International Journal of Nutrition and Food Sciences
2019; 8(2): 40-45
http://www.sciencepublishinggroup.com/j/ijnfs
doi: 10.11648/j.ijnfs.20190802.13
ISSN: 2327-2694 (Print); ISSN: 2327-2716 (Online)

Application of ε-poly-L-lysine·HCl on Extending the Shelf-Life of Spiced Meats

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To cite this article:

Gao Xiang, Hao Yu, Wu Zhumeng, Wang Fang, Pang Meixia, Qi Jinghua. Application of ε-poly-L-lysine·HCl on Extending the Shelf-Life of Spiced Meats. *International Journal of Nutrition and Food Sciences*. Vol. 8, No. 2, 2019, pp. 40-45. doi: 10.11648/j.ijnfs.20190802.13

Received: July 3, 2019; **Accepted**: August 22, 2019; **Published**: August 27, 2019

Abstract: In order to prolong the shelf life of spiced meats, ε-poly-L-lysine·HCl (ε-p-L·HCl) was used as preservative, and four kinds of corruption bacterium (Staphylococcus, Proteus, Bacillus and Serratia) were used as indicator bacterium. The minimum inhibitory concentration of preservatives against them was 500 ug/mL. The suitable concentration, temperature and pH were determined by single factor experiment. On the basis of single factor experiment, orthogonal experiment was used to get the best bacteriostasis conditions. The optimum condition for Staphylococcus and Proteus ware 1.2 mg/mL, 80°C and pH 3. The optimum condition for Bacillus and Serratia were 1.2 mg/mL, 80°C and pH4. According to the national standard, preservatives were added to the spiced meat with the use of 0.25 g/kg in the experimental group, and no preservatives were added in the control group. The total number of bacteria and the sensory score were used as evaluation indexes to explore the effect of preservatives on shelf life. Compared with the control group, at the same storage time, the sensory score was higher and the total number of colonies was less. It was concluded that added ε-p-L·HCl could extend the shelf-life of the spiced pork for 8 days, added ε-p-L·HCl could extend the shelf-life of the sauced bath chap for 7 days.

Keywords: ε-p-L·HCl, Spiced Meats, Shelf-Life

1. Introduction

The spiced meat product is a kind of special food, cooked with salt, soy sauce and spices in water. So It has unique flavor, attractive color and deeply loved by consumers [1]. In traditional processing technology, the temperature of cooking is generally less than 100°C, which could only kill the nutrient bodies of pathogenic bacteria with low heat resistance, but some bacteria and spores still live. [2] When the conditions were suitable, the spores would germinate and multiply rapidly. Therefore, sauce and marinated meat was easy to deteriorate in the sales process, and the shelf life was very short [3]. In order to prolong the shelf life of products, many factories used high temperature and pressure sterilization to sterilize, but it reduced the elasticity of products and produced a larger cooking taste, which greatly influenced the flavor and taste [4]. In order to retain the unique flavor and prolong the shelf life, the most economical and effective way was to add preservatives [5]. In recent years, the application of natural preservatives in meat products has gradually risen. Zhang Xuan and Li Xinwei studied the effects of several natural preservatives on the inhibiting effect of traditional fermented ham [6]. Chen Nannan and other researchers discussed the effects of different preservatives on the micro-production of Western low-temperature sausage [7]. Zhang Panjing researched the effects of E-PL on the preservation of chilled pork and selected the optimum conditions [8-10]. XuBaocai studied the bacteriostasis effect of compound preservatives on low-temperature ham slices, compound preservatives mainly composed of natural preservatives—Streptococcus lactis [11-12]. And it was applied to the industrialized production of meat products. But the temperature of ham processing was higher than 72°C, Streptococcus lactis exerts the best bacteriostasis effect was less than 72°C, so it was easy to reduce the bacteriostasis effect in processing [13-14]. Epsilon-polylysine (epsilon-PL) is a polymer of L-lysine with a degree of polymerization of 25-30. It was a new type of natural preservative, extracted natural metabolite of Streptomyces, Beilisporium and ergot fungi [15]. Compared with traditional preservatives, it had a broad spectrum of antimicrobial activity. Under neutral and slightly acidic conditions, it had bacteriostatic effect on most Gram-positive bacteria, Gram-negative bacteria and fungi [16]. In Japan, Korea and the United States, E-PL was widely used in food industry. China's "national food safety standards stipulated that the maximum addition of ε-p-L·HCl in spiced meats was 0.25 g/kg [17]. Therefore, its unique effects and application prospects had attracted great attention. The purpose of this paper was to explore the bacteriostatic effect of ε-p-L·HCl on spiced meats, so as to provide some guidance for improving shelf life and quality of similar meat products.

