A Research Note

Survival of *Yersinia enterocolitica* in Fermented Sausages Manufactured With Different Levels of Nitrite and Different Starter Cultures

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ABSTRACT

The ability of *Yersinia enterocolitica* 0:3 to grow and survive during the manufacture of fermented sausages made with 0, 50, 80, and 120 mg/kg added sodium nitrite and three different commercial starter cultures was determined. The sausage mass was inoculated to contain 1.7 x 10^5 of *Y. enterocolitica* per g. *Yersinia* was not detected after 28 d in sausages made with 80, 100, or 120 mg/kg of sodium nitrite. All sausages manufactured without or with 50 mg/kg sodium nitrite harbored *Yersinia* during the test period of 35 d. The highest level of *Yersinia* (5.9 log_{10} CFU/g) was detected in sausages made with no sodium nitrite and with *Pediococcus acidilactici* (C). In sausages made with *Lactobacillus pentosus* (A), the level of *Yersinia* was less than 2.0 log_{10} CFU/g. In sausages made with *Lactobacillus plantarum* (B) and 50 mg/kg sodium nitrite, the level of *Yersinia* was 2.9 log_{10} CFU/g. The pH values made with starters A, B, and C reached the pH values of 4.9, 5.2, and 5.4, respectively.

*Yersinia enterocolitica* is a ubiquitous bacterium which has been isolated from many foods and raw materials (1,3,4,7,9,11,16). It is obvious that pork plays an important role as a source of infection. Tauxe et al. (17) found a correlation between pork consumption and *Y. enterocolitica* infections. The virulent plasmids of both porcine and human isolates have also been reported to have identical restriction patterns (9,11,16). It is obvious that pork plays an important role as a source of infection. Tauxe et al. (17) found a correlation between pork consumption and *Y. enterocolitica* infections. The virulent plasmids of both porcine and human isolates have also been reported to have identical restriction patterns (9,11,16).

Preparation of the sausages

Sausages were prepared from frozen meat (beef 42 kg, cow 100 kg, pork and pork fat 64 kg). The meat was ground at 4°C in a Sedemmann cutter K41 (Stuttgart, Germany) to which spices and salt were added. In the second cutting glucose and ascorbic acid were mixed with the sausage mass. The prepared mass was then divided into 15-kg batches to which the resuspended starters and *Y. enterocolitica* were mixed with a Baku type 900 mixer (Hugo Kunzi, Stuttgart, Germany). The mass was divided into 3-kg batches to which sodium nitrite was added. After that the sausage masses were stuffed into 70-mm fibrous casings. The sausages (250-300 g) were tied by hand and then kept for 2 h at 24°C at the relative humidity (RH) of 80-85%. Preripening was carried out for 2 d at 23°C at a RH of 90-95%. During the next 5 d, sausages were smoked in the Autoterm (Waxweiler, Germany) at 18-20°C at a RH of 80-90%. The ripening was completed by keeping the sausages at 15°C and at 75-80% RH for 14 d. The prepared sausages were stored chilled at 10°C for another 14 d.

Yersinia enterocolitica strain

*Yersinia enterocolitica* NVIF 1016, serovar 0:3, biotype 4 was isolated from pig tonsils. It was biotyped by Wauters et al. (19) and serotyped by slide agglutination, Wauters (18). *Y. enterocolitica* was grown overnight in brain heart infusion broth (Merck) at 30°C and added to the sausage mix at 1.7 x 10^5 cells per g.

Starter cultures and food additives

Three different commercial freeze-dried starter cultures (Rudolf Müller & Co., Giessen, Germany) were used in preparing the sausages. The starter cultures were combinations of microorganisms as follows: A) *Lactobacillus pentosus* O 3a DSM No. 3402 and *Staphylococcus carnosus* DSM No. 1952 (Pentoferment 85); B) *Lactobacillus plantarum* L 74 DSM No. 154 and *S. carnosus* DSM No. 1952 (Duploferment 66); and C) *Pediococcus acidilactici* DSM No. 2536, *S. carnosus* DSM No. 1952, and *Streptomyces griseus*. Lactobacilli and *P. acidilactici* concentrations in sausages were 10^5 CFU/g.

