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Nisin: a Possible Alternative or Adjunct to Nitrite in the Preservation of Meats

M. K. RAYMAN,* B. ARIS, AND A. HURST

Bureau of Microbial Hazards, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario K1A 0L2, Canada

Nisin at 75 ppm (75 μg/g) was superior to 150 ppm of nitrite in inhibiting outgrowth of *Clostridium sporogenes* PA3679 spores in meat slurries, which had been heated to simulate the process used for cooked ham. The inhibitory activity of nisin decreased as the spore load or pH of the slurries increased. Unlike nitrite, inhibition by nisin was unaffected by high levels of iron either as a constituent of meats or when added as an iron salt. In slurries treated with 75 ppm of nisin, refrigerated storage for 56 days resulted in depletion of nisin to a level low enough to allow outgrowth within 3 to 10 days if the slurries were subsequently abused at 35°C. In contrast, a combination of 40 ppm of nitrite and either 75 or 100 ppm of nisin almost completely inhibited outgrowth in these slurries. The nisin-nitrite combination appeared to have a synergistic effect, and the low concentration of nitrite was sufficient to preserve the color in meats similar to that of products cured with 150 ppm of nitrite.

Over the past decade, much attention has been focused on the presence in foods of *N*-nitrosamines, many of which have been shown to be carcinogenic (19, 20; P. Issenberg, Fed. Proc. **35**:1322, 1976). These compounds are formed by reaction of naturally occurring amines with nitrites that are either added to foods or are produced by bacterial reduction of nitrates.

Although nitrites are of little concern as a source of nitrosamines in meats, except in fried bacon (Nitrite Safety Council, Food Technol. **34**:45, 1980), there is intent to eliminate or reduce its use in foods, as soon as a suitable alternative is found that would afford as good or better protection against botulism as traditional nitrite cures.

Among the alternative preservative methods which have shown promise are the use of *Lactobacillus* cultures to reduce the pH of the product to a safe level (3, 4, 21, 25, 28), use of α-tocopherol in conjunction with ascorbate and low levels of nitrite (23), use of sorbic acid or sorbate with and without low levels of nitrite (14, 26, 27, 32), use of sulfur dioxide (31), and irradiation (5, 24; A. Brynjolfsson, Tech. Rep., Natick/TR-79/022, U. S. Army, Natick Research and Development Command, Natick, Mass., 1979; F. J. Ley, Tech. paper no. 16, Atomic Energy Canada Ltd. seminar, 1980).

Another possible alternative to nitrite in food preservation is nisin. It is a polypeptide antibiotic produced by group N lactic streptococci. Nisin is unique in that it is the only antibiotic presently used as a food preservative. It was used in foods for the first time in 1951 to prevent "blowing" of Swiss-type cheese caused by *Clostridium butyricum* (12). *Bacillus* and *Clostridium* spores are sensitive to nisin (17), and the sensitivity appears to be more pronounced if the spores are heated (1, 11, 22). Nisin is used extensively in European and other countries as a preservative in dairy products, vegetables, soups and sauces (13, 15, 16); it has also been used in semipreserved meats but with limited success (2, 9).

We now report on the preservative effect of nisin in meat slurries inoculated with *Clostridium sporogenes* PA3679. These experiments indicate that the use of nisin may permit reduction in the current levels of nitrite without affecting the safety of the food.

MATERIALS AND METHODS

Organism. *C. sporogenes* PA3679 was used throughout this study. Spores of the organism were prepared by the method of Uehara et al. (35). Spores were enumerated on liver veal (LV) agar after they were heat shocked at 80°C for 15 min.

Preparation of meat slurries. Fresh meats purchased from local supermarkets were minced or cut up into small pieces and blended with NaCl solution so that the final concentration of NaCl was 2%. All additives to the slurry, such as spores, nisin, or nitrite, were made before blending. Nisin was added in the form of Nisaplin (Aplin and Barrett, Ltd., Throwbridge, Wiltshire, England), a commercial product containing 2.5% pure nisin in skim milk powder. Nisaplin contains 10^6 IU/g.