2. Materials

ε-p-L·HCl, purchased from Zhejiang Yinxiang Bioengineering Pharmacy

Spiced meats, purchased at WuMei supermarket.

3. Methods

3.1. Single Factor Experiment

3.1.1. Oxford Cup Method for Minimum Inhibitory Concentration (MIC)

Staphylococcus, Proteus, Bacillus and Serratia were selected as indicator bacteria. The ε-p-L·HCl was added into water to prepare 6 different concentrations of bacteriostatic liquid: 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL. Then prepared a control group with distilled water. In each Petri dish, 7 Oxford cups were placed evenly, and 100 μL of different concentration of bacteriostatic solution and sterile water were added to the Oxford cup, cultured in constant temperature incubator at 37°C for 12 h. Then determined the diameter of the bacteriostasis circle, paralleled operation for 3 times and taken the average.

3.1.2. Effect of Different Temperatures on Antibacterial Activity of ε-p-L • HCl

The antimicrobial solution of 1 mg/mL was removed from five tubes and soaked in water bath at 20°C, 40°C, 60°C, 80°C and 100°C for 30 min. The diameter of bacteriostatic circle was measured by oxford cup method. The average value was obtained after three parallel operations.

3.1.3. Effect of Different pH on Antibacterial Activity of ε-p-L • HCl

Seven gradients of pH 3, 4, 5, 6, 7, 8 and 9 were prepared with buffer solution, and the concentration of bacteriostatic solution was 1 mg/L. Then determined the diameter of bacteriostasis circle of bacteriostasis solution with different pH. The average value was obtained after three parallel operations.

3.2. Orthogonal Experiments

On the basis of single factor experiment, concentration, temperature and pH, were selected to carry out orthogonal experiment with three factors and three levels. The method of bacteriostasis experiment was the same as the above. The orthogonal experimental design of three factors and three levels was as follows.

Table 1. *Three factors and three levels of orthogonal test.*

Factor	level		
	A	B	C
Concentration (mg/mL)	1.0	1.2	1.4
Temperature (°C)	70	80	90
pH	3	4	5

3.3. Data Analysis

Statistical analysis of data by Excel.

3.4. Effect of ε-p-L • HCl on the Shelf Life of Spiced Pork

3.4.1. Technological Process

Control group: 1 cleaning raw material; 2 draining; 3 slicing; 4 pickling; 5 cooling; 6 packaging

Experimental group: 1 cleaning raw material; 2 draining; 3 slicing; 4 pickling; 5 spraying preservative; 6 cooling; 7 packaging

3.4.2. Sensory Evaluation

Two groups of samples from the first day of storage were collected and judged by sensory evaluation. According to the GBT23586-2009, the characteristics of the sensory evaluation test were designed as shown in Table 2.

Table 2. *Sensory characteristic of spiced pork.*

Project	Indexes
Appearance	Tidy, clean
Color	Caramel or brown
Taste and flavor	Delicious, Special flavor of meats
Form	Tightness
Impurity	Non-existent or less

3.4.3. Determination of the Total Number of Colony

The total number of colony was determined according to GB 4789.2-2016 "national food safety standard - food microbiology test - total colony determination".

3.4.4. Determination of Coliform

The number of coliform was determined according to GB 4789.3-2016 "national food safety standard - food microbiology test - coliform.

3.5. Effect of ε-p-L • HCl on Shelf Life of Bath Chap

3.5.1. Technological Process

Control group: 1 cleaning raw material; 2 draining; 3 slicing; 4 pickling; 5 cooling; 6 packaging

Experimental group: 1 cleaning raw material; 2 draining; 3 slicing; 4 pickling; 5 spraying preservative; 6 cooling; 7 packaging

3.5.2. Sensory Evaluation

Two groups of samples from the first day of storage were collected and judged by sensory evaluation. According to the GBT23586-2009, the characteristics of the sensory evaluation test were designed as shown in Table 3.

Table 3. *Sensory characteristic of bath chap.*

Project	Indexes
Appearance	Tidy, clean
Color	Caramel or brown
Taste and flavor	Delicious, Special flavor of meats
Form	Tightness
Impurity	Non-existent or less

3.5.3. Determination of the Total Number of Colony

The total number of colony was determined according to GB 4789.2-2016 "national food safety standard - food microbiology test - total colony determination".

