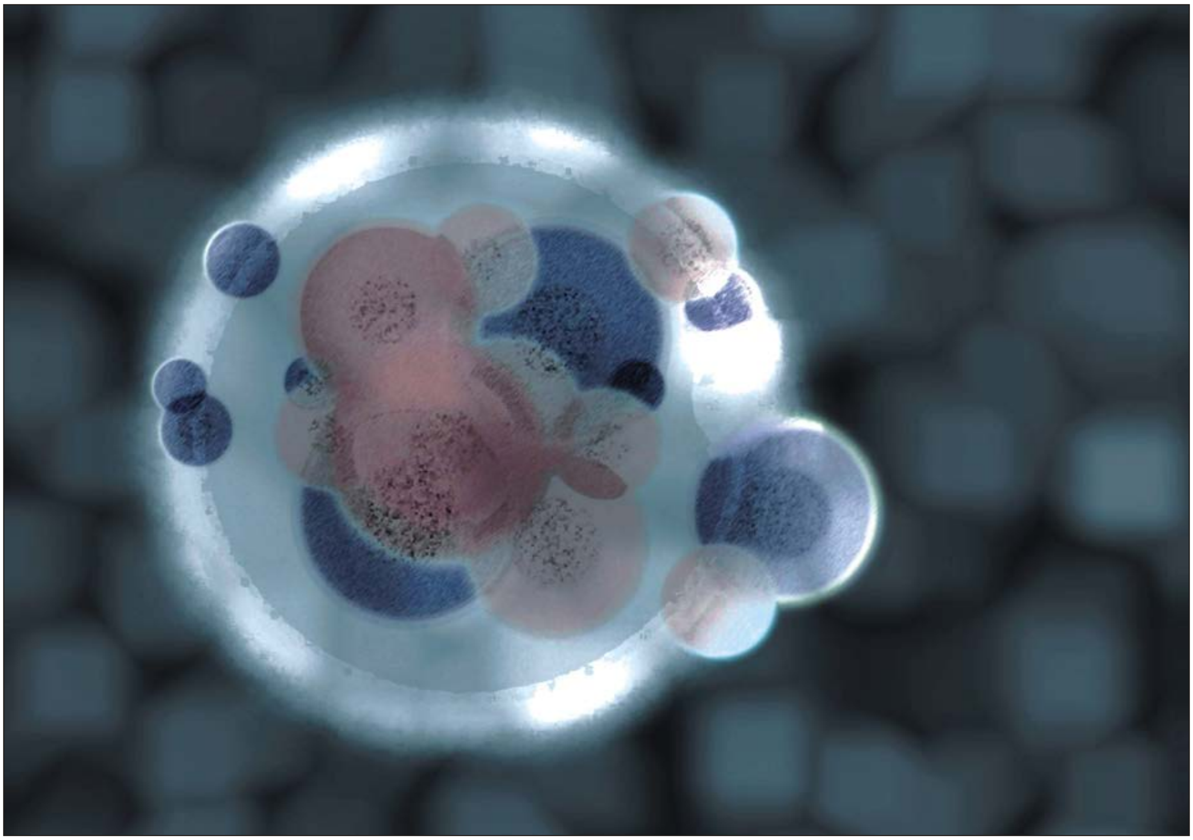

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Shelf Life Extension of Cheddar Processed Cheese Using Polyethylene Coating Films of Nisin against *Bacillus cereus*

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Abstract: Antimicrobial food packaging is vital issue. This study aimed to test shelf life extension of Cheddar Processed Cheese at room temperature using low-density polyethylene (LDPE) films incorporating nisin against *Bacillus cereus*. In particular, the efficiency of different treatments was tested against *Bacillus cereus*. Different concentrations (0, 500, 1000, 2000, 4000, 8000, 16000 IU mL^{-1}) of nisin were used in activating (coating) LDPE films. There was no mainly clear difference in inhibition zone from 2000 to 16000 IU, so that a concentration of 2000 IU was used for active packaging. Four different treatments were tested in cheese: samples without coating; samples without coating and inculcated by *Bacillus cereus*; samples coating by polyethylene films that activated by nisin without inoculation and samples coating by the same films and inoculated by *Bacillus cereus*. Microbiological analyses including total count, spore forming bacteria and yeast and moulds of the cheese samples were performed during 8 months. Results indicated that the cheese which coated with polyethylene films that treated with nisin regardless whether it was inculcated or not were free of *Bacillus cereus* bacteria.