Processing conditions. Meat slurries (approximately 10 ml) were pipetted into test tubes (16 by 150

mm), taking care to avoid trapping of air bubbles. Unless otherwise stated, the tubes were placed in a water bath at 40°C which was allowed to rise to 70°C. The time to reach 70°C was 2.5 to 3 h. This time-temperature combination simulated that used for processing of cooked ham. The tubes were then removed from the water bath, chilled in ice-cold water, and capped with a 1 cm layer of Vaspar (two parts of melted paraffin wax to one part of mineral oil).

Abuse conditions. Meat slurries were abused either immediately after heat processing or after storage at 4°C for 56 days. In either case, tubes containing the slurry were incubated at 35°C and examined at daily intervals over 56 days for growth, as evidenced by gas production.

Nisin assay. Nisin concentrations in meat slurries were determined by the plate diffusion method of Tramer and Fowler (34), using *Micrococcus flavus* as the assay organism. Nisin standards, prepared from Nisaplin, were run with each assay.

RESULTS AND DISCUSSION

Effect of pH on nisin activity. It is known that the solubility and stability of nisin decreases as the pH increases and that the effectiveness of nisin diminishes at high pH (13, 33). To investigate this effect on outgrowth of *C. sporogenes* spores, preliminary experiments were done in which nisin was added at 5 IU (0.8 ppm [0.8 μg/g])/ml of LV agar which had been adjusted to different pH values. Approximately 10^9 *C. sporogenes* spores heated for 40 min at 92°C were enumerated on the nisin-containing LV agar and on LV agar without nisin as a control. Figure 1 shows that as the pH of LV agar increased, the effectiveness of nisin in preventing outgrowth of spores decreased in an almost linear fashion. In further preliminary experiments, we determined that approximately 75 ppm of nisin was sufficient to prevent outgrowth of 10^3 *C. sporogenes* spores per ml of pork slurry (pH 6.0) containing 2% NaCl and processed to an internal temperature of 70°C (data not shown). This concentration of nisin and the above conditions were therefore used to determine the effect of pH on inhibition of outgrowth of spores in pork slurries. For comparison, slurries containing 150 ppm of nitrite were prepared and processed in a similar manner. All tubes of slurry were abused by placing them in an incubator at 35°C for up to 56 days. Table 1 shows that no obvious growth occurred in the nisin-treated slurries up to pH 6.5. However, in the slurry adjusted to pH 6.6, the preservative effect of nisin was lost. This phenomenon is probably more gradual than the data indicate. In contrast, 150 ppm of nitrite prevented outgrowth in slurries adjusted up to pH 6.0, but above this pH value, outgrowth was evident in some or all of the tubes in the trial. Growth occurred in all control tubes which contained neither nisin nor nitrite.

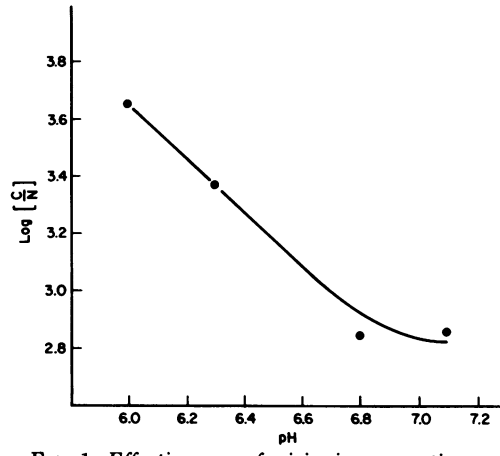

FIG. 1. *Effectiveness of nisin in preventing outgrowth of C. sporogenes PA3679 spores on LV agar at different pH values. Abbreviations: C, counts per milliliter on LV agar; N, counts per milliliter on nisin-containing LV agar.*

TABLE 1. *Effect of pH on ability of nisin or nitrite to inhibit outgrowth of C. sporogenes spores in pork slurries*[a]

pH of pork slurry	No. of growth-positive tubes/no. of tubes in trial	
	Nisin[b]	Nitrite[c]
5.7	0/5	0/5
5.8	0/10	0/10
6.0	0/10	0/10
6.1	0/5	2/5
6.3	0/10	8/10
6.4	0/10	3/10
6.5	0/10	10/10
6.6	10/10	10/10

[a] Tubes of pork slurry containing 2% NaCl were inoculated with about 10^3 spores per g, heated to 70°C, and incubated at 37°C for 56 days. All control tubes containing inoculum but no nisin or nitrite showed growth.