3.5.4. Determination of Coliform

The number of coliform was determined according to GB 4789.3-2016 "national food safety standard - food microbiology test - coliform".

4. Results and Discussion

4.1. Minimum Inhibitory Concentration

Table 4. *Bacteriostatic results of ε-p-L • HCl with different concentrations.*

Bacteria	Concentrations of ε-p-L·HCl (μg/mL)					
	37.25	62.5	125	250	500	1000
Bacillus	++	++	++	+-	-	-
Proteus	++	++	++	++	-	-
Staphylococcus	++	++	++	++	-	-
Serratia	++	++	++	+−	-	-

Notes:"-"indicates that fungus didn't grow,"+-"indicates that a slight growth of fungus,"++"indicates that growth of fungus

According to the results in the table 4, it can be seen that at 250 ug/mL, the bacteriostatic effect of preservatives on four kinds of bacteria was slightly different, but when ≥ 500 ug/mL, there was no bacteriostatic circle, so the MIC was 500 ug/mL.

4.2. Effect of Temperature on Bacteriostasis of ε-p-L • HCl

Table 5. *Bacteriostatic effect of temperature on bacillus.*

Temperature (°C)	20	40	60	80	100
Bacteriostasis circle of bacillus (mm)	15.0	13.5	13.0	12.0	11.0
Bacteriostasis circle of proteus (mm)	15.0	14.0	15.0	16.5	14.0
Bacteriostasis circle of staphylococcus (mm)	11.6	10.5	9.5	10.0	11.0
Bacteriostasis circle of serratia (mm)	12.5	12.0	12.0	13..5	11..5

It can be seen from the table that ε-p-L·HCl has a high bacteriostatic effect on four kinds of bacteria in different temperature range. The optimum bacteriostasis temperature of Bacillus and Staphylococcus were 20°C, Proteus and Serratia were 80 C.

4.3. Effect of Different pH on Bacteriostatic Effect of ε-p-L • HCl

Table 6. *Bacteriostatic effect of different pH.*

pH	3	4	5	6	7	8	9
Bacteriostasis Circle of Bacillus (mm)	21.0	22.0	23.0	19.0	17.0	17.0	17.0
Bacteriostasis Circle of Proteus (mm)	16.0	14.0	18.0	16.0	16.0	15.0	14.0
Bacteriostasis Circle of Staphylococcus (mm)	17.0	16.0	16.0	11.5	13.0	12.0	12.0
Bacteriostasis Circle of Serratia (mm)	21.5	22.0	23.0	18.0	18.5	18.0	18.0

It can be seen from the table that ε-p-L·HCl had high bacteriostatic effect on four strains at different pH, but it had different optimal bacteriostatic pH for different strains. The optimal bacteriostatic pH of Bacillus, Proteus and Staphylococcus were 5, and that of Serratia was 3.

4.4. Results of Orthogonal Experiments

4.4.1. Results of Orthogonal Experiments of Bacillus

Table 7. *Orthogonal experiments of bacillus.*

code \ factor	A	B	C	Circle (mm)
1	1	70	3	20
2	1	80	4	21
3	1	90	5	21
4	12	70	4	24
5	1.2	80	5	22
6	1.2	90	3	21
7	1.4	70	5	23
8	1.4	80	3	23
9	1.4	90	4	21
K1	62	67	65	
K2	67	66	64	
K3	67	63	67	
R	1.6	1.3	0.7	

Table 8. *Variance analysis.*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
A	5.56	2	2.78	1.64	0.02
B	2.89	2	1.445	0.85	0.18
C	1.34	2	0.67	0.395	0.57
Error	3.77	2	1.885		

According to the data in Table 7, the order of factors influenced the bacteriostasis effect was A (concentration)>B (temperature)>C (pH), and the optimal levels were A3, B3 and C3. Table 8 was the result of the difference analysis of the orthogonal test. The results were shown that the P value of A is less than 0.05 and it was the main factor, B (temperature) and C (pH) were the secondary factors. The optimal level chosen according to actual production needs was A3B3C3.

