Journal of Food Protection, Vol. 50, No. 12, Pages 1037-1043 (December 1987)

Effects of Hot Boning and Various Levels of Salt and Phosphate on Microbial, TBA, and pH Values of Preblended Pork during Cooler Storage

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(Received for publication February 25, 1987)

ABSTRACT

Five pork carcasses were used to determine the effects of hot boning and various combinations of salt (0, 1.5 or 3.0%) and a phosphate mixture (0 or 0.5%) on microbial, TBA and pH values of preblended pork (preblends). In both HB (hot boned within 2 h postmortem) and CB (conventionally boned at 24 h postmortem) preblends, salt increased ($P<0.05$) TBA values and decreased ($P<0.05$) psychrotrophic counts, whereas phosphate increased ($P<0.05$) pH and decreased TBA values. Salt level could be reduced from 3.0 to 1.5% in preblends without any storage problems if phosphate (0.5%) was included. Phosphate (mixture pH 7.2) seemed to have little influence on microbial growth of preblends during cooler storage.

Hot boning of pork before carcass chilling has several economic advantages stemming from savings in energy, space, labor, materials and supplies, and product shrinkage as well as improved product functional properties and quality *(19)*. Several researchers *(2,3,13)* have reported that prerigor processed cuts of pork generally had acceptable bacterial counts. However, Pulliam and Kelly *(24)* reported significantly higher microbial counts before smoking for hot-processed hams than for those normally processed hams. Davidson et al. *(8)* found the mesophilic counts for hot-processed pork sausage samples to be significantly greater than those for conventionally processed sausage samples after storage for 10 d. They also found that differences in psychrotrophic counts were not significant between hot-processed and conventionally processed sausage samples after storage of 10 d.

According to Reagan et al. *(25)*, preblending with salt (3 or 5%) was necessary for maintenance of desirable sausage-making characteristics of hot-boned beef stored at 2°C for 7 d. However, they concluded that off-flavor problems might develop in products from preblended raw materials stored at –10°C beyond 14 d postmortem. Lipid oxidation in meat is enhanced by presence of added salt, and the degree of lipid oxidation generally increases as the level of added salt increases *(9,30)*. Acton and Saffle *(1)* reported that preblending either prerigor or postrigor meat with salt and nitrite decreased total bacterial counts of these raw materials. Whiting et al. *(31)* inoculated cooked corned beef (1.5 or 2.5% salt) with either clostridial spores or staphylococci and incubated it at temperatures ranging from 5 to 30°C. Growth of indigenous microflora, staphylococci, and clostridia was similar at both salt levels at a given incubation temperature. However, increasing the temperature greatly increased growth of all organisms.

Phosphate can inhibit lipid oxidation because of its chelating effect on metal ions. Froning *(10)* reported that sodium polyphosphate-treated, mechanically deboned, poultry meat had significantly lower TBA values than untreated controls during 8 weeks of frozen storage at –29°C. However, Schwartz and Mandigo *(27)* found that added salt and sodium tripolyphosphate (STP) increased TBA values in a restructured pork product. Alkaline phosphate can also inhibit lipid oxidation as it increases the pH of ground meat. According to Chen and Waimaleongora-Ek *(6)*, adjusting the pH values of ground raw poultry meat to neutral or alkaline slowed the increase in TBA values during refrigeration storage. The effect of phosphate on the microbial characteristics of meat products is still a subject of some controversy. In studies with vacuum packed, sliced, Berliner sausage, Mol et al. *(21)* found that the presence of 0.45% polyphosphates did not affect growth of unclassified *Streptobacterium* LL3 during storage at 12°C. Nielsen and Zeuthen *(23)* reported that addition of low pH phosphate had a pronounced influence on growth of *Brochothrix thermosphacta* and *Serratia liquefaciens* in vacuum packed, bologna-type sausage, but that STP addition had little influence on growth of those bacteria. Wagner and Busta *(29)* found that addition of sodium acid pyrophosphate (SAPP) inhibited growth of *Clostridium botulinum* in peptone-yeast extract-glucose broth primarily because of the low pH and the sequestering of heavy metal ions

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essential for cell development. Molins et al. *(22)* observed no significant bacterial inhibition by any phosphate during refrigerated storage, nor was there appreciable growth in the control cooked, vacuum packaged bratwurst. However, there was significant inhibition of aerobic and anaerobic bacteria by SAPP upon temperature abuse, and some inhibition by tetrasodium pyrophosphate and STP. They postulated enzymatic hydrolysis of phosphate as a major factor in loss of antimicrobial properties of phosphate in processed meats.