Key words: Shelf life, cheddar processed cheese, polyethylene coating films, nisin, *Bacillus cereus*

INTRODUCTION

Nisin is an antibacterial substance produced by *Lactococcus lactis* sp. *lactis*. It is considered as a bacteriocin against Gram-positive bacteria such as *Lactobacillus*, *Listeria*, *Bacillus* and *Clostridium* (Brewer *et al*., 2002; Lopez-Pedemonte *et al*., 2003; Sobrino-Lopez and Martin-Belloso, 2006). It is particularly effective against bacterial spores (Delves-Broughton, 2005; Morency *et al*., 2001). It is classified as a 'lantibiotic' because it includes unusual compounds β-methyl lanthionine and lanthionine (Hancock and Rozek, 2002).

Nisin is widely used as a food preservative and has been shown to be able to be incorporated into various food packaging films (Quintavalla and Vicini, 2002; Cooksey, 2005; Chollet *et al*., 2008; Kristo *et al*., 2008; Manikantan and Varadharaju, 2012); it has successfully been used as preservative in dairy and meat products (Hampikyan and Ugur, 2007). In the United States, nisin has received GRAS (generally recognized as safe) status and it is approved for use in some processed cheese spreads to prevent the growth of spoilage organisms and to extend the shelf life (Hurst, 1981). Antimicrobials may be directly coated onto the surface of food or incorporated into packaging materials. These antimicrobial films can preserve their activity, thus inhibition bacterial growth during extended storage after packaging (Hoffman *et al*., 2001).

Polymers such as Low-Density Poly Ethylene (LDPE) constitute a majority of primary packages for foods and beverages and a great deal of research has been devoted to the development of active polymer packaging. Polymers can be activated by the addition of components such as antioxidants, antibacterial agent (Rooney, 1995). Cheese is a ready-to-eat product which is considered as a "potentially dangerous food, so that insuring its safety is very important to human being. This study aimed to test shelf life extension of Cheddar Processed Cheese at room temperature using LDPE films incorporating nisin against *Bacillus cereus*.

MATERIALS AND METHODS

The study extended for 8 month starting from December 2011 to August 2012. LDPE was used as a packaging material. Nisin is produced from *Lactococcus Lactis* sp. *lactis* and obtained from SIGMA. *Bacillus cereus* was the target microorganism.

Preparation of antimicrobial LDPE: LDPE (1.5×1.5 cm) was saturated in nisin diluted in phosphate buffer 60 mL mol^{-1}, pH 6.8 at concentration of 0, 500, 1000, 2000, 4000, 8000 and 16000 IU mL^{-1} for 20 min. Thereafter, the films were dried under sterilized conditions. Then the developed films were investigated against the target microorganism. The nisin activated films were tested for antibacterial activity against *Bacillus cereus*. Individual samples of the treated films were put on the surface of Nutrient agar media. Agar plats were seeded with 25% of 18-h culture of *Bacillus cereus*. The treated films were in connecting with agar. The plates were incubated at 30°C/24 h. The activity of nisin treated LDPE was estimated by observing growth inhibition as a clear zone around the films.

Cheese manufacturing: Processed cheese was manufactured by adding water, emulsifying salt and butter to ragged natural Cheddar cheese then heating the blind up to 90°C with a constant stir until a homogenous mass was obtained. Thereafter, pouches of activated and non-activated LDPE were filled with Cheddar processed cheese. Before closing some pouches, the *Bacillus cereus* was put on the face by 2.5 mL (equivalent to 2.2×10^3 CFU $mL^{-1}/30\ cm^2$) where as the other pouches left without inculcation. All used pouches were stored at room temperature. Overall, four different treatments were prepared in cheese: samples without coating; samples without coating and inculcated by *Bacillus cereus*; samples coating by polyethylene films that activated by nisin without inoculation and samples coating by the same films and inoculated by *Bacillus cereus*. Microbial analysis was then performed on the following day of manufacturing and with an interval of 1-8 months.

