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Effect of reducing nitrite levels on the physicochemical, microbiological, proteolytic, and volatile profile of Cantonese sausage

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Abstract

The aim of the present work was to evaluate the effect of reducing nitrite content on the physicochemical, microbiological, proteolytic, and volatile properties of Cantonese sausages during fermentation and storage. The Cantonese sausages were divided into six groups based on the amount of nitrite added (0, 30, 60, 90, 120, and 150 mg/kg). Results showed that among the physicochemical parameters, moisture, weight loss, *a** value, thiobarbituric acid reactive substance value, and nitrite residue levels were significantly affected ($p < 0.05$) along with nitrite reduction. In addition, the total viable counts and Gram-positive cocci increased with the reduction of nitrite, while lactic acid bacteria decreased. The band densities of actin (48 kDa) increased with the reduction of nitrite, while no major change in sarcoplasmic protein bands was observed. The results of volatile compounds suggested that the reduction of nitrite mainly affected compounds originating from carbohydrate fermentation, esterase activity, and lipid oxidation.

Introduction

As one of the most important food additives, nitrite is widely used in meat products. Nitrite contributes considerably to the quality of meat products through the actions on colour, flavour, microorganisms, and lipid oxidation (EFSA, 2003; Alahakoon *et al.,* 2015). However, questions have been raised regarding the safety of nitrite since it can form the carcinogen N-nitrosamine *in vivo* (Wang *et al.,* 2013; Sallan *et al.,* 2020). Despite many studies being conducted, no ideal substitute for nitrite has been developed (Li *et al.,* 2013; Alahakoon *et al.,* 2015; Gou *et al.,* 2019). Historically, the permitted levels of nitrite for addition to meat products have continually decreased. To date, the maximum level of nitrite permitted to be added to cured meat products by the European Union (2011) is 150 mg/kg, which is the same as that permitted in China (NFSS, 2014). In addition, consumers also tend to purchase meat products with low nitrite content (Hung *et al.,* 2016).

The effect of reducing nitrite content on the quality of different meat products has been studied previously (Bozkurt and Erkmen, 2004; Thomas *et al.,* 2013; Hospital *et al.,* 2015; Cardinali *et al.,* 2018; Christieans *et al.,* 2018). In dry fermented sausages, the pH, water activity, moisture, colour parameters (*L**, *a**, *b**), and residual nitrite were all affected by the nitrate and nitrite content (Marco *et al.,* 2006; Hospital *et al.,* 2015; Perea-Sanz *et al.,* 2018; 2019). Moreover, the reduction of nitrate and nitrite levels in dry fermented sausages significantly affected the Gram-positive catalase-positive cocci and Enterobacteriaceae (Hospital *et al.,* 2015), but not coagulase-negative cocci (Cardinali *et al.,* 2018). In addition, the survival of *Listeria monocytogenes, Salmonella* spp., and *Clostridium botulinum* was also affected by reducing the concentrations of nitrate and nitrite (Hospital *et al.,* 2016; Christieans *et al.,* 2018).

Nitrite reduction affects not only the physicochemical parameters and microbial survival, but also the proteolytic and volatile profiles. Protein hydrolysis mainly involves myofibrillar and sarcoplasmic protein (Sun *et al.,* 2011a; Cheng *et al.,* 2021). The degree of hydrolysis is affected by many factors such as nitrite content (Feng *et al.,* 2016), enzymes (Zhang *et al.,* 2017), microbial species (Mauriello *et al.,* 2010), phenols (Cheng *et al.,* 2021), and protein types (Sun *et al.,* 2011a). Depending on the nitrite concentration, the effect of nitrite on

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protein oxidation can be promoted or inhibited (Feng *et al.,* 2016). Nitrite is essential for the development of the cured aroma in fermented meat products (Flores, 2018). As high as 25% reduction of nitrate increased the unpleasant volatile compounds, while 15% reduction produced pleasant volatile compounds (Perea-Sanz *et al.,* 2019). However, 25% reduction had a minor effect on microbial growth, and increased the volatile compounds derived from amino acid degradation (Perea-Sanz *et al.,* 2018).

Cantonese sausage is a traditional Chinese sausage famous for its unique flavour and attractive colour. Cantonese sausage is different from other dry fermented sausages due to its simple formula (containing only meat, salt, sugar, wine, soy sauce, and nitrite), high manufacturing temperature (45 - 50°C), and lack of the inoculation of starter cultures (Sun *et al.,* 2010). This renders nitrite as an important factor in preserving the Cantonese sausage quality characteristics. Therefore, the objective of the present work was to evaluate the changes in physicochemical, microbial, proteolytic, and volatile properties of Cantonese sausages during processing and storage with a gradient reduction in nitrite concentration.

