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Antimicrobial effect of nisin on *Bacillus cereus* isolated from some meat products

Mohamed A. Hassan [1], Reham A. Amin.[2], Nesreen Z. Eleiwa. [2],Fatema A. Hussien[1]

[1]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Egypt.

[2]Food Hygiene Department, Animal Health Research Institute Tanta Lab., Egypt

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ABSTRACT

Processed ready to eat meat products are considered the main source of infection with *Bacillus cereus*(*B. Cereus*)which is an aerobic spore former bacteria commonly found in raw and processed foods. So, more cautions are needed to be considered in order to minimize the contamination of such products. Nisin is a natural preservative for many food products and can be used to inhibit the germination and outgrowth of spores. This study was designed to determine the antimicrobial effect of nisin on *B. cereus* inoculated into minced beef with an inoculation dose of 3×10^6cfu/g. Nisin was inoculated in the artificial infected minced meat at different concentrations 10,30 and 50 ppm, resulted in a decrease in *B. cereus* count after five days from $5.0\times10^7\pm0.82\times10^7$(initial bacterial load) to $2.46\times10^6\pm0.35\times10^6$ with a reduction percentage 95.1%,100% and 100% at concentration 10,30 and 50ppm, respectively. In conclusion, Nisin can be used as a safe anti-microbial food preservative for meat products

1. INTRODUCTION

Bacillus cereus (*B. cereus*) is an aerobic spore former commonly found in raw and processed foods. Food borne illness associated with this pathogen is caused primarily by consumption of cooked foods with inadequate refrigeration (Peng et al.,2001).Lack of the sanitary measures during processing, handling and storage may act as the main source of food contamination with aerobic spore formers (Torky-Amal,2004). *Bacillus cereus* is one of the potential spoilage bacteria associated with red meat (Nel et al.,2004). The risk in its transmission through processed, pasteurized, sterilized, and heat-treated food products is due to its resistant endospores (Kotiranta et al.,2000).The occurrence of *B. cereus* as a meat contaminant was reported by some investigators, not only in raw meat, but also in meat products (Rather et al., 2012). Processed meat products are considered the main source of infection with *B. cereus* and more caution need to be taken in order to minimize the contamination of such products. The selection of fresh and clean flesh, decontamination of the mincing machine, grinders, equipment and knives used in the processing of such products will decrease the incidence of *B. cereus* foodborne illness cases among the consumers(FDA,2012).

Nisin is a natural preservative used to inhibit the germination and outgrowth of spores. Other antimicrobials commonly used to inhibit the growth of *B. cereus* include benzoate, sorbates and ethylenediaminetetraacetic acid (Jenson and Moir,2003).Nisin is the most commercial bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, which exhibits antimicrobial activity against a wide range of Gram-positive vegetative cells and spores. Nisin has been used for processed cheese in Korea (Ministry of Food and Drug Safety, 2015). Bacteriocin has already been used in more than 50 countries in the food industry as an antagonistic additive (Ray,1992). In addition, nisin has been permitted in processed meat include limits of 12.5 mg/kg in USA(Food and Drug Association, 2015),and has mainly been applied to dairy and meat products as a target of Gram-positive pathogen (Balciunas et al., 2013).

Sensory evaluation of meat provides methods for understanding human perceptions products. The three basic methods of sensory evaluationare discrimination, descriptive analysis, and consumer. Different tools available to meat scientists to assist in selection and conducting the appropriate their sensory objectives(Miller, 2012).

The current study aimed to detect the effect of nisin on *B. cereus* as well as the organoleptic characteristics of meat.

2. MATERIAL AND METHODS

Fresh minced meat (1500 g) put under the UV light in the cabinet for20 min in order to reduce the number of microorganisms attached to its surface.

2.1. Preparations of inoculate:

Bacillus cereus(*B. Cereus) strains* were obtained from Food Analysis Center, Faculty of Veterinary Medicine, Benha University. The bacteria was subcultured on Brain–Heart Infusion (BHI) broth and incubated for 24 hours at 37°C. The cells were harvested by centrifugation (3000×*g*, 15 min), washed twice, and resuspended with saline (NaCl, 0.85%, w.v.)(Tassue et al., 1995).