Microbial analysis: Total microbial count was detected by nutrient agar medium. Plates were incubated for 48 h at 32°C. Same medium was used for detecting spore former bacteria count and incubated for 72 h at 32°C. However, potato dextrose agar was used to detect Yeast and moulds. Plates were incubated for 7 days at room temperature. All media were prepared according to Difco Laboratories (1984).

RESULTS AND DISCUSSION

The results indicated the importance of nisin as a food preserving material. The cheese which coated with polyethylene films that treated with nisin show the vital role of nisin in inhibition the growth of microorganism including total count, yeast and moulds and spore former bacteria specifically *Bacillus cereus*. Figure 1 demonstrates that there was no inhibition zone around the control film. The films which were activated with nisin, in its all concentrations, effectively prevent the growth of *Bacillus cereus*. As concentration of nisin duplicated within the range of 500-2000 IU, the inhibition zone increased. However, similar inhibition zone was found when the concentration ranges between 2000 and 16000. Henning *et al.* (1986) explained the mechanism in which nisin works. They provided evidence showing that the antimicrobial effect of nisin is attributable to its interaction with the phospholipid component of the cytoplasmic membrane. They confirmed that isolated cytoplasmic membrane fragments could irritate the inhibitory effect of nisin and that nisin would combine with phospholipids to form nisin-phospholipid complexes. The initial electrostatic attraction between the target cell membrane and the bacteriocin peptide is considered to be the main factor for succeeding events. Deegan *et al.* (2006) argued that nisin forms pores that disrupt the proton motive force and the pH equilibrium causing leakage of ions and hydrolysis of ATP resulting in cell death.

Table 1 shows the effect of nisin on total bacterial count of Cheddar processed cheese. There were no reported growths in the samples that were packaged with films activated by nisin. Specifically, no bacterial cells were found in 1 g of Cheddar processed cheese. On the other hand, the samples that were not treated with nisin

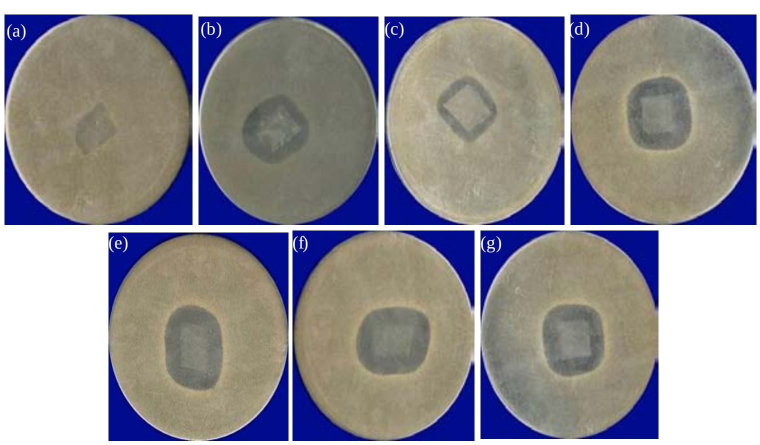

Fig. 1(a-g): Effect of nisin films (a), on *Bacillus cereus* Control, (b) 500 IU, (c) 1000 IU, (d) 200 IU, (e) 4000 IU, (f) 8000 IU and (g) 1600 IU concentration showed statistically significant continuous increase in the total bacterial count over the storage period. The control sample indicated significant count of bacteria after the fifth month of storage while the other samples inculcated by *Bacillus cereus* showed significant count of bacteria from the third month of storage and thereafter. Table 2 shows the effect of nisin activated films on spore former count (log CFU g^{-1}) of Cheddar processed cheese.

The results were consistent with those in Table 1. There were no documented growths in the samples that were packaged with films activated by nisin. In particular, no bacterial cells were found in 1 g of Cheddar processed cheese. Conversely, a statistically significant increase in spore former bacteria count was found in the samples that were free of nisin. No yeast and moulds were found in all study samples.

