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Research Article

Nisin retarded *L*-arginine (or *L*-lysine)-caused the augmented total volatile basic nitrogen content of Cantonese sausage by inhibiting the activity of amino acid decarboxylase

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Abstract: The study investigated whether the inhibitory effects of nisin on the increase in total volatile basic nitrogen (TVB-N) content caused by *L*-arginine (Arg) or *L*-lysine (Lys) in Cantonese sausage. Nisin had minimal influence on water activity and pH. However, it significantly reduced the content of non-protein nitrogen from 5.23 (or 5.32) to 4.57 (or 4.53) mg/g, amino acid nitrogen content from 3.41 (or 3.38) to 2.96 (or 2.93) mg/g, TVB-N content from 55.92 (or 48.21) to 36.46 (or 36.14) mg/100 g, and biogenic amines content from 58.69 (or 57.28) to 51.89 (or 47.36) mg/100 g. In contrast, it increased the contents of trichloroacetic acid-soluble peptide from 5.55 (or 4.99) to 6.54 (or 6.40) mg/g during 28 days of storage in Cantonese sausages containing Arg (or Lys). In these two sausage samples, nisin also decreased the total bacterial count from 6.13 (or 6.10) to 5.31 (or 5.36) (lg (CFU/g)), the count of lactic acid bacteria from 6.08 (or 6.09) to 5.24 (or 5.13) (lg (CFU/g)), and the activity of amino acid decarboxylase from 0.63 (or 0.65) to 0.57 (or 0.60) U/g. Lactic acid bacteria were predominant in all the sausage samples. Thus, nisin effectively suppressed the Arg- or Lys-induced increase in TVB-N content by inhibiting the growth of lactic acid bacteria, which secrete amino acid decarboxylase. The results provide technical support for using Arg or Lys for producing low-sodium and low-phosphorus Cantonese sausages.

Keywords: nisin; *L*-arginine; *L*-lysine; total volatile basic nitrogen content; protein degradation; Cantonese sausages

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1 Introduction

L-Arginine (Arg)/*L*-lysine (Lys) has been shown to enhance the texture and color of low-sodium Cantonese sausage, while also inhibiting its lipid and protein oxidation in the absence of exogenous phosphate, thus improving its overall quality attributes[1]. However, Arg/Lys addition also increases total volatile basic nitrogen (TVB-N) content in Cantonese sausage. Volatile basic nitrogen comprises various volatile nitrogen-containing compounds (such as biogenic amines) that result from extensive protein degradation and are detrimental to human health[2]. Volatile basic nitrogen accumulation not only diminishes the sensory quality of Cantonese sausage but also poses food safety risks, thereby limiting the application of Arg/Lys in low-sodium and low-phosphorus Cantonese sausage formulations. Hence, exploring strategies to mitigate the increase in TVB-N content induced by Arg/Lys is necessary.

The degradation of meat proteins is a complex biochemical process involving enzymes secreted by various microorganisms[3]. Given that fermentation is a crucial step in Cantonese sausage production[1], it can be inferred that microorganisms and their secreted enzymes actively participate in protein degradation, thereby influencing volatile basic nitrogen accumulation. Studies have demonstrated that some specific microorganisms, such as lactic acid bacteria (LAB) and yeast, can secrete proteases such as amino acid decarboxylase (AAD), which decompose peptides and amino acids, leading to the formation of derivatives such as biogenic amines[4–6].

Additionally, raw meat contains various endogenous and exogenous proteases that participate in the early stages of protein degradation[7]. These enzymatic processes are primarily responsible for improving the tenderness and other desirable quality attributes of meat products[8]. Consequently, the activities of such proteases and their associated protein degradation are beyond the scope of the present study.

Nisin, a well-known natural preservative, is widely recognized for its safety and broad-spectrum antimicrobial activity. It can inhibit multiple microorganisms, including *Staphylococcus aureus*, *Clostridium botulinum*, LAB, and yeast, by disrupting the structure and function of the bacterial cell membrane[9–10]. Therefore, it is hypothesized that nisin could lower TVB-N content in Cantonese sausage by inhibiting the reproduction and growth of microorganisms that secrete meat protein-degrading enzymes.

Accordingly, this study aims to investigate the inhibitory effects of nisin on TVB-N content in low-sodium and low-phosphorus Cantonese sausage containing Arg (or Lys). For this purpose, we measured the contents of various protein degradation products, including non-protein nitrogen (NPN), trichloroacetic acid (TCA)-soluble peptide, amino acid nitrogen (AAN), TVB-N, and biogenic amines during storage. Furthermore, microbial

composition of Cantonese sausage and AAD activity involved in the deep degradation of meat proteins were also analyzed to elucidate the underlying mechanism. The study findings provide a reference for delaying volatile basic nitrogen accumulation in low-sodium and low-phosphorus Cantonese sausage containing Arg (or Lys).

2 Material and methods

2.1 Materials and chemicals

Chevon was obtained from 24 legs of 6 Huanghuai White Goat carcasses. These animals are approximately 6 months old and weighed 30–40 kg (Baisiyuan Food Co., Ltd., Lu'an, China). The slaughter procedure was conducted in accordance with the GB/T 9961–2008[11]. The chevon was removed of all skin, bone, connective tissues, and internal visible fat and delivered to the laboratory within 24 h. Chevon fat was supplied by Luoming Trading Co., Ltd. (Shangqiu, China). Both chevon and chevon fat were stored at −18 °C until use.

