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Influence of partial replacement of NaCl by KCl, L-histidine and L-lysine on the lipase activity and lipid oxidation in dry-cured loin process

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ABSTRACT

An attempt to decrease the NaCl content in dry-cured meat product through the use of amino acids, the effect of a salt substitute containing L-histidine (L-his) and L-lysine (L-lys) on lipolysis and lipid oxidation was compared with that of NaCl throughout processing. In the final product, the sodium content of the loin with salt substitute (SS-loin) was 53.79% less than that of the loin treated with NaCl (S-loin). A significant increase in phospholipase activity was observed in SS-loin from the end of drying-ripening, which was 8–18 days ($P < 0.05$). The activities of acid lipase in porcine loin were markedly activated by 0.05–0.4 M L-his. Compared to the control, higher phospholipase activity was observed for 0.2–0.4 M L-lys and 0.05–0.4 M L-his in porcine loin. The Aw in the SS-loin was 3.51% more than that in the S-loin, and the TBARS was 5.19% lower ($P < 0.05$) in the SS-loin compared to the S-loin. The results suggested that the decreased TBARS content of the dry-cured loins was might mainly due to the inhibition of lipid oxidation by L-lys and L-his in salt substitute.

1. Introduction

Sodium chloride is essential and affects some physicochemical phenomena, such as lipolysis, lipid oxidation and proteolysis, which contribute to the development of the typical texture and flavour of dry-cured meat products (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004; Guàrdia, Guerrero, Gelabert, Gou, & Arnau, 2006). Nevertheless, high levels of sodium intake might contribute to the development of hypertension and the rise of blood pressure (Armenteros, Aristoy, Barat, & Toldrá, 2012; McCarty, 2004).

Different strategies have been attempted for reduction of sodium content in dry-cured meat products, mainly by replacing the NaCl with other chloride salts such as KCl, $MgCl_2$, $CaCl_2$ (Aliño, Grau, Fuentes, & Barat, 2010; Comaposada, Arnau & Gou, 2007; Corral, Salvador, & Belloch, 2015; Lorenzo et al., 2015; Zanardi, Ghidini, Conter, & Ianieri, 2010) or K-lactate or Glycine (Costa-Corredor, Muñoz, Arnau, & Gou, 2010; Fulladosa, Serra, Gou, & Arnau, 2009; Gou, Guerrero, Gelabert, & Arnau, 1996). Several combinations of salts as partial replacers of sodium chloride could affect the activity of lipolytic enzymes and change the free fatty acid (FFA) profile. Ripollés, Campagnol, Armenteros, Aristoy, and Toldrá (2011) studied the replacement of NaCl with KCl, $CaCl_2$ and $MgCl_2$ in dry-cured ham. They found that the 100% NaCl formulation was the treatment that most inhibited acid lipase activity, and formulation III (55% NaCl, 25% KCl, 15% $CaCl_2$ and 5% $MgCl_2$) was the least inhibitory, which resulted in a slightly higher lipolysis in formulation III. In dry-cured loin, Armenteros, Aristoy, Barat, and Toldrá (2009) reported that the replacement of 50% NaCl by KCl did not promote significant differences in either total amounts of saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA) fatty acid content. Ripollés et al. (2011) observed that replacing dry-cured ham with 50% KCl or 25% KCl, 15% $CaCl_2$ and 5% $MgCl_2$ did not produce a significant change in the TBARS value compared to the 100% NaCl treatment at the end of processing (330 days).

Some studies have been performed to investigate the influence of a salt substitute containing amino acids on physicochemical or biochemical properties of meat. Gou et al. (1996) replaced NaCl with glycine in dry-cured loin and found a significant reduction in springiness at the 40% substitution level. In a recent study of Zhang et al. (2014), L-lys, L-his and L-arginine might contribute to the salty taste of NaCl through interactions between the components. Further, L-lys and L-his were found to cause transformations in secondary structures and increases in the solubility of porcine

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myosin and chicken (Guo, Peng, Zhang, Liu, & Cui, 2015; Hayakawa, Ito, Wakamatsu, Nishimura, & Hattori, 2009, 2010). Nevertheless, amino acids as salt substitute components have not been used in dry-cured meat products. Therefore, the aim of the present was to compare the effect of NaCl and a salt substitute containing L-his and L-lys on lipid fractions, lipase activity, free fatty acid composition and lipid oxidation of dry-cured loin throughout processing. The influence of NaCl, KCl, L-his and L-lys on lipase activity (neutral, acid and phospholipase) in fresh loin were also determined.

