Validation of Acidified Calcium Sulfate for Control of *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* serotype Enteritidis in Shelf-Stable Pumpkin Pies

A. Tanus[1], R. K. Phebus[1], G. R. Huber[2], A. Nutsch[1], and L. Franken[1]

[1]Dept. of Animal Sci. & Ind. and Food Sci. Inst., Kansas State University, Manhattan, KS 66506; [2]The XIM Group, LLC, Sabetha, KS 66534

ABSTRACT

This study was designed to validate the effectiveness of XIM's antimicrobial ACS formulation (trade name ***pHresh R17110LW-RX1***) as a functional ingredient to control the outgrowth of pathogens in shelf stable pumpkin pies. Pies formulated with either 1.5 or 2.1% ACS in the filling (both treatments contained 1.26% ACS in the crust) were inoculated with *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* serotype Enteritidis and stored at 25 ± 2°C for 8 days. Non-inoculated pies of each formulation were stored similarly and analyzed for naturally occurring bacterial populations, pH, and water activity. Preliminary sensory evaluations were performed on the pie formulation containing 1.5% ACS. This study demonstrated that the addition of ACS to pie formulations at a level of 1.5% or greater restricted the growth of these three associated pathogens providing a margin of safety for shelf-stable pumpkin pies. Additionally, both levels of ACS provided a significant extension in microbial shelf life compared to control pies, especially in prevention of surface mold appearance. Preliminary sensory evaluations indicated no negative impacts on the appearance or taste of pies.

MATERIALS & METHODS

Experimental design. This study evaluated the effect of two concentrations of ACS incorporated into shelf-stable pumpkin pies on pathogen populations and populations of naturally-occurring bacteria when pies were stored at 25 ± 2°C for 8 days.

Preparation of pumpkin pies. Pumpkin pies (9-in. diameter) were prepared and baked by technicians at the KSU Grain Science & Industry Baking Laboratory according to standard baking industry procedures. Pie crust and filling recipes are provided in Table 1. In addition to the base ingredients, pies were formulated with acidified calcium sulfate (ACS; trade name ***pHresh R17110LW-RX1***, composed of acidified calcium sulfate, cultured whey, whey solids, maltodextrin, gluconic acid, natural flavor, XIM Group LLC, Sabetha, KS) at one of three concentrations: 0% (no ACS in filling or crust), 1.5% (1.5% in filling and 1.26% in crust), or 2.1% (2.1% in filling and 1.26% in crust). Pies were baked at 425°F for 40 minutes. After baking the pies were allowed to cool to room temperature prior to transferring to the inoculation laboratory.

TABLE 1. *Base formulation for pumpkin pie crust and filling.*

Ingredient (yield: 10 pies)	grams	%
Crust		
Pastry flour	890.05	52.36
Nonfat dry milk	4.45	0.26
Dextrose	17.80	1.05
Salt	17.80	1.05
All-purp. veg. shortening	578.53	34.03
Water (2°C)	191.36	11.26
Total	1,700.00	100.00
Filling		
Pumpkin (canned)	1,517.10	29.75
Liquid whole eggs	485.47	9.52
Pastry flour	53.10	1.04
Tapioca starch	30.34	0.59
Pregelatinized tapioca starch	22.76	0.45
Granulated sugar	834.41	16.36
Nonfat dry milk	144.12	2.83
Salt	22.76	0.45
Cinnamon	15.17	0.30
Nutmeg	2.53	0.05
Water (38°C)	1,972.24	38.67
Total	5,100.00	100.00

Bacterial cultures & inoculum preparation. Bacterial cultures used in this study included three strains of *L. monocytogenes* (101M, 109, and serotype 4c [ATCC]), three strains of *Salmonella* serotype Enteritidis (USDA-FSIS 15060, Ames 15060, and ATCC 13076), and three strains of *S. aureus* (ATCC 25923, ATCC 25178, and ATCC 12606). Cultures were serially propogated in tryptic soy broth (TSB; Difco, Sparks, MD) with incubation at 35°C for 24 h. After three successive propogations, 5 ml aliquots of each culture in TSB were combined to provide 45 ml of a mixed-strain cocktail containing 9 to 10 log CFU/ml of each pathogen.

