Effective Use of Nisin To Control Bacillus and Clostridium Spoilage of a Pasteurized Mashed Potato Product

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ABSTRACT

Heat-resistant spore-forming bacteria such as Bacillus and Clostridium can survive and grow in cooked potato products. This situation represents both a public health problem and an economic problem. The natural food preservative nisin is used in heat-treated foods to prevent the growth of such bacteria. A cocktail of Clostridium sporogenes and Clostridium tyrobutyricum spores was inoculated into cooked mashed potatoes, which were vacuum packed, pasteurized, and incubated at 8 and 25°C. The shelf life of the mashed potatoes at 25°C was extended by at least 58 days with the addition 6.25 µg of nisin per g. At 8°C, in control samples not containing nisin, the natural contaminant Bacillus grew, but the inoculated Clostridium strains did not until the temperature was raised to 20°C after 39 days. No bacterial growth occurred in nisin-containing samples. The shelf life of the mashed potatoes was extended by at least 30 days with 6.25 µg of nisin per g. In trials involving a cocktail of Bacillus cereus and Bacillus subtilis strains, 6.25 µg of nisin per g extended the shelf life of mashed potato samples that were not vacuum packed by at least 27 days at 8°C. At 25°C, 25 µg of nisin per g extended shelf life by a similar period. Shelf life extension was also observed at lower nisin levels. Microbiological analysis of the mashed potato ingredients showed that a high spore level was associated with the onion powder. It is emphasized that the preservative and the ingredients must be well mixed to ensure good nisin efficacy. Nisin remained at effective levels after pasteurization, and good retention was observed throughout the shelf life of the mashed potatoes.

Food manufacturers are now producing more and varied product lines incorporating potatoes as a main ingredient. Although these products usually contain cooked potatoes, the cooking process is not a sterilization procedure. Consequently, gram-positive endospore-forming bacteria such as Bacillus and Clostridium species can survive and grow, even at temperatures of ≤5°C ([15, 27]). The potential growth of the pathogen Clostridium botulinum is a major concern. Several foodborne outbreaks involving both Bacillus cereus and C. botulinum have been reported in the United States, The Netherlands, and the United Kingdom. Products implicated in these outbreaks have included mashed potatoes, potato dip, and potato salad ([1, 3, 5–9, 12, 17, 21, 25, 26]).

When harvested from the soil, raw potatoes may be contaminated with C. botulinum ([18, 28]). To prevent the spores from growing and producing toxin in a cooked potato product, either the potatoes should be given a heat treatment equivalent to the F0.3 process (equivalent to 3 min at 121°C) or a combination of preservative hurdles must be incorporated. In one study, a large proportion of C. botulinum spores reportedly survived a double pasteurization of peeled, inoculated, vacuum-packed potatoes stored at 25°C ([20]). In another study, the inoculation of baked potatoes with C. botulinum spores prior to baking resulted in toxin production after 3 to 7 days at 25°C ([28]). In a study of both proteolytic and nonproteolytic C. botulinum strains, thermal inactivation of the spores was slower in vacuum-packed cooked potatoes than in phosphate buffer ([23]), and toxin production was found to occur in this potato product during storage at 4 to 20°C. At temperatures above 10°C, toxin production occurred before the product spoiled, even though spoilage was rapid at 15 to 20°C, with obvious gas production.

The prevalence and growth of B. cereus in a range of commercial cooked, chilled foods containing vegetables has also been investigated ([10]). After 5 to 12 days of incubation at room temperature, 60% of pasteurized potato purées were found to contain B. cereus, and 80% of these samples incubated for 20 days at 10°C were found to contain this pathogen. B. cereus strains were also found in puréed broccoli, carrots, zucchini, and split peas. Other species of Bacillus were also identified. Thomas and Masters ([31]) investigated the growth of bacteria on precooked potato-topped pies stored at 4 and 37°C. Bacillus, Streptococcus, and Staphylococcus were the predominant organisms isolated. It was concluded that such food products represented a potential public health risk if they were stored at inappropriate temperatures.