Materials and methods

Materials

Fresh lean pork (73.6% moisture, 21.5% protein, 4.2% fat, and 1.1% ash) and back fat were obtained from the Jilin Huazheng Meat Processing Co. (Changchun, China). Salt (China National Salt Industry Group Co., Ltd.), sugar (Taikoo), soy sauce (Haitian), wine (Beijing Hongxing), and sodium nitrite (Sichuan Jinshan) were of food-grade, and purchased from a local supermarket.

Preparation of Cantonese sausage

Lean pork was ground through a 6 mm plate, and back fat was diced into 6 mm cubes. Cantonese sausage was prepared according to the following formulation: lean pork (80 g), back fat (20 g), salt (1.2 g), sugar (7 g), soy sauce (2.0 g), and wine (4.0 g). Sodium nitrite was added at different concentrations (0, 30, 60, 90, 120, and 150 mg/kg), and these samples were named N0, N30, N60, N90, N120, and N150, respectively. Each sample was produced in triplicate batches, and each batch size was 6 kg. After mixing, the mixture was stuffed into natural collagen casings with a diameter of 35 mm. Then, these were dried at 50°C for 3 h and 45°C for 69 h (Sun *et al.,* 2010). The dried Cantonese sausages were vacuum-packed and stored at 18 ± 2°C. Samples were periodically taken from the centre of the Cantonese sausages from each batch at 0, 1, 2, 3, and 30 d for further analyses.

Physicochemical parameters

pH was determined using a portable pH meter (Testo206, Germany). Water activity (a_w) was measured using a water activity meter (Rotronic, Switzerland) by weighing 1 g of sample (accurate to 0.001 g) into the sample dish, and response values were recorded under the conditions of 18 - 25°C and 50 - 80% relative humidity. Weight loss was determined by subtracting the initial sample weight at each stage. Moisture content was determined using a rapid moisture content analyser (HB43-S Halogen, Mettler Toledo, Switzerland). Colour differences of samples were determined using a HunterLab colorimeter (ColorFlex®, USA) with an Illuminant D65 10° observer. A white standard plate (L^* = 94.52, a^* = -0.86, and b^* = 0.68) was used for calibration. The degree of lipid oxidation was determined using thiobarbituric acid-reactive substances (TBARS), as described by Riazi *et al.* (2016). Briefly, 10 g of sample was mixed with 20 mL of trichloroacetic acid (10%, w/v), and homogenised. The homogenate was then centrifuged at 5,000 *g* for 30 min, and filtered using Whatman No.1 filter paper. The filtrate (2 mL) was mixed with 2 mL of thiobarbituric acid solution (300 mg thiobarbituric acid/100 mL distilled water), and kept in a water bath at 97°C for 20 min. Then, the absorbance of samples was measured at 532 nm using a spectrophotometer (TU-1901, Persee, China), and the results were expressed as mg malondialdehyde/kg meat sample. Residual nitrite content was determined according to the standard method (NFSS, 2016). All values were analysed in triplicates.

Microbiological analysis

Samples from each replicate (25 g) were homogenised with 225 mL of peptone water (0.1%, w/w) in a stomacher bag. The decimal dilutions were then prepared and spread on appropriate agar media. The colony count was expressed as log CFU/g. Total viable counts were enumerated on Plate Count agar incubated at 36°C for 48 h. Lactic acid bacteria were enumerated on de Man, Rogosa, and Sharpe agar incubated at 30°C for 72 h. Gram-positive cocci were enumerated on Mannitol Salt agar at 30°C for 3 - 5d and Baird-Parker agar at 37°C for 48 h. Enterobacteriaceae were enumerated on Violet Red Bile agar with glucose at 37°C for 48 h. *Listeria monocytogenes* were enumerated on Agar *Listeria* according to Ottaviani and Agosti (ALOA) incubated

at 37°C for 48 h. Yeasts and moulds were quantified on Rose Bengal agar incubated at 28°C for 5 d. All viable counts were enumerated in triplicate.

Electrophoretic analysis

To determine the alteration of sarcoplasmic and myofibrillar proteins in Cantonese sausages with nitrite reduction, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. The sarcoplasmic and myofibrillar proteins were extracted following the method of Mauriello *et al.* (2010). Briefly, 10 g of chopped Cantonese sausage samples were mixed with 100 mL of 20 mM phosphate buffer (pH 6.5), and homogenised for 3 min. The homogenate was centrifuged at 10,000 *g* for 20 min (4°C), and the supernatant filtered through a 0.22 μm membrane was defined as myosin. The obtained particles were resuspended in 30 mM of phosphate buffer (pH 7.4; containing 0.1% (v/v) Triton X-100), and homogenised for 2 min. The homogenate was centrifuged at 10,000 *g* for 20 min (4°C), and the particles were washed three times with the same buffer. The precipitate was then mixed with a 0.1 M phosphate buffer (pH 6.5) solution (containing 0.7 M KI) at a ratio of 1:9 (w/v). After homogenisation at 3,000 rpm for 8 min and centrifugation at 10,000 *g* for 20 min, the supernatant filtered with a 0.22 μm membrane was defined as myofibrillar protein. The protein concentration of all supernatants was determined using the Pierce™ BCA Protein Assay Kit (Catalogue Number 23225, Thermo Scientific, USA). Finally, 12% polyacrylamide gel was used to separate the protein bands, and 10 μL samples were added to each well.

