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Research Article

Changes in Lipase and Antioxidant Enzyme Activities during Processing of Cantonese Sausage with D-Sodium Erythorbate

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Pork was used as raw material to produce Cantonese sausage, with additions of 0.05% or 0.1% D-sodium erythorbate. The oxidation indices (peroxide value, TBARS value, carbonyl value, and conjugated dienes value) and enzyme activity (total phospholipase, acid lipase, neutral lipase, superoxide dismutase, and glutathione peroxidase) were measured in the sausages at different processing periods. The results showed slowed lipid oxidation in the presence of D-sodium erythorbate, inhibition of total phospholipase, acid lipase, and neutral lipase activities, and increased superoxide dismutase activity, with little change in glutathione peroxidase activities. When increasing D-sodium erythorbate, the superoxide dismutase activity was negatively correlated with the peroxide value at 5 h ($P < 0.01$), the neutral lipase activity was positively correlated with the conjugated dienes value at 15 h ($P < 0.01$), and the total phospholipase activity was positively correlated with the peroxide value at 30 h ($P < 0.01$). This study explored the antioxidant effect of D-sodium erythorbate and provides a theoretical foundation to improve the quality of Cantonese sausage.

1. Introduction

Cantonese sausage is a traditional Chinese semidry sausage that is made in Guangdong Province, a region in the south of China. Cantonese sausage can be fancy grade, grade one, or grade two depending on the ratio of lean pork to pork back fat, with ratios of 8 : 2, 7 : 3, and 6 : 4, respectively [1]. Lipids in Cantonese sausage play an important role in the quality of the product. The unique flavor of this sausage is mainly derived from lipid degradation and lipid oxidation [2]. Proper lipid oxidation promotes flavor formation, but excessive lipid oxidation causes the formation of unpleasant flavor compounds [3], which can seriously affect the quality of the sausage. Therefore, the control of excessive lipid oxidation is a key goal of sausage processing.

Lipid oxidation can be controlled by vacuum-packaging or by adding antioxidants. Ascorbic acid is a commonly used antioxidant in food systems as an oxygen scavenger (e.g., 3.5 mg of ascorbic acid will scavenge the oxygen within in $1\,cm^{-3}$ of headspace). In addition, the metal-sequestering activity of ascorbic acid provides antioxidant activity, by the formation of metal-ascorbate complexes that are less reactive with oxygen than with metal ions alone [4].

Sodium erythorbate (sodium isoascorbate) is an optical isomer of sodium ascorbate. It lacks the physiological activity of sodium ascorbate, but has a higher antioxidant activity than that of sodium ascorbate. Because its production cost is only 1/3 that of sodium ascorbate, it is widely used in the production of meat products. The application of sodium erythorbate in meat products has been studied by various researchers. Xu et al. [5] studied the effect of different concentrations of sodium erythorbate on the peroxide value of sausage, and the results showed that 650 mg/kg reduced 90% of the peroxide value. Choe et al. [6] found that for a storage period of 12 days, the peroxide value and degree of lipid oxidation as measured by the 2-thiobarbituric acid extraction (TBARS value) of pork pie were effectively inhibited by addition of sodium erythorbate.

Anjum et al. [7] observed that flax seed oil resulted in an increase in the superoxide dismutase (SOD) activity of Broiler Serum, from 2.80 to 3.22 (50% pyrogyllol auto-oxidation/min/mg) and increased glutathione peroxidase

(GSH-Px) activity, from 2.33 to 3.23 (nmol NADPH/min/mg protein). Zhao et al. [8] observed that SOD activity of the longest muscle increased from 5.84 to 6.3 U/mg tissue by adding 5% of wine grape residue to lamb feed, and SOD activity of the sample was only 6.17 U/mg tissue when 10% of wine grape residue was added.

Most studies on the effects of antioxidants on lipase and antioxidant enzymes in meat products have reported similar effects to those reported by Anjum et al. [7] and Zhao et al. [8]. Animals were fed with antioxidants for a period of time and slaughtered, and then the lipase and antioxidant enzymes of the animals were analyzed. However, few researchers have focused on the direct addition of sodium erythorbate to meat to make meat products, with analysis of the changes of lipase and antioxidant enzymes. The main objective of this study was to investigate the effects of D-sodium erythorbate on the activity of lipase and antioxidant enzymes during the processing of Cantonese sausage and analyze the correlation of oxidation indices and enzyme activities, providing a theoretical foundation to improve the quality of Cantonese sausage.

