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

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Key word: Chitosan-acetic acid-spoilage bacteria-S.aureus-S.typhimurium-L.monocytogenes-Pork.

ABSTRACT

Fresh meat and ones inoculated with S.aureus. S.typhimurium and L.monocytogenes were separately dipped for 10s in 10% chitosan num acetic acid mixture (CA mixture), 20% acetic acid solution (A solution) and sterile distilled water, and allowed to drip for 3-5 min be packing in polyethylere bag, then stored at 10°C or 20°C. 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, Enteroccus, Enterococcus, Enteroccus, Enter S.aurens, S.typhimurium and L.monocytogenes was examined, and compared with the antibacterial activity of CA mixture and A solution. 31, results showed that during the entire experiment, CA mixture had definite inhibition of various spoilageand pathogenic becteria including lactobacillus. Inhibition of Enterobacteriaceac and Enterococrus was strongest by CA mixture. Although CA mixture was as effective ter removing bacteria from the surface of fresh meat as A solution on O day, but the effectiveness of CA mixture against bacteria was grea 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°C than that at 20°C.

At the same time, the outgrowth of Clostridium of fresh meat was also examined, the result indicated that CA mixture or A Solution Sci 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 Pairs countries, especially industrialized countries, hygiene management of fresh meat slaughted has been greathy strengthened. The shelflife on fresh meat has been apparenty extended by many methods such as refrigeration, modified atmosphere packaging and vaccum packaging(1). Beogin re of the worldwide lack of energy, researchers are developing new saving-evergy technology of fresh meat preservation. Especially (sh developing countries, the shortage of energy and refrigerating equipmentis more serious. So the research is considered more important [#] 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 asp^oFI which are mainly focused on studing how to prolong the storagelife of beef, mutton and chicken. Only a few papers on extending the shelling of pork are found. But there is increasing tendency to study how to strengthen the shelflife of pork by acetic acid () in recent y^{ed} nis Acetic acid. Generally Regarded as Safe Substance (GRAS) with strong bactericidal action, has received approval for use as a san^{itid} of red meat carcasses (). Anderson et al () reported that a 30% acetic acid solution used as a meat surface sanitizer can Mer effectively reduce microbial population in meat than sodium hypochlorite (200-250 mg/L). Eustance et al () reported the bacterial co⁰the were decreased by 96% and 99% when lamb carcasses were sprayed with 1.5% and 3.0% acetic acid, respectively. The reduction of the bacterivan population on beef treated with acetic acid is 1 to 2 log cycles. Biemuller et al () also proved that acetic acid can reduce the bachas ial counts on the pork carcasses. The storagelife of fresh meat could be increased because acetic acid reduced its surface bacterial court Cacciarelli et al () obtained that bacterial counts in fresh pork loins Spray-washed with a 20% acetic acid solution were lower sign and cantly before vaccum packaging and storage at 4°C for 28 days Shay et al () showed the shelflife of pork treated with acetic acid phace to vaccum packaging was increased from 3 weeks to 6 weeks previous research showed that more than 20% acetic acid concentration would effsta organoleptic properties of meat (). Recent reports have proved that the antibacterial activity of acetic acid can be increased by pithe with other organic acid such as lactic acid, formic acid or with other preservatives such as sorbic acid. It supports a possibility isho fresh meat treated with acetic acid can have relative long shelflife under non-refrigerating condition. Leisfner et al () sprayed b and mutton carcasses with a mixture containing 20% acetic, 1% lactic, 0.25% citric and 0.1% ascorbic acid and made their shelflife incidio one time under 15°C. Authors () has observed when pork was dipped with a mixture containing 20% acetic, 1% lactic, 0.25% citric and 0the , its shelflife can be kept for at least 7 days at 30°C(). In offer ascorbic acid and 3.0% potassium sorbate as well as 4.0% to increase the antibacterial effectiveness of acetic acid to extend the shelflife of fresh meat under non-refrigerating condition" very necessary to study and develop some new, high-effective nontoxic preservatives.

 $Chitosan \ is \ a \ polymer \ composed \ of \ glucosamine \ residues \ linked \ by \ \beta \ , \ 1-4 \ glucosidic \ bonds. \ It \ is \ a \ deacetylated \ derivative \ of \ chitip^{(\beta)}$ recent years, the effectiveness of chitosan against microorganism has been concerned in the field of food inclustry. There are some rep that objective against microorganism has been concerned in the field of food inclustry. that chitosan can inhibit the growth of E.coli S.aureus, P.aeruginosa and B.subtilis. Authors have found that chitosan can effection 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 is found that only a patent concerning chitosan has been used to fresh meat preservative to extend its storage life under non-refrigers, a condition.

