Survival of Selected Indicator and Pathogenic Bacteria in Refrigerated Pizzas

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ABSTRACT

This study was conducted to determine the shelf life of previously frozen pizzas stored at refrigeration temperatures. Pizzas were prepared using meat inoculated with Escherichia coli, Staphylococcus aureus, and Salmonella typhimurium. The pizzas were frozen, then stored at 3 and 10°C. Samples were analyzed every 2 d for 14 d. Sensory analysis was conducted every day for 8 d using uninoculated product. There was a significant (P<0.05) increase in the population of E. coli between 8 and 10 d at 10°C. There were no significant (P>0.05) differences in the populations of S. typhimurium or S. aureus with either time or temperature. The sensory shelf life of the pizzas was approximately 5 d at 10°C and 6 d at 3°C. The pizzas were unacceptable after 7 d at either temperature.

There has been an increase in the sale of previously frozen convenience items from retail refrigeration cases. A typical product will be prepared at a central processing facility and distributed frozen. The frozen product will then be placed in a refrigerated display case for retail sale. The microbiological and sensory shelf life of this product is an obvious concern from both public health and marketing aspects.

Pizzas are currently being marketed in this manner from grocery and convenience stores. A pizza can be divided into four subgroups: the crust, sauce, meat, and cheese. Of these subgroups, the meat and cheese are usually the most sensitive ingredients from a microbiological standpoint.

The refrigerated shelf life of meat has been reviewed by several authors (9,14). The microbiological hazards with cooked meat include survival of Salmonella and other pathogenic bacteria, either from improper cooking or post-processing contamination (15). Since Salmonella can survive the freezing process (3), there is the potential for growth once the product is thawed.

The primary concern with cheese is Staphylococcus aureus, although there has been some concern with enteropathogenic Escherichia coli (16). Although there has generally not been a public health problem with cheese in normal commercial channels (5), it has still been classified as a “moderate direct health threat” by the ICMSF (10). The possibility for food poisoning does exist under abuse conditions (19).

This study was undertaken to evaluate the sensory and microbiological shelf life of pizzas stored at refrigeration temperatures.

MATERIALS AND METHODS

Bacterial cultures

Cultures of E. coli (ATCC 25922), S. aureus (ATCC 25923), and Salmonella typhimurium (ATCC 14028) were grown and maintained in Tryptic soy broth (Difco) at 32°C. Bacterial cells were harvested in late logarithmic growth phase by centrifugation (5000 X g, 10 min). The pellets were washed once in 10 ml of cold (3°C) 2% sodium citrate buffer (12), and the washed cells were resuspended in 10 ml of citrate buffer.

Inoculated meat

The washed cells of all three bacteria were diluted into the same 300 ml of citrate buffer. Cooked beef and textured vegetable protein (TVP) (250 g) was added to the buffer and the mixture was gently agitated for 2 min. The mixture was allowed to stand for an additional 2 min, then was drained through several layers of cheesecloth for approximately 1 min. The remaining buffer was hand-expressed from the meat/TVP mixture.

Preparation of pizzas

Individual pizzas were assembled on 10 x 15 cm crusts, using 28 g each of pizza sauce, inoculated meat, and a 90:10 blend of imitation and natural cheeses. The pizzas were placed in individual sterile sample bags and frozen to -20°C.

Microbiological analysis

Samples were homogenized for 1 min. in a Stomacher 400 (Tekmar, Inc., Cincinnati, OH) in appropriate volumes of sterile citrate buffer. E. coli was enumerated using Violet Red Bile agar (BBL) and incubated for 24 h at 32°C. S. aureus was enumerated using Baird-Parker agar (BBL) with Egg Yolk Tellurite supplement (Gibco, Madison, WI) and incubated for
48 h at 32°C. Both of these bacteria were enumerated using an automatic Spiral Plater (Spiral Systems, Inc., Bethesda, MD) (13). S. typhimurium was enumerated using a modified 3-tube MPN method, similar to that of Kent et al. (11). One ml of the appropriate dilution was added to individual tubes each containing 9 ml of lactose broth (BBL). These tubes were incubated for 24 h at 32°C. A 1-ml portion from each positive (turbid) tube was transferred to individual tubes each containing 9 ml of tetrathionate broth (Difco). After incubation for 24 h at 32°C contents of the tubes were streaked for isolation on XLD agar (Difco) with 1.25 ml of 1% aqueous brilliant green (Difco)/L. Plates were incubated for 24 h at 32°C, then 2 typical Salmonella colonies were transferred to TSI and L1A (Difco) slants for presumptive identification. The populations were determined using 3-tube MPN tables (1).