2. Materials and methods

2.1. Processing of the dry-cured loins and samplings

Nine fresh loins (Longissimus dorsi), three for raw material, six for each processing step, with an average weight of 1.5 ± 0.2 kg and a mean pH value of 5.66 ± 0.01 measured 24 h post-mortem were selected in the local slaughterhouse (Sushi Co., Ltd., Nanjing, Jiangsu, China). All the loins were frozen at −35 °C and stored at −20 °C for 30 days in vacuum packaging. Then, the frozen loins were thawed at 4 °C for 3 days. Three of the loins were used in order to characterise the raw material analysis. The six of loins for each of other processing steps were divided into S-loin group and SS-loin group, with three loins in each group. Dry-cured loins were produced as follows: After chilling, the exterior fascia of the loins was removed. Then, the loins' surfaces were rubbed with 3% salt (NaCl, g/100 g loin) and 3% salt substitute (a low-sodium salt substitute, Rolys Co., Ltd, Nanjing, China). The salt substitute contained 39.7 g/100 g of NaCl, 51.3 g/100 g of KCl and the mixture of L-his and L-lys (9.0 g/100 g). Then, the loins were stuffed into artificial casings and immediately held at 4 °C with a relative humidity (RH) of 80–90% for 3 days in order to allow the salt to penetrate. After the salting stage, the loins were dried for ripening at 12–14 °C with a relative humidity of 90% for 3 days. Subsequently, the loins were dried for ripening at 20–22 °C and 80% RH for 15 days.

Samples were measured at six process points (raw, at the end of 3 days salting, and during drying-ripening at 3, 8, 13 and 18 days). At each processing point, three loins of each group were randomly sampled for analysis. The loins were cut into cubes (approximately 2 cm × 2 cm × 2 cm) and ground in a rotary screw mincer (Model X 70; Scharfen GmbH & Co. Maschinenfabrik KG, Witten, Germany). Samples were randomly obtained from the minced meat to analyse. Samples that remained after each processing step were vacuum-packaged and stored at −20 °C prior to further analysis.

2.2. Moisture and Aw determination

Moisture content was determined by oven drying to constant weight at 100 °C (ISO Norm R-1442, 1979). The Aw of each sample was determined by using an HP23-Aw-set-40 hygrometer (Rotronic Devices, Inc., Switzerland).

2.3. Total lipid extraction and separation

Total lipids were extracted from 5 g of minced muscles according to the method described by Folch, Lees, and Sloane-Stanley (1957) using 25 ml of chloroform: methanol (2:1) as the solvent. The extracted lipid was expressed as g/100 g dry matter. One hundred milligrams of the dry extracted lipids was separated using NH_2-aminopropyl minicolumns, following the method of Kaluzny, Duncan, Merritt, and Epps (1985). Neutral lipids (NL) were eluted with 3.0 ml of $CHCl_3$: iso-proponal (2:1), free fatty acids (FFAs) were eluted with 3.0 ml of diethyl ether: acetic acid 2%, and phospholipids (PL) were eluted with 3.0 ml of MeOH. The amounts of NL, FFAs and PL were expressed as g/100 g total lipids obtained.

2.4. Lipase extraction and activity assay

2.4.1. Lipase extraction

Crude lipases were extracted according to the method described by Toldrá, Flores, Aristoy, Virgili, and Parolari (1996), with some modifications. Five grams of chopped samples were homogenised in 25 ml of 50 mM phosphate buffer (pH 7.5) using a Polytron (4 × 10s, 20,000 rpm) homogenizer IKA T18 basic (IKA, Germany). During homogenisation, the tubes were kept in ice to avoid heating. The homogenate was stirred for 30 min and then centrifuged at 4 °C, 10,000 g for 20 min. The supernatant was filtered and finally diluted to 25 ml with an extraction buffer for enzyme activity assays.

2.4.2. Lipases activities assay

2.4.2.1. Acid lipase activity. The acid lipase activities were assayed according to the method of Vestergaard, Schivazappa, and Virgili (2000). 0.1 ml of lipase 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) TritonX-100 and 0.8 mg/ml bovine serum albumin. 0.1 ml of 1.0 mM 4-methylumbelliferyl-oleate was added into this mixture as substrate. After incubation at 37 °C for 30min, this reaction was stopped with 0.5 ml of 1 N HCl. The fluorescence was monitored at $\lambda_{ex} = 328$ nm and $\lambda_{em} = 470$ nm.

2.4.2.2. Neutral lipase activity. The neutral lipase activities were assayed according to the method of Vestergaard et al. (2000). 0.1 ml of lipase extract was diluted with 2.8 ml of 0.22 M Tris/HCl buffer, pH 7.5, containing 0.05% (w/v) TritonX-100. 0.1 ml of 1.0 mM 4-methylumbelliferyl-oleate was added into this mixture as substrate. After incubation at 37 °C for 30min, the samples were immediately cooled in ice-water mixture and measured at $\lambda_{ex} = 328$ nm and $\lambda_{em} = 443$ nm.

