temperature and time. RSM optimization yielded conditions that significantly reduced CML and CEL, i.e., yeast addition at 0.6 %, sugar addition at 23 %, baking temperature at 210 °C, and baking duration at 15 min. Under these conditions, CML and CEL levels were reduced by 27.07 % and 18.67 %, respectively, compared to bread produced under standard conditions. The study holds promise for application in large-scale industrial production, aiming to achieve feasibility in producing bread with low AGEs content that is also acceptable in terms of taste and flavor to consumers, thereby promoting health.

Ethical Statement

The Ethics Committee of Tongji Medical College concluded that the edible bread tasting program of the study doesn't require ethical approval. Besides, all volunteers have signed informed consent forms before the bread tasting.

CRediT authorship contribution statement

Guanhua Jiang: Writing – original draft, Visualization, Validation, Software, Methodology. **Haili Yu:** Visualization, Resources, Methodology, Data curation. **Liangkai Chen:** Writing – review & editing, Supervision, Resources, Investigation, Formal analysis. **Yan Zhang:** Writing – review & editing, Supervision, Resources, Investigation. **Liegang Liu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

All authors declare no competing interests, and certify that they have participated sufficiently in work to take public responsibility for the conception and design of the study, acquisition, analysis and interpretation of data. All authors have read and approved the manuscript, which neither has been published previously nor is being considered by any other peer-reviewed journal.

Acknowledgment

We gratefully acknowledge the financial support provided by the National Natural Science Foundation of China (Project No. 81773423, 82473625).

Data availability

The data that has been used is confidential.

References

Ahrne, L., Andersson, C.-G., Floberg, P., et al., 2007. Effect of crust temperature and water content on acrylamide formation during baking of white bread: Steam and falling temperature baking. Lwt-Food Sci. Technol. 40 (10), 1708–1715. https://doi.org/10.1016/j.lwt.2007.01.010.

Chen, G., 2021. Dietary N-epsilon-carboxymethyllysine as for a major glycotoxin in foods: a review. Compr. Rev. Food Sci. Food Saf. 20 (5), 4931–4949. https://doi.org/10.1111/1541-4337.12817.

Cheng, W., Wang, X., Zhang, Z., et al., 2021. Development of an isotope dilution UHPLC-QqQ-MS/MS-based method for simultaneous determination of typical advanced glycation end products and acrylamide in baked and fried foods. J. Agric. Food Chem. 69 (8), 2611–2618. https://doi.org/10.1021/acs.jafc.0c07575.

Dobi, A., Bravo, S.B., Veeren, B., et al., 2019. Advanced glycation end-products disrupt human endothelial cells redox homeostasis: new insights into reactive oxygen

