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ORIGINAL PAPER

Effects of ε-Polylysine on Physicochemical Characteristics of Chilled Pork

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Abstract The aim of this paper was to research effects of ε-polylysine (ε-PL) on physicochemical characteristics of chilled pork during storage. Chilled pork was treated with different concentrations of ε-PL. Sensory properties, total bacterial counts (TBC), pH, total volatile base nitrogen (TVB-N), metmyoglobin (MetMb) content, and α-thiobarbituric acid (TBA) of treated and control samples were analyzed periodically during refrigerated storage. Compared to control, significant reductions ($p<0.05$) for TBC, pH, TVB-N, and MetMb content of chilled pork treated with ε-PL during storage were found in this study. However, there were significant increments in sensory scores, and TBA almost did not change. TVB-N, pH, and $\log_{10}$CFU/g showed a positive linear relationship among them. These results indicated that 1.25 % ε-PL could effectively inhibit the growth and reproduction of bacteria and enhance preservative effects of chilled pork. Thus, ε-PL has good potential to be a preservative and extends shelf life of chilled pork.

Keywords ε-PL · Chilled pork · Physicochemical characteristics

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Introduction

There is a growing recognition that the continued widespread use of chemical synthetic preservatives in food industry may cause various hazards to human being health (Ho et al. 2000). Thus, safe and efficient natural food preservatives have become the research priority to improve the safety of food products and increasingly stringent requirements for microbial control in many countries for decades (Acuña et al. 2011; Attouchi and Sadok 2011; Nirmal and Benjaku 2012). Najjar et al. (2007) also reported that all natural food preservatives would play a crucial role in food industry in the future.

ε-polylysine (ε-PL), produced from aerobic bacterial fermentation by *Streptomyces albulus*, is a cationic homopolymer of 25 to 30 L-lysine residues, with molecular weight of 5,000 roughly (Chang et al. 2010), and connected by a peptide bond between the carboxyl and ε-amino groups (Hiraki et al. 1998; Yoshida and Nagasawa 2003; Zinoviadou et al. 2010). As a nutritional ingredient, ε-PL is edible, water soluble, stable at high temperatures, non-toxic to humans, and environmentally friendly due to its biodegradability (Hiraki et al. 2003; Shih et al. 2006; Geornaras et al. 2007; Jung et al. 2009). Food and Drug Administration (FDA) confirmed that ε-PL had GRAS (generally recognized as safe) status in 2004 and was approved for the usage of ε-PL in cooked or sushi rice at levels up to 50 mg/kg (Chang et al. 2010).

In early findings by some scientists, as a food additive, ε-PL, decomposed into lysine in the stomach, was acknowledged in secure area by toxicological experiments using rats (Hiraki 1995, 2000; Neda et al. 1999). Low concentrations of ε-PL could be used as preservative in foods due to its strong antibacterial property (Yoshida and Nagasawa 2003; Geornaras and Sofos 2005). Hiraki (2000) as well as Otsuka et al. (1992) reported that levels of 1,000–5,000 ppm ε-PL showed excellent preservation effects on sliced fish, fish sushi, boiled rice, noodles, and cooked vegetable, respectively.

Generally, meat and meat products are highly susceptible to microbiological contamination, due to their high water activity, high proteins, and relatively large quantities of free amino acids (Jeyasekaran et al. 2006; Duan et al. 2010). Microbiological and physicochemical characteristics, such as, sensory properties, total bacterial counts (TBC), pH, total volatile base nitrogen (TVB-N), metmyoglobin (MetMb), and α-thiobarbituric acid (TBA), play important roles in evaluation of meat and meat products deterioration. Souza et al. (2010) measured the indexes such as TBC, pH, TVB-N, and TBA of samples to study the preservation effect of additive on shelf-life extension of salmon fillets. Duan et al. (2010) evaluated the application of fish oil incorporated chitosan coatings for improving physicochemical and microbial qualities of fresh lingcod fillets by observing the changes in pH, TBA, and TBC of lingcod. To elucidate that chitosan film was suitable for extending the shelf life of bonito fish, Alak et al. (2010) determined some indicators of samples, consisting of TBC, pH, TVB-N, and TBA. In addition, Fan et al. (2009) reported that chitosan coating on fish samples could retain their good quality characteristics and extend the shelf life during cold storage by analysis of sensory properties, TBC, pH, TVB-N, and TBA. Besides, Rodríguez-Carpena et al. (2011) investigated the changes in MetMb and TBA of raw porcine patties, indicating that avocado by-products could be as outstanding inhibitors of color deterioration, and lipid and protein oxidation in these patties subjected to chilled storage.

To date, almost no literatures related with the physicochemical characteristic's effects on foods were found when ε-PL was added to foods, especially for meat products. The main purpose of this paper was to determine indexes (sensory properties, TBC, pH, TVB-N, MetMb, and TBA) of physicochemical and microbiological characteristics of chilled pork treated with ε-PL, and to evaluate preservative effects of ε-PL on chilled pork at 4 °C storage.

