Effect of Chitosan-acetic acid mixture on the growth of bacteria in non-vaccum packaged pork stored at 10℃ or 20℃

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ABSTRACT

Fresh meat and ones inoculated with S.aureus, S.typhimurium and L.monocytogenes were separately dipped for 10s in 10% chitosan-acetic acid mixture (CA mixture), 20% acetic acid solution (A solution) and sterile distilled water, and allowed to drip for 3-5 min before packing in polyethylere bag, then stored at 10℃ or 20℃. Effect of CA mixture and A solution on the growth of variousbacteria growing fresh meat including the total bacterial count, Pseudomonas, Enterobacteriaceae, Lactobacillus, Staphylococcus, Micrococcus, Enterococcus, S.aurens, S.typhimurium and L.monocytogenes was examined, and compared with the antibacterial activity of CA mixture and A solution. results showed that during the entire experiment, CA mixture had definite inhibition of various spoilageand pathogenic becteria including lactobacillus. Inhibition of Enterobacteriaceae and Enterococrus was strongest by CA mixture. Although CA mixture was as effective removing bacteria from the surface of fresh meat as A solution on 0 day, but the effectiveness of CA mixture against bacteria was greater than that of A solution. So the shelf life of the meat treated with CA mixture was correspondinglylonger than that treated with A solution. We also found CA mixture had much greater inhibition of bacteria at 10℃ than that at 20℃.

At the same time, the outgrowth of Clostridium of fresh meat was also examined, the result indicated that CA mixture or A Solution had inhibition effect on the bacteria, but CA mixture had greater inhibition effect on the bacteria than A Solution.

It is a problem noticed generally by meat industry to reduce the microbial contamination of fresh meat and extend its shelflife. In many countries, especially industrialized countries, hygiene management of fresh meat slaughted has been greathy strengthened. The shelflife of fresh meat has been apparenty extended by many methods such as refrigeration, modified atmosphere packaging and vaccum packaging(1). Because of the worldwide lack of energy, researchers are developing new saving-evergy technology of fresh meat preservation. Especially in developing countries, the shortage of energy and refrigerating equipmentis more serious. So the research is considered more important if fresh meat is sprayed or dipped by acetic acid to extedn its shelflife.

Fresh meat is usually sprayed or dipped by edible organic acid such as acetic acid, lactic acid. There are many reports on this aspect which are mainly focused on studing how to prolong the storagelife of beef, mutton and chicken. Only a few papers on extending the shelflife of pork are found. But there is increasing tendency to study how to strengthen the shelflife of pork by acetic acid ( ) in recent years. Acetic acid. Generally Regarded as Safe Substance (GRAS) with strong bactericidal action, has received approval for use as a sanitizer of red meat carcasses ( ). Anderson et al ( ) reported that a 30% acetic acid solution used as a meat surface sanitizer can more effectively reduce microbial population in meat than sodium hypochlorite (200-250 mg/L). Eustance et al ( ) reported the bacterial counts were decreased by 96% and 99% when lamb carcasses were sprayed with 1.5% and 3.0% acetic acid, respectively. The reduction of the bacterial population on beef treated with acetic acid is 1 to 2 log cycles. Biemuller et al ( ) also proved that acetic acid can reduce the bacterial counts on the pork carcasses. The storagelife of fresh meat could be increased because acetic acid reduced its surface bacterial count. Cacciarelli et al ( ) obtained that bacterial counts in fresh pork loins Spray-washed with a 20% acetic acid solution were lower significantly before vaccum packaging and storage at 4℃ for 28 days Shay et al ( ) showed the shelflife of pork treated with acetic acid prior to vaccum packaging was increased from 3 weeks to 6 weeks previous research showed that more than 20% acetic acid concentration would affect organoleptic properties of meat ( ). Recent reports have proved that the antibacterial activity of acetic acid can be increased by mixing with other organic acid such as lactic acid, formic acid or with other preservatives such as sorbic acid. It supports a possibility that fresh meat treated with acetic acid can have relative long shelflife under non- refrigerating condition. Leisfner et al ( ) sprayed beef and mutton carcasses with a mixture containing 20% acetic, 1% lactic, 0.25% citric and 0.1% ascorbic acid and made their shelflife increase one time under 15℃. Authors ( ) has observed when pork was dipped with a mixture containing 20% acetic,1% lactic, 0.25% citric and 0.1% ascorbic acid and 3.0% potassium sorbate as well as 4.0% , its shelflife can be kept for at least 7 days at 30℃( ). In order to increase the antibacterial effectiveness of acetic acid to extend the shelflife of fresh meat under non-refrigerating condition, it was very necessary to study and develop some new, high-effective nontoxic preservatives.

