Effects of substitution of NaCl with KCl, L-histidine, and L-lysine on instrumental quality attributes of cured and cooked pork loin

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ABSTRACT
This study evaluated the effect of substituting NaCl with varying amounts of a combination of KCl, L-histidine (L-his), and L-lysine (L-lys) on the instrumental characteristics of cooked loin. Fifteen cooked loins were produced by replacing 0%, 25%, 50%, 75%, and 100% of NaCl with the salt substitute. Experiments were conducted in triplicate to determine the water-holding capacity (WHC), and T2 relaxation time, and a texture profile analysis (TPA) and color determination test were also performed. T2 relaxation time analysis indicated that substitution affected the distribution of water by increasing the proportion of immobilized water and improving the WHC. Results of TPA and color tests showed no adverse effect from using the experimental treatments. Results showed that 50% was the most suitable substitution ratio (actual 30.15% substitution level of NaCl), for which the Na content was approximately 27% lower than that of the control for cooked loin.

1. Introduction
Almost 20% of dietary sodium comes from meat and meat products (Desmond, 2006). The demand for low-sodium foods, especially meat products, continues to increase (Pietrasik & Gaudette, 2014; Ruusunen & Puolanne, 2005) due to increasing awareness that excessive sodium intake can increase the risk of hypertension and cardiovascular disease (Cook et al., 2007; Dickinson & Havas, 2007; Paik, Wendel, & Freeman, 2005). However, reducing the content of salt/sodium in meat products has become a major challenge for the meat industry, as salt/sodium contributes to many important attributes within meat and meat processing, such as the WHC, texture, and flavor (Aliño, Grau, Fuentes, & Barat, 2010; Tobin, O’Sullivan, Hamill, & Kerry, 2013).

An effective approach for reducing the amount of sodium in meat products is to substitute NaCl with other salts that have similar functionalities, such as KCl, MgCl2, CaCl2, or K-lactate (Aliño, Grau, Toldrá, & Barat, 2010; Costa-Corredor, Muñoz, Arnau, & Gou, 2010; Horita, Morgano, Celeghini, & Pollonio, 2011; Lorenzo et al., 2015) In the study of Gou, Guerrero, Gelabert, and Arnau (1996), substitution of NaCl with KCl at levels of 10%-60% did not affect the texture and colour of dry-cured loin, but a noticeable bitter taste was detected in substitution levels of 50% and 60%. Lorenzo et al. (2015) demonstrated that treatment III (45% NaCl, 25% KCl, 20% CaCl2 and 10% MgCl2) and IV (30% NaCl, 50% KCl, 15% CaCl2 and 5% MgCl2) was not significantly different from the control (100% NaCl) in terms of color, odor and hardness of dry-cured lacón, but there was lower saltiness and higher bitterness in treatment III and IV compared to the control. In Lee et al.’s study (2012), although no significant changes were observed for sensory characteristics below 50% KCl substitution in marinated broiler, replacement at greater than 50% of NaCl with KCl resulted in significant decreases in tenderness and WHC. It was suggested that substitution NaCl with other salts caused flavour defects or meat product quality deterioration.

Recently, some studies have investigated the influence of a salt substitute containing amino acids on the physico-chemical or biochemical properties of meat. Campagnol, Dos Santos, Morgano, Terra, and Pollonio (2011) found that a significant reduction in the cohesiveness and sensory qualities of fermented sausages when 50% of NaCl was replaced...
by KCl, lysine, taurine, and disodium salts. Different from it, physicochemical properties were promoted in the study of Zhang, Zhang, Hui, Guo, and Peng (2015), who used a salt substitute containing L-histidine and L-lysine to replace 50% of salt in dry-cured ham. The addition of L-lysine could lead to 80% of soluble chicken breast myosin in low salt concentration solution (1 mmol/L KCl) (Hayakawa, Ito, Wakamatsu, Nishimura, & Hattori, 2009), resulting in fine gel properties (Hayakawa et al., 2012). Furthermore, L-lysine and/or L-histidine may contribute to the salty taste of NaCl through various interactions (Zhang et al., 2014). Nevertheless, there have been few attempts to use amino acids as salt substitutes in cooked meat products. In this study, we hypothesize that L-lysine and L-histidine as a salt substitute component may not only produce a reduction in the sodium content but may also affect the physicochemical properties of cooked meat products.

