Effect of marinating with green tea extract on the safety and sensory profiles of oven-baked oyster

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Abstract

Baked oyster is a popular seafood dish around the world. The present study investigated the effect of various concentrations of a green-tea extract (GTE) marinade on the safety and sensory profiles of oysters baked for different durations. The results showed 10 g/L of GTE and 10-min baking time was the optimal combination, as supported by significantly attenuated lipid oxidation (35.29%) and Nε-(carboxyethyl)lysine (CEL) content (48.51%) without appreciable negative impact on the sensory or nutritional quality of the oysters. However, high concentrations of the marinade or prolonged baking promoted protein oxidation and Nε-(carboxymethyl)lysine (CML) formation likely through the pro-oxidative action of the GTE phytochemicals. Correlation analysis further revealed the main factors that affected CML, CEL, and fluorescent AGEs generation, respectively. These findings provide theoretical support for the protective effect and mechanism of GTE against quality deterioration of baked oysters and would help broaden the application of GTE in the food industry.

Keywords: Oyster; Green tea extract; Lipid oxidation; Protein oxidation; Advanced glycation end-products
1. Introduction

Oyster is one of the most consumed marine bivalves worldwide, and its global production has exceeded 5.5 million tons per year in recent decades (Fu et al., 2024). It is worth noting that South China produces the world’s fourth-largest amount of edible oyster meat (the main species being *Crassostrea hongkongensis* (Loaiza, Wong, & Thiagarajan, 2023). Some marine foods including oysters are also known as “superfoods” owing to their rich content of high-quality proteins, lipids, vitamins, and other nutrients (Loaiza et al., 2023). In particular, omega-3 polyunsaturated fatty acids (n-3 PUFAs) like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported to possess multiple beneficial properties in major chronic disorders such as cardiovascular disease (Tan, Huang, Tan, Lim, Peng, & Cheong, 2023). In China and other Asian regions, steaming, boiling, barbecuing, and baking are common home cooking methods for oysters (Zhou, Dong, & Zeng, 2022). These methods not only bring out the unique flavor of oysters but also help ensure microbiological safety by eliminating pathogenic bacteria in raw oysters (C. Liu et al., 2021). Nonetheless, cooking at high temperatures may also have adverse impact due to heat-induced reactions, especially protein and lipid oxidation and the formation of hazardous substances, that would eventually compromise the physicochemical, nutritional, and safety properties of the food product concerned (Nawaz et al., 2022). Because of the high content of lipids and proteins, advanced glycation end-products (AGEs) have been identified to be among the most important types of the above mentioned heat-induced harmful by-products in oysters (Uribarri et al., 2010). AGEs are a large class of compounds formed at the later stages of the Maillard reaction. In addition
to glycosylation of proteins (or amino acids), AGE formation has also been reported to be closely associated with other reactions in food such as lipid oxidation, sugar autoxidation, and polyol degradation (Nawaz et al., 2022; Shi et al., 2024). Based on their structures and properties, AGEs can be categorized into fluorescent cross-linked AGEs (e.g., pentosidine), non-fluorescent cross-linked AGEs (e.g., methyl glyoxal (MGO)-lysine dimer (MOLD)), and non-fluorescent non-cross-linked AGEs (e.g., Nε-(carboxymethyl)lysine (CML) and Nε-(carboxyethyl)lysine (CEL)) (Yeh, Hsia, Lee, & Wu, 2017). Accumulating evidence suggests that high intake of dietary AGEs may be associated with elevated risk of developing certain diseases, such as type-2 diabetes and cardiovascular disease (Q. Zhang, Wang, & Fu, 2020). Therefore, the development of strategies to effectively reduce AGE content in food products are essential to mitigate the health risks associated with dietary AGEs exposure.

Marinating prior to heat processing is an effective and widely used pretreatment method to improve the quality of the final food products (Khan et al., 2024). Green tea and its extract have received much attention as a natural seasoning ingredient in many cuisines not only because of its beneficial phytochemicals, particularly phenolic antioxidants, but also because of its potential to impart the characteristic green tea flavor on food products. Studies have also demonstrated that marinating with green tea prior to heat treatment could effectively inhibit the formation of hazardous substances, including polycyclic aromatic hydrocarbons (PAHs) (Shen, Huang, Tang, Zhan, & Liu, 2022; C. Wang et al., 2018) and heterocyclic amines (HAs) in meat (Park, Pyo, Kim, & Yoon, 2017; Quelhas, Petisca, Viegas, Melo, Pinho, & Ferreira, 2010; Yao, Khan, Cheng, Ang,
Zhou, & Huang, 2020). In another study that investigated the effect of different tea infusions on charcoal-grilled chicken wings, green tea was found to be the most efficient in inhibiting the formation of PAH8, with a 57% inhibition rate (C. Wang et al., 2018). Besides the unique catechins and catechin-derived phenolic antioxidants, the distinct advantages of green tea as a culinary ingredient and for application in the food industry over other types of tea rest on its light color and wider consumer acceptance, the latter may be attributed to its long history of consumption, especially in Asian diets, and the ample literature data on its nutraceutical and pharmacological properties (Cunha, Gadelha, Mello, Marmelo, Marques, & Fernandes, 2022; Dai, Sameen, Zeng, Li, Qin, & Liu, 2022; Passos et al., 2022; Xing, Zhang, Qi, Tsao, & Mine, 2019).

The unique protein, lipid, and micronutrient profiles of oysters endow them extraordinary popularity as a culinary ingredient in many cuisines. Paradoxically, these favorable characteristics also render them susceptible to deteriorative changes, especially under thermal and/or pro-oxidative conditions. Although the inhibitory activity of tea extract against the formation of toxic Maillard by-products PAHs and HAs in meat products has been well documented (Park et al., 2017; Quelhas et al., 2010; Shen et al., 2022; C. Wang et al., 2018; Yao et al., 2020), limited information is available regarding its effectiveness in improving key quality attributes of seafood. Thus far, most of the research on oysters has focused on their sensory attributes (Fu et al., 2024; Loaiza et al., 2023) and biological functions (Lee et al., 2021; D. Zhu, Yuan, Wu, Wu, El-Seedi, & Du, 2023). While increasing attention has also been centered on the potential of green tea extract (GTE) as a postharvest treatment agent to extend the shelf life of oysters (Xi, Liu,
& Su, 2012), the effect of marinating with GTE on the formation of AGEs as well as key parameters associated with lipid and/or protein oxidation in high-temperature processed oysters has not been reported. Therefore, the present study aimed to evaluate the inhibitory activity of different concentrations of a GTE marinade against the formation of AGEs in baked oysters. Considering the phenolic antioxidant-rich nature of GTE, total phenolic content, antioxidant activity, lipid (thiobarbituric acid-reactive substance (TBARS)) and protein (protein carbonyl) oxidation, free amino acid and fatty acid composition, as well as color and texture of baked oysters pretreated with the GTE marinade were also analyzed.