Chitosan is a polymer composed of glucosamine residues linked by β, 1-4 glucosidic bonds. It is a deacetylated derivative of chitin. In recent years, the effectiveness of chitosan against microorganism has been concerned in the field of food inclustry. There are some reports that chitosan can inhibit the growth of E.coli S.aureus, P.aeruginosa and B.subtilis. Authors have found that chitosan can effectively inhibit the growth of five type pathogenic bacteria (S.aureus, E.coli, Y.enterocolitica, S.typhimuriumand L.monocytogenes) (data published). In Japan, there are some patents that chitosan can be used as food preservative to extend the shelflife of food. But so far, it is found that only a patent concerning chitosan has been used to fresh meat preservative to extend its storage life under non-refrigerated condition.

The objective of this study is to analyze the inhibition of chitosan-acetic acid mixture on various spoilage and pathogenic bacteria which grow on non-vaccum packaged pork stored at 10℃ or 20℃, and compare the antibacterial effectiveness of chitosan-acetic acid mixture with that of acetic acid.

MATERIALS and METHODS

Organism: all tested species including L.monocytogenes (Serotype 46), S.aureus and S.typhimurium were supported by Dr.Y.kokubo, who worked in Dept, of Food Hygiene & Nutrition, Tokyo Metropolitan Research Laboratory public Health. Before experiment, these bacteria were incubated under a specific condition (shown TABLE Ⅰ) and were harvested by washing with sterile 0.85% saline buffer and centrifugated for 10 min 3000×g. The pellets resuspended in 2000ml sterile 0.85% saline buffer, which must be used within 2hrs.

Preparation and storage of samples: Fresh pork belly were obtained from HONGQIAO free market in Beijing, and cut into about 100g lumbs aseptically mixed to facilitate distribution of bacteria over the surface and to insure randomness in assigning lumps to the various treatments. This experiment was divided into two parts. One is to analyze the effect of chitosan-acetic acid mixture on the growth of spoilage bacteria on meat stored at 10℃ or 20℃, another is to study the influence of chintosan-acetic acid mixture on the growth of pathogenic bacte

(S.aureus, S.typhimurium and L.monocytogenes) inoculated on the meat. The procedure of treatment of samples is as follow.

Part I: The prepared lumps were dipped for 10s in 10% chitosan-2% acetic acid mixture (CA mixture), 2% acetic acid (A solution) and steril distilled water, respectively and were allowed to drip for 3 or 5 min before wrapping in polyethylene bag, Then stored at 10℃ or 20℃.

part II: The prepared clumps separately transferred to these three types of pathogeric bacteria resuspension, and allowed to standat room temperature 15 S. The lumps were then incubated at 30℃ for 2 or 3 hrs to make these bacteria firmly attach to the procedure of part I.

Microbiological analysis: According to schedule, the bacterial change of the samples stored at 20℃ was analyzed on 0, 1, 2, 3, 4, 5 and 6 days. The bacterial change of the samples stored at 10℃ was examined on 0, 2, 4, 6, 8, 10 and 12 days during the entire experiment.

20 ground samples were placed aseptically into a bottle containing 180ml sterile 0.85% saline buffer. The appropriate dilutions were made in tubes containing 9ml sterile 0.85% saline buffer. Except that the Clostridium was examined by pour-plate method, other bacteria were numbered by spreading method. Plates were incubated as in TABLE Ⅰ.

RESULTS and DISCUSSION

PART Ⅰ: Bacterial population found in pork belly on 0 day was 4.11 log CFU/g, it indicated that the microbial contamination of fresh meat slaughtered was relatively low. The result agreed with ourlastest investigation on bacterial contamination from retail meats in Beijing(data not being published). Initial bacterial numbers of the meat had definite influence on its shelflife ( ). But our result showed that the temperature of stored meat had greater effect on its shelflife than initial bacterial population did (shown in TABLE Ⅱ, Ⅲ).