Volatile compounds analysis

The volatile compounds in Cantonese sausages were analysed by headspace solid phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (Agilent 5975-6890N, Palo Alto, CA, USA), as described by Hospital *et al.* (2015) with minor modifications. Samples (4 g) were placed into 15 mL vials, and sealed with PTFE/silicon septum. After heating at 45°C for 30 min, the SPME fibre was exposed to the headspace at 50°C for 30 min. The absorbed compounds were desorbed at the injection port at 250°C in the splitless mode. The HP-INNOWax polyethylene glycol column (30 m × 0.25 mm × 0.25 μm, Agilent 19091N-133, Palo Alto, USA) was used to separate the molecules, and the temperature programs were 37°C for 30 min, then increased to 280°C at a rate of 4°C/min, and held for 30 min. The volatile compounds were identified by comparison with the mass spectra in the NIST'08 database (National Institute of Standards and Technology, MD, USA), and linear retention index (LRI) values of authentic standards or literature. Quantification was determined by the total extracted area, and each of the results was presented as the mean of three different replications.

Statistical analysis

All data were expressed as mean ± standard deviation. The SPSS Statistics software was used to analyse the experimental data with one-way ANOVA, followed by Duncan's multiple range tests. $p < 0.05$ was considered significant, and $p < 0.01$ was considered extremely significant.

Results and discussion

pH, aw, moisture, and weight loss

The pH values decreased considerably during 72 h of processing, but no significant differences ($p > 0.05$) were observed in the six experimental groups on day 30 (Figure 1A). The a_w decreased rapidly and increased slightly during the storage period (Figure 1B). Although there were no significant differences ($p > 0.05$), the higher nitrite concentration corresponded to the faster decline rate of a_w. Interestingly, this result was also found in other dry-fermented sausages (Hospital *et al.,* 2015; Christieans *et al.,* 2018; Perea-Sanz *et al.,* 2019). The moisture content of the samples was negatively correlated with the nitrite concentration (Figure 1C). With the increase in nitrite levels, the moisture content of the Cantonese sausages gradually decreased, which suggested that the water loss rate increased. Weight loss decreased sharply during the drying process, and stabilised after 72 h (Figure 1D). The relationship between nitrite addition and weight loss was not strictly linear. When the additive amount was 150 mg/kg, the weight loss of Cantonese sausage was the highest (59.79 ± 2.63%), while at 90 mg/kg, weight loss was the lowest (56.68 ± 3.20%). In addition, the four typical evolution curves (pH, a_w, moisture, and weight loss) of the Cantonese sausage did not change with nitrite reduction, which indicated that these parameters were mainly affected by the processing technology.

Colour, lipid oxidation, and nitrite residue

During processing and storage, the L^* and b^* values of all groups decreased significantly ($p < 0.05$) regardless of the amount of nitrite added, while the a^* values increased gradually. In addition, the a^*