2. Materials and Methods

2.1. Preparation of Cantonese Sausage. Fresh pork was purchased from a local supermarket and then chilled for 24 h at 4°C in a refrigerator. The raw pork was then cut into piece of 8–10 mm, and the ratios of lean pork and pork back fat were 8 : 2. The ingredients for the sausage include sugars (5 g/100 g pork), salt (2 g/100 g pork), raw soy sauce (5 g/100 g pork), Chinese liquor (3 g/100 g pork), nitrate (10 mg/100 g pork), and water (15 g/100 g pork). Next, sodium erythorbate was added to the mixture at 0%, 0.05%, or 0.1% (DSE-0, DSE-0.05, or DSE-0.1, respectively). The mixture was then mixed evenly and stuffed into natural pork casing of variable diameters. The sausages hung vertically in drying chambers (50°C) for 72 h. The index values were measured at 0 h, 5 h, 10 h, 15 h, 20 h, 30 h, 48 h, and 72 h.

2.2. Peroxide Value (PV) Assay. The PVs of all samples were determined according to the AOAC method (AOAC, 965.33) and expressed in milliequivalent (meq) peroxide per kg of sample [9].

2.3. Thiobarbituric Acid Reactive Substances (TBARS) Assay. Lipid oxidation was measured by the 2-thiobarbituric acid extraction method of Vyncke [10]. TBARS values were calculated based on the absorbance of each sample at 532 nm and a standard curve (8–50 nmol) of malondialdehyde (MDA) that was constructed using freshly prepared MDA by the acidification of 1,1,3,3-tetraethoxypropane. TBARS values were expressed as mg MDA/kg sausage.

2.4. Carbonyl Value and Conjugated Dienes Value Assay. The carbonyl value and conjugated dienes value were determined according to the method of Klein [11] with slight modifications. First, 2 g of meat sample was shredded and added into 40 ml of a 2 : 1 mixture (v/v) of trichloromethane/methanol, the mixture was homogenized for 60 s at 4000 rpm using an ultrahomogenizer (T25, IKA, Germany). The homogenate was then allowed to stand for 1 h and then passed through a layer of filter. Next, 8 ml 0.7% NaCl was added, and the reaction was oscillated and stated for 3 h. The lower liquid was steamed in a rotary evaporator (R202-2, Heidolph, Germany) in a 44°C water bath. The obtained oil was dissolved in cyclohexane and diluted to 200 ml/L. Absorbance was recorded at 215 nm, 232 nm, and 275 nm using a UV/Vis spectrophotometer (SP-2102UV, Spectrum, China). The A_{275}/A_{215} value corresponded to the carbonyl value, and the A_{232}/A_{215} value corresponded to the conjugated dienes value.

2.5. Preparation of Crude Enzyme Solution and Determination of Enzyme Activity. Five grams of minced muscles were homogenized in 25 ml of 50 mM phosphoric acid buffer, pH 7.0, containing 5 mM of EGTA. The mixture was homogenized at 25,000 rpm using an ultrahomogenizer (4×10 s, cooling with ice) and then stirred for 30 min in a cool bath. The homogenates were centrifuged at 4°C and 10,000 g for 20 min (CTH 2050R, Thermo Fisher, USA) and then filtered through glass wool. The filtrate was then subjected to enzyme assays [12].

2.6. Assay of Total Phospholipase Activity. Total phospholipase activities were assayed according to previously described methodologies [13] and described briefly below. First, 0.1 ml of muscle extract was diluted with 2.8 ml of 0.1 M disodium phosphate/0.05 M citric acid buffer, pH 5.0, containing 0.05% (w/v) Triton X-100, 0.15 M sodium fluoride and 0.8 mg/ml bovine serum albumin (BSA). To this mixture, 0.1 ml of 1.0 mM 4-methylumbelliferyl-oleate (Sigma) was added as substrate. After incubation at 37°C for 30 min, the reaction was stopped with the addition of 0.5 ml of 1 M·HCl, and the fluorescence was monitored at $\lambda_{ex} = 328$ nm and $\lambda_{em} = 470$ nm using a fluorescence spectrophotometer (RF-5301, SHIMADZU, Japan).

2.7. Acid Lipase Activity and Neutral Lipase Activity. Muscle acid and neutral lipase activities were assayed as described in [3] and as described below.

2.7.1. Acid Lipase. To assay acid lipase, 0.1 ml of muscle extract was diluted with 2.8 ml of 0.1 M disodium phosphate/0.05 M citric acid buffer, pH 5.0, containing 0.05% (w/v) Triton X-100 and 0.8 mg/ml bovine serum albumin (BSA). To this mixture, 0.1 ml of 1.0 mM 4-methylumbelliferyl-oleate (Sigma) was added as substrate. After incubation at 37°C for 30 min, the reaction was stopped with 0.5 ml of 1 M HCl and the fluorescence was monitored at $\lambda_{ex} = 328$ nm and $\lambda_{em} = 470$ nm.

2.7.2. Neutral Lipase. To measure neutral lipase, 0.1 ml of muscle extract was diluted with 2.8 ml of 0.22 M Tris/HCl

buffer, pH 7.5, containing 0.05% (w/v) Triton X-100. To this mixture, 0.1 ml of 1.0 mM 4-methylumbelliferyl-oleate (Sigma) was added as substrate. After incubation at 37°C for 30 min, the incubated samples were immediately cooled in an ice-water mixture and then measured within a minute. The fluorescence was monitored at $\lambda_{ex} = 328$ nm and $\lambda_{em} = 433$ nm.

