The Use of Nisin as a Preservative in Crumpets

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ABSTRACT

Crumpets, a high moisture flour based product, have been implicated in food poisoning due to growth and toxin production by naturally occurring Bacillus cereus during 5-day storage at ambient temperature. Bacillus cereus isolates from untreated crumpets at the end of their shelf-life were shown to be sensitive to nisin. Addition of nisin to the batter at levels of 3.75 μg/g and above effectively prevented the growth to levels capable of causing food poisoning. The fate of nisin during the production and shelf-life of the crumpet was determined.

Key Words: Bacillus cereus, bacteriocin, nisin, preservative, baked food.

Crumpets are high moisture, flour-based products that are particularly popular in the United Kingdom and Australia. They are produced on a hot plate from flour batter and contain yeast, an aerating agent or both to give them a raised profile and open texture. They are cooked on the griddle to cook in a mold for 3 to 5 min then cooling and packing. During cooking carbon dioxide escapes, leaving an open aerated texture. They are reheated on a grill or in the front of a fire, and eaten hot (2). Crumpets have a non-acid pH (6 to 8), high moisture (48 to 54%) and high water activity (a_w) (0.95 to 0.97). Consequently, they have a short shelf-life of usually under 6 days. Although some manufacturers suggest that the product should be kept refrigerated such practice would be costly, limit market opportunities and be unacceptable to many retailers. The product is therefore traditionally stored at ambient temperatures.

Spoilage of crumpets, due to gas-producing bacteria (14) and molds (15), has been observed and attempts have been made to improve shelf-life stability by modified atmosphere packaging (14) or combinations of modified atmosphere, pH, storage temperature and sorbate (15). No attention has been given to the prevention of spoilage, due to Bacillus sp. There have been a number of outbreaks of food poisoning due to the growth of B. cereus in crumpets or similar products (12,13). Bacillus cereus causes two distinct types of food poisoning characterized either by diarrhea and abdominal pain, or by nausea and vomiting. The first type occurs 8 to 16 h and the second type 1 to 5 h after ingesting contaminated food. In most instances large numbers of B. cereus cells have been isolated from the foods implicated in food poisoning incidents (11).

Flour used in the manufacture of crumpets will invariably contain a low number of B. cereus spores (4,7,10). In the cooking process the bottom of the crumpet receives a high heat treatment but the rest of the crumpet receives a lower heat treatment, which Bacillus spores easily survive. Thus, at the time of production crumpets will routinely contain a low level of B. cereus spores. During the 3 to 5-day shelf-life of the product the levels of B. cereus may increase from undetectable levels to greater than 10^7/g, which can be sufficient to cause food poisoning (11).

This work describes the evaluation of nisin as a preservative for use in crumpets to control the growth of B. cereus. Nisin is a bacteriocin produced by Lactococcus lactis and has been widely used as a food preservative for many years. It is accepted as safe for food use in many countries. Nisin has generally regarded as safe (GRAS) status in the United States and is allowed in pasteurized processed cheese spreads. It is active against gram-positive bacteria particularly those that produce spores, e.g., Bacillus and Clostridium spp. Foods in which it is used as a preservative include pasteurized processed cheese products and canned foods where there is a need to control the outgrowth of bacterial spores (3,5,9). Work carried out to evaluate the effectiveness of nisin as a preservative in crumpets involved sensitivity testing of B. cereus isolates found in crumpets, determination of the fate of nisin added to crumpet batter and determination of an effective addition level to control B. cereus.

MATERIALS AND METHODS

The nisin used in the study was the commercial preparation, Nisaplin. This preparation contains 25 mg (1 x 10^6 IU) nisin/g.

Isolation and enumeration.

Bacillus cereus was isolated or enumerated in crumpets using the method of Hollbrook and Anderson (8). For calculation of the statistics, counts of less than 5.0 x 10^2/g were treated as 5.0 x 10^2/g and counts of greater than 1 x 10^2/g were treated as 1 x 10^2/g.
Nisin sensitivity testing.