When fresh meat was stored at 10℃, the storage life of the control was about 1 or 2 days. A solution could increase its shelflife about 5 or 7 days. The total bacterial counts of the meat treated with CA mixture was less than 6.00log CFU/g (ca.5.52log CFU/g) at the end of the experiment;When fresh meat was stored at 20℃,it took only 1 day when the control and the meat treated with A solution spoiled.But CA mixture could increase its shelflife two times. It seems that CA mixture had greater antibacterial activity than A solution (shown in TABLEⅡ, Ⅲ)

The reduction of the bacterial counts on the surface of the meat was about 1 log CFU/g, when the meat was treated with A solution or CA mixture on 0 day. Of the examined spoilage bacteria, the number of pseudomonas, Enterobacteriaceae, Lactobacillus and Staphylocouus was also correspondingly reduced 1 log CFU/g. It indicated that CA mixture was as effective as A solution in removing bacteria from the surface of fresh meat. This result coincided with previous conclusion that acetic acid could reduce the microbial population of fresh meat ( ) (shown in TABLE Ⅱ, Ⅲ)

When fresh meat was stored at 10℃, the maximum bacterial population(9.72 log CFU/g) of the control was achieved after 4 days of storage. Pseudomonas and Exterobacteriaceal were the dominant organisms. The bacterial number of the meat treated with A solution reached 8.56 log CFU/g at the end of the experiment. Inhibition of Exterobacteriaceae and Enteroccus were greatest. The grouth of other spoilage bacteria except lactobacillus were also inhibited to a greater or less degree during the storage of the meat. Leistner et al ( ) reported a acid mixture containing acetic, lactic, citric and ascorbic acid Was more effective against Enterobacteriaceae. But Acuft et al( ) noted no difference in the bactericidal effectiveness betweenthe acid mixture of Leistner and solutions of either 1% acetic acid or 1% lactic acid. Mendonca et al( ) also reported 1.0 or 1.5% acetic acid could inhibit the growth of Enterobacteriaceae. Until the end of the experiment, the bacterial count of the meat treated with CA mixture had not reached 6.00 log CFU/g. During the entire experiment, The Inhibition of various spoilage bacteria by CA mixture was relatively strong, the effectiveness of CA mixture against Enterobacteiaceae and Enterococcus was greatest, and their numbers were undetected at the end of the experiment (shown in TABLE Ⅱ).

When fresh meat was stored at 20℃,the control had reached its maximum population (Ca9.41 log CFU/g) after 3 days. Similarly,pseudomonas and Enterobacteriaceae were predominart orgarism. Compared to the control, A solution had definite inhibition against pseudomonas, Enterobacteriaceae, Staphylococcus and Enterococms, inhibition of Enterobacteriaceae was strongest, following by Enterococcus, Oseudomonas, Staphylococcus and Micrococcus. But at the same time, A solution hadn't inhibited the growth of lactobacillus. Compared to the control and the meat treated with A solution, CA mixture had greater inhibition on various spoilage bacteria incluling Lactobacillus. Theresult also showe that the effectiveness of CA mixture or A solution at 10℃ was stronger than at 20℃(shown in TABLE Ⅲ).

At the same time, the outgrowth of Clostridium on fresh meat also was examined. When the meat was stored at 20℃, Clostridium was found to be present in the control after 1 day.The meat treated with A solution was positive for clostrediumafter 4 day. But the meat treated with CA mixture was free at the end of the experiment.When the meat was stored at 10℃,the control was found to be positive for Clostrichium after 4 days, A solution could keep the meat free of clostridium until 10 days, similarly the meat treated with CA mixture was tree until the end of the experiment. It indicated that CA mixture and A solution had effectiveness against clostridium, and CA mixture had much greater inhibition of Clostridium than A solution (shown in TABLE Ⅶ).