There have been other studies that have examined the incidence and growth of nonpathogenic spoilage strains of Bacillus and Clostridium. The growth of spore-forming bacteria in a range of commercial, cooked, pasteurized, and chilled vegetable purées including potatoes was investigated ([4]). Rapid spoilage occurred at room temperature, but
growth was also observed at 10°C. Isolates were identified as Bacillus species, predominantly Bacillus cereus, Bacillus subtilis, and Bacillus polymyxa. In a study of mayonnaise-based salads, including potato salads, contamination with Clostridium species was found (2). In two studies of the microbial spoilage of fully or partially cooked vacuum-packaged potatoes (29, 30), it was found that the natural contaminant spoilage microflora was dominated by B. cereus, B. polymyxa, and Bacillus licheniformis. The growth of these strains was rapid at 22°C, with swelling of the package occurring after 17 to 21 days because of gas production.

Products such as cooked mashed potatoes form part of the growing range of convenience foods offered to consumers. Such foods are becoming increasingly popular. The reports described above indicate that there is a need for further preservative measures for these products. The present study reports on trials undertaken to investigate the efficacy of nisin in controlling the growth of Bacillus and Clostridium spores, which survive the cooking process and grow in mashed potatoes. The bacteriocin nisin is a safe, natural antimicrobial preservative that would be suitable for use in such products (11, 31, 33). Nisin can protect against temperature abuse, extend shelf life, and provide a further safety measure to protect against the growth of heat-resistant food-poisoning bacteria.

MATERIALS AND METHODS

Bacterial cultures. Bacterial cultures originally isolated from a variety of spoiled foods were obtained from the Danisco culture collection. The test strains included B. cereus 204, B. cereus 3046, B. cereus Campden strain (obtained from the Campden and Chorleywood Food Research Association), Bacillus subtilis Campden strain, Clostridium sporogenes 1.003, C. sporogenes 1.120, C. sporogenes 4.439, C. sporogenes 4.440, C. sporogenes Campden strain, and Clostridium tyrobutyricum 12573. The Clostridium strains were grown anaerobically at 37°C overnight on reinforced clostridial agar (Oxoid, Basingstoke, UK) and then left at ambient temperature for 1 week to sporulate. Bacillus strains were grown on milk plate count agar (Oxoid) overnight at 30°C and then left at ambient temperature for 1 week to sporulate. When spores were detected microscopically, spore suspensions were created in maximum recovery diluent (Oxoid) and heat treated (80°C for 20 min) to kill vegetative cells. Spores were enumerated by viable counts, and the suspensions were adjusted to 10^6 spores per ml. Mixed inocula were prepared by combining spore suspensions in equal concentrations. Spores were inoculated into the mashed potatoes to give a predicted level of 10^3 CFU/g.

Nisin. Nisin was added to the experimental samples in the form of Nisaplin, a commercial preparation of nisin A (Danisco, Beaminster, Dorset, UK). Nisaplin has a standard activity of 1 × 10^6 IU/g. Pure nisin has an activity of approximately 4 × 10^6 IU/g. Throughout this paper, nisin measurements are given in units of pure nisin (µg/g). Where applicable, the equivalent activity units (IU/g) or Nisaplin levels (mg/kg) are given. For example, 12.5 µg of pure nisin per g is equivalent to 500 mg of Nisaplin per kg and 500 IU/g.

Preparation of mashed potatoes. The mashed potatoes comprised (wt/wt) 80% potatoes (Wilja or Cypress), 14% skimmed milk (prepared as 2% solution with dried skimmed milk powder), 5% unsalted butter, 0.75% sodium chloride (BDH, Poole, UK), 0.2% onion powder (Schwartz, Aylesbury, UK), and 0.075% black pepper (Millstone, Bracknell, UK, or Creative Cuisine, Bury St. Edmunds, UK). All ingredients were purchased from local retail outlets. All ingredients were microbiologically analyzed by viable count enumeration on a range of agar (milk plate count agar, reinforced clostridial agar, and deMan Rogosa Sharpe agar [Oxoid]) before and after heat treatment. In this manner, the total viable cell and spore counts for contaminating aerobic, anaerobic, and lactic acid bacteria were obtained.

