

## Research Article

### Effects of L-Arginine on Physicochemical and Sensory Characteristics of Pork Sausage

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**Abstract:** The objective of this study is to investigate the effects of L-arginine on physicochemical and sensory properties of pork sausage. CL decreased while pH increased with L-arginine levels ( $p < 0.05$ ). WHC increased at 0.8% L-arginine, but decreased at 0.2% L-arginine, compared with the control.  $L^*$  decreased while  $a^*$  increased at 0.4-0.8% L-arginine, compared with the control. Hardness, springiness and chewiness increased at 0.2-0.8% L-arginine ( $p < 0.05$ ), compared with the control. SEM illustrated that the addition of 0.6% L-arginine induced myofibrillar proteins to form a more smooth, compact and uniform gel matrix. DSC disclosed that the addition of 0.6% L-arginine increased the two thermal transition temperatures (Tp). The sample containing 0.6% L-arginine had higher sensory color, flavor, mouthfeel and slice traits than the control. Therefore, L-arginine showed a potential for improvement of yield, texture and sensory qualities of pork sausage.

**Keywords:** L-Arginine, pork sausage, physicochemical properties, sensory qualities

## INTRODUCTION

Water binding capacity is a very important in terms of product yield and eating quality (Cheng and Sun, 2008). Also, textural property that affects eating quality is important (Nishinari *et al.*, 2008). In meat industry, phosphate is a traditional curing agent for improvement of Water Holding Capacity (WHC) and texture (Wang *et al.*, 2009). However, phosphate has also been reported to cause the deteriorations of color (Ranken, 2000) and flavor (Fernández-López *et al.*, 2004) of meat product to which it is added. Moreover, an excessive dietary intake of phosphate is potential health risk, especially for kidney disease patients (Sherman and Mehta, 2009). Therefore, considerable attentions have been paid to development of safe meat product.

Polysaccharides have been attempted to produce the meat products for their contribution to desirable binding characteristics, texture and appearance of the meat products (Luruena-Martinez *et al.*, 2004). However, polysaccharides are not considered to be pronounced effective in binding water, stabilizing emulsions and increasing firmness of meat products, especially, in the case of high salt concentration (Xiong *et al.*, 1999). Also, non-meat proteins such as soy protein isolate (Fang *et al.*, 2008), blood plasma (Cofrades *et al.*, 2000) and milk proteins (Hung and Zayas, 1992) have been used as additives in meat product without losing yield or sensory quality. However, non-meat proteins such as egg, milk, soya and wheat proteins may have potential health risks since there are possibilities for these proteins to be allergens (Hurtado *et al.*, 2012). The

application of additives is still an important means to improve flavor and/or enhance texture of meat products (Sun and Holley, 2011). Therefore, the corresponding researches still continue.

Application of amino acids in meat industry has attracted wide interests because amino acids can improve flavor (Turk, 1993) and color (Apple *et al.*, 2004) and increase solubility of myofibrillar proteins of vertebrate skeletal muscle (Ito *et al.*, 2003). More recently, we have reported that L-lysine is effective in improvement of water binding capacity, texture and microstructures (Zhou *et al.*, 2014). Similar to L-lysine, L-arginine can also adjust pH (Baker, 2007), coordinate with metallic ions (Remko *et al.*, 2008) and prevent fat and protein from oxidation (De Nigris *et al.*, 2003). Theoretically, these properties indicated that L-arginine might have a potential for improvement of water binding capacity, texture and microstructures of meat product. Besides these common properties, L-arginine can serve as nerve conduction factor, which is related to brain memory (Kathleen, 2006). Additionally, it is able to dilate blood vessels (Ignarro *et al.*, 2007) and inhibit proliferation of tumor cell (Singh *et al.*, 2000). In China, L-arginine has been approved for food used as flavoring essence and spice since 2011 (<http://wsb.moh.gov.cn/zwgkzt/psp/wsbz.shtml>). However, up to date, no document has been reported on the effects of L-arginine on color, texture and water binding capacity of meat product. The objective of this study is to clarify that L-arginine decreased Cooking Loss (CL) and improved color, textural property, microstructures and sensory qualities of pork sausage.

