Effect of Chitosan on Meat Preservation

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ABSTRACT

The effect of chitosan as preservative on the qualities of meat including microbiological, chemical, sensory and color qualities were examined. In liquid medium chitosan 0.01% inhibited the growth of some spoilage and pathogenic bacteria such as ***Bacillus subtilis, Escherichia coli, Pseudomonas fragi*** *and* ***Staphylococcus aureus****. At 0.1% concentration it also inhibited the growth of meat starter cultures,* ***Lactobacillus plantarum, Pediococcus Pentosaceus*** *and* ***Micrococcus varians****. In meat, during incubation at 30°C for 48 hours or storage at 4°C for 10 days, chitosan inhibited the growth of spoilage bacteria, reduced lipid oxidation, putrefaction and resulted in better sensory test. Chitosan also had a good effect on the development of red color of meat during storage.*

INTRODUCTION

Chitin [ß (1-4)-acetyl D-glucosamine] and chitosan (deacetylated chitin) are currently available in large quantities as waste products and by products of the shellfish industry (Knorr, 1991).

The production of chitosan from crustacean shell waste consists of a number of processing steps. Firstly, removal of protein from ground shell by treatment with 1-20% NaOH followed by removal of calsium by treatment with 10% HCl, deacetylation of chitin by treatment with 40-50% NaOH, rinsing, pH adjustment and drying (Knorr, 1984). The preparation of chitosan is usually designed to ensure a product completely soluble in acid solvents but insoluble in neutral or alkaline solvents. Chitosan is usually not soluble in a common organic solvents.

Experimental evidence regarding the toxicity of chitosan has shown that it is not toxic (Arrai et al., 1968), safe (Hirano et al., 1990) and increase IgM production in human hybridoma cells (Maeda et al., 1992). Chitosan already has a number of uses in the food industry. Furda (1980) patented its use as a lipid-binding food additive. Knorr (1982, 1983) reported its emulsification property, dye absorption capacity and the other properties of chitosan such as water binding capacity, bioactivity and toughness makes it an attractive material for food industry. In the preservation of fruits, it has been used as a coating and antifungal agent, resulting in increase quality and storageability (Ghaouth et al., 1991). Imery and Knorr (1988) also reported the use of chitosan for the reduction of titritable acidity and color index of carrot and apple juice.

In the management of food processing waste, chitosan has been used as an effective agent for coagulation of suspended solids (Bough, 1975; Bough and Landes, 1976) and recovery of organic compounds from crayfish processing waste water (No and Meyers, 1989). Besides the favorable physical and chemical properties, the antagonistic action of chitosan against fungal plant pathogens has been established by Allan and Hadwiger (1979) and in addition, the bacteriostatic action of water soluble chitosan was investigated by Papineau et al. (1991).

There is little documentation on use of chitosan as a preservative agent in meat products. The present investigation described a study on chitosan as a preservative agent for meat products.

MATERIALS AND METHODS

A. Assessment of the antimicrobial activity of chitosan in liquid media

The bacteria examined in this study were *Lactobacillus plantarum* IAM 1216. *Pediococcus pentosaceus*

IAM 1011, *Escherichia coli* RB, *Bacillus subtilis* IFO 3025 and *Pseudomonas fragi* IFO 3458. The strains were incubated in Yeast extract Peptone Glucose (YPG) broth which contained years extract, 5 g; peptone, 10 g; glucose, 10 g; Twen 80,1 g; L-cystein, 0.1 g in distilled water 1000 ml, pH 6.8 (Nakae et al., 1987).

The effect of chitosan on the growth of bacterial cultures was determined in YPG broth media. Each was cultured in a series of tubes containing a broth medium with 0%, 0.001%, 0.01% and 0.1% of hitosan. All bacteria were incubated with slow shaking at 30°C. After 24 h, growth was measured as turbidity by spectrophotometer at 660 nm.

B. Effect of chitosan on the storage life of minced beef Preparation of meat samples

Fresh beef was purchased from a local market on the day of preparation. Meat was cut and minced through a 4 mm plate diameter with a meat grinder. Each 10 g sample of the minced meat was mixed either 0%, 0.2%, 0.5% and 1 % of chitosan in three replications. Each sample was then wrapped with saran wrap, and divided into two batches. One batch was placed in a chiller operating at 4°C for 0, 3, 5 and 10 days. These samples were used for microbial analyses, TBA (thiobarbituric acid) and VBN (Volatile Basal Nitrogen) value determination.