2. Materials and methods

2.1. Materials and chemicals

Green tea (C. sinensis Longjing 43) was purchased from Hangzhou (Hangzhou Tea Factory Co. Ltd, China). Oysters (Crassostrea hongkongensis) were from a wet market in Taishan (Guangdong Province, China). Hydrochloric acid, phosphoric acid, sodium hydroxide, sodium borohydride, and trichloromethane were procured from Sinopharm Chemical Reagent Company (Shanghai, China). $N^\epsilon$-carboxymethyl-lysine-d4 (CML-d4) and $N^\epsilon$-carboxyethyl-lysine-d4 (CEL-d4) with 98% purity were obtained from Toronto Research Chemicals Inc. (TRC, Toronto, ON, Canada). HPLC grade formic acid and acetonitrile were obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA) and Merck KGaA (Darmstadt, Germany), respectively. Amino acid reference standards were purchased from Waters Corporation (Waters, Milford, MA, USA). Standards of fatty acid methyl esters (FAMEs) were from Anple Laboratory Technologies Inc. in
China. All other analytical grade chemicals and solvents were from Macklin Biochemical Technology Co., Ltd (Shanghai, China).

2.2. Preparation of GTE and oven-baked oyster samples

Tea leaves were ground to powders and passed through a 40-mesh sieve before extraction. A series of GTE marinade of different concentrations were prepared by respectively extracting 2.5 g, 5 g, 10 g, and 20 g of the powders with 500 mL of ultrapure water (90 °C) for 30 min in a sonicator maintained at 45 °C. The supernatant was collected after centrifugation and used for marinating oysters.

Fresh oysters of similar weight (~25 g) were selected for this study. They were randomly divided into five groups (15/group) for the following marinating treatments: control (ultrapure water), and GTE treatment groups (5 g, 10 g, 20 g, and 40 g/L of the GTE marinade, respectively). The ratio of oyster to marinade was 1:1 (w/vol.) and the marination was performed at 4 °C for 4 h. After marination, the oysters were removed from the marinades, dried slightly with a paper towel, and baked in a BAKLON-58M oven (Germany) at 200 °C for 10, 15, and 20 min, respectively (Fig. 1). After baking, the samples were freeze-dried and stored at −20 °C until analysis.

2.3. Color and texture profile analysis (TPA)

A calibrated Hunter Lab instrument (LX 18964, Hunter Associates Lab Inc., VA, USA) with D65/10° illuminant, 25-mm aperture, and standard observer settings was used for the color analysis of the baked oysters. For TPA, an oven-baked oyster was placed on the platform of a texture analyzer (TA. newplus, Shanghai Rui Fen Co. Ltd, China). A
spherical probe (TA/0.25S) was used with the following parameters: force = 40 gf, trigger = 5 gf, and test speed = 1 mm/s. Five oysters from each group were analyzed.

2.4. Preparation of hydrophilic and lipophilic extracts from oven-baked oysters

Hydrophilic and lipophilic extracts of the oyster samples were prepared for the determination of phenolic contents and antioxidant activity according to the method in a recent study (Q. Wang et al., 2020) with minor modifications. In brief, approximately 0.5 g of each oyster sample was homogenized (IKA T25, Digital Ultra-Turrax, Germany) in 9 mL of phosphate buffered solution (PBS, 50 mM, pH 7.5). The homogenate was centrifuged (3,780 × g, 20 min) to obtain the supernatant. The supernatant (1 mL) was mixed with 5 mL of ethanol to precipitate proteins, and the supernatant after centrifugation (3,780 × g, 20 min) was used as the hydrophilic extract. The residue was suspended in 9 mL of acetone/ethanol (50 : 50, v/v), vortexed, and incubated in darkness at room temperature for 30 min. The mixture was centrifuged to collect the supernatant as the lipophilic extract.

2.5. Analysis of antioxidant activity

Antioxidant activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay (Chen et al., 2019). A standard curve was constructed using a series of Trolox solutions in a concentration range of 50 – 400 μM. The activity of samples was expressed as μmol Trolox equivalent (TE)/g oyster.

2.6. Analysis of total phenolic contents (TPC)

TPC was determined by the Folin-Ciocalteau assay as described by Manful et al.
Briefly, 25 μL of each sample extract was added to a well of a 96-well plate with 125 μL of Folin-Ciocalteu reagent (0.2 M). For the hydrophilic extracts, 50 μL of PBS was added, while for the lipophilic extracts, 50 μL of acidified ethanol (0.7% v/v) was added. The resulting mixtures were incubated in darkness at room temperature for 60 min, and their absorbance was measured at 760 nm on a Biotek Agilent™ H1 multimode reader (Agilent Technologies, Santa Clara, CA, USA). The data are expressed as mg gallic acid equivalent (GAE)/g oyster.

2.7. TBARS assay

The determination of TBARS content was based on the method of Kerth and Rowe (Kerth & Rowe, 2016). A freeze-dried oyster sample (0.5 g each) was placed in a 50-mL centrifuge tube with 15 mL of trichloroacetic acid (TCA, 7.5%, v/v) extraction solution. After homogenization at 7,200 rpm for 1 min, the homogenate was centrifuged at 3,780 × g for 20 min. Each supernatant (5 mL) was mixed with an equal volume of 80 mM 2-thiobarbituric acid (TBA) in a 50-mL centrifuge tube and then incubated for 130 min in a thermostatic water-bath shaker (Model: FWS-30, FAITHFUL Co., Ltd, China) set at 40 °C. After incubation, 250 μL of each sample was transferred to a well in a 96-well plate, and the absorbance was measured at 540 nm in a microplate reader. The contents of TBARS were calculated using malondialdehyde (MDA) as a reference standard and expressed as mg MDA /kg sample.

2.8. Analysis of protein carbonyl content

Soluble proteins were extracted from the freeze-dried oyster samples according to
the method of Wang and co-workers (Q. Wang et al., 2020) with slight modifications. Briefly, 0.2 g of each sample was dissolved in 5 mL of \(n\)-hexane to remove fat. The defatted sample was added to PBS (15 mL), homogenized at 10,000 rpm for 2 min, and then centrifuged at 9,500 \(\times\) g for 10 min. Protein concentration of the supernatant was determined using a Bradford Protein Assay Kit (Beyotime Biotechnology Co., Ltd, China).

Protein carbonyl contents of the oyster samples were determined using the procedure of Mesquita’s study (Mesquita, Oliveira, Bento, Geraldo, Rodrigues, & Marcos, 2014). An equal volume (400 \(\mu\)L) of 2,4-dinitrophenylhydrazine (DNPH, 10 mM in 0.5 M \(H_3PO_4\)) and protein solution obtained from each of the oyster samples were mixed and incubated for 10 min at room temperature. Then 400 \(\mu\)L of NaOH (6 M) was added and incubated for 10 min in the dark. Absorbance was read at 450 nm against a blank, which contained an equal volume of PBS solution in place of the protein solution. The molar absorption coefficient of 22,000 mol\(^{-1}\) cm\(^{-1}\) was utilized to quantify the concentration of protein carbonyls.