[b] Nisin was used at a final concentration of 75 ppm (3,000 IU/ml).

[c] Nitrite was used at a final concentration of 150 ppm.

TABLE 2. *Effect of spore load on ability of nisin to prevent outgrowth of C. sporogenes spores in pork slurries*[a]

Nisin concn (ppm)	No. of growth-positive tubes/no. of tubes in trial at spore load (per g of pork slurry) of:				
	10^6	10^5	10^4	10^3	10^2
75	10/10	6/10	1/10	ND[b]	ND
50	10/10	6/10	1/10	ND	ND
25	ND	ND	5/10	2/10	0/10

[a] Conditions were as in Table 1 except that the inoculum was varied.

[b] ND, Not determined.

Effect of spore load on nisin activity. Results of Table 2 show that the effectiveness of nisin in preventing outgrowth decreased with increasing spore load in pork slurries. These results are similar to those obtained by others (6, 7). This inverse relationship between inhibition by nisin and spore load, and the observation by Gibbs and Hurst (9) that nisin is ineffective in preventing spoilage when raw materials of inferior quality are used, should dismiss any concern about the use of nisin as a cover-up for poor manufacturing practices.

Nisin in different meats. The inhibitory effect of nisin on outgrowth of *C. sporogenes* spores in different meats is shown in Table 3. Over a 56-day incubation period, nisin inhibited growth in all but dark turkey meat. We have no explanation for this; pH could not be the reason because growth in minced pork at a similar pH was completely inhibited. Nitrite at 150 ppm performed poorly in all but white turkey meat and minced pork. Tompkin et al. (29, 30) showed reduced effectiveness of nitrite in preventing outgrowth of *C. botulinum* spores in heart muscle which contains a relatively high level of iron. They proposed that iron in these meats competed for nitrite, making it unavailable to interact with some vital iron containing protein(s) in the bacterial cell. If nitrite does inhibit outgrowth in the manner proposed by Tompkin et al. (30), then inhibition by nisin seems to be by a different mechanism, because it was equally effective in preventing outgrowth of spores in meats containing both low and high levels of iron.

The ineffectiveness of iron in reducing the inhibitory capacity of nisin is further demonstrated by the results in Table 4. Addition of 75 ppm of Fe^{2+} to pork slurries had no effect on nisin, but inhibition by nitrite was abolished.

Processing temperature and nisin inhibition. Pork slurries prepared at two pH values with 75 ppm of nisin and 2% NaCl were inoculated with approximately 10^3 heat-shocked spores per ml (80°C for 15 min) and incubated at 35°C either without processing or after processing to various temperatures. For comparison, similar experiments were performed with 150 ppm of nitrite. Neither nisin nor nitrite prevented outgrowth in slurries processed to 50°C or without heating; only partial inhibition of outgrowth was achieved with nisin at a processing temperature of 60°C, and as shown in previous experiments, complete inhibition was obtained at 70°C (Table 5). With nitrite, only partial inhibition of outgrowth was attained even at 70°C. These results confirm that 75 ppm of nisin is superior to 150 ppm of nitrite in preventing outgrowth of *C. sporogenes* spores, but they also show that lowering of the processing temperature results in reduced inhibition by nisin. The ability of nisin to completely inhibit outgrowth only in slurries processed to 70°C was not due to a die-off of spores. There was an 80%

TABLE 3. *Effect of nisin and nitrite on outgrowth of C. sporogenes spores in slurries of different meats*[a]

Meat type	No. of growth-positive tubes/no. of tubes in trial		
	pH	Nisin[b]	Nitrite[c]
Minced pork	6.4	0/10	3/10
Pork heart	6.1	0/10	10/10
Minced beef	5.8	0/10	10/10
Beef heart	6.0	0/10	10/10
White turkey meat	5.8	0/10	0/10
Dark turkey meat	6.4	10/10	10/10

[a] Conditions were as in Table 1.
[b] As in Table 1.
[c] As in Table 1.

