Food Control 23 (2012) 48–53

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Inactivation of *Listeria innocua* in brined white cheese by a combination of nisin and heat

Murad A. Al-Holy [a,*], Anas Al-Nabulsi [b], Tareq M. Osaili [b], Mutamed M. Ayyash [c], Reyad R. Shaker [b]

[a] Department of Clinical Nutrition and Dietetics, Faculty of Allied Health Sciences, Hashemite University, P.O Box 150459, Zarqa 13115, Jordan
[b] Department of Nutrition and Food Technology, Faculty of Agriculture, Jordan University of Science and Technology, P.O. Box 3030, Irbid 22110, Jordan
[c] School of Biomedical and Health Sciences, Faculty of Health, Engineering and Science, Victoria University – Werribee Campus, P.O. Box 14428, Victoria 8001, Australia

ARTICLE INFO

Article history:
Received 18 October 2010
Received in revised form
31 May 2011
Accepted 7 June 2011

Keywords:
Listeria innocua
White cheese
Nisin
Heat
Synergy

ABSTRACT

The objective of this study was to investigate the effect of nisin alone and in combination with heat (63 °C/5 min) on the inactivation of *Listeria innocua* in white cheese. Nisin was added at different concentrations (500, 1000, and 1500 IU ml^{-1}) to pasteurized milk before curd formation. The curd was soaked for 24 h in 10% solution of brine containing ca 10^6 CFU ml^{-1} of a cocktail mixture of three strains of *L. innocua*. Part of the nisin treated samples were heat treated at 63 °C/5 min. Total mesophilic count (TMC), *L. innocua* survivors and changes in the pH of white cheese were monitored each 2 d for a period of 12 d of storage at 4 or 10 °C. Nisin at 500 IU ml^{-1} did not diminish TMC in white cheese compared to the control. The combination of heat and nisin (1000 or 1500 IU ml^{-1}) exhibited a bacteriostatic effect on TMC throughout the storage period at 4 or 10 °C. Nisin at 500 IU ml^{-1} had a marginal inhibitory activity against *L. innocua*. However, nisin at 1000 and 1500 IU ml^{-1}l resulted in a more than 2 log_{10} reduction in *L. innocua* count and the effect was more prominent at 10 °C. In comparison, the combination of nisin (1000 or1500 IU ml^{-1}) and heat treatment exhibited a synergistic inhibitory activity against *L. innocua*, where a complete elimination of the organism was accrued after 6 and 8 d of storage at 10 and 4 °C. Therefore, nisin and heat combination could be used as a prudent hurdle to preclude the growth of *Listeria* in white cheese, especially under the condition of abused refrigeration conditions.

1. Introduction

Listeria monocytogenes is the causative agent of sporadic but life-threatening outbreaks of listeriosis, a severe disease with high hospitalization and case fatality rates, especially among people with compromised immune system (Mead et al., 1999). *L. monocytogenes* can survive and grow over a wide range of environmental conditions such as refrigeration temperatures (2–4 °C), low pH and high salt concentrations (Gandhi & Chikindas, 2007). This allows the pathogen to overcome food preservation and safety barriers, and pose a potential risk to human health (Gandhi & Chikindas, 2007).

White cheese is unripened rennet-coagulated type and is usually consumed fresh or after storing into brine solution. White cheese may serve as an ideal medium for bacterial proliferation because of the absence of competing starter culture, high water activity, protein and fat content. *L. monocytogenes* is a microorganism of a ubiquitous nature that is likely to contaminate and grow in white cheese (Bell & Kyriakides, 1998). *L. monocytogenes* was responsible for several outbreaks associated with dairy food consumption, especially soft cheeses (De Buyser, Dufour, Maire, & Lafarge, 2001; Kozak, Balmer, Byrne, & Fisher, 1996). Consequently, eradicating this microorganism from dairy products is a major concern for the dairy industry. White cheese is prone to rapid bacterial deterioration, particularly if handled under abusive storage temperatures or poor hygienic conditions.Several studies elucidated the remarkable capability of *Listeria* spp. to survive and grow in different types of cheeses. *Listeria innocua* was able to survive during the manufacture and storage of Turkish white cheese kept in 16% brine solution due to inadequate pasteurization or post-process contamination (Öztürkoğ, Gürakan, & Alpas, 2006). Other studies indicated that *L. monocytogenes* can survive for more than a year in Cheddar cheese (Ryser & Marth, 1987), and for more than 140 days in Colby cheese (Yousef & Marth, 1988). *L. monocytogenes* also survived in white pickled cheese containing 8% of salt and stored at 4 °C for more than 28 days (Abdalla, Christen, & Davidson, 1993).

