Study on The Inhibition Effect of Nisin

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Abstract: Nisin is a kind of bacterial toxin produced by *Lactococcus lactic*. It is of wide inhibition effect on Gram positive bacteria, and is widely accepted as a safe biological preservative. The inhibition effect of Nisin by itself and Nisin combined with Ethylene Diamine Tetraacetic Acid (EDTA) on *Micrococcus*, *Bacillus subtilis*, and lactic acid bacteria was studied. The effect of pH value and high temperature on the inhibitory activity was discussed. [The Journal of American Science. 2005;1(2):33-37].

Key words: *Lactococcus lactic,* inhibition; Nisin

1 Introduction

As food preservative, Nisin is widely used in over fifty countries and areas in the world up to date. The study of Nisin in China began in 1989, and was normally authorized as a kind of food preservative in 1990 (He, 2004). Nisin is hypogenous metabolic products produced by *Lactococcus*, which is consist of polypeptides, and can be hydrolyzed into amino acids in the intestine by α-chromotrypsin (Mattick, 1944). It has no effect on the normal flora in the intestine and is not toxic to human. It is safe for people to eat and is the only antibiotic which is permitted to be used in food at present. It was suggested that Nisin has inhibitory effect on some gram positive bacteria, and had little effect on gram negative bacteria, fungi and virus (Benkerroum, 1981; Scott, 1981; Stevens, 1991; Brotz and Sahl, 2000). But Nisin, connected with chemical preservatives, can reduce the dosage of the latter, lower the temperature of sterilization in the food processing, shorten the time of heat treatment, and so the destruction of nutrients, color, odor and flavor in food can be reduced or the shelf life of food can be prolonged. Moreover Nisin was of the characteristics of acid tolerance, high temperature tolerance, and low temperature storage, and these make it be widely used in milk and milk products, meat and egg products, and canned food (Tian, 2003). The inhibitory effect of self-made Nisin was tested, and the characteristics of high temperature tolerance and low pH value tolerance were examined.

2 Materials and Methods

2.1 Materials

Stains: *Micrococcus*, *Salmonella, Bacillus subtilis*, *Lactobacillus Bulgaricus acidophilus*, *Lactococcus lactic* subsp. *cremoris* (provided by Food Science Department of Northeast Agricultural University, China).

Medium: glucose broth agar, general nutritive agar, whey agar, trypsinized soybean protein broth (sterilized at 115℃, 30 min) (Jiang, 2002).

Chemicals: phosphate buffer of pH 6.0, physiological salt solution 0.85%, hydrochloric acid 0.02 M, EDTA 0.02 M, standard sample of Nisin, purchased from Aplin and Barrett Corp. Ltd., British (1,000,000 IU/mL), self made Nisin provided by Food Science Department of Northeast Agricultural University of China.

2.2 Methods

2.2.1 Comparative testes between standard and self made Nisin

Micrococcus, was inoculated on the slant tube of glucose broth agar, cultured 20~22 hours, 37℃; the lawn was flushed down with physiological salt solution. The glucose broth agar, melt and cooled to 50℃, about 20 mL, was poured to the plate. This was the bottom plate solid agar. The bacterial suspension, 0.2 mL, is mixed with glucose broth agar 10 mL (about 50℃) quickly, and was poured over the bottom plate agar, waiting for solidification.

Four oxford cups ware put into the plate prepared above, with the asepsis manipulation. Nisin, standard and self made, 200 IU/mL, 400 IU/mL, were injected in separately. The Nisin solution should not overflow, incubating 16~18 hours, 37℃, the diameter of the inhibitory circle was measured precisely, and the result

was recorded.

2.2.2 Tests of inhibitory effect of self made Nisin

The suspensions of *Micrococcus*, *Salmonella* and *Bacillus subtilis* were prepared. For *Bacillus subtilis*, the incubation time was 7 days, 37℃. The number of spores was over 85% in gram staining, and the lawn flushed down was heated at 65℃ for 30 min. For the lactic acid bacteria, 5 mL fat-free milk was used as medium, incubating 10~12 hours, at 37℃, then the culture was diluted with 5 mL physiological salt solution to get the stain suspension. Pouring of the plate was as above. The concentrations of self made Nisin were 100 IU/mL, 200 IU/mL, 300 IU/mL, 400 IU/mL, and 500 IU/mL separately.

