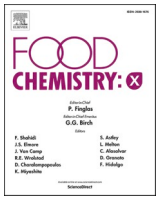
Food Chemistry: X 15 (2022) 100387

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Heat-induced formation of advanced glycation end-products in ground pork as affected by the addition of acetic acid or citric acid and the storage duration prior to the heat treatments

Hui Lin [a], Keqiang Lai [b], Juanjuan Zhang [a], Faxiang Wang [a], Yongle Liu [a], Barbara A Rasco [c], Yiqun Huang [a,*]

[a] School of Food Science and Bioengineering, Changsha University of Science and Technology, 960, 2nd Section, Wanjiali South Rd, Changsha, Hunan 410114, China
[b] College of Food Science and Technology, Shanghai Ocean University, No. 999 HuchengHuan Road, Nanhui New City, Shanghai 201306, China
[c] College of Agriculture and Natural Resources, University of Wyoming, Dept 3354, 1000 E University Ave, Laramie, WY 83071, United States

ARTICLE INFO

Keywords:
Carboxymethyllysine
Carboxyethyllysine
Meat
pH
Marinate
Lipid oxidation

ABSTRACT

The heat-induced (121 °C, 10 or 30 min) formation of two potentially hazardous advanced glycation end-products (AGEs), protein-bound N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL), in pork as affected by citric or acetic acid (0.5, 1 g/100 pork) and the storage duration (0 °C, 0 – 8 d) prior to the heating was investigated. A longer storage time of raw pork resulted in higher levels of AGEs produced during the later heating, likely due to the accumulation of some AGE precursors during the storage. Depending on the acid level and heating time, adding acid in pork led to 30 – 54% (citric acid) or 14 – 48% (acetic acid) average reduction of heat-induced production of CML/CEL, which corresponded to the reduction of thiobarbituric acid reactive substances and Schiff bases. The marinating time of raw pork with an acid did not significantly affect ($P = 0.959$ – 0.998) the acid's inhibition effect on heat-induced formation of CML/CEL.

Introduction

Acetic acid and citric acid are generally recognized as safe (GRAS) based upon the US Food and Drug Administration (FDA, 2021). These two organic acids are widely used as acidifiers, acidifier regulators, and/or preservatives to improve sensory characteristics (such as color, flavor, tenderness, and juiciness), inhibit the growth of microorganisms, and extend the shelf life of various muscle food products (Braïek & Smaoui, 2021; Ke, Huang, Decker, & Hultin, 2009). In addition, citric acid and acetic acid have been used to inhibit lipid oxidation due to their ability to chelate iron ions in muscle foods (Ke et al., 2009; Kim et al., 2019). However, the addition of acid in meat could also promote the release of iron ions from myoglobin and hemoglobin as well as accelerate the oxidization of these heme-binding proteins, which consequently expedite lipid oxidation (Chen & Waimaleongora-EK, 1981; Richards & Hultin, 2000; Sharedeh, Gatellier, Astruc, & Daudin, 2015). Furthermore, lowering the pH of meat could reduce the nucleophilicity of the free amino groups in muscle proteins, and thus reduce their reactivity with reducing sugars during the initial step of the Maillard reaction (Lund & Ray, 2017; O'Brien, Morrissey, & Ames, 1989). The change in pH of meat also influences the degradation pathways of Amadori compounds during the Maillard reaction. The acidic condition favors the production of furfural and related derivatives through 1,2-eneaminol pathway, while the alkaline condition favors the production of various fission products like reductones and α-dicarbonyls through 2,3-enediol pathway (O'Brien et al., 1989).

Although the effects of acids on lipid oxidation and the Maillard reaction have been recognized, there is a general lack of study regarding the effects of acids on the generation of advanced glycation end-products (AGEs), a class of potentially toxic chemicals mainly produced through the Maillard reaction and lipid oxidation in meat products (Chen, 2021; Srey et al., 2010). Consuming foods high in AGEs have negative impacts on the gut microbiota and have been associated with some chronic and degenerative diseases like diabetes, atherosclerosis, and cognitive impairment (Zhang, Wang, & Fu, 2020). Protein-bound N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL) are two lysine-derived AGEs found in various food products, which are commonly used as markers for AGEs in foods. Generally, meat products contain high levels of CML and CEL, since they are rich in proteins, fat, and some other compounds (such as iron ions) that favor the Maillard

* Corresponding author.
E-mail address: yiqunh@csust.edu.cn (Y. Huang).

https://doi.org/10.1016/j.fochx.2022.100387
Received 15 April 2022; Received in revised form 3 July 2022; Accepted 4 July 2022
Available online 6 July 2022

reaction and/or lipid oxidation, especially for meat products subjected to relatively intense heat treatments such as commercial sterilization (Li, Xue et al., 2021; Sun et al., 2017; Sun et al., 2021; Yu et al., 2016; Zhu et al., 2019; Zhu, Huang, Cheng, Khan, & Huang, 2020).

Since organic acids could act as a prooxidant or antioxidant and affect the speed and the pathways of the Maillard reaction, their effects on AGEs formation could be quite complicated, depending on the pH, food matrix, the type and concentration of the acid (Ke et al., 2009; Lund & Ray, 2017; O'Brien et al., 1989; Sharedeh et al., 2015). There are a very few reported studies involving the effects of organic acids on the formation of AGEs in food matrices, while the results are inconsistent. Uribarri et al. (2010) found that marinating beef in lemon juice or vinegar (25 g meat in 10 mL liquid, 1 h) prior to the roasting (150 °C, 15 min) led to significantly less CML produced during the heating as compared to the beef without being marinated, although no actual value of CML was reported due to the limitation of the enzyme-linked immunosorbent assay used for the quantification of CML in the study. However, Li, Kong et al. (2021) showed that heating ground pork added with 0.5% acetic acid (without marinating) at 121 °C for 10 min resulted in an average of 33% more CML produced as compared to that without acid; but when the heating time was extended to 30 min, the corresponding level of CML produced in the acid treated pork was 23% less. Systematic studies are needed to fully understand whether the addition of an organic acid affects the levels of AGEs in meat products, particularly the effects of marinating time of raw meat with an acid on the levels of AGEs in the raw meat and the later heat-treated meat products, which has not been reported in the literature.

Therefore, this study was to understand the effects of acetic acid and citric acid on the formation of protein-bound CML and CEL in raw pork during storage and subsequently commercial sterilization. In addition, the corresponding changes in the levels of thiobarbituric acid reactive substances (TBARS), an indicator for lipid oxidation, and Schiff bases, intermediates that could be formed during the initial stage of the Maillard reaction, were investigated to evaluate their possible links with the changes of AGEs levels in pork.