Materials and Methods

Preparation of ε-PL Solution and Treatment of Chilled Pork with ε-PL Solution

ε-PL powder (Silver Elephant Biological Co., Ltd., Zhejiang, China) was dispersed in sterile distilled water to obtain ε-PL concentrations (*w*/*v*) of 0.50, 0.75, 1.00, 1.25, and 1.50 %. Chilled pork was purchased from a local supermarket in Jinan, China. Using clean knives, chilled pork was cut into uniform size block for 1×1×1 cm, and evenly divided into six parts (approximately 170 g per part). The six parts were dipped into 0, 0.50, 0.75, 1.00, 1.25, and 1.50 % ε-PL solutions for 30 s, respectively, and drained out for 1 min on sterile stainless steel mesh screen. Then, the samples were loaded into sterile plastic bags in the refrigerator (Haier Co., Ltd., Qingdao, China) at 4 °C. Chilled pork was minced using a mincer (Joyoung Co., Ltd., Jinan, China) before pH, MetMb contents, TVB-N, TBA, and TBC of samples were detected.

Sensory Evaluation

After chilled pork was baked by microwave for 3 min at 700 w, the sensory properties of chilled pork were evaluated by using the modified method of Küçükgülmez et al. (2013) by a seven member trained panel who were familiar with meat characteristics. Panelist scored sensory properties, such as appearance, odor, texture, taste, and overall acceptability, using a nine-point descriptive scale (1, dislike extremely, to 9, like extremely).

Microbiological Analysis

TBC of minced pork was determined as described by Chounou et al. (2013) with some modifications. Approximately 5 g of minced pork was homogenized with 25 ml phosphate buffer solution using a homogenizer (Seward Medical, Worthing, UK) at 8,000–10,000 r/min for 2 min. Samples (0.1 ml) of serial dilutions of homogenates were spread on the surface of the appropriate dry medium in Petri dishes for determination of TBC on plate count agar (0.5 g tryptone, 0.25 g yeast extract, 1 g glucose, and 1.5 g agar dissolved in 100 ml distilled water), and incubated at 30 °C for 8 days. Furthermore, before the experiment, plate count agar and Petri dishes need to be autoclaved using pressure steam sterilizer (Shenan Medical Instrument Factory, Shanghai, China) at 121 °C for 22 min. The total bacterial counts of the samples were counted at 0, 1, 2, 3, 4, 6, 7, and 8 days, respectively. Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g).

Determination of pH

For pH measurement as described by Duan et al. (2010) and Souza et al. (2010) with slight modifications, 5 g of treated chilled pork was placed into a 100-ml beaker, and blended with 50 ml sterile water by a blender (Blessed Experimental Equipment Co., Ltd., Shanghai, China) at 3,000 rpm for 30 s. Then the mixture was filtered through qualitative filter paper which was obtained from Special Paper Co., Ltd. (Hangzhou, China). Depending on a precision pH meter (Sartorius, Goettingen, Germany), the pH of sample filtrate was determined.

Determination of TVB-N

TVB-N values of minced pork were measured using the modified method of Goulas and Kontominas (2005). Approximately 5 g of minced pork was mixed with 50 ml sterile water

for 30 min by magnetic stirring in a 100-ml beaker. The mixture was filtrated through qualitative filter paper, and the filtrate was stored in a refrigerator. An aliquot (5 ml) of absorbing solution (boric acid, 20 g/ml, AR) and three to four drops of mixed indicator solution (methyl red-ethanol, 2 g/l and methylene blue, 1 g/l, AR) were blended evenly and placed into a conical flask. The conical flask was placed at bottom of condenser of distilling device. An aliquot (2 ml) of previous filtrate mixed evenly with equal volume of magnesia suspension (10 g/l) was distilled for 5 min in the reaction chamber of distiller. Meanwhile, distillate was dropped into the previous conical flask (including absorbing solution and mixed indicator solution). Then, the mixture of the conical flask was titrated using hydrochloric acid (HCl, 0.01 M) to convert into bluish violet, recording the volume of HCl. Experiment of reagent blank was performed simultaneously. The contents of TVB-N in samples were calculated according to the formula:

$$\text{TVB-N}\left(\text{mg}\Big/100\text{g}\right) = \frac{(V_1 - V_2) \times c \times 14}{m \times 0.05} \times 100$$

V_1—volume of HCl for samples (ml); V_2—volume of HCl for reagent blank (ml); c—concentration of HCl (M); m—samples weigh (g).

Determination of metmyoglobin contents

Metmyoglobin contents of minced pork were determined as described by Maqsood and Benjakul (2010) with slight modifications. Three grams of minced pork was mixed evenly with an aliquot (10 ml) of 40 mM phosphate buffer solution (pH 6.8, disodium hydrogen phosphate and sodium dihydrogen phosphate, AR), and centrifuged at 6,000×g for 15 min using a centrifuger (Jing Li centrifuge Ltd., Beijing, China) to obtain the supernatant. Absorbance (A) of the supernatant was measured at 572, 565, 545, and 525 nm, respectively, using a spectrophotometer (Beauty Noda Instrument Co., Ltd., Shanghai, China). MetMb% was defined as:

$$\text{MetMb}\% = (2.541R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100\%$$

R_1, R_2, and R_3 indicated $A_{572\text{nm}}/A_{525\text{nm}}$, $A_{565\text{nm}}/A_{525\text{nm}}$, and $A_{545\text{nm}}/A_{525\text{nm}}$, respectively.