The objective of this study was to determine the effects of hot boning and various levels of salt and phosphate on the microbial, TBA, and pH values of preblended pork during cooler storage.

MATERIALS AND METHODS

Sample preparation

Five pigs were slaughtered at the Kansas State University Meat Laboratory. One side of each carcass was hot boned within 2 h postmortem (HB). The other side was conventionally boned after chilling at 2-4°C until 24 h postmortem (CB). Triceps brachii muscle portions were excised (HB at 2 h; CB at 24 h postmortem) and trimmed of exterior fat and connective tissue. Conventionally and hot-boned meat samples were coarsely ground, and divided into 6 subsamples (150 g each). Each subsample was blended with one of six different combinations of salt (3 levels of NaCl; 0, 1.5, or 3%) and phosphate mixture (2 levels; 0 or 0.5%) using an aseptic spatula. Each subsample was placed in a different aseptic Stomacher Lab Blender bag, and stored in a 4°C cooler for 6 d. Salt was chemical grade NaCl and phosphate was the mixture of sodium acid pyrophosphate (SAPP, pH 4.2), sodium tripolyphosphate (STP, pH 9.8) and sodium hexametaphosphate (SHMP, pH 7.0) at a ratio of 1:5:4, respectively (mixture pH 7.2). Sampling was done for pH, microbial counts and TBA analysis at 4 times (0, 2, 4 and 6 d of cooler storage).

pH measurement

Samples (1-2 g) of the preblended meat (preblends) were blended with 10 ml of 5 m*M* sodium iodoacetate in 150 m*M* KCl solution *(5)*. The pH values were measured just after preblending and during storage.

Microbial counts

At each sampling time during storage, an 11-g sample of each preblend was placed in a Stomacher bag and mixed with 99 ml of Standard Plate Count buffer solution for 1 min in the Stomacher Lab-Blender 400 (Dynatech Lab. Inc.). Samples were withdrawn from the Stomacher bag for viable cell counts using standard plate count agar by standard methods *(20)*. One set of plates (10^{-2} and 10^{-3}) was incubated for 48 h at 32°C for the mesophilic count; another set (10^{-1} and 10^{-2}) was incubated for 10 d at 7°C for the psychrotrophic count. The counts were reported as $\log_{10}$ organisms per g of preblends.

2-Thiobarbituric acid (TBA) analysis

At each sampling time, a 40-g sample of each preblend was placed in a polyethylene bag (air was expressed) and stored at –80°C in an ultra-freezer for not over 3 months. For TBA analysis, the extraction method of Witte et al. *(32)* was used with some modifications. Just before analysis, samples were thawed at 0°C for 8-12 h, then cut into approximately 0.25-0.5-cm cubes. A 10-g sample was mixed with 15 ml of 10% cold perchloric acid and 20 ml of cold distilled water, and then blended in a Virtis homogenizer jar at about 20,000 rpm for 15 s. This precipitated protein and extracted malonaldehyde. The short blending time allowed filtrate clarity and prevented further oxidation from heat generated during longer blending. The slurry was filtered through Whatman #2 filter paper. Distilled water (5 ml) was used to rinse the jar and rinse water was added to the slurry. Five ml of filtrate was transferred to 5 ml of 0.02 *M* TBA reagent in a test tube. The mixture was covered with parafilm, mixed with a Vortex mixer, and stored in the dark for 18 h to develop the color. Three ml of the resulting pink solution was then transferred to a 5-ml disposable cuvette, which was placed in the spectrophotometer sample chamber, and absorbance was determined at 529.5 nm. Blanks prepared from 5 ml of distilled water in 5 ml of 0.02 *M* TBA reagent were stored in the dark with samples.

TBA standard curve

A standard curve for TBA analysis was prepared from appropriate dilutions of 2×10^{-5} moles 1,1,3,3-tetraethoxypropane (TEP)/ml stock solution to give the required concentrations, namely 2×10^{-9} to 8×10^{-8} moles TEP/5 ml (total of 7 standards). This range of concentration was predetermined by subjecting muscle samples to the TBA analysis together with TEP concentration standards. Five ml of each standard was quantitatively transferred to 5 ml of 0.02 *M* TBA reagent in a test tube and treated the same as samples. Recovery of the same concentration of standard added to authentic samples of fresh meat was 95%. The absorbance values obtained from standards were plotted against malonaldehyde amounts, and a regression equation was used to calculate TBA values of samples. TBA values were expressed as μg of malonaldehyde per 5 ml of filtrate (which represented 1 g of sample) or mg of malonaldehyde per 1000 g of preblended sample. All determinations were in duplicate.