The objective of this study is to analyze the inhibition of chitosan-acetic acid mixture on various spoilage and pathogenic bac which grow on non-vaccum packaged pork stored at 10°C or 20°C, and compare the antibacterial effectiveness of chitosan-acetic acid pix the some store acid pix the source store stor with that of acetic acid. lls

MATERIALS and METHODS

Organism: all tested species including L.monocytogenes (Serotype 48), S.aureus and S.typhimurium were supported by Dr.Y.kokubo, who work that Dept, of Food Hygiene & Nutrition, Tokyo Metropolitan Research Laboratory public Health. Before experiment, these bacteria were includer under a specific condition (shown TARLE I) and see the second under a specific condition (shown TABLE I) and were harvested by washing with sterile 0.85% saline buffer and centrifugated for 10 "that the sterile of the $3000 \times g$. 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 HONCOIAO free market in Beijing, and cut into about 100g lumber of a sentical mixed to facilitate distribution of the sentical mixed to facilitate distribution o asepticallmixed to facilitate distribution of bacteria over the surface and to insure randomness in assigning lumps to the various treature are the surface and to insure randomness in assigning lumps to the various treature are the surface and to insure randomness in assigning lumps to the various treature are the surface and to insure randomness in assigning lumps to the various treature are the surface and to insure randomness in assigning lumps to the various treature are the surface and to insure randomness in assigning lumps to the various treature are the surface and to insure randomness in assigning lumps to the various treature are the surface and to insure randomness in assigning lumps to the various treature are the surface and to insure randomness in assigning lumps to the various treature are the surface ar This experiment was divided into two parts. One is to analyze the effect of chitosan-acetic acid mixture on the growth of spoilage bar on meat stored at 10°C or 20°C, another is to study the influence of chintosan-acetic acid mixture on the growth of pathogenic bach

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(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 disture distilled water, respectively and were allowed to drip for 3 or 5 min before wrapping in polyethylene bag, Then stored at 10°C or 20°C. part II: The prepared clumps separately transferred to these three types of pathogenic bacteria resuspension, and allowed to standat room temperature 15 S. The lumps were then incubated at 30°C 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°C was analyzed on 0, 1, 2, 3, 4, 5 and 6 days

days. The bacterial change of the samples stored at 10°C 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 n numbered by spreading method. Plates were incubated as in TABLE I. beli

RESULTS and DISCUSSION RESULTS and DISCUSSION slauphtered that the microbial contamination of fresh meat slauphtered slaughtered was relatively low. The result agreed with our lastest investigation on bacterial contamination from retail meats in Beijing(data not being multi-^{aughtered} was relatively low. The result agreed with ourlastest investigation on bacterial contamination. But our result showed that the ^{bot} being published). Initial bacterial numbers of the meat had definite influence on its shelflife (). But our result showed that the ^{bot} temperature of the meat had definite influence on its shelflife (). But our result showed that the i^{ve} 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°C, the storage life of the control was about 1 or 2 days. A solution could increase its shelflife about ^{nument} fresh meat was stored at 10°C, the storage life of the control was about 1 or 2 ways. A solution (a, b, b, c) or 7 days. The total bacterial counts of the meat treated with CA mixture was less than 6.00 log CFU/g (ca.5.52 log CFU/g) at the end of the experiment mutual bacterial counts of the meat treated with CA mixture and the meat treated with A solution spoiled. But CA mixture experiment; When fresh meat was stored at 20°C, it took only 1 day when the control and the meat treated with A solution spoiled. But CA mixture

^{10/} ould increase its shelflife two times. It seems that CA mixture had greater antibacterial activity than A solution (shown in TABLEII, III) The mode 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 The reduction of the bacterial counts on the surface of the meat was about I log Crurg, when the meat $if_{20}respondingly reduced 1 \log CFU/g$. It indicated that CA mixture was as effective as A solution in removing bacteria from the surface of resh meat π_1 . epsychologingly reduced 1 log CFU/g. It indicated that CA mixture was as effective as a solution that acetic acid could reduce the microbial population of fresh meat (If (shown in Times) is the solution of the solution of