2. Materials and methods

2.1. Preparation of cooked pork loin

Fifteen fresh pork loins (longissimus lumborum) with an average weight of 1.5 ± 0.2 kg and a mean pH of 5.66 ± 0.01 measured 24 h post-mortem were selected from the local slaughterhouse (Sushi Co., Ltd, Nanjing, Jiangsu, China). The loins were frozen at −35°C, stored at −20°C for 30 days in vacuum packaging, then thawed at 4°C for 72 h and randomly divided into five treatments comprising three loins in each treatment. Loins from one treatment were used with the traditional NaCl content (100% NaCl). The other treatments were salted using the same processing conditions, but with a partial substitution of NaCl with a salt substitute (Roly’s Co., Ltd, Nanjing, China) (Table 1). The salt substitute contained 39.7% of NaCl, 51.3% of KCl and a mixture of L-his and L-lys (9.0%).

For each treatment, the formulation of cooked loin included 2.3% salt (NaCl or NaCl + salt substitute), 0.0125% sodium nitrite, 0.32% polyphosphates with sodium tripolyphosphate (STP), sodium pyrophosphate (SPP), and sodium hexametaphosphate (SHMP) in proportions of 3:3:4, and L-lysine (9.0%).

The samples were injected using injector equipment (Model B225-JI, EXPRO Machinery Engineering Co., Ltd, Jiaxing, China) to the required injection level of 20%, and then placed in a tumbler (Model 125ESK STL, VAKONA GmbH, German) and tumbled at 15 rpm for 20 h with alternating tumbling and resting times of 20 min and 5 min at 4°C in an 85% vacuum. The five treatments were tumbled in separate tumbler batches. After tumbling, all samples from each treatment were simultaneously cooked in a cooking chamber (Model BYXX-50, EXPRO Machinery Engineering Co., Ltd, Jiaxing, China) for 70 min at 125°C. The loins were then cooled to 25°C, vacuum packaged and stored at −20°C prior to further analysis.

2.2. Water-holding capacity (WHC)

The WHC was determined according to the method described by Trout (1988). Samples obtained from the central portion of the loin were cut parallel to the muscle fibers into 10 mm × 5 mm × 5 mm cubes and centrifuged for 20 min at 6000 g and 4°C (Model Allegra 64R Centrifuge, Beckman Coulter, Inc. 250 S.Kraemer Boulevard, Brea, CA 92821, USA). The released water was dried from the surface of the sample with filter paper. The WHC was expressed as the ratio of the sample weight after centrifugation to the initial weight:

\[
\text{WHC} (\%) = \frac{(B - 100)}{A}
\]

where A is the initial weight of the sample and B is the weight of sample after centrifugation. Each determination was performed in triplicate.

2.3. Texture profile analysis

Texture profile analysis was performed using a texture analyzer (Model TMS-PRO, ENSOUL TECHNOLOGY LTD, Beijing, China). The test speed was 60.00 mm/min, and the starting force was 0.6 N. Samples (25 mm in diameter, 20 mm height) were compressed to 50% of their original height with a 1000 N probe. The interval time between pressing was 30 s, and the rise height was 20 mm. The following main attributes were calculated: hardness (peak force on first compression, Hd[N]), springiness (distance the sample recovered after the first compression, Sp [mm]), cohesiveness (ratio of the active work done under the first compression curve, Ch), gumminess (the product of hardness and cohesiveness, Gm [N] and chewiness (the product of gumminess and springiness, Cw [mJ]) (Desmond & Kenny, 2005). All measurements were performed at room temperature.

2.4. Color determination

Color determination was conducted with a CR-400 color chromatic meter (Konica Minolta, Inc., Japan) with an 8 mm diameter measurement aperture, illuminant D 65 and 2° Standard Observer at room temperature. Color was measured just after cutting the surface. The chosen analytic indexes were: lightness (L*), redness (a*) and yellowness (b*), and the instrument was calibrated against a white plate (L* = 99.28, a* = 0.19, b* = 0.85) prior to conducting measurements.