Statistical analysis

Data were analyzed as a split-split-plot design using the Statistical Analysis System of Barr and Goodnight *(4)*. Significance ($P<0.05$) was determined by the F-test and Fisher's LSD multiple comparison *(28)*.

RESULTS AND DISCUSSION

Mean values for pH, mesophilic and psychrotrophic counts, and TBA of preblended pork for two boning methods and the six salt and phosphate treatments are shown in Table 1. Without addition of salt and phosphate, there was no difference ($P>0.05$) in pH between HB and CB preblended pork (preblends). Judge and Aberle *(16)* and Yasosky et al. *(33)* reported that prerigor ground pork had higher ultimate pH than postrigor ground pork, when prerigor muscle was removed within 45 min postmortem. Addition of salt (3.0%) maintained higher ($P<0.05$) pH values in HB preblends when compared to CB preblends, regardless of phosphate levels. This result agrees with the observations of other researchers *(7,12)*, in which addition of salt to prerigor muscle may have inhibited the complete breakdown of glycogen in the muscle. In both HB and CB preblends, addition of phosphate (0.5%) maintained higher ($P<0.05$) pH

TABLE 1. *Mean values for pH, mesophilic and psychrotrophic counts, and TBA of preblended pork by boning method and salt (S) and phosphate (P) treatment*[h].

Treatment	pH value[c]		Mesophilic count[a,c]		Psychrotrophic count[a,c]		TBA[b,c]	
	HB	CB	HB	CB	HB	CB	HB	CB
S 0 ,P 0	5.59[g]	5.55[e]	4.05[e]	3.36[e]	2.03[d]	1.65[d]	0.428[g]	0.162[f]
S 1.5 ,P 0	5.60[g]	5.58[e]	3.88[f]	3.48[d]	1.88[e]	1.55[e]	1.846[e]	1.407[d]
S 3.0 ,P 0	5.68[f]	5.58[e]	4.14[d]	3.27[f]	1.68[f]	1.59[e]	2.593[d]	1.683[d]
S 0 ,P 0.5	5.78[e]	5.76[d]	4.15[d]	3.36[e]	1.94[e]	1.68[d]	0.278[g]	0.082[f]
S 1.5 ,P 0.5	5.80[e]	5.79[d]	4.05[e]	3.37[e]	1.75[f]	1.44[f]	1.348[f]	0.554[e]
S 3.0 ,P 0.5	5.84[d]	5.78[d]	3.93[f]	3.34[e]	1.59[g]	1.38[f]	1.749[e]	0.789[e]

[a]Log_{10} organisms per g of preblends.
[b]Mg of malonaldehyde per 1000 g of preblends.
[c]Mean values of HB and CB samples, underscored by a common line, are not different ($P>0.05$).
[defg]Mean values in the same column bearing common superscript letters are not different ($P>0.05$).
[h]Data combined from 4 sampling periods (0, 2, 4 and 6 d of cooler storage).

values than no phosphate, regardless of salt levels. This result was expected because a high pH phosphate mixture was used.