Microbiological analysis

One ml of each homogenate sample was aseptically diluted stepwise throught a series of tube containing 9 ml sterile buffer saline. Appropriate diluents of each tube were placed on the following media in duplicate; Plate Count Agar (PCA)(Difco Co.Ltd.) supplemented with 10% NaCl for Micrococci (Steele and Stiles, 1981), Crystal Violet Tetrazolium (CVT) agar (Eiken Ltd.) for Gram-negative bacteria (Reddy et al., 1970), Vogel Johnson agar (Eiken Ltd.) for Staphylococci; OF Basal Medium (Difco Co.LTD.) Which contains OF Basal medium, 9.4g; yeast extract, 2.0 g; Tween 80, 1.0 g; agar, 13 g in 1000 ml distilled water and supplemented with Nalidixic acid, 7.5 mg and Novobiocin 30 mg in 10 ml sterile distilled water for Pseudomonas; and Desoxycholate agar (Nissui Co. Ltd.) for E. coli (Anonim, 1983).

PCA duplicate pour plates were incubated at 35°C and Vogel Johnson duplicate surface plates were incubated at 35°C for 48 h. CVT surface plates were incubated at 30°C for 48 hours and all black colonies were counted. OF Basal medium surface plates were incubated at 25°C for 48 hours, and white colonies were counted. For E. coli, duplicate pour plate were made of each dilution using 10 ml in Desoxycholate agar. After the pour plate solidified, they were covered with additional 5 ml of Desoxycholate agar, incubated at 30°C for 24 hours and red colonies were counted (Becton Dickinson, 1983).

Chemical Analysis

Thiobarbituric Acid (TBA value) was determined by steam distillation method (Ando and Yamauchi, 1968) as modified by Izumimoto et al., (1990) and expressed as mg malonaldehyde/kg meat. VBN value was measured by Conway's method (Kousaka, 1983).

Color Analysis

Each 40 g of sample was placed into plastic petri dish with 60 x 15 mm size (Corning, Iwaki Co.Ltd.), presses and wrapped with saran wrap before closing with petri cap. The color of meat was measured using Minolta Colorimeter (CM-1000, Minolta Co.ltd.) and expressed as L* (lightness), a* (redness) and b* (yellowness) values.

Sensory Analysis

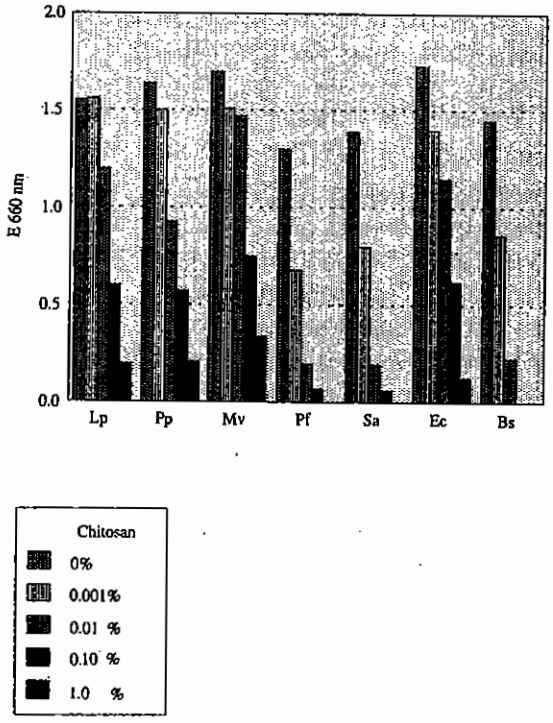
Each control sample and samples containing chitosan were formed into 100 g patty having diameter of 10 cm and a thickness of 1 cm. The samples were fried at 190°C in an oil hot plate for 4 min each side. Samples were cut into 1.5 cm cubes, labeled with random numbers and served in random order. Panelist evaluated each sample for degree of difference and acceptability in accordance with a standard questionnaire for Triangle Test (Larmond, 1977).

RESULTS AND DISCUSSION

Microbiological

The inhibitory effect of Chitosan on the growth of some meat spoilage bacteria and some meat starter

culture in liquid medium during incubation at 30°C for 24 h is shown in Fig. 1. *Pseudomonas fragi, Staphylococcus aureus, Bacillus subtilis* were inhibited by 0.01% of chitosan. *E. coli* was more resistant than the other bacteria, and was inhibited at 0.1%. Some meat starter culture *including Lactobacillus plantarum* and *Pediococcus pentosaceus* were inhibited at 0.1% with no growth at 0.5% of chitosan. *Micrococcus varians was slightly resistant than both lactic acid* bacteria.



micro

The mode of inhibition of chitosan on the growth of some spoilage bacteria and some meat starter culture might be due to the interaction of chitosan with membranes or cell wall components (Young et al., 1982) resulting in increased permeability of the membranes and leakage of cell material from tissue, or due to water binding capacity and inhibition of various enzymes by chitosan (Young et al., 1982). Chitosan also has bioabsorbant activity (Knorr, 1991) and can absorb nutrients of bacteria and may inhibit their growth.