Table 1. Replacement level of salt substitute and estimated content of Na and K in cooked loin.

| Treatment | NaCl (%) | Substitute ratio of the salt substitute (%) | Actual substitution levels of NaCl (%) | Na | K |
|-----------|----------|------------------------------------------|--------------------------------------|----|----|
| A         | 100      | -                                        | 0                                    | 1.011 | 0.2 |
| B         | 75       | 25                                       | 15.225                               | 0.874 | 0.35 |
| C         | 50       | 50                                       | 30.15                                | 0.738 | 0.51 |
| D         | 25       | 75                                       | 45.225                               | 0.602 | 0.66 |
| E         | -        | 100                                      | 60.3                                 | 0.465 | 0.82 |

Table 1. Nivel de reemplazo del sustituto de sal y contenido estimado de Na y K en el lomo cocido.
2.5. **Low-field NMR relaxation time ($T_2$) measurement**

The $T_2$ relaxation time measurement was conducted on an NMR Analyser (Model Niumag Benchtop Pulsed PQ001, Niumag Electric Corporation, Shanghai, China). The method was performed according to Bertram and Andersen (2007) with minor adjustments. The experiment was performed at 23.2 MHz, 25°C. Approximately 2.0 g of sample, obtained from the central portion of the cooked loin, was placed into a chromatographic tube (2-mL volume), and the entire unit was placed into a cylindrical glass tube (18-mm length) and inserted in the NMR probe. The $T_2$ relaxation time was measured using the Carr-Purcell-Meiboom-Gill sequence (CPMG) with 4096 echoes, 16 scans repetitions, a TR (repetition delay) of 2000 ms and a $\tau$ value (time between 90° pulse and 180° pulse) of 150 $\mu$s.

Relaxation curves were fitted to a multi-exponential shape with MultiExpInv Analysis software (Niumag Electric Corporation, Shanghai, China). The following parameters were assessed: $T_{2b}$, $T_{21}$, $T_{22}$ and $T_{23}$, corresponding to a peak value of the different states of water. $T_{2b}$ and $T_{21}$ referred to the water that existed in macromolecular structures, $T_{22}$ represented immobilized water and $T_{23}$ referred to free water. $P_{2b}$, $P_{21}$, $P_{22}$, and $P_{23}$ corresponding to peak area, represented the relative content of the four components of water (Shao et al., 2016).

2.6. **Statistical analysis**

The data were analyzed using repeated-measures ANOVA and the PRO MIXED procedure of the SAS program 8.01 (SAS Institute, Inc., Cary, NC, USA). Three replications of samples were used for each analysis. The differences in the mean values were compared by Duncan’s multiple range test, and the mean values and standard error of the means (SEM) were reported ($P < 0.05$). Graphs were plotted with OriginPro 8 (Originlab Corporation, Northampton, MA01060, USA).

3. Results and discussion

3.1. **Water holding capacity (WHC)**

Compared to the 100% NaCl treatment, the WHC was 6.79%, 8.64% and 4.94% higher when NaCl was substituted with salt substitute at levels of 25%, 50% and 75%, respectively ($P < 0.05$), while no significant difference was found among these three treatments ($P > 0.05$). At a 100% level of substitution, the WHC was not significantly different from the 0%, 25% and 75% replacement ($P > 0.05$). This positive result was considered to be due to the introduction of L-his and L-lys into the salt substitute, as it has been reported L-his and/or L-lys enhanced the WHC of porcine myosin gels (Zhang, Wu, Jamali, Guo, & Peng, 2017) and chicken breast myofibril gel (Chen et al., 2016). L-His could dissociate myosin filament and increase the solubility of myosin at low ionic strength solution (Hayakawa et al., 2009; Hayakawa, Ito, Wakamatsu, Nishimura, & Hattori, 2010), which affected intermolecular interactions (Chen et al., 2014) and increased water entrapment of myofibrils. The present data indicated that a maximum substitution level of 50% should be applied to improve the WHC of cooked loin.

3.2. **Texture profile analysis**

With an increased substitution ratio, there was no significant difference ($P > 0.05$) in hardness, springiness and chewiness between experimental treatments and the control (Table 2), while salt substitute caused an increase in cohesiveness and gumminess ($P < 0.05$). At a 50% level of substitution, the cohesiveness and gumminess were 10.87% and 33.55% higher than the control, respectively. In contrast, Campagnol et al. (2011) found that replacement of 50% NaCl with KCl had no effect on the cohesiveness of fermented cooked sausage. Lee, Zhekov, Owens, Kim, and Meullenet (2012) also found that replacing up to 50% of NaCl with KCl did not affect the marinated broiler meat texture. This might be due to the introduction of L-His and L-Lys into the salt substitute in the present study. L-His and L-Lys caused an increase of the solubility of porcine myosin at low ionic strength solution (Guo, Peng, Zhang, Y. Liu, & Cui, 2015). During cooked ham manufacturing, muscle tissue is disrupted and protein extractability is facilitated by tumbling, ensuring better textural properties (Maddock, 2014). Furthermore, L-His was found to promote the formation of myofibril gel structure with small pores at 1 mM NaCl (Chen et al., 2016). The results in the present study indicated that introduction of L-His and L-Lys contributed to protein extractability, resulting in excellent textural characteristics.