When fresh meat was stored at 10°C, the maximum bacterial population(9.72 log CFU/g) of the control was achieved after 4 days of storage. Seudomonas and Exterobacteriaceal were the dominant organisms. The bacterial number of the meat treated with A solution reached 8.56 log sport last the end of the experiment. Inhibition of Exterobacteriaceae and Enteroccus were greatest. The grouth of other spoilage bacteria Il accept lactobacillus were also inhibited to a greater or less degree during the storage of the meat. Leistner et al () reported a acid penixture containing acetic, lactic, citric and ascorbic acid Was more effective against Enterobacteriaceae. But Acuft et al () noted no difference in the section of the i¹¹ ifference 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 what et al () also reported 1.0 or 1.5% acetic acid could inhibit the growth of Enteropacter factor. whether bacterial count of the meat treated with CA mixture had not reached 6.00 log CFU/g. During the entire experiment, The Inhibition of tevarious spoilage to the meat treated with CA mixture had not reached 6.00 log CFU/g. During the entire experiment, The Inhibition of te^{various} spoilage bacteria by CA mixture was relatively strong, the effectiveness of CA mixture against Enterobacteiaceae and Enterococcus ac^{twas} greatert ^{spontiage} bacteria by CA mixture was relatively strong, the criterian (shown in TABLE II). ^{at When for the formulation (Ca9.41 log)}

When fresh meat was stored at 20°C, the control had reached its maximum population (Ca9.41 log CFU/g) after 3 days. Similarly, pseudomonas d Enterohaeter in Tresh meat was stored at 20°C, the control had reached its maximum population (cas.m. is cross) and Enterobacteriaceae were predominart orgarism. Compared to the control, A solution had definite inhibition against pseudomonas, Entero-placteriaceae for the predominart orgarism. Compared to the control, A solution had definite inhibition against pseudomonas, Enterop^{1)acteriaceae}, Staphylococcus and Enterococcus, inhibition of Enterobacteriaceae was strongest, following by Enterococcus, Oseudomonas, [[Staphylococcus]] (Staphylococcus] and Enterococcus, inhibition of Enterobacteriaceae was strongest, following by Enterococcus, Oseudomonas, ^{[[Staphylococcus} and Micrococcus and Enterococms, inhibition of Enterobacteriaceae was strongest, forforme of and Micrococcus. But at the same time, A solution hadn't inhibited the growth of lactobacillus. Compared to the control and spithe meat treated out of the same time, A solution hadn't inhibited the growth of lactobacillus. Theresult also $g^{\rm photococcus}$ and Micrococcus. But at the same time, A solution hadn't inhibited the growth of factobaction. The same time, A solution hadn't inhibited the growth of factobaction. The solution, the same time, A solution on various spoilage bacteria including Lactobacillus. The solution is the same that the same time had greater inhibition on various spoilage bacteria including Lactobacillus. The solution is the same time that the same time had greater inhibition on various spoilage bacteria including Lactobacillus. The solution is the same time that the same time had greater inhibition on various spoilage bacteria including Lactobacillus. The solution is the same time that the same time had greater inhibition on various spoilage bacteria including Lactobacillus. $y^{\rm showe}$ that the effectiveness of CA mixture or A solution at 10°C was stronger than at 20°C (shown in TABLE III). At the component of CA mixture or A solution at 10°C was stronger than at 20°C (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°C, proclostridium was a fine time, the outgrowth of Clostridium on fresh meat also was examined with A solution was positive for close

pthe meat treated the be present in the control after 1 day. The meat treated with A solution was positive for clostrediumafter 4 day. But pthe meat treated with A solution was positive for clostrediumafter 4 day. But othe meat treated with CA mixture was free at the end of the experiment. When the meat was stored at 10°C, the control was found to be positive for Clostriching of the control was free at the end of the experiment. When the meat was stored at 10°C, the control was found to be positive of Clostrichium after 4 days, A solution could keep the meat free of clostridium until 10 days, similarly the meat treated with CA mixture (1943 tree until the solution had effectiveness against clostridium, and CA mixture j^{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 interesting the experiment. and much greater inhibition of Clostridium than A solution (shown in TABLE VI). From metioned above.We found that CA mixture was effective in removing bacteria from the surface of fresh meat on O day as A solution, and bacterial above.We found that CA mixture was effective in removing bacteria from the surface of fresh meat on O day as A solution,