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## METASYNTHESIS

**Materials:** L-Arginine ( $\geq 99\%$ , biological reagent) was purchased from Shanghai Yuanju biotechnology Co., Ltd. (Shanghai, China). Sodium chloride ( $\geq 99.5\%$ , analytical reagent) was purchased from Dongxing Salt Chemical Co. Ltd. (Dingyuan, China). Ginger powder (food grade) and white pepper (food grade) were purchased from Hefei Runwu Industry and Trade Co., Ltd. (Hefei, China). Monosodium glutamate (food grade) was purchased from Shanghai Totole Food Co., Ltd. (Shanghai, China).

Frozen pork legs were purchased from Carrefour, a local supermarket in Hefei. The proper amount of frozen pork legs was thawed for 24 h at  $2-4^{\circ}\text{C}$  and then removed of skin, bone, visible fat and connective tissue. The trimmed pork legs were ground with a meat grinder (YEEKAI, MGB-120, Guangdong, China) fitted with a plate of 6 mm diameter holes. The proximate compositions of the ground meat were measured to be moisture  $75.03\pm 0.16\%$ , protein  $21.51\pm 0.17\%$ , fat  $2.46\pm 0.16\%$  and ash  $1.07\pm 0.07\%$  (in triplicates).

**Preparation of pork sausages:** The trimmed lean tissue and fresh pork backfat in accordance with the ratio of 9:1 were mixed and then ground with a chopper (YEEKAI, MGB-120, Guangdong, China). To the meat mixture, 5% water, 2.5% sodium chloride, 0.05% monosodium glutamate, 0.2% ginger powder and 0.2% white pepper powder were added. Additionally, the pre-assigned levels of L-arginine (A: 0% L-arginine, B: 0.2% L-arginine, C: 0.4% L-arginine, D: 0.6% L-arginine and E: 0.8% L-arginine) were also added. The added ingredients were based on meat mixture. The meat mixture was mixed thoroughly for another 15 min and then stuffed into polyethylene casing ( $\phi$ : 30 mm). Each sample was approximately 20 g. The raw sausages were stored at  $4^{\circ}\text{C}$  for 24 h and heated to an internal temperature of  $85\pm 1^{\circ}\text{C}$  in a water bath for 40 min. Cooled with tap water, the sausages were stored at  $4^{\circ}\text{C}$  for 15 h without packaging for analysis.

**Cooking Loss (CL) and Water Holding Capacity (WHC):** CL was determined according to the protocol described by Ma *et al.* (2012). The chilled samples were removed from the casing. The gels were immediately wiped with qualitative filter paper and weighted. CL was expressed as a percentage based on the raw stuffed net weight. All measurements were carried out in triplicates.

Water holding capacity (WHC) was determined according to the protocol described by Ma *et al.* (2012). The known weight of samples (19 mm in diameter, 10 mm height) were wrapped with qualitative filter paper and then placed in a centrifuge tube (25 mm in inner diameter) filled with degreasing cotton in the bottom. The samples were centrifuged at 3800 g and  $25^{\circ}\text{C}$  for 10 min. WHC was expressed as the ratio of gel weight after

centrifugation to the initial gel sample weight. All measurements were carried out in triplicates.

**Measurement of pH:** pH measurement was performed according to the method described by Deng *et al.* (2002) with some modifications. Sausage samples ( $5.0\pm 0.1$  g) were blended with 45 mL of distilled water for 1 min with a shearer (FA25, Fluko, German). pH values of the mixture were immediately determined with a pH meter (model PHS-3C, Shanghai Sanxin Instrumentation Inc.) equipped with a combination pH electrode calibrated to pH from 0 to 14. All measurements were carried out in triplicates.

**Color measurement:** The color of pork sausages were measured using the Hunter scale with an automatic colorimeter WB-2000 IXA (Beijing Kangguang Instrument Co., Ltd, China) according to the procedure described by Zhou *et al.* (2012). Specifically, the samples were cut to 1.0 cm lengths and measured immediately. Six measurements for each of three replicates were expressed as  $L^*$  value,  $a^*$  value and  $b^*$  value. The overall lightness or darkness was determined by  $L^*$  value (0 = black, 100 = white). The higher  $L^*$  value of the sample has the higher brightness. Red (positive =  $a^+$ ) and green (negative =  $a^-$ ) intensity are represented on the  $a^*$  scale and yellow (positive =  $b^+$ ) and blue (negative =  $b^-$ ) intensity on the  $b^*$  scale.