2.8. Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px) Activities. Superoxide dismutase (SOD) activity and glutathione peroxidase (GSH-Px) activity were determined using the corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu Province, China) according to the instructions of the manufacturer.

2.9. Statistical Analysis. All the data are expressed as means ± standard deviations of triplicate determinations. The statistical package SPSS 16.0 was used for one-way ANOVA. The statistical differences between treatments were determined by Tukey's multiple range test, and the significance was established at $P < 0.05$. In addition, the Pearson's two-tailed correlation analysis was performed to assess the relationship between oxidative index and enzyme activities.

3. Results and Discussion

3.1. Changes of PV in Cantonese Sausage Processing. Peroxide value (PV) is used to evaluate the amount of hydrogen peroxide produced by lipid oxidation, which could form primary oxidation products. As shown in Table 1, PV of Cantonese sausage with DSE-0 significantly increased ($P < 0.05$) at 0–10 h and then decreased in 15–72 h. This may reflect the accumulation of hydroperoxide of 0–10 h changed into small molecules,with increased TBARS value, which is consistent with Qiu et al. [14]. Choe et al. [6] observed reduced PV in pork with addition of 0.05% of ascorbic acid, resulting in a dramatic and continual increase after 12 days or storage. Compared with samples prepared with only 0% of ascorbic acid, PVs of samples prepared with 0.05% of ascorbic acid were lower, indicating that ascorbic acid played a sustained antioxidant role in the oxidation of lipid. In our present study, PVs of samples with DSE-0.05 decreased without significant differences ($P > 0.05$) from 0 h to 10 h and increased without significant differences ($P > 0.05$) from 15 h to 72 h. This PV change trend of samples with DSE-0.1 was similar to the trend for samples with DSE-0.05, but lower than those with DSE-0.05 which is probably due to higher concentration.

3.2. Changes of TBARS Value in Cantonese Sausage Processing. Secondary oxidation products of meat products are usually reflected by the determination of TBARS value, and the TBARS value is expressed as the amount of malondialdehyde (MDA). Wood et al. [15] predicted that meat would have a sour taste when the concentration of MDA exceeded 0.5 mg/kg, but Ferreira et al. [16] forecasted meat would have a bad flavor when the concentration of MDA was over 2–3 mg/kg. As shown in Table 1, the TBARS value of all the Cantonese sausage in our present study did not exceed 2 mg/kg. With increased processing time, the TBARS value of samples with DSE-0 increased significantly ($P < 0.05$), and samples with DSE-0.05 and DSE-0.1 increased as well, though to a lesser extent as samples with DSE-0.

3.3. Changes of Carbonyl Value and Conjugated Dienes Value in Cantonese Sausage Processing. The carbonyl values of DSE-0 (Table 1) increased initially (0–5 h) and thereafter decreased during the rest stage (10–72 h), possibly due to formation of ester compounds and some volatile molecules that are produced by organic acids and alcohols in 0–5 h. The carbonyl values of sausages with DSE-0.05 increased at the early stage (0–5 h) and thereafter decreased at the rest stage (10–72 h). Compared with the sausages with DSE-0, there were lower carbonyl values with DSE-0.05 for each treatment (except 30 h), indicating that D-sodium erythorbate acted as an antioxidant role in the oxidation processing of Cantonese sausage. The carbonyl value at 5 h with DSE-0.1 increased without significant differences ($P > 0.05$) and decreased significantly ($P < 0.05$) at the rest stage.

The conjugated dienes values reflect the number of conjugated double bonds formed in the initial stage of oxidation of unsaturated fatty acids. The number of conjugated dienes decreased significantly ($P < 0.05$) with prolonged processing time (Table 1), possibly due to the conversion of diene to other substances. Compared with samples with DSE-0, the conjugated dienes values of DSE added were lower, exhibiting effective inhibition of lipid oxidation by the addition of DSE. Compared with the sausages prepared with DSE-0.05, the conjugated dienes values of DSE-0.1 decreased without significant differences ($P > 0.05$) in 5–15 h but increased without significant differences ($P > 0.05$) at 30 h.

3.4. Changes of Total Phospholipase Activity in Cantonese Sausage Processing. The formation of Cantonese sausage flavor is mainly dependent on lipids. During the processing period of Cantonese sausage, lipids undergo two processes: lipid degradation and lipid oxidation. Lipid degradation is the processing of glycerides into diesters, monoglycerides, and free fatty acids. Lipase was thought to play an important role in lipid degradation. These enzymes can be derived from muscle, adipose tissue, or microorganisms [17].