Regardless of salt and phosphate levels, HB preblends had higher ($P<0.05$) mesophilic and psychrotrophic counts than CB preblends, but these differences were not practically important because both samples were in the microbiologically acceptable range (all values lower than 10^5 organisms/g; *11*). Davidson et al. *(8)* found that hot-processed pork sausage had higher mesophilic ($P<0.05$) and psychrotrophic counts than conventionally processed pork sausage for up to 10 d of storage. According to Judge and Cousin *(17)*, the numbers of psychrotrophic bacteria in prerigor ground pork were slightly greater than in postrigor ground pork throughout an 11-d storage period at 2°C. For the HB preblends, addition of salt (1.5%) decreased ($P<0.05$) mesophilic counts, regardless of phosphate levels. In CB preblends, addition of salt (3.0%) decreased ($P<0.05$) mesophilic count in the absence of phosphate. In both HB and CB preblends, addition of salt (1.5 or 3.0%) decreased ($P<0.05$) psychrotrophic counts, regardless of phosphate levels. Acton and Saffle *(1)* reported that total bacterial counts of sausage meat were decreased by preblending with salt and nitrite and by freezing. Addition of phosphate (0.5%) alone increased ($P<0.05$) the mesophilic counts in HB preblends or maintained similar values in CB preblends in the absence of salt. Addition of phosphate (0.5%) with salt (1.5 or 3.0%) decreased ($P<0.05$) psychrotrophic counts in both HB and CB preblends, regardless of salt levels (1.5 or 3.0%). This result was probably due to salt and phosphate interaction. Since a high pH phosphate mixture (pH 7.2) was used in this study, an antimicrobial effect by phosphate was not expected. If microbial count determinations had been conducted during longer storage, the effect of salt and phosphate might have been more pronounced. Nielsen and Zeuthen *(23)* reported that addition of low pH phosphate had a noticeable influence on growth of *B. thermosphacta* and *S. liquefaciens* in vacuum packed, bologna-type sausage, but that STP addition had little influence on growth of those bacteria.

For TBA results, without addition of salt, there was no difference ($P>0.05$) between HB and CB preblends regardless of phosphate levels. Jacobs and Sebranek *(14)* reported that TBA values were not significantly different between prerigor and postrigor beef patties. Juhl *(18)* found little difference in TBA values of beef ground at 3 and 72 h after slaughter. However, other researchers *(16,33)* reported that prerigor ground pork had a higher ultimate pH and was less susceptible to lipid oxidation than postrigor ground pork. Chen and Waimaleongora-Ek *(6)* found that increasing the pH values slowed down the lipid oxidation of ground raw poultry meat as measured by the TBA test. In this study, since there was no difference ($P>0.05$) in pH value between HB and CB preblends in the absence of salt, the inhibitory effect of a high ultimate pH on lipid oxidation was not realized. Temperature can affect lipid oxidation in many ways. Janky and Froning *(15)* reported that the oxidation rate of both heme protein and lipid was increased by increased storage temperature in mechanically deboned turkey meat and that catalytic effect of heme on lipid oxidation was observed best from 10 to 15°C. Since the HB preblends were boned within 2 h postmortem and coarsely ground before storage at 4°C, temperature of the HB preblends was higher during grinding than that of the CB preblends. This relatively higher temperature of HB preblends during grinding could enhance the catalytic effect of heme and nonheme iron on lipid oxidation, resulting in a numerically higher TBA value in HB preblends when compared to CB preblends. This temperature effect on lipid oxidation would be more apparent in HB preblends when pro-oxidant salt was added. When salt (1.5 or 3.0%) was added, HB preblends had higher ($P<0.05$) TBA values than CB preblends, regardless of phosphate levels. In both HB and CB preblends, addition of salt (1.5 or 3.0%) increased ($P<0.05$) TBA values, regardless of phosphate levels, but addition of phosphate (0.5%) decreased ($P<0.05$) these values when salt was added. This result agrees with the observations of Ellis et al. *(9)* and Waldman et al. *(30)*, who reported that lipid oxidation in meat was increased by the presence of added salt and

the degree of lipid oxidation generally increased as the level of added salt increased. Rhee et al. *(26)* also found that addition of salt (1.25 or 2.5%) to ground pork increased ($P<0.05$) TBA values, regardless of microorganism inoculation. According to Froning *(10)*, sodium polyphosphate-treated, mechanically deboned poultry meat had lower ($P<0.05$) TBA values than untreated controls during 8 weeks of frozen storage at –29°C.

Mean values for pH, mesophilic and psychrotrophic counts, and TBA of preblended pork for two boning methods during 6 d of cooler storage are shown in Table 2. Except for day 2, HB preblends had higher ($P<0.05$) pH values than CB preblends during storage. In mesophilic and psychrotrophic counts, there were no differences ($P>0.05$) between HB and CB preblends during the 6-d storage, even though HB preblends had numerically higher values than CB preblends. With careful control of storage temperature, both samples showed relatively constant microbial counts for 6 d, and were in the microbiologically acceptable range (all values lower than 10^5 organisms/g; *11*). This result agrees with the observation of Judge and Cousin *(17)* that the numbers of psychrotrophic bacteria were slightly greater in prerigor ground pork than in postrigor ground pork throughout an 11-d storage period at 2°C. Except for day 4, there were no differences ($P>0.05$) in TBA values between HB and CB preblends during 6 d of storage. However, both samples showed a sharp increase in TBA values after 2 d. According to Jacobs and Sebranek *(14)*, TBA values were not significantly different between prerigor and postrigor processed patties, but there was a significant increase in the values during storage.