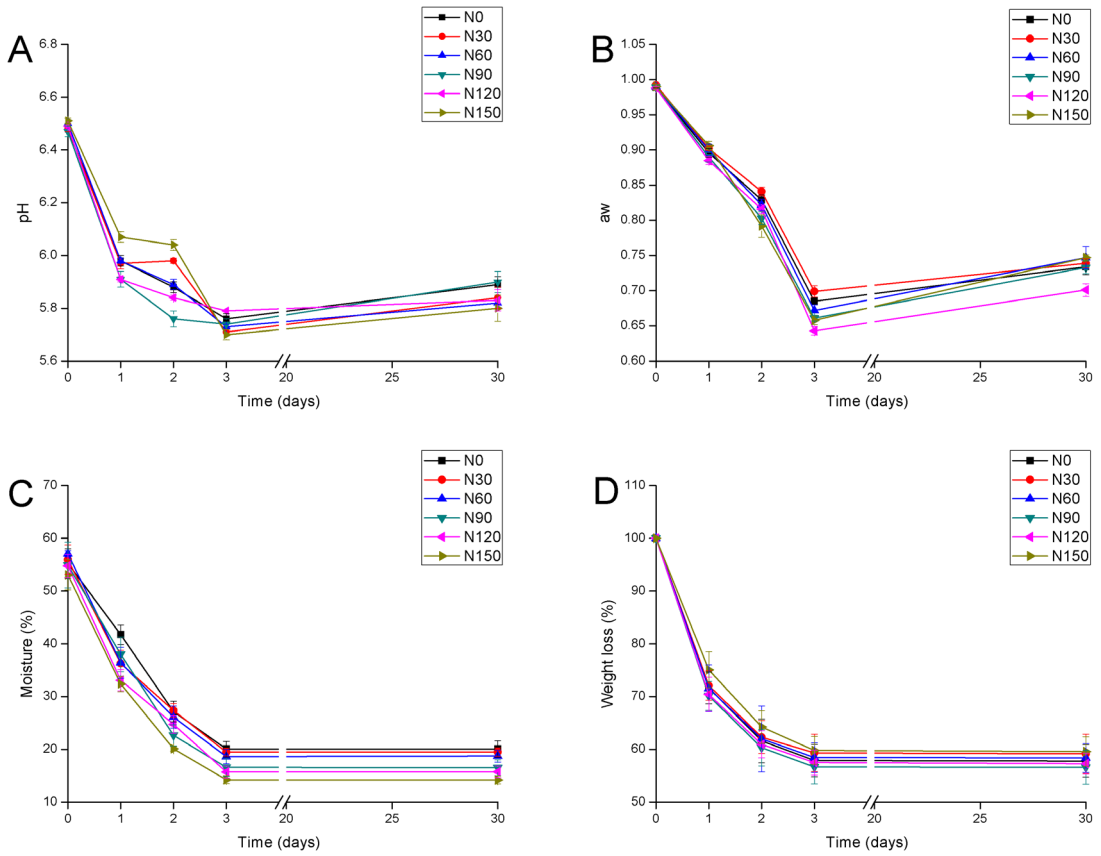

Figure 1. Effect of nitrite concentrations (0, 30, 60, 90, 120, and 150 mg/kg) on the parameters of pH (A), a_w (B), moisture (C), and weight loss (D) during the processing and storage of Cantonese sausage.

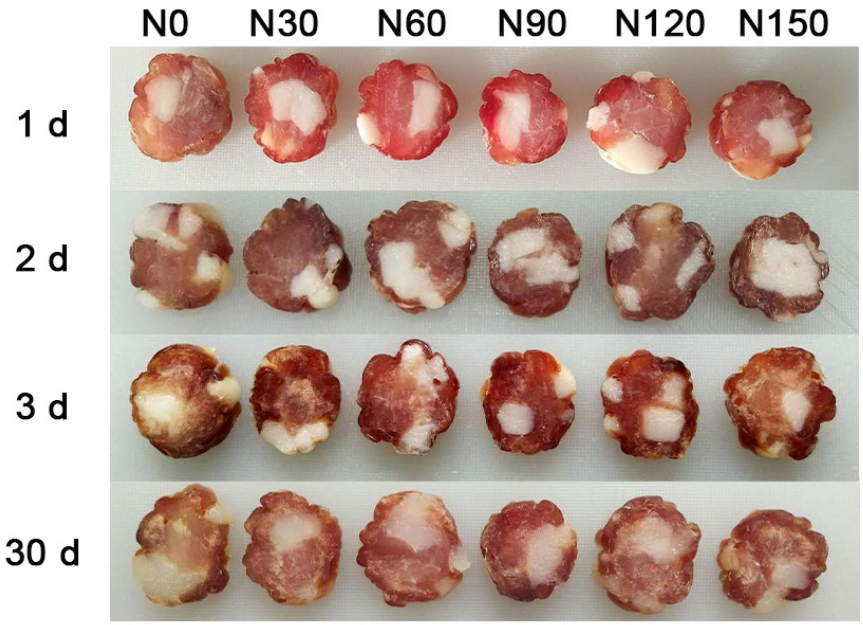

Figure 2. The colour of Cantonese sausages in different experimental groups during processing (1, 2, and 3 d) and storage (30 d).

values significantly decreased with the reduction of nitrite, which is in accordance with the results of Song *et al.* (2015) and Perea-Sanz *et al.* (2019). Nitrosomyoglobin, a characteristic of reddish pigment, is responsible for these results (Li *et al.,* 2013). Moreover, these colorimetric results were in good accordance with the visual colour effects presented in Figure 2.