Microorganisms have little influence on lipid degradation, as shown by Montel et al. [18] and Johansson et al. [19]. Montel et al. [18] found that the content of free fatty acids in sausages prepared under sterile conditions were only slightly lower than those in the sausages with fermentation using microorganisms. Johansson et al. [19] found that the addition of antibiotics to dry fermented sausages could not reduce lipid degradation, and the lipid degradation of dry fermented sausage was mainly derived from the lipase and phospholipase activity of pork itself.

Toldrá and Flores [20] determined lower lipase activity of adipose tissue than the lipase activity of muscle in dry

Table 1: Changes of oxidation indices in Cantonese sausage processing.

		Peroxide value (meq/kg)	TBARS (mg MDA/kg sausage)	Carbonyl value	Conjugated dienes value
0 h	0% DSE	0.171 ± 0.015^{Acd}	0.770 ± 0.019^{Ae}	0.939 ± 0.103^{Ab}	13.214 ± 0.952^{Aa}
	0.05% DSE	0.168 ± 0.015^{Aa}	0.764 ± 0.010^{Ad}	0.889 ± 0.032^{Ab}	12.914 ± 1.235^{Aa}
	0.1% DSE	0.166 ± 0.018^{Aa}	0.757 ± 0.019^{Ad}	0.874 ± 0.011^{Aa}	12.814 ± 0.387^{Aa}
5 h	0% DSE	0.225 ± 0.010^{Ab}	0.881 ± 0.078^{Ade}	1.727 ± 0.023^{Aa}	10.372 ± 0.587^{Ab}
	0.05% DSE	0.157 ± 0.005^{Ba}	0.791 ± 0.049^{Ad}	1.557 ± 0.088^{Aa}	7.486 ± 0.499^{Bb}
	0.1% DSE	0.130 ± 0.017^{Bb}	0.777 ± 0.029^{Ad}	1.002 ± 0.081^{Ba}	5.886 ± 0.238^{Bb}
10 h	0% DSE	0.294 ± 0.012^{Aa}	0.915 ± 0.049^{Acde}	0.343 ± 0.015^{Ac}	1.211 ± 0.152^{Ac}
	0.05% DSE	0.159 ± 0.002^{Ba}	0.887 ± 0.068^{Acd}	0.196 ± 0.049^{Bc}	1.056 ± 0.020^{Ac}
	0.1% DSE	0.121 ± 0.005^{Cb}	0.805 ± 0.029^{Acd}	0.139 ± 0.004^{Bb}	0.852 ± 0.029^{Ac}
15 h	0% DSE	0.228 ± 0.005^{Ab}	1.059 ± 0.019^{Abcd}	0.136 ± 0.023^{Ad}	0.963 ± 0.398^{Ac}
	0.05% DSE	0.167 ± 0.015^{Ba}	0.970 ± 0.010^{Bbc}	0.105 ± 0.014^{Ac}	0.834 ± 0.017^{Ac}
	0.1% DSE	0.159 ± 0.013^{Bab}	0.832 ± 0.029^{Ccd}	0.128 ± 0.015^{Ab}	0.703 ± 0.296^{Ac}
20 h	0% DSE	0.207 ± 0.010^{Abc}	1.156 ± 0.078^{Abc}	0.179 ± 0.020^{Ad}	1.032 ± 0.171^{Ac}
	0.05% DSE	0.169 ± 0.002^{Ba}	0.997 ± 0.049^{ABbc}	0.088 ± 0.041^{Ac}	0.611 ± 0.087^{Ac}
	0.1% DSE	0.157 ± 0.003^{Bab}	0.860 ± 0.049^{Bcd}	0.137 ± 0.006^{Ab}	0.803 ± 0.142^{Ac}
30 h	0% DSE	0.191 ± 0.012^{Abc}	1.307 ± 0.097^{Ab}	0.133 ± 0.006^{Ad}	0.977 ± 0.002^{Ac}
	0.05% DSE	0.169 ± 0.012^{Aa}	1.135 ± 0.049^{ABab}	0.170 ± 0.005^{Bc}	0.822 ± 0.109^{Ac}
	0.1% DSE	0.157 ± 0.005^{Aab}	0.908 ± 0.019^{Bbc}	0.120 ± 0.010^{Bb}	0.889 ± 0.080^{Ac}
48 h	0% DSE	0.169 ± 0.012^{Acd}	1.568 ± 0.078^{Aa}	0.106 ± 0.008^{Ad}	0.743 ± 0.339^{Ac}
	0.05% DSE	0.157 ± 0.005^{Aa}	1.190 ± 0.029^{Ba}	0.070 ± 0.001^{Ac}	0.658 ± 0.034^{Ac}
	0.1% DSE	0.139 ± 0.005^{Aab}	0.984 ± 0.029^{Bab}	0.073 ± 0.019^{Ab}	0.640 ± 0.070^{Ac}
72 h	0% DSE	0.128 ± 0.010^{Ad}	1.747 ± 0.058^{Aa}	0.122 ± 0.005^{Ad}	0.625 ± 0.035^{Ac}
	0.05% DSE	0.142 ± 0^{Aa}	1.266 ± 0.039^{Ba}	0.103 ± 0.004^{Ac}	0.625 ± 0.035^{Ac}
	0.1% DSE	0.123 ± 0.002^{Ab}	1.080 ± 0.029^{Ca}	0.096 ± 0.008^{Ab}	0.625 ± 0.035^{Ac}