All sausages had as food additives 3% NaCl, 0.03% ascorbic acid, 0.6% glucose, and 0.2% spice extract (wt/wt). The concentrations of added sodium nitrite (NaNO_2) were 0, 50, 80, 100, and 120 mg/kg.
Preparation of the samples and isolation of Yersinia enterocolitica

Y. enterocolitica was isolated both by direct plating on cesulfodin irgasan novobiocin agar (CIN, Merck) and by using method No. 117 of the Nordic Committee on Food Analysis (12) which was supplemented with KOH treatment (2) at the last enrichment step. One sausage from each combination of starter culture and level of nitrite was cut into two halves and examined separately as duplicate samples on days 1, 3, 7, 14, 28, and 35 after preparation. Samples (10 g) were diluted in sterile plastic bags (Steward Medical) containing 90 ml peptone solution (10 g peptone and 0.85 g NaCl in 1,000 ml distilled water). From each dilution and from each sample 10 g were transferred to 90 ml of phosphate sorbitol buffer (PSB) for enrichment. Diluted samples (0.1 ml) were plated on CIN agar. The PSB broths were tested by plating 0.1 ml of PSB broth after 3 h at 20°C and after 28 d at 4°C. After 8 d of incubation 0.1 ml of PSB broth was also transferred to modified Rappaport broth (Merck) without carbenicillin, which was then incubated at 20°C for 4 d and tested for Y. enterocolitica on CIN agar. Typical colonies were confirmed biochemically by cultivation into triple sugar iron agar (Oxoid) and on urea agar (BBL) and tested serologically.

Determination of pH value, water activity (a_w), and levels of nitrite (-N02) and nitrate (-N03)

The pH of the sausages was measured after being sampled for Y. enterocolitica examinations. At the same time samples were taken for a_w determinations which was carried out for 10 min using a Humicap HMP 31UT (Vaisala Oy, Helsinki, Finland) after equilibrating the samples at 20°C for 2 h. Nitrate and nitrite were determined spectrophotometrically according to the methods of the Finnish Standards Association-International Organization for Standardization (5,6).

RESULTS AND DISCUSSION

In our study Y. enterocolitica could not be detected after the 35-d monitoring period in sausages with a level of NaN02 of 80 mg/kg or more. All sausages made without NaN02 or with only 50 mg/kg NaN02 harbored Y. enterocolitica to the end of 35-d follow-up (Table 1). The levels of Y. enterocolitica in sausages A were less than 100 CFU/g. Sausages B without any nitrite or with 50 mg/kg sodium nitrite harbored Y. enterocolitica at 2.5 log10 CFU/g and 2.9 log10 CFU/g, respectively. Sausages C with no NaN02 harbored Y. enterocolitica at 5.9 log10 CFU/g. The difference between sausages can partly be explained with the growth temperatures of the lactic acid producers of the combinations used in this study. At the temperatures used, P. acidilactici (C) has a slower rate of souring than L. pentosus (A) and L. plantarum (B). The final pH levels were reached in all sausages between days 3 and 7. The pH values decreased during ripening in sausages made with L. pentosus (A) from 5.6 to 4.9, in sausages made with L. plantarum (B) from 5.9 to 5.2, and in sausages made with P. acidilactici (C) from 5.7 to 5.4. The a_w values decreased is sausages A, B, and C from 0.98 to 0.86, 0.86, and 0.85, respectively. Only small amounts of nitrate (<3.5 mg/kg) and nitrite (<28.0 mg/kg) were found in sausages after ripening for 3 d.

Earlier studies have shown that the addition of 150 mg/kg sodium nitrite to fermented sausages can decrease the population of Y. enterocolitica from 10^2-10^3 CFU/g to less than 10^2 CFU/g (15). Raccach and Henning (14) found that both 3% sodium chloride and 156 mg/kg sodium nitrite added to heated meat could control the growth of Y. enterocolitica 0.3 at 27°C better than the lactic acid bacteria alone. The best effect was achieved by combining curing and the use of lactic acid bacteria. They found P. acidilactici to be less inhibitory than L. plantarum at 27°C, but at 35°C they controlled the growth of Y. enterocolitica 0.3 equally. The results of our study show that even lower concentrations of sodium nitrite have an inhibitory effect on Y. enterocolitica. The addition of 80, 100, and 120 mg/kg sodium nitrite decreased Y. enterocolitica below the level of detection in fermented sausages.

In conclusion, this study shows that sodium nitrite can control Y. enterocolitica in fermented sausages at levels even lower than are currently permitted. However, in this case the importance of the selection of starter culture especially in respect of the manufacturing temperatures and the control of the manufacturing process becomes even more important than when using high levels of nitrite.

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REFERENCES


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