species production. Free Radic. Res. 53 (2), 150–169. https://doi.org/10.1080/10715762.2018.1529866.
Fu, Q., Li, L., Li, B., 2012. Formation of N epsilon-(Carboxymethyl)lysine in saccharide-lysine model systems by different heat treatments. Int. J. Food Eng. 8 (3). https://doi.org/10.1515/1556-3758.2724.
Gill, V., Kumar, V., Singh, K., et al., 2019. Advanced glycation end products (AGEs) May Be a striking link between modern diet and health. Biomolecules. (12), 9. https://doi.org/10.3390/biom9120888.
Heitmann, M., Zannini, E., Arendt, E., 2018. Impact of Saccharomyces cerevisiae metabolites produced during fermentation on bread quality parameters: a review. Crit. Rev. Food Sci. Nutr. 58 (7), 1152–1164. https://doi.org/10.1080/10408398.2016.1244153.
Hull, G.L.J., Woodside, J.V., Ames, J.M., et al., 2012. N-epsilon-(carboxymethyl)lysine content of foods commonly consumed in a Western style diet. Food Chem. 131 (1), 170–174. https://doi.org/10.1016/j.foodchem.2011.08.055.
Jiao, Y., He, J., He, Z., et al., 2019. Formation of N(ε)-(carboxymethyl)lysine and N(ε)-(carboxyethyl)lysine during black tea processing. Food Res. Int. 121, 738–745. https://doi.org/10.1016/j.foodres.2018.12.051.
Jing, H., Kitts, D.D., 2002. Chemical and biochemical properties of casein-sugar Maillard reaction products. Food Chem. Toxicol. 40 (7), 1007–1015. https://doi.org/10.1016/s0278-6915(02)00070-4.
Jost, T., Henning, C., Heymann, T., et al., 2021. Comprehensive analyses of carbohydrates, 1,2-dicarbonyl compounds, and advanced glycation end products in industrial bread making. J. Agric. Food Chem. 69 (12), 3720–3731. https://doi.org/10.1021/acs.jafc.0c07614.
Koska, J., Saremi, A., Howell, S., et al., 2018. Advanced glycation end products, oxidation products, and incident cardiovascular events in patients with type 2 diabetes. Diabetes Care 41 (3), 570–576. https://doi.org/10.2337/dc17-1740.
Laroque, D., Inisan, C., Berger, C., et al., 2008. Kinetic study on the Maillard reaction. Consideration of sugar reactivity. Food Chem. 111 (4), 1032–1042. https://doi.org/10.1016/j.foodchem.2008.05.033.
Li, L., Han, L., Fu, Q., et al., 2012. Formation and inhibition of Nε-(carboxymethyl)lysine in saccharide-lysine model systems during microwave heating. Molecules. 17 (11), 12758–12770. https://doi.org/10.3390/molecules171112758.
Liang, Z., Li, L., Fu, Q., et al., 2016. Formation and elimination of pyrraline in the Maillard reaction in a saccharide-lysine model system. J. Sci. Food Agric. 96 (7), 2555–2564. https://doi.org/10.1002/jsfa.7376.
Liu, T., Li, Y., Sadiq, F.A., et al., 2018. Predominant yeasts in Chinese traditional sourdough and their influence on aroma formation in Chinese steamed bread. Food Chem. 242, 404–411. https://doi.org/10.1016/j.foodchem.2017.09.081.
Ma, S., Wang, Z., Guo, X., et al., 2021. Sourdough improves the quality of whole-wheat flour products: mechanisms and challenges-a review. Food Chem. 360, 130038. https://doi.org/10.1016/j.foodchem.2021.130038.
Nakra, S., Tripathy, S., Srivastav, P.P., 2025. Green and sustainable extraction of bioactive compounds from Centella asiatica leaves using microwave pretreatment and Ultrasonication: kinetics, process optimization, and biological activity. Food Biophys. 20 (1), 56. https://doi.org/10.1007/s11483-025-09948-9.
Nguyen, H.T., van der Fels-Klerx, H.J., van Boekel, M.A., 2016. Kinetics of N(ε)-(carboxymethyl)lysine formation in aqueous model systems of sugars and casein. Food Chem. 192, 125–133. https://doi.org/10.1016/j.foodchem.2015.06.110.
Nie, C., Li, Y., Qian, H., et al., 2022. Advanced glycation end products in food and their effects on intestinal tract. Crit. Rev. Food Sci. Nutr. 62 (11), 3103–3115. https://doi.org/10.1080/10408398.2020.1863904.
Nováková, L., Vlcková, H., 2009. A review of current trends and advances in modern bio-analytical methods: chromatography and sample preparation. Anal. Chim. Acta 656 (1-2), 8–35. https://doi.org/10.1016/j.aca.2009.10.004.