2.4.2.3. Phospholipase activity. The phospholipase activity was determined following the method of Toldrá et al. (1996). 0.1 ml of lipase extract was diluted with 2.8 ml of 0.1 M disodium phosphate/0.05 M citric acid buffer, pH 5.0, containing 150 mM sodium fluoride, 0.05% (w/v) TritonX-100 and 0.8 mg/ml bovine serum albumin. 0.1 ml of 1.0 mM 4-methylumbelliferyl-oleate was added into this mixture as substrate. After incubation at 37 °C for 30min, this reaction was stopped with 0.5 ml of 1 N HCl. The fluorescence was monitored at $\lambda_{ex} = 328$ nm and $\lambda_{em} = 470$ nm.

Enzymatic activity was expressed as nmol of released 4-methylumbelliferone $h^{-1} \times$ g $protein^{-1}$.

2.5. Effect of salt substitute components (NaCl, KCl, L-lys and L-his) on the lipase activity

The effect of NaCl, KCl, L-lys and L-his on lipase activity was evaluated by using the assay buffer containing the individual component (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35 and 0.4 M). The lipase extractions were obtained as described in Section 2.4.1 and the activity was determined as described in Section 2.4.2. In all cases, activity was compared with the control, which was simultaneously measured in the absence of any salt or amino acids.

2.6. Oxidation

The extent of lipid oxidation was evaluated by 2-thiobarbituric acid-reactive substances (TBARS) and peroxide value (POV). The TBARS in meat samples was determined according to the method of Salih, Smith, Price, and Dawson (1987). The TBARS was expressed as mg of malondialdehyde (MDA) per kg of muscle. The POV of the lipid samples was measured as the Chinese national standard (GB/T

5538-1995) described by Jin et al. (2010) and was expressed as g of peroxide per 100 g of lipid.

2.7. Free fatty acids analysis

The fatty acid composition of free fatty acids was determined by gas–liquid chromatography. The fatty acids methyl esters were prepared as described by Morrison and Smith (1964). The analysis was carried out in a gas chromatograph (Trace GC Ultra, Thermo Electron Corporation, Waltham, USA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 (50 m × 0.25 mm) (Chrompack Inc., Middleburg, The Netherlands), with nitrogen as the carrier gas (flow rate = 1 ml/s). The oven temperature was maintained at 90 °C for 2 min, increased to 180 °C at 10 °C/min and maintained for 5 min, then increased to 240 °C at 5 °C/min and maintained for 12 min. The split ratio was 1:70. The detector and injector temperatures were 240 °C. The fatty acids were identified by comparing their retention times with those of standards. Amounts of individual fatty acids were quantified using C21:0 as internal standard. The results were expressed as mg/g lipid.

2.8. Measurement of sodium and potassium

All glassware was washed overnight in a solution of 0.3 M HCl in deionised distilled water prior to use. The nitration of samples was carried out according to the method of Tahergorabi, Beamer, Matak, and Jaczynski (2012). Then, the samples were allowed to cool and diluted with 50 ml of deionised distilled water for analysis. The contents of sodium and potassium were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 2100DV, Perkin Elmer) with an axially viewed configuration. The ICP-OES operational parameters were listed as follows: 1300 W radio frequency power, 15 L/min plasma gas flow rate, 0.2 L/min auxiliary gas flow rate, 0.8 L/min nebuliser gas flow rate, and 1.5 L/min sample uptake rate. The emission lines that were employed were Na 589.592 and K 769.896.

2.9. Statistical analysis

The data were analysed using repeated-measures ANOVA (SAS 8.2, SAS Inst. Inc. 2001). Significant differences ($P < 0.05$) between the treatments were assessed using Duncan's multiple-range tests. Each parameter was analysed in three replicates of loins.

3. Results and discussion

3.1. Sodium, potassium, moisture content and Aw comparisons

In the final products, the Na content in the SS-loin was 53.79% less than that in the S-loin (Fig. 1). On the contrary, the K content in the SS-loin increased 3.43% of dry matter, which was 59.45% more than that in the S-loin. The final SS-loin product contained 1715 mg/100 g of K, which is much lower than the recommended level (4700 mg/day, McGuire & Beerman, 2007). The typical diet contains 3000 mg of K/day/person according to Skrabal, Aubock, Hortnagl, and Braunsteiner (1980).

No significant difference in moisture content was observed between the S-loin group and SS-loin group ($P > 0.05$) during processing (Fig. 1). Similar results were observed in dry-cured ham salted with mixtures of NaCl, KCl and $CaCl_2$ in Armenteros' work (2012), who found that moisture content was not significantly influenced by the type of salt substitution during manufacturing. However, the Aw in SS-loin was 2.45% and 3.51% more than that in S-loin ($P < 0.05$) at 13 days and 18 days of drying-ripening, respectively. However, no significant difference in Aw was found between SS-loin and S-loin until 8 days of drying-ripening ($P > 0.05$). This could be explained by the reduction of the Na ion in the SS-loin. Water molecules have a very strong interaction with the Na ion and a weak interaction with the K ion, i. e. the hydration level between NaCl and KCl was in the order of NaCl > KCl (Haard & Simpson, 2000).