A–C: means within D-sodium erythorbate concentration with different superscript letters are significantly different ($P < 0.05$). a–h: means within time with different superscript letters are significantly different ($P < 0.05$).

cured ham, and the main enzyme related to the flavor formation of cured products is intramuscular lipase. Enzymes related to lipid oxidation include phospholipase, fatty hydrolase, and lipoxygenase. Phospholipase is an important enzyme that acts in both fat hydrolysis and flavor formation. The total phospholipase activities (Table 2) with DSE-0 were significantly increased from 0 to 15 h ($P < 0.05$), which could be due to the decrease of water activity and increase of salt content with prolonged processing time, and a certain amount of salt can promote the oxidation reaction [21]. The total phospholipase activities decreased significantly ($P < 0.05$) from 20 to 72 h, which might be attributed to the decrease in water activity because the salt content of sausage may not suitable for enzyme activities. The total phospholipase activities of samples with addition of DSE increased without significant differences in 0–5 h ($P > 0.05$) and then decreased significantly during the rest stage ($P < 0.05$), which could be due to a reaction between D-sodium erythorbate and phospholipase.

3.5. Changes of Acid Lipase and Neutral Lipase Activity in Cantonese Sausage Processing. Lipase mainly consists of acid lipase and neutral lipase. Pigs of different genotypes have different fat hydrolase activities [22]. The acid lipase activity with DSE-0 increased significantly ($P < 0.05$) from 0 to 15 h and then decreased significantly ($P < 0.05$) in 15–72 h. This is consistent with the results from the study of Toldrá [23]. The water activity decreased when sausages were dried for 15 h, which promotes the increase of acid lipase activity, but the acid lipase activity continued to decrease after 15 h, which could be related to excessive content of salt [24]. The acid lipase activity in the sausage prepared with addition of DSE was highest at 5 h and then decreased during the rest stage, which was consistent with changes of phospholipase activity.

The optimum enzyme activity temperature of acid lipase is 37°C, and the optimum temperature of neutral lipase is 45°C [13]. Compared with acid lipase activity, neutral lipase activity is higher, which could reflect similarity of the sausage processing temperature (50°C) to the optimum temperature of neutral lipase, which is beneficial to the release of its activity. In addition, the pH is near neutral during sausage processing [17], which promotes neutral lipase activity. The neutral lipase activity of sausages with DSE-0 was highest at 15 h and then decreased, while sausages with DSE-0.05 and DSE-0.1 reached the highest point at 5 h and then decreased, effects that were consistent with acid lipase activity.

3.6. Changes of Superoxide Dismutase (SOD) Activity and Glutathione Peroxidase (GSH-Px) Activity in Cantonese Sausage Processing. Dashdorj et al. [22] found that different genotypes of pork not only had different lipase activities but also had different SOD and GSH-Px activities. Compared with DSE-0, the activities of SOD with DSE-0.05 and DSE-0.1 were significantly increased ($P < 0.05$) at 5 h, 10 h, 15 h, 20 h, 30 h, and 72 h (Table 2), but SOD activities with DSE-0.05 decreased without significant differences ($P > 0.05$) and increased significantly ($P < 0.05$) with DSE-0.1 at 48 h.

Table 2: Changes of total phospholipase, acid lipase, neutral lipase, and SOD and GSH-Px activities in Cantonese sausage processing.