2.2 Manufacture of Cantonese sausages

The collected chevon was fully ground in a 5-mm-diameter meat grinder after thawing for 24 h at 4 °C. Meanwhile, the chevon fat was segmented into cubes of 5 mm. Next, the chevon batter along with fat cube were mixed well and assigned to six treatment groups: control group: neither Arg, Lys nor nisin; A group: 0.6% (*m*/*m*, the same as below) Arg; L group: 0.6% Lys; AN group: 0.6% Arg and 0.25% nisin; and LN group: 0.6% Lys and 0.25% nisin. The contents of Arg, Lys, or nisin were decided based on our previous results[1] and GB 2760–2024[12]. In addition to these additives, 1.875% NaCl, 0.625% KCl, 20% ice water, 14% sugar, 4% Baijiu, 2.5% soy sauce, 0.4% monosodium glutamate, and 0.002 4% sodium nitrite were added. The remaining steps were the same as detailed in our previous study[1]. The sausage samples were analyzed on days 1, 14, and 28, respectively.

2.3 Water activity (a_w) and pH value determination

The a_w was measured as described elsewhere[13]. After cutting into small pieces, 5 g of the sausage sample was homogeneously spread in the box of the a_w meter (GYW-1W, Guanya Technology Co., Ltd., Shenzhen, China) for detection.

The pH value of the sausage sample was monitored according to the protocol of Körmendy et al.[14] with appropriate modifications. The chopped sample (5 g) was homogenized with 50 mL of distilled water. The pH value of the mixture was immediately monitored by using a pH meter (pH-3E, Leici, Shanghai, China).

2.4 Determination of contents of various protein degradation products

2.4.1 NPN content

The sausage sample (5 g) was completely homogenized with 50 mL of distilled water and filtered. The filtrate was mixed with an equal volume of 10 g/100 mL TCA solution to remove water-soluble protein. Finally, 10 mL of the filtrate was used for the determination of NPN content by using the Association of Official Analytical Chemists Kjeldahl method[15].

In addition, the presence of Arg or Lys (A, L, AN, and LN) resulted in higher NPN detection values compared to the control, which does not truly reflect the amount of NPN produced through protein degradation during storage. Therefore, the corresponding amount of Arg or Lys (labeled as CA and CL, respectively) was added individually to eliminate this effect when the sausage (the control) was homogenized.

2.4.2 TCA-soluble peptide content

The sausage sample (5 g) was mixed and homogenized with 50 mL of 5 g/100 mL TCA solution, centrifuged at 12 000 × *g* and 4 °C for 15 min, followed by collection of the supernatant. The content of TCA-soluble peptide was monitored by using the Lowry method[16].

2.4.3 AAN content

AAN content was monitored by using the formaldehyde titration method[17]. First, the sausage sample (5 g) was mixed and homogenized in 100 mL of distilled water and then centrifuged at 5 000 × *g* under 4 °C for 10 min to obtain the supernatant. Second, the supernatant (20 mL) was diluted with distilled water to 80 mL. The pH value of the diluted supernatant and distilled water (80 mL, designated as a reference) was adjusted to pH 8.2 with 0.05 mol/L NaOH solution, to which 10 mL of formaldehyde was added. Finally, the pH value of the mixture was adjusted to 9.2 with a 0.05 mol/L NaOH solution, and the volume of NaOH solution consumed was recorded (V_1 for the sample to be tested (mL), and V_0 for the reference (mL)).

Similar to the detection of NPN content, the presence of Arg or Lys (A, L, AN, and LN) was interfered with based on the detection of ANN content. To eliminate this effect, the sausage sample (the control) was added individually with the corresponding amount of Arg or Lys (labeled as CA and CL, respectively) before homogenization. The ANN content is calculated as follows:

$$\text{AAN content}\ (\text{mg/g}) = (V_1 - V_0) \times c \times 1.400\ 8$$

Where c represent the concentration of NaOH standard solution (mol/L).

2.4.4 TVB-N content

TVB-N content was monitored by using an automatic Kjeldahl nitrogen analyzer based on the method proposed elsewhere[18]. Briefly, the sausage sample (2 g) was homogenized with 20 mL of distilled water. To which, 0.2 g of MgO was added, followed by steam distillation. The distillate was absorbed with 25 mL of 20 g/L boric acid solution containing 1 mL/100 mL of indicator and titrated with 0.1 mol/L HCl, using 0.1 g/100 mL methyl red ethanol and 0.1 g/100 mL bromomethyl green solution as indicator.

2.4.5 Biogenic amines contents

The sausage sample (5 g) was homogenized with 20 mL of 5 g/100 mL TCA solution for 3 min. The mixture was subjected to ultrasonic treatment for 30 min, followed by centrifugation for 10 min at 2 800 × *g* under 4 °C. The resulting residue was further extracted with the TCA solution by vortex mixing for 30 min, followed by centrifugation under the same conditions. The supernatant was combined and diluted to a final volume of 50 mL. Next, 10 mL of the supernatant, 0.5 g of NaCl, and 10 mL of *n*-hexane were added. After that, the mixture was subjected to ultrasonic treatment for 10 min, followed by static separation. The lower layer (water phase layer) was collected for subsequent derivatization.