Park, J. J., Olawuyi, I. F., & Lee, W. Y., 2023. Effect of combined UV-thermosonication and Ecklonia cava extract on advanced glycation end-products in soymilk. 46(1), e14208. doi:https://doi.org/10.1111/jfpe.14208.
Pešić, M.B., Pešić, M.M., Bezbradica, J., et al., 2023. Okara-enriched gluten-free bread: nutritional, antioxidant and sensory properties. Molecules. (10), 28. https://doi.org/10.3390/molecules28104098.
Pétel, C., Onno, B., Prost, C., 2017. Sourdough volatile compounds and their contribution to bread: a review. Trends. Food Sci. Technol. 59, 105–123. https://doi.org/10.1016/j.tifs.2016.10.015.
Pico, J., Bernal, J., Gómez, M., 2015. Wheat bread aroma compounds in crumb and crust: a review. Food Res. Int. 75, 200–215. https://doi.org/10.1016/j.foodres.2015.05.051.
Poulsen, M.W., Hedegaard, R.V., Andersen, J.M., et al., 2013. Advanced glycation endproducts in food and their effects on health. Food Chem. Toxicol. 60, 10–37. https://doi.org/10.1016/j.fct.2013.06.052.
Qin, R., Wu, R., Shi, H., et al., 2022. Formation of AGEs in fish cakes during air frying and other traditional heating methods. Food Chem. 391, 133213. https://doi.org/10.1016/j.foodchem.2022.133213.
Scheijen, J., Clevers, E., Engelen, L., et al., 2016. Analysis of advanced glycation endproducts in selected food items by ultra-performance liquid chromatography tandem mass spectrometry: Presentation of a dietary AGE database. Food Chem. 190, 1145–1150. https://doi.org/10.1016/j.foodchem.2015.06.049.
Sergi, D., Boulestin, H., Campbell, F.M., et al., 2021. The role of dietary advanced glycation end products in metabolic dysfunction. Mol. Nutr. Food Res. 65 (1), e1900934. https://doi.org/10.1002/mnfr.201900934.
Sharma, A., Weber, D., Raupbach, J., et al., 2020. Advanced glycation end products and protein carbonyl levels in plasma reveal sex-specific differences in Parkinson's and Alzheimer's disease. Redox. Biol. 34, 101546. https://doi.org/10.1016/j.redox.2020.101546.
Singh, S. M., Tripathy, S., Ghodki, B. M., et al., 2025. Optimization of wall material composition for encapsulating bioactive compounds from Giloy (Tinospora cordifolia) stems. 2025(1), 6459848. doi:https://doi.org/10.1155/jfpp/6459848.
Srey, C., Hull, G.L., Connolly, L., et al., 2010. Effect of inhibitor compounds on Nε-(carboxymethyl)lysine (CML) and Nε-(carboxyethyl)lysine (CEL) formation in model foods. J. Agric. Food Chem. 58 (22), 12036–12041. https://doi.org/10.1021/jf103353e.
Sun, X., Tang, J., Wang, J., et al., 2015. Formation of advanced glycation endproducts in ground beef under pasteurisation conditions. Food Chem. 172, 802–807. https://doi.org/10.1016/j.foodchem.2014.09.129.
Uribarri, J., del Castillo, M.D., de la Maza, M.P., et al., 2015. Dietary advanced glycation end products and their role in health and disease. Adv. Nutr. 6 (4), 461–473. https://doi.org/10.3945/an.115.008433.
Yu, L., Li, Y., Gao, C., et al., 2022. N(ε)-carboxymethyl-lysine and N(ε)-carboxyethyl-lysine contents in commercial meat products. Food Res. Int. 155, 111048. https://doi.org/10.1016/j.foodres.2022.111048.
Yuan, X., Nie, C., Liu, H., et al., 2021. Comparison of metabolic fate, target organs, and microbiota interactions of free and bound dietary advanced glycation end products. Crit. Rev. Food Sci. Nutr. 1–22. https://doi.org/10.1080/10408398.2021.1991265.
Yuan, X., Nie, C., Liu, H., et al., 2023. Comparison of metabolic fate, target organs, and microbiota interactions of free and bound dietary advanced glycation end products. Crit. Rev. Food Sci. Nutr. 63 (19), 3612–3633. https://doi.org/10.1080/10408398.2021.1991265.
Zhang, Q., Wang, Y., Fu, L., 2020. Dietary advanced glycation end-products: Perspectives linking food processing with health implications. Compr. Rev. Food Sci. Food Saf. 19 (5), 2559–2587. https://doi.org/10.1111/1541-4337.12593.
Zhao, D., Sheng, B., Wu, Y., et al., 2019. Comparison of free and bound advanced glycation end products in food: a review on the possible influence on human health. J. Agric. Food Chem. 67 (51), 14007–14018. https://doi.org/10.1021/acs.jafc.9b05891.