Determination of TBA

TBA values of samples were determined using the method described by Rodríguez-Carpena et al. (2011) with some modifications. About 10 g of minced pork was soaked in 50 ml sterile water for 5 min. The suspension was shifted into 500-ml kjeldahl flask and adjusted pH to 1.5 by adding, dropwise, 4 M HCl aqueous solution. Using liquid paraffin as defoamer, the suspension was distilled for 10 min. An aliquot (5 ml) of distillate was blended evenly with equal volume of TBA reagents (2.883 g α-thiobarbituric acid dissolved in 100 ml 95 % glacial acetic acid), and placed into 25-ml colorimetric tube with stopper, and bathed with boiling water for 35 min. With 5 ml distilled water as control, the absorbance (A) of mixture was measured at 532 nm and TBA was obtained according to the formula:

$$\text{TBA}\left(\text{mg}\Big/\text{kg}\right) = A \times 7.8$$

Statistical Analysis

Every experiment was performed in triplicates and average values with standard errors were reported. Using SPSS17.0, statistical analysis was carried out according to analysis of variance (ANOVA) which was adopted Duncan's method, and graphs were performed with Excel 2010, and linear regression analysis was used to determine the significant difference at 5 % confidence intervals ($p < 0.05$).

Results and Discussion

Sensory Evaluation

The sensory properties of chilled pork treated with different concentrations ε-PL during 8 days were given in Table 1. The samples were not considered acceptable for human consumption until the sensory score reached 4.0. As observed in Table 1, there was a notable decline in all sensory scores of control with increasing storage time. At the fourth day of cold storage, control had approximately 4.2 of sensory properties scores and deteriorated right away. The control was assessed as "putrefaction" achieving approximately 2.0 of sensory scores at the sixth day. - 0.50, 0.75, and 1.00 % ε-PL-treated samples appeared with similar result at the sixth day to those of control at the fourth day, with approximately 4.4 of sensory scores. However, all sensory scores of samples treated with 1.25 and 1.50 % ε-PL were approximately 6.8 and 7.2 at the sixth day of storage with well-acceptable evaluation. At the eighth day of storage, appearance, odor, texture, taste, and overall acceptability of all samples presented "unacceptable" scores except 1.50 % ε-PL-treated sample. These results showed ε-PL could improve sensory properties during storage and 1.25 and 1.50 % ε-PL presented significantly ($p < 0.05$) preservative effect on chilled pork with 6 days of shelf life. ANOVA demonstrated that there were no differences between 1.25 and 1.50 % ε-PL in sensory properties of chilled pork. Thus, 1.25 % ε-PL was optimal concentration with the maximum 6.8 score of overall acceptability at the sixth day for the use of chilled pork.

Table 1 Sensory evaluation of pork treated with different concentrations ε-PL at 4 °C of storage

Sensory properties	Treatment	Storage time (days)				
		0	2	4	6	8
Appearance	Control	9.0±0.21^{a}	7.1±0.17^{c}	4.2±0.13^{de}	2.3±0.09^{e}	1.0±0.06^{f}
	0.5 % ε-PL	9.0±0.19^{a}	7.6±0.21^{b}	6.0±0.15^{cd}	4.0±0.13^{d}	1.2±0.09^{e}
	0.75 % ε-PL	9.0±0.18^{a}	8.0±0.20^{b}	6.8±0.14^{c}	4.2±0.12^{d}	1.7±0.10^{e}
	1 % ε-PL	9.0±0.19^{a}	8.5±0.19^{b}	7.1±0.15^{c}	4.4±0.14^{d}	2.1±0.08^{e}
	1.25 % ε-PL	9.0±0.22^{a}	8.8±0.21^{a}	8.1±0.16^{b}	6.8±0.16^{c}	3.0±0.12^{de}
	1.5 % ε-PL	9.0±0.17^{a}	9.0±0.24^{a}	8.5±0.17^{b}	7.3±0.16^{c}	4.8±0.11^{d}
Odor	Control	9.0±0.16^{a}	7.0±0.22^{c}	4.4±0.11^{d}	2.1±0.10^{e}	1.1±0.09^{ef}
	0.5 % ε-PL	9.0±0.18^{a}	7.5±0.18^{bc}	6.0±0.12^{c}	4.0±0.11^{d}	1.3±0.08^{e}
	0.75 % ε-PL	9.0±0.15^{a}	8.0±0.19^{b}	6.7±0.14^{c}	4.5±0.13^{d}	1.6±0.09^{e}
	1 % ε-PL	9.0±0.15^{a}	8.3±0.17^{b}	7.1±0.16^{c}	4.5±0.14^{d}	2.2±0.11^{e}
	1.25 % ε-PL	9.0±0.16^{a}	8.7±0.20^{ab}	8.3±0.17^{b}	6.9±0.14^{c}	3.2±0.12^{de}
	1.5 % ε-PL	9.0±0.17^{a}	9.0±0.19^{a}	8.6±0.16^{b}	7.3±0.16^{c}	4.6±0.14^{d}
Texture	Control	9.0±0.14^{a}	7.0±0.16^{c}	4.3±0.12^{d}	2.0±0.09^{e}	1.0±0.08^{ef}
	0.5 % ε-PL	9.0±0.20^{a}	7.6±0.14^{b}	6.1±0.13^{c}	4.2±0.12^{d}	1.1±0.07^{ef}
	0.75 % ε-PL	9.0±0.22^{a}	8.1±0.15^{b}	6.5±0.12^{c}	4.4±0.13^{d}	1.5±0.09^{e}
	1 % ε-PL	9.0±0.15^{a}	8.4±0.17^{b}	7.0±0.15^{c}	4.5±0.15^{d}	2.0±0.11^{e}
	1.25 % ε-PL	9.0±0.17^{a}	8.8±0.16^{a}	8.3±0.17^{b}	6.8±0.14^{c}	2.9±0.09^{de}
	1.5 % ε-PL	9.0±0.18^{a}	8.9±0.16^{a}	8.5±0.19^{b}	7.1±0.15^{c}	4.6±0.13^{d}
Taste	Control	9.0±0.21^{a}	7.2±0.14^{c}	4.1±0.10^{d}	2.1±0.10^{e}	1.0±0.08^{ef}
	0.5 % ε-PL	9.0±0.24^{a}	7.8±0.19^{b}	6.1±0.14^{c}	4.0±0.10^{d}	1.2±0.09^{e}
	0.75 % ε-PL	9.0±0.19^{a}	8.2±0.18^{b}	6.6±0.12^{c}	4.5±0.11^{d}	1.3±0.09^{e}
	1 % ε-PL	9.0±0.17^{a}	8.5±0.17^{ab}	7.0±0.15^{c}	4.6±0.14^{d}	1.8±0.11^{e}
	1.25 % ε-PL	9.0±0.15^{a}	8.8±0.22^{a}	8.5±0.17^{b}	6.7±0.15^{c}	2.9±0.10^{de}
	1.5 % ε-PL	9.0±0.23^{a}	9.0±0.23^{a}	8.7±0.19^{b}	7.0±0.13^{c}	4.7±0.15^{d}
Overall acceptability	Control	9.0±0.18^{a}	7.1±0.17^{c}	4.2±0.11^{d}	2.1±0.08^{e}	1.0±0.08^{ef}
	0.5 % ε-PL	9.0±0.16^{a}	7.6±0.17^{bc}	6.1±0.13^{c}	4.1±0.09^{d}	1.2±0.07^{e}
	0.75 % ε-PL	9.0±0.21^{a}	8.1±0.18^{b}	6.7±0.15^{c}	4.4±0.12^{d}	1.5±0.09^{e}
	1 % ε-PL	9.0±0.19^{a}	8.4±0.19^{b}	7.0±0.17^{c}	4.5±0.13^{d}	2.1±0.09^{e}
	1.25 % ε-PL	9.0±0.19^{a}	8.8±0.17^{ab}	8.4±0.18^{b}	6.8±0.15^{c}	3.0±0.11^{de}
	1.5 % ε-PL	9.0±0.18^{a}	8.9±0.18^{a}	8.6±0.19^{b}	7.2±0.15^{c}	4.7±0.12^{d}