**Texture profile analysis:** Texture profile analysis was carried out according to the protocol reported by Ma *et al.* (2012) with a slight modification. The analysis was achieved by using a TA-XT plus Texture Analyzer (Stable Micro System Co., England) at room temperature. The samples were compressed twice with a cylindrical 6 probe (P/36R, stainless steel) at trigger type Button, pre-assigned speed 2.0 mm/s, test speed 1.0 mm/s, post-test speed 1.0 mm/s, distance 4.0 mm, trigger force 0.05 N and time lag 5 s between two compressions. The TPA parameters were expressed as hardness (N), springiness (dimensionless), cohesiveness (dimensionless) and chewiness (N). All measurements were carried out in triplicates.

**Scanning Electron Microscopy (SEM):** The microstructures of two samples (A: without L-arginine; B: with 0.6% L-arginine) were investigated according to the protocol described by Chen *et al.* (2010) with some modifications (in triplicates). Sample blocks ( $1\times 1\times 1$  mm) were cut with a razor blade from the inside of each treatment. These blocks were fixed with a mixture of equal volume of formaldehyde (4%) and glutaraldehyde (2.5%) for 2 h and rinsed 5-10 times in 0.1 M phosphate buffer (pH = 7.2) (10 min each). The samples were dehydrated with ethanol in a graded series of 30, 50, 70, 80, 90 and 95% at each ethanol concentration for 10 min. Dehydration was completed with three 10 min washes in 100% ethanol. Subsequently, the samples

were dried for 15-16 h with an Alpha 1-4 LSC Christ lyophilizer and sputter-coated (Jeol JFC-1600 Tokyo, Japan) using gold/palladium and observed with a Scanning Electron Microscope (Jeol, JSM 6490LV, Tokyo, Japan) at 20 kV.

**Differential Scanning Calorimeter (DSC):** DSC was carried out according to the procedure described by Ma *et al.* (2012) with some modifications (two replicates). Two samples (A: without L-arginine; B: with 0.6% L-arginine, unheated and stored for about 24 h at 4°C) were determined by a Q200 DSC calorimeter (TA, USA) calibrated against indium. The sample (10-15 mg) was sealed into the DSC pan hermetically. The sample pan and reference pan were placed in the DSC sample holder and allowed to equilibrate at the initial scanning temperature (20°C) for 5 min and then heated from 20 to 100°C at a rate of 5°C/min. Sample holders were filled with 100% nitrogen before and during heating. The temperature at each endothermic peak was recorded using Universal Analysis 2000 software (TA Instruments). The thermal transition temperature ( $T_p$ ) was described as the maximum transition temperature of the main endothermic peak and the apparent enthalpy ( $\Delta H$ , J/g of meat mixture) was described as the global area under the DSC curve.

**Sensory evaluation:** Nine food science and engineering-majored master graduate students of Hefei University of Technology were chosen as the members of sensory panel. The criteria for panelists were who have normal or superior visual and taste acuity, a good grasp of the knowledge of food sensory evaluation and are willing to evaluate the color, flavor, mouthfeel and slice traits of the L-arginine-treated pork sausages. Before conducting the experiment, the panelists were trained for the development of a sensory memory with respect to each descriptive term. The method described by Wang and Zhao (2008) was used to prepare the list of descriptive terms. The panelists were asked to list the differences and similarities of the samples being presented in pairs. The following scales were used: color (10-8 = red and bright, 7-5 = pale and slightly red, 4-1 = pale and slightly dark); flavor (10-8 = tasty, 7-5 = slightly irritating smell and basically acceptable, 4-1 = strongly irritating smell and not acceptable); mouthfeel (10-8 = good, 7-5 = moderate, 4-1 = poor) and slice traits (10-8 = solid and smooth; 7-5 = slightly softening along peripheral and slight fissures, 4-1 = strongly softening). The samples were cooled to the room temperature and stored at 4°C until sensory evaluation. Three samples for each treatment were coded with random numbers. The samples in polyethylene casing were submitted to each panelist, subsequently, opened and evaluated individually. All the sensory evaluations were carried out at room temperature under natural light.

**Statistical analysis:** The data, expressed as mean±Standard Deviation (SD), were analyzed by Excel 2003 (Microsoft official Excel 2003 for Windows).