Based on the texture and WHC data from the present study, it was considered that a 50% salt substitute could be used to replace NaCl content in cooked pork loin.

3.3. **Color determination**

The color of cooked loin with respect to the substitution ratios used is presented in Table 3. No significant difference was obtained for lightness value ($L^*$) or yellowness value ($b^*$) among the five treatments ($P > 0.05$). For redness ($a^*$), salt

### Table 2. Effects of different substitution ratios on the TPA of cooked loin (n = 3).

| Substitute ratio (%) | Hardness (N) | Cohesiveness | Springiness (mm) | Gumminess (N) | Chewiness (mJ) |
|----------------------|--------------|--------------|-----------------|---------------|---------------|
| 0                    | 32.06        | 0.46$^b$     | 3.74            | 15.05$^b$     | 56.80         |
| 25                   | 31.89        | 0.50$^a$     | 3.92            | 19.57$^a$     | 54.72         |
| 50                   | 30.33        | 0.51$^a$     | 3.98            | 20.10$^a$     | 56.99         |
| 75                   | 31.61        | 0.49$^a$     | 3.60            | 18.93$^a$     | 55.64         |
| 100                  | 33.82        | 0.50$^a$     | 3.78            | 16.74$^a$     | 54.87         |
| SEM                  | 3.31         | 0.01         | 0.12            | 0.17          | 7.35          |
| P value              | 0.438        | < 0.01       | 0.127           | 0.024         | 0.072         |

Means in the same column with no superscript letters after them or with a common superscript letter following them were not significantly different ($P > 0.05$).
This could be related to a higher amount of extracted protein, including myoglobin pigment in the 25%, 50%, 75% and 100% substitution treatments; this result was supported by the findings of Youssef and Barbut (2009), who found that a higher protein level significantly increased the redness (*P* < 0.05) of meat products.

Furthermore, the color determination results were consistent with WHC and textural properties data in the present study. Bowker and Zhuang (2013) also reported that high-WHC broiler breast fillets were redder than low-WHC broiler breast fillets.

### 3.4. Sodium and potassium contents

The sodium and potassium contents of cooked pork loin are presented in Figure 2. A significant reduction in Na and an increase in the K content were obtained through the substitution of NaCl with the salt substitute (P < 0.05). The final cooked loin product made with salt substitute at 0%, 25%, 50%, 75% and 100% contained 330, 460, 630, 770, and 900 mg/100 g of K, respectively. It was shown that the measured values of K content were higher than those of estimated content in Table 1, which might be due to the low estimated K content in the control. On the contrary, the measured values of Na content (1050, 790, 660, 580 and 430 mg/100 g at 0%, 25%, 50%, 75% and 100% substitution level, respectively) were lower than those of estimated content (Table 1) because of a little loss sample during the measurement. According to the Dietary Reference Intakes from the Institute of Medicine, an adequate intake of K was 4700 mg/day (McGuire & Beerman, 2007), and thus such a cooked meat product only contributed one fifth of the 4700 mg/day adequate intake when the NaCl was substituted with 100% salt substitute. In addition, it has been shown that a 600 mg/day increase in dietary K intake lowers blood pressure by 133 Pa (ICRG, 1988).