2.2. Inoculation of minced meat with tested bacteria

* Corresponding author: Prof. **Hassan M. A** .Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Egypt.

For inoculation of the minced meat, one ml of the dense suspension 3×10^6cfu/g for *B. cereus* was employed. Then the inoculated samples were subjected to bacteriological examination after 30 minutes for counting initial bacteriological load of *B. cereus*

2.3. Nisin preparation

Nisin was prepared at concentrations 10, 30 and 50 ppm according to Hassan (1999)

2.4. Mixing minced meat with nisin

The inoculatedsamplesweredividedinto4groups;the1stwasuntreated control (+ve), the 2ndwas mixed with nisin 10 ppm, the 3rd group was mixed with nisin 30 ppm and the 4th group was mixed with nisin 50 ppm. Nisin was added by spraying and mixed for15 minutes. The control and treated minced meat samples were labeled and packaged as triplicates, then stored at 2±1°C in refrigerator. All groups (either control or treated) were subjected to *B. cereus* count at zero day (within 2 hours after application) and periodically every 24 hours(until the samples spoil).

2.5. Sensory evaluation of the inoculated groups

The overall acceptability of all samples was carried out using nine-point standardized numerical scale, where ten corresponded to components characteristic of the highest quality. The panel consisted of 9 staff members from Animal Health Research Institute, Tanta Branch and all were experienced in sensory evaluation of various food products. Panelists were asked to evaluate each group for overall acceptability(color, odour and texture) as described by Kanatt *et al.* (2010)as following:

Sensory properties trademarks	Overall acceptability
9-10	Excellent
8	Very very good
7	Very good
6	Good
5	Medium
4	Fair
3	Poor
2	Very poor
1	Very very poor

The descriptions of sensory properties and how to rate a sample for the particular sensory property were on the evaluation form.

2.6. Enumeration of the tested bacteria

In order to enumerate *B. cereus*, 100µl of a suitable dilution of the bacteria grown on Brain Heart Infusion (BHI)broth were surface plated on Mannitol Egg Yolk Polymyxin (M.Y.P) media. Enumerations were carried out after incubating of the plates at 37°C for 24 hours on M.Y.P. Typical colonies of *B. cereus* were characterized by pink color and surrounded by a white halo (Tallent et al., 2012).

2.7. Statistical Analysis

The obtained results were statistically evaluated by application of ANOVA test according to Feldman et al. (2003).

3. RESULTS

3.1. Sensory evaluation

Results obtained in table(1) revealed that the organoleptic characteristics respecting the color, odor and texture of minced meat treated with nisin 10,30and 50ppm the treated and control have excellent score at zero day and the first day of treatment while in second day the treated and control were very good with starting the decrease the quality of texture and odor of control meanwhile the third day the control score start to decrease with medium score while the treated groups have very good to good overall acceptability. At the fourth day, the control group decomposed,10 ppm had medium overall acceptability while 30ppm,50ppm had very good score of overall acceptability. At last the fifth day, 10ppm group was decomposed while 30ppm was fair overall acceptability otherwise 50ppm was good to medium.

3.2. Antibacterial activity of nisin on viability of *B. cereus* inoculated into minced meat.

The results recorded in table (2) indicated that nisin (10 ppm) reduced *B. cereus* count (cfu/g) artificially inoculated into minced meat samples from $5.0\times10^7\pm0.82\times10^7$ to$3.12\times10^7\pm0.44\times10^7$, $1.39\times10^7\pm0.23\times10^7$,$9.02\times10^6\pm1.87\times10^6$ and $2.46\times10^6\pm 0.35\times10^6$ after 1st day, 2nd day, 3rddayand 4th day, respectively, with reduction percentages 37.6 %, 72.2%, 81.9%and 95.1%, respectively.

The results recorded in table (3) indicated that nisin (30ppm) reduced *B. cereus* count (cfu/g) artificially inoculated into minced meat samples from $5.0\times10^7\pm 0.82\times10^7$ to $1.97\times10^7\pm0.30\times10^7$,$3.19\times10^6\pm0.45\times10^6$,$5.38\times10^5\pm0.69\times10^5$, $2.38\times10^4\pm 0.31\times10^4$ after 1st day , 2nd day , 3rd day, 4th day, respectively, and no bacilli were detected at the5th day, with reduction percentages 60.6%, 93.6%, 98.9%, 99.9 % and 100%,respectively.