p^{the} antibacterial activity of CA mixture or A solution at 10°C was greater than at 20°C. and CA mixture had much greater, antibacterial p^{the antibacterial} activity of CA mixture or A solution at 10°C was greater than at 20°C. and CA mixture had much greater. Previous p^{activity} than A solution did. It should be noted that CA mixutre could inhibit the growth of Lactobacillus to a definite degree. Previous p^{cesearch} showed that presearch 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 ^{supported} a chance to extend the shelflife of vaccum packaged meat. part II. Fresh meat inoculated with L.monocytogenes, S.typhimurium and ^a³-aureus was separately treated with CA mixture, A solution and sterile distilled water to observe effect of CA mixture on the growth of ^{these} pathogenic to the second sterile distilled water to observe effect of CA mixture on the growth of these pathogenic to the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to observe effect of the second sterile distilled water to obse

Then fresh meat was inoculated with S.typhimurium, the initial bacterial number of the control was 5.80 log CFU/g, and the reduction of bacterial count inoculated with S.typhimurium, the initial bacterial number of the control was 5.80 log CFU/g, and the reduction of bacterial count inoculated with S.typhimurium, the initial bacterial number of the control was 5.80 log CFU/g, and the reduction of bacterial count inoculated with S.typhimurium, the initial bacterial number of the control was 5.80 log CFU/g, and the reduction of bacterial count in the initial bacterial count in the initial bacterial count in the initial bacterial bacterial count in the initial bacterial count in the initial bacterial count in the initial bacterial bacterial count in the initial bacterial bacterial count in the initial bacterial bacterial bacterial count in the initial bacterial bacte ^{14 und} fresh meat was inoculated with S.typhimurium, the initial bacterial number of the control was 5.80 log CF0/g, and the sector is 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 of a solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g. Anderson et al () repoted a 3% acid mixture of A solution was 2 log CFU/g Sontaining acetic, lactic, citric and ascorbic acid would reduce the number of S.typhimurium inoculated on Beef 2.3 log CFU/g. Wang() Iso found PH2 acetic Iso 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°C or 20°C, S.typhimurium inoculated on beef reduce 4 log CFU/g. When the meat was stored at 10°C $A_{\rm mixture or A}$ solution against the pathogen was greater at 10°C than at 20°C, and CA mixture had greater inhibition against the pathogen was greater at 10°C than at 20°C.