2.2.3 The inhibitory effect of Nisin and EDTA together

The bacterial suspensions of *Salmonella*, *Bacillus subtilis* and lactic acid bacteria were used. The concentrations of Nisin were 100 IU/mL, 200 IU/mL, 300 IU/mL, 400 IU/mL, and 500 IU/mL separately, together with EDTA and phosphate buffer, pH6.0. The standard Nisin 200IU/mL was used as a comparison. The test method was the same as in 2.2.2.

2.2.4 The effect of pH on the inhibitory function of Nisin

Micrococcus suspension was used, and the number of bacteria was in the level of 10 000/mL. Four tubes of 9 mL trypsinized soybean broth medium, with the pH values of 4.0, 5.0, 6.0, 7.0 separately, were added with 1 mL of suspension above, 10,000/mL; another group of tubes with different pH were added with 1 mL of suspension above too, at the same time. Nisin was added to each tube, and the terminal concentration was 250 IU/mL. A group of tubes, the same as above, were used as a blank. Three groups of tubes were cultivated at 37℃. Standard plate counts were carried out in the days of 0, 3, 6, and 9, and the results were recorded.

2.2.5 The high temperature tolerance test of Nisin

Four tubes of Nisin solution, 100 IU/mL, 10 mL, were heated for 10 min at 120℃, 100℃, and 80℃, and 30 min at 63℃ separately. *Micrococcus* suspension was used. The tests were carried out, using the Nisin solution after heat treatment above, and the method was the same as in 2.2.1.

2.2.6 Volatility test of Nisin

The powder of Nisin was put into weight bottles, dried about 4 to 5 hours at 60℃ to remove water, and weighted. Then the bottles were heated at 110℃, for 2 hours and 4 hours separately, and the weights were recorded.

3 Results and Discussion

3.1 The comparison of inhibition effect between standard Nisin and self-made Nisin

The results are in Table 1. The effect of self-made Nisin was better than the standard under the same conditions.

3.2 Test of bacterial inhibition effect of self-made Nisin

The results are in Table 2. *Micrococcus* was sensitive to Nisin, Nisin of 50 IU/mL inhibits the growth of it, and inhibitory circle was 8.3 mm. The inhibitory circle increases with the increase of Nisin concentration. Other bacteria were not sensitive to Nisin, even if the concentrations of Nisin were 1000 IU/mL and 1500 IU/mL. It was clear that *Micrococcus* was very sensitive to Nisin, at the same time Nisin had no inhibitory effect on *Salmonella, Bacillus subtilis, Lactobacillus bulgaricus, Lactobacillus acidophilus, Steptococcus thermophilus, Lactococcus lactic subsp. Cremoris.*

3.3 Tests of the inhibitory effect of Nisin and EDTA together

The results was in Table 3. For the comparison groups, Nisin by itself, phosphate buffer pH6.0 or EDTA, have no inhibitory effect on the test strains. While Nisin connected with EDTA had inhibitory effect on *Bacillus subtilis* and *Salmonella*. It was reported that EDTA had the ability to chelate Mg^{2+} of liposaccharides in the out member of cell wall, which resulted in the destroy of liposaccharides in the out member of cell wall, and the increasing of osmotic pressure in cell, so the sensitivity of cell to antibiotics and bactericides was promoted. When Nisin function together with EDTA, the inhibitory function was accelerated, the inhibitory circle was expanded, and Nisin even inhibited the growth of some gram negative bacteria (Stevens, 1991).

Table 1. Results of Comparison of Bacterial Inhibition Effect

	C (IU/mL)[b]			1	2	3	4	5[c]	average diameter	results comparison
D (mm)[a]	standard	SL	200	8.2	9.0	9.0	9.0	8.8	8.8	$\overline{UL}-\overline{SL}$ =4.3 mm
		SH	400	11.0	11.2	10.2	13.5	10.5	11.3	
	self-made	UL	200	12.0	12.5	13.0	13.0	15.0	13.1	$\overline{UH}-\overline{SH}$ =2.7 mm
		UL	400	13.0	14.0	14.0	13.0	16.0	14.0	

a D is the diameter of inhibition circles.
b C is the concentration of Nisin.
c parallel sample number

Table 2. Results of Inhibition Effect of Nisin with Different Concentration on Different Strains

	C (IU/mL)	100	200	300	400	500
D (mm)	*Micrococcus*	14.0	15.5	16.8	17.0	17.5
	Salmonella	—	—	—	—	—
	Bacillus subtilis	—	—	—	—	—
	Lactobacillus bulgaricus	—	—	—	—	—
	Lactobacillus acidophilus	—	—	—	—	—
	Steptococcus thermophilus	—	—	—	—	—
	Lactococcus lactic subsp. cremoris	—	—	—	—	—

— represents no inhibitory circle.