Inoculation & storage of pies. Pies were inoculated using a sterile tuberculin syringe to inject 0.0625 ml of the mixed-strain inoculum at a depth of 1/4 in. at 12 symmetrically distributed locations. The total inoculum applied per pie was 0.75 ml, which provided an initial pathogen concentration of about 6 log CFU/pie without affecting water activity. Inoculated and non-inoculated pies of each formulation were placed in standard cardboard pie boxes and stored at 25 ± 2°C with samples analyzed on days 0, 3, 6, and 8 of storage. Inoculated pies were analyzed for pathogen populations; non-inoculated pies were analyzed for naturally-occurring microbial populations, pH, and water activity (a_w).

Sample collection & analysis. Each pie was cut into four equal quarters and each quarter was individually weighed, placed in a sterile filter stomacher bag, and homogenized with an equivalent volume of 0.1% sterile peptone diluent using a stomacher lab blender. Ten ml of filtrate from each quarter were combined in a sterile 50-ml centrifuge tube, vortexed for 1 min, and this combined sample was serially diluted in sterile peptone diluent. Appropriate serial dilutions were spiral plated using a Whitley automatic spiral plater (Don Whitley Scientific Ltd., Shipley, West Yorkshire, England). Samples from inoculated pies were plated on modified Oxford agar, Baird Parker agar, and xylose lysine desoxycholate agar to enumerate *L. monocytogenes*, *S. aureus*, and *Salmonella* Enteritidis, respectively, with incubation at 37°C for

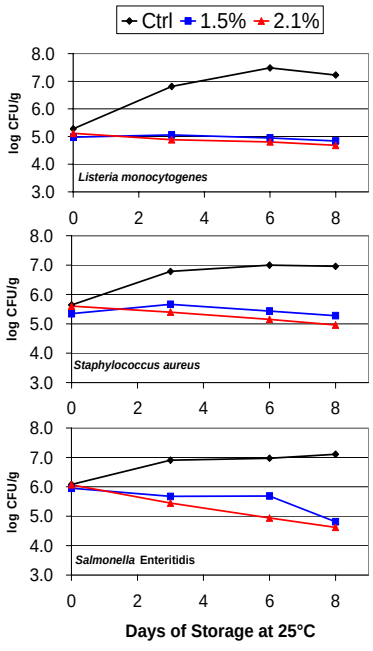
24 h. Samples from non-inoculated pies were plated on plate count agar to determine total aerobic plate counts (incubation at 37°C for 24 h), E. coli/Coliform Petrifilm (3M Microbiology, St. Paul, MN; incubation at 37°C for 24 h) to determine generic *E. coli* and coliform populations, and potato dextrose agar (pH 3.5) incubated at 25°C for 5 days to determine yeast and mold populations.

The pH of non-inoculated pies was determined using a Corning Pinnacle 530 pH meter equipped with a glass electrode. The pH (average of measurements from four quadrants) was obtained by penetrating the pie surface with the glass electrode to a depth of 0.5 cm. To determine water activity, a core sample representing both filling and crust portions was collected from the center of non-inoculated pies. Water activity of these samples was determined using an AquaLab CX-2 water activity meter (Decagon Devices, Inc., Pullman, WA).

Sensory evaluation. The overall organoleptic acceptability of the finished pumpkin pie formulated with 1.5% ACS was evaluated after one day of storage by a panel of six persons (three experienced taste panelists and three with no previous experience). Each panelist was asked to identify the type of pie, rate the pie's appearance (poor, average, very good, excellent) and taste (poor, average, very good, excellent), and indicate whether they would purchase the pie for a holiday dinner.