TBARS values increased with the reduction of nitrite, indicating the antioxidant activity of nitrite. Antioxidant activity is predominantly attributed to the potential of nitrite to form nitric oxide (Alahakoon *et al.,* 2015). In addition, Al-Shuibi and Al-Abdullah (2002) reported that the lowest nitrite level to work was 40 mg/kg. Feng *et al.* (2016) also suggested that 50 mg/kg nitrite significantly inhibited lipid oxidation in sausages ($p < 0.05$). In fact, the concentration of sodium nitrite, which can inhibit lipid oxidation, is affected by other additives such as sodium ascorbate (Berardo *et al.,* 2016). In the present work, 30 mg/kg of nitrite addition in Cantonese sausage significantly inhibited lipid oxidation as compared to that of the control group N0 (except day 0). Results also showed that the addition of 90 mg/kg nitrite produced a very good effect of inhibiting lipid oxidation, which was comparable to that of 120 and 150 mg/kg.

After 72 h of processing, the nitrite residue level in the N90 group (13.01 mg/kg) was the lowest, except for the control group; while those of the N60 and N150 groups were the highest (16.25 mg/kg). Residual levels of nitrite are affected by many factors including pH, storage temperature, heat process, and addition of reducing agents (EFSA, 2003). In addition, no direct relationship was found between the residual level of nitrite and the initial level added (EFSA, 2003). The lower pH value of the product, higher storage temperature, higher heat treatment temperature, and addition of reducing agents all contributed to the reduction of nitrite residue (EFSA, 2003). Within 48 h of Cantonese sausage processing, the pH of the N90 group decreased the fastest, while that of the N150 group decreased the slowest; pH may be the main reason for the difference in nitrite residue levels. After 30 days storage, the nitrite residue decreased significantly ($p < 0.05$) in the N30, N60, N90, and N150 groups. The reduction of nitrite residues was also observed in other dry fermented sausages during storage (Hospital *et al.,* 2015; Christieans *et al.,* 2018), and nitrate reductase/nitrite reductase activity was the major contributor (Papamanoli *et al.,* 2002).

Microbiological quality

The total viable counts decreased with increasing nitrite concentration (Figure 3A), which

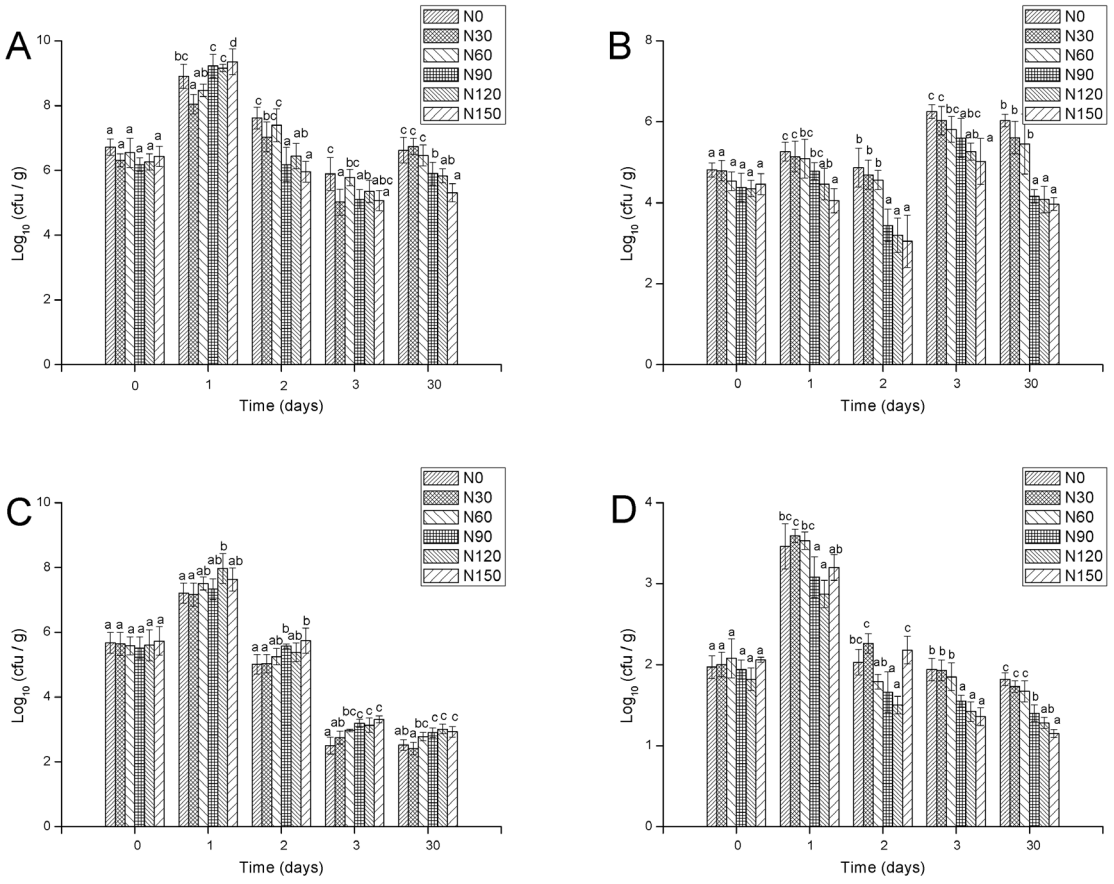

Figure 3. Effect of nitrite concentrations (0, 30, 60, 90, 120, and 150 mg/kg) on the growth of total viable counts (A), micrococci (B), lactic acid bacteria (C), and yeast and mould count (D) during the processing and storage of Cantonese sausage.