Table 3. Results of The Double Bacteria Inhibition Effect of Nisin and EDTA

D (mm)	0.02M EDTA	solution of Nisin and EDTA IU/mL				phosphate buffer pH6.0	200 IU/mL Nisin solution
		200	300	400	500		
Bacillus subtilis	—	11.0	11.3	11.8	11.7	—	—
Salmonella	—	11.3	11.6	11.8	12.0	—	—
Lactobacillus bulgaricus	—	—	—	—	—	—	—
Lactobacillus acidophilus	—	—	—	—	—	—	—
Steptococcus thermophilus	—	—	—	—	—	—	—
Lactococcus lactic subsp. cremoris	—	—	—	—	—	—	—

Table 4. Inhibitory effect of Nisin in Different pH

Days			0	3	6	9
bacterial number/mL	pH with Nisin	4	2.9×10^2	0	0	0
		5	2.9×10^2	2.1×10	0	0
		6	2.9×10^2	3.5×10	0	0
		7	2.9×10^2	1.9×10^2	0	0
	pH without Nisin	4	2.9×10^2	2.2×10	0	0
		5	2.9×10^2	1.0×10^2	1.0×10	0
		6	2.9×10^2	8.8×10^3	1.6×10^4	9.6×10^6
		7	2.9×10^2	1.7×10^4	4.0×10^4	1.4×10^7

3.4 Effect of pH on the bacterial inhibition function

The results were in Table 4.

In the group without Nisin, the living bacterial amount reduced markedly at the third day, and the

living bacterial cell reduced to zero at the sixth and the ninth day. It was apparent that the lower the pH it was, the more strongly *Micrococcus* were inhibited. While in the group with Nisin, the living cell amounts dropped faster at pH4.0 and pH5.0. It was clear that Nisin and low pH values functioned together. That was, Nisin functioned better at lower pH. In the group without Nisin, the living cell amounts increased remarkably at pH6.0 and pH7.0. At the same time, in the group with Nisin, the living cell amounts reduced remarkably at pH6.0 and pH7.0. It was suggested that the growth of *Micrococcus* was well inhibited by Nisin under moderate pH value.

3.5 High temperature tolerance test

Many foods must experience heat treatment during processing, but some food preservatives can not tolerate high temperature, during which they may be denatured or decomposed under high temperature so much that they will lose their function as a food preservative. From the results of Table 5, the inhibitory circles had no difference. It was explained that high temperature had no apparent effect on inhibitory effect. That was, Nisin was a high-temperature-tolerance preservative. This provided the basement for Nisin to be used in heat-treated food.

3.6 Volatility test of Nisin

The results were in Table 6. The weights changed in the range that error permitted after heat treatment at 110℃, 2 hours and 4 hours. It was thought that Nisin did not volatilize at the high temperature of 110℃.

Table 5. Results of Nisin Inhibition Effect on *Micrococcus* after Different Heat Treatments

Temperature and time of heat treatment		120℃×10 min	100℃×10 min	80℃×10 min	63℃×30 min
D (mm)	1	12.5	12.2	13.5	13.0
	2	13.2	11.8	13.0	13.0
	Average	12.8	12.0	13.2	13.0

Table 6. Results of Volatility Tests

drying conditions		60℃, 4~5 hours	110℃, 2 hours	110℃, 4 hours
weight after drying (g)	1	29.8240	29.8175	29.8095
	2	34.2972	34.2945	34.2915

4 Conclusions

(1) The effect of self-made Nisin was better than that of standard Nisin under the same conditions.

(2) *Micrococcus* was very sensitive to Nisin, and Nisin with the concentration of 50 IU/mL still exist inhibitory function.

(3) Nisin by itself had no inhibitory effect on *Bacillus subtilis*, *Salmonella*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Steptococcus thermophilus*, and *Lactococcus lactic* subsp. *Cremoris*; but Nisin added with EDTA inhibitted the growth of *Bacillus subtilis* and *Salmonella*, while had no effect on other bacteria.

(4) The inhibitory effect of Nisin was better under acidic conditions, and it increased with the increasing of acidification.

(5) Nisin in pH6.0 was stable to heat treatment, can retain inhibitory effect at the temperature 120℃, and did not volatilize under high temperature.

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