Effects of various levels of salt and phosphate on pH values of HB and CB preblended pork during cooler storage are shown in Fig. 1 and 2, respectively. Addition of salt (1.5 or 3.0%) to HB preblends maintained higher pH values regardless of phosphate levels after 2 d of storage. In both HB and CB preblends, addition of phosphate (0.5%) maintained higher pH values regardless of salt levels during 6 days of storage. This result was expected because high pH phosphate was used. Effects of various levels of salt and phosphate on mesophilic and psychrotrophic counts of HB and CB preblended pork during cooler storage are shown in Fig. 3, 4, 5 and 6, respec-

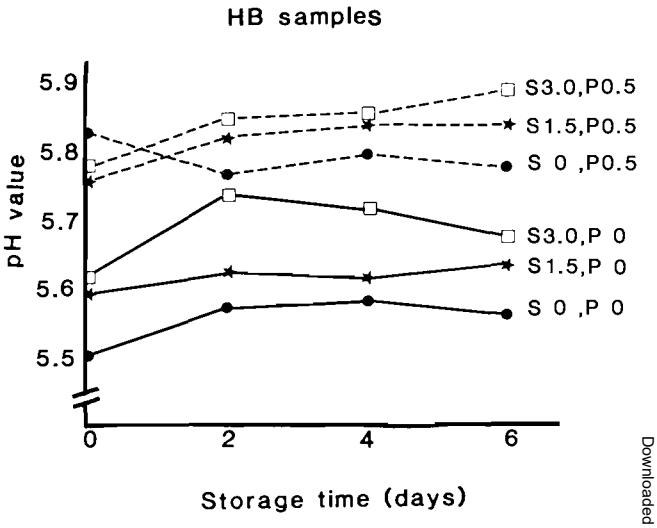

Figure 1. *Effects of various levels of salt and phosphate on pH values of HB preblended pork during cooler storage (LSD, least significant difference at 5% level, for comparison of storage time means within a treatment = 0.069; LSD for comparison of treatment means within a storage time = 0.081).*

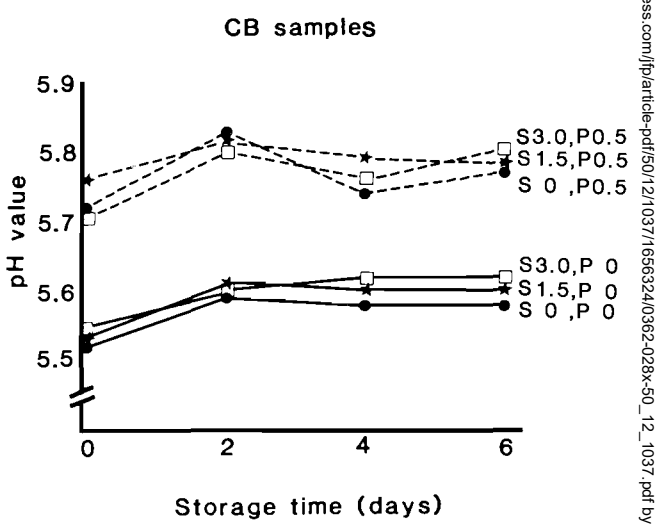

Figure 2. *Effects of various levels of salt and phosphate on pH values of CB preblended pork during cooler storage (LSD for comparison of storage time means within a treatment = 0.069; LSD for comparison of treatment means within a storage time = 0.081).*

TABLE 2. *Mean values for pH, mesophilic and psychrotrophic counts, and TBA of preblended pork by boning method during 6-day cooler storage*[d].

Storage time (d)	pH value[c]		Mesophilic count[ac]		Psychrotrophic count[ac]		TBA[bc]	
	HB	CB	HB	CB	HB	CB	HB	CB
0	5.68	5.63	4.12	3.40	1.90	1.62	0.36	0.11
2	5.72	5.71	4.06	3.34	1.79	1.46	0.92	0.38
4	5.73	5.68	3.96	3.33	1.78	1.46	1.99	0.94
6	5.73	5.69	3.97	3.38	1.77	1.64	2.23	1.69

[a]Log_{10} organisms per g of preblends.
[b]Mg of malonaldehyde per 1000 g of preblends.
[c]Mean values of HB and CB samples, underscored by a common line, are not different ($P>0.05$).
[d]Data combined from 6 treatments.