Mean values followed by different letters represent significant difference at $p<0.05$

According to sensory evaluation in this study, peculiar smell was not found in chilled pork treated with ε-PL of all concentrations. This study was in support of Hiraki et al. (2003) who reported that there were no notable adverse effects even when ε-PL at high dose levels of 20,000 ppm was given in the diet in a chronic feeding study in rats. Similarly, Chang et al. (2011) showed that certain ε-PL-pectin complexes (1 % ε-PL; pectin: ε-PL≥2) could be incorporated into green tea beverages without adversely affecting their appearance or physical stability.

Effect of ε-PL on TBC of Chilled Pork

TBC of chilled pork was affected by the addition of different concentrations ε-PL during 8 days as presented in Table 2. The values of $\log_{10}$CFU/g of all samples appeared to an upward trend with the increase of the days of storage. The values of $\log_{10}$CFU/g of control increased from 3.48 to 10.62 during the storage period. However, the value of $\log_{10}$CFU/g of all treated samples increased slower than that of control. Moreover, the value of $\log_{10}$CFU/g of chilled pork decreased with the increase of ε-PL concentrations during the same storage period. At the sixth day, chilled pork treated with 0.50, 0.75, 1.00, 1.25, and 1.50 % ε-PL had 7.08, 6.82, 6.04, 5.06, and 4.95 $\log_{10}$CFU/g, respectively, whereas the value of control already reached 8.80 $\log_{10}$CFU/g. Analysis of variance (as observed in Table 2) showed that 1.25 and 1.50 % ε-PL had the more notable antibacterial property than the other three concentrations (0.50, 0.75, and 1.00 %; $p<0.05$). Moreover, there was no significant difference between 1.25 and 1.50 % ε-PL in the decrease of

Table 2 Effect of ε-PL with different concentrations on TBC of pork at 4 °C of storage