		Total phospholipase activity (U/g protein)	Acid lipase activity (U/g protein)	Neutral lipase activity (U/g protein)	SOD activity (U/mg protein)	GSH-Px activity (U/g protein)
0 h	0% DSE	1.126 ± 0.048^{Acd}	2.534 ± 0.046^{Ade}	8.542 ± 0.299^{Abc}	21.209 ± 0.769^{Ad}	1.896 ± 0.063^{Aa}
	0.05% DSE	1.123 ± 0.043^{Aab}	2.494 ± 0.102^{Abc}	8.462 ± 0.261^{Abc}	21.709 ± 0.062^{Ad}	1.871 ± 0.027^{Aa}
	0.1% DSE	1.115 ± 0.032^{Aab}	2.514 ± 0.018^{Aab}	8.596 ± 0.224^{Ab}	21.609 ± 0.628^{Ad}	1.846 ± 0.008^{Aa}
5 h	0% DSE	1.217 ± 0.073^{Abcd}	2.459 ± 0.163^{Be}	9.588 ± 0.312^{Cab}	25.534 ± 0.413^{Bc}	1.407 ± 0.111^{Ab}
	0.05% DSE	1.218 ± 0.038^{Aa}	3.531 ± 0.270^{Aa}	19.128 ± 0.053^{Aa}	31.078 ± 1.032^{Ab}	0.279 ± 0.010^{Bd}
	0.1% DSE	1.218 ± 0.058^{Aa}	2.694 ± 0.197^{ABa}	14.808 ± 1.532^{Ba}	33.121 ± 1.032^{Ab}	1.140 ± 0.036^{Ac}
10 h	0% DSE	1.384 ± 0.021^{Aabc}	2.539 ± 0.163^{Bde}	8.970 ± 0.209^{Abc}	33.097 ± 0.828^{Ba}	1.098 ± 0.039^{Bc}
	0.05% DSE	1.172 ± 0.016^{Bab}	3.001 ± 0.114^{Aab}	9.114 ± 0.347^{Ab}	36.933 ± 1.103^{Aa}	1.034 ± 0.051^{Bc}
	0.1% DSE	1.149 ± 0.016^{Bab}	2.445 ± 0.115^{ABab}	8.241 ± 0.190^{Abc}	39.404 ± 0.736^{Aa}	1.556 ± 0.056^{Aa}
15 h	0% DSE	1.607 ± 0.133^{Aa}	5.237 ± 0.210^{Aa}	11.197 ± 0.528^{Aa}	15.275 ± 0.874^{Be}	1.296 ± 0.016^{Abc}
	0.05% DSE	1.115 ± 0.071^{Bab}	2.668 ± 0.157^{Bbc}	5.934 ± 0.375^{Bd}	19.514 ± 0.125^{Ad}	1.161 ± 0.039^{Bbc}
	0.1% DSE	1.007 ± 0.029^{Bbc}	2.497 ± 0.234^{Bab}	5.845 ± 0.446^{Bcd}	20.485 ± 0.999^{Ad}	1.246 ± 0.029^{ABc}
20 h	0% DSE	1.468 ± 0.005^{Aab}	4.474 ± 0.212^{Ab}	7.681 ± 0.529^{Ac}	21.946 ± 0.601^{Cd}	1.132 ± 0.037^{Bc}
	0.05% DSE	0.987 ± 0.074^{Bbcd}	2.388 ± 0.024^{Bc}	7.395 ± 0.169^{Ac}	28.743 ± 1.001^{Bbc}	1.233 ± 0.004^{Bb}
	0.1% DSE	0.967 ± 0.047^{Bbcd}	2.174 ± 0.072^{Bab}	4.656 ± 0.283^{Bd}	33.132 ± 0.200^{Ab}	1.443 ± 0.024^{Ab}
30 h	0% DSE	1.437 ± 0.068^{Aab}	3.483 ± 0.150^{Ac}	7.797 ± 0.727^{Ac}	10.327 ± 0.356^{Cf}	0.830 ± 0.042^{Ad}
	0.05% DSE	1.030 ± 0.061^{Babc}	2.685 ± 0.090^{ABbc}	9.188 ± 0.544^{Ab}	13.727 ± 0.178^{Be}	0.250 ± 0.000^{Bd}
	0.1% DSE	0.800 ± 0.076^{Bd}	1.861 ± 0.308^{Bb}	3.956 ± 0.534^{Bd}	18.891 ± 0.356^{Ad}	0.700 ± 0.071^{Ad}
48 h	0% DSE	1.100 ± 0.074^{Ad}	1.989 ± 0.046^{Be}	4.059 ± 0.049^{Bd}	30.455 ± 0.205^{Bb}	0.374 ± 0.000^{Be}
	0.05% DSE	0.885 ± 0.046^{Acd}	2.446 ± 0.014^{Ac}	5.560 ± 0.147^{Ad}	28.714 ± 0.205^{Bbc}	0.230 ± 0.041^{Cd}
	0.1% DSE	0.869 ± 0.064^{Acd}	2.147 ± 0.158^{ABab}	5.321 ± 0.387^{Ad}	33.427 ± 0.923^{Ab}	0.694 ± 0.036^{Ad}
72 h	0% DSE	0.820 ± 0.038^{Ae}	3.120 ± 0.141^{Acd}	2.818 ± 0.058^{Bd}	23.517 ± 0.663^{Bcd}	0.137 ± 0.010^{Bf}
	0.05% DSE	0.794 ± 0.022^{Ad}	2.716 ± 0.149^{ABbc}	4.280 ± 0.238^{Ae}	25.850 ± 0.718^{ABc}	0.165 ± 0.021^{Bd}
	0.1% DSE	0.785 ± 0.012^{Ad}	2.381 ± 0.031^{Bab}	3.511 ± 0.279^{ABd}	28.783 ± 0.976^{Ac}	0.517 ± 0.053^{Ad}

A–C: means within D-sodium erythorbate concentration with different superscript letters are significantly different ($P < 0.05$). a–h: means within time with different superscript letters are significantly different ($P < 0.05$).