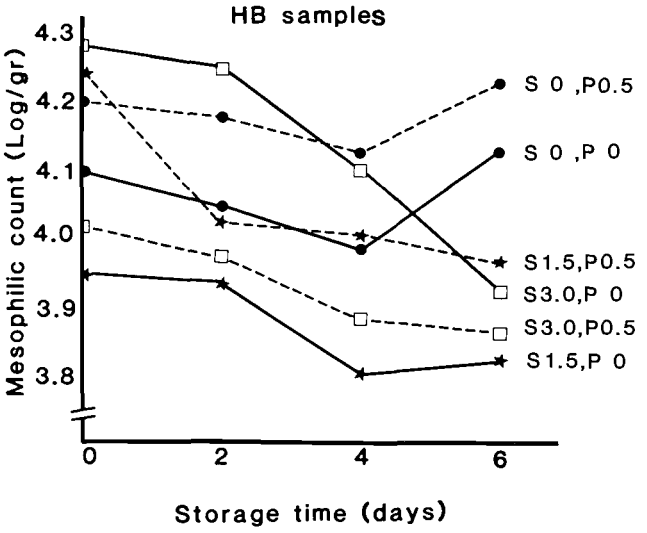

Figure 3. *Effects of various levels of salt and phosphate on mesophilic counts of HB preblended pork during cooler storage (LSD for comparison of storage time means within a treatment = 0.079; LSD for comparison of treatment means within a storage time = 0.204).*

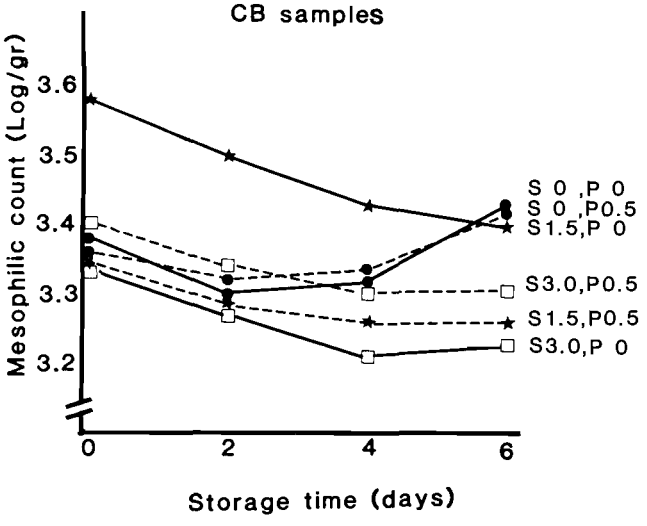

Figure 4. *Effects of various levels of salt and phosphate on mesophilic counts of CB preblended pork during cooler storage (LSD for comparison of storage time means within a treatment = 0.079; LSD for comparison of treatment means within a storage time = 0.204).*

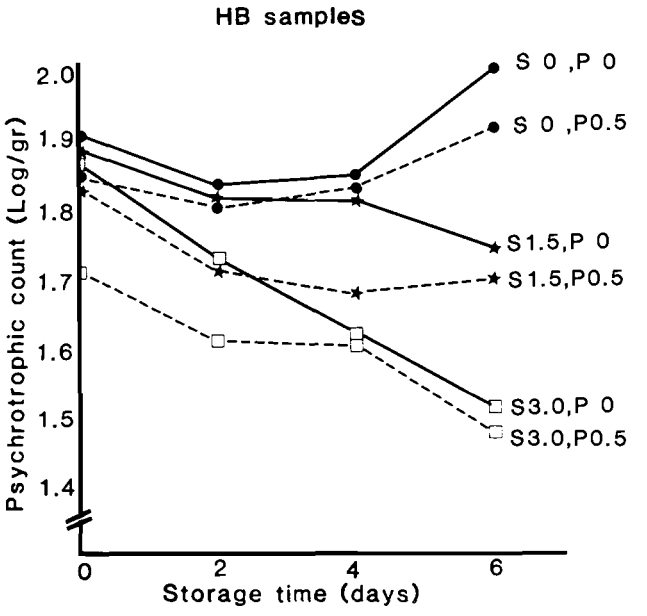

Figure 5. *Effects of various levels of salt and phosphate on psychrotrophic counts of HB preblended pork during cooler storage (LSD for comparison of storage time means within a treatment = 0.095; LSD for comparison of treatment means within a storage time = 0.294).*