2.9. Analysis of amino acid composition

The method of hydrolysis, derivatization, and determination of amino acids was adopted from the study of Loaiza et al. (Loaiza et al., 2023). Samples were hydrolyzed in 5 mL of 6 M HCl in sealed tubes at 110 \(^\circ\)C for 24 h. The hydrolyzed samples were then derivatized with AccQ-Tag ultra and analyzed by high-performance liquid chromatography (HPLC, Model: 1260, Agilent Technology, Santa Clara, CA, USA) with a ZORBAX SB-C18 (4.6 mm \(\times\) 250 mm, 5 \(\mu\)m) column. The samples were filtered with
a 0.22-μm microfiltration membrane prior to HPLC analysis. The absorbance was monitored at 254 nm.

2.10. Analysis of fatty acid composition

Fatty acid methyl esters (FAMEs) were generated using a one-stage transmethylation method (Perez-Palacios, Solomando, Ruiz-Carrascal, & Antequera, 2022). GC-MS analysis was performed on an Agilent model 7890B gas chromatograph equipped with a model 5977B selective mass selection detector and a model 7693A automatic sampler. An Agilent HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm; Waldbronn, Germany) was used for separation. Injection was in split mode (5:1 split ratio) and injection volume was 1 μL. The injector was maintained at 225 °C. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The column temperature was programmed from 45 °C (isothermal for 1.5 min) to 150 °C at 15 °C/min, then increased at a rate of 6 °C/min to 240 °C (isothermal for 12 min). The FAMEs were identified by using the Agilent Mass Hunter Workstation Quantitative Analysis Software and quantified against calibration curves of standard FAMEs.

2.11. Extraction and determination of CML and CEL

The extraction and determination of CML and CEL were based on previously reported methods with slight modifications (Cheng et al., 2021). First, 0.5 g of each sample was defatted with n-hexane prior to acid hydrolysis. The sample was reduced using 5 mL of 0.2 M sodium boric buffer and 2.5 mL of 1 M sodium borohydride at 4 °C overnight. The reducing solution was vigorously mixed with 4 mL of
chloroform/methanol (2:1, v/v) and centrifuged at 3,780 × g for 15 min to precipitate proteins. The precipitate was then mixed with 5 mL of 6 M HCl and hydrolyzed at 110 °C for 24 h. After cooling to room temperature, the protein hydrolysate was filtered and diluted to 25 mL with ultrapure water, from which 1.5 mL was taken and spiked with CML-d4 and CEL-d4 to a final concentration of 25 ng/mL. The solution was subjected to solid-phase extraction. The eluate was dried under nitrogen and reconstituted with 4 mL of acetonitrile/water (2:98, v/v). Lastly, the sample solution was filtered through a 0.22-μm filter and analyzed by UPLC-MS.

The UPLC-MS analysis was conducted in an ACQUITY UPLC H-Class Plus XEVO TQ-XS system equipped with an electrospray ionization (ESI) interface (Waters, Milford, MA, USA). For chromatographic separation, a Waters ACQUITY HSS T3 column (1.8 μm, 2.1 mm × 100 mm) was employed. The column temperature was set at 30 °C, and the injection volume was 1 μL. The mobile phase (0.3% formic acid) was set at a flow rate of 0.08 mL/min. The detection of parent and fragment ions of CML and CEL was performed using positive ESI in multiple reaction monitoring (MRM) mode. The conditions of ESI and gradient elution were based on those of our recent study (Cheng et al., 2021).

2.12. Measurement of total fluorescent AGEs

The extraction of fluorescent AGEs from the baked oysters was based on the method of a previous study with slight modifications (W. Wang et al., 2023). Five milliliters of extraction buffer (0.05% Tween 20, 1% sodium dodecyl sulfate, 5% β-mercaptoethanol, and 50 mM Tris-HCl at pH 7.4) and 250 mg of each freeze-dried oyster sample were
gently mixed in a water-bath shaker at room temperature for 12 h. The supernatant was collected after centrifugation at 21,380 × g for 20 min. The levels of fluorescent AGES were measured at excitation/emission wavelengths of 325/440 nm in a microplate reader.

2.13. Statistical analysis

Statistical analysis of the data was conducted using SPSS version 26 (SPSS Inc., Chicago, IL, USA) and one-way analysis of variance (ANOVA) at a confidence level of 0.05. Duncan’s multiple-range test for the population with equal variances and Tamhane’s test for those with unequal variances were employed to carry out multiple comparisons at \( P < 0.05 \), and the findings are presented as mean ± SD. Pearson’s correlation was performed using SPSS.

3. Results and Discussion

3.1. Effect of GTE on total phenolic content and antioxidant activity of oven-baked oysters

Phenolic antioxidants have been well established to be among the most important bioactive phytochemicals in tea (S. Li, Zhang, Wan, Zhan, & Ho, 2022). To investigate whether the GTE marinade could introduce phenolic compounds and thus antioxidant capacity to the oysters, total phenolic content (TPC) and total antioxidant activity (TAA) of the baked oysters were measured by the Folin-Ciocalteu assay and DPPH radical scavenging assay, respectively. TPC of the baked oysters with or without marinating with different concentrations of the GTE ranged from 6.93 to 9.86 mg of GAE per g of sample (Fig. 2). Their TPCs also exhibited a somewhat linear relationship with GTE
concentrations. To better understand the antioxidant property/nature of the oyster samples, hydrophilic and lipophilic oyster extracts were prepared. It appeared that baking time played an important part in the relative contribution of hydrophilic (HPC) and lipophilic phenolic content (LPC) to the TPC. For the 10- and 15-min baking time, the control (GTE 0) and low concentrations of the GTE marinade (5 and 10 g/L) presented significantly higher HPC than LPC and vice versa for the higher concentrations of the GTE marinade. For the 20-min baking time, however, the HPC and LPC of the oyster samples did not seem to have any significant difference in their relative contribution to the TPC. It was also noted that baked oysters without pretreatment with the GTE marinade possessed considerable TPC. This may originate from their phenolic constituents, such as 3,5-dihydroxy-4-methoxybenzyl alcohol, gallic acid, and chlorogenic acid (Lee et al., 2021; Watanabe et al., 2012).

Subsequently, TAA of the oyster samples with or without marinating with the GTE were compared (Fig. 3). Consistent with the TPC data, the control oyster samples also exhibited good free radical scavenging capacities (67.15, 65.70, and 63.65 μmol TE/g at 10-, 15-, and 20-min baking time, respectively), and the GTE-pretreated samples showed significantly higher capacities. Of note, TAA of the 40 g/L GTE-pretreated group reached 74.26, 80.90, and 73.73 μmol TE/g after 10, 15, and 20 min of baking, respectively. The enhanced free radical scavenging activity could be attributed to the phenolic compounds in the GTE, especially catechins (e.g., epigallocatechin gallate, EGCG) (C. Li et al., 2023; C. Wang et al., 2018). The TPC and TAA data together support the successful transfer of the GTE phenolic compounds to the oysters during marination. The enhanced antioxidant
capacity could in turn be translated into a protective effect against heat-induced adverse oxidative reactions during thermal treatment (i.e. baking), apart from the presumably improved dietary antioxidant intake upon consumption of the oysters.