	Treatment	Storage time (days)							
		0	1	2	3	4	6	7	8
$\log_{10}$ CFU/g	Control	3.48 ± 0.13^{a}	5.30 ± 0.18^{d}	6.40 ± 0.19^{e}	7.30 ± 0.23^{f}	7.87 ± 0.26^{g}	8.80 ± 0.31^{h}	9.80 ± 0.32^{i}	10.62 ± 0.36^{ij}
	0.50 % ε-PL	3.48 ± 0.12^{a}	4.55 ± 0.17^{bc}	4.98 ± 0.17^{d}	5.14 ± 0.19^{cd}	6.54 ± 0.21^{def}	7.08 ± 0.24^{ef}	8.36 ± 0.30^{h}	9.03 ± 0.31^{h}
	0.75 % ε-PL	3.48 ± 0.12^{a}	4.01 ± 0.15^{b}	4.57 ± 0.15^{cd}	5.03 ± 0.16^{cd}	6.00 ± 0.19^{de}	6.82 ± 0.21^{ef}	7.74 ± 0.31^{fg}	8.95 ± 0.27^{h}
	1.00 % ε-PL	3.48 ± 0.14^{a}	3.97 ± 0.16^{b}	4.18 ± 0.16^{c}	4.60 ± 0.15^{c}	5.39 ± 0.16^{d}	6.04 ± 0.22^{e}	7.33 ± 0.25^{fg}	7.73 ± 0.29^{g}
	1.25 % ε-PL	3.48 ± 0.11^{a}	3.70 ± 0.14^{ab}	3.85 ± 0.12^{b}	3.95 ± 0.16^{b}	4.90 ± 0.17^{c}	5.06 ± 0.19^{d}	6.50 ± 0.22^{ef}	7.69 ± 0.24^{g}
	1.50 % ε-PL	3.48 ± 0.13^{a}	3.47 ± 0.13^{a}	3.51 ± 0.14^{ab}	3.69 ± 0.12^{b}	4.69 ± 0.18^{c}	4.95 ± 0.17^{d}	6.07 ± 0.23^{e}	6.56 ± 0.22^{f}

Mean values followed by different letters represent significant difference at $p<0.05$

$\log_{10}$CFU/g of chilled pork ($p>0.05$). In addition, sensory analysis also demonstrated that chilled pork treated with 1.25 and 1.50 % ε-PL was well acceptable to panelist at the sixth day. At the eighth day, the values of $\log_{10}$CFU/g of all samples exceeded 6.56 $\log_{10}$CFU/g with "unacceptable" scores (lower than 4.0). These results indicated that 1.25 and 1.50 % ε-PL could inhibit notably the growth and reproduction of bacteria and extend shelf life of chilled pork to 6 days.

This was supported by the observation of Zhou et al. (2011) that ε-PL solution was highly effective at killing various microorganisms. Similarly, those results were in agreement with the conclusion of Chang et al. (2010) that confirmed the effectiveness of ε-PL against bacteria and that antibacterial property depended on the ε-PL concentration. Additionally, Geornaras and Sofos (2005) and Najjar et al. (2007) also reported that treatments of ε-PL appeared to be very effective in inhibiting growth of pathogens.

Effect of ε-PL on pH of Chilled Pork

Changes in pH of control and treated pork with ε-PL of different concentrations during 8 days storage at 4 °C were shown in Table 3. The values of pH of all samples appeared to an upward trend with the increment of storage time, however, those of all treated with ε-PL increased more slowly than the control which was consistently higher than all-treated pork during the whole storage period. At the third and fourth day, pH of control reached 6.12 and 6.60, showing forthcoming deterioration of pork, while the highest pH of treated sample with 0.50 % ε-PL was just 5.99, indicating being fresh pork yet (sensory evaluation). At the sixth day, pH of all samples was 7.00, 6.42, 6.30, 6.00, 5.78, and 5.60, respectively, showing pH decreased with increasing ε-PL concentrations from 0 to 1.50 %. The result of ANOVA (Table 3) was that pH was reduced significantly by 1.25 and 1.50 % ε-PL ($p<0.05$) and the difference of preservative effect between 1.25 % and 1.50 % ε-PL was not significant ($p>0.05$). This was consistent with the sensory evaluation and TBC.

The rise of pH has a notable effect on the quality of the meat during storage, especially, in aspects of sensorial characteristics such as odor, color, and texture, which has negative effects (Shenderyuk and Bykowski 1990). This rise of pH is attributed to alkaline substance such as ammonia and amines degraded from meat protein due to bacterial growth and production of metabolites with the extension of storage time (Souza et al. 2010; Campos et al. 2005). The rising tendency of pH in this research was in agreement with the finding of Jay et al. (2005) who declared that the increase in pH was due to the growth of bacteria in the fish. These results demonstrated that 1.25 and 1.50 % ε-PL could inhibit the growth of bacteria,

Table 3 Effect of ε-PL with different concentrations on pH of pork at 4 °C of storage

	Treatment	Storage time (days)						
		1	2	3	4	6	7	8
pH	Control	5.66 ± 0.16^{c}	5.79 ± 0.18^{d}	6.12 ± 0.18^{e}	6.60 ± 0.20^{f}	7.00 ± 0.21^{g}	7.20 ± 0.23^{g}	7.40 ± 0.22^{h}
	0.50 % ε-PL	5.62 ± 0.17^{b}	5.63 ± 0.16^{bc}	5.74 ± 0.17^{cd}	5.99 ± 0.19^{e}	6.42 ± 0.18^{ef}	6.80 ± 0.20^{f}	7.04 ± 0.20^{g}
	0.75 % ε-PL	5.54 ± 0.15^{b}	5.55 ± 0.15^{bc}	5.70 ± 0.15^{c}	5.80 ± 0.17^{de}	6.30 ± 0.20^{ef}	6.70 ± 0.21^{f}	7.00 ± 0.19^{g}
	1.00 % ε-PL	5.45 ± 0.14^{ab}	5.50 ± 0.15^{bc}	5.58 ± 0.16^{c}	5.66 ± 0.18^{d}	6.00 ± 0.19^{e}	6.70 ± 0.18^{f}	7.00 ± 0.19^{g}
	1.25 % ε-PL	5.37 ± 0.13^{a}	5.42 ± 0.12^{ab}	5.47 ± 0.14^{b}	5.56 ± 0.15^{c}	5.78 ± 0.17^{d}	6.10 ± 0.19^{e}	6.80 ± 0.17^{f}
	1.50 % ε-PL	5.36 ± 0.14^{a}	5.39 ± 0.13^{a}	5.45 ± 0.14^{b}	5.50 ± 0.16^{c}	5.60 ± 0.15^{d}	5.90 ± 0.18^{e}	6.70 ± 0.18^{f}