4.4.2. Results of Orthogonal Experiments of Proteus

Table 9. *Orthogonal experiments of proteus.*

code \ factor	A	B	C	Circle (mm)
1	1	70	3	25
2	1	80	4	20
3	1	90	5	18
4	12	70	4	16
5	1.2	80	5	17
6	1.2	90	3	27
7	1.4	70	5	23
8	1.4	80	3	23
9	1.4	90	4	17
K1	63	64	57	
K2	60	60	53	
K3	63	62	58	
R	1	1.3	1.1	

Table 10. *Variance analysis.*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
A	2	2	1	0.017	0.20
B	2.6	2	1.3	0.022	0.03
C	4.3	2	2.15	0.930	0.32
Error	117.1	2	58.55		

According to the data in Table 9, the order of factors influenced the bacteriostasis effect was B (temperature)>C (pH) >A (concentration), and the optimal levels were A2, B3 and C1. Table 10 was the result of the difference analysis of the orthogonal test. The results were shown that the P value of B was less than 0.05 and it was the main factor, A (concentration) and C (pH) were the secondary factors. The optimal level chosen according to actual production needs was A2B3C1.

4.4.3. Results of Orthogonal Experiments of Staphylococcus

Table 11. *Orthogonal experiments of staphylococcus.*

code \ factor	A	B	C	Circle (mm)
1	1	70	3	22
2	1	80	4	20
3	1	90	5	18
4	12	70	4	20
5	1.2	80	5	20
6	1.2	90	3	23
7	1.4	70	5	20
8	1.4	80	3	20
9	1.4	90	4	20
K1	60	62	65	
K2	63	60	60	
K3	60	61	58	
R	1	0.7	2.4	

Table 12. *Variance analysis.*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
A	2	2	1	0.427	0.42
B	0.66	2	0.33	0.141	0.36
C	8.66	2	4.33	1.850	0.03
Error	4.68	2	2.34		

4.4.4. Results of Orthogonal Experiments of Serratia

According to the data in Table 11, the order of factors influenced the bacteriostasis effect was C (pH) >A (concentration)>B (temperature), and the optimal levels were A2, B3 and C1. Table 10 was the result of the difference analysis of the orthogonal test. The results shown that the P value of C (pH) was less than 0.05, and it was the main factor, A (concentration) and B (temperature) were the secondary factors. The optimal level chosen according to actual production needs was A2B3C1.

Table 13. *Orthogonal experiments of serratia.*

code \ factor	A	B	C	Circle (mm)
1	1	70	3	17
2	1	80	4	16
3	1	90	5	17
4	12	70	4	18
5	1.2	80	5	17
6	1.2	90	3	19
7	1.4	70	5	20
8	1.4	80	3	18
9	1.4	90	4	16
K1	50	57	54	
K2	54	52	54	
K3	54	52	50	
R	1.3	1.7	1.3	

Table 14. *Variance analysis.*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
A	3.56	2	1.78	2.08	0.41
B	5.40	2	2..70	3.16	0.02
C	3.56	2	1.78	2.08	0.41
Error	1.71	2	0.855		

According to the data in Table 13, the order of factors influenced the bacteriostasis effect was B (temperature)>C (pH) =A (concentration), and the optimal levels were A3, B1 and C3. Table 14 was the result of the difference analysis of the orthogonal test. The results were shown that the P value of C (pH) was less than 0.05 and it was the main factor, A (concentration) and B (temperature) were the secondary factors. The optimal level chosen according to actual production needs was A2B3C1.

4.5. Effect of ε-p-L • HCl on Shelf-Life of Spiced Pork

4.5.1. Sensory Evaluation Results of Spiced Pork

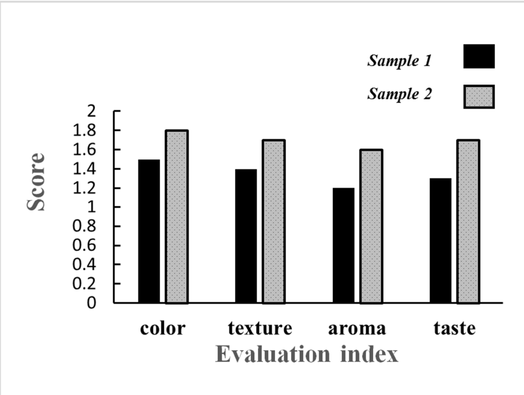

Figure 1. *Sensory evaluation.*

The sensory evaluation results were shown in Figure 1, Compared with No. 1 sample without adding ε-p-L･HCl, the sample with ε-p-L･HCl did not have nausea taste and had a light flavoring taste, so that the score of experiment group was higher.