Materials and methods

Chemicals

Except for the HPLC grade methyl alcohol (Tedia Company, Inc., Fairfield, OH USA), formic acid and ammonium acetate (Sigma Chemical Co., St. Louis, MO, USA), as well as AGEs standards (Toronto Research Chemicals Inc., Toronto, Ontario, Canada), all chemicals used were analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Sample preparation

Fresh pork (*longissimus thoracis et lumborum*) bought from a Metro AG store in Changsha (Hunan, China) were cut into small pieces and chopped in a machine (ZB-5G, Zhucheng Huagang Machinery Co., Ltd, Zhucheng, China) for a total of four min, and then divided into five portions (about 500 g each). Four portions of pork were mixed with acetic or citric acid solutions (20 or 50 g acid/100 g water) at a ratio of 3 g solution per 100 g of pork so that the final samples contained 0.5 g or 1 g acid per 100 g of pork. The selection of these acid levels was based upon the possible amounts of the acids added in pork during food preparation and the final pH for low-acid foods (pH $>$ 4.6). The fifth portion of ground pork was added with water at a ratio of 3 g water per 100 g pork and used as the control. The pork from each of the five treatments was further portioned and sealed into three LDPE ziplock bags (each bag contained about 150 g pork), stored at 0 °C for 0, 4, and 8 days, respectively. After each storage period, one bag of sample from each treatment was used to analyze for its levels of protein-bound CML, CEL, pH, TBARS and Schiff bases, and also used for the following heat treatments.

The above sample preparation and all following heat treatments were repeated three times for each relevant measurement, using pork purchased at different time points.

Thermal treatments

After each storage period, ground pork from each of the five treatments was sealed in six cylindrical aluminum cells (12.20 ± 0.01 g pork/cell) originally designed by Kong, Tang, Rasco, Crapo, and Smiley (2007). Three of the cells with samples were heated at 121 °C for 10 min, while the other three were heated for 30 min in an oil bath (HAAKE PC 300-S7; Thermo Fisher Scientific Inc., Waltham MA), and then immersed in an ice-water mixture for 20 min. Since it took about 4 – 5.5 min for the cold spot of the pork in the aluminum cell to reach the target temperature, the remaining 4.5 – 6 min of 10 min heating at 121 °C was to meet the basic requirement for sterilization of meat products with a margin of safety (Sun et al., 2016). The use of 30 min heat treatment was to simulate the common time used for retort sterilization of commercially canned meat products (Tang, 2015). The heated meat samples from all three cells were mixed with a pestle in a mortar, and immediately used for triplicate analysis of the levels of protein-bound CML and CEL, TBARS and Schiff bases in the sample.

Determination of proximate composition, pH, TBARS, and Schiff bases of pork

The moisture, fat, and protein content of raw pork were analyzed via the oven drying, acid hydrolysis, and Kjeldahl methods, respectively, based upon the National Food Safety Standards of China (GB 5009, 2016). The pH values of raw pork with and without acid added were measured with a digital pH meter (DELTA 320, Mettler Toledo (Shanghai) Co. Ltd, Shanghai, China) based upon a standard from the GB 5009 (2016).

The method of Vyncke (1975) was used to determine the levels of TBARS in both raw and sterile pork. In short, TBARS were extracted from pork in the mixed solution of trichloroacetic acid, propyl gallate and EDTA-2Na, and then reacted with 2-thiobarbituric acid to form a pink product, which was quantified based upon its absorbance intensity at 532 nm with a spectrophotometer (TU-1901, Beijing Persee General Instrument Co., Ltd, Beijing, China). The TBARS level was calculated as malondialdehyde (mg/kg pork) based upon its standard curve.

The Schiff bases in pork was analyzed according to the method described by Utrera, Parra, and Estévez (2014) with some modifications. In brief, pork sample (0.50 g) was homogenized with sodium phosphate (20 mM, 20 mL) buffer solution (pH 6.5, containing 0.6 M NaCl) for 20 s, and then centrifuged (6945*g* force, 4 °C) for 15 min. After this, the fluorescence intensity of the supernatant was determined with a fluorescence spectrophotometer (F-7100, Hitachi High-tech Science Co., Ltd, Ibaraki, Japan). Both the excitation (360 nm) and emission (380 – 600 nm) slit widths were set as 5 nm. The voltage of photomultiplier tubes was 700 v, and the scanning speed was 1200 nm/min.

For each sample, duplicate analysis was conducted for its proximate composition and pH, while triplicate analysis was conducted for the levels of TBARS, Schiff bases, protein-bound CML and CEL.

Analysis for protein-bound CML and CEL

The protein-bound CML and CEL were first extracted from raw or heated pork via an acid hydrolysis approach (Niquet-Léridon & Tessier, 2011) before being quantified with a verified HPLC-MS/MS method (Sun et al., 2015). In short, pork sample was reduced with sodium borohydride in a borate-boric buffer system (4 °C, 8 h), followed by being defatted with chloroform–methanol. The precipitated protein was acid hydrolyzed (110 °C, 24 h), diluted with water, added with the isotopes of CML (d_4-CML) and CEL (d_4-CEL) as the internal standards,

and dried in a vacuum oven (60 °C, 8 h). The dried sample was added with water, and further purified with an MCX solid phase extraction cartridge (Shanghai ANPEL Scientific instrument Co., Ltd, Shanghai, China), dried under nitrogen gas, dissolved in methanol–water solution, and filtered via a membrane filter before being analyzed for its CML and CEL content with HPLC-MS/MS.

All test conditions for the HPLC-MS/MS method, including the instruments used and the settings for both HPLC and mass spectrometer, were the same as those reported by Wu et al. (2020), which were similar to that described in detail by Sun et al. (2015), except for the desolvation temperature (500 °C), the composition of the mixture of AGE standards (each of the four standards was 200 ng/mL), and two internal standards (d_4-CML, d_4-CEL) instead of one (d_4-CML) used for calculating the response factors (RFs) of the four AGE standards. The RFs of four AGE standards were determined each day prior to the analysis of sample extracts. The ratios of the RFs of CML and CEL to the RFs of their isotopes (considered as constants), the concentrations of the internal standards, together with the peak areas of two AGEs and their isotopes of the sample extract, were used for the calculation of CML and CEL levels in the sample.

Data analysis

Linear mixed model was employed to analyze whether there were significant ($\alpha = 0.05$) effects of two fixed factors (acid treatments or storage duration), their interaction, and random term (different batches of pork samples) on the mean values of TBARS and Schiff bases in either raw or heat-treated pork, the average amounts of CML and CEL in raw pork and that were formed during the commercial sterilization (Biffin, Smith, Bush, Morris, & Hopkins, 2020). The amount of CML or CEL formed during the heating was calculated via subtracting the amount of CML or CEL in raw pork from the heated pork. Bonferroni adjustment was selected for multiple comparison of means ($\alpha = 0.05$). All statistical analysis were conducted with SPSS (Version 26, IBM Corp., Armonk, NY).

Results and discussion

Proximate composition, pH of raw pork

The raw pork used in this study contained 74.7 – 74.8% moisture, 22.2 – 24.3% protein, and 1.1 – 1.9% fat based on the sample weight (w/w).