Mean values followed by different letters represent significant difference at $p<0.05$

Table 4 Effect of ε-PL with different concentrations on TVB-N of pork at 4 °C of storage

	Treatment	Storage time (days)							
		0	1	2	3	4	6	7	8
TVB-N	Control	9.0 ± 0.13^{a}	12.0 ± 0.17^{bc}	17.3 ± 0.19^{d}	20.3 ± 0.18^{ef}	23.2 ± 0.19^{fg}	24.3 ± 0.24^{g}	25.2 ± 0.23^{h}	26.5 ± 0.28^{i}
	0.50 % ε-PL	9.2 ± 0.15^{a}	9.4 ± 0.15^{ab}	11.0 ± 0.16^{c}	16.0 ± 0.19^{de}	19.0 ± 0.18^{e}	20.9 ± 0.21^{f}	22.0 ± 0.21^{g}	23.0 ± 0.22^{gh}
	0.75 % ε-PL	9.0 ± 0.16^{a}	9.3 ± 0.15^{ab}	12.0 ± 0.17^{c}	15.2 ± 0.17^{de}	17.2 ± 0.18^{e}	20.0 ± 0.20^{f}	21.1 ± 0.19^{g}	22.0 ± 0.20^{gh}
	1.00 % ε-PL	9.1 ± 0.14^{a}	9.3 ± 0.18^{ab}	10.1 ± 0.15^{c}	12.2 ± 0.15^{d}	16.0 ± 0.17^{e}	18.0 ± 0.21^{f}	19.0 ± 0.20^{g}	21.8 ± 0.23^{gh}
	1.25 % ε-PL	9.0 ± 0.17^{a}	9.0 ± 0.17^{a}	9.5 ± 0.14^{b}	10.4 ± 0.16^{c}	13.0 ± 0.17^{d}	15.8 ± 0.17^{e}	17.0 ± 0.19^{f}	21.4 ± 0.21^{g}
	1.50 % ε-PL	8.9 ± 0.16^{a}	8.9 ± 0.15^{a}	9.2 ± 0.15^{ab}	9.9 ± 0.15^{c}	12.1 ± 0.15^{d}	14.6 ± 0.15^{e}	16.4 ± 0.18^{f}	20.5 ± 0.19^{g}

Mean values followed by different letters represent significant difference at $p<0.05$

decrease the degradation of meat protein, and prolong the storage time of chilled pork.

Effect of ε-PL on TVB-N of Chilled Pork

As a parameter that quantifies the compounds composed of ammonia and primary, secondary, and tertiary amines, generally, TVB-N was regarded as an important indicator of pork deterioration, and the lower the content of TVB-N the higher the freshness of meat (Fan et al. 2009).

The impact of ε-PL with different concentrations on TVB-N of chilled pork during 8 days storage at 4 °C was shown in Table 4. The initial TVB-N value of samples was approximately 9.0 mg/100 g, demonstrating the chilled pork was of good quality (sensory evaluation). TVB-N values of control rose from 9.0 to 26.5 mg/100 g with increasing storage time. TVB-N values of treated samples increased more slowly than that of control during 8 days storage ($p<0.05$). At the third day, TVB-N values of treated samples with 1.50, 1.25, and 0.50 % ε-PL were 9.9, 10.4, and 16.0 mg/kg, respectively, whereas, the value of control already reached 20.3 mg/100 g. At the sixth day, TVB-N values of 1.50, 1.25, 1.00, 0.75, and 0.50 % ε-PL-treated samples were 14.6, 15.8, 18.0, 20.0, and 20.9 mg/100 g (Table 4), respectively, however, control 24.3 mg/100 g. ANOVA indicated that 1.50 and 1.25 % ε-PL decreased TVB-N value of pork more significantly than the other concentrations of ε-PL (1.00, 0.75, and 0.50 %, 0; $p<0.05$). These results showed that 1.50 and 1.25 % ε-PL could prevent the deterioration of chilled pork remarkably.

Similar rising trend about TVB-N values with increasing storage time had been found for salmon (Souza et al. 2010; Ibrahim Sallam 2007). This result was in agreement with the finding of El Bassi et al. (2009) that TVB-N values for samples treated with antimicrobial agent were lower than untreated samples, consistently. This finding was supported by the observation of Chung et al. (2004) and Souza et al. (2010) that TVB-N values of fresh salmon increased gradually, attaining apparent differences between control and coated samples. The significant difference for TVB-N between each treatment sample and control was attributed to the effective antimicrobial activity and the ability of reducing protein decomposition of ε-PL. These results of TVB-N were in agreement with that of pH.

Correlation Among pH, TVB-N and TBC of Chilled Pork

During the storage period, pork deterioration occurred due to the growth and reproduction of spoilage bacteria, which contributes to degradation of pork protein into alkaline autolysis compounds (nitrogenous compounds) and production of bacterial metabolites (Campos et al. 2005; Fan et al. 2009; Souza et al. 2010), thus, pH and TVB-N of pork rise. Therefore, the relationship of TBC and pH and TVB-N would be positive relevant.

