INFLUENCE OF TEMPERATURE, PH VALUE AND STORAGE CONDITIONS ON NISIN ACTIVITY

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Introduction

Over the last decade, much attention has been focused on the chemical preservatives in foods, many of which have been shown to be harmful. There is a tendency to eliminate or reduce their uses in foods and suitable alternatives such as some natural biopreservatives are increasingly applied at the same time. Nisin is a bacteriocin produced by *Lactococcus lactis subsp. lactis* that exhibits a broad inhibitory spectrum against gram-positive bacteria, including bacterial endospores. Many studies have showed nisin is a safe substance for use in foods and it has no negative effect on food sensory quality. Therefore, nisin has been widely used in more than 50 countries and areas of the world, as a new kind of natural food preservative (Delves-Broughton, 1996; Linda 1992). In China, however, the study on nisin just began in 1989. And until 2000, it was not produced industrially first by Zhejiang Silver Elephant Bioengineering Ltd. The application of nisin product in modern food industry hasn't been popularized widely in China.

The inhibition effect of biopreservatives can be affected by many environmental factors in food processing, packaging, storing, and others. It is necessary to research the effects of environmental factors on the inhibitory activity of biopreservatives for their reasonable applications. Such information is very limited for nisin products in China. Our objective was to determine the influences of temperature, pH value and storage conditions on the activity of nisin made in China product by the method of filter paper scrip.

Materials and Methods

Nisin powder, a commercial product (10^6IU/g), was provided by Zhejiang Silver Elephant Bioengineering Ltd. (China). Serial solutions of nisin were prepared with 0.02N hydrochloric acid and filtered with 0.22μm filter membrane. Assay medium for the estimation of nisin activity (Trame and Fowler, 1964; Wu, 1999) was prepared, sterilized and cooled to 55-60℃, poured 20ml into each flat-bottomed sterile Petri dishes and allowed to solidify. *Micrococcus flavus* was suspended with sterile saline solution and made into homogenous bacterium suspension, diluted the concentration into 10^7 bacteria per milliliter suspension. 0.1 ml of the bacterium suspension was removed to the ready Petri dishes and smeared well-distributed with L-type glass stick (Shen et al., 1999).

Some sterilized filter paper pieces of 6.35mm diameter round scrip were soaked respectively in different concentrations of nisin solution for 10 minutes, the control scrip was soaked in 0.02N hydrochloric acid. The scrip was taken out with sterile tweezers, dripped dry and then put on the above mentioned prepared solidified medium, six pieces of scrip were put equal-distantly in each dish. The plates were then converted，and transferred to a 30℃ incubator and left overnight. The next day the diameters of the inhibition zones were measured to the nearest 0.1mm by means of calipers (Shen et al. 1999).

Nisin solutions were adjusted to difinated pH values and then/or kept at different temperature for 20 minutes and then determined the inhibitory activity.The nisin solution and the dry nisin powder were kept at 4℃、20℃、30℃ respectively for 6 weeks, and the inhibitory activity was examined respectively weekly.

All analyses were performed triplicates. One-way analysis of variance (ANOVA) was performed on the data using SPSS11.0. Duncan's new multiple range method was calculated to compare the means for the different data.

Results and Discussion

The relationship between nisin activity and inhibition zone diameters. No clear zones were formed around the 0.02N hydrochloric acid. This indicated that the formation of inhibition zone was not caused by hydrochloric acid but by nisin inhibition. The inhibition zone diameter was enlarged with the increase of the concentration of nisin solution and the enlargement slowed down when the concentration was higher than 100mg/L (Figure1). A straight-line response was produced when the log nisin concentrations (2.5-100mg/L) were plotted against linear zone diameters, and the linear equation was y=0.4389x+0.6042 (Figure 2).

Figures 1 and 2 showed that the nisin activity could be estimated according to the inhibition zone diameters by the method of filter paper scrip under concentrations between 2.5 and 100mg/L, and 100mg/L could be determined as the concentration of nisin for further experiments.

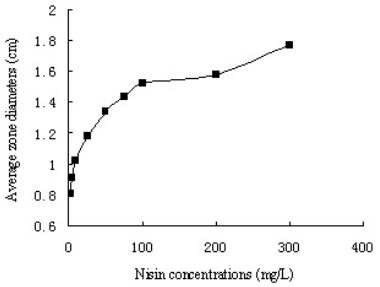

Figure 1 Zones of inhibition produced by different concentrations of nisin.