Significance level of  $p < 0.05$  was used to determine differences among treatments throughout the study.

## RESULTS AND DISCUSSION

**CL, WHC and pH:** CL, WHC and pH measurement was carried out in order to reveal the effects of L-arginine on CL, WHC and pH of pork sausages. Figure 1 illustrated the effects of L-Arginine on CL and WHC of pork sausage. As showed in Fig. 1a, L-arginine affected CL of pork sausages, significantly ( $p < 0.05$ ). The addition of less than 0.6% L-arginine decreased CL values ( $p < 0.05$ ). Further addition of L-arginine did not affect CL values ( $p > 0.05$ ). The result indicated that L-arginine had the benefit of increasing pork sausage productivity. Compared with the control, the sample treated with 0.2% L-arginine had lower WHC value, while that treated with 0.8% L-arginine had higher WHC value, significantly ( $p < 0.05$ ). WHC value of the sample treated with 0.4% or 0.6% L-arginine was not different from that of the control, significantly ( $p > 0.05$ ) (Fig. 1b). The behaviors that the addition of L-arginine caused the reductions of CL are similar to those of L-lysine (Zhou *et al.*, 2014). Puolanne *et al.* (2001) revealed that the water binding capacity of meat product was related to its pH value. It is well known that the isoelectric point (pI) of the major proteins, especially myosin is 5.4 (Puolanne and Halonen, 2010). Theoretically, the addition of L-arginine can lead to the increase of pH values of meat and meat product since it is a basic amino acid (Baker, 2007), which resulted in the changes in CL and WHC. It was also reported that the changes in pH values of meat and meat product led to the changes in CL and WHC (Young *et al.*, 2005). Subsequently, pH values of the samples treated with 0, 0.2, 0.4, 0.6 and 0.8% L-arginine were measured respectively (Fig. 2). These results showed that the addition of L-arginine increased significantly pH ( $p < 0.05$ ) and thus affecting CL values of pork sausages. Additionally, there are many other factors such as moisture content, electrostatic, hydrogen and hydrophobic bonds that affect water binding capacity (Ma *et al.*, 2012). Moisture content might be dominant factor that affect WHC of pork sausage treated with low levels of L-arginine. Therefore, compared with the control, the sample treated with 0.2% L-arginine had lower WHC might be due to higher moisture content (lower CL, Fig. 1a). However, electrostatic might become dominant factors and resulted in the increase of WHC when the level of L-arginine increased.

**Color:** Color measurement was performed in order to disclose the effects of L-arginine on color of pork sausages. As shown in Fig. 3, L-arginine affected the color of pork sausages. The samples which had more L-arginine added tended to have lower  $L^*$ , significantly ( $p < 0.05$ ). Hong *et al.* (2006) found that the changes in  $L^*$  values of meat product were associated with its moisture content and that the meat product that had

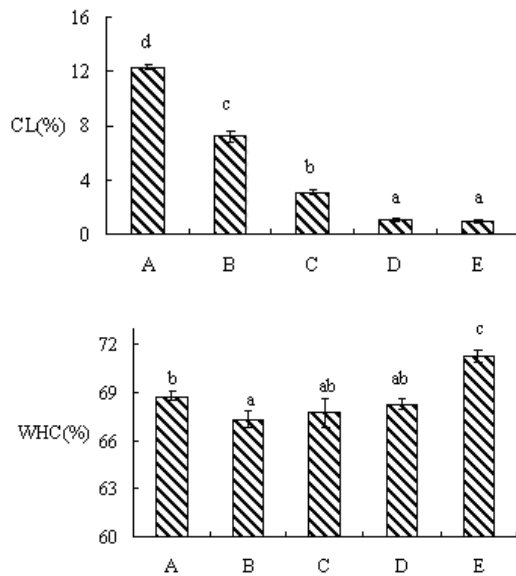


Fig. 1: CL and WHC of pork sausages, (a) and (b) illustrate CL and WHC, respectively; A: Control, without L-arginine; B: 0.2% L-arginine; C: 0.4% L-arginine; D: 0.6% L-arginine; E: 0.8% L-arginine; All samples were heated to an internal temperature of  $85\pm 1^\circ\text{C}$  for 40 min, subsequently, cooled and stored at  $4^\circ\text{C}$  for 15 h without packaging for analysis. Means $\pm$ SD having different letters (a-e) means that the five treatments are significantly different ( $p < 0.05$ )