Nisin, a bacteriocin produced by strains of *Lactococcus lactis* subsp. *Lactis*, is generally recognized as safe (GRAS) natural

* Corresponding author. Tel.: +9625390 3333; fax: +962 390 3368.
E-mail address: murad@hu.edu.jo (M.A. Al-Holy).

0956-7135/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2011.06.009

preservative that is permitted for use in foods, including dairy products (Delves-Broughton & Gasson, 1994). The use of nisin goes back to 1956, when it was first used to inactivate spores of *Clostridium botulinum* and *Clostridium sporogenes* in cheese (Mattick & Hirsch, 1956). It was indicated that applying nisin at a level of 2.5 mg l^{-1} to the milk used in the production of ricotta-type cheese could effectively inhibit the growth of *L. monocytogenes* for a period of at least 8 weeks (Davies, Bevis, & Delves-Broughton, 1997). Nisin has an inhibitory effect against a wide variety of Gram positive food-borne pathogens and spoilage microorganisms (Rodriguez, 1996). The underlying mode of action of nisin is through disruption of membrane function instigated by formation of pores in the bacterial cell membrane followed by leakage of the cellular material (Winkowski, Ludescher, & Montville, 1996).

The United States Food and Drug Administration has set a zero tolerance policy for the presence of *L. monocytogenes* in ready-to-eat foods because of its serious health implications (Klima & Montville, 1995). White cheese is a ready-to-eat food that requires no additional heat treatment before consumption. Therefore, necessary measures should be applied during processing and handling of cheese to impede the growth of pathogens such as *L. moncocytogenes* in white cheese. Using nisin alone or in combination with other hurdles may serve as a potential effective method to eliminate *L. monocytogenes* and other pathogens form white cheese. The present study aimed to investigate the effect of different nisin concentrations against *L. innocua* (a surrogate bacterium for *L. monocytogenes*) inoculated into white cheese. Additionally the combined effect of nisin with mild heat treatment against *L. innocua* was also studied under proper (4 °C) and abusive (10 °C) storage temperature conditions.

2. Material and methods

2.1. Bacterial strains and culture media

All cultures used in this study were obtained from the culture collection of the Food Microbiology Lab at the Hashemite University (Zarqa-Jordan). All tested cultures were maintained as frozen stocks at −20 °C in 15% glycerol. A stationary-phase culture of a cocktail of three *L. innocua* strains (ATCC 51742, ATCC 33090, and ATCC 33091) was used to inoculate pasteurized milk that was used to prepare white cheese because cells in this stage are most resistant to preservation stresses (Jydegaard, Gravesen, & Knochel, 2000; Ueckert, ter Steeg, & Coote, 1998). *L. innocua* was selected for the study as a surrogate alternative for *L. monocytogenes* to prevent the introduction of the pathogenic organism to the dairy plant and because *L. innocua* is even more resistant to environmental stresses such as nisin and heat compared to *L. monocytogenes* strains (Friedly et al., 2008; Kamat & Nair, 1996). *Listeria* strains were individually cultured into brain heart infusion (BHI) broth (Oxoid, Basingstoke, Hampshire) at 37 °C for 24 h, harvested by centrifugation at 4000×*g* for 20 min and washed three times with 0.9% saline solution. The resulting pellet was 10-fold serially diluted in 0.9% saline solution and inoculated at a level of ca 1×10^6 CFU ml^{-1} in 10% sterile brine solution.