When fresh meat was inoculated with L.monocytogenes, there was no differenece in the initial number of the pathogen on the control and at treated with CA minter ^{men} fresh meat was inoculated with L.monocytogenes, there was no differenece in the initial number of the pathogen of that treated with CA mixture or A solution. When the meat was stored at 10°C or 20°C, although L.monocytogenes of the control grew rapidly, s^{A solution} inhibited it. ^{1/2} ^{solution} inhibited its growth. During the storage of the meat, there was tendency for the pathogen to grow, but its growth was very slow. ^{a far and an inhibited its growth. During the storage of the meat, there was tendency for the pathogen to grow, out its growth viable cells ^{a far adually} decreased. But at 2000 mixture was stronger, the growth of L.monocytogenes on the meat stored at 10°C halted and the viable cells} p^{or action} of the pathogen by CA mixture was stronger, the growth of L.monocytogenes on the meat stored at 100 martee and the experiment(shown p^{or action} decreased. But at 20°C, 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°C 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 bacterial count on the meat treated with CA mixture was still detectable at the end of the experiment similarly. At 10°C, S.^{al} 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 g^{ri} stop during the entire experiment. The reason is that other bacteria on the Control grow rapidly and lead to inhibition of S.aureus, A solution 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 experiⁱ 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 $\pi^{i}t^{i}$ mixture and A solution, and CA mixture and A solution had difinite inhibition of these pathogen. Effectiveness of CA mixture or A solution against these bacteria at 10°C was stronger than at 20°C, 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 pathogenicbactive. The 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 experif. 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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T	ount			17 200 19		T i	me(h)	Temp(°C)	Referer			
pseudomon	terial count	plate	e count	Agar			48	30	(8)			
Enterobac	as	plate	e count	Agar			48	30	(8)			
Lactobaci	llus	DHL					24	30	(8)			
Micrococc	us	MRS					40	30	(8)			
Enteropoloc	occus	Manni	itol Sal	t phenol	-red Aga:	r	72	37	(8)			
S. aureus	CUS	Azide	e Esculi	n Agar			24	4 5	(12)			
Styphimur	ium	Manni	itol Sal	f phenol	-red Aga	r	72	37	(8)			
L.monocyt	ogenes	PALCA	A M				48	30	(8)			
ABLE II. Ef	fect of CA mixture	or A se	olution or	the grow	th of spoi	lage hact	teria on f	resh meat st	ored at 10°C			
ype of	Type of				logio	CFU/g						
reatment	spoilage bactlria			0 1 1 90 1 1	Con Frank	an man in						
			0	2	4	6	8	1 0	12			
	Total bacterial c	ount	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			
Control	Enterobacteriacea	е	3.04	6.81	7.81	8.11	8.56	8.52	8.89			
	Micrococours		3.20	6.67	6.62	7.26	7.66	7.94	7.41			
	Staphylococcus		2.85	5.00	5.12	0.08	0.51 7 19	0.23	6.30 7.49			
	Enterococcus		<2.00	3.56	3.80	5.74	6.18	5.97	6.34			
	Total bacterial c	ount	3.23	5.11	6.98	8.94	7.71	7.91	8.56			
٨	Enterobant		2.30	3.51	5.00	6.15	6.30	6.48	7.30			
solution	Lactobacillus	e	2.00	2.00	<2.00	2.00	2.00	5.45	7.85			
	Micrococcus		<2.00	3.30	5.00	6.81	1.60	6 43	6 BO			
	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			