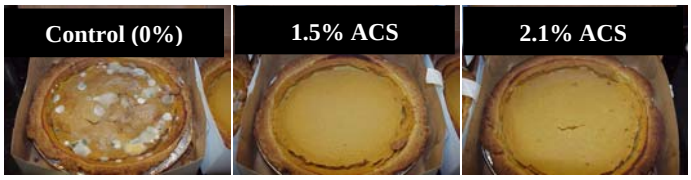
RESULTS & DISCUSSION

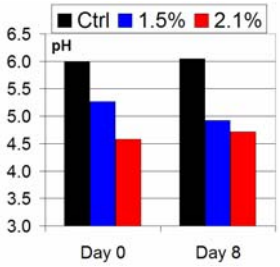
Effect of ACS on pathogen populations. Pies containing no ACS allowed significant increases in pathogen populations during the 8 days of room temperature storage. *L. monocytogenes* counts increased from 5.3 on day 0 to 6.8 log CFU/g by day 3, and further to 7.3 log CFU/g by day 6. *S. aureus* counts increased from an initial 5.6 to 6.8 log CFU/g by day 3, and to 7 log CFU/g by day 6. *Salmonella* counts were initially 6.1 log CFU/g and increased to 6.8 logs by day 3. *Salmonella* counts reached 7.1 log CFU/g by day 8.



Both levels of ACS (1.5 and 2.1%) were highly effective in preventing growth of the three inoculated pathogen populations throughout the entire 8-day storage period. *Salmonella* was the most susceptible to ACS addition with populations declining by 1.2 and 1.5 log cycles, respectively, during storage. *L. monocytogenes* population levels remained stable during storage, with slight reductions (0.2 and 0.4 log cycles) observed by day 8. *S. aureus* populations in 1.5% ACS pies increased slightly (0.4 log cycles) by day 3, but returned to inoculation level by day 6. In 2.1% ACS pies, *S. aureus* counts decreased by 0.6 log CFU/g during storage.

Effect of ACS on naturally occurring bacterial populations. Total aerobic bacterial counts increased rapidly from 4.8 to 7.7 log CFU/g in control (no ACS) pies during the first 3 storage days and reached 8.7 log CFU/g by day 6. Counts in pies containing ACS remained at ~5 log CFU/g during the first 6 days of storage. By day 8, total aerobic counts were greater than 8.0 log CFU/g. Coliform populations were 0.6 CFU/g in control pies on day 0 and were undetectable (<0.3 log CFU/g detection limit) in pies containing ACS. By day 3, pies from all three formulations demonstrated 0.5 log CFU/g. The coliform populations increased gradually and reached 3.3, 2.6 and 2.1 log CFU/g in control, 1.5% ACS and 2.1% ACS pies, respectively. The primary difference observed in the microbial quality of pies was in the growth of surface mold. Control pies demonstrated slight to moderate molding by day 3 and virtually complete mold coverage by day 6. Pies containing ACS at either level showed no molding by day 3 and only an occasional mold spot on day 6.





Effect of ACS on pH and water activity. The water activity of all pie formulations at all sampling times was 0.97-0.98. The pH of pies containing no ACS was 6.0 on day 0 and remained stable at this level throughout storage. The pH of pies containing 1.5% ACS on day 0 was 5.3 and declined to 4.9 by day 8. For pies containing 2.1% ACS, pH values of 4.6 and 4.7 were observed on days 0 and 8, respectively.

Sensory evaluation. All panelists identified the type of pie as pumpkin and rated the appearance as excellent. Taste was rated as very good by five panelists and as average by one panelist. All subjects indicated they would purchase the pie for a holiday dinner. In addition, Brian Strouts, Head of Experimental Baking with the American Institute of Baking (AIB) evaluated the pies formulated with 1.5% ACS for commercial salability. Following subjective taste testing, Mr. Strouts indicated the pie tasted less acidic than other shelf stable pumpkin pies currently selling well in the market place.

ACKNOWLEDGEMENTS

The authors thank the KSU Grain Science & Industry Baking Laboratory for assistance in preparing the pumpkin pies used in this study. Additionally, our appreciation is extended to Dr. Maureen Olewnik and Brian Strouts at the American Institute of Baking for their assistance and guidance in this project.

CONTACT INFORMATION:

Gordon R. Huber, Principal
The XIM Group, LLC
Sabetha, KS 66534
Phone: 785 547-6038

Food Science Institute
KANSAS STATE UNIVERSITY