Present results are consistent with other studies which found an effective impact of the nisin incorporated into various kinds of films in reducing the undesirable bacteria in different foodstuff. Some studies focused on antimicrobial packaging of cheese. Ko *et al*. (2001) showed that the combined action of nisin and galactomannan edible coatings over *Listeria monocytogenes* growth on Ricotta cheese samples stored at 4°C resulted in a reduction of the microbial count at least during 14 days of storage. Cao-Hoang *et al.* (2010) provided evidence of the effectiveness of sodium caseinate films containing nisin (1000 IU cm^{-2}) in controlling *Listeria innocua* in an artificially contaminated cheese, specifically, Mini red Babybel cheese was chosen as a model semi-soft cheese. Thereby extending the shelf life and possibly enhancing the microbial safety of cheeses. Martins *et al*. (2010) demonstrate that a novel galactomannan-based edible coating, when combined with nisin, provides consumer-friendly alternatives to reduce *Listeria monocytogenes* post contamination on cheese products during storage. Suppakul *et al*. (2008) showed that the use of polymeric films containing the principal constituents of basil as the AM components enhances quality and safety of cheeses. However, other researchers studied antimicrobial packaging of different foodstuff. Grower *et al.* (2004) found that the antimicrobial coated films containing nisin were effective in inhibition of *Listeria monocytogenes* in hotdogs, particularly when 7, 500 and 10,000 IU cm^{-2} of nisin concentrations were used. Consistently, Franklin *et al*. (2004) find that packaging films containing nisin is also effective in controlling *Listeria monocytogenes* in individually packaged hot dogs. They also found that nisin alone is more effective than nisin-rosemary blend for hot dogs performed. Millette *et al*. (2007) documented a *Staphylococcus aureus* reduction on sliced beef covered with palmitoylated alginate-based films containing nisin at 500 IU mL^{-1}. Moreover, Ercolini *et al*. (2010) studied the microbial populations causing the spoilage of chilled beef during storage through examining the effect of nisin activated antimicrobial packaging on meat storage. They found that the use of the nisin activated packaging reduced the number of spoilage populations of chilled beef but did not

Table 1: Effect of nisin activated films on total bacterial count of Cheddar processed cheese

Storage (month)	Control	Control+*Bacillus cereus* (2.2×10^3 CFU mL^{-1}/30 cm^2)	Nisin coated film	Nisin coated film+*Bacillus cereus* (2.2×10^3 CFU mL^{-1}/30 cm^2)
Fresh	ND	ND	ND	ND
1	1.20	1.45	ND	ND
2	1.98	2.00	ND	ND
3	2.55	3.19*	ND	ND
4	3.09	3.79	ND	ND
5	4.03*	4.09*	ND	ND
6	4.85*	5.11*	ND	ND
7	5.52*	5.90*	ND	ND
8	6.00*	6.23*	ND	ND

ND: Not detected in 1 g of cheese, *Significant values at a critical value of p<5%

Table 2: Effect of nisin activated films on spore former count of Cheddar processed cheese

Storage (month)	Control	Control+*Bacillus cereus* (2.2×10^3 CFU mL^{-1} /30 cm^2)	Nisin coated film	Nisin coated film+*Bacillus cereus* (2.2×10^3 CFU mL^{-1}/30 cm^2)
Fresh	ND	ND	ND	ND
1	ND	1.01	ND	ND
2	ND	1.20	ND	ND
3	1.16	1.65	ND	ND
4	1.5	2.01	ND	ND
5	1.98	2.77*	ND	ND
6	2.35*	3.22*	ND	ND
7	2.90*	3.90*	ND	ND
8	3.88*	4.35*	ND	ND

ND: Not detected in 1 g of cheese, ND: Not detected in 1 g of cheese, *Indicate significant values at a critical value of (p<5%)

affect the species diversity. Finally, Jin and Zhang (2008) found that biodegradable polylactic acid polymer incorporated with nisin showed an antimicrobial effectiveness against *Listeria monocytogenes, Escherichia coli* and *Salmonella* in liquid foods (orange juice and liquid egg).

CONCLUSION

Nisin was found to be an effective antimicrobial packaging material and the package coating solution successfully used as a carrier of the substance. LDPE films activated by a concentration of 2000 IU of nisin inhabit the growth of microorganism, specifically *Bacillus cereus*, on the surface of Cheddar processed cheese.

Overall, it could be concluded that the main source of spoilage of many foods is the microbial growth; accordingly the application of antimicrobial material to packaging these foods could be functional to prevent the growth of microorganisms on the product surface and hence may lead to an extension of the shelf-life and improved microbial safety of the products.

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