### 3.5. Low-field NMR relaxation (*T*2) time measurements

Low-field NMR can provide information about water mobility in meat or meat products (Bertram & Ersen, 2004; Marcone et al., 2013). In the present study, four peaks were detected by multi-exponential fitting of a *T*2 distribution used to measure the relaxation time of hydrogen protons (Figure 3).
T\textsubscript{22} and T\textsubscript{23} represent immobilized water (inside the myofibrillar network) and free water (outside the myofibrillar structure), respectively (Bertram & Ersen, 2004). Compared to the control group (T\textsubscript{22} 50.19 ms and T\textsubscript{23} 316.82 ms), a significant decrease was obtained in T\textsubscript{22} (41.41 ms) and T\textsubscript{23} (266.9 ms) for the 50% replacement treatment (P < 0.05, Figure 4), indicating that both immobilized and free water were more restricted. Correspondingly, a distinct increase in P\textsubscript{2b} (97.17%) and a decrease in P\textsubscript{2i} (1.14%) (P < 0.05, Fig. 5) were found with the 50% replacement treatment compared to the control group, which was consistent with the WHC results obtained in this study. This suggested that more immobilized water was trapped inside the myofibrillar protein and myosin network when using the salt substitute than with the control. The strongest water holding capacity of cooked loin with 50% replacement might be caused by this difference.

There were no significant differences (P < 0.05) in values of T\textsubscript{2b}, T\textsubscript{21} and P\textsubscript{2b}+ P\textsubscript{21} between the control and experimental treatments (where T\textsubscript{2b} and T\textsubscript{21} reflect water bound to macromolecular structures and P\textsubscript{2i} represents the relative content of the four components of water); these results
were consistent with the results of Bertram, Dønstrup, Karlsson, and Andersen (2002) and Pearce, Rosenvold, Andersen, and Hopkins (2011), who reported these factors had only a minor influence on the physicochemical properties. As a result, $T_{2b}$, $T_{21}$, and $P_{2b}$ for the five groups are not be discussed further in this study.

For the 25% and 100% replacement groups, both values of $T_{2s}$ (265.61 ms of 25% and 248.31 ms of 100%) were significantly lower than in the control (316.82 ms) ($P < 0.05$), indicating that free water was more restricted in these two treatments. Moreover, $P_{22}$ (95.31%) was significantly higher with 25% replacement than for the 100% (93.40%) replacement and the control (93.27%) ($P < 0.05$), which was a reflection of the differences in the WHC.

For the 75% replacement treatment, no difference was detected in $T_{2b}$ (46.07 ms) and $T_{2s}$ (294.64 ms), but a significantly higher $P_{22}$ (5.65%) was observed compared to the control (4.19%) ($P < 0.05$). This may account for the somewhat higher WHC of 75% compared to the control. Therefore, it is suggested that both $P_{22}$ and $P_{23}$ are directly correlated with WHC in cooked loin, in accordance with the study by Bertram et al. (2002), who reported that the populations of immobilized and free water were related to the WHC in pork longissimus dorsi. In the control, the higher $T_{2s}$ (50.19 ms) and $T_{2b}$ (316.82 ms) among the five groups implied a high mobility of immobilized and free water. Moreover, cooked loin in the control group had a low $P_{22}$ (93.27%) and a poor WHC.

Therefore, increased substitution likely plays an important role in altering the water distribution in cooked loin, which reduced water mobility and transformed varying amounts of free water into immobilized water depending on the substitution ratio. During tumbling, native muscle tissue fiber texture is destroyed and is blurred by degrees, and the substitute gradually diffuses to the interior of the muscle fiber. With an increase in the substitution ratio, an increasing amount of dissociated myosin was constantly shifted outward in the vacuum state to form a network with other proteins during cooking. The substitute increased the protein content extracted from muscle and the probability of interactions between proteins. For the 75% and 100% replacement groups, the quantity of KCl increased by approximately 40% and 50% compared to the control.

The diffusion rate of potassium is faster than sodium due to a smaller hydrate ion radius (Jr, 1958; Volkov, Paula, & Deamer, 1997). As a result, higher replacement could require a shorter tumbling time to achieve a similar effect. Excessive destruction of the structure was too severe to hold water with a redundant tumbling time for ham (Mueller, 1990), which may account for the poor WHC and uneven texture for the 75% and 100% replacement groups.

### 4. Conclusion

The present work indicated that using a salt substitute consisting of a mixture of NaCl, KCI, L-his, and L-lys induced a distinct decrease in the Na content of the cooked loin, reduced the water mobility and free water content of the loins, and ultimately resulted in improvements in the WHC and textual characteristics. An acceptable color property was also obtained, and it thus implied that the salt substitute could be used to replace 50% of the NaCl (actual 30.15% substitution level) without causing any significant adverse effects on the instrumental properties.
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