For the derivatization process: 1 mL of saturated $NaHCO_3$ solution, 100 μL of 1 mol/L NaOH solution, and 1 mL of DNS-Cl

were added to 1 mL of the extract. The mixture was vortex-mixed for 1 min and incubated in the dark at 60 °C for 15 min. Next, 200 μL of 25 g/100 mL ammonia solution was added to the mixture. The mixture was fully mixed and incubated for 30 min in the dark to terminate the reaction.

To the mixture, 0.5 g of NaCl was added, and the mixture was fully dissolved. Subsequently, 5 mL of diethyl ether was added, and the mixture was subjected to ultrasonic treatment for 2 min. After static separation, the upper layer (ether layer) was collected and dried in a nitrogen evaporator. Finally, 1 mL of acetonitrile was added to re-dissolve the residues. Finally, the solution was filtered through a 0.22-μm organic phase filter membrane and stored until the next analysis. Concurrently, standard biogenic amine solutions were derivatized under similar conditions and used for analysis.

The samples were analyzed by high performance liquid chromatograph equipped with the Zorbax Eclipse XDB-C_{18} column (4.6 mm × 250 mm, 5 μm) at 254 nm, and the contents of biogenic amines was calculated based on the standard curve method[19].

2.5 Microbial count determination

The total bacterial count, yeast count, *Escherichia coli* count, and LAB count in the sausage sample were determined as per the national standard of China[20–23]. The sausage (10 g) was homogenized with 0.9 g/100 mL NaCl solution (90 mL) and then subjected to gradient dilution, inoculated onto corresponding agar plates, and incubated at 36, 28, 37, and 36 °C, respectively.

2.6 AAD activity determination

The AAD activity was determined as per a previously reported method[24] with some modifications. Especially, the sheep AAD linked immunosorbent assay kit (REF: YJ235805; Shanghai Yuanju Biotechnology Center, Shanghai, China) was used in this work.

2.7 Statistical analysis

The results (as presented as mean ± standard error) were analyzed using SPSS (version 27.0 for Windows, SPSS Inc., IL, USA). A one-way analysis of variance (ANOVA) was performed to assess the significant differences. The different treatments and the repeated experiments were considered as fixed factors and random factors, respectively. Each sample was analyzed three times. Duncan's test was introduced to compare the differences, and $P < 0.05$ was considered to indicate statistical significance. Correlations were analyzed by using the correlation plot application in Origin software.

3 Results and discussion

3.1 Analysis of a_w and pH value

The a_w is a key factor influencing microbial growth. As shown in Table 1, slight differences in a_w were noted among the different sausage samples, consistent with previous results that the addition of Arg or Lys does not significantly alter the a_w of sausage products[1]. Except that sausage sample control whose a_w decreased significantly ($P < 0.05$) on day 28, the a_w of the other sausage samples remained relatively stable throughout prolonged storage, indicating minimal fluctuation in a_w during the entire storage period. Overall, the a_w of all sausage samples remained approximately 0.77 during the whole storage period.

As presented in Table 1, the pH values of all sausage samples decreased significantly ($P < 0.05$) with prolonged storage, which may be attributed to the continuous production of lactic acid by LAB. A similar trend was observed in the prepared pork chops injected with Arg or Lys solution, where pH decreased with extended storage[25]. In general, compared with the control, the addition of Arg and Lys, regardless of whether or not combined with nisin, resulted in significantly higher pH values ($P < 0.05$). This effect can be explained by the basic nature of Arg and Lys, whose isoelectric points are 10.76 and 9.74, respectively[26]. Samples receiving only single treatments exhibited significantly lower ($P < 0.05$) pH values than those subjected to combined treatments. This phenomenon might be related to the inhibitory action of nisin on the growth and reproduction of LAB, which produce lactic acid (to be discussed in section 3.3).

3.2 Analysis of meat protein degradation products

During storage, meat proteins undergo enzymatic degradation mediated by microbial enzymes, which results in several protein-derived products during storage. Therefore, protein degradation indices were analyzed to assess the individual and combined effects of Arg, Lys, and nisin on meat protein breakdown and volatile basic nitrogen accumulation during storage.

Table 1 Effects of different treatments on a_w, pH, the total bacterial count, and LAB count.