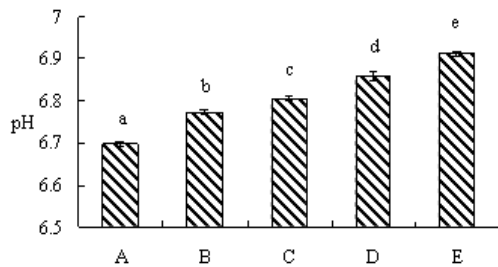


Fig. 2: pH of different treatment; The conditions were the same as Fig. 1

higher moisture content had lower  $L^*$  values. The L-arginine-treated samples had lower  $L^*$  values since the addition of L-arginine caused the decrease of CL (higher moisture content) (Fig. 1a). The samples that had 0.4% and more than 0.4% L-arginine added had higher  $a^*$  values than the control ( $p < 0.05$ ). The changes in  $a^*$  values reflect the changes in deoxy-myoglobin (Mb) and/or oxy-myoglobin ( $\text{MbO}_2$ ) portion (Jung *et al.*, 2003) by an oxidation of ferrous myoglobin to ferric met-myoglobin (Carlez *et al.*, 1995). L-arginine is reported to have the capacities of antioxidant activity (De Nigris *et al.*, 2003) and binding with endogenous metallic ions (Remko *et al.*, 2008). These metallic ions can fasten Mb and/or  $\text{MbO}_2$  oxidation (Yamashita *et al.*,

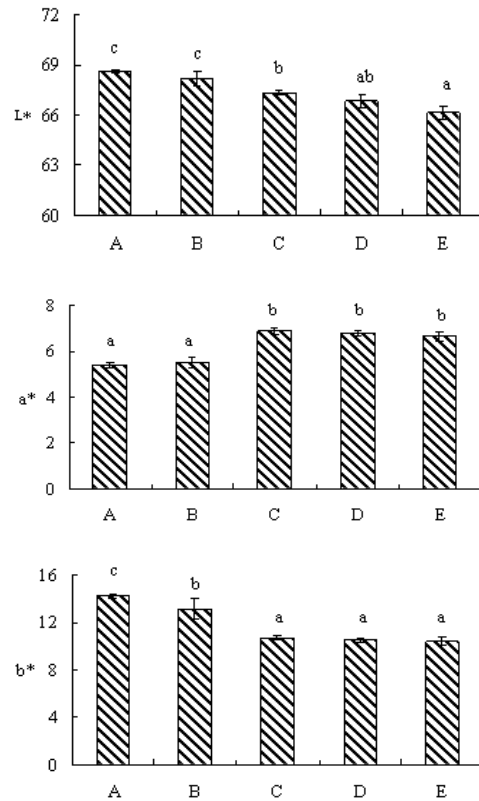


Fig. 3: Color of different treatments, (a), (b) and (c) illustrate  $L^*$  values,  $a^*$  values and  $b^*$  values, respectively. The conditions were the same as Fig. 1

1990). Therefore, it was possible that the addition of L-arginine to pork sausages prevented Mb and/or  $\text{MbO}_2$  from oxidation and thereby enhancing  $a^*$  values. The changes in  $b^*$  values were similar to those of  $L^*$  values, that is, the samples treated with L-arginine had low  $b^*$  values, significantly ( $p < 0.05$ ). Bekhit *et al.* (2005) reported that the infusion of calcium to fresh lamb led to reductions in  $b^*$  values along with increase in met-myoglobin accumulation rate. They speculated that the result could be related to the lower amounts of unbound water. The addition of L-arginine caused the decrease of CL (Fig. 1a), which was the possible reason that L-arginine-treated sausages had low  $b^*$  values. These results showed that the L-arginine-treated samples were less dark, more reddish and less yellowish than the control.