Sensory analysis
Additional pizzas were assembled using uninoculated beef and TVP, and handled in the same manner as the inoculated pizzas. These pizzas were evaluated by 5 trained sensory analysts. The pizzas were rated on a three-point hedonic scale with 3 as acceptable, 2 as borderline, and 1 as unacceptable.

Experimental design
The frozen pizzas were transferred to the appropriate storage temperature, 3 or 10°C. Sufficient pizzas were used so that one pizza from each temperature could be removed every 2 d for microbiological analysis and daily for sensory analysis. The experiments were repeated four times.

Statistical analysis
The bacterial populations were transformed from colony forming units/g to log10 numbers. The data were analyzed using Minitab (Minitab Project, Penn State University) on an IBM PC.

RESULTS AND DISCUSSION

Escherichia coli
The coliform counts of pizzas held at 3 and 10°C are shown in Fig. 1. There was a decrease in the population at 3°C during the 14-d storage, although this decrease was not significant (P>0.05). There was an increase in the population at 10°C, and this increase became significant (P<0.05) between 8 and 10 d of storage. There was no significant (P>0.05) difference between the populations at 3 and 10°C between 0 and 8 d. These results are similar to those of Skelton and Harmon (17), who reported on growth patterns of coliforms in cottage cheese stored at 4 and 10°C.

Staphylococcus aureus
The results of the S. aureus counts are shown in Fig. 2. There was an overall decrease in the populations at both temperatures during storage, although this decrease was not significant (P>0.05). There was no significant (P>0.05) difference between storage at 3 and 10°C.

The temperature range for growth of S. aureus is 6.5 to 46°C, with an optimum of 30 to 37°C (4). Toxin production at 10°C has been reported (18), although the quantities were very small. It is generally assumed that a population of 10⁶ S. aureus per gram is required to produce sufficient toxin to cause illness (2), and the results presented here do not indicate that this level would be reached under these storage conditions and inoculum levels.

Salmonella typhimurium
The Salmonella counts are shown in Fig. 3. There was an increase in population at 10°C and a decrease at 3°C, although neither was statistically significant (P>0.05). There was no significant (P>0.05) difference between the populations at 3 or 10°C.

Goepfert and Kim (8) reported that Salmonella failed to grow in ground beef at 3°C. However, there was growth reported in vacuum packaged ground beef at 12°C (7). It has been shown that Salmonella can survive up to 8 months in Cheddar cheese stored at 5°C (6). If salmonellae are present in frozen pizza, they clearly will survive during refrigerated storage and could present a potential public health hazard.
Sensory analysis

Results of the sensory analysis are shown in Fig. 4. Length of storage was a significant (P<0.05) factor at both temperatures. The pizzas became substandard after 5 d at 10°C and 6 d at 3°C. The primary defect was a sour taste in the meat. Most consumers would notice this and many would discard the substandard or spoiled product. The taste of the product declined during the next 2 d to unacceptable. It was the consensus of the sensory analysts that an unacceptable product tasted “spoiled” and would not be consumed under normal circumstances.

CONCLUSIONS

The single most important factor in determining the microbiological safety of pizza during storage is the initial product quality. Although there was no growth of either S. typhimurium or S. aureus during storage, there was also no decrease in the populations. Therefore, it is essential to eliminate or minimize the populations of these bacteria during production. There was growth of E. coli at 10°C, but this growth coincided with the unacceptable sensory ratings. The refrigerated products tasted “spoiled” before there was any growth of this indicator bacterium.

REFERENCES