The pH values for raw pork ranged from 5.54 to 5.65 (mean: 5.60 ± 0.08). The addition of acetic acid resulted in the decrease of pH to 4.85 ± 0.01 (0.5 g acid/100 g pork) or 4.59 ± 0.01 (1 g acid/100 g pork), while the addition of citric acid led to the decrease of pH to 4.82 ± 0.01 (0.5 g acid/100 g pork) or 4.55 ± 0.02 (1 g acid/100 g pork). The pork samples added with acetic acid (p*Ka* = 4.76) and that with citric acid (p*Ka*: 3.13, 4.76, 6.40) (Braïek & Smaoui, 2021) at either level had similar pH values. As expected, the changes of pH values for raw pork samples with or without an acid added during the cold storage showed similar trends (Fig. 1a), decreasing during the first 4 days of storage because of the anaerobic degradation of glycogen to lactic acid caused by endogenous enzymes, and then slightly increased due to the decomposition of muscle proteins and release of some basic compounds caused by the activities of microorganisms and enzymes (Kalahrodi, Baghaei, Emadzadeh, & Bolandi, 2021).

Effects of acids on the levels of TBARS, Schiff bases, CML and CEL in raw pork during storage

Based upon the results from linear mixed models, both TBARS and Schiff bases of ground pork were significantly affected by the storage time ($P = 0.001$ for both parameters) and acid treatment (TBARS, $P = 0.006$; Schiff bases, $P = 0.000$); and there was no significant interaction

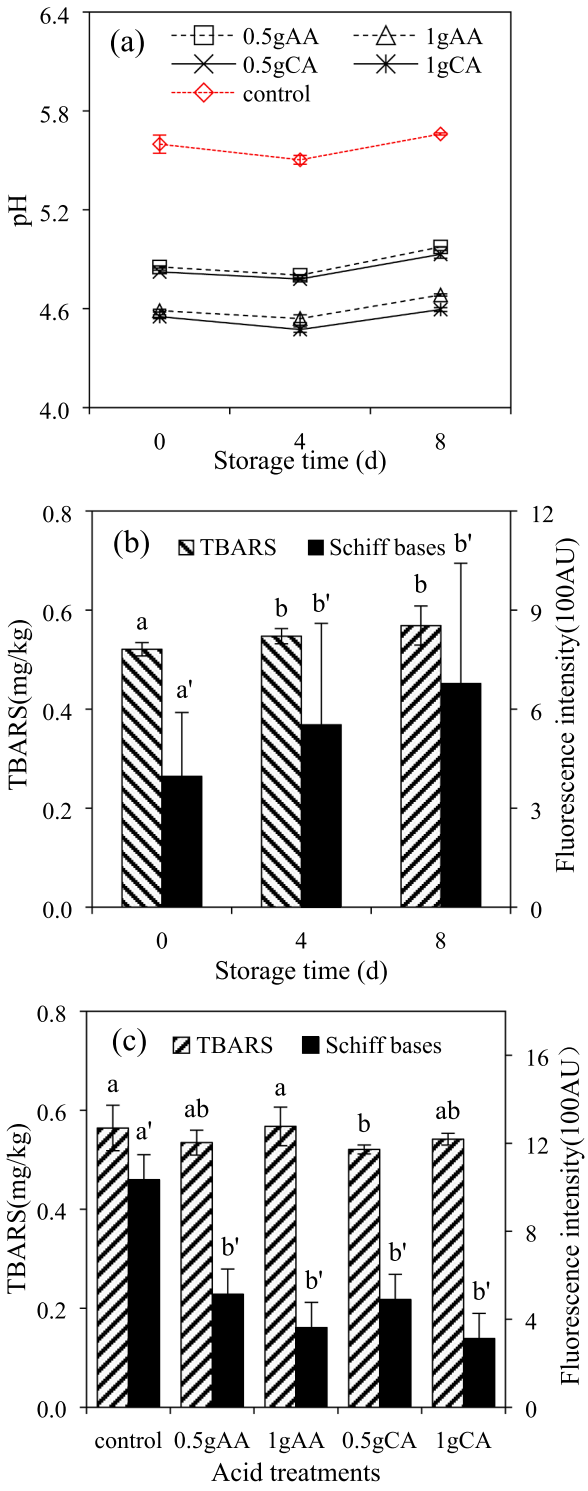


Fig. 1. Changes of (a) pH ($n = 3$), and the effects of (b) storage duration ($n = 15$) and (c) the addition of acetic acid (AA) or citric acid (CA) at the level of 0.5 or 1 g/100 g pork ($n = 9$) on the amounts of TBARS and Schiff bases (expressed as fluorescence intensity) in raw pork during storage (0 °C, 0 – 8 d). Data were shown as mean and standard deviation. Different letters (ab or a′b′) above the columns indicate significant difference ($P < 0.05$).

effect between the two factors for either TBARS ($P = 0.067$) or Schiff bases ($P = 0.365$). The levels of TBARS and Schiff bases in raw pork with or without acid added significantly ($P < 0.05$) increased during the cold storage (Fig. 1b). The increase of TBARS in pork during storage indicates the presence of lipid oxidation. Since Schiff bases could be formed

between proteins and reducing sugars during the initial stage of the Maillard reaction as well as between proteins and other carbonyl compounds, the increase of Schiff bases in muscle foods during cold storage was generally tied to the increased extent of protein oxidation (Chelh, Gatellier, & Santé-Lhoutellier, 2007; Shen et al., 2022). Moreover, the addition of two different levels of either acid led to 41 – 55% (0.5 g/100 g pork) or 63 – 71% (1 g/100 g pork) reduction of Schiff bases in pork (Fig. 1c), implying the inhibiting effect of the organic acids on protein oxidation or the promoting effect of the acids on the reversion of the Schiff bases (Ge & Lee, 1997). However, the effects of these two acids on the levels of TBARS in pork were more complicated. The pork added with 0.5 g citric acid/100 g pork (average 7.6% less) had significantly ($P = 0.020$) lower amounts of TBARS compared to that of the control (0.564 ± 0.042 mg/kg), but the average TBARS level in pork added with 0.5 or 1 g/100 g pork of acetic acid ($P = 0.265$ or 1.000) or 1 g/100 g citric acid/ ($P = 0.673$) was not significantly different from that of the control. The results indicated that there may be some minor inhibiting effect of organic acids for lipid oxidation in raw pork during cold storage, but this effect depends on the concentration and the type of the acid applied.

