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

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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 oxidation 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 Nε-carboxymethyllysine (CML) and Nε-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

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**ABSTRACT**

The heat-induced (121 °C, 10 or 30 min) formation of two potentially hazardous advanced glycation end-products (AGEs), protein-bound Nε-carboxymethyllysine (CML) and Nε-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.
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. Urribarri 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 Huangag 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 cans (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 300-57; 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 (Tong, 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 Estevez (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 (6945g 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 isopotes of CML (d1-CML) and CEL (d4-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 (d4-CML, d4-CEL) instead of one (d4-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 (α = 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 (α = 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 (pKa = 4.76) and that with citric acid (pKa: 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 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, & Sante-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 = 0.28; 3.99 ± 0.23 mg/kg protein), although not significantly higher than that stored for 4 days (4.11 ± 0.23 mg/kg protein), was generally tied to the increased extent of protein oxidation (Chelh, Gatellier, & Sante-Lhoutellier, 2007; Shen et al., 2022).

### Table 1

|                  | 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         | 6.95 ± 0.54         |
| AA-1 g           | 2.63 ± 0.14         | 4.22 ± 0.18         | 4.71 ± 0.34         | 10.84 ± 0.72        | 9.65 ± 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 ± 0.99         |
| 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         |

* Samples were stored at 0 °C for up to 8 d. Data were presented as mean ± standard error of triplicate analysis.
The amounts of N\textsuperscript{-}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) \textsuperscript{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 ± 1.14   | 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 ± 2.32 |
| 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  |

\textsuperscript{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.

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 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 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.

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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 ($0^\circ C$, 0 – 3 wk) and pork ($0^\circ C$, 0 – 8 d; or $-18^\circ C$, 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. 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 ($121^\circ C$), 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 $0^\circ C$ 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 ($P < 0.05$).
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 (a or a’) 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.

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