### 3.2. Effect of GTE on protein oxidation and lipid oxidation in oven-baked oysters

During heat treatment, lipids and proteins are susceptible to oxidative degradation, leading to the production of reactive intermediary compounds, such as reactive carbonyl species (RCS), which may exacerbate the formation of harmful Maillard products such as AGEs (Nawaz et al., 2022). In this study, protein oxidation and lipid oxidation were assessed by measurement of the content of protein carbonyls and TBARS, respectively.

In line with the widely accepted notion that prolonged heating at elevated temperatures can promote oxidative modifications of various molecules in food, the protein carbonyl content of the oyster samples increased with baking time (Fig. 4A). Traore et al. also noted that an increase in heating time resulted in higher carbonyl content in pork, which was significantly correlated with drip loss (Traore et al., 2012). Interestingly, none of the GTE marinades demonstrated any appreciable inhibition of the formation of protein carbonyls in the oysters. In contrast, the GTE-marinated oysters showed consistently higher contents of protein carbonyls than the control at the three baking time points assayed. The degree of protein oxidation depends on factors such as the type of heat treatment, exogenous regulators (e.g., polyphenolic phytochemicals), and metal ions (Nawaz et al., 2022). In parallel with the present research, Zhu et al. reported that the carbonyl content of deep-fried and air-fried chicken breast increased with increasing concentration of grape seed extract beyond the addition level of 0.5 g/kg (Z. This preprint research paper has not been peer reviewed. Electronic copy available at: https://ssrn.com/abstract=4719853
Zhu, Fang, Yang, Khan, Huang, & Huang, 2021). In another study, the addition of black pepper (1.5%, w/w) to fish fillets promoted protein oxidation, and the authors ascribed the phenomenon to the pro-oxidant effect of high concentrations of antioxidants (Bao, Miao, Fan, & Lai, 2020).

The TBARS values of the baked oysters in our study were close to those described in the literature (Zhou et al., 2022). Different than the effect on protein oxidation, the GTE dose-dependently inhibited lipid oxidation in the oysters during baking (Fig. 4B). The effect was more prominent for the 20-min time point at which the TBARS values of the samples marinated with different concentrations of the GTE (5 – 40 g/L) were significantly lower than that of the control (1.08 – 1.48 mg MDA/kg vs. 1.67 mg MDA/kg). However, none of the experimental groups, especially the GTE-marinated ones, showed a clear relationship between baking time and the degree of lipid oxidation.

The protective effect of tea or tea extract against lipid oxidation has also been reported in previous studies. For example, green tea and yerba mate tea marinade strongly inhibited the formation of TBARS in pancetta (Park et al., 2017). Phenolic compounds have been shown to reduce lipid oxidation and aldehyde production through several pathways, including scavenging free radicals, reacting with early lipid oxidation intermediates, and trapping lipid oxidation-derived carbonyls (Fan et al., 2023). Nevertheless, the changes in TBARS content/value over time during heat treatment in food added with phytochemicals have remained inconclusive. On one hand, the protective capacity of phytochemicals from the added plant material/extract against lipid oxidation may get weaker along the heating process primarily due to structural changes that subsequently
resulted in attenuated antioxidant activity (C. Li et al., 2023). On the other hand, prolonged heating may result in lower TBARS values, probably due to the consumption of certain aldehydes through reactions with free amino groups of amino acids or proteins (Bao et al., 2020; Q. Liu, Wang, Wang, Dong, Zhao, & Zeng, 2022; Zhou et al., 2022).

In summary, the data on protein carbonyls and TBARS content together indicate that marinating with GTE is an effective strategy against heat-induced lipid oxidation, but not protein oxidation, in baked oysters.

3.3. Effect of GTE on free amino acid and free fatty acid composition in oven-baked oysters

Subsequent to the evaluation of protein and lipid oxidation, the impact of marinating with the GTE on the composition of free amino acids (FAAs) and free fatty acids (FFAs) in baked oysters was analyzed (Table S1). The GTE marinade did not cause any significant changes in the composition or content of FAAs (24.90 – 28.05 g/100 g dry weight) in the oysters for the 10- and 20-min baking time. For the 15-min time point, however, an increase in the concentration of the GTE resulted in a successive decrease in the FAA content of the oysters. It was noted that the values of individual and total FAAs in the baked oysters were generally lower than those described in other studies (Loaiza et al., 2023). Furthermore, cysteine was not detectable in any groups, suggesting that none of the processing conditions would give rise to or promote the cysteine-associated off-flavor. FAA profiles in oysters depend on species, harvest location, reproduction cycle, microalgal diet, thermal processing condition, etc. (C. Liu et al., 2021). Aspartic acid (Asp) and glutamic acid (Glu) are major contributing amino acids to the fresh flavor of oysters.
food. Consistent with other studies on seafood including oysters (C. Liu et al., 2021) and shrimp (Duppeti, Manjabhatta, Martin, & Kempaiah, 2022), Asp and Glu were most abundant in all the groups. Lysine (Lys) and Arginine (Arg) have been identified to be among the most reactive amino acids in the formation of AGEs (Wei et al., 2022). Through the comparison of various combinations of baking time and GTE concentration, 15 min was established to be optimal, where all the four tested levels of the GTE could significantly ($P < 0.05$) reduce the contents of these two amino acids (Arg by 11.32% – 24.53% and Lys by 9.09% – 24.24%) relative to the control group. In a recent study, tea polyphenols were found to compete with glucose for binding to Lys and Arg, resulting in significantly reduced levels of these amino acids and thus inhibition of protein glycation and AGE formation, although the study was performed under low-temperature vacuum-heating conditions using sturgeon fillets (Wei et al., 2022).

In total, 17 medium- and long-chain FFAs were detected (Table S2). Of these, ~45% were saturated fatty acids (SFAs), ~15% were monounsaturated fatty acids (MUFAs), and the rest were polyunsaturated fatty acids (PUFAs), consistent with the data from a previous study (Futagawa, Yoshie-Stark, & Ogushi, 2011). Palmitic acid (C16:0) was the major fatty acid, accounting for 28.30% – 34.26% of the total fatty acids (3.77 – 4.59 mg/g dry weight), followed by EPA (C20:5n3, 10.12% – 14.44%) and DHA (C22:6n3, 10.11% – 14.87%). The relative levels of these fatty acids resemble those in other studies (Felici, Vittori, Meligrana, & Roncarati, 2020; Futagawa et al., 2011). Compared to the control, it appeared that the medium-level (10 g/L and 20 g/L) GTE marinades were most effective in protecting or even enhancing the levels of unsaturated fatty acids (UFAs,
MUFAs + PUFAs) in the oysters of the 10- and 20-min groups. In contrast, the low (5 g/L) and high (40 g/L) concentrations of the GTE marinade caused a decrease in the content of UFAs.