3.2. Lipid fraction comparisons

During the whole processing, no significant difference in total lipid content was found between S-loin and SS-loin ($P > 0.05$, Table 1). At the end of drying-ripening 13 days, the content of neutral lipids in the SS-loin group were 1.78% more than that in S-loin, whereas phospholipids were significantly decreased, 8.58%, in the SS-loin group compared to the S-loin group. In the final products, no marked differences in neutral lipids were obtained

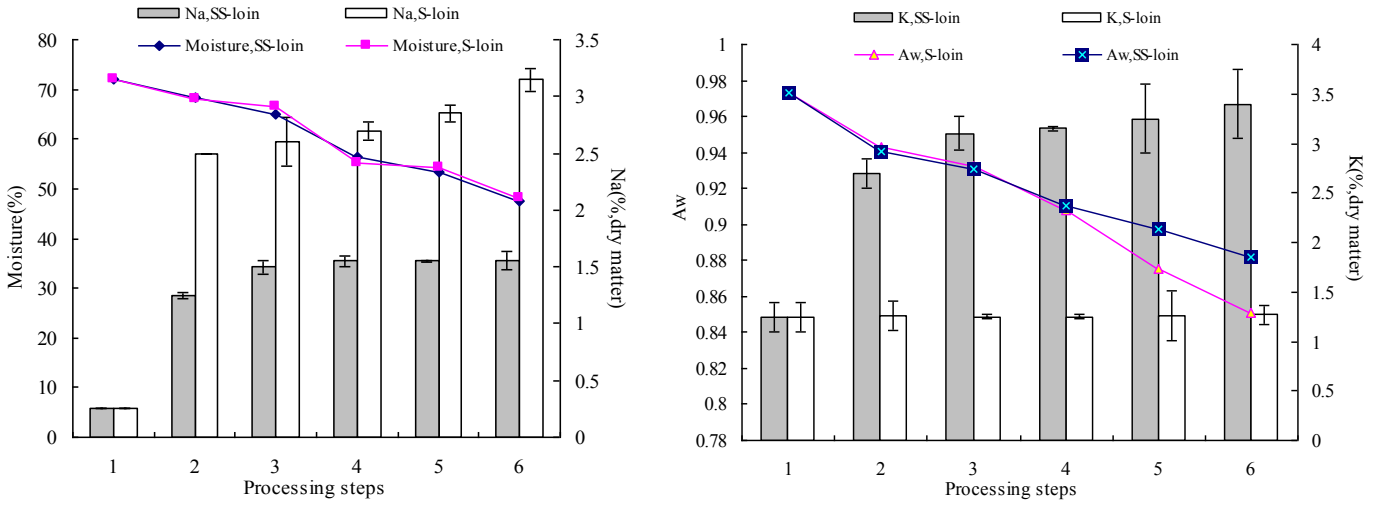

Fig. 1. Changes of Aw, sodium, potassium and moisture content of dry-cured loin during processing. Note: Moisture was expressed as the g/100 g muscle. Processing step 1 = raw; Processing step 2 = end of salting; Processing step 3 = drying-ripening 3 days; Processing step 4 = drying-ripening 8 days; Processing step 5 = drying-ripening 13 days; Processing step 6 = drying-ripening 18 days.

Table 1
Changes in lipid composition of dry-cured loin during processing.

Lipid fractions	Processing steps										
	Raw	End of salting		Drying-ripening 3 days		Drying-ripening 8 days		Drying-ripening 13 days		End of drying-ripening	
		S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin
Total lipids[A]	15.09^{a} ± 1.12	13.77^{b} ± 1.06	13.52^{b} ± 1.71	13.23^{b} ± 1.39	14.00^{b} ± 1.31	11.21^{c} ± 0.98	10.97^{c} ± 1.17	11.15^{c} ± 0.92	10.28^{c} ± 1.25	11.14^{c} ± 0.89	10.33^{c} ± 1.09
Neutral lipids[B]	74.78^{g} ± 2.28	75.49^{f} ± 6.75	75.67^{f} ± 5.58	76.70^{e} ± 6.26	77.11^{de} ± 6.77	77.44^{d} ± 5.96	77.69^{cd} ± 6.62	78.16^{c} ± 7.18	79.55^{b} ± 8.21	81.48^{a} ± 8.01	81.74^{a} ± 8.36
Phospholipids[B]	21.60^{a} ± 2.08	20.65^{b} ± 1.87	20.46^{b} ± 1.79	18.89^{c} ± 1.72	18.53^{c} ± 1.88	17.79^{d} ± 1.81	17.49^{de} ± 1.75	16.89^{e} ± 1.72	15.44^{f} ± 1.33	13.18^{g} ± 1.09	12.91^{g} ± 1.27
Free fatty acids[B]	3.61^{i} ± 0.39	3.86^{h} ± 0.51	3.87^{h} ± 0.46	4.41^{f} ± 0.61	4.36^{g} ± 0.52	4.77^{e} ± 0.38	4.82^{d} ± 0.49	4.95^{c} ± 0.41	5.01^{b} ± 0.56	5.33^{a} ± 0.61	5.35^{a} ± 0.66