The total phospholipase and acid lipase activity (Table 2) with DSE-0 had highest value at 15 h, while the SOD activity had a low value. The SOD activity had a lowest value at 30 h, with higher total phospholipase and acid lipase activities. This could be explained by the following two points: (1) lipase may have an inhibitory effect on SOD activity; (2) Lee et al. [25] considered that in the 1.5%–2% range of NaCl concentration, there was a slow decrease in the activity of GSH-Px and catalase in the longissimus dorsi of pork, while SOD activity decreased rapidly with the increase of NaCl concentration. In our present study, water activity decreased and NaCl concentration increased with increased processing time, resulting in a rapid decrease in SOD activity. There were different effects of different antioxidants on SOD. Deng et al. [26] added perilla seed (an antioxidant) to longissimus dorsi of lamb and found decreased SOD activity, which was different from the results of our study.

GSH-Px activities of samples with DSE-0 decreased significantly ($P < 0.05$) in 0–10 h and then increased without significant differences ($P > 0.05$) at 15 h, before decreasing significantly ($P < 0.05$) in 20–72 h. GSH-Px activity at 15 h was lower than that at 0 h, but GSH-Px activity and SOD activity of 15 h still were at the highest level. GSH-Px activities of samples with DSE-0.05 decreased rapidly, then increased, and then decreased. It had a high value in 10–20 h.

3.7. Correlation of Oxidation Indices (Peroxide Value, TBARS Value, Carbonyl Value, and Conjugated Dienes Value) and Total Phospholipase, Acid Lipase, Neutral Lipase, SOD, and

Table 3: Correlation of oxidation indices and total phospholipase, acid lipase, neutral lipase, and SOD and GSH-Px activities as processing time proceeded.

	0% DSE	0.05% DSE	0.1% DSE
PV			
Total phospholipase	0.685	0.232	−0.081
Acid lipase	0.115	−0.270	−0.289
Neutral lipase	0.720*	−0.014	−0.078
SOD	0.269	−0.230	−0.725*
GSH-Px	0.431	0.700	0.361
TBARS value			
Total phospholipase	−0.493	−0.902**	−0.872**
Acid lipase	−0.032	−0.422	−0.466
Neutral lipase	−0.863**	−0.638	−0.792*
SOD	−0.010	−0.242	0.025
GSH-Px	−0.957**	−0.652	−0.857**
Carbonyl value	−0.151	0.614	0.711*
Total phospholipase	−0.402	0.700	0.641
Acid lipase	0.387	0.879**	0.805*
Neutral lipase	0.174	0.165	−0.065
SOD	0.593	0.056	0.458
GSH-Px			
	−0.225	0.488	0.544
Conjugated dienes value	−0.390	0.205	0.478
Total phospholipase	0.333	0.470	0.530
Acid lipase	0.035	−0.054	−0.268
Neutral lipase	0.685	0.232	−0.081
SOD	0.115	−0.270	−0.289
GSH-Px	0.720*	−0.014	−0.078

* $p < 0.05$. ** $p < 0.01$.

Table 4: Correlation of oxidation indices and total phospholipase, acid lipase, neutral lipase, and SOD and GSH-Px activities with different additions of DSE.

	0 h	5 h	10 h	15 h	20 h	30 h	48 h	72 h
PV								
Total phospholipase	0.929	−0.800	0.993	0.999*	0.980	1.000**	0.844	−0.019
Acid lipase	0.655	−0.439	−0.158	0.998*	0.988	0.987	−0.244	0.212
Neutral lipase	−0.217	−0.734	0.656	0.917	0.749	0.595	−0.754	0.920
SOD	−0.866	−1.000**	−0.982	−0.998*	−0.987	−0.964	−0.700	−0.326
GSH-Px	0.982	0.456	−0.580	0.703	−0.884	0.358	−0.745	−0.662
TBARS value								
Total phospholipase	0.982	−0.698	0.755	0.889	0.901	0.972	0.957	0.999*
Acid lipase	0.500	−0.573	0.425	0.831	0.921	0.998*	−0.493	0.981
Neutral lipase	−0.397	−0.830	0.967	0.993	0.888	0.762	−0.551	0.407
SOD	−0.756	−0.990	−0.919	−0.892	−0.997	−0.999*	−0.484	−0.950
GSH-Px	1.000**	0.588	−0.938	0.250	−0.971	0.135	−0.541	−0.760
Carbonyl value								
Total phospholipase	0.882	−0.995	0.984	0.582	0.823	0.095	0.992	0.999*
Acid lipase	0.735	0.088	−0.095	0.672	0.794	0.261	−0.808	0.980
Neutral lipase	−0.107	−0.277	0.702	0.260	0.039	0.861	0.158	0.404
SOD	−0.916	−0.848	−0.992	−0.577	−0.566	−0.365	−0.080	−0.949
GSH-Px	−0.955	−0.069	−0.630	0.994	−0.274	−0.892	−0.146	−0.757
Conjugated dienes value								
Total phospholipase	0.866	−0.846	0.872	0.937	0.875	0.688	0.994	NS
Acid lipase	0.756	−0.365	0.233	0.891	0.849	0.557	−0.649	NS
Neutral lipase	−0.075	−0.676	0.896	1.000*	0.137	−0.183	−0.383	NS
SOD	−0.929	−0.995	−0.980	−0.939	−0.643	−0.463	−0.310	NS
GSH-Px	0.945	0.383	−0.848	0.363	−0.367	0.927	−0.372	NS

* $p < 0.05$; ** $p < 0.01$. NS, not significant.