suggested the antibacterial effect of nitrite (Alahakoon *et al.,* 2015). Similarly, Cardinali *et al.* (2018) proved that a high nitrite concentration could reduce the microbial diversity of Fabriano-like fermented sausages. It should also be noted that the total viable count in Cantonese sausage (72 h) was 5.37 log CFU/g, which was lower than that (6.5 - 7.6 log CFU/g) reported by Perea-Sanz *et al.* (2019) and that (approximately 9.0 log CFU/g) obtained by Hospital *et al.* (2015). This could be attributed to the high manufacturing temperature, non-inoculation of the starter cultures, and limited fermentation of Cantonese sausage.

Micrococci, lactic acid bacteria, and yeasts and moulds were the typical microbiota in Cantonese sausages (Figure 3B, 3C, and 3D). Several studies have reported on the effect of nitrate and nitrite concentration on the microbiological characteristics of dry fermented sausages, demonstrating the inhibition of nitrate and nitrite against Gram-positive cocci (Hospital *et al.,* 2015; Christieans *et al.,* 2018; Perea-Sanz *et al.,* 2019). However, regarding the effect of nitrate and nitrite concentration on lactic acid bacteria, study results have not been entirely consistent. Marco *et al.* (2006) observed that dry fermented sausages supplemented with nitrate had a higher population of lactic acid bacteria than sausages with added nitrite, while Perea-Sanz *et al.* (2019) reported that 25% sodium nitrate reduction (187.5 mg/kg) had no influence on the counts of lactic acid bacteria. In addition, when nitrate and nitrite were used alone or in combination (with different concentration gradients), no significant changes in lactic acid bacteria were observed (Christieans *et al.,* 2018). However, other studies concluded that nitrite had a promotional effect on lactic acid bacteria, although there was no significant difference (Hospital *et al.,* 2015; Cardinali *et al.,* 2018). In the present work, the promotional effect of nitrite could be explained by the relatively lower amount of lactic acid bacteria, since they were the dominant flora in other dry fermented sausages, except for the Cantonese sausage (Cardinali *et al.,* 2018). Regarding the effect of nitrite on yeasts and moulds, the promotional effect is supported by a study by Bozkurt and Erkmen (2004).

Overall, the reduction in nitrite content affected the microbial community of Cantonese sausages. When the content changed considerably, this effect was significant. Therefore, to ensure the flavour of Cantonese sausages, 90 mg/kg was selected because its microbiological quality was close to that of 150 mg/kg. For dry fermented sausage, nitrate and nitrite reduction did not affect the microbial growth, but affected their metabolic activity (Perea-Sanz *et al.,* 2020). Dry fermented sausage and Cantonese sausage differ from each other in terms of the production process and formula; thus, their microbial groups are also different.

Listeria monocytogenes and Enterobacteriaceae were only investigated in the final product (3 and 30 d), and were not detected, thus suggesting good hygiene practices. However, microbial food safety involves many microorganisms in fermented sausages, such as *L. monocytogenes, Salmonella spp., Escherichia coli*, and *Staphylococcus aureus* (Gürbüz and Çelikel Güngör, 2020). The reduction of nitrite may cause the presence of potentially dangerous *Salmonella* spp. (Christieans *et al.,* 2018). In fact, the monitoring of nitrite residues in products is of limited value. In particular, the level of nitrite residue does not indicate an inhibitory effect of the product on microorganisms, especially *Clostridium botulinum* (EFSA, 2003). Therefore, to guarantee microbial safety, the reduction of nitrite can be combined with antibacterial techniques such as supercritical carbon dioxide (Jauhar *et al.,* 2020) or some natural antibacterial ingredients from plants (Ijabdeniyi *et al.,* 2019). Therefore, the relationship between reducing nitrite content and the microbial safety of Cantonese sausage should be studied in more detail in the future.