The PUFA content in the 20-min control group was significantly lower than that in the 10- and 15-min groups, although their total EPA and DHA contents did not significantly differ. Among the GTE-marinated groups, however, there was not a clear trend, except for the 20-min baking time where high GTE concentration seemed to be able to retain more of the PUFAs in the oysters against heat-induced degradation. Compared to the control group, the GTE marinade did not demonstrate effective protection of PUFAs for the 10- and 15-min baking time. It was also noted that some of the GTE-pretreated groups from the 15-min time point even showed lower PUFA contents than the control. In general, high temperatures and prolonged heat treatment led to a significant reduction in the UFA content of fish and shellfish (Felici et al., 2020; Tan et al., 2023). For the 20-min baking time, it appeared that the 5 g/L GTE-marinated group had reduced content of PUFAs relative to the control, and their contents were not significantly different than the control for the 10 – 40 g/L GTE-marinated groups. Many studies have reported the preventive effect of phenolic compounds on lipid peroxidation in food products. For example, quercetin prevented the oxidation of UFAs and reduced the release of aldehydes in DHA-fortified soybean oil (Fan et al., 2023). Hu et al. demonstrated that antioxidant-rich extracts from bamboo leaves could significantly reduce the loss of PUFAs, EPA, and DHA in roasted scallops (Hu et al., 2022). However, no similar pattern was observed in our study, which could be attributed to the different
nature of the food matrix and constituents in oysters as well as the interaction of the GTE with these components. Our data suggest that under prolonged heating conditions, GTE somewhat prevented the decomposition of UFAs in oysters, which may be beneficial in inhibiting the formation of harmful by-products.

3.4. Effect of GTE on CML, CEL, and fluorescent AGEs formation in oven-baked oysters

Fig. 5 presents the effects of baking time and different concentrations of the GTE on the contents of CML, CEL, and fluorescent AGEs in oysters. In broad agreement with the literature, AGE contents of the oysters were significantly higher after baking for longer durations (Zhou et al., 2022). This was particularly obvious with regard to CEL. For CML, the 15-min baked oysters had higher contents (except for the 40 g/L GTE-marinated group) than the 20-min ones, which might be attributed to its degradation during prolonged heating and that the GTE at lower concentrations was unable to confer sufficient inhibitory effect (Fig. 5A). The GTE marinade in general promoted CML formation in the oysters (especially at 10 g/L in combination with 15- and 20-baking time, respectively), although without a linear concentration-dependent trend. Weaker enhancing effect was observed for the higher GTE concentration groups. In fact, previous studies have also found that GTE (mainly tea polyphenols) could promote the formation of CML both in food systems and chemical models. Yang and co-workers showed that 800 μM catechin significantly increased CML generation in a histone H1 glycation system likely through its promoting action on H₂O₂ production (Yang et al., 2018). In another study, GTE was found to increase the level of CML in milk by promoting the oxidation of Amadori products (Poojary, Zhang, Olesen, Rauh, & Lund, 2020). Although there has
not yet been a unified conclusion on the mechanism by which catechins promote the
formation of CML, it is generally accepted that catechins may have a pro-oxidant effect
under certain conditions (e.g., Fenton’s conditions in the presence of iron II or copper I)
that are favorable for their autoxidation to generate H$_2$O$_2$ or hydroxyl radicals, which in
turn could participate in the pathways of CML formation (Fujiwara et al., 2011; Han et
al., 2013). Meanwhile, some studies have reported significantly higher CML content in
processed green tea than in fresh green tea due to higher temperature, longer heating time,
and lower moisture content (Jiao et al., 2017; Jiao et al., 2019). This green tea-derived CML may also add to the CML content of the GTE-marinated baked oyster samples.

Utterly different from CML, the GTE marinade inhibited CEL production in a
roughly concentration-dependent manner (Fig. 5B). The inhibitory effect was strongest
(46.28% relative to control) with 10 g/L of the GTE after baking for 10 min and with 40
g/L of the GTE after baking for 15 min (48.51% relative to control). Our findings are
consistent with some previous studies. The addition of tea polyphenols reduced CEL
content of sturgeon fillets in the study of Wei et al. (Wei et al., 2022). Similar results were
found by Xue et al. on the inhibition of CEL in roasted beef patties by ginger and
curcumin (Xue et al., 2022). The anti-glycation activity of green tea may be attributed to
the presence of polyphenols (e.g., caffeine, epigallocatechin, epigallocatechin gallate, and
epicatechins) (Zawada et al., 2022). These compounds have electron-rich conjugated
systems and electron-donating phenolic groups, giving them potent antioxidant activity
as well as the ability to attack electron-deficient RCS to form adducts (S. Li et al., 2022).
A series of investigations in recent years have led to partial elucidation of the RCS-
trapping mechanism of tea polyphenols. It was reported that both green and black tea
extracts potently reduced acrolein (ACR) and MDA (> 96%) levels, and for EGCG, the
C-8 and C-6 positions of the A-ring were identified to be primary ACR-capture sites (Q.
Zhu et al., 2009). It was also shown that a variety of catechins could covalently bind to
methylglyoxal (MGO) and/or glyoxal (GO) to form different adducts in aqueous Maillard
system (138 °C) and physiological conditions (37 °C, pH 7.4) (Lo, Li, Tan, Pan, Sang, &
Ho, 2006; Sang, Shao, Bai, Lo, Yang, & Ho, 2007; Totlani & Peterson, 2006). It has been
established that the precursor dicarbonyls of CML and CEL are primarily GO and MGO,
respectively (Z. Zhang, Cheng, Wang, Wang, Chen, & Cheng, 2022). In this regard, MGO
has been found to be more easily captured by tea polyphenols than GO and 3-
deoxyglucosone (3-DG), as supported by their different reaction constants (H. Zhu,
Poojary, Andersen, & Lund, 2020). In addition, it has been reported that metal ions could
significantly promote the formation of CML and GO, while inhibiting the formation of
CEL and MGO (Jiao et al., 2019). Our data and these previous studies have therefore
emphasized that polyphenolic antioxidants, especially tea polyphenols, could manifest
completely opposite influence on CML and CEL formation in thermally processed food
despite their similar formation pathways.