Table 1 exhibits the amounts of two tested AGEs in raw pork (with or without acid) stored for different lengths of time. The levels of CML and CEL in the control pork were 2.13 – 6.91 mg/kg protein and 4.96 – 13.17 mg/kg protein, respectively, depending on the storage duration and the batch of pork samples used. Adding 0.5 – 1 g/100 g pork of either acetic or citric acid did not lead to any significant effect on the average amount (all in mg/kg protein) of CML ($P = 0.172$; control: 4.14 ± 0.28; acetic acid, 0.5 g: 4.24 ± 0.52; acetic acid, 1 g: 4.90 ± 0.65; citric acid, 0.5 g: 4.30 ± 0.57; citric acid, 1 g: 3.97 ± 0.30) or CEL ($P = 0.396$; control: 9.41 ± 0.52; acetic acid, 0.5: 11.78 ± 0.92; acetic acid, 1 g: 11.58 ± 0.90; citric acid, 0.5 g: 10.40 ± 1.25; citric acid, 1 g: 10.13 ± 1.37) in ground pork during the cold storage. The storage duration significantly influenced the mean CML ($P = 0.015$) in raw pork, although the mean CEL ($P = 0.244$) was not significantly affected. The average amount of CML in raw pork stored for 8 days (4.83 ± 0.51 mg/kg protein) was significantly higher than that without storage ($P = 0.021$; 3.99 ± 0.23 mg/kg protein), although not significantly higher than that stored for 4 days ($P = 0.059$; 4.11 ± 0.36 mg/kg protein). This implies that lipid oxidation (as indicated by the increase of TBARS level, Fig. 1b) and/or protein oxidation (as indicated by the increase of Schiff bases, Fig. 1b) may promote the formation of CML in raw pork during storage. The studies of Niu et al. (2018) on refrigerated pork with salts (sodium chloride, sodium nitrite), Yu et al. (2016) on frozen pork, and Niu et al. (2017a) on refrigerated fish showed that the average levels of protein-bound CML and CEL in raw muscle food matrices were not significantly affected by the storage duration based upon analysis of variance (ANOVA). However, the study of Niu et al. (2017a) also showed that the average amount of CML in the white muscle of 12 grass carp continuously increased from 8.8 ± 1.2 mg/kg protein to 10.4 ± 0.9 mg/kg protein during the three weeks of cold storage, although the change was not significant based upon one-way ANOVA.

Influences of acids and cold storage on the formation of CML and CEL in pork during heating

The 10 min and 30 min heat-treated pork (with and without an acid) contained 6.55 – 33.70 mg/kg protein (1.59 – 7.90 mg/kg sample) and 20.98 – 63.86 mg/kg protein (5.09 – 14.98 mg/kg sample) of CML, respectively (Table 2), and contained 9.48 – 55.23 mg/kg protein (2.30 – 12.95 mg/kg sample) and 42.84 – 182.89 mg/kg protein (10.39 – 42.89 mg/kg sample) of CEL, respectively (Table 3). During the 10 min of heat treatment, 4.41 – 26.79 mg/kg protein of CML and 3.89 – 42.05 mg/kg protein of CEL were produced in pork, while the corresponding data for that of the 30 min of heat treatment were 18.83 – 58.74 mg/kg protein and 38.42 – 169.72 mg/kg protein, depending on the batch of the pork used, the storage duration and the acid added. The amounts of CML and CEL in raw and heat-treated pork from three different batches varied greatly, which is commonly found in animal-source foods like pork, fish, and table eggs (Li, Kong et al., 2021; Niu et al., 2017b; Wu et al., 2020). In addition, more CEL was produced than CML in pork during the commercial sterilization, particularly for the 30 min heat treatment, which resulted in 142 – 384% more CEL formed than that of CML, implying that probably a much higher level of methylglyoxal (precursor for CEL) in pork was formed during the 30 min heating than that of glyoxal (precursor for CML) (Sun et al., 2021; Treibmann, Hellwig, Hellwig, & Henle, 2017). Similarly, Yu et al. (2016) found that a severe heat treatment (121 °C, 30 min) resulted in more CEL produced than CML in lean pork previously cured in 2% NaCl for 2 d prior to the heating.

Based upon the results from mixed linear models, both acid

Table 1
The amounts of N^{ε}-carboxymethyllysine (CML) and N^{ε}-carboxyethyllysine (CEL) in raw ground pork added with 0.5 or 1 g/100 g pork of acetic acid (AA) or citric acid (CA) and that without acid (control). [a]

	CML(mg/kg protein)			CEL(mg/kg protein)		
	0 day	4 days	8 days	0 day	4 days	8 days
1st-batch						
control	2.13 ± 0.11	3.69 ± 0.21	3.16 ± 0.32	7.98 ± 0.98	6.17 ± 0.48	4.96 ± 0.30
AA-0.5 g	2.35 ± 0.05	3.58 ± 0.15	3.41 ± 0.44	9.71 ± 0.53	8.11 ± 0.07	9.69 ± 0.54
AA-1 g	2.63 ± 0.14	4.22 ± 0.18	4.71 ± 0.34	10.84 ± 0.72	6.95 ± 0.26	10.54 ± 0.28
CA-0.5 g	2.37 ± 0.19	3.42 ± 0.08	4.02 ± 0.17	5.39 ± 0.62	4.56 ± 0.67	4.32 ± 0.63
CA-1 g	2.10 ± 0.03	2.84 ± 0.23	3.51 ± 0.30	5.09 ± 0.01	4.42 ± 0.77	4.81 ± 0.41
2nd-batch						
control	4.66 ± 0.14	3.08 ± 0.07	3.32 ± 0.08	10.02 ± 1.25	9.34 ± 0.28	11.78 ± 1.75
AA-0.5 g	3.98 ± 0.25	2.84 ± 0.36	3.91 ± 0.02	12.88 ± 0.10	16.70 ± 4.21	12.99 ± 0.97
AA-1 g	4.69 ± 0.46	3.42 ± 0.25	4.59 ± 0.36	11.14 ± 1.36	14.80 ± 1.36	11.94 ± 1.85
CA-0.5 g	3.83 ± 0.74	3.16 ± 0.05	3.94 ± 0.46	12.16 ± 0.55	13.40 ± 0.46	15.98 ± 1.79
CA-1 g	3.98 ± 0.33	3.09 ± 0.09	3.69 ± 0.28	12.77 ± 0.85	13.19 ± 0.46	15.37 ± 0.81
3rd-batch						
control	5.13 ± 0.34	5.14 ± 0.09	6.91 ± 0.25	9.91 ± 0.64	11.31 ± 1.14	13.17 ± 0.38
AA-0.5 g	5.54 ± 0.57	5.39 ± 0.15	7.22 ± 0.19	9.55 ± 1.26	12.05 ± 0.87	14.35 ± 1.41
AA-1 g	5.77 ± 0.25	6.47 ± 0.17	7.57 ± 0.59	10.75 ± 0.53	11.92 ± 0.66	15.31 ± 0.89
CA-0.5 g	5.21 ± 0.16	5.93 ± 0.09	6.83 ± 0.32	10.30 ± 2.20	12.54 ± 1.21	14.97 ± 1.43
CA-1 g	5.39 ± 0.35	5.40 ± 0.11	5.75 ± 0.21	9.63 ± 0.38	10.95 ± 1.01	14.90 ± 1.74

[a] Samples were stored at 0 °C for up to 8 d. Data were presented as mean ± standard error of triplicate analysis.

Table 2
The amounts of N^{ε}-carboxymethyllysine (mg/kg protein) in commercially sterilized (121 °C, 10 min or 30 min) pork added with 0.5 or 1 g/100 g pork of acetic acid (AA) or citric acid (CA) and that without acid (control) [a.]