S-loin = the loins treated with salt; SS-loin = the loins treated with salt substitute.
[a–i] Means in the same row with different superscripts differ significantly ($p < 0.05$).
[A] Expressed as the g/100 g dry matter.
[B] Expressed as g/100 g Total lipids.

Table 2
Changes of free fatty acids composition from dry-cured loin during processing.

Free fatty acids	Processing steps										
	Raw	End of salting		Drying-ripening 3 days		Drying-ripening 8 days		Drying-ripening 13 days		End of drying-ripening	
		S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin
C14:0	0.257^{i} ± 0.001	0.268^{h} ± 0.001	0.267^{h} ± 0.002	0.341^{g} ± 0.002	0.346^{f} ± 0.002	0.402^{c} ± 0.001	0.378^{d} ± 0.001	0.341^{g} ± 0.001	0.358^{e} ± 0.001	0.446^{b} ± 0.001	0.453^{a} ± 0.001
C16:0	8.809^{g} ± 0.016	9.295^{f} ± 0.033	9.327^{f} ± 0.005	10.869^{e} ± 0.060	10.812^{e} ± 0.009	12.136^{d} ± 0.012	12.262^{c} ± 0.009	12.243^{c} ± 0.065	12.422^{b} ± 0.062	13.862^{a} ± 0.018	13.927^{a} ± 0.014
C18:0	4.820^{i} ± 0.010	5.165^{i} ± 0.018	5.181^{i} ± 0.003	6.095^{g} ± 0.034	6.032^{h} ± 0.001	6.616^{f} ± 0.006	6.735^{e} ± 0.005	6.791^{d} ± 0.036	6.866^{c} ± 0.034	7.573^{b} ± 0.010	7.625^{a} ± 0.008
∑SFA	13.886^{h}	14.728^{g}	14.778^{g}	17.305^{f}	17.190^{f}	19.154^{e}	19.375^{d}	19.376^{d}	19.645^{c}	21.882^{b}	22.005^{a}
C16:1	0.865^{i} ± 0.002	0.921^{h} ± 0.003	0.924^{h} ± 0.001	1.040^{f} ± 0.006	1.022^{g} ± 0.080	1.050^{e} ± 0.001	1.067^{d} ± 0.001	1.087^{c} ± 0.006	1.098^{b} ± 0.005	1.178^{a} ± 0.001	1.181^{a} ± 0.001
C18:1	13.141^{g} ± 0.025	13.822^{f} ± 0.049	13.819^{f} ± 0.008	15.504^{bcd} ± 0.951	14.667^{e} ± 0.010	15.001^{de} ± 0.014	15.117^{cde} ± 0.011	15.712^{bc} ± 0.083	15.888^{b} ± 0.067	17.216^{a} ± 0.022	17.212^{a} ± 0.051
C20:1	0.216^{g} ± 0.001	0.229^{f} ± 0.001	0.229^{f} ± 0.001	0.264^{b} ± 0.001	0.257^{d} ± 0.002	0.249^{e} ± 0.001	0.251^{e} ± 0.001	0.261^{c} ± 0.001	0.265^{b} ± 0.003	0.282^{a} ± 0.002	0.282^{a} ± 0.002
∑MUFA	14.22^{h}	14.975^{g}	14.972^{g}	16.194^{e}	15.946^{f}	16.299^{de}	16.435^{d}	17.061^{c}	17.260^{b}	18.676^{a}	18.650^{a}
C18:2	5.970^{i} ± 0.011	6.639^{h} ± 0.024	6.661^{h} ± 0.020	7.911^{f} ± 0.044	7.837^{g} ± 0.056	9.175^{e} ± 0.038	9.250^{d} ± 0.067	9.737^{b} ± 0.052	9.881^{a} ± 0.049	9.503^{c} ± 0.012	9.551^{c} ± 0.039
C18:3	0.209^{i} ± 0.003	0.229^{h} ± 0.001	0.231^{h} ± 0.001	0.244^{f} ± 0.003	0.242^{g} ± 0.002	0.264^{e} ± 0.001	0.265^{e} ± 0.001	0.272^{d} ± 0.001	0.279^{c} ± 0.001	0.334^{a} ± 0.004	0.330^{b} ± 0.003
C20:4	1.847^{i} ± 0.013	2.054^{h} ± 0.037	2.067^{h} ± 0.021	2.447^{f} ± 0.014	2.425^{g} ± 0.035	2.837^{e} ± 0.033	2.858^{d} ± 0.029	3.007^{b} ± 0.016	3.050^{a} ± 0.015	2.950^{c} ± 0.042	2.965^{c} ± 0.036
∑PUFA	8.025^{h}	8.923^{g}	8.977^{g}	10.602^{f}	10.504^{f}	12.276^{e}	12.373^{d}	13.016^{b}	13.210^{a}	12.787^{c}	12.846^{c}