Proteins

Myofibrillar and sarcoplasmic proteins analysed by SDS-PAGE are presented in Figure 4. The reduction of nitrite had no significant effect on the 63, 35, and 17 - 25 kDa band densities of myofibrillar protein, but the 48 kDa band density of actin increased with the decrease in nitrite addition (Figure 4A). In addition, bands near 140 kDa were observed in groups with low nitrite addition. The 140 kDa band might have been derived from the hydrolysis of myosin heavy chain during processing (Candogan *et al.,* 2009). The protein bands near 105, 75, 35, and 10 kDa might have been the rupture of hydrophobic interactions of myosin and actin (Candogan *et al.,* 2009). Hydrogen bonds, hydrophobic interactions, disulphide bonds, and ionic bonds all contribute to the three-dimensional network structure of proteins (Sun *et al.,* 2011a). Similar distribution patterns of myofibrillar protein bands were obtained from Cantonese bacon manufactured with flavourzyme (Zhang *et al.,* 2017), and cooked sausages containing different concentrations of sodium nitrite (Feng *et al.,* 2016). The results indicated that the hydrolysis of Cantonese sausages occurred during processing. Moreover, the

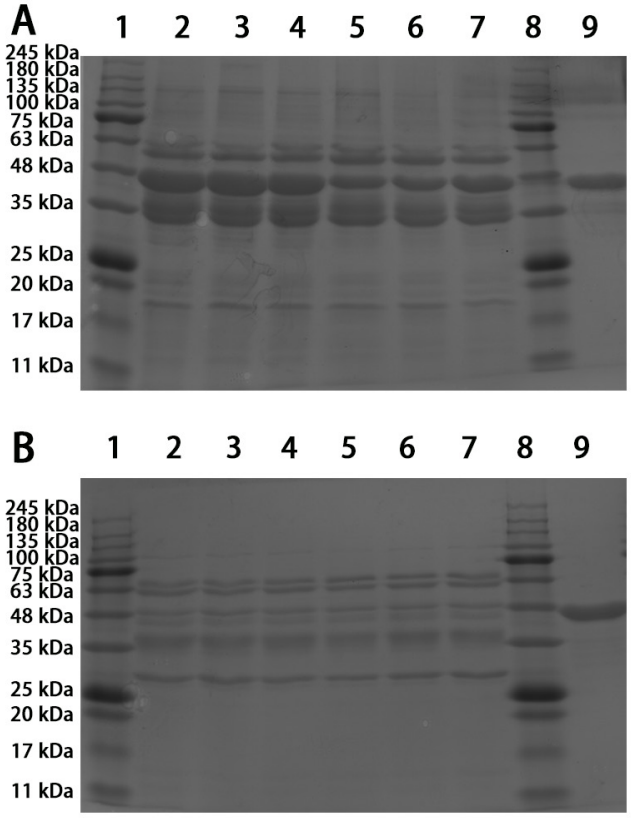

Figure 4. SDS-PAGE of myofibrillar proteins (a), and sarcoplasmic proteins (b) from different sausage samples. Lanes 1 and 8: molecular weight markers (245, 180, 135, 100, 75, 63, 48, 35, 25, 20, 17, and 11 kDa); lane 2: N0 group; lane 3: N30 group; lane 4: N60 group; lane 5: N90 group; lane 6: N120 group; lane 7: N150 group; and lane 9: actin.

amount of nitrite added affected the hydrolysis process, and the group with lower nitrite content tended to have a higher degree of myofibrillar protein hydrolysis. Feng *et al.* (2016) supported the conclusion that the intensity of myosin heavy chain and actin was dose-dependent with nitrite, and the bands of actin and myosin were wider at low nitrite dosages. The proteolytic susceptibility of Cantonese sausages was influenced by the degree of protein oxidation (Sun *et al.,* 2011a), while protein oxidation was common in dry fermented sausages (Candogan *et al.,* 2009; Mauriello *et al.,* 2010; Sun *et al.,* 2011b; Berardo *et al.,* 2016). Protein oxidation during processing results in an increase in the carbonyl and S-S groups, and a decrease in the SH group (Sun *et al.,* 2011a; 2011b; Cheng *et al.,* 2021). The increase in nitrite concentration could inhibit the oxidation of this protein (Feng *et al.,* 2016). Moreover, phenols could also inhibit the myofibrillar protein oxidation of Cantonese sausages in different ways (Cheng *et al.,* 2021). The electrophoretogram of sarcoplasmic proteins of Cantonese sausages is shown in Figure 4B. However, no major change in the sarcoplasmic protein bands was observed with nitrite reduction. The low molecular weight bands (65 and 35 kDa) of the sarcoplasmic proteins were produced by proteolysis (Zhang *et al.,* 2017), and hydrolysis was identified by proteomic methods (Picariello *et al.,* 2006). Moreover, the SDS-PAGE profile of sarcoplasmic proteins was changed by the addition of flavourzyme. Candogan *et al.* (2009) and Mauriello *et al.* (2010) also reported notable changes in sarcoplasmic protein patterns by inoculating different starter cultures. The results demonstrated that nitrite affected protein hydrolysis, mainly depending on the activity of endogenous and microbial enzymes (Picariello *et al.,* 2006).