Parameters	Storage time (day)	Control	A	L	AN	LN
a_w	1	0.78 ± 0.01^{Ba}	0.78 ± 0.01^{Aa}	0.78 ± 0.01^{Aa}	0.77 ± 0.01^{Aa}	0.78 ± 0.02^{Aa}
	14	0.77 ± 0.01^{Ba}	0.78 ± 0.01^{Aa}	0.78 ± 0.01^{Aa}	0.78 ± 0.01^{Aa}	0.78 ± 0.01^{Aa}
	28	0.76 ± 0.02^{Aa}	0.77 ± 0.01^{Aa}	0.78 ± 0.01^{Ab}	0.77 ± 0.01^{Aa}	0.78 ± 0.01^{Ab}
pH	1	6.11 ± 0.01^{Ca}	6.37 ± 0.02^{Cb}	6.39 ± 0.01^{Cb}	6.50 ± 0.02^{Cd}	6.45 ± 0.02^{Cc}
	14	5.86 ± 0.01^{Ba}	6.07 ± 0.02^{Bb}	6.09 ± 0.01^{Bb}	6.20 ± 0.02^{Bc}	6.22 ± 0.02^{Bc}
	28	5.66 ± 0.02^{Aa}	5.80 ± 0.01^{Ab}	5.82 ± 0.02^{Ab}	5.95 ± 0.01^{Ac}	5.98 ± 0.02^{Ad}
Total bacterial count (lg (CFU/g))	1	6.14 ± 0.04^{Cc}	6.23 ± 0.04^{Ccd}	6.30 ± 0.04^{Cd}	5.80 ± 0.05^{Cb}	5.37 ± 0.15^{Ca}
	14	6.08 ± 0.02^{Bc}	6.17 ± 0.02^{Bd}	6.15 ± 0.04^{Bd}	5.51 ± 0.05^{Bb}	5.41 ± 0.04^{Ba}
	28	5.83 ± 0.01^{Ab}	6.13 ± 0.05^{Ac}	6.10 ± 0.05^{Ac}	5.31 ± 0.01^{Aa}	5.36 ± 0.06^{Aa}
LAB count (lg (CFU/g))	1	6.02 ± 0.07^{Bb}	6.33 ± 0.07^{Cc}	6.36 ± 0.04^{Cc}	5.58 ± 0.03^{Ba}	5.59 ± 0.04^{Ba}
	14	6.02 ± 0.04^{Bb}	6.15 ± 0.03^{Bb}	6.21 ± 0.02^{Bb}	5.30 ± 0.19^{Aa}	5.28 ± 0.11^{Aa}
	28	5.81 ± 0.08^{Ac}	6.08 ± 0.08^{Ad}	6.09 ± 0.01^{Ad}	5.24 ± 0.06^{Ab}	5.13 ± 0.05^{Aa}

Note: Different lowercase letters in the same row indicate significant differences ($P < 0.05$) among the different treatments at the same storage time. Different capital letters in the same column indicate significant differences ($P < 0.05$) in different storage times for the same treatment group.

3.2.1 NPN content analysis

NPN refers to nitrogen-containing compounds not bound in the protein form, including soluble peptides, AAN, and non-AAN (e.g., ammonia, amine, nitrate, and nitrite)[27]. With the extension of storage, NPN contents gradually increased in all sausages ($P < 0.05$) (Figure 1A), likely due to continuous protein degradation and NPN accumulation during sausage storage.

After 1 day of storage, no significant ($P > 0.05$) differences in NPN contents were observed between the control samples (CA and CL) and sausage samples treated with Arg or Lys alone; However, samples subjected to combined treatments (AN and LN) exhibited lower NPN content ($P < 0.05$) (3.46 and 3.38 mg/g, respectively) than those subjected to single treatments (3.74 mg/g in A and 3.71 mg/g in L). After 14 and 28 days of storage, sausage samples treated with Arg or Lys alone (4.47 and 4.44 mg/g on day 14; 5.23 and 5.32 mg/g on day 28, respectively) showed higher NPN contents than the controls (4.29 and 4.72 mg/g in CA; 4.32 and 4.80 mg/g in CL, respectively), whereas the combination of Arg (or Lys) and nisin (4.17 and 4.57 mg/g in AN; 4.12 and 4.53 mg/g in LN, respectively) resulted in slightly lower NPN content than the controls. For comparison, equal amounts of Arg or Lys were added to the control samples (CA and CL) at each sampling time when NPN contents were determined. These results suggest that the addition of Arg or Lys alone promotes protein degradation, leading to higher NPN accumulation. This may be because Arg and Lys provide additional nitrogen sources for microorganisms[28], stimulating their growth and reproduction and enzymatic activity, including the secretion of proteases and AAD. These enzymes accelerate protein decomposition and gradual NPN accumulation. In samples subjected to combined treatments, nisin inhibited microbial growth and reproduction and enzyme secretion, ultimately slowing protein decomposition and resulting in lower NPN contents.

3.2.2 TCA-soluble peptide content analysis

The content of TCA-soluble peptides (primarily water-soluble peptides) serves as an important indicator of protein degradation[17]. Overall, TCA-soluble peptide content in Cantonese sausages sample A (or L) increased significantly ($P < 0.05$) from 3.88 (or 4.00) to 5.55 (or 4.99) mg/g, respectively, with the prolonged storage period (Figure 1B). Soluble peptides are the initial products of protein degradation, followed by amino acids and biogenic amines. These results indicate continuous degradation of proteins in sausage samples throughout storage to generate TCA-soluble peptides, with the formation rate of peptides exceeding their subsequent degradation rate. The similar results show that proteolysis during sausage fermentation causes an increase in TCA-soluble peptides content[29].

Notably, TCA-soluble peptide content increased rapidly during the early storage period and plateaued later, a trend consistent with changes observed in NPN content (Figure 1A). This might be due to increased acidity (decreased pH value) over time, which can reduce protease activity[30].