	121 °C, 10 min			121 °C, 30 min		
	0 day	4 days	8 days	0 day	4 days	8 days
1st-batch						
control	13.21 ± 0.89	13.07 ± 2.10	15.37 ± 0.74	29.42 ± 0.72	38.58 ± 2.86	38.85 ± 3.13
AA-0.5 g	10.26 ± 0.50	12.29 ± 0.48	15.07 ± 0.21	25.74 ± 1.40	27.15 ± 3.72	32.53 ± 0.96
AA-1 g	11.57 ± 1.94	12.58 ± 0.54	16.87 ± 0.57	21.90 ± 1.41	28.87 ± 1.29	34.73 ± 1.24
CA-0.5 g	8.37 ± 0.53	9.94 ± 0.19	12.06 ± 0.37	23.55 ± 2.29	30.65 ± 0.31	30.84 ± 1.29
CA-1 g	6.55 ± 0.25	9.22 ± 0.21	10.79 ± 0.32	20.98 ± 0.82	26.07 ± 1.68	25.04 ± 1.03
2nd-batch						
control	15.85 ± 0.62	18.39 ± 0.92	20.76 ± 0.56	35.43 ± 1.60	41.47 ± 0.39	43.99 ± 3.85
AA-0.5 g	12.80 ± 1.24	14.38 ± 0.38	15.53 ± 1.25	32.52 ± 3.72	35.92 ± 3.72	40.69 ± 4.52
AA-1 g	13.22 ± 1.12	13.56 ± 0.43	15.73 ± 0.47	30.13 ± 2.82	34.39 ± 3.87	36.89 ± 3.87
CA-0.5 g	11.13 ± 0.65	9.76 ± 0.50	13.81 ± 0.83	24.76 ± 2.53	31.58 ± 0.53	32.96 ± 0.53
CA-1 g	9.50 ± 0.95	9.13 ± 1.28	12.74 ± 0.81	24.32 ± 1.18	29.31 ± 2.17	32.13 ± 3.24
3rd-batch						
control	24.77 ± 0.06	32.13 ± 0.31	33.70 ± 0.34	56.18 ± 1.03	63.86 ± 4.63	62.96 ± 3.09
AA-0.5 g	19.35 ± 0.52	21.21 ± 0.19	25.26 ± 0.41	46.69 ± 4.72	53.42 ± 2.54	57.55 ± 1.76
AA-1 g	18.28 ± 0.35	17.64 ± 0.80	20.82 ± 0.88	36.76 ± 5.05	43.18 ± 3.15	46.11 ± 0.96
CA-0.5 g	12.13 ± 0.10	19.17 ± 0.70	21.08 ± 0.52	37.69 ± 1.03	39.74 ± 0.89	43.23 ± 3.02
CA-1 g	12.24 ± 1.52	15.39 ± 0.75	18.25 ± 0.68	28.85 ± 4.48	32.92 ± 1.73	41.22 ± 0.49

[a] Samples were stored at 0 °C for up to 8 d prior to the heat treatments. Data were presented as mean ± standard error of triplicate analysis.

Table 3
The amounts of N^{ε}-carboxyethyllysine (mg/kg protein) in commercially sterilized (121 °C, 10 min or 30 min) pork added with 0.5 or 1 g/100 g pork of acetic acid (AA) or citric acid (CA) and that without acid (control) [a.]

	121 °C, 10 min			121 °C, 30 min		
	0 day	4 days	8 days	0 day	4 days	8 days
1st-batch						
control	16.76 ± 0.27	19.20 ± 1.33	22.07 ± 0.85	85.04 ± 9.72	85.67 ± 6.89	102.75 ± 5.03
AA-0.5 g	13.86 ± 0.39	13.70 ± 0.33	21.40 ± 0.76	54.76 ± 7.97	54.96 ± 5.24	68.82 ± 4.00
AA-1 g	14.74 ± 0.62	13.57 ± 0.89	24.10 ± 1.28	54.64 ± 2.89	49.94 ± 2.47	53.14 ± 2.27
CA-0.5 g	12.81 ± 0.99	17.51 ± 1.84	16.91 ± 3.62	59.94 ± 2.80	66.73 ± 1.72	64.40 ± 1.83
CA-1 g	9.48 ± 0.06	11.26 ± 1.00	15.72 ± 3.08	43.67 ± 2.90	42.84 ± 0.85	49.57 ± 0.02
2nd-batch						
control	31.27 ± 3.47	32.65 ± 0.63	40.53 ± 0.59	128.33 ± 4.89	146.57 ± 7.88	155.44 ± 5.01
AA-0.5 g	22.50 ± 0.81	28.59 ± 1.39	31.99 ± 0.70	99.04 ± 3.02	119.82 ± 7.30	109.30 ± 0.54
AA-1 g	21.51 ± 0.67	25.94 ± 1.02	35.06 ± 1.41	75.22 ± 5.03	81.51 ± 4.57	83.41 ± 1.92
CA-0.5 g	21.66 ± 0.91	26.99 ± 0.59	36.85 ± 3.43	68.27 ± 3.60	102.25 ± 2.47	103.54 ± 6.60
CA-1 g	18.87 ± 2.73	22.62 ± 0.39	31.23 ± 0.57	58.10 ± 7.72	77.19 ± 4.60	79.42 ± 1.42
3rd-batch						
control	36.81 ± 1.04	46.29 ± 5.12	55.23 ± 3.26	166.46 ± 1.79	173.03 ± 9.90	182.89 ± 3.23
AA-0.5 g	27.87 ± 0.61	32.40 ± 0.38	38.64 ± 2.29	123.34 ± 1.45	123.35 ± 1.70	132.10 ± 2.43
AA-1 g	25.54 ± 1.60	27.18 ± 1.78	38.96 ± 1.60	85.15 ± 7.73	109.81 ± 1.10	109.38 ± 6.42
CA-0.5 g	24.21 ± 0.52	27.80 ± 2.45	37.11 ± 1.68	107.15 ± 5.03	108.39 ± 1.94	113.07 ± 2.19
CA-1 g	24.06 ± 3.18	26.72 ± 2.67	32.27 ± 3.33	73.17 ± 2.42	97.32 ± 2.97	99.07 ± 1.97

[a] Samples were stored at 0 °C for up to 8 d prior to the heat treatments. Data were presented as mean ± standard error of triplicate analysis.

treatments (CML: $P = 0.000 – 0.005$; CEL: $P = 0.000$) and storage duration (CML: $P = 0.000 – 0.020$; CEL: $P = 0.000 – 0.008$) significantly affected the amount of CML or CEL produced in pork during the commercial sterilization. As shown in Fig. 2a&b, the addition of citric acid led to significant reduction of heat-induced formation of CML and CEL. Regardless of the storage duration of the raw pork, adding 0.5 g citric acid/100 g pork resulted in average reduction of 30 – 45% CML and 34 – 38% CEL that were formed during the two commercial sterilization treatments, while adding 1 g citric acid/100 g pork resulted in 38 – 53% less CML and 52 – 54% less CEL formed during the heat treatments. Acetic acid generally had less of inhibiting effects on the heat-induced formation of CML and CEL in pork compared to the citric acid, and did not result in significant difference on the average amounts of CML formed in pork during the 10 min heat treatment (Fig. 2a). Still, adding 0.5 g acetic acid/100 g pork could reduce average of 14 – 24% CML and 33 – 44% CEL formed during the commercial sterilization, while adding 1 g acetic acid/100 g pork could reduce 27 – 30% CML and 44 – 48% CEL. Although the pork samples added with the same level of acetic acid and citric acid had similar pH (Fig. 1a), the inhibiting effects of the two acids on heat-induced formation of CML and CEL were not quite the same, implying that the acetate and citrate ions played an important role on these inhibiting effects.