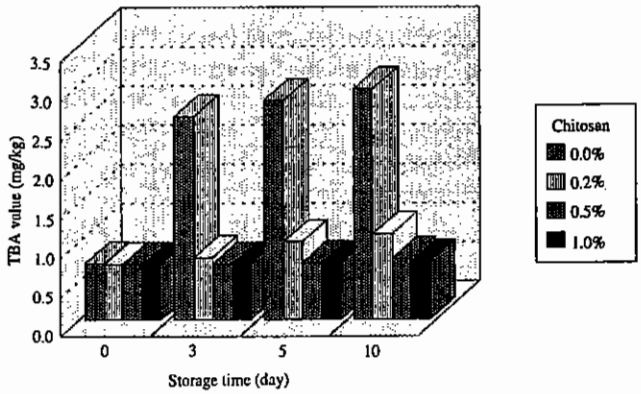
The growth of Staphylococci, coliform, Gram-negative bacteria, Micrococci and Pseudomonas in meat during storage at 30°C for 48 h and 4°C for 10 days were also inhibited by presence of chitosan as shown in Table 1 and Table 2.

Table 1. **Changes of Microbial count (log CFU/g) of Meat with Added Chitosan during Storage at 30°C for 48 h**

Microorganisms	Storage time (h)	Chitosan (%)			
		0.0	0.2	0.5	1.0
Total Bacteria	0	5.98	5.98	5.67	5.45
	12	7.48	7.46	7.46	7.32
	24	9.04	9.00	9.95	8.90
	48	9.30	9.43	9.28	7.90
Pseudomonas	0	3.65	3.61	3.60	3.60
	12	5.32	5.17	4.94	4.81
	24	5.66	5.46	4.95	4.27
	48	8.50	8.50	6.81	5.30
Staphylococci	0	3.17	3.08	3.00	2.84
	12	5.00	5.46	4.15	3.60
	24	6.04	5.78	5.00	4.78
	48	7.30	7.60	6.90	5.50
Coliform	0	3.40	3.22	3.30	2.84
	12	5.36	5.43	5.40	4.38
	24	8.84	8.66	8.40	7.48
	48	9.28	9.46	9.08	8.04
Gram(-) bacteria	0	4.25	4.25	4.26	4.18
	12	5.98	5.87	5.71	5.49
	24	8.49	8.00	7.90	6.86
	48	9.08	8.46	8.11	7.62
Micrococci	0	4.15	4.23	4.20	3.43
	12	6.08	5.08	4.60	3.60
	24	6.28	6.20	4.74	5.50
	48	7.46	7.50	5.90	5.30

Table 2. **Changes of Microbial count (log CFU/g) of Meat with Added Chitosan during Storage at 4°C for 10 days**

Microorganisms	Storage time (day)	Chitosan (%)			
		0.0	0.2	0.5	1.0
Total Bacteria	0	5.98	5.98	5.67	5.48
	3	8.04	7.66	7.60	7.34
	5	8.94	8.93	8.46	7.89
	10	9.81	9.43	9.11	8.87
Pseudomonas	0	3.65	3.61	3.60	3.60
	3	6.00	6.20	5.58	5.04
	5	6.81	6.46	5.88	5.38
	10	7.66	7.75	6.28	2.85
Staphylococci	0	3.17	3.08	3.00	2.84
	3	4.80	4.77	4.20	4.00
	5	5.74	4.87	5.62	4.77
	10	6.08	5.36	5.30	5.04
Coliform	0	3.40	3.32	3.30	2.84
	3	5.88	5.70	5.23	5.08
	5	6.81	6.63	6.48	5.25
	10	8.15	7.83	7.15	6.73
Gram(-) bacteria	0	4.26	4.25	4.26	4.17
	3	6.94	6.58	6.41	5.30
	5	7.98	7.82	7.75	6.08
	10	9.61	8.36	8.20	7.25
Micrococci	0	4.15	4.23	4.20	3.43
	3	5.28	4.60	4.23	3.78
	5	5.81	4.61	4.30	3.85
	10	6.04	4.65	4.45	4.00



tba 4

The exact mechanisms of the chitosan's inhibitory effect on TBA value of meat is unknown, and is currently under investigation in greater detail in our laboratory. As shown in the 0 h incubation at 30°C and 0 day storage at 4°C that TBA of samples treated with and without chitosan were the same value, it suggest that chitosan does not interfere with TBA reactive substance. It could be implied that chitosan has antioxidative properties as reported by Pasquel and Babbits (1991) that shrimp meat contained natural antioxidant, and shrimp meat maybe contains a small amount of chitosan.