At the 28th day of storage, single treatments resulted in lower TCA-soluble peptide contents (5.55 and 4.99 mg/g) compared with combined treatments (6.54 and 6.40 mg/g), whereas the contents following both treatments were lower than those with the controls (7.24 mg/g) ($P < 0.05$). The results suggest that Arg or Lys alone either slowed down the conversion of TCA-soluble peptides and/or promoted their further degradation, while their separate combinations with nisin produced the opposite effect. Some studies have shown that LAB can secrete peptidases that hydrolyze peptides[30]. Microbiological analyses unveiled higher LAB counts in

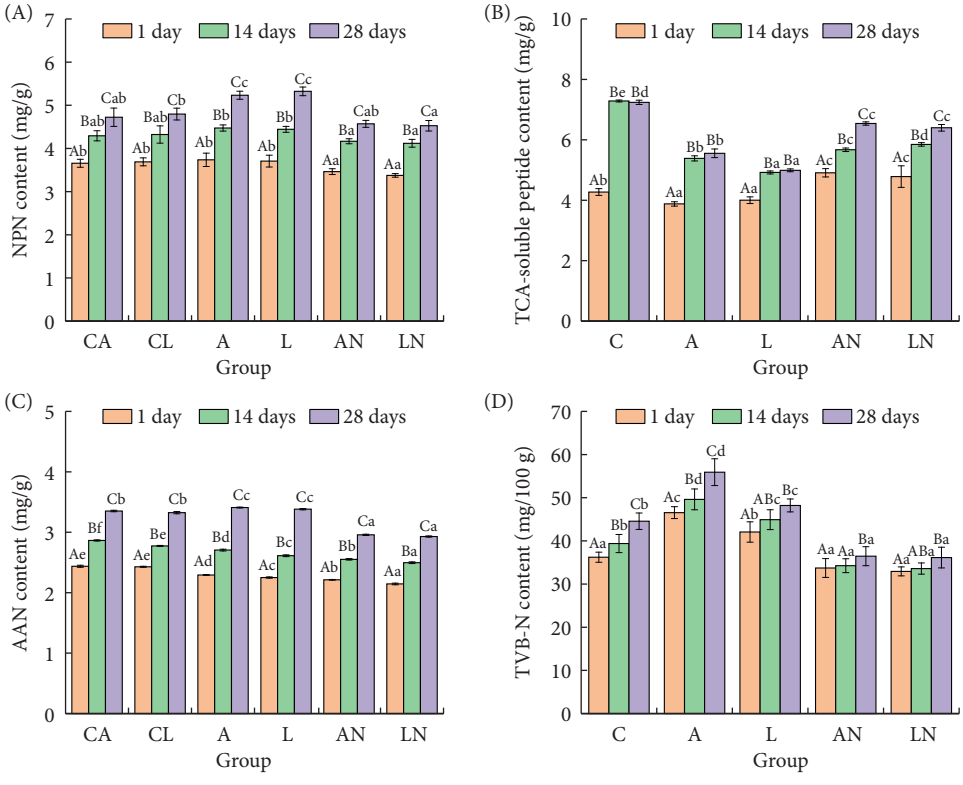

Figure 1 Effects of different treatments on (A) NPN, (B) TCA-soluble peptide, (C) AAN and (D) TVB-N contents. Different lowercase letters indicate significant differences ($P < 0.05$) among the different treatments at the same storage time. Different capital letters indicate significant differences ($P < 0.05$) in different storage times for the same treatment group.

sausage samples A and L (6.08 and 6.09 (lg (CFU/g)) and lower counts in sausage samples AN and LN (5.24 and 5.13 (lg (CFU/g))), compared with the controls (5.81 (lg (CFU/g))) (Table 1). Therefore, it can be speculated that Arg or Lys alone stimulated LAB growth and reproduction and peptidase secretion (higher AAD activity), leading to accelerated degradation of soluble peptides. However, the presence of nisin inhibited LAB growth and reproduction, resulting in reduced peptidase activity (notably lower on day 14, Table 1) and decreased degradation of TCA-soluble peptides.

3.2.3 AAN content analysis

AAN represents a class of protein degradation products in the form of amino acids[17]. As shown in Figure 1C, AAN contents gradually increased with storage time, indicating continuous degradation of proteins and peptides, as well as the sustained formation and accumulation of amino acids. Moreover, the accumulation rate of AAN exceeded its rate of consumption.

On the 1st and 14th days of storage, the AAN contents increased in the following order: LN < AN < L < A < CA < CL. Compared with samples A and L (2.29 and 2.25 mg/g on day 1; 2.71 and 2.61 mg/g on day 14, respectively), the control samples CA and CL exhibited higher AAN contents (2.44 and 2.43 mg/g on day 1; 2.86 and 2.77 mg/g on day 14, respectively). Of note, the timing of Arg and Lys addition differed among the sausage samples L, A, CA, and CL: Arg and Lys were incorporated into the two samples A and L during sausage preparation, whereas in samples CA and CL, they were added when AAN contents were measured. These results suggest that some amino acids in the sausage samples were degraded into low-molecular-weight nitrogenous compounds during sample processing and storage. Samples AN and LN (2.96 and 2.93 mg/g, respectively) had lower AAN contents than samples A and L (3.41 and 3.38 mg/g, respectively) on day 28, showing slower AAN formation and/or faster amino acid consumption in samples subjected to combined treatments. Microbiological analysis further supported this observation, as samples AN and LN exhibited lower total bacterial counts (5.31 and 5.36 (lg (CFU/g)), respectively) and LAB counts (5.24 and 5.13 (lg (CFU/g)) on day 28, respectively) than the other groups (Table 1), which further supported lower amino acid generation in sausage samples subjected to combined treatments.

After 28 days of storage, AAN contents increased in the following order: AN ≈ LN < CA ≈ CL < A ≈ L. These results further indicated that nisin inhibited the increase in AAN contents, which might be related to its antibacterial effect. AAN accumulation progressed in samples A and L (3.41 and 3.38 mg/g, respectively), although the total count of bacterial and LAB colonies showed a downward trend in the late storage period (5.31 and 5.36 (lg (CFU/g)) for total count of bacterial colonies; 5.24 and 5.13 (lg (CFU/g)) for LAB colonies, respectively) (Table 1). In general, nisin inhibits protein degradation and delays the increase of AAN contents. This is consistent with the variation trend of NPN contents (Figure 1A).