The inhibition effects of citric and acetic acids for reducing heat-induced formation of CML and CEL were basically corresponding to their effects on reducing the levels of TBARS (Fig. 2c) and Schiff bases (Fig. 2d) in sterile pork. The reduction of TBARS in sterile pork due to the addition of acetic acid or citric acid indicates that the acetic acid or citric acid could slow down lipid oxidation of pork during the

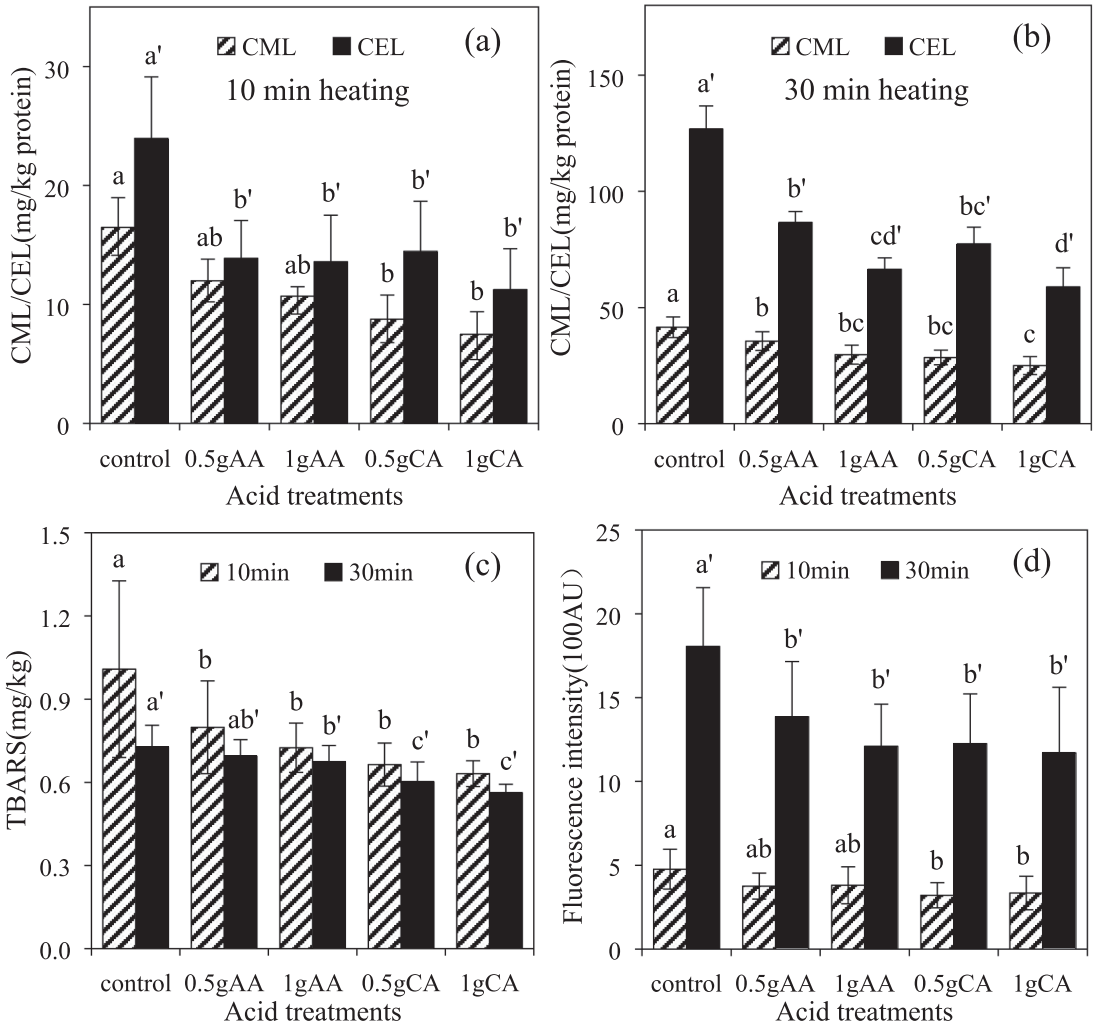


Fig. 2. Effects of acetic acid (AA) or citric acid (CA) (0.5 or 1 g acid/100 g pork) on the amounts of (a) CML and CEL formed in ground pork during the 10 min or (b) 30 min of heating (121 °C), and (c) TBARS and (d) Schiff bases (expressed as fluorescence intensity) in the heat-treated pork. The pork samples with or without acid were stored at 0 °C for 0 – 8 d prior to the heat treatments. Data were shown as mean (n = 9) ± standard deviation. Different letters (abc or a′b′c′d′) above the columns indicate significant difference ($P < 0.05$).

commercial sterilization, which was likely due to the ability of these organic acids to chelate some prooxidants such as iron ions in muscle foods (Ke et al., 2009). Also, since the Schiff bases could be formed via the reaction of free amino groups in proteins/peptides and aldehyde groups of reducing sugar during the initial stage of the Maillard reaction, the addition of an acid in pork could lead to the reduction of nucleophilicity of the free amino groups at the acidic condition, and therefore slow down the initial step of the Maillard reaction (Lund & Ray, 2017; O'Brien et al., 1989). As CML and CEL are mainly formed through the Maillard reaction and lipid oxidation (Srey et al., 2010), the inhibiting effects of acetic and citric acids on lipid oxidation and the initial stage of the Maillard reaction at least partly accounted for their inhibiting effects on heat induced CML and CEL in pork formed during the commercial sterilization. Furthermore, the addition of acid in pork could affect the pathways of the Maillard reaction during heating, favoring the production of furfural and related derivatives from the Amadori compounds instead of the reductone and other fission products (*e.g.* precursors for AGEs) that are mainly formed under the alkaline condition (Martins, Jongen, & Van Boekel, 2001; O'Brien et al., 1989).

The results from the mixed linear models also showed that the acid treatments and storage time of ground pork (with and without acid) had no significant interaction effects on the amounts of CML (P = 0.974 – 0.998) and CEL (P = 0.959 – 0.980) produced during the commercial sterilization. This indicated that the length of marinating time of pork with an acid did not influence the acid's inhibition effects for heat-induced production of AGEs.

The raw pork (with and without acid) stored longer in general led to more CML and CEL produced during the subsequently commercial sterilization, although it may not be significantly different (Fig. 3). Compared to the raw pork without storage, the raw pork (with and without acid) stored for 8 d prior to the heat treatments resulted in average increases of 29 – 45% CML and 19 – 111% CEL, while the raw pork stored for 4 d led to average increases of 19 – 22% CML and 15 – 36% CEL that were produced during the commercial sterilization, depending upon the length of heating time. Similarly, a few reported studies on muscle foods including fish white meat (0 °C, 0 – 3 wk) and pork (0 °C, 0 – 8 d; or −18 °C, 4 mon) revealed that the raw meat stored longer had more CML and CEL produced during the subsequently thermal processing (Niu et al., 2017a, 2018; Yu et al., 2021). This indicated that the lipid oxidation and/or increase levels of Schiff bases in raw pork during the storage (as discussed in 3.1, Fig. 1b) likely resulted in the production of some intermediate products (including but not limited to the Schiff bases, glyoxal, methylglyoxal) that promoted the formation of protein-bound CML and CEL during the later heat treatments, although the storage duration may not influence the CML and CEL levels in raw pork.