	Total bacterial c	ount	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			
-A mixture	Lactobacteriacea	е	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00			
	Micrococcus		2.30	2.30	3.68	3.90	4.48	4.04	4.41			
	Staphylococcus		2.70	2.40	3.57	3.97	4.38	4.04	4.32			
	Enterococcus	1111	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00			
TABLE III. Et	ffect of CA mixtur	e or As	solution o	on the grou	wth of spoi	lage bac	steria on	fresh meat s	tored at 20°			
Type of	Tuno		log10 CFU/g									
	Type OI				logic	CFU/g						
treatment	spoilage bactlri	a			logic	CFU/g	0					
treatment	spoilage bactlri	a		1	logic Storage	e time (c	1)	5	6			
treatment	spoilage bactlri	a	0	1	logic Storage 2	cFU/g e time (c 3	1) 4	5	6			
treatment	spoilage bactlri Total bacterial Pseudomonas	a count	0 4.11 3.00	1	log10 Storage 2 8.79 8.46	9.41	1) 4 9.1	5 9.20	6 8.99 8.48			
Control	spoilage bactlri Total bacterial Pseudomonas Enterobacteriace	a count ae	0 4.11 3.00 3.04	1 7.51 5.78 6.97	logio Storage 2 8.79 8.46 8.11	CFU/g e time (c 3 9.41 8.43 8.65	1) 4 9.1 8.8 8.7	5 3 8.95 5 8.63	6 8.99 8.48 8.81			
Control	Total bacterial Pseudomonas Enterobacteriace: Lactobacillus	a count ae	0 4.11 3.00 3.04 3.20	1 7.51 5.78 6.97 6.40	logic Storage 2 8.79 8.46 8.11 7.30	CFU/g time (c 3 9.41 8.43 8.65 8.62	4 9.1 8.8 8.7 9.1	5 5 9.20 3 8.95 5 8.63 1 8.08	6 8.99 8.48 8.81 8.40			
Control	Total bacterial Pseudomonas Enterobacteriace: Lactobacillus Micrococcus Stanbulco	a count ae	0 4.11 3.00 3.04 3.20 3.85	1 7.51 5.78 6.97 6.40 4.99	logic Storage 2 8.79 8.46 8.11 7.30 6.55	CFU/g time (c 3 9.41 8.43 8.65 8.62 6.38	4) 9.1 8.8 8.8 7 9.1 6.8	5 9.20 3 8.95 5 8.63 1 8.08 3 6.76	5 8.99 8.48 8.81 6.40 6.52			
Control	Total bacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Enterococcus	a count ae	0 4.11 3.00 3.04 3.20 3.85 3.75 <2.00	1 7.51 5.78 6.97 6.40 4.99 6.04 5.00	log10 Storage 2 8.79 8.46 8.11 7.30 6.55 7.60 5.93	CFU/g time (c 3 9.41 8.43 8.65 8.65 8.65 8.65 8.62 6.38 7.36 6.26	4 9.1 8.8 9.7 8.8 8.7 9.1 6.8 7.4 6.8	5 9.20 3 8.95 5 8.63 1 8.08 6 6.76 9 7.30 8 6.99	6 8.99 8.48 8.81 6.40 6.52 8.04 6.66			
Control	Total bacterial Pseudomonas Enterobacteriace. Lactobacillus Micrococcus Staphylococcus Enterococcus Total bacterial	a count ae count	0 4.11 3.00 3.04 3.20 3.85 3.75 <2.00 3.23	1 7.51 5.78 6.97 6.40 4.99 6.04 5.00 7.26	log10 Storage 2 8.79 8.46 8.11 7.30 6.55 7.60 5.93 7.81	CFU/g time (c 3 9.41 8.43 8.65 8.62 6.38 7.36 6.26 7.15	4 9.1 8.8 8.7 9.1 6.8 7.4 6.8 8.8	5 5 9.20 3 8.95 5 8.63 1 8.08 6 6.76 9 7.30 8 6.99 1 8.94	6 8.99 8.48 8.81 6.40 6.52 8.04 6.66 8.54			
Control	Total bacterial Pseudomonas Enterobacteriace. Lactobacillus Micrococcus Staphylococcus Enterococcus Total bacterial Pseudomonas Enterit	a count ae count	0 4.11 3.00 3.04 3.20 3.85 3.75 <2.00 3.23 <2.00	1 7.51 5.78 6.97 6.40 4.99 6.04 5.00 7.28 4.94	log10 Storage 2 8.79 8.46 8.11 7.30 6.55 7.60 5.93 7.81 5.90	CFU/g time (c 3 9.41 8.43 8.65 8.62 6.38 7.36 6.26 7.18 6.30	4 9.1 8.8 9.7 9.1 6.8 7.4 6.8 8.4 9.7.4	5 9,20 3 8,95 5 8,63 1 8,08 6 6,76 9 7,30 8 6,99 1 8,94 8 7,00	6 8.99 8.48 8.81 6.40 6.52 8.04 6.66 8.54 6.94			