GSH-Px in Cantonese Sausage. The correlation of peroxide value, TBARS value, and antioxidant enzyme activity in the dry-salted bacon processing period was analyzed by Jin et al. [27], who found only very few correlations between antioxidant enzyme activities and oxidation indices. There was a significant correlation between TBARS value and GSH-Px activity ($P < 0.05$) and a significant correlation between TBARS value and SOD activity ($P < 0.01$) for samples that were salted with 1.0% salt and ripened at an initial temperature of 16°C. Chen et al. [28] studied the potential correlation of phospholipase A2 and GSH-Px activity in normal pork and PSE pork and found no correlation between the total phospholipase A2 activity and GSH-Px activity. sPLA2 + cPLA2 and GSH-Px activities were significantly correlated ($P < 0.05$), and calcium-independent phospholipase A2 was also significantly related to GSH-Px activity.

The correlations of oxidation indices and total phospholipase, acid lipase, neutral lipase, SOD, and GSH-Px activity with increasing processing time were determined and are shown in Table 3. The neutral lipase activity and GSH-Px activity with DSE-0 were negatively correlated to TBARS value ($P < 0.01$), neutral lipase activity was positively correlated to PV ($P < 0.05$), and the conjugated dienes value was positively correlated to GSH-Px activity ($P < 0.05$). The total phospholipase activity of DSE-0.05 was significantly negatively correlated to the TBARS value ($P < 0.01$), and neutral lipase activity and carbonyl value were also negatively correlated. The SOD activity of DSE-0.1 was significantly negatively correlated to PV ($P < 0.05$), the TBARS value was significantly negatively correlated to neutral lipase activity ($P < 0.05$), and the correlation of total phospholipase activity and TBARS value as well as GSH-Px activity and TBARS value were significantly negative ($P < 0.01$). There was a significant correlation between total phospholipase activity and carbonyl value ($P < 0.05$) as well as neutral lipase activity and carbonyl value ($P < 0.05$).

The correlations of oxidation indices and total phospholipase, acid lipase, neutral lipase, SOD, and GSH-Px activity with different additions of DSE are shown in Table 4. At 0 h, the TBARS value and GSH-Px activity were significantly positively correlated ($P < 0.01$). At 5 h, PV and SOD activity were significantly negatively correlated ($P < 0.01$). At 15 h, acid lipase activity and PV, total phospholipase activity, and PV were significantly negatively correlated ($P < 0.05$) with a significantly positive correlation of SOD activity and PV ($P < 0.05$) and a significantly positive correlation of neutral lipase activity and conjugated dienes value ($P < 0.01$). At 30 h, total phospholipase activity and PV showed a significantly positive correlation ($P < 0.01$), acid lipase activity and TBARS value showed a significantly positive correlation ($P < 0.05$), but SOD activity and TBARS value showed a significantly negative correlation ($P < 0.05$). At 72 h, the total phospholipase activity and TBARS value showed a significantly positive correlation ($P < 0.05$) as there was a strong relationship between total phospholipase activity and carbonyl value ($P < 0.05$).

4. Conclusions

The rates of lipid degradation and oxidation were slowed down by addition of D-sodium erythorbate. The increase of

peroxide value during the early stage (0–10 h) of sausage processing might be due to formation of hydroperoxide, while the increase of TBARS value during the resting stage (15–72 h) might be due to the formation of small molecular flavors. Neutral lipase activity was the highest among the three lipases, followed by acid lipase activity, with total phospholipase activity as the lowest. This was closely related to the processing temperature and pH of the sausage. The addition of D-sodium erythorbate increased SOD activity but showed no clear effect on GSH-Px activity. With increased content of D-sodium erythorbate, SOD activity and peroxide value were significantly negatively correlated ($P < 0.01$) at 5 h, neutral lipase activity and conjugated dienes value showed a significantly positive correlation ($P < 0.01$) at 15 h, and the total phospholipase activity and peroxide value showed a significantly positive correlation ($P < 0.01$) at 30 h.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors have declared no conflicts of interest regarding the publication of this article.

Authors' Contributions

Xiaolan Shang designed the experiment, contributed to the acquisition of data, and interpreted the data. Jie Qiao performed the enzyme activity determination experiments. Zhanxiong Chen performed the measurements of peroxide value, TBARS value, carbonyl value, and conjugated dienes value.

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