The potatoes (2.5 kg) were washed in cold water, peeled, and cut into 1-cm³ cubes. They were then steamed in a vegetable steamer for 30 min, allowed to cool, and weighed into a sterile container and the other ingredients were added to produce 2 kg of mashed potatoes. The ingredients were mixed and then separated into four portions of 450 g, and the appropriate quantity of nisin, dissolved in 5 ml milk, was added to each portion. Next, the mashed potatoes were stomached for 1 min in a Colworth stomacher (Seward, London, UK). The four portions were then inoculated with a Bacillus or Clostridium spore cocktail to give a final level of approximately 10^6 spores per g. The portions were stomached for a further minute and then distributed as 15-g portions into vacuum bags (The Vacuum Pouch Company, Printpack Europe Ltd., Bury, UK). Samples inoculated with Clostridium spores were vacuum packed at 1.5 kPa with a Multivac vacuum-packing machine (Multivac UK Ltd., Swindon, UK), heated in a water bath for 10 min at 80°C, and cooled in a water bath (Grant Instrument Cambridge Ltd., Cambridge, UK) at ambient temperature for 10 min. Samples inoculated with Bacillus strains were similarly processed but were not vacuum packed. The potato samples were then incubated at 8, 25, or 30°C and sampled at regular intervals. Sampling was carried out with the dilution of the total contents of a single bag.

Sample analysis. The analysis of the samples consisted of the enumeration of total aerobic bacteria at 30°C and the enumeration of total anaerobic bacteria anaerobically at 37°C on reinforced clostridial agar. For initial analysis only, the determination of the total aerobic spore count (on milk plate count agar after heat treatment of the sample at 80°C for 20 min) and the anaerobic spore count (on reinforced clostridial agar after heat treatment of the sample at 80°C for 20 min) was undertaken. The minimum detection level was 100 CFU/g. The pHs of the samples were recorded at sampling time, and the nisin concentrations in the mashed potatoes were assayed initially and after incubation. These assays were carried out by the plate diffusion procedure using Micrococcus luteus NCIB8166 as the test organism according to the method described by Fowler et al. (14).

RESULTS

Investigation of nisin control of Clostridium spoilage in mashed potatoes. For the first experiment (experiment 1), nisin was added at 0, 12.5, and 25 µg/g and potato samples were incubated at 25°C. The raw ingredients (excluding potatoes) were found to be of satisfactory bacteriological quality. The total bacterial count of the salt and black pepper was <10^3 CFU/g. The milk contained Bacillus (1.6 × 10^2 CFU/g), and the butter contained lactic acid bacteria (7.0 × 10^5 CFU/g). The onion powder contained gram-positive bacteria consisting mostly of Bacillus spores (3.1 × 10^4 CFU/g). Analysis of the nisin in the samples showed no substantial drop in levels as a result of the pasteurization heat treatment. After 40 days of incubation at
TABLE 1. Initial and final nisin levels in vacuum-packed mashed potatoes

<table>
<thead>
<tr>
<th>Initial nisin level</th>
<th>Experiment 1</th>
<th>Experiment 3</th>
<th>Experiment 4</th>
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<tr>
<td>µg/g</td>
<td>IU/g</td>
<td>µg/g</td>
<td>µg/g</td>
</tr>
<tr>
<td>6.25</td>
<td>250</td>
<td>260 (104)</td>
<td>178 (71)</td>
</tr>
<tr>
<td>12.5</td>
<td>500</td>
<td>490 (98)</td>
<td>340 (68)</td>
</tr>
<tr>
<td>25</td>
<td>1,000</td>
<td>960 (96)</td>
<td>610 (61)</td>
</tr>
</tbody>
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Nisin level (IU/g) for:
- Experiment 1: Initially After 40 days at 25°C
- Experiment 3: Initially After 42 days at 8°C and 26 days at 20°C
- Experiment 4: Initially After 59 days at 8°C

<table>
<thead>
<tr>
<th>Nisin level (IU/g) for*:</th>
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<tbody>
<tr>
<td>6.25</td>
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<td>12.5</td>
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* Percentages are shown in parentheses.