The results recorded table (4) indicated that nisin (50ppm) reduced *B. cereus* count (cfu/g) artificially inoculated into minced meat samples from $5.0\times10^7\pm 0.82\times10^7$ to $9.74\times10^6\pm 2.03\times10^6$, $7.36\times10^5\pm 1.15\times10^5$, after 1st day, 2nd day, 3rd day, respectively, and no bacilli were detected at the 4th and 5th days, with reduction percentages 80.5%, 98.5%, 99.9%, 100% and 100%, respectively.The statistical evaluation indicates high significant differences ($P<0.01$)

4. DISCUSSION

Meat additives used in meat products during processing such as rice and flour have been considered a source of *B. cereus* (Giffel et al.,1996).The contamination of meat products probably occurred during handling and preparation or post-processing contamination. In addition, keeping the products unrefrigerated for several hours enhances the multiplication of *B. cereus* and hence the liberation of enterotoxin (Shawishand *Tarabees*, 2017).Furthermore, improper handling of meat products after cooking allow the spores of *B. cereus* to germinate resulting in vegetative cells which multiply and lead to food poisoning (Torky-Amal,2004).Additionally, additives, seasoning, and spices area added, and these additives are considered a potential risk factor can increase the number of Bacillus spores and hence magnitude the incidence of food poisoning (Shawish and Tarabees, 2017).

Nisin is a natural preservative for many food products. This bacteriocin is mainly used in dairy and meat products. Nisin

inhibits pathogenic food borne bacteria such as *Listeria monocytogenes* and many other Gram-positive food spoilage microorganisms. Nisin can be used alone or in combination with other preservatives or also with several physical treatments (Gharsallaoui et al., 2016).The obtained results were nearly similar to those reported by (Ibrahim et al.,2014), who mentioned that nisin had bactericidal effect against *B. cereus* when used at concentrations 20, 40 and 60 ppm.

Table 1 Organoleptical properties of tested minced meat treated with different concentration of nisin

item	Treatment	Days of treatment					
		Zero day	1st day	2nd day	3rd day	4th day	5th day
Color	Control +ve	10±0.00	9.83±0.21	8.16±0.28	5.16±0.17	----------	----------
	Nisin 10ppm	10±0.00	10±0.00	8.16±0.16	6.3±1.7	5.1±.21	----------
	Nisin 30ppm	10±0.00	10±0.00	8.6 ± 0.18	6.4±0.17	6.33±0.17	5±0.17
	Nisin50ppm	10±0.00	10±0.00	8.8± 1.6	7.88±1.66	6.5±0.05	5.56±0.12
Odeur	Control +ve	10±0.00	10±0.00	7.8±0.16	6±0.00	----------	----------
	Nisin 10ppm	10±0.00	10±0.00	8.16±0.16	6.3±1.7	5.83±0.16	----------
	Nisin 30ppm	10±0.00	10±0.00	8.16±0.16	7.5±0.00	6.3±.17	4.76±0.14
	Nisin50ppm	10±0.00	10±0.00	9±0.00	7.9±.26	6.4±0.21	6.2±0.12
Texture	Control +ve	10±0.00	10±0.00	7.33±0.7	5.3±.16	----------	----------
	Nisin 10ppm	10±0.00	10±0.00	7.66±0.16	7.16±.08	5.3±.33	----------
	Nisin 30ppm	10±0.00	10±0.00	8.33±0.17	7.16±.17	6±0.00	4.76±0.14
	Nisin50ppm	10±0.00	10±0.00	8.3±1.6	8.06±.06	6.1±.12	6.2±0.12

Table 2 Antibacterial activity of nisin (10ppm) on viability of *B. cereus* inoculated into minced meat by intensity of 5.0×10^7