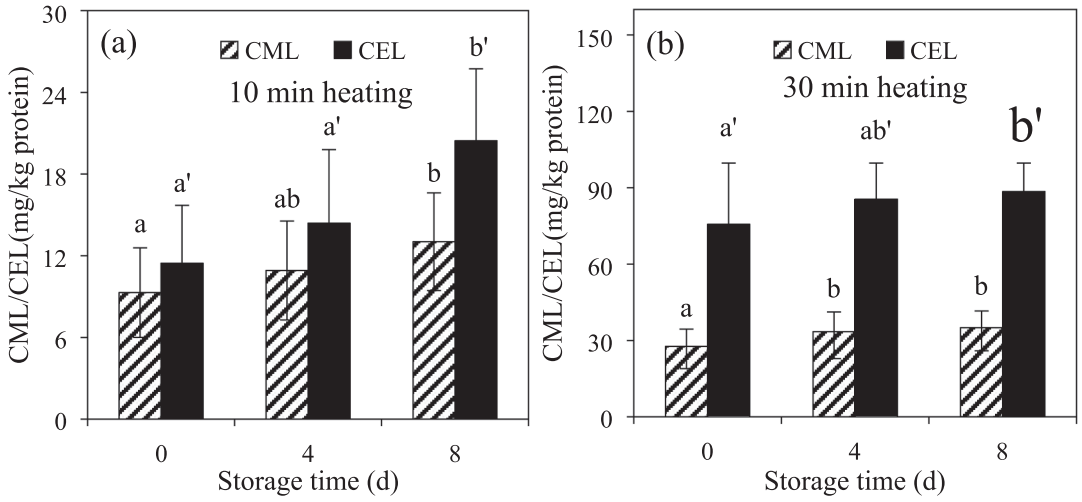


Fig. 3. Effects of storage duration (0 °C, 0 – 8 d) of pork prior to the heat treatments on the amounts of CML and CEL formed in ground pork during (a) 10 min and (b) 30 min of heating (121 °C). Data were shown as mean ($n = 15$) ± standard deviation. Different letters (ab or a′b′) above the columns indicate significant difference ($P < 0.05$).

Conclusions

The effects of citric and acetic acids for reducing heat-induced formation of CML and CEL corresponded to their effects on reducing the levels of TBARS and Schiff bases in pork, suggesting that the inhibiting effects of these two acids on lipid oxidation and/or the initial stage of the Maillard reaction likely contribute to their inhibiting effects on CML and CEL production in pork during heating. In addition, raw pork stored longer in general led to more CML and CEL produced during the subsequently commercial sterilization, which was likely due to more intermediate products (such as Schiff bases) produced from lipid and protein oxidation that promoted the formation of CML and CEL. However, the inhibiting effect of acetic or citric acid on heat-induced formation of either CML or CEL was not significantly affected ($P = 0.959$ – 0.998) by the length of marinating time for the pork with acid before commercial sterilization. Furthermore, the inhibiting effects of citric and acetic acids on heat-induced formation of CML and CEL were affected by the acetate and citrate ions, but the underlying mechanisms need to be further explored.

CRediT authorship contribution statement

Hui Lin: Investigation, Data curation, Formal analysis, Writing – original draft. **Keqiang Lai:** Methodology. **Juanjuan Zhang:** Investigation, Data curation. **Faxiang Wang:** Formal analysis. **Yongle Liu:** Conceptualization, Funding acquisition. **Barbara A Rasco:** Conceptualization, Writing – review & editing. **Yiqun Huang:** Conceptualization, Project administration, Funding acquisition, Writing – review & editing.

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.

Acknowledgement

This study was funded by the National Natural Science Foundation of China (31871733).

References

Biffin, T. E., Smith, M. A., Bush, R. D., Morris, S., & Hopkins, D. L. (2020). The effect of whole carcase medium voltage electrical stimulation, tenderstretching and *longissimus* infusion with actinidin on alpaca meat quality. *Meat Science, 164*, Article 108107.

Braïek, O. B., & Smaoui, S. (2021). Chemistry, safety, and challenges of the use of organic acids and their derivative salts in meat preservation. *Journal of Food Quality, 2021*. https://doi.org/10.1155/2021/6653190

Chelh, I., Gatellier, P., & Santé-Lhoutellier, V. (2007). Characterisation of fluorescent Schiff bases formed during oxidation of pig myofibrils. *Meat Science, 76*, 210–215.

Chen, G. (2021). Dietary N-epsilon-carboxymethyllysine as for a major glycotoxin in foods: A review. *Comprehensive Reviews in Food Science and Food Safety, 20*, 4931–4949.

Chen, T. C., & Waimaleongora-EK, C. (1981). Effect of pH on TBA values of ground raw poultry meat. *Journal of Food Science, 46*, 1946–1947.

FDA, 2021. CFR - Code of Federal Regulations Title 21, Part 184, direct food substances affirmed as generally recognized as safe. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=184. Accessed October 30, 2021.

GB 5009. (2016). National food safety standard of China. National and Family Planning Commission of the People's Republic of China. Method GB 5009.3-2016, determination of moisture in food; Method GB 5009.5-2016, determination of protein in food; Method GB 5009.6-2016, determination of fat in food; Method GB 5009.237-2016, determination of pH value of foods.

Ge, S.-J., & Lee, T.-C. (1997). Kinetic significance of the Schiff base reversion in the early-stage Maillard reaction of a phenylalanine-glucose aqueous model system. *Journal of Agricultural and Food Chemistry, 45*, 1619–1623.

Kalahrodi, M. M., Baghaei, H., Emadzadeh, B., & Bolandi, M. (2021). The combined effect of asparagus juice and balsamic vinegar on the tenderness, physicochemical and structural attributes of beefsteak. *Journal of Food Science and Technology, 58*, 3143–3153.

Ke, S., Huang, Y., Decker, E. A., & Hultin, H. O. (2009). Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle. *Meat Science, 82*, 113–118.

Kim, T. K., Hwang, K. E., Lee, M. A., Paik, H. D., Kim, Y. B., & Choi, Y. S. (2019). Quality characteristics of pork loin cured with green nitrite source and some organic acids. *Meat Science, 152*, 141–145.

Kong, F., Tang, J., Rasco, B., Crapo, C., & Smiley, S. (2007). Quality changes of salmon (Oncorhynchus gorbuscha) muscle during thermal processing. *Journal of Food Science, 72*, S103–S111.

Li, L., Kong, S., Liu, Y., Huang, Y., Li, Y., & Lai, K. (2021). Effects of acetic acid, ethanol, and sodium chloride on the formation of N^{ε}-carboxymethyllysine, N^{ε}-carboxyethyllysine and their precursors in commercially sterilized pork. *Journal of Food Measurement and Characterization, 15*, 5337–5344.