3.2.4 TVB-N content analysis

TVB-N content is often used to evaluate the extent of protein degradation in food[18]. As shown in Figure 1D, TVB-N contents of all samples gradually increased with the storage period, indicating that proteins were continuously degraded during this period. This observation is consistent with previous observations reported by Xue et al.[31]. At the 28th of storage, compared with the controls (44.56 mg/100 g), single treatments (55.92 and 48.21 mg/100 g) led to higher TVB-N contents, whereas combined treatments (36.46 and 36.14 mg/100 g) led to lower contents ($P < 0.05$). Among the individually treated samples, sample A (55.92 mg/100 g) exhibited higher TVB-N contents than sample L (48.21 mg/100 g), and no significant differences in TVB-N contents were found between sample AN (36.46 mg/100 g) and sample LN (36.14 mg/100 g) ($P > 0.05$). These results indicate that Arg or Lys addition alone promotes the increase in TVB-N content, consistent with previous results[1]. Conversely, the addition of nisin hindered the increase in TVB-N content. The variation trend of TVB-N contents in sausage samples closely paralleled that of NPN contents.

As volatile basic nitrogen encompasses various volatile nitrogen-containing compounds, such as biogenic amines, TVB-N content serves as an important indicator of spoilage in meat products[2]. At the end of storage, the lower TVB-N contents observed in the combined treatments (36.46 and 36.14 mg/100 g) than in the controls (44.56 mg/100 g) suggest reduced spoilage and improved sensory quality of these samples.

3.2.5 Biogenic amines content analysis

Biogenic amines are primarily formed due to amino acid decarboxylation. Total biogenic amine contents of all samples increased with storage (Table 2), indicating continuous degradation of amino acids to produce biogenic amines. Compared with the controls (49.04 mg/100 g), samples with individual treatments exhibited similarly significantly elevated total biogenic amine contents (58.69 mg/100 g in A and 57.28 mg/100 g in L) ($P < 0.05$), whereas samples with combined treatments (51.89 mg/100 g in AN and 47.36 mg/100 g in LN) did not have statistically significant differences in the total biogenic amine contents ($P > 0.05$) during the entire storage period.

Histamine and spermidine contents remained very low (< 0.60 mg/100 g) in all samples during the entire storage period, whereas spermine contents were significantly ($P < 0.05$) higher in the samples containing Arg or Lys alone than in the other samples at the end of storage. In addition, samples A and L overall had higher putrescine and tyramine content than the other samples. Some studies have shown that Arg serves as a precursor of putrescine and spermine[32], which may explain the higher total biogenic amine content observed in the Arg-treated sample. Histamine is known to adversely affect human health, with excessive intake causing symptoms such as headache, nausea, vomiting, and diarrhea. Putrescine is a key indicator of food spoilage, and its excessive intake can cause gastrointestinal discomfort or other adverse reactions[33]. The present findings indicate that nisin effectively inhibited the increase in biogenic amine contents caused by Arg and Lys in Cantonese sausage, thereby contributing to the improvement in product safety.

These findings imply the presence of microorganisms in sausages that can produce putrescine, tyramine, and spermine. Research has indicated that yeast[5], *E. coli*[33], and LAB[4] can utilize amino acids to produce biogenic amines through amino acid decarboxylation. Consequently, a further analysis was conducted to investigate the potential microbial populations and AAD activity within the sausage samples.

3.3 Microbiological analysis

Microorganisms play a dual role in fermented meat products. They contribute to the characteristic flavor and unique texture of the products, while also potentially posing food safety risks. Studies have demonstrated that *E. coli*, yeast, and LAB produce various enzymes during their reproduction and growth that degrade

Table 2 Effects of different treatments on biogenic amines contents (mg/100 g).

Biogenic amines	Storage time (day)	Control	A	L	AN	LN
Putrescine	1	12.09 ± 1.46^{Aab}	14.30 ± 1.11^{Abc}	17.37 ± 0.88^{Ac}	9.92 ± 1.07^{Aa}	11.23 ± 0.98^{Aab}
	28	22.10 ± 1.49^{Ba}	24.49 ± 1.21^{Ba}	25.33 ± 0.90^{Ba}	22.05 ± 2.17^{Ba}	22.09 ± 1.18^{Ba}
Histamine	1	0.44 ± 0.06^{Ba}	0.25 ± 0.13^{a}	0.17 ± 0.01^{a}	0.21 ± 0.06^{a}	0.09 ± 0.01^{Aa}
	28	0.05 ± 0.02^{Aa}	–	–	–	0.06 ± 0.02^{Aa}
Tyramine	1	1.33 ± 0.15^{Aab}	3.05 ± 0.55^{Ab}	2.98 ± 0.83^{Ab}	0.99 ± 0.49^{Ac}	0.25 ± 0.01^{Ac}
	28	5.28 ± 0.83^{Ba}	8.48 ± 1.12^{Ba}	7.01 ± 1.55^{Ba}	9.30 ± 2.20^{Ba}	5.22 ± 0.67^{Ba}
Spermidine	1	0.18 ± 0.08^{Aa}	0.20 ± 0.05^{Aa}	0.27 ± 0.01^{Aa}	0.19 ± 0.02^{Aa}	0.26 ± 0.02^{Aa}
	28	0.23 ± 0.02^{Aa}	0.23 ± 0.04^{Aa}	0.52 ± 0.04^{Bb}	0.26 ± 0.01^{Ba}	0.57 ± 0.10^{Bb}
Spermine	1	13.31 ± 0.71^{Aa}	15.56 ± 1.35^{Aa}	14.35 ± 0.74^{Aa}	13.04 ± 0.42^{Aa}	12.28 ± 0.99^{Aa}
	28	21.38 ± 0.78^{Ba}	25.49 ± 0.88^{Bb}	24.42 ± 0.74^{Bb}	20.28 ± 0.91^{Ba}	19.41 ± 0.78^{Ba}
Total biogenic amines	1	27.37 ± 0.49^{Aa}	33.36 ± 0.58^{Ab}	35.15 ± 1.00^{Ab}	24.35 ± 1.89^{Aa}	24.11 ± 1.01^{Aa}
	28	49.04 ± 3.17^{Ba}	58.69 ± 0.94^{Bb}	57.28 ± 1.75^{Bb}	51.89 ± 0.88^{Ba}	47.36 ± 2.66^{Ba}