From metioned above,We found that CA mixture was effective in removing bacteria from the surface of fresh meat on 0 day as A solution, the antibacterial activity of CA mixture or A solution at 10℃ was greater than at 20℃. and CA mixture had much greater. antibacterial activity than A solution did. It should be noted that CA mixutre could inhibit the growth of Lactobacillus to a definite degree. Previous research showed that one of the main bacteria Which caused the spoilage of vaccum packaged meat was Lactobacillus( ). Our finding perhaps supported a chance to extend the shelflife of vaccum packaged meat. part Ⅱ. Fresh meat inoculated with L.monocytogenes, S.typhimurium and S.aureus was separately treated with CA mixture, A solution and sterile distilled water to observe effect of CA mixture on the growth of these pathogeric bacteria.

When fresh meat was inoculated with S.typhimurium, the initial bacterial number of the control was 5.80 log CFU/g, and the reduction of the bacterial count on the meat treated with CA mixture or A solution was 2 log CFU/g. Anderson et al( ) repoted a 3% acid mixture containing acetic, lactic, citric and ascorbic acid would reduce the number of S.typhimurium inoculated on Beef 2.3 log CFU/g. Wang( ) also found PH2 acetic acid solution made the number of S.typhimurium inoculated on beef reduce 4 log CFU/g. When the meat was stored at 10℃ or 20℃, S.typhimurium on the control grew well, the growth of the pathogen was inhibited by CA mixture or A solution. The effectiveness of CA mixture or A solution against the pathogen was greater at 10℃ than at 20℃, and CA mixture had greater inhibition against the pathogen than A solution (shown in TABLE Ⅴ).

When fresh meat was inoculated with L.monocytogenes, there was no differenece in the initial number of the pathogen on the control and that treated with CA mixture or A solution. When the meat was stored at 10℃ or 20℃, although L.monocytogenes of the control grew rapidly, A solution inhibited its growth. During the storage of the meat, there was tendency for the pathogen to grow, but its growth was very slow. Inhibition of the pathogen by CA mixture was stronger, the growth of L.monocytogenes on the meat stored at 10℃ halted and the viable cells gradually decreased. But at 20℃,the pathogen nearly stop grouing, only there was tendency for it to grow at the end of the experiment(shown

in TABLE Ⅳ).

When fresh meat was inoculated with S.aureus, A solution or CA mixture could reduce the initial bacterial count 1 log CFU/g ,compare the control. S.aureus of the control stored at 20℃ grew well on the first day, following other days of storage, the number of the path was undetected, though these meat treated with A solution stop growing and after the first fourth dayits number also was less than 2.00 CFU/g,only the bacterialcount on the meat treated with CA mixture was still detectableat the end of the experiment similarly,At 10℃,S.a of the control was undetectable after the third days the number of the pathogen on the meat treated with A solution was less than 2.00 CFU/g after sixth days. Its count on the meat treated with CA mixture was still detectable at the end of the experiment, although its gr stop during the entire experiment.The reason is that other bacteria on the Control grow rapidly and lead to inhibition of S.aureus,A sol or CA mixture also inhibited the growth of S.aureus, but A solution or CA mixture had stronger inhibition effect on other bacteria, So survival of the pathogen on the treatments could keep much more time than one on the control.In order to prove our inference,this experi was repeated, the same results were obtained (shown in TABLE Ⅵ)

From mentioned above, we found that there were no differences in removing these pathogen from the surface of fresh meat treated wit mixture and A solution, and CA mixture and A solution had difinite inhibition of these pathogen. Effectiveness of CA mixture or A solu against these bacteria at 10℃ was stronger than at 20℃, and CA mixture had greaterinhibition of these pathogen than A solution.

Through the above two experiments, we found CA mixture or A solution had definite inhibition of various spoilage and pathogenicbact except that A solution didn't inhibit the growth of Lactobacillus. Because CA mixture had stronger antibacterial activitythan A solution the shelflife of the meat treated with CA mixture was longer than that treated with A solution. The antibacterial mechanism of chitosan not been clear yet, but the antibacterial activity of chitosan does exist. Some researches ( ) reported that effectiveness of chit against bacteria growing on protein food such as meat and milk is relatively weak. But our findings show that inhibition of bacteria on meat by CA mixture is stronger.It should be noted that effectiveness of CA mixture against fungi is relatively weak,in the latter experi Some samples treated with either CA mixture or A solution were found moulds and yeasts.But in general, It is possible for chitosan to be a new, non-toxic natural meat preservative.