4.5.2. Determination of Microbial Indexes in Spiced Pork During Storage

Table 15. *The results of microbial index determination on sample (1).*

	Day 0-6	Day 7	Day 8
total numbers of colony (CFU/g)	<8000	9800	14000
Coliform population (MPN/100g)	0	0	0

Table 16. *The results of microbial index determination on sample (2).*

	Day 0-10	Day 11-13	Day 13-15	Day 16
total numbers of colony (CFU/g)	<7000	8600	9800	12000
Coliform population (MPN/100g))	0	0	0	0

On the 8th days of storage, the total number of colony and coliform exceeded the national standards, respectively. The shelf life of No. 1 sample was 7d. On the 16th day of storage, the total number of colony exceeded the requirements of the national standard, so the shelf life of No. 2 sample was 15 days.

4.6. Effect of ε-p-L • HCl on Shelf-Life of Bath Chap

4.6.1. Sensory Evaluation Results of Bath Chap

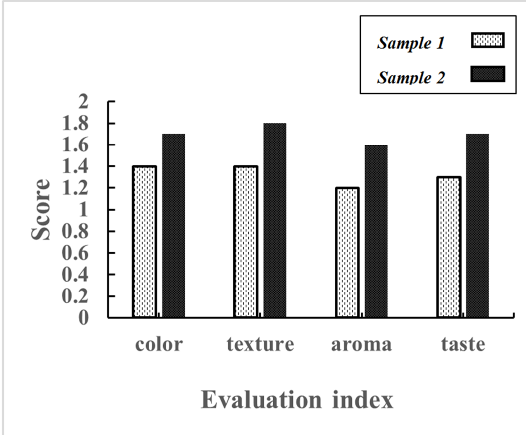

Figure 2. *Sensory evaluation.*

The sensory evaluation results were shown in Figure 2, compared with No. 1 sample without adding ε-p-L･HCl, the sample with ε-p-L･HCl had no nausea taste and had a light flavoring taste, so that the sauced bath chap did not taste greasy.

4.6.2. Determination of Microbial Indexes in Bath Chap During Storage

Table 17. *The results of microbial index determination on sample (3).*

	Day 0-5	Day 6	Day 7
total numbers of colony (CFU/g)	<6000	8400	12000
Coliform population (MPN/100g)	0	0	110

Table 18. *The results of microbial index determination on sample (4).*

	Day 0-8	Day 9-10	Day 11-12	Day 13
total numbers of colony (CFU/g)	<10	7800	55000	98000
Coliform population (MPN/100g))	0	0	300	330

On the 7th and 8th days of storage, the total number of colony and coliform exceeded the national standards respectively. The shelf life of No. 1 sample was 9d. On the 10th day of storage, the total number of colony and coliform bacteria exceeded the requirements of the national standard, so the shelf life of No. 2 sample was 9 days.

5. Conclusion

As a good preservative, ε-p-L･HCl has many advantages, such as good bacteriostasis effect, broad bacteriostasis spectrum, non-toxic and safe. It has good bacteriostasis effect on spoilage bacteria in meat at low concentration. For Staphylococcus, Proteus, Bacillus and Serratia, the minimum inhibitory concentration of preservatives was 500 ug/mL. The thermal stability of ε-p-L･HCl is fine, and it could withstand high temperature of 100 C. The bacteriostasis effect of ε-p-L·HCl after heating was almost unaffected. Therefore, in meat production and processing, the addition of ε-p-L·HCl could be combined with food for heat treatment and secondary sterilization, so as to prolong the shelf life of meat. The antimicrobial activity of ε-p-L·HCl has a wide range of pH value, and has a good antimicrobial effect in the weak acidic environment of meat processing. In addition to the national standard, the shelf-life of the pork elbow was increased by 9 days compared with the blank control. Compared with the blank control, the shelf life of pork head meat was prolonged by 7 days after adding ε-p-L·HCl. Through the study of this experiment, we would provide experimental basis for antisepsis and preservation of meat products.

Acknowledgements

We thank the Teaching Group of Food Chemistry, Faculty of Food Science and Engineering of Beijing University of Agriculture for laboratory assistance. Molecular analyses were supported by Beijing Key Laboratory of Agricultural, Product Detection and Control of Spoilage Organisms and Pesticide Residue. We thank Beijing Innovation Consortium of Swine Researeh System and Graduation Design of the Practical Training Program for the Cross-Cultivation of High-Level Talents in Beijing Colleges and Universities for support.

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