Sensory characteristic

Sensory evaluation was carried out on meat prepared with chitosan and stored at 4°C after 0, 3, 5 and 10 days storage. As shown in Table 3 meat containing chitosan was significantly different from control sample ($p < 0.05$) and was more acceptable ($p < 0.05$) The lower TBA and VBN value indicates depression of rancid and spoilage flavor of meat prepared with chitosan thereby causing in more acceptable taste. It has been reported by Furda (1980) and Knorr (1983) that chitosan demonstrated its lipid binding and water binding capacity, so the sample containing chitosan has a better sensory appearance that control sample, that was similar with the result of sensory test at 0 time storage. This observation suggest that chitosan has a good effect on the sensory attributes of meat.

Table 3. **Triangle Test for sensory Evaluation of Meat with 0 and 0.5% Added Chitosan during Storage at 4°C for 10 days**

Storage time (day)	Number of tester	Correct answer		Acceptability	
		Chitosan (%)		Chitosan (%)	
		0	5	0	5
0	18	2	16**	4	12***
3	18	7	11*	4	7***
5	18	7	11*	4	7***
10	18	5	13**	5	8***

* and ** : indicate a significant difference at 5 and 1% level respectively
*** : indicate a more acceptable at 5%

Color

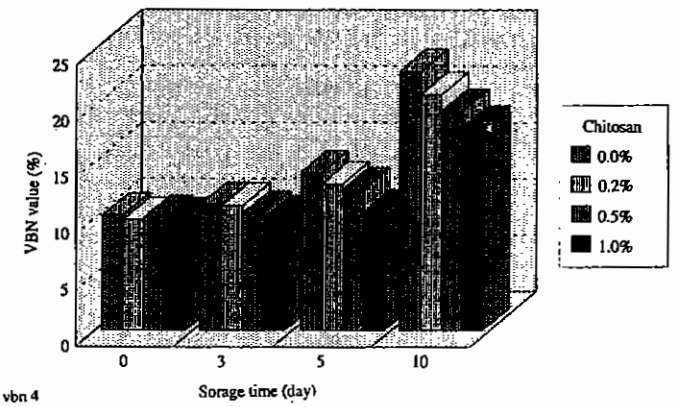
The effect of chitosan on the color of beef patties during storage at 30°C for 24 h and 4°C for days are shown in Table 4 and Table 5. The result of student-t analysis indicated no effect of chitosan on L*, a*, and b* value at 0 time of storage ($p < 0.05$). During storage the L* value of control sample increased compared with the sample containing chitosan. It may be caused by water binding capacity, so it could repress the drip loss of meat resulting in increased transparency and lowering of lightness. The a* and b* values of all meat samples increased during storage and the increase in control sample was lower compared with samples containing chitosan. The greater of a* value corresponded to the increasing met-form heme pigment proportion during storage (Izumimito et al., 1982). This observation suggest that meat prepared with chitosan resulted in a good color of meat.

Table 4. **Result for t-Student Method for Effect of Chitosan on Color of Meat during Incubation for 30°C for 24 hours**

Storage time (h)	Chitosan (%)	L* value	a* value	b* value
0	0.0	29.3 a	13.7 a	8.2 a
	0.2	29.7 a	14.3 a	7.8 a
	0.5	30.2 a	12.9 ab	8.4 a
	1.0	29.2 a	13.3 b	8.9 a
12	0.0	30.3 ab	15.4 a	7.9 a
	0.2	29.6 ab	15.4 a	7.9 a
	0.5	30.5 a	15.1 a	7.8 a
	1.0	28.5 b	16.6 b	7.7 b
24	0.0	30.8 ab	14.9 a	7.9 a
	0.2	31.9 ab	15.1 a	8.7 a
	0.5	32.0 a	15.7 ab	8.8 a
	1.0	30.5 b	16.7 b	7.7 b

The different letters within column are significantly different ($p<0.05$).