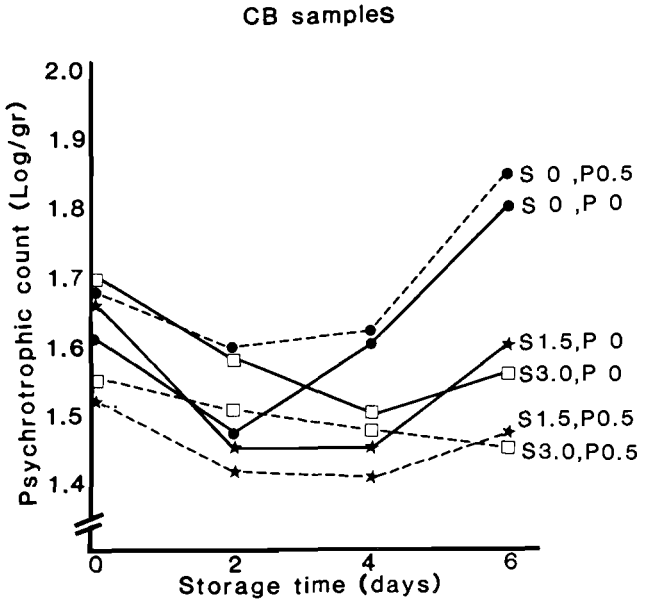

Figure 6. *Effects of various levels of salt and phosphate on psychrotrohic counts of CB preblended pork during cooler storage (LSD for comparison of storage time means within a treatment = 0.095; LSD for comparison of treatment means within a storage time = 0.294).*

tively. In both HB and CB preblends, addition of salt (1.5 or 3.0%) generally decreased mesophilic and psychrotrophic counts regardless of phosphate levels during the 6-d storage period, but addition of phosphate (0.5%) alone increased these values after 4 d of storage. Although each treatment showed acceptable microbial count values during 6 d of storage, no salt treatment tended to increase this value very quickly after 4 d of storage. Effects of various levels of salt and phosphate on TBA values of HB and CB preblended pork during cooler storage are shown in Fig. 7 and 8, respectively. In both HB and CB preblends, addition of salt (1.5 or 3.0%) increased TBA values during 6 d of storage regardless of phosphate levels, but addition of phosphate (0.5%) decreased these values regardless of salt levels. Effect of phosphate as an antioxidant was apparent with presence of salt.

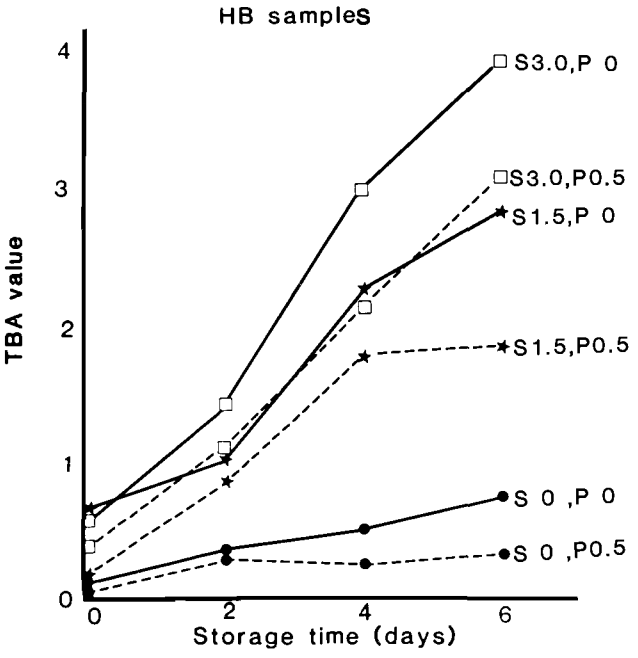

Figure 7. *Effects of various levels of salt and phosphate on TBA values of HB preblended pork during cooler storage (LSD for comparison of storage time means within a treatment = 0.641; LSD for comparison of treatment means within a storage time = 0.65).*