**Texture:** Texture profile analysis was carried out in order to illustrate the effects of L-arginine on textural properties of pork sausages. As shown in Table 1, the addition of L-arginine increased significantly hardness ( $p < 0.05$ ). The L-arginine samples had significant higher springiness than the control had. The changes in chewiness had the similar tendency to those in hardness, that is, the L-arginine samples had significantly higher chewiness than the control ( $p < 0.05$ ). Basically, the

Table 1: Texture profile analysis of different treatments

Treatment	A	B	C	D	E
Hardness (N)	18.148±0.346a	19.165±0.302b	19.092±0.359b	19.584±0.405b	18.766±0.303b
Springiness	0.910±0.006a	0.922±0.004b	0.932±0.003c	0.940±0.005c	0.944±0.008c
Cohesiveness	0.841±0.010b	0.841±0.005b	0.830±0.005ab	0.823±0.004a	0.830±0.011ab
Chewiness (N)	13.88±0.154a	14.86±0.206bc	14.77±0.192bc	15.15±0.385c	14.70±0.156bc

The conditions were the same as Fig. 1. Means ± SD (n = 3) within the row having unlike letters (a-c) means that each treatment is significantly different (p<0.05).

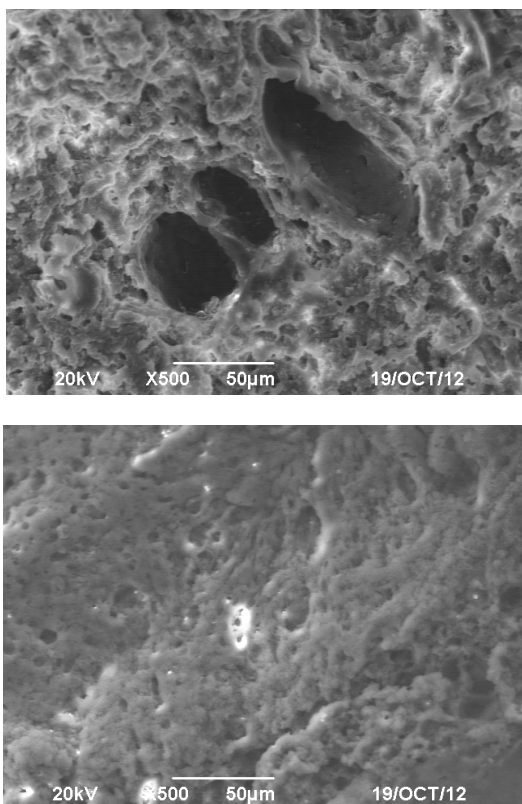


Fig. 4: SEM images of different treatments; A: Control, without L-arginine; and B: 0.6% L-arginine. All samples were heated to an internal temperature of 85±1°C for 40 min, subsequently, cooled and stored at 4°C for 15 h without packaging for analysis

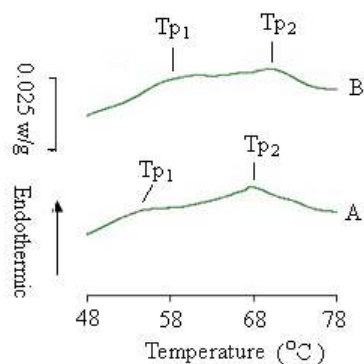


Fig. 5: Typical DSC normalized traces of different treatments; A: without L-arginine; B: with 0.6% L-arginine; The samples were unheated and stored for about 24 h at 4°C and then determined by a Q200 DSC calorimeter (TA, USA) calibrated against indium

addition of L-arginine decreased cohesiveness, but the differences of cohesiveness were not so great to be significant (p>0.05). Also, Barbut (1997) found that gel strength of turkey breast batters increased from pH 4.5 to pH 7.5. Lesiów and Oziemblowski (2003) disclosed that the gel strength was positively correlated to pH value. Most proteins aggregate and are least soluble at their pI (Sun and Holley, 2011). Aggregation of proteins prior to heating inhibits gel formation by myofibrillar proteins and aggregation extent depends basically upon the electrostatic charge on the molecules (Boyer *et al.*, 1996), which in turn is affected by pH (Sun and Holley, 2011). Therefore, the addition of L-arginine increased pH and deviated from pI of myofibrillar proteins, which might enhance gel strength.