The correlation among pH, TVB-N and TBC of chilled pork treated with different concentrations ε-PL at the sixth day of storage was shown in Fig. 1. The values of pH of chilled pork increased from 5.60 to 7.00 with the increase of $\log_{10}$CFU/g values from 4.95 to 8.80. A plot of pH as a function of $\log_{10}$CFU/g showed a positive linear relationship with 0.9904 of R^2, as expressed in Eq. 1:

$$\mathrm{pH} = 0.3481\log_{10}\mathrm{CFU/g} + 3.9353 \quad (1)$$

The correlation between TVB-N and $\log_{10}$CFU/g presented similar trend to that between pH and $\log_{10}$CFU/g. A plot of

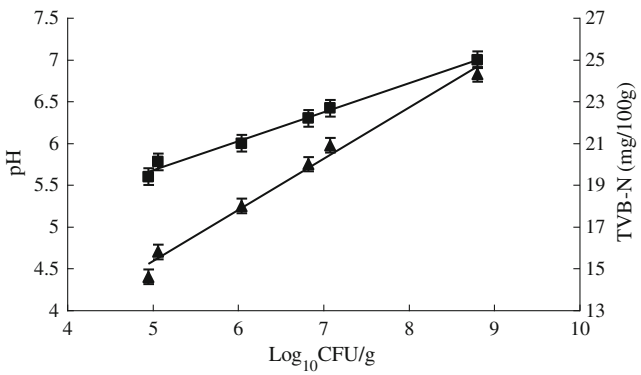

Fig. 1 Correlation among pH, TVB-N and TBC of chilled pork. TVB-N and pH were stood for by *triangles* and *squares*, respectively

Table 5 Effect of ε-PL with different concentrations on content of MetMb of pork at 4 °C of storage

	Treatment	Storage time (days)							
		0	1	2	3	4	6	7	8
MetMb%	Control	8.1 ± 0.13^{a}	14.2 ± 0.15^{d}	20.1 ± 0.17^{e}	31.0 ± 0.21^{f}	39.2 ± 0.24^{g}	43.3 ± 0.24^{h}	58.9 ± 0.27^{k}	62.1 ± 0.26^{k}
	0.50 % ε-PL	8.0 ± 0.14^{a}	12.3 ± 0.16^{c}	17.0 ± 0.15^{d}	28.9 ± 0.18^{ef}	33.1 ± 0.20^{f}	40.2 ± 0.21^{gh}	51.0 ± 0.23^{ij}	58.0 ± 0.24^{j}
	0.75 % ε-PL	8.2 ± 0.11^{a}	11.1 ± 0.14^{bc}	16.4 ± 0.16^{d}	26.0 ± 0.19^{ef}	28.9 ± 0.21^{f}	33.3 ± 0.21^{g}	45.3 ± 0.24^{ij}	54.8 ± 0.22^{j}
	1.00 % ε-PL	7.9 ± 0.12^{a}	10.0 ± 0.15^{b}	12.0 ± 0.13^{cd}	19.1 ± 0.16^{de}	21.3 ± 0.19^{ef}	29.1 ± 0.19^{g}	35.2 ± 0.21^{i}	44.1 ± 0.23^{i}
	1.25 % ε-PL	8.1 ± 0.14^{a}	10.2 ± 0.13^{b}	10.9 ± 0.14^{c}	14.8 ± 0.17^{d}	18.0 ± 0.17^{e}	20.1 ± 0.20^{f}	28.1 ± 0.22^{h}	30.0 ± 0.20^{h}
	1.50 % ε-PL	8.0 ± 0.15^{a}	9.4 ± 0.13^{b}	10.5 ± 0.12^{c}	13.1 ± 0.14^{d}	16.2 ± 0.18^{e}	18.2 ± 0.19^{f}	22.1 ± 0.19^{g}	27.2 ± 0.21^{h}

Mean values followed by different letters represent significant difference at $p<0.05$

TVB-N and $\log_{10}$CFU/g showed a positive linear relationship ($R^2=0.9863$) in Eq. 2:

$$\text{TVB-N} = 2.4489\log_{10}\text{CFU/g} + 3.1176 \quad (2)$$

Effect of ε-PL on MetMb Content of Chilled Pork

The color of pork is a key indicator for consumers for predicting the freshness and wholesomeness (Mancini and Hunt 2005; Chun et al. 2013). Change of fleshy color is due to interconversion of oxymyoglobin, myoglobin, and metmyoglobin during the storage of meat products. And change of pork color from bright to darkness was attributed to increasing MetMb contents (Bou et al. 2008; Maqsood and Benjakul 2010).