Spores of *B. cereus* cultures isolated from crumpets were tested for their sensitivity to nisin. Isolates were grown in nutrient broth for 72 h and then heated to 80°C for 10 min in order to prepare a spore suspension. The spore suspension was diluted in sterile Ringers solution and 0.1 ml of each dilution was used to inoculate nutrient broth. Nisaplin was suspended in sterile water and added to broth cultures to give 0, 1.25, 2.5, 6.25 and 12.5 µg nisin/ml. Each isolate was tested with several levels of spores and nisin. The broth cultures were incubated at 37°C for 7 days and the presence or absence of growth noted. The control tube containing no nisin was used to estimate the number of spores added to each tube. The highest dilution for which growth was noted was deemed to have 10 spores/ml and the spore numbers in other tubes were calculated on this basis.

Nisin assay.

Samples of batter and crumpet were assayed for nisin by the horizontal agar plate diffusion method using *Micrococcus luteus* as the indicator organism (6). For assay, a 10 g disc was cut through the depth of the crumpet.

Laboratory preparation of crumpets.

Crumpet batter was supplied by the manufacturer. The batter was dispensed in 500 g portions and nisin was added at levels of 1.25, 2.5 and 4.5 µg nisin/g batter. Batters were blended in a Waring Blender™ for 30 s. Crumpets were baked from each batch of batter using a domestic electric frying pan. Each crumpet was made from 25 ml of batter poured into an egg ring and cooked for 3 min with the lid on the frying pan.

Moisture determination.

Moisture levels of crumpet batter and crumpets were determined so that nisin assay figures could be corrected for moisture loss and to indicate that crumpets were produced in a uniform manner with respect to the heat treatment received. Moisture levels were determined gravimetrically using an oven at 100°C and drying to constant weight.

Stability of nisin in batter and crumpets.

Nisin was added to batter at a level of 3.75 µg/g. Factory crumpets and batter were assayed as soon as possible after manufacture and at the end of shelf-life. Samples were collected, refrigerated overnight, assayed then stored at room (20 to 25°C) temperature for 5 days before repeating the assay on a crumpet from the same packet.

Commercial crumpet production.

Batter (800 kg) was produced using plain flour, salt, a leavening compound and water at 60°C. After mixing, the batter was dispensed onto a gas heated hot plate at 176°C where it remained for about 3 min. The cooking was sufficient to set the batter. In the last stage of cooking, a heating bar above the crumpet removed excess moisture from its surface. After cooking, the crumpets were cooled for about 20 min at 4°C before packing into wrappers.

Addition of nisin.

Nisin, as Nisaplin, was suspended in a litre of water and added to the batter before the major solid constituent (flour) to ensure good mixing. Nisin was added to give the stated concentration, without taking into account moisture losses or nisin losses during cooking.

Trial protocol.

Four batches of crumpets were produced before the trial commenced. The last of these was designated a control batch containing no nisin. The next four batches were produced with nisin concentrations of 1.25, 2.5, 3.75 and 5 µg nisin/g. A further control batch was produced after the nisin containing batches. Two packets were removed every two minutes midway through processing of each batch until eight packs were sampled. For the control samples post nisin treatment 14 packs were sampled.

Crumpet storage and testing.

Crumpets were stored at room temperature (20 to 25°C) until the 5th day and then were tested quantitatively for *B. cereus*.

RESULTS AND DISCUSSION

Nisin sensitivity of *B. cereus* isolated from crumpets.

Five isolates of *B. cereus* were selected from naturally contaminated crumpets 5 days after they were produced. Nisin sensitivity test results are presented in Table 1. The *B. cereus* spores were found to be sensitive to nisin at the levels tested with sensitivity of each isolate appearing to be similar. The greater minimum inhibitory concentration seen with higher spore levels is consistent with the results of other workers (5). The level of spores used in the test was consistent with those found in crumpets.

Nisin assay.