Data of the levels of fluorescent AGEs are presented in Fig. 5C. Marinating with
low concentrations of the GTE (5 g/L and 10 g/L) inhibited the production of fluorescent
AGEs in oysters baked for different durations, whereas high concentrations (20 g/L and
40 g/L) enhanced it. There lacked a clear trend in the effect of baking time on fluorescence
intensity. The inhibition rates achieved with 5 g/L GTE ranged from 10.18% to 22.74%.
A previous study reported that a polyphenol-enriched fraction from decaffeinated tea powder was able to significantly \( (P < 0.05) \) decrease the levels of fluorescent AGEs in glycoxidation model systems and bread (Culetu, Fernandez-Gomez, Ullate, del Castillo, & Andlauer, 2016). Wang et al. observed concentration-dependent reduction in fluorescent AGE contents by green tea powder and one-year-stored GTE in fried potato chips, reaching up to 42.9% and 63.8% relative to control, respectively (W. Wang et al., 2023). Due to the high bioaccumulation capacity of bivalves such as oysters, metallic substances may accumulate in their body over time (Sun, Ji, Li, & Wu, 2023). Thus, depending on the degree of accumulation, these metal ions may contribute to the establishment of the so-called a Fenton system, which could compromise the purportedly antioxidant activity of the polyphenols present. This implies the existence of a complex relationship between the characteristic of the oysters in question and the concentration of phenolic compounds in the system. Specifically, AGE formation could be inhibited within suitable concentration range of phenolic compounds through their antioxidant activity such as metal ion chelation and RCS scavenging. In a somewhat Fenton-like environment, high concentrations of polyphenols could undergo autoxidation and promote AGE formation (Jiao et al., 2019; S. Li et al., 2022; Yang et al., 2018). A similar opposite modulatory pattern has also been demonstrated for other thermal processing-derived harmful products. For instance, 0.5% and 1% oolong tea marinade were found to reduce and increase the content of total HAs in chicken thighs, respectively (Caliskan, Gumus, & Kizil, 2023). In another study, low levels of quercetin significantly reduced the acrylamide content in a model bread system (Mildner-Szkudlarz, Różańska,
Piechowska, Waśkiewicz, & Zawirska-Wojtasiak, 2019). On the contrary, a promoting effect was noted at higher levels.

The data from our study and previous studies reinforced the notion that caution should be taken when extrapolating the modulatory capability of tea extracts or polyphenols in the formation of different types of AGEs. Noticeably, the lack of a consistent or unified modulatory pattern highlights some important factors when evaluating the efficiency of an intervention strategy. These include the type of food, characteristics of the food matrix, form and redox properties of plant extract or phytochemicals added to the food, and processing conditions (Shi et al., 2024).

3.5. Effect of GTE on color and texture of oven-baked oysters

Having observed significant modulating effects of the GTE marinade on important parameters pertinent to the safety profile of oven-baked oysters, we proceeded to assess its impact on the sensory characteristics of the oyster samples. Color is the first attribute that affects consumer acceptance of food products. In general, baked oysters marinated with the GTE had a darker color than the control as reflected by their \( L^* \) values, although no clear concentration-response relationship was observed (Table 1, Fig. 1). As expected, the \( L^* \) values of all the experimental groups exhibited a decreasing trend with increasing baking time. This could be explained by the progression of the Maillard reaction and caramelization reactions (Bao et al., 2020). Similar to a recent study (Zhou et al., 2022), the \( a^* \) values of the baked oysters increased with baking time. Of note, the GTE marinade resulted in significantly (\( P < 0.05 \)) higher \( a^* \) values than the control and in a concentration-dependent manner, indicating a tendency of the oysters to lose their
greenish color while developing a reddish hue that gradually intensified with increasing GTE concentration and baking time. The generally lower $L^*$ and higher $a^*$ values of the GTE-marinated samples might be partly ascribed to oxidative changes of the polyphenols adsorbed on or taken up into the oysters during marination (Mau, Lu, Lee, Lin, Cheng, & Lin, 2015). Meanwhile, the GTE-derived polyphenols may help prevent or slow down the oxidation of metmyoglobin and thus hinder discoloration of oyster tissue through their antioxidant activity (Sha & Liu, 2022). This could be evidenced by the observation that neither the baking time nor the GTE concentrations had a significant effect on the $b^*$ value. $\Delta E^*$, representing the total color difference, ranged from 32.23 to 39.16. Only after 20 min of baking did the GTE significantly ($P < 0.05$) increase the $\Delta E^*$ values of the oysters.

Texture is an essential indicator for evaluating the quality of oysters. As shown in Table 2, marinating with GTE had almost no effect on the oysters’ hardness, chewiness, and gumminess after baking for 10 and 15 min, respectively. When extended to 20 min, the hardness, chewiness, and gumminess values were significantly ($P < 0.05$) higher in the GTE marinated groups than in the control, but no statistical significance was observed among the GTE groups. Interestingly, the GTE did not seem to affect the springiness or cohesiveness of the baked oysters. Among the five indices, hardness was the least affected by baking time, followed by chewiness and gumminess. Springiness and cohesiveness values significantly increased when baking time was increased from 10 to 15 or 20 min, indicating the highly sensitive nature of these two parameters. It was reported that phenolic compounds in marinades could interact with or bind to proteins and other
components of food, which may help to protect them from heat-induced structural alteration or damage (Khan et al., 2024). The above data together suggest that short to medium heating times (10 and 15 min) in conjunction with the GTE marinade would not have any significant negative impact on the color or texture of oven-baked oysters.

3.6. Correlation analysis

The above experiments have enabled a comprehensive evaluation of the potential of the GTE marinade for improving the quality of oven-baked oysters. In order to get a clear picture of the relationship among the parameters, Pearson’s correlation analysis of TPC, TAA, protein carbonyl, TBARS, CML, CEL, and fluorescent AGEs of the oven-baked oyster samples was performed, from which plausible mechanisms underlying the modulatory effect of the strategy could also be inferred (Fig. 6). The result showed a strong correlation between TPC and TAA, supporting that phenolic compounds were the major contributor to the antioxidant activity of the GTE, and that the marinating treatment was effective in improving the antioxidant property of the baked oysters. For protein and lipid oxidation products and AGEs, a strong positive correlation ($P < 0.001$) existed between CML and carbonyls. This agreed well with the theory that oxidative stress plays a critical role in the carbonylation process of proteins, and interaction between the Maillard reaction and protein oxidation may significantly enhance the formation of CML (Nawaz et al., 2022; Yang et al., 2018). Moreover, CEL was positively correlated with protein carbonyls ($r = 0.65, P < 0.01$) and TBARS ($r = 0.85, P < 0.001$). The stronger correlation with TBARS suggests that lipid oxidation was likely a more important factor that influenced CEL formation under the experimental conditions. It was reported that
oxidation and pyrolysis of lipids could give rise to α-dicarbonyl compounds (e.g., MGO) and free radicals, the former of which is capable of cross-linking with Lys to form CEL, while the latter can be involved in the Maillard reaction and thus further contributing to the generation of CEL (Gao, Qin, Wu, Xiong, Huang, & Liu, 2023). The lack of a significant positive correlation between CEL and TAA revealed that the GTE’s inhibitory effect on CEL was mainly mediated through alternative pathways (e.g., trapping of RCS) instead of scavenging free radicals. In contrast, fluorescent AGEs correlated positively and significantly with TPC and TAA but not with carbonyl or TBARS, suggesting that the pro-oxidant effect of high concentrations of the GTE rather than protein or lipid oxidation was likely a main influential factor for their formation during the baking process.