Li, Y., Xue, C., Quan, W., Qin, F., Wang, Z., He, Z., et al. (2021). Assessment the influence of salt and polyphosphate on protein oxidation and N^{ε}-(carboxymethyl) lysine and N^{ε}-(carboxyethyl) lysine formation in roasted beef patties. *Meat Science, 177*, Article 108489.

Lund, M. N., & Ray, C. A. (2017). Control of Maillard reactions in foods: Strategies and chemical mechanisms. *Journal of Agricultural and Food Chemistry, 65*, 4537–4552.

Martins, S. I. F. S., Jongen, W. M. F., & Van Boekel, M. A. J. S. (2001). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology, 11*, 364–373.

Niquet-Léridon, C., & Tessier, F. J. (2011). Quantification of N^{ε}-carboxymethyl-lysine in selected chocolate-flavoured drink mixes using high-performance liquid

chromatography-linear ion trap tandem mass spectrometry. *Food Chemistry, 126*, 655–663.
Niu, L., Sun, X., Tang, J., Wang, J., Rasco, B. A., Lai, K., et al. (2017a). Formation of advanced glycation end-products in fish muscle during heating: Relationship with fish freshness. *Journal of Food Composition and Analysis, 63*, 133–138.
Niu, L., Sun, X., Tang, J., Wang, J., Rasco, B. A., Lai, K., et al. (2017b). Free and protein-bound N^{ε}-carboxymethyllysine and N^{ε}-carboxyethyllysine in fish muscle: Biological variation and effects of heat treatment. *Journal of Food Composition and Analysis, 57*, 56–63.
Niu, L., Sun, X., Tang, J., Wang, J., Wang, J., Rasco, B. A., et al. (2018). Combination effects of salts and cold storage on the formation of proteinbound N^{ε}-(carboxymethyl)lysine and N^{ε}-(carboxyethyl)lysine in raw and subsequently commercially sterilized ground pork. *Food Chemistry, 264*, 455–461.
O'Brien, J., Morrissey, P. A., & Ames, J. M. (1989). Nutritional and toxicological aspects of the Maillard browning reaction in foods. *Critical Reviews in Food Science & Nutrition, 28*, 211–248.
Richards, M. P., & Hultin, H. O. (2000). Effect of pH on lipid oxidation using trout hemolysate as a catalyst: A possible role for deoxyhemoglobin. *Journal of Agricultural and Food Chemistry, 48*, 3141–3147.
Sharedeh, D., Gatellier, P., Astruc, T., & Daudin, J. D. (2015). Effects of pH and NaCl levels in a beef marinade on physicochemical states of lipids and proteins and on tissue microstructure. *Meat Science, 110*, 24–31.
Shen, X., Li, T., Li, X., Wang, F., Liu, Y., & Wu, J. (2022). Dual cryoprotective and antioxidant effects of silver carp (*Hypophthalmichthys molitrix*) protein hydrolysates on unwashed surimi stored at conventional and ultra-low frozen temperatures. *LWT - Food Science and Technology, 153*, Article 112563.
Srey, C., Hull, G. L. J., Connolly, L., Elliott, C. T., Del Castillo, M. D., & Ames, J. M. (2010). Effect of inhibitor compounds on N^{ε}-(carboxymethyl)lysine (CML) and N^{ε}-(carboxyethyl)lysine (CEL) formation in model foods. *Journal of Agricultural and Food Chemistry, 58*, 12036–12041.
Sun, X., Li, X., Tang, J., Lai, K., Rasco, B. A., & Huang, Y. (2021). Formation of protein-bound N^{ε}-carboxymethyllysine and N^{ε}-carboxyethyllysine in ground pork during commercial sterilization as affected by the type and concentration of sugars. *Food Chemistry, 336*, Article 127706.
Sun, X., Tang, J., Wang, J., Rasco, B. A., Lai, K., & Huang, Y. (2015). Formation of advanced glycation end-products in ground beef under pasteurisation conditions. *Food Chemistry, 172*, 802–807.
Sun, X., Tang, J., Wang, J., Rasco, B. A., Lai, K., & Huang, Y. (2016). Formation of free and protein-bound carboxymethyllysine and carboxyethyllysine in meats during commercial sterilization. *Meat Science, 116*, 1–7.
Sun, X., Tang, J., Wang, J., Rasco, B. A., Lai, K., & Huang, Y. (2017). Formation of N^{ε}-carboxymethyllysine and N^{ε}-carboxyethyllysine in ground beef during heating as affected by fat, nitrite and erythorbate. *Journal of Food Measurement and Characterization, 11*, 320–328.
Tang, J. (2015). Unlocking potentials of microwaves for food safety and quality. *Journal of Food Science, 80*, E1776–E1793.
Treibmann, S., Hellwig, A., Hellwig, M., & Henle, T. (2017). Lysine-derived protein-bound Heyns compounds in bakery products. *Journal of Agricultural and Food Chemistry, 65*, 10562–10570.
Uribarri, J., Woodruff, S., Goodman, S., Cai, W., Chen, X., Pyzik, R., et al. (2010). Advanced glycation end products in foods and a practical guide to their reduction in the diet. *Journal of the American Dietetic Association, 110*, 911–916.
Utrera, M., Parra, V., & Estévez, M. (2014). Protein oxidation during frozen storage and subsequent processing of different beef muscles. *Meat Science, 96*, 812–820.
Vyncke, W. (1975). Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (Scomber scombrus L.). *Fette, Seifen, Anstrichmittel, 77*, 239–240.
Wu, S., Huang, Y., Chen, M., Li, X., Xiang, X., & Lai, K. (2020). Protein-bound N^{ε}-carboxymethyllysine and N^{ε}-carboxyethyllysine in raw and heat treated whites and yolks of hen eggs. *Journal of Food Composition and Analysis, 90*, Article 103491.
Yu, L., Gao, C., Zeng, M., He, Z., Wang, L., Zhang, S., et al. (2016). Effects of raw meat and process procedure on N^{ε}-carboxymethyllysine and N^{ε}-carboxyethyl-lysine formation in meat products. *Food Science and Biotechnology, 25*, 1163–1168.
Yu, L., Li, Q., Li, Y., Yang, Y., Guo, C., & Li, M. (2021). Impact of frozen storage duration of raw pork on the formation of advanced glycation end-products in meatballs. *LWT-Food Science and Technology, 146*, Article 111481.
Zhang, Q., Wang, Y., & Fu, L. (2020). Dietary advanced glycation end-products: Perspectives linking food processing with health implications. *Comprehensive Reviews in Food Science and Food Safety, 19*, 2559–2587.
Zhu, Z., Cheng, Y., Huang, S., Yao, M., Lei, Y., Khan, I. A., et al. (2019). Formation of N^{ε}-carboxymethyllysine and N^{ε}-carboxyethyllysine in prepared chicken Breast by pan frying. *Journal of Food Protection, 82*, 2154–2160.
Zhu, Z., Huang, M., Cheng, Y., Khan, I. A., & Huang, J. (2020). A comprehensive review of N^{ε}-carboxymethyllysine and N^{ε}-carboxyethyllysine in thermal processed meat products. *Trends in Food Science & Technology, 98*, 30–40.