Note: Different lowercase letters in the same row indicate significant differences ($P < 0.05$) among the different treatments at the same storage time. Different capital letters in the same column indicate significant differences ($P < 0.05$) in different storage times for the same treatment group. – Means not detected.

proteins and amino acids into biogenic amines such as histamine, tyramine, putrescine, and spermine[33]. Therefore, bacterial populations, including total viable counts and counts of *E. coli*, yeast, and LAB in the sausage samples, were analyzed. Neither *E. coli* nor yeast was detected in the samples. As *E. coli* is known to cause diarrhea and other health issues, its absence underscores the high level of safety of the fermented sausages produced.

Throughout storage, the number of LAB colonies in all samples was approximately equal to the total bacterial counts (Table 1), indicating that LAB dominated the microbial community in all samples. Both the total bacterial and LAB counts gradually decreased in all samples during storage, likely due to the unfavorable low-temperature conditions that inhibited microbial reproduction and growth[34], as well as the self-limiting effects of LAB metabolites, particularly lactic acid production and pH reduction (Table 1), on microbial proliferation.

At the 28th day of storage, samples treated with Arg or Lys alone (6.13 and 6.10 (lg (CFU/g)) for total bacterial colonies; 6.08 and 6.09 (lg (CFU/g)) for LAB counts, respectively) exhibited significantly higher bacterial and LAB counts than the controls (5.83 (lg (CFU/g)) for total bacterial colonies; 5.81 (lg (CFU/g) for LAB counts) ($P < 0.05$). By contrast, samples treated with Arg (or Lys) combined with nisin (5.31 and 5.36 (lg (CFU/g)) for total bacterial colonies; 5.24 and 5.13 (lg (CFU/g)) for LAB counts, respectively) displayed notably lower bacterial and LAB counts than the controls ($P < 0.05$). The addition of Arg and Lys likely served as a rich nitrogen source for LAB growth and reproduction in sausages[35], resulting in increased microbial counts. Furthermore, nisin, a broad-spectrum antimicrobial agent, effectively inhibited LAB[36], leading to reduced microbial growth in the samples subjected to combined treatments. Thus, the experimental results offer an explanation for the changes in protein degradation products of sausage samples during storage.

3.4 AAD activity analysis

LAB were found to be the dominant microorganisms in all samples. Studies have shown that LAB secretes AAD, an enzyme that catalyzes the decarboxylation of amino acids to form biogenic amines[34]. Therefore, AAD activity was measured in the present study.

As shown in Figure 2, AAD activity gradually decreased in all samples over the storage period. This decline could be attributed to environmental conditions, such as a_w and pH value of the samples, might have been non-conducive for LAB proliferation, thus reducing AAD secretion (Table 1 and Figure 2). AAD may have gradually lost activity during prolonged storage[34].

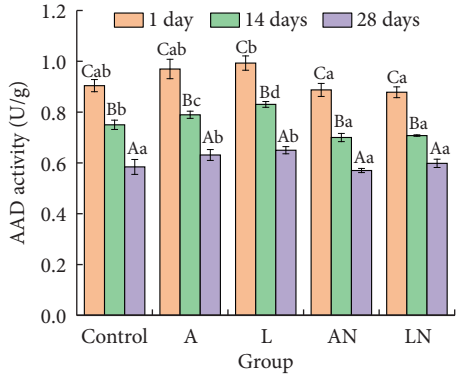

Figure 2 Effects of different treatments on AAD activity. Different lowercase letters indicate significant differences ($P < 0.05$) among the different treatments at the same storage time. Different capital letters in the same column indicate significant differences ($P < 0.05$) in different storage times for the same treatment group.

At the 28th day of storage, compared with the controls (0.58 U/g), AAD activity was higher in samples A and L (0.63 and 0.65 U/g, respectively) ($P < 0.05$). No significant differences in AAD activity were observed between samples A and L or between samples AN and LN ($P > 0.05$). The results suggest that these two amino acids provided a nitrogen source that promoted LAB growth and reproduction, thereby increasing AAD activity. Compared with the controls (0.58 U/g), AAD activity was almost equal in AN and LN groups (0.57 and 0.60 U/g, respectively), as nisin effectively inhibited LAB growth, thus reducing AAD secretion.