4. Conclusion

In conclusion, the present study comprehensively investigated the effect of baking time in conjunction with various concentrations of a polyphenol-rich GTE marinade on the quality of oven-baked oysters. Taking into consideration the safety (CML, CEL, fluorescent AGEs, lipid and protein oxidation products), functional (TPC and TAA), and sensory characteristics of the GTE-marinated versus the control oysters, 10 g/L of the GTE and 10-min baking time seemed to be the optimal among the combinations tested. This processing condition was able to significantly ($P < 0.05$) suppress lipid oxidation (TBARS) and CEL accumulation without any appreciable negative consequences on the sensory, TPC, TAA, amino acid, and fatty acid composition of the baked oysters. The findings together with the correlation analysis revealed that during the baking process, CML formation was strongly influenced by carbonyl (protein oxidation) while CEL
formation by TBARS (lipid oxidation) content. Furthermore, free radical scavenging capacity was not a major contributor to the modulatory effect of the GTE on CEL formation in oysters during baking. The concentration-response analysis also indicated that high concentrations of the GTE might result in a pro-oxidative effect that eventually promoted fluorescent AGEs formation. The findings from our study together with previous studies emphasize the complex context of the intricate relationship among various types of heat-induced reactions, including those that are essential for the development of favorable sensory characteristics and those that give rise to hazardous by-products. Therefore, although green tea has been well established as one of the most promising dietary ingredients for improving the safety profiles of an array of food products, careful adjustment of the sample preparation and heat processing parameters is critical to accomplish desirable outcomes. Apart from these considerations, further studies should be conducted to optimize and broaden the application of GTE in the food industry through comprehensive investigation of factors including food type, food form or characteristic of food matrix, form and redox property of plant extract or phytochemicals added to the food, and processing condition.

**CRediT authorship contribution statement**

Huaixu Wang: Conceptualization, Methodology, and Writing – Original draft, review & editing; Baoping Shi, Weitao Wang & Yajie Zhang: Writing – review and editing; Ka-Wing Cheng: Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Project administration, Resources, and Supervision.
Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgement

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Fig. 1. Representative images of oysters pre-marinated with different concentrations of green tea extract and baked for 10, 15, and 20 min, respectively.
Fig. 2. Effect of baking time (A, 10 min; B, 15 min; C, 20 min; D, all time) and different concentrations of green tea extract on the phenolic content of oven-baked oysters. Values are expressed as mean ± standard deviation (SD) (n = 3). Bars denoted with different letters are significantly different (P < 0.05). TPC = HPC + LPC. TPC: total phenolic content; HPC: hydrophilic phenolic content; LPC: lipophilic phenolic content; GAE: gallic acid equivalent.
**Fig. 3.** Effect of baking time (A, 10 min; B, 15 min; C, 20 min; D, all time) and different concentrations of green tea extract on the antioxidant activities of oven-baked oysters. Values are expressed as mean ± standard deviation (SD) (n = 3). Bars denoted with different letters are significantly different ($P < 0.05$). TAA = HAA + LAA. TAA: total antioxidant activity; HAA: hydrophilic antioxidant activity; LAA: lipophilic antioxidant activity; TE: Trolox equivalent.
Fig. 4. Effect of green tea extracts on the content of protein carbonyl (A) and TBARS (B) in oven-baked oysters. Values are expressed as mean ± standard deviation (SD) (n = 3). Different lowercase letters (a - d) indicate significant differences ($P < 0.05$) between different extract concentrations. Different capital letters (X - Z) indicate significant differences ($P < 0.05$) between different baking times.
Fig. 5. Effect of green tea extracts on CML (A), CEL (B) and fluorescent AGEs (C) formation in oven-baked oysters. Values are expressed as mean ± standard deviation (SD) (n = 3). Different lowercase letters (a - e) indicate significant differences ($P < 0.05$) between different extract concentrations. Different capital letters (X - Z) indicate significant differences ($P < 0.05$) between different baking times.
Fig. 6. Pearson correlation analysis between TPC, TAA, protein carbonyl, TBARS, CML, CEL, and fluorescent AGEs in oven-baked oysters. *P < = 0.05, **P < = 0.01, ***P < = 0.001.
Table 1
Effect of green tea extract and baking time on the color of oven-baked oysters.

<table>
<thead>
<tr>
<th>Baking time</th>
<th>Green tea extract concentrations</th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
<th>$\Delta E^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>0 g/L</td>
<td>65.38 ± 1.35$^{abcX}$</td>
<td>1.27 ± 0.34$^{cX}$</td>
<td>15.37 ± 0.49$^{Y}$</td>
<td>32.23 ± 1.09$^{Y}$</td>
</tr>
<tr>
<td></td>
<td>5 g/L</td>
<td>63.49 ± 2.93$^{abcX}$</td>
<td>2.39 ± 0.35$^{bY}$</td>
<td>15.45 ± 1.20$^{X}$</td>
<td>34.45 ± 2.80$^{abY}$</td>
</tr>
<tr>
<td></td>
<td>10 g/L</td>
<td>63.14 ± 2.02$^{abcX}$</td>
<td>2.87 ± 0.22$^{bY}$</td>
<td>16.04 ± 2.03$^{X}$</td>
<td>35.63 ± 2.30$^{Y}$</td>
</tr>
<tr>
<td></td>
<td>20 g/L</td>
<td>66.90 ± 1.54$^{abcX}$</td>
<td>2.97 ± 0.35$^{bY}$</td>
<td>16.63 ± 1.22$^{X}$</td>
<td>32.39 ± 2.89$^{abX}$</td>
</tr>
<tr>
<td></td>
<td>40 g/L</td>
<td>65.13 ± 0.94$^{abcX}$</td>
<td>3.13 ± 0.12$^{aY}$</td>
<td>15.89 ± 0.71$^{X}$</td>
<td>33.08 ± 1.31$^{abY}$</td>
</tr>
<tr>
<td>15 min</td>
<td>0 g/L</td>
<td>63.82 ± 0.83$^{XY}$</td>
<td>1.76 ± 0.16$^{aX}$</td>
<td>16.61 ± 0.96$^{abY}$</td>
<td>34.55 ± 1.73$^{abcX}$</td>
</tr>
<tr>
<td></td>
<td>5 g/L</td>
<td>60.98 ± 0.66$^{XY}$</td>
<td>2.98 ± 0.38$^{bX}$</td>
<td>15.53 ± 1.16$^{abX}$</td>
<td>35.60 ± 1.11$^{abcX}$</td>
</tr>
<tr>
<td></td>
<td>10 g/L</td>
<td>63.04 ± 1.74$^{abcX}$</td>
<td>2.87 ± 0.32$^{bX}$</td>
<td>15.13 ± 0.72$^{bcX}$</td>
<td>34.36 ± 2.06$^{Y}$</td>
</tr>
<tr>
<td></td>
<td>20 g/L</td>
<td>62.29 ± 3.37$^{abcY}$</td>
<td>3.04 ± 0.40$^{bXY}$</td>
<td>16.19 ± 1.24$^{abX}$</td>
<td>35.83 ± 3.49$^{abX}$</td>
</tr>
<tr>
<td></td>
<td>40 g/L</td>
<td>61.32 ± 2.46$^{abcY}$</td>
<td>4.35 ± 0.23$^{aX}$</td>
<td>16.97 ± 1.14$^{aX}$</td>
<td>37.49 ± 1.71$^{abcX}$</td>
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<tr>
<td>20 min</td>
<td>0 g/L</td>
<td>62.91 ± 1.36$^{aY}$</td>
<td>1.55 ± 0.44$^{aX}$</td>
<td>17.09 ± 0.46$^{X}$</td>
<td>35.75 ± 0.97$^{abcX}$</td>
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<td>5 g/L</td>
<td>58.63 ± 1.94$^{bcY}$</td>
<td>3.41 ± 0.40$^{bX}$</td>
<td>15.21 ± 0.58$^{X}$</td>
<td>38.91 ± 1.74$^{abX}$</td>
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<tr>
<td></td>
<td>10 g/L</td>
<td>57.52 ± 1.55$^{cY}$</td>
<td>3.63 ± 0.40$^{bX}$</td>
<td>14.74 ± 0.59$^{bX}$</td>
<td>39.15 ± 1.20$^{abX}$</td>
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<tr>
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<td>20 g/L</td>
<td>61.20 ± 1.88$^{abcY}$</td>
<td>3.65 ± 0.59$^{bX}$</td>
<td>17.02 ± 0.83$^{aX}$</td>
<td>37.08 ± 0.59$^{bcX}$</td>
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<tr>
<td></td>
<td>40 g/L</td>
<td>59.19 ± 2.61$^{bcY}$</td>
<td>4.41 ± 0.37$^{aX}$</td>
<td>17.07 ± 1.30$^{abX}$</td>
<td>39.16 ± 1.91$^{abcX}$</td>
</tr>
</tbody>
</table>