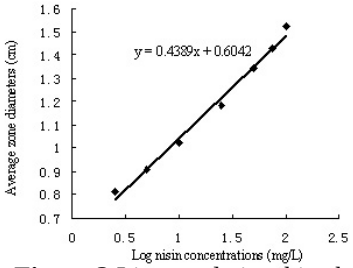

Figure 2 Linear relationships between log nisin concentrations and zone diameters.

Effect of temperature and pH value on nisin activity. The inhibition zone diameter shortened ($P<0.01$) along with the rise of temperature (Figure 3) or the drop of pH value (Figure 4). The activity of nisin was lost completely after 121℃ heat treated for 20min in pH6 and pH7 conditions (Figure 5).

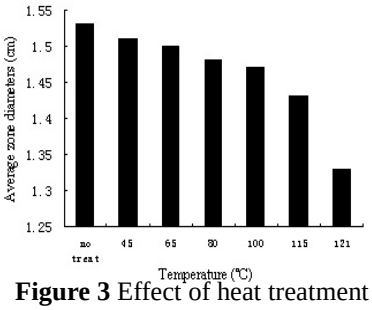

Figure 3 Effect of heat treatment on inhibition zone. Diameters.

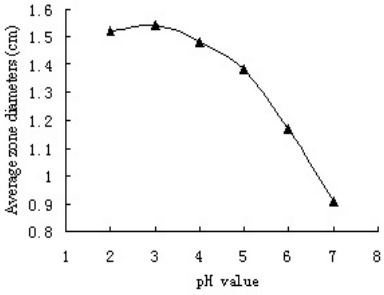

Figure 4 Effect of pH value on inhibition zone. Diameters.

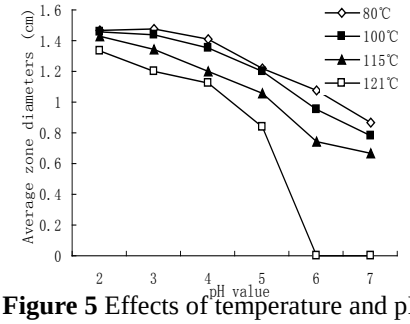

Figure 5 Effects of temperature and pH value on inhibition zone diameters.

Effect of storage conditions on nisin activity. The inhibition zone diameters produced by nisin solution or nisin powder were becoming smaller and smaller ($P<0.01$) along with the storage time prolonging, meanwhile, the higher the storage temperature was, the lower the activity was (Figure 6). However, the inhibition zone diameters produced by nisin solution decreased faster as a whole than those produced by nisin powder.

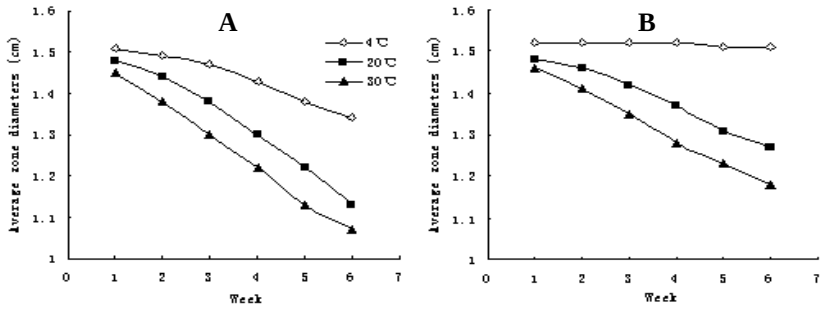

Figure 6 Changes of inhibition zone diameters produced by nisin solution (A) or nisin powder (B).

Conclusions

Using the method of filter paper scrip, nisin activity could be estimated according to the inhibition zone diameters. Chinese nisin activity was affected significantly by temperature, pH value and storage conditions. Nisin was stable to temperature under acidic condition; however, as pH was increased, the higher the temperature was, the more losses of nisin activity were. Meanwhile, the activity of nisin solution was different from that of nisin powder, which indicated that the effectiveness of nisin used in liquid food may be different from that in solid food. It is reconmanded that all these factors should be considered when use nisin in food processing and preserving.

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