TABLE 4. *Effect of iron on ability of nisin or nitrite to prevent outgrowth of C. sporogenes spores in pork slurries*[a]

Iron[b]	No. of growth-positive tubes/no. of tubes in trial	
	Nisin[c]	Nitrite[d]
−	0/10	4/10
+	0/10	10/10

[a] Conditions were as in Table 1.
[b] Final concentration of iron in the form of $FeSO_4$ was 75 ppm.
[c] As in Table 1.
[d] As in Table 1.

TABLE 5. *Effect of heat treatment on ability of nisin or nitrite to prevent outgrowth of C. sporogenes spores in pork slurries*[a]

pH of pork slurry	Maximum temp of treatment (°C)	Time to attain maximum temp (min)	No. of growth-positive tubes/no. of tubes in trial	
			Nisin[b]	Nitrite[c]
5.7	Room temp		5/5	5/5
	50	60	5/5	5/5
	60	120	3/5	5/5
	70	160	0/5	1/5
6.1	Room temp		5/5	5/5
	50	60	5/5	5/5
	60	120	1/5	5/5
	70	160	0/5	2/5

[a] Tubes of pork slurry containing 2% NaCl were inoculated with about 10^3 spores per g and heated to the desired temperature. All other conditions were the same as in Table 1.
[b] As in Table 1.
[c] As in Table 1.

reduction in viable spores to 2×10^2/ml as a result of heating to 70°C, but in pork slurries inoculated with this low level of spores, 75 ppm of nisin was also incapable of completely preventing outgrowth in slurries processed to 50°C (data not shown). It appears that heating of spores to a certain temperature is a prerequisite for inhibition by nisin. As early as 1962, Hawley noted that heated spores were more sensitive to nisin (10). It has been suggested that heating of spores with nisin causes sublethal damage, but the mechanism by which the damage occurs is unknown (A. Hurst, Adv. Appl. Microbiol., in press).

Depletion of nisin during refrigerated storage. Tubes containing slurries of pork or white turkey meat formulated with 2% NaCl and 75 ppm of nisin were inoculated with 10^3 spores per ml and processed to 70°C. One half of the tubes incubated immediately at 35°C showed no growth over a 56-day period, but the other half which was stored at 4°C for 56 days and then incubated at 35°C showed growth in most of the tubes within 3 to 10 days. These unexpected results were presumably due to the depletion of nisin during refrigerated storage. To test this hypothesis, we determined residual nisin in slurries stored at 4°C. Figure 2 is a typical plot of storage time against residual nisin. With an initial concentration of 100 ppm of nisin, the amount remaining after processing was 73 ppm, and from the slope of the line, the rate of depletion was calculated to be 1.2 ppm per day. From similar graphs, residual nisin was estimated in pork slurries with different treatments immediately after processing, and the rates of depletion were calculated (Table 6). Approximately 25 to 30% of added nisin is lost as a result of processing to 70°C, and the rate of depletion averaged about 1.1 ppm per day. There were negligible differences due to the presence or absence of nitrite or at the two pH values tested. Fowler and McCann (7) reported an 82 to 88% loss of nisin activity in canned chocolate milk stored at 4°C for 3 to 24 months, and Fowler (6) demonstrated a less than 15% loss of nisin in cheese processed at 80 to 95°C for up to 15 min and a decrease in nisin of 65 to 70% over 6 months of storage at 30°C. McClintock et al. (18) observed a 75% decline in nisin activity in processed cheese held at 37°C for 4 to 6 months, and Caserio et al. (2) reported a 57% decline in nisin content in frankfurters stored at 2 to 4°C for 28 days. The indications are that loss of nisin due to processing and the rate of depletion depends on the product and conditions of processing and storage. These parameters will have to be determined for each product before suitable nisin levels could be recommended.