Control A solution	Total bacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Enterococcus Total bacterial Pseudomonas Enterobacteriace Lactobaciti	a count ae count ae	0 4.11 3.00 3.04 3.20 3.85 3.75 3.75 3.23 3.23 3.23 3.23 3.23 3.20 3.23 3.23	1 7.51 5.78 6.97 6.40 4.99 6.04 5.00 7.26 4.94 <2.00	log10 Storage 2 8.79 8.46 8.11 7.30 6.55 7.60 5.93 7.81 5.90 <2.00	CFU/g time (c 3 9.41 8.43 8.65 8.62 6.38 6.38 6.38 6.38 6.38 6.38 6.38 6.38	4 9.1 8.8 9.1 6.8 7.4 6.8 8.4 7.4 7.4	5 5 5 5 5 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 1 8 0 8 9 7 3 0 8 9 7 5 8 6 3 1 8 0 8 9 7 5 8 6 3 1 8 0 8 9 7 3 0 8 9 7 3 0 8 9 7 3 0 8 9 7 3 0 8 9 7 3 0 8 9 7 3 0 8 9 7 3 0 8 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 9 1 8 9 9 9 1 8 9 9 9 1 8 9 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 8 9 1 1 8 9 1 1 1 1 1 1 1 1 1 1 1 1 1	6 8.99 8.48 8.81 6.40 6.52 8.04 6.66 8.54 6.94 8.04			
Control A solution	Total bacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Enterococcus Total bacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcue	a count ae count ae	0 4.11 3.00 3.04 3.20 3.85 3.75 3.23 <2.00 <2.00 <2.00 <2.00 <2.00	1 7.51 5.78 6.97 6.40 4.99 6.04 5.00 7.26 4.94 <2.00 6.71 4.00	log10 Storage 2 8.79 8.46 8.11 7.30 6.55 7.60 5.93 7.81 5.90 <2.00 6.80 6.80	CFU/g time (c 3 9.41 8.43 8.65 6.36 6.26 6.36 7.18 6.30 4.69 7.00 7.00 7.00	4 9.1 8.8 9.1 8.8 9.1 8.8 9.1 8.8 9.1 8.8 9.1 8.7 9.1 8.8 9.1 8.6 8.7 9.1 8.7 9.1 8.7 9.1 8.8 9.7 9.7 9.7 9.7 9.7 9.7 9.7 9.7 9.7 9.7 9.7 9.7 9.7 9.7 9.7	5 5 9.20 3 8.95 5 8.63 1 8.08 6.76 9 7.30 8 6.99 1 8.94 8 7.00 0 7.30 8 8 8 8 8 8 8 8 9 7.30 8 8 9 7.30 8 8 9 7.30 8 8 9 7.30 8 8 9 7.30 8 8 9 7.30 8 8 9 7.30 8 8 9 7.30 8 8 9 7.30 8 8 9 7.30 8 8 8 9 7.30 8 8 9 7.30 8 8 8 9 7.30 8 8 8 9 7.30 8 8 8 9 7.30 8 8 8 9 7.30 8 8 8 9 7.30 8 8 8 9 7.30 8 8 9 7.30 8 8 8 9 7.30 8 8 8 9 7.30 8 8 8 9 7.30 8 8 8 9 9 7.30 8 8 8 9 7.30 8 8 8 8 8 8 7 8 9 8 8 8 7 7 8 9 8 8 8 8 8 8 8 8 8 8 8 8 8	6 8.99 8.48 8.81 6.40 6.52 8.04 6.66 8.54 6.94 8.04 7.99 6.60			
Control A solution	Total bacterial Pseudomonas Enterobacteriace: Lactobacillus Micrococcus Staphylococcus Enterobacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Staphylococcus	a count ae count ae	0 4.11 3.00 3.04 3.20 3.85 3.75 <2.00 3.23 <2.00 <2.00 <2.00 <2.00 <2.00	$\begin{array}{c} 1\\ \hline 7.51\\ 5.78\\ 6.97\\ 6.40\\ 4.99\\ 6.04\\ 5.00\\ \hline 7.26\\ 4.94\\ <2.00\\ 6.71\\ 4.00\\ 5.67\\ \end{array}$	log10 Storage 2 8.79 8.46 8.11 7.30 6.55 7.60 5.93 7.81 5.90 <2.00 6.80 5.98 8.04	CFU/g time (c 3 9.41 8.43 8.65 6.38 7.36 6.26 7.18 6.30 4.65 7.00 6.70 5.95	4 9.1 8.8 8.7 9.1 8.8 9.1 8.8 9.1 8.8 9.1 8.8 9.1 8.8 9.1 8.8 9.1 8.8 9.1 8.8 9.1 8.8 8.8 8.8 8.8 8.4 9.7 9.7 9.8 9.7 9.8 9.7 9.8 9.7 9.8 9.7 9.8 9.8 9.8 9.7 9.8 9.8 9.8 9.8 9.8 9.8 9.8 9.8 9.8	5 9.20 3.8.95 5.8.63 1.8.08 8.6.76 9.7.30 8.6.99 1.8.94 8.7.00 0.7.30 8.8.32 2.7.00 5.7.20	6 8.99 8.48 8.81 6.40 6.52 8.04 6.66 8.54 6.94 8.04 7.99 6.86 7.20			
Control A solution	Total bacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Enterobacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Staphylococcus Enterococcus	a count ae count ae	0 4.11 3.00 3.04 3.20 3.85 3.75 3.23 <2.00 <2.00 <2.00 <2.00 <2.00 <2.00 <2.00 <2.00	$\begin{array}{c} 1\\ \hline 7.51\\ 5.78\\ 6.97\\ 6.40\\ 4.99\\ 6.04\\ 5.00\\ \hline 7.26\\ 4.94\\ <2.00\\ 6.71\\ 4.00\\ 5.67\\ 3.00\\ \end{array}$	log10 Storage 2 8.79 8.46 8.11 7.30 6.55 7.60 5.93 7.81 5.90 <2.00 6.80 5.98 6.04 3.81	CFU/g time (c 3 9.41 8.43 8.65 8.62 6.38 7.36 6.26 6.30 4.30 7.18 6.30 4.30 4.26	$\begin{array}{c} 4 \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ &$	5 5 3 5 8 6 9 7 3 8 6 9 7 3 8 6 9 7 3 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 7 3 0 8 6 9 9 7 3 0 8 6 9 9 7 3 0 8 6 9 9 7 3 0 8 6 9 9 7 3 0 8 6 9 9 7 3 0 8 6 9 9 7 3 0 8 6 9 9 7 3 0 8 6 9 8 8 8 8 8 8 8 8 8 8 8 8 8	6 8.99 8.48 8.81 6.40 6.52 8.04 6.66 8.54 6.94 8.04 7.99 6.86 7.20 6.78			