Free fatty acid was expressed as mg/g total lipid.
[a–j] Means in the same row with different superscripts differ significantly ($p < 0.05$).

between the S-loin and SS-loin. Similar results were found for the content of phospholipids. Correspondingly, the free fatty acids content in SS-loin was more than that in S-loin after 8 and 13 days of drying-ripening. The results indicated that free fatty acids were derived from hydrolysis of phospholipids, and the degree of lipolysis in the SS-loin was higher than that in the S-loin during the late stage of processing, suggesting that 50% replacement of KCl (Ripollés et al., 2011), L-his and L-lys in the salt substitute might promote lipase activity and consequently lipolysis.

3.3. Free fatty acid composition comparisons

As was shown in Table 2, no significant differences in either total amounts of saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA) fatty acids were found between the SS-loin and S-loin at the end of salting. After 8 and 13 days of drying-ripening, the free fatty acid contents were significantly higher in SS-loin than in S-loin ($P < 0.05$). In the final products, no significant differences ($P > 0.05$) were observed in the contents of MUFA and PUFA between the SS-loin and S-loin, whereas the SFA of SS-loin was 0.56% more than that of S-loin ($P < 0.05$), suggesting a slight trend toward lipolysis in the SS-loin at the end of processing. In contrast to this observation, Armenteros et al. (2009) found that the dry-cured loins salted with 50% NaCl/50% KCl and with 55% NaCl, 25% KCl, 15% $CaCl_2$ and 5% $MgCl_2$ did not show differences in the SFA, MUFA or PUFA content. This can be explained by the introduction of L-his and L-lys into the salt substitute. The present result was supported by the study of Los and Murata (1998), who found that histidine and iron ions constituted the catalytic centre of the fatty acid desaturases. The addition of histidine might cause a competitive binding to iron ions at the catalytic centre, resulting in a reduction of fatty acid desaturase activity.

3.4. Comparison of lipase activities between SS-loin and S-loin

Though the lipase activities were markedly decreased with time during the whole process (Table 3), the SS-loin showed a higher neutral lipase activity than that of S-loin at the end of salting. After 8 days of drying-ripening, the activity of neutral lipase in the SS-loin group was 26.42% more than that in the S-loin group. Thereafter, no significant differences were found between SS-loin and S-loin. Similar changes were observed in acid lipase activities (Table 3). The results were in accordance with Ripollés et al. (2011), who reported that no significant difference in acid lipase activity was observed between the alternative formulation (50% NaCl and 50% KCl) and control formulation (100% NaCl) for dry-cured ham.

As for phospholipase, the activity in SS-loin was significantly higher than that in S-loin from 8 to 18 days of drying-ripening ($P < 0.05$). Furthermore, the phospholipase activity in the final SS-loin product was 79.63% more than that in S-loin ($P < 0.05$). This increased phospholipase activity in SS-loin might be responsible for the decreased phospholipids and increased free fatty acid content described before (Table 1).

3.5. Effect of NaCl, KCl, L-his and L-lys on lipase activities

NaCl and KCl exhibited similar influence on phospholipase, acid lipase and neutral lipase activities (Fig. 2a, b). The acid lipase and neutral lipase activities were inhibited by NaCl and KCl, whereas the activity of phospholipase was increased with NaCl or KCl up to 0.1 M. This result was in accordance with the findings of Meijer, Ter Riet, Van Himbergen, Musgrave, and Munnik (2002), who reported that phospholipase D was

Table 3
Changes of lipase activities in dry-cured loin during processing.