Effect of nisin-nitrite combination in pork slurries refrigerated before abuse. We tested a combination of 40 ppm of nitrite and either 75 or 100 ppm of nisin to determine whether these treatments would be more effective than nisin alone in inhibiting outgrowth of spores in slurries which had been refrigerated before abuse. Nine and five tubes containing 75 and 100 ppm of nisin, respectively, showed growth within the 56-day incubation period, following storage at 4°C for 56 days (Table 7).

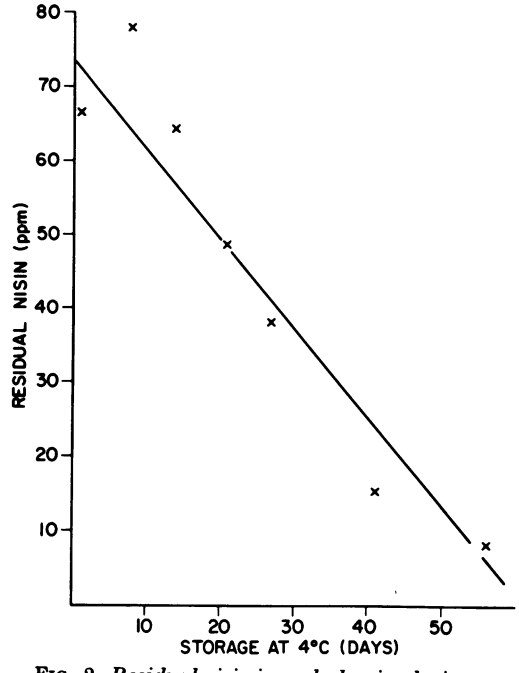

FIG. 2. *Residual nisin in pork slurries during storage at 4°C. The line was drawn by using the least-squares method.*

TABLE 6. *Residual nisin after processing and the rates of depletion in pork slurries stored for 56 days at 4°C*[a]

Composition of pork slurry			Residual nisin after processing (%)	Rate of nisin depletion (ppm/day)
Nisin (ppm)	Addition of 40 ppm of nitrite	pH		
100	−	6.3	73	1.2
100	+	6.3	76	1.0
100	−	5.8	73	0.9
100	+	5.8	69	0.8
150	−	6.3	67	1.1
150	+	6.3	70	1.3
150	−	5.8	79	1.4
150	+	5.8	75	1.2

[a] Conditions were as in Table 1, except that tubes were not incubated at 37°C.

TABLE 7. *Combined nisin-nitrite effect on outgrowth of C. sporogenes spores in pork slurries refrigerated before abuse*[a]

Nisin concn (ppm)	Nitrite added (ppm)	No. of growth-positive tubes/no. of tubes in trial
75	0	9/10
75	40	1/10
100	0	5/10
100	40	1/10
0	40	5/5
0	150	10/10

[a] Conditions of preparation were as in Table 1. Tubes were stored at 4°C for 56 days and then incubated at 25°C for 56 days.

Nitrite alone at 40 ppm or even at 150 ppm did not prevent outgrowth in any of the tubes. In contrast, only one tube in either of the combined nisin-nitrite treatments allowed outgrowth to occur. We have shown that nitrite did not affect the rate of depletion of nisin during storage. It must therefore be assumed that there is a synergistic effect of nisin and nitrite in preventing outgrowth of *C. sporogenes* spores. These results show that under the severe abuse conditions used in this work, 75 to 100 ppm of nisin alone was at least as good as 150 ppm of nitrite alone, and the combination of 75 ppm of nisin with 40 ppm of nitrite was much superior to 150 ppm of nitrite alone.

Nisin is nontoxic (8) and may be a useful preservative for some cured meats. Assuming that *C. botulinum* spores are as susceptible as those of *C. sporogenes*, then 75 to 100 ppm of nisin in combination with low levels of nitrite may be a suitable alternative to higher levels of nitrite in the preservation of meats which have received some degree of heat treatment. To confirm this assumption, we shall be continuing this line of study with *C. botulinum* spores.

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