This observation is important with regard to manufacture of intermediate fermented meats, as the starter culture has been used to enhance the preservative quality of these product (Darmadji et al., 1990). A combination of chitosan and meat starter cultures may result in greater enhancement of meat preservative quality. This is attributed to its high water binding capacity. In addition, its ability to control lactis acid bacteria would prevent excessive acid production (Imeri and Knorr, 1988) besides inhibition of undesirable organisms.



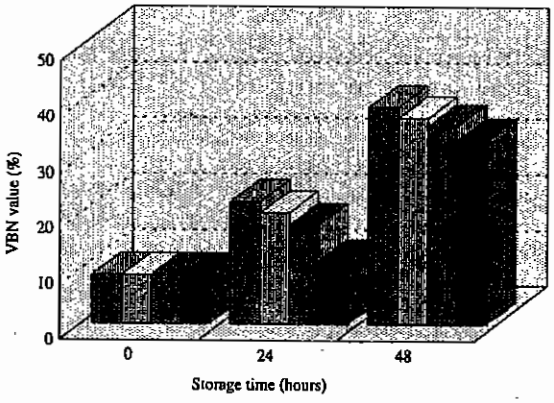
vbn 4

VBN value

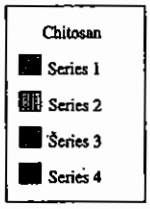
During storage at 30°C for 24 and 48 h, the VBN value of meat containing chitosan was lower than the control as shown in Fig. 2. The same observation was obtained during storage at 4°C for 0, 3, 5 and 10 days as shown in Fig. 3. some spoilage bacteria such as Pseudomonas secrete proteolytic enzymes which play a role in the degradation of protein. This process may affect meat putrefaction and production of volatile nitrogen. However, chitosan showed its ability to inhibit the growth of these bacteria.

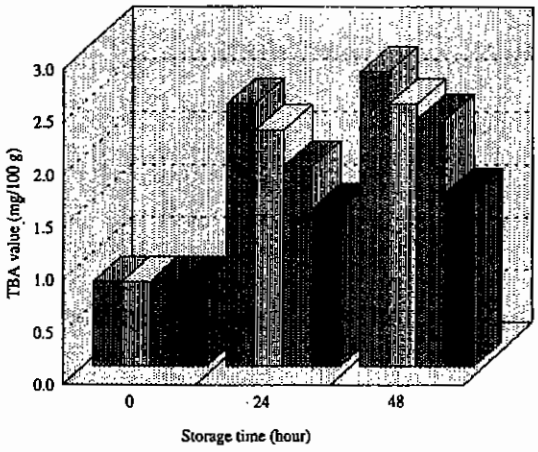
TBA value

The rate of lipid oxidation in meat was reduced by addition of chitosan as indicated by changes in TBA value of meat during storage at 30°C (Fig. 4). The decrease in TBA value depended on chitosan concentration. At concentration of 0.2, 0.5 and 1.0% TBA value decreased by 10,25 and 40% respactively. At 4°C, the effect of chitosan on TBA value of meat was distinct and declined by about 70% after 3 days of storage. After 10 days of storage the TBA value of meat containing chitosan was the same as at day 0, whereas TBA value of control samples increase sharply as shown in Fig. 5.



vbn 30





tba30

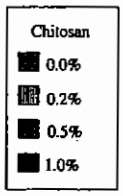


Table 5. **Result for t-Student Method for Effect of Chitosan on Color of Meat during storage for 4°C for 10 days**

Storage time (h)	Chitosan (%)	L* value	a* value	b* value
0	0.0	30.3 a	13.9 a	8.4 ac
	0.2	30.2 a	14.5 a	7.6 ab
	0.5	30.1 a	11.5 b	7.5 b
	1.0	30.2 a	12.2 b	9.0 c
3	0.0	30.4 a	14.7 a	7.9 a
	0.2	29.1 b	15.6 a	7.6 ab
	0.5	29.1 b	15.4 a	7.2 a
	1.0	27.9 c	15.2 a	6.9 b
5	0.0	31.7 a	17.2 a	12.6 a
	0.2	28.9 b	19.3 c	12.0 ab
	0.5	27.5 c	19.8 b	11.4 b
	1.0	26.8 c	20.8 a	11.3 b
10	0.0	31.2 a	16.5 a	13.3 a
	0.2	29.3 a	18.3 b	12.2 b
	0.5	27.5 b	19.1 bc	11.3 b
	1.0	25.4 c	19.5 c	11.1 c

The different letters within column are significanttly different ($p < 0.05$)

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