Lipolytic enzymes	Processing steps											
	Raw	End of salting		Drying-ripening 3 days		Drying-ripening 8 days		Drying-ripening 13 days		End of drying-ripening	
		S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin
Neutral lipase	$150.362^{a} \pm 4.005$	$118.051^{c} \pm 0.711$	$128.511^{b} \pm 13.592$	$113.824^{cd} \pm 0.643$	$108.041^{d} \pm 3.068$	$74.889^{f} \pm 0.927$	$94.673^{e} \pm 6.472$	$66.750^{f} \pm 1.758$	$71.950^{f} \pm 2.248$	$47.862^{g} \pm 0.716$	$52.544^{g} \pm 0.100$
Acid lipase	$94.509^{ab} \pm 6.629$	$84.110^{b} \pm 17.732$	$100.420^{a} \pm 24.198$	$56.198^{de} \pm 3.129$	$58.553^{dc} \pm 4.008$	$46.466^{de} \pm 5.638$	$65.205^{c} \pm 4.392$	$43.828^{dfe} \pm 3.436$	$42.346^{gfe} \pm 3.692$	$28.192^{g} \pm 1.299$	$31.126^{gf} \pm 1.220$
Phospholipase	$29.387^{a} \pm 3.190$	$20.470^{bc} \pm 2.745$	$21.840^{b} \pm 1.138$	$18.180^{cd} \pm 1.680$	$16.761^{d} \pm 1.341$	$13.509^{e} \pm 1.875$	$18.829^{cd} \pm 1.337$	$9.243^{g} \pm 2.683$	$11.335^{f} \pm 2.058$	$3.747^{i} \pm 1.087$	$6.731^{h} \pm 1.451$

Lipolytic enzyme activity was expressed as nmol of released 4-methylumbelliferone $h^{-1} \times g$ protein^{-1}.
a–i Means in the same row with different superscripts differ significantly ($p < 0.05$).

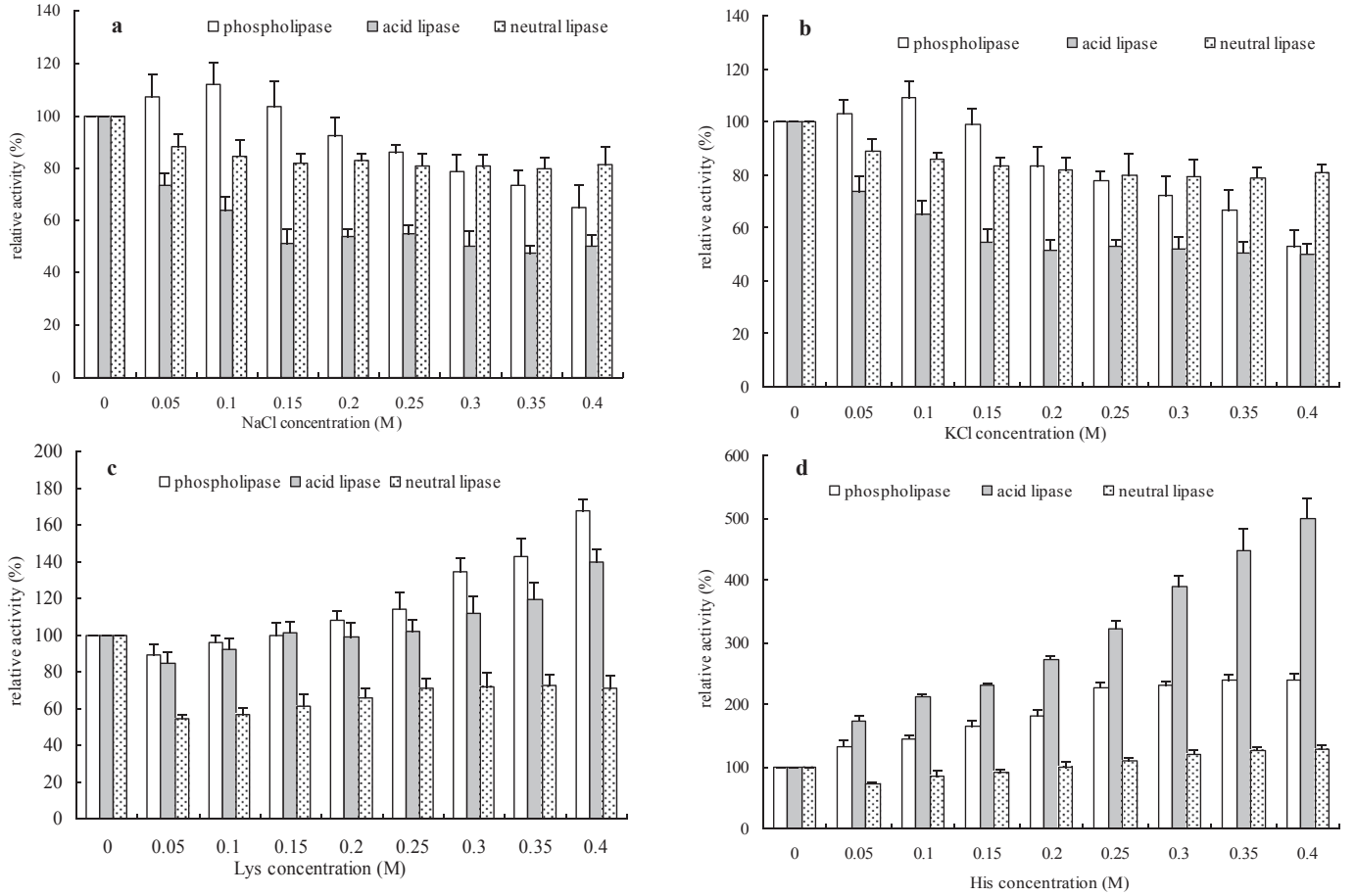

Fig. 2. Effect of salt substitute components on the activities of phospholipase, acid lipase and neutral lipase of dry-cured loin.

activated more strongly by low (0.05 M) than by high (0.4 M) KCl concentration.