Different lowercase letters (a - c) indicate the significant differences ($P < 0.05$) between different extract concentrations. Different capital letters (X, Y) indicate the significant differences ($P < 0.05$) between different baking times.
Table 2
Effect of green tea extract and baking time on the texture of oven-baked oysters.

<table>
<thead>
<tr>
<th>Baking time</th>
<th>Green tea extract concentrations</th>
<th>Hardness/gf</th>
<th>Springiness</th>
<th>Chewiness/gf</th>
<th>Gumminess/gf</th>
<th>Cohesiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 g/L</td>
<td>10 min</td>
<td>256.50 ± 26.00&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>0.77 ± 0.00&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>136.10 ± 15.30&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>173.84 ± 17.02&lt;sup&gt;abXY&lt;/sup&gt;</td>
<td>0.67 ± 0.01&lt;sup&gt;Z&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 g/L</td>
<td>258.78 ± 35.16&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>0.76 ± 0.03&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>124.59 ± 26.25&lt;sup&gt;abY&lt;/sup&gt;</td>
<td>166.42 ± 23.31&lt;sup&gt;abY&lt;/sup&gt;</td>
<td>0.68 ± 0.04&lt;sup&gt;Y&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>10 g/L</td>
<td>253.75 ± 22.4&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>0.76 ± 0.04&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>123.54 ± 15.74&lt;sup&gt;abY&lt;/sup&gt;</td>
<td>162.76 ± 18.72&lt;sup&gt;abY&lt;/sup&gt;</td>
<td>0.67 ± 0.06&lt;sup&gt;Y&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>20 g/L</td>
<td>317.55 ± 39.47&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>0.75 ± 0.02&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>149.71 ± 13.13&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>198.88 ± 18.93&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>0.62 ± 0.06&lt;sup&gt;Y&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>40 g/L</td>
<td>265.88 ± 22.98&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>0.77 ± 0.02&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>130.66 ± 14.81&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>170.82 ± 16.05&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>0.67 ± 0.05&lt;sup&gt;Y&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0 g/L</td>
<td>15 min</td>
<td>249.14 ± 17.37&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.82 ± 0.01&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>151.70 ± 10.58&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>188.50 ± 7.60&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.76 ± 0.01&lt;sup&gt;aX&lt;/sup&gt;</td>
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<td>5 g/L</td>
<td>259.51 ± 17.67&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.82 ± 0.01&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>166.47 ± 18.08&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>201.68 ± 22.71&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.76 ± 0.03&lt;sup&gt;aX&lt;/sup&gt;</td>
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<tr>
<td>10 g/L</td>
<td>273.55 ± 24.13&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.83 ± 0.01&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>165.98 ± 18.75&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>203.79 ± 23.65&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.75 ± 0.02&lt;sup&gt;aX&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>20 g/L</td>
<td>264.90 ± 22.28&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.82 ± 0.03&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>167.61 ± 20.17&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>202.98 ± 24.57&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.75 ± 0.03&lt;sup&gt;aX&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>40 g/L</td>
<td>264.08 ± 14.83&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.81 ± 0.02&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>157.85 ± 20.95&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>192.84 ± 22.42&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.73 ± 0.03&lt;sup&gt;aX&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>0 g/L</td>
<td>20 min</td>
<td>226.53 ± 18.35&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>0.83 ± 0.02&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>136.92 ± 10.73&lt;sup&gt;abX&lt;/sup&gt;</td>
<td>166.49 ± 8.11&lt;sup&gt;abY&lt;/sup&gt;</td>
<td>0.74 ± 0.01&lt;sup&gt;aY&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 g/L</td>
<td>252.67 ± 7.87&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.84 ± 0.01&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>160.81 ± 9.7&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>193.19 ± 8.26&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.75 ± 0.02&lt;sup&gt;aX&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>10 g/L</td>
<td>254.71 ± 7.43&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.86 ± 0.01&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>169.25 ± 2.43&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>197.13 ± 3.02&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.77 ± 0.01&lt;sup&gt;aX&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>20 g/L</td>
<td>267.10 ± 24.21&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.84 ± 0.01&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>172.81 ± 20.68&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>204.97 ± 24.17&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.76 ± 0.02&lt;sup&gt;aX&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>40 g/L</td>
<td>273.44 ± 15.84&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.84 ± 0.02&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>174.34 ± 15.78&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>205.90 ± 13.60&lt;sup&gt;aX&lt;/sup&gt;</td>
<td>0.76 ± 0.03&lt;sup&gt;aX&lt;/sup&gt;</td>
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Different lowercase letters (a, b) indicate the significant differences ($P < 0.05$) between different extract concentrations.
Different capital letters (X - Z) indicate the significant differences ($P < 0.05$) between different baking times.