Storage time	Control		Nisin (10 ppm)	
	Count	R %*	Count	R %
Zero time	$5.0\times10^7\pm 0.82\times10^7$	------	$5.0\times10^7\pm 0.82\times10^7$	------
1st day	$4.84\times10^7\pm 0.61\times10^7$	3.0	$3.12\times10^7\pm 0.44\times10^7$	37.6
2nd day	$4.71\times10^7\pm 0.68\times10^7$	5.8	$1.39\times10^7\pm 0.23\times10^7$	72.2
3rd day	$4.59\times10^7\pm 0.53\times10^7$	8.2	$9.02\times10^6\pm 1.87\times10^6$	81.9
4th day	----------	----------	$2.46\times10^6\pm 0.35\times10^6$	95.1
5th day	----------	----------	----------	----------

R %*= Reduction %

Table 3 Antibacterial activity of nisin (30ppm) on viability of *B. cereus*inoculated into minced meat by intensity of 5.0×10^7.

Storage time	Control		Nisin (30 ppm)	
	Count	R %*	Count	R %
Zero time	$5.0\times10^7\pm 0.82\times10^7$	------	$5.0\times10^7\pm 0.82\times10^7$	------
1st day	$4.84\times10^7\pm 0.61\times10^7$	3.0	$1.97\times10^7\pm 0.30\times10^7$	60.6
2nd day	$4.71\times10^7\pm 0.68\times10^7$	5.8	$3.19\times10^6\pm 0.45\times10^6$	93.6
3rd day	$4.59\times10^7\pm 0.53\times10^7$	8.2	$5.38\times10^5\pm 0.69\times10^5$	98.9
4th day	----------	-------	$2.38\times10^4\pm 0.31\times10^4$	99.9
5th day	----------	---------	ND	100

R %*= Reduction %. ND= Not detected

Table 4 Antibacterial activity of nisin (50ppm) on viability of *B. cereus* inoculated into minced meat by intensity of 5.0×10^7.

Storage time	Control		Nisin (50 ppm)	
	Count	R %*	Count	R %
Zero time	$5.0\times10^7\pm 0.82\times10^7$	------	$5.0\times10^7\pm 0.82\times10^7$	------
1st day	$4.84\times10^7\pm 0.61\times10^7$	3.0	$9.74\times10^6\pm 2.03\times10^6$	80.5
2nd day	$4.71\times10^7\pm 0.68\times10^7$	5.8	$7.36\times10^5\pm 1.15\times10^5$	98.5
3rd day	$4.59\times10^7\pm 0.53\times10^7$	8.2	$3.83\times10^4\pm 0.46\times10^4$	99.9
4th day	----------	10.1	ND	100
5th day	----------	12.6	ND	100

R %*= Reduction %. ND= Not detected.Analysis of variance (ANOVA) indicates high significant differences (P<0.01).

Roberts and Hoover(1996)found that 10^5,10^6/ml initial *Bacillus* spores concentration were reduced by $3\times10^2$when nisin concentration was 1.0 I.U/ml. While, Lee et al., (2015)studied the effect of nisin(100 IU/g and 500 IU/g)onthe growth of *Bacillus cereus* inoculated in beef jerky during storage and the results suggest that nisin could be an effective approach to extend the shelf-life, and improve the microbial safety of beef jerky, during storage

The obtained result revealed that the organoleptic characteristics of minced meat treated with nisin 50ppm was not affected during experimental time and this agree with results obtained by Sumonsiri(2019), who used nisin (25-75

ppm) the samples treated with 50 and 75 ppm nisin had significantly lower aerobic microbial counts than the control without affecting color, turbidity and sensory acceptability. The treated samples 50 ppm Nisin without effects on color, turbidity, and sensory acceptability by a reduction in changes of the microbial growth during the refrigerated storage.

Nisin inhibits outgrowth of germinating spores of *B. cereus* through either covalent binding to a spore target or loss of membrane integrity. Nisin causes lysis of vegetative cells (Ian *et al*., 2011).Nisin was first commercially marketed in England in 1953. In 1969, a joint commission between the Food and Agriculture Organization of the United Nation (FAO) and the World Health Organization (WHO) recognized nisin as a safe and legal biological food preservative. In United States, the use of nisin in food has been legally approved by the American Food and Drug Administration (FDA) since 1988 (Zacharof and Lovitt, 2012).

5. CONCULOSION

Nisin is safe anti-microbial food preservative in meat products without affecting the sensory characteristics and acceptability to consumer.

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