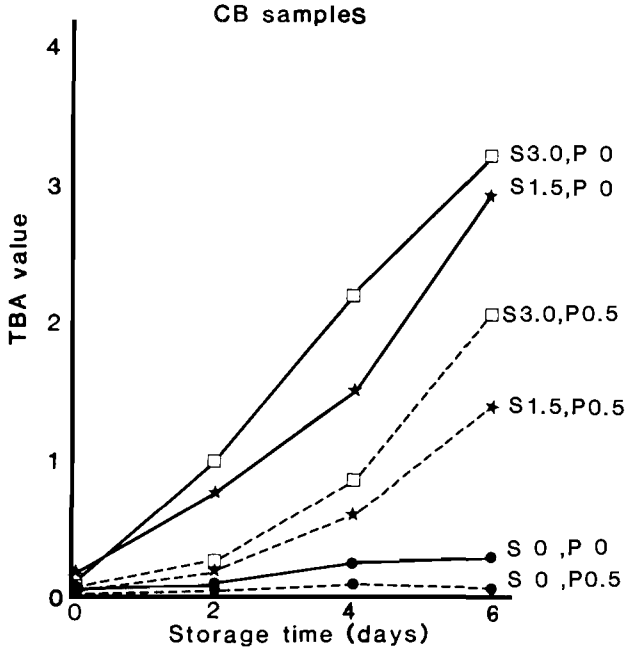

Figure 8. *Effects of various levels of salt and phosphate on TBA values of CB preblended pork during cooler storage (LSD for comparison of storage time means within a treatment = 0.641; LSD for comparison of treatment means within a storage time = 0.65).*

In conclusion, without addition of salt and phosphate, HB preblended pork (preblends) had higher pH, TBA, and mesophilic and psychrotrophic counts ($P<0.05$) than CB preblends. During 6 d of cooler storage, reduced level of salt (1.5%) with phosphate (0.5%) maintained higher pH, lower TBA, and acceptable microbial counts in both HB and CB preblends when compared to high salt (3.0%) alone. Addition of phosphate (0.5%) appeared to have little influence on microbial growth during storage. Although each treatment had microbiologically acceptable levels under careful control of storage temperature, TBA values quickly increased after 2 d of cooler storage when salt (1.5 or 3.0%) was added.

ACKNOWLEDGMENTS

Contribution No. 87-308-J, from the Kansas Agricultural Experiment Station, Manhattan, Kansas 66506. The authors thank Dr. M. McNulty, Department of Statistics for assistance in data analysis and Dr. D. Y. C. Fung, Department of Animal Sciences and Industry for facilities used to obtain microbial counts.

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DISCUSSION

The SPCB simulated the situation in commercial pickled cheese brine which is difficult to standardize. Our studies show that toxigenic *S. aureus* from bovine sources can grow in pickled cheese brine under the proper conditions (temperature 10 or 20°C, salt content 9% and pH above 5.0). Presence of growing yeast cells increased the pH of the medium, thus spurring the growth of *S. aureus*. Both *S. aureus* and yeasts showed continued growth in the presence of each other. The yeasts were not identified at this stage. Our studies show that acid-consuming yeasts are quite prevalent in Israeli pickled cheese brine.

It is interesting to note that Minor and Marth *(7)* stressed the possible role of *S. aureus* from bovine source in recontaminating dairy products and practically predicted part of the results of the present study. We see no reason why *S. aureus* from human sources will behave differently. A reintroduction of human *S. aureus* into pickled cheese brine is quite possible as much of this cheese is "fished" by the vendor from the brine in which it is marketed.

Hofi et al. *(3)* graded Egyptian market Domiati cheese and showed that there was a good relationship between pH and organoleptic quality good cheese, average pH 4.2 (4.0-4.4); medium quality, pH 4.6 (4.5-4.8); fair, pH 4.9 (4.6-5.6). Their cheese was much saltier than our system. Nevertheless, it seems that their grading system is valid also from the point of view of staphylococcus food poisoning.

It has been shown here that growth of yeasts in pickled cheese brine will increase the pH, thus stimulating the growth of *S. aureus*. The levels of *S. aureus* counts reached in our study may be below the numbers reported to produce damaging levels of toxins *(10)*. However, the model suggested here seems to be a valid avenue by which previously considered safe products may become a vehicle of *S. aureus*-produced enterotoxin.

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