Control A solution	Total bacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Enterobacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Staphylococcus Enterobacteriace Lactobacillus Micrococcus Staphylococcus Enterococcus Total bacterial Pocod bacterial	a count ae count ae count	0 4.11 3.00 3.04 3.20 3.85 3.75 3.23 <2.00 <2.00 <2.00 2.00 <2.00 3.30	1 7.51 5.78 6.97 6.40 4.99 6.04 5.00 7.26 4.94 <2.00 6.71 4.00 5.67 3.00 5.89	log10 Storage 2 8.79 8.46 8.11 7.30 6.55 7.60 5.93 7.81 5.90 <2.00 6.80 5.98 6.04 3.81 6.54	CFU/g time (c 3 9.41 8.43 8.65 8.62 6.38 7.36 6.26 6.26 6.30 4.65 7.00 4.65 7.00 5.95 4.26 7.51	4 9.11 8.8 9.11 8.8 9.11 6.8 7.4 6.8 8.4 9.7.2 9.8.1 9.7.2 9.8.4 9.7.2 9.8.4 9.7.2 9.8.4 9.7.2 9.8.4 9.8.4 9.8.4 9.8.4 9.8.4 8.4 8.4 8.4 8.4 9.8.4 9.8.4 8.8.4 8.4 8.4 8.4 8.4 8.4 8.4 8.4 8.4	5 5 9 ,20 3 8 .95 5 8 .63 9 7 .30 8 6 6 .76 9 7 .30 8 6 .99 1 8 .94 8 7 .00 0 7 .30 8 8 .99 1 8 .94 8 .32 2 7 .00 5 7 .20 8 8 .32 1 .20 7 .20 8 8 .32 1 .20 1 .	6 8.99 8.48 8.81 6.40 6.52 8.04 6.66 8.54 8.94 8.99 8.54 6.94 8.04 6.94 8.04 7.99 6.86 7.20 6.78 8.18			
Control A solution	Total bacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Enterobacterial Pseudomonas Enterobacteriace Lactobacillus Micrococcus Staphylococcus Staphylococcus Enterococcus Total bacterial Pseudomonas Enterobacterial Pseudomonas Enterobacterial	a count ae count ae count	0 4.11 3.00 3.04 3.20 3.85 3.75 <2.00 <2.00 <2.00 <2.00 <2.00 <2.00 <2.00 <2.00 <3.30 <2.00	$\begin{array}{c} 1\\ \hline 7.51\\ 5.78\\ 6.97\\ 6.40\\ 4.99\\ 6.04\\ 5.00\\ \hline 7.26\\ 4.94\\ <2.00\\ 6.71\\ 4.90\\ <5.67\\ 3.00\\ \hline 5.89\\ 4.67\\ \end{array}$	log10 Storage 2 8.79 8.46 8.11 7.30 6.55 7.60 5.93 7.81 5.93 7.81 5.98 6.04 3.81 8.54 5.48	CFU/g time (c 3 9.41 8.43 8.65 6.36 6.26 6.36 7.18 6.30 4.30 7.18 6.30 4.26 7.51 6.00	$\begin{array}{c} 4\\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & $	5 9 20 3 8.95 5 8.63 1 8.08 6 6.76 9 7.30 8 6.99 1 8.94 8 7.00 0 7.30 8 8.322 2 7.00 5 7.20 8 6.95 1 7.99 5 6.75	6 8.99 8.48 8.81 6.40 6.52 8.04 6.66 8.54 6.94 8.04 7.99 6.86 7.20 6.78 8.18 7.95			
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-7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & -7.5\\ & 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Type of	Temperature of storage (°C)				1	ogio CFU/	g			A. State	Terr		
treatment			Storage time (d)										
		0	1	2	3	4	5	6	8	10	12		
Control A solution	10 10	5.80	14.5	6.62		7.41 3.64		8.11 4.48	8.00 4.76	8.32	7.95		
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 V. Effect of CA mixture or A solution on the growth of S.typhimurium inoculated on fresh meat stored at 10°C or 20°C

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

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

TABLE VI. Effect of CA mixture or A solution on the Incidence of Clostridium on fresh meat stored at 10°C or 20°C

Type of treatment	Temperature of storage (°C)	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 log₁₀ CFU/g ** means the number of clostridium per sample(g) is not less than 1.00 log₁₀ CFU/g Mea