3.5 Correlation analysis

Figure 3 demonstrates that the correlations among sample indicators showed no appreciable variations from day 1 to 28 of storage. This stability indicates that the relationships among indicators in the sausage samples were robust across the storage period, thus effectively reflecting the regularity and underlying mechanisms governing TVB-N content variation.

On the first day of storage, TVB-N content was significantly positively correlated with biogenic amine content, LAB count, and

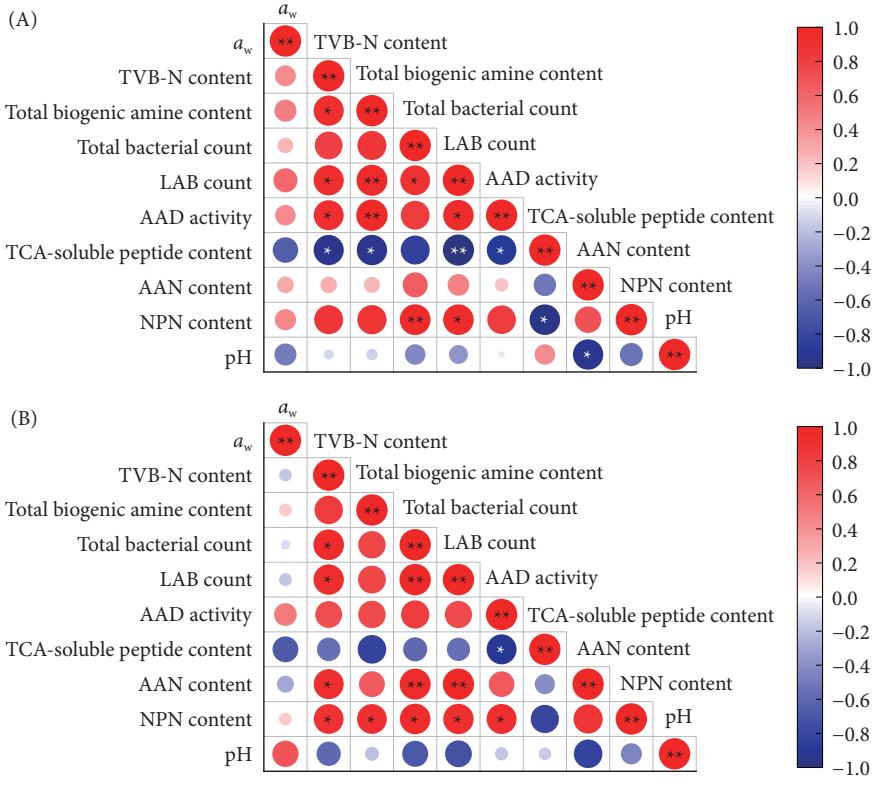

Figure 3 Correlation analysis of various measurement indexes of sausages with different treatments on the (A) 1st and (B) 28th day. *$P < 0.05$, **$P < 0.01$.

AAD activity, whereas it was negatively correlated with TCA-soluble peptide content. Biogenic amines were significantly positively correlated with LAB count and AAD activity, whereas negatively correlated with TCA-soluble peptide content. A significant positive correlation was observed between total bacterial count and LAB count ($P < 0.01$) as well as between total bacterial count and NPN content ($P < 0.05$). LAB count were positively correlated with AAD activity and NPN content but negatively correlated with TCA-soluble peptide content. AAD activity was negatively correlated with TCA-soluble peptide content, and TCA-soluble peptide content was negatively associated with NPN content. At last, AAN was negatively correlated with pH value.

These findings further demonstrated that Arg and Lys served as a nitrogen source for LAB growth and reproduction. This enhanced AAD secretion, thereby accelerating the decomposition of meat proteins and their derivatives. Conversely, the addition of nisin inhibited LAB growth and reproduction and AAD activity. This effectively suppressed the accumulation of volatile basic nitrogen and biogenic amines in the sausage samples. Thus, the addition of nisin improved the edible safety of the final sausage products.

4 Conclusion

Nisin effectively suppressed the degradation of meat proteins (as evidenced by the lower contents of NPN, AAN, TVB-N, and biogenic amines) in Cantonese sausage containing Arg/Lys, although causing the higher TCA-soluable peptides, thus enhancing their edible safety. This inhibition is attributed to nisin's antimicrobial action, which reduced LAB growth and reproduction (as evidenced by the lower total bacterial count, especially LAB) and decreased AAD secretion (as evidenced by the lower AAD activity). These findings provide technical support for the production of low-sodium and low-phosphorus Cantonese sausage. However, further research is required to evaluate whether nisin also effectively inhibits the Arg-/Lys-induced increase in TVB-N content of other meat products.

Conflict of interest

The authors declare no conflict of interest.

Data availability statement

The data presented in this study are available on request from the corresponding author.

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Declaration of animal subjects

All procedures for chevon and fat used in the work complied with relevant laws and institutional guidelines, and have been approved by the relevant institutional committees.

Authors contribution

Axiang Li: Data curation (lead); investigation (lead); methodology (lead); writing-original draft (lead). Xun Gao: Writing-review and editing (supporting). Yang Cheng: Writing-review and editing (supporting). Huihui Liang: Writing-review and editing (supporting). Cunliu Zhou: Conceptualization (lead); methodology (supporting); writing-review and editing (lead). Hongmei Fang: Funding acquisition (lead).

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