As is shown in Fig. 2c, the activities of phospholipase, acid lipase and neutral lipase increased with the concentrations of L-lys. Compared to the control, the phospholipase activity was inhibited by 0.05 M and 0.1 M L-lys, whereas the activity was promoted by L-lys ranging from 0.2 to 0.4 M ($P < 0.05$). The acid lipase activity was inhibited by L-lys in the range of 0.05–0.25 M, whereas it was promoted from 0.3 to 0.4 M. A significant inhibition of neutral lipase by L-lys was observed in the range of 0.05–0.4 M ($P < 0.05$).

The activities of phospholipase, acid lipase and neutral lipase were increased with the concentrations of L-his (Fig. 2d). The phospholipase activity in 0.05 M L-his was higher than the control and increased with the concentration of L-his up to 0.25 M. However, a significant decrease in neutral lipase activity was found in the range from 0.05 to 0.4 M L-his ($P < 0.05$). It was a remarkable finding that the activity of acid lipase was significantly increased with L-his concentrations compared to the control. The introduction of L-his in salt substitute might influence the catalytic activity of lipase in the dry-cured loin. It was known that L-his was part of the active centre of acid lipase and phospholipase in lysosomes and bacteria (Du, Duanmu, Witte, & Grabowski, 1998; Verheij et al., 1980). This notable present result is supported by the work of Han and Rhee (1985), who observed that addition of histidine substantially increased the activity of lipase-catalysed hydrolysis of olive oil in reverse micelles.

3.6. Lipid oxidation comparisons

The POV markedly increased ($P < 0.05$) during salting and the first stages of drying-ripening and reached the peak after 3 days of drying-ripening. Thereafter, POV decreased until the end of the drying-ripening process (Table 4). After 3 days of drying-ripening, the POV value in SS-loin was 4.54% less than that in S-loin ($P < 0.05$).

TBARS values in the final S-loin and SS-loin products were 107.69% and 96.92% more than that in raw loin, respectively. Furthermore, the TBARS in the final SS-loin was 5.19% lower than that in S-loin ($P < 0.05$). In the work of Zhai, Zhou, and Ashraf (1995), histidine, being as a single oxygen scavenger, was found to significantly inhibit TBARS formation and prevent lipid peroxidation of rat myocardial membranes. It seemed that the histidine or/and lysine in the salt substitute contributed to decelerate lipid peroxidation in dry-cured loin.

4. Conclusion

In summary, the present work showed that using a salt substitute containing L-his and L-lys induced a decrease of 53.79% in Na content of the dry-cured loins, delayed lipid oxidation and produced a slightly higher lipolysis in the loins, resulting in larger content of free fatty acids and higher phospholipase activity at the end of the ripening stage. Furthermore, the activities of phospholipase and acid lipase in porcine loin were activated by L-his and L-lys. It was demonstrated that the introduction of L-his and L-lys not

Table 4
Changes in lipid oxidation indices during processing of dry-cured loin.

Lipid oxidation indices	Processing steps											
	Raw	End of salting		Drying-ripening 3 days		Drying-ripening 8 days		Drying-ripening 13 days		End of drying-ripening	
		S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin	S-loin	SS-loin
POV	$0.033^{d} \pm 0.004$	$0.045^{c} \pm 0.004$	$0.046^{c} \pm 0.004$	$0.066^{a} \pm 0.007$	$0.063^{b} \pm 0.006$	$0.064^{ab} \pm 0.006$	$0.065^{a} \pm 0.006$	$0.061^{b} \pm 0.006$	$0.063^{b} \pm 0.006$	$0.041^{d} \pm 0.004$	$0.040^{d} \pm 0.004$
TBARS	$0.065^{e} \pm 0.006$	$0.126^{d} \pm 0.011$	$0.121^{d} \pm 0.013$	$0.128^{c} \pm 0.013$	$0.129^{c} \pm 0.012$	$0.136^{ab} \pm 0.014$	$0.132^{b} \pm 0.014$	$0.141^{a} \pm 0.016$	$0.138^{a} \pm 0.015$	$0.135^{b} \pm 0.013$	$0.128^{c} \pm 0.012$

POV was expressed as milliequivalents peroxide/kg lipid; TBARS was expressed as mg MDA/kg muscle.
[a–e] Means in the same row with different superscripts differ significantly ($p < 0.05$).

only produced a reduction in sodium content but also promoted physicochemical properties in dry-cured loin.

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