A standard solution of nisin was added to batter and precooked crumpets containing no nisin at various levels directly before assay (Table 2). The data indicate that some of the nisin becomes bound to a component of the batter and crumpet in such a way that it becomes undetectable in the assay. In batter, 24% of the nisin added became undetectable and in the crumpet 41% of the nisin added became undetectable by the assay. These results suggest that a moderately high level of nisin addition to batter would be necessary to attain a concentration in excess of the minimal inhibitory concentration in a freshly produced crumpet.

| TABLE 1. Nisin sensitivity of *B. cereus* spores. |
|-----------------------------|-----------------------------|
|                              | Minimal inhibitory concentration of nisin (µg/ml) |
|                              | Number of spores/ml          |
| Isolate | 10 | 10² | 10³ | 10⁴ | 10⁵ |
| 1       | 1.25 |    |    |    |     |
| 2       | 1.25 | 1.25 |    |    |     |
| 3       | 1.25 | 1.25 | 1.25 |    |     |
| 4       | 1.25 | 1.25 | 1.25 | 2.5 | 6.25 |
| 5       | 1.25 | 1.25 |    |    |     |

The blank areas of the table indicate that spore suspensions were not sufficiently dense to inoculate broth with that level of spores.

| TABLE 2. Retention of nisin in crumpets and batter. |
|-----------------------------|-----------------------------|
| Level of Nisin Added to Sample | Residual Nisin |
|                              | Batter | Crumpets |
| µg/g | µg/g | % recovery | µg/g | % recovery |
| 1.25 | 0.93 | 74.4 | 0.81 | 64.8 |
| 2.5 | 2.06 | 82.4 | 1.51 | 60.4 |
| 4.5 | 3.28 | 72.9 | 2.32 | 51.6 |
Stability of nisin in batter and crumpets.

The nisin content of the batter assayed on the first day was 4.6 µg/g and on the 5th day was less than 0.25 µg/g. This represents a loss of greater than 95% over 5 days. Crumpets with an initial residual nisin content of 2.36 µg/g had 0.75 µg/g remaining after 5 days. This represents a loss of 69%. At the normal storage temperature of crumpets, the level of nisin is reduced over the 5-day shelf-life. Nisin is almost completely degraded in the batter under room storage conditions, probably due to the high nisinase activity of contaminant organisms.

During cooking, the bottom of a crumpet receives more intense heating than the top. Residual nisin in the top layer of a laboratory produced crumpet was found to be 1.63 µg/g whereas the bottom layer contained only 0.63 µg/g. The additional heating of the bottom appears to significantly reduce the residual nisin level.

Effect of nisin concentration on the control of B. cereus in commercial crumpets.

_Bacillus cereus_ counts in the crumpets after cooking prior to storage were low with levels of 50/g or less than 50/g. Eight crumpets each from different packs were analyzed for _B. cereus_ content after 5 days' storage at room temperature. Geometric mean counts were calculated and expressed as a histogram (Fig. 1). Thus, control samples before and after nisin treatments showed growth of _B. cereus_ to average levels of $2.5 \times 10^6$ and $1.2 \times 10^6$/g, respectively. A number of the control crumpets had counts of greater than $10^7$/g which is significantly above the level at day 5 significantly (t-test, p 0.05) and to a level that would not cause food poisoning. A level of 5 µg/g was not significantly more effective than 3.75 µg/g. No crumpet after 5 days' storage treated with 3.75 and 5 µg/g nisin had a _B. cereus_ count of greater than $5.4 \times 10^6$/g. From these results it may be concluded that the use of nisin at a level of 3.75 µg/g appears to be the minimum level, which results in a significant inhibitory effect on the growth of _B. cereus_.

More extensive trials were performed using 3.75 µg/g nisin. Crumpets were produced for several days with half of the production receiving nisin treatment. Seventy control and the same number of treated crumpets were tested. Of the treated crumpets, 93% were found to contain less than 100 _B. cereus/_g on day 5, whereas 55% of untreated crumpets contained $10^7$/g or greater after the same time. Furthermore, similar studies have been carried out in wholemeal crumpets, pikelets and flapjacks with similar results. A higher level of nisin of 6.25 µg/g was found to be required in wholemeal crumpets whereas nisin levels of 3.75 µg/g were found to be effective in both pikelets and flapjacks. The higher level required in wholemeal crumpets is due mainly to higher spore levels in wholemeal flour (4) or possibly due to an interaction between nisin and wholemeal flour.

This is the first time the use of nisin in a flour-based product has been reported. Additionally, we have shown that nisin may be effective when used alone against _B. cereus_. The work described here resulted in regulations in Australia allowing the use of nisin in crumpets and similar high moisture flour based products (1).

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REFERENCES


Figure 1. Effect of nisin concentration on presumptive _B. cereus_ per gram at day 5. Bars indicate the standard deviation from the mean.