2.2. White cheese preparation and antimicrobial treatment

Whole fat cows milk (40 kg) from Jordan University of Science and Technology Dairy plant was pasteurized at 72 °C for 15 s and cooled to 35 °C. A nisin powder of 650 international unit (IU) mg^{-1} (2.5% nisin content) ((ICN Biomedicals, Inc; Aurora, OH) was used in the current study. A stock solution of nisin (10000 IU ml^{-1}) was prepared by dissolving an appropriate amount of nisin in 0.02 M HCl. The solution was heated at 80 °C for 7 min, and kept at −20 °C until use. Nisin was added to obtain final concentrations of 0, 500, 1000 and 1500 IU ml^{-1} of pasteurized milk. A single-strength calf rennet extract (Chr. Hansen, Milwaukee, WI) was diluted (1:10) with sterile distilled water and added to nisin-inoculated milk and kept for 35 min until curd formed. Curd was cut into cubes (~1 cm^3) and stirred for 5 min to increase whey separation. Whey was removed using cheesecloth and pressed by stainless steel plate to form a cheese layer with thickness of 1 cm. After 30 min of pressing, the cheese was cut into pieces that weighed ca 15 g (4 × 3 × 1 cm in size). Sterile brine solution (10%) was cooled (4 °C) and inoculated with *L. innocua* strains mentioned earlier (ca 1×10^6 CFU ml^{-1}) and white cheese pieces were added and kept for 24 h and stirred occasionally to achieve an initial population of ca 1×10^4 - 1×10^5 CFU g^{-1} of cheese. After soaking for 24 h, cheese pieces were vacuum-packaged into polyethylene bags (3 pieces per bag). For each nisin treatment, cheese pieces were randomly divided into two equal parts. One part was heated at 63 °C for 5 min and the other part was left unheated. Each treatment in this study was stored at either 4 °C or at abused refrigeration temperature of 10 °C for 12 d. The process of white cheese preparation and inoculation with *L. innocua* is described in Fig. 1. All treatments in this study were prepared in triplicate.

2.3. Microbiological enumeration

Three cheese pieces were sampled at 0, 2, 4, 6, 8, 10, and 12 d of storage per each treatment. Five-gram samples of white cheese pieces were placed in sterile stomacher bags with 45 ml of sterile 0.1% peptone water, and homogenized for 1.5 min with a model 400 stomacher (Seward Ltd., London, UK) and then 10-fold serially diluted with 9 ml of sterile 0.1% peptone water. TMC was determined after dilution by spread plating 100 μl of diluted sample onto plates of tryptic soy agar (TSA) (Oxoid) which were incubated at 37 °C for 36 h. Surviving and injured *L. innouca* cells were enumerated using the overlay method, which was designed specifically to improve the recovery of injured cells. TSA spread plates were incubated at 37 °C for 2 h to allow the injured *L. innocua* cells to repair and resuscitate and then about 7 ml of the *Listeria* selective PALCAM medium base (Himedia, LBS Marg, Mumbai, India) containing the antimicrobic supplement (Difco Laboratories, Sparks, MD) was overlaid onto the TSA (Al-Holy, Ruiter, Lin, Kang, & Rasco, 2004). The plates were incubated for an additional 34 h at 37 °C and typical black colonies were enumerated. Experiments were conducted in triplicate and the results were expressed as $\log_{10}$ CFU g^{-1}.

2.4. Chemical analysis of white cheese

Moisture, fat, protein, and ash contents of cheese were determined according to the AOAC methods (1995). Salt concentration of cheese samples was determined as per the method of Kosikowski and Mistry (1997). The pH of cheese samples was measured using pH-meter (Cyberscan 500, Eutech instruments, Singapore). All measurements were the average of three replicates.

2.5. Statistical analysis

Each reported value is a mean of three replicate experiments. Data were analyzed with a computer software package (SAS Institute, Cary, N.C) using and analysis of variance and Fisher's least significant difference (LSD) test for mean separations ($P \leq 0.05$).