**SEM:** SEM was carried out in order to investigate the effects of L-arginine on microstructure of pork sausages. Meat proteins without L-arginine formed a three-dimensional gel network. However, there existed many cavities and large clusters that were distributed in a three-dimensional gel network (Fig. 4a). The addition of L-arginine caused large clusters/cavities to disperse into small clusters/cavities, thereby forming the dense and uniform networks when 0.6% L-arginine was added (Fig. 4b). As shown in Fig. 2, L-arginine caused the increase in pH values and the deviation from pI of myofibrillar proteins (Puolanne and Halonen). Theoretically, myofibrillar proteins became more soluble and were, easily extracted from meat tissue (Ito *et al.*, 2004), thereby affecting microstructure of pork sausages. In addition, such difference of microstructure can explain the changes in the CL as well as textural parameters. Mao *et al.* (2001) thought that small and uniform pores were responsible for the water holding capacity. Since L-arginine can induce the loss of the large and uneven cavities to form continuous and uniform gel matrix, the L-arginine-treated pork sausages might have higher hardness and lower CL. The gels with small and uniform pores was also observed to have higher hardness and lower CL in duck muscle gels containing 1% curdlan (Chen *et al.*, 2010). So the advantages of L-arginine are not only to improve the textural properties, but to increase potentially the yield of pork sausages.

**DSC:** DSC tests were performed in order to disclose the effects of L-arginine on thermal characteristic of meat protein (Fig. 5). Typical thermal transition temperatures for meat proteins range from 43 to 67°C for myosin and its subunits, 67 to 69°C for sarcoplasmic proteins and 71

Table 2: Sensory evaluation of different treatments

Treatment	A	B	C	D	E
Sensory color	3.33±0.58a	5.00±0.01b	6.00±0.01c	5.67±0.58ab	5.67±0.58ab
Flavor	6.00±0.58a	7.33±0.60b	7.67±0.58bc	8.00±0.00c	6.00±0.01a
Mouth feel	3.67±0.58a	6.67±0.58b	7.00±0.01b	7.33±0.56b	7.67±0.58b
Slice traits	3.67±0.58a	6.33±0.62b	7.33±0.608bc	7.33±0.56bc	8.00±0.58c

The conditions were the same as Fig. 1. Means±SD (n = 3) within the row having unlike letters (a-c) means that each treatment is significantly different (p<0.05).

to 83°C for actin (Smyth *et al.*, 1996). As for the control, two typical thermal transition peaks were located at the temperatures (Tp<sub>1</sub> and Tp<sub>2</sub>) of 54.4°C and 67.6°C, respectively. Therefore, these two peaks were assigned for myosin and its subunits and sarcoplasmic proteins, respectively. In the present work, the peak for actin was not observed. Tp<sub>1</sub> and Tp<sub>2</sub> were increased by 3.1°C and 2.9°C, respectively when 0.6% L-arginine was added to meat mixture. Ma *et al.* (2012) found that locust bean gum induced shifts in salt-soluble meat protein transition temperatures. These authors speculated that interactions between protein and locust bean gum would be the likely indicator of interactions between protein and locust bean gum. Therefore, the present results indicated that interactions between protein and L-arginine might occur during heating process.

**Sensory characteristics:** Sensory qualities of the samples were also evaluated, as summarized in Table 2. Compared with pale exhibited in the internal parts of the control, slight red was exhibited and more acceptable in the same parts of the L-arginine-treated samples. Therefore, the addition of L-arginine increased sensory color (p<0.05). This more red color by sensory evaluation could also be observed by the subjectively color evaluation that indicated increased a\* values. The addition of less than 0.6% L-arginine increased significantly flavor (p<0.05), which is possibly resulted from L-arginine fragrance since it has been approved for food use as flavoring essence and spice in China. Increasing further L-arginine caused irritating flavor and thus decreasing significantly flavor scores (p<0.05). Compared with the control, the L-arginine-treated samples had better palatability. It was found that the slice of the control was rough and obviously exudative, while that of L-arginine-treated sample was smooth and less exudative. Therefore, the addition of L-arginine enhanced both slice trait scores and mouthfeel scores (p<0.05). These results indicated that the addition of L-arginine had the positive influence on sensory characteristics of pork sausage and thereby showing a potential in the manufacture of pork sausage.

## CONCLUSION

The application of L-arginine decreased CL, favored to form compact and uniform gel matrix and had the positive influence on textural properties and sensory qualities. These results showed that L-arginine had the

potential benefit of increasing products yield as well as improving quality characteristics of pork sausage.

## ACKNOWLEDGMENT

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