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TABLE Ⅰ.Type of count, medium used and time and temperature of incubation

Type of count		Time(h)	Temp(℃)	Reference
Total bacterial count	plate count Agar	48	30	(8)
pseudomonas	plate count Agar	48	30	(8)
Enterobacteriaceae	DHL	24	30	(8)
Lactobacillus	MRS	48	30	(8)
Micrococcus	MRS	48	30	(8)
Staphylococcus	Mannitol Salt phenol-red Agar	72	37	(8)
Enterococcus	Azide Esculin Agar	24	45	(12)
S.aureus	Mannitol Salf phenol-red Agar	72	37	(8)
Styphimurium	DHL	24	30	(8)
L.monocytogenes	PALCAM	48	30	(8)

TABLE Ⅱ. Effect of CA mixture or A solution on the growth of spoilage bacteria on fresh meat stored at 10℃

Type of treatment	Type of spoilage bactlria	log_{10} CFU/g						
		Storage time (d)						
		0	2	4	6	8	10	12
Control	Total bacterial count	4.11	7.54	9.72	9.15	8.73	9.48	9.36
	Pseudomonas	3.00	6.20	8.90	8.28	8.15	8.60	8.48
	Enterobacteriaceae	3.04	6.81	7.81	8.11	8.56	8.52	8.89
	Lactobacillus	3.20	6.67	6.62	7.26	7.66	7.94	7.41
	Micrococcus	2.85	5.00	5.72	6.08	6.51	6.23	6.30
	Staphylococcus	3.75	6.68	7.62	7.69	7.18	7.72	7.49
	Enterococcus	<2.00	3.56	3.80	5.74	6.18	5.97	6.34
A solution	Total bacterial count	3.23	5.11	6.98	6.94	7.71	7.91	8.56
	Pseudomonas	2.30	3.51	5.00	6.15	6.30	6.48	7.30
	Enterobacteriaceae	2.00	2.00	<2.00	2.00	2.00	5.45	7.85
	Lactobacillus	2.00	5.08	6.15	6.81	7.60	7.65	7.04
	Micrococcus	<2.00	3.30	5.00	6.81	6.53	6.43	6.60
	Staphylococcus	2.30	4.28	5.48	5.67	6.20	6.30	6.34
	Enterococcus	<2.00	<2.00	<2.00	<2.00	<2.00	4.15	5.53
CA mixture	Total bacterial count	3.30	3.48	4.93	5.23	5.32	5.59	5.52
	Pseudomonas	<2.00	3.00	3.95	4.48	4.30	4.90	4.70
	Enterobacteriaceae	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
	Lactobacillus	2.30	2.30	3.68	3.90	4.48	4.04	4.41
	Micrococcus	<2.00	2.00	3.57	3.97	4.38	4.04	4.32
	Staphylococcus	2.70	2.40	3.70	3.95	4.15	4.51	5.20
	Enterococcus	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00

TABLE Ⅲ. Effect of CA mixture or A solution on the growth of spoilage bacteria on fresh meat stored at 20℃

Type of treatment	Type of spoilage bactlria	log_{10} CFU/g						
		Storage time (d)						
		0	1	2	3	4	5	6
Control	Total bacterial count	4.11	7.51	8.79	9.41	9.15	9.20	8.99
	Pseudomonas	3.00	5.78	8.46	8.43	8.83	8.95	8.48
	Enterobacteriaceae	3.04	6.97	8.11	8.65	8.75	8.63	8.81
	Lactobacillus	3.20	6.40	7.30	8.62	9.11	8.08	6.40
	Micrococcus	3.85	4.99	6.55	6.38	6.86	6.76	6.52
	Staphylococcus	3.75	6.04	7.60	7.36	7.49	7.30	8.04
	Enterococcus	<2.00	5.00	5.93	6.26	6.88	6.99	6.66
A solution	Total bacterial count	3.23	7.26	7.81	7.18	8.41	8.94	8.54
	Pseudomonas	<2.00	4.94	5.90	6.30	7.48	7.00	6.94
	Enterobacteriaceae	<2.00	<2.00	<2.00	4.69	7.20	7.30	8.04
	Lactobacillus	2.00	6.71	6.80	7.00	8.18	8.32	7.99
	Micrococcus	<2.00	4.00	5.98	6.70	7.32	7.00	6.86
	Staphylococcus	2.30	5.67	6.04	5.95	6.45	7.20	7.20
	Enterococcus	<2.00	3.00	3.81	4.26	6.88	6.95	6.78
CA mixture	Total bacterial count	3.30	5.89	6.54	7.51	8.41	7.99	8.18
	Pseudomonas	<2.00	4.67	5.48	6.00	6.85	6.75	7.95
	Enterobacteriaceae	<2.00	<2.00	<2.00	<2.00	<2.00	4.20	7.79
	Lactobacillus	<2.00	5.38	6.08	6.91	7.11	7.51	7.79
	Micrococcus	<2.00	4.30	4.83	5.23	5.88	6.66	6.51
	Staphylococcus	2.70	3.30	4.81	4.70	4.94	5.85	6.26
	Enterococcus	<2.00	<2.00	<2.00	3.59	5.52	4.18	4.28