25°C, the nisin levels had dropped, but in most samples these levels remained at effective concentrations (Table 1).

The initial aerobic spore level in the samples was below the detectable level (10² spores per g). The anaerobic spore count was approximately 10³ spores per g. The initial pH was approximately 5.9. The pH fell to 5.4 to 5.6 in control samples after 12 days of incubation, whereas all of the nisin samples had pHs of approximately 5.8 after 33 days of incubation. The results for anaerobic viable counts at 25°C are shown in Table 1. No significant *Clostridium* growth occurred in potato samples containing nisin over the course of the experiment. After only 2 days, control samples had counts of >10⁷ CFU/g and showed visible evidence of spoilage. The main spoilage organism was *Bacillus*, which was a natural contaminant of the mashed potatoes and had not been inoculated. There were no detectable aerobic counts for mashed potato samples containing nisin during the experimental period, except at 15 days for the sample containing 12.5 µg of nisin per g. A count of 5.0 × 10⁴ CFU of *Bacillus* per g was recorded after 15 days of incubation. Upon subsequent sampling, counts were found to be at their previous low levels. It was presumed that this aberrant result could have been due to poor mixing either of the ingredients (resulting in a localized high concentration of spores) or of the nisin (resulting in a localized area of low nisin concentration). Therefore, this point was omitted from Figure 1.

Due to the successful results obtained above, the efficacy of a lower level of nisin (6.25 µg/g) was investigated in a repeat experiment (experiment 2). Samples were prepared as before, inoculated with a *Clostridium* cocktail, and incubated at 25°C. A bioassay of the initial samples found 68 to 76% of the initial nisin level. After 61 days at 25°C, the nisin levels had dropped to 49 IU/g (20% of the original level, which was equivalent to 250 IU/g). The initial pH of the potatoes was 5.73, and the pH dropped to 5.21 after 6 days of incubation in the control samples not containing nisin. After 61 days at 25°C, the pH of the nisin samples was 5.62. After 3 days of incubation, bacterial counts (total aerobic viable counts and total anaerobic viable counts) for control samples not containing nisin reached >10⁸ CFU/g. Spoilage was clearly evidenced by the production of gas and free liquid within the bags and a color change from cream to yellow-orange. The distinctive odor of *C. sporogenes* was very evident. After 61 days at 25°C there was no evidence of growth in the nisin samples; total aerobic viable counts were <10² CFU/g, and total anaerobic viable counts remained at approximately 10³ CFU/g.

A further *Clostridium* experiment (experiment 3) was set up to investigate spoilage at 8°C. Nisin levels before and after incubation are shown in Table 1. The onion powder contained approximately 10⁴ spores per g. There was no detectable growth of the inoculated *Clostridium* strains after 39 days at 8°C, although natural *Bacillus* species had grown to a level of 10⁶ CFU/g by this time (aerobic viable counts are shown in Fig. 2A, and anaerobic viable counts are shown in Fig. 2B). On day 39, the incubation temperature of all of the samples was raised to 20°C, whereupon the *Clostridium* strains grew to a level of 10⁸ CFU/g after 9 days in control samples not containing nisin (Fig. 2B). After 35 days of incubation at the higher temperature, the growth of the inoculated *Clostridium* and that of the natural *Bacillus* species were controlled by nisin at all levels tested.