The MetMb contents of pork not treated or treated with different concentrations ε-PL during 8 days storage at 4 °C were depicted in Table 5. MetMb content of sample was approximately 8.0 % at the beginning and those of them increased progressively with the increment of storage time. Compared to control, MetMb contents of treated samples took on the trend of slight increase during the whole storage time. Furthermore, MetMb contents of samples decreased with the increase of ε-PL concentrations during the same storage period. At the sixth day, all samples treated with 0, 0.50, 0.75, 1.00, 1.25, and 1.50 % ε-PL had 43.3, 40.2, 33.3, 29.1, 20.1, and 18.2 % of MetMb, respectively. MetMb content of control (43.3 %) was two times more than that of 1.25 and 1.50 % ε-PL (20.1 and 18.2 %). Moreover, 1.25 and 1.50 % ε-PL affected MetMb content of chilled pork significantly by an ANOVA ($p<0.05$). At the eighth day of storage, there was similar change phenomenon of MetMb content to those at the sixth day. Sensory analysis indicated also all sensory properties including appearance, odor, texture, taste, and overall acceptability had "unacceptable" scores at the eighth day. These results showed that 1.25 and 1.50 % ε-PL could effectively limit color change of chilled pork with a significant dependency of concentrations ($p<0.05$).

Tang et al. (2006) also exhibited the increase in MetMb content of minced beef patties held in different conditions as the time of storage increased. This study was coincidental with the finding of Chun et al. (2013) who announced formation of negative characteristics due to MetMb appearance. Besides, Rodríguez-Carpena et al. (2011) reported that the plant extracts could retard MetMb formation in patties.

Effect of ε-PL on TBA of Chilled Pork

TBA is widely used as a critical index of lipid oxidation in processed meat and meat products. The increase of TBA indicates

Table 6 Effect of ε-PL with different concentrations on TBA of pork at 4 °C of storage

	Treatment	Storage time (days)							
		1	2	3	4	5	6	7	8
TBA	Control	0.83 ± 0.05^{a}	0.91 ± 0.07^{b}	1.12 ± 0.09^{c}	1.19 ± 0.07^{d}	1.20 ± 0.09^{d}	1.34 ± 0.10^{e}	1.38 ± 0.07^{f}	1.53 ± 0.11^{g}
	0.50 % ε-PL	0.76 ± 0.03^{a}	0.98 ± 0.05^{b}	1.05 ± 0.07^{c}	1.07 ± 0.06^{d}	1.27 ± 0.10^{d}	1.24 ± 0.09^{e}	1.47 ± 0.10^{f}	1.56 ± 0.10^{g}
	0.75 % ε-PL	0.66 ± 0.04^{a}	0.72 ± 0.04^{b}	0.78 ± 0.05^{c}	0.78 ± 0.04^{c}	0.94 ± 0.05^{d}	1.13 ± 0.09^{e}	1.41 ± 0.09^{f}	1.51 ± 0.11^{g}
	1.00 % ε-PL	0.54 ± 0.03^{a}	0.56 ± 0.02^{a}	0.74 ± 0.05^{c}	0.94 ± 0.05^{d}	1.05 ± 0.07^{d}	1.25 ± 0.10^{e}	1.54 ± 0.11^{f}	1.58 ± 0.10^{g}
	1.25 % ε-PL	0.44 ± 0.02^{a}	0.61 ± 0.05^{a}	0.80 ± 0.04^{c}	1.01 ± 0.07^{d}	1.03 ± 0.06^{d}	1.07 ± 0.07^{de}	1.15 ± 0.09^{ef}	1.40 ± 0.12^{g}
	1.50 % ε-PL	0.69 ± 0.04^{a}	0.78 ± 0.06^{b}	0.90 ± 0.06^{c}	0.96 ± 0.04^{d}	1.14 ± 0.09^{d}	1.73 ± 0.12^{ef}	1.76 ± 0.13^{fg}	1.80 ± 0.13^{g}

Mean values followed by different letters represent significant difference at $p<0.05$

that lipid oxidation become serious by measuring the malonaldehyde content (Srikar and Hiremath 1972; Souza et al. 2010).

Changes in TBA of control and samples treated with ε-PL of different concentrations during 8 days storage at 4 °C were shown in Table 6. Slight rising tendency of TBA for all samples was presented throughout the storage of 8 days, less than a value of 0.9 at the beginning. At the eighth day, TBA of chilled pork was 1.53, 1.56, 1.51, 1.58, 1.40, and 1.80 mg/kg for all samples treated with 0, 0.50, 0.75, 1.00, 1.25, and 1.50 % ε-PL, respectively. Statistical analysis revealed that there was no significant difference in TBA of samples with increasing ε-PL concentrations ($p > 0.05$), exhibiting ε-PL did not have anti-oxidation effect on chilled pork.

Similar results were found by other researchers. Maqsood and Benjakul (2010) reported that rapid increase of TBA values for ground beef with increasing storage time. According to Fan et al. (2009) and Connell (1990), usually, fish flesh would behave an objectionable odor when TBA values were beyond 1–2 mg/kg. Possibly, no antioxidation of ε-PL in this research was attributed to interference from other operating conditions for instance pH.

Conclusion

As can be seen from this study, the application of ε-PL on chilled pork during storage at 4 °C resulted in significant effects ($p < 0.05$) of sensory scores, TBC, pH, TVB-N, and MetMb with increasing concentrations of ε-PL when compared to control. However, ε-PL did not affect TBA of chilled pork. These results revealed that 1.25 and 1.50 % ε-PL could inhibit notably the growth and reproduction of microorganisms, decrease the decomposition of pork protein, and prevent the change of color of chilled pork. Thus, 1.25 and 1.50 % ε-PL retained good quality attributes of chilled pork. Analysis of sensory properties demonstrated that 1.25 % ε-PL exhibited optimal effects when used in chilled pork. Therefore, ε-PL has the potential to be used as nature preservative and to extend the shelf life of chilled pork.

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