Volatile compounds

A total of 75 volatile compounds were identified by HS-SPME, and classified based on their origin. These results agree with those of Sun *et al.* (2010), and most of the compounds had very similar contents. These results also showed that the types and contents of these volatile compounds were quite different from those of other dry fermented sausages (Tabanelli *et al.,* 2013; Coloretti *et al.,* 2014). In Milano-type dry fermented sausages, aldehydes were the main components, and accounted for approximately 60% of the total volatile compounds (Tabanelli *et al.,* 2013). In the Cantonese sausages, ethanol was the most abundant volatile compound, accounting for 61.63 - 70.92% of the total. A large amount of ethanol was added to produce Cantonese sausage, which is also a unique feature of Chinese-style sausage. Ethanol not only has a unique flavour, but also has an inhibitory effect against undesirable microflora (Coloretti *et al.,* 2014). Even without the addition of ethanol, the sausage could still produce ethanol due to microbial fermentation (Hospital *et al.,* 2015). In the determination of volatile compounds, the added ethanol and ethanol produced by microbial fermentation were calculated together for the total content. Therefore, without considering the large amount of ethanol added artificially, the contents of volatile compounds in Cantonese sausage were in the order of esterase activity > carbohydrate fermentation > lipid oxidation > amino acid degradation > lipid β oxidation.

With the decrease in nitrite addition, the content of compounds originating from carbohydrate fermentation decreased, while the compounds originating from esterase activity and total lipid oxidation increased. Carbohydrate fermentation is mainly a function of lactic acid bacteria, which

produce acids that contribute to the sourness of meat products (Flores, 2018), and provide part of the substrate for ester formation. The promotional effect of nitrite on lactic acid bacteria might explain the decrease in carbohydrate fermentation (Figure 3C). However, esterase activity was overestimated because some esters were not produced by microbial activity but by direct esterification of acids and alcohols at higher production temperatures and longer drying times (Sun *et al.,* 2010). The esterase activity of staphylococci affects ester formation, while nitrite inhibits the growth of *Staphylococcus* (Hospital *et al.,* 2015; Christieans *et al.,* 2018). Therefore, the reduction in nitrite content resulted in the decrease of esterase-derived compounds. In addition, the levels of ethyl hexanoate were the highest of all esters in Cantonese sausages, followed by ethyl hexadecanoate and ethyl benzoate. However, ethyl acetate was the most abundant ester for other dry fermented sausages (Marco *et al.,* 2006; Perea-Sanz *et al.,* 2019). Nitrite can inhibit the oxidation of lipids in sausages (Alahakoon *et al.,* 2015; Berardo *et al.,* 2016), which might explain the increase in lipids caused by the reduction of nitrite. Aldehydes were the most interesting lipid-derived volatiles, and had a wide aroma contribution and a low threshold (0.01 - 0.1) (Flores, 2018). The inhibition of nitrite on aldehydes has also been reported in previous studies (Thomas *et al.,* 2013; 2014), but this may be due to the change in the sulphide compound. In the present work, sulphides were not detected because of the small amounts of spices used.

The relationships between the physicochemical parameters and volatile compounds were evaluated by principal component analysis. Two principal components explained 51.5% of the total variability. PC1 accounted for 36.8% of the variability, and distinguished samples by the nitrite addition amounts, as seen from right to left. Most of the volatile compounds (except ethyl nonanoate, ethyl pentanoate, ethyl hexanoate, ethyl 2-methylbutyrate, ethyl lactate, ethyl butanoate, ethyl acetate, and ethanol), physicochemical parameters (except residual nitrite contents), and microbiological parameters (except lactic acid bacteria counts) were located in the positive part of PC1, thus suggesting that the lower amount of nitrite (0 - 60 mg/kg) had a significant effect on these parameters of Cantonese sausages. The higher amounts of nitrite (90 - 150 mg/kg) were located in the negative part of PC1, thus indicating their lower effect on the flavour of Cantonese sausage. These results are consistent with the findings that the optimal addition amount for nitrite should be 90 mg/kg.

Conclusion

The changes in physicochemical, microbial, proteolysis, and volatile profiles of Cantonese sausages with different nitrite amounts added during processing and storage were defined. Nitrite reduction greatly affected the microbial and volatile properties, while the physicochemical and proteolytic properties were less affected. In conclusion, 90 mg/kg of nitrite was a suitable choice for Cantonese sausage to allow reduced nitrite levels, and ensure product quality. Further studies regarding the sensory and overall acceptability are necessary before commercial application.

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