FIGURE 1. Growth of *Clostridium* strains in cooked mashed potatoes incubated at 25°C. Total anaerobic viable counts for samples containing nisin at (O) 0 µg/g, (▲) 12.5 µg/g, and (■) 25 µg/g are shown. Counts below the minimum detection level of 100 CFU/g are indicated by the vertical arrow.
Investigation of nisin control of Bacillus spoilage in mashed potatoes. For experiment 4, nisin was added at 0, 6.25, 12.5, and 25 μg/g and potato samples were incubated at 8 and 25°C. Analysis of the nisin in the samples after the pasteurization heat treatment showed satisfactory levels, and in most cases these levels had not fallen greatly after incubation at 8°C for 59 days (Table 1). Anaerobic and aerobic spore counts at the start of the experiment ranged from undetectable levels to a maximum of 9.0 × 10^2 spores per g. The initial pH was approximately 5.7. After 3 days at 25°C, control samples not containing nisin had total bacterial counts of >10^9 CFU/g. For mashed potato samples containing 25 μg of nisin per g incubated at 25°C, counts ranged from undetectable to a maximum of 10^3 CFU/g for the 45-day period of the experiment. The 17-day sample containing 6.25 μg of nisin per g contained 10^5 CFU/g, although counts for subsequent samples collected for 7 days after this point with the same nisin level had counts of only 10^2 CFU/g. At this point, sampling for the 6.25-μg/g level was discontinued. It was postulated, as before, that poor mixing was the cause of this apparently spurious result. The high spore count for the onion powder suggested that inadequate mixing of this ingredient could cause a localized high spore concentration.

Growth in control samples incubated at 8°C was slower, but counts reached 10^6 CFU/g at 12 days, peaking at >10^7 CFU/g after 18 days of incubation (Fig. 3). Sampling for all nisin-containing samples continued for another 27 days. At all nisin levels (6.25, 12.5, and 25 μg/g), counts were generally undetectable for the 45-day incubation period; a maximum level of 10^3 CFU/g was recorded.

DISCUSSION

The experiments presented here demonstrated the efficacy of nisin, even at low levels (6.25 μg/g), in controlling the growth of Bacillus and Clostridium spores in cooked mashed potatoes stored at refrigeration and ambient temperatures. Analysis of the ingredients showed that onion powder and spices in particular may be a significant source of contamination. These ingredients may have been responsible for the few sporadic spoiled samples observed in the present study. To ensure good nisin efficacy, it is always recommended that the preservative be homogeneously mixed throughout the matrix of the food system. Food manufacturers should also ensure that their ingredients are of good bacteriological quality and are also mixed thoroughly to ensure that there is no concentrated locus of contamination. Nisin is an effective preservative in numerous types of heat-treated foods, including a wide range of pasteurized products and canned vegetables (11, 32). Studies such as that of Penna and Moraes (24) have also demonstrated the
effectiveness of nisin in reducing the thermal resistance of gram-positive bacterial spores. Between 80 and 100°C, a 40% mean reduction in the \( D \)-values of \( B. \) cereus in milk due to the addition of nisin (25 \( \mu \)g/ml) was reported. A recent review has suggested that the shelf life and safety of potato cakes, a cooked potato product similar to mashed potatoes, could be increased by nisin (31). These baked foods have higher moisture contents than crumpets, which have been implicated in outbreaks due to the growth of \( B. \) cereus in the warm climate of Australia (19, 22). This problem has been solved by the addition of nisin to crumpet dough before baking (16).

Nisin is authorized for use in several countries; some of these countries allow general nisin usage (32). The U.S. Food and Drug Administration has affirmed that nisin is generally recognized as safe, and broader nisin usage has recently been allowed in this country by a process of self-affirmation (13). The results of the present study indicate that nisin could extend the shelf life of cooked mashed potato products and confer an additional safety measure, protecting the consumer against the possibility of food poisoning caused by heat-resistant bacterial pathogens.

REFERENCES