TABLE Ⅳ. Effect of CA mixture or A solution on the growth of L.monocytogenes inoculated on fresh meat stored at 10℃ or 20℃

Type of treatment	Temperature of storage (℃)	log_{10} CFU/g									
		Storage time (d)									
		0	1	2	3	4	5	6	8	10	12
Control	10	4.15		6.69		7.20		7.40	7.18	7.28	6.98
A solution	10	4.11		4.86		4.04		4.28	5.32	5.34	5.96
CA mixture	10	4.00		4.15		4.00		3.58	3.30	3.78	3.60
Control	20	4.15	6.95	7.04	6.85	6.62	6.82	5.74			
A solution	20	4.11	4.36	4.63	5.08	5.51	5.66	6.00			
CA mixture	20	4.00	4.15	4.36	4.89	4.68	4.70	5.79			

TABLE V. Effect of CA mixture or A solution on the growth of S.typhimurium inoculated on fresh meat stored at 10℃ or 20℃

Type of treatment	Temperature of storage (℃)	log_{10} CFU/g Storage time (d) 0	1	2	3	4	5	6	8	10	12
Control	10	5.80		6.62		7.41		8.11	8.00	8.32	7.95
A solution	10	3.88		3.70		3.64		4.46	4.76	6.82	7.11
CA mixture	10	3.63		2.90		3.62		3.70	4.57	5.49	7.38
Control	20	5.80	7.78	8.00	7.95	7.78	7.30	7.30			
A solution	20	3.88	6.87	8.28	8.38	8.57	8.60	8.95			
CA mixture	20	3.63	5.98	7.51	7.97	8.48	8.75	8.76			

TABLE VI. Effect of CA mixture or A solution on the growth of S.aureus inoculated on fresh meat stored at 10℃ or 20℃

Type of treatment	Temperature of storage (℃)	log_{10} CFU/g Storage time (d) 0	1	2	3	4	5	6	8	10	12
Control	10	4.90		5.00		<2.00		<2.00	<2.00	<2.00	<2.00
A solution	10	3.34		3.70		3.38		3.00	<2.00	<2.00	<2.00
CA mixture	10	3.00		3.08		3.82		3.36	3.32	3.78	3.30
Control	20	4.90	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00			
A solution	20	3.34	5.04	5.75	5.00	<2.00	<2.00	<2.00			
CA mixture	20	3.00	4.49	3.15	4.79	4.30	3.32	4.58			

TABLE VII. Effect of CA mixture or A solution on the Incidence of Clostridium on fresh meat stored at 10℃ or 20℃

Type of treatment	Temperature of storage (℃)	log_{10} CFU/g Storage time (d) 0	1	2	3	4	5	6	8	10	12
Control	10	−*		+**		+		+	+	+	+
A solution	10	−		−		−		−	−	−	+
CA mixture	10	−		−		−		−	−	−	−
Control	20	−	+	+	+	+	+	+			
A solution	20	−	−	−	−	+	+	+			
CA mixture	20	−	−	−	−	−	−	−			

* means the number of clostridium per sample(g) is less than 1.00 log_{10} CFU/g
** means the number of clostridium per sample(g) is not less than 1.00 log_{10} CFU/g