

Research Note

Validating the Inhibition of *Staphylococcus aureus* in Shelf-Stable, Ready-to-Eat Snack Sausages with Varying Combinations of pH and Water Activity

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ABSTRACT

Shelf-stable, ready-to-eat meat and poultry products represent a large sector of the meat snack category in the meat and poultry industry. Determining the physiochemical conditions that prevent the growth of foodborne pathogens, namely, *Staphylococcus aureus* postprocessing, is not entirely clear. Until recently, pH and water activity (a_w) criteria for shelf stability has been supported from the U.S. Department of Agriculture training materials. However, concern about the source and scientific validity of these critical parameters has brought their use into question. Therefore, the objective of this study was to evaluate different combinations of pH and a_w that could be used for establishing scientifically supported shelf stability criteria defined as preventing *S. aureus* growth postprocessing. Snack sausages were manufactured with varying pH (5.6, 5.1, and 4.7) and a_w (0.96, 0.92, and 0.88) to achieve a total of nine treatments. The treatments were inoculated with a three-strain mixture of *S. aureus*, with populations measured at days 0, 7, 14, and 28 during 21°C storage. Results revealed treatments with a pH \leq 5.1 and $a_w \leq$ 0.96 did not support the growth of *S. aureus* and thus could be considered shelf stable for this pathogen. The results provide validated shelf stability parameters to inhibit growth of *S. aureus* in meat and poultry products.

Snack sausages represent a significant and growing sector of the meat and poultry industry. Because refrigerated storage is not required during the product shelf life, these products offer tremendous consumer flexibility and convenience. When the product is manufactured to achieve conditions that do not support the growth of microorganisms, the consumer receives a shelf-stable and safe product. Thermal processing effectively eliminates vegetative pathogenic bacteria, but formulation and storage product conditions must be sufficient to inhibit pathogen growth to dangerous levels in the event of any post-thermal processing contamination.

Between 1998 and 2008, 458 outbreaks of foodborne illnesses caused by *Staphylococcus aureus* were reported, with 55% of them caused by food worker contamination, resulting in an estimated cost of \$1.2 billion annually (4, 5). Producers of shelf-stable, ready-to-eat (RTE) meat and poultry products consider *S. aureus* a major concern because of the pathogen's ability to survive and grow in environments with a pH as low as 4.0 and a water activity (a_w) as low as 0.86 and in anaerobic conditions (16). Further, the RTE meat system may provide a nutrient-rich environment with ideal conditions that can allow *S. aureus* to grow to dangerous levels and produce enterotoxins if not refrigerated (2). An

estimated population of 10^6 CFU/g of the bacteria within a contaminated food can produce 1 ng of enterotoxin, a dose sufficient to cause human illness (4, 8). Due to this potential food safety hazard, the U.S. Department of Agriculture–Food Safety and Inspection Service (USDA-FSIS) has focused special attention on those products considered shelf stable and, hence, not refrigerated after manufacture.

The USDA-FSIS requires manufacturers of shelf-stable, RTE meat and poultry products to have validated evidence (that is, a safe harbor) outlining the critical parameters needed to prevent the growth of any pathogenic bacteria postprocessing to ensure this bacterial growth is prevented when stored without refrigeration. Previously, the maximum moisture:protein ratio (MPR) was widely used to define shelf stability; however, scientific studies have demonstrated that a_w , pH, or both are more appropriate physiochemical properties to define shelf stability for preventing bacterial growth (12, 15, 17). Since this realization, the meat and poultry industries have transitioned to utilizing a_w , pH parameters, or both as critical limits in their food safety programs. Minimum water activities required for growth are readily available for a variety of pathogenic bacteria in the literature and textbooks and can be effectively used for certain dried products such as jerky (11). However, in other products, such as fermented, semidry sausages, reducing the water content low enough to achieve shelf-stable conditions based on a_w alone could detrimentally impact product

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TABLE 1. Experiment pH and water activity (a_w) parameters and compositional data of snack sausage treatments

Treatment	pH ^a		a_w ^b	Moisture (%) ^c	Protein (%) ^d	MPR ^e	Fat (%) ^f	NaCl (%) ^g	Nitrite (ppm) ^h
	Target	Actual							
1	5.6	5.6	0.957 ± 0.002	51.01 ± 1.42	27.41	1.86	21.58 ± 1.00	2.21 ± 0.14	51.9 ± 2.8
2	5.6	5.6	0.919 ± 0.006	36.81 ± 1.62	36.39	1.01	26.80 ± 0.76	2.64 ± 0.30	51.9 ± 2.8
3	5.6	5.6	0.879 ± 0.003	29.39 ± 1.93	43.69	0.67	26.92 ± 1.88	3.02 ± 0.26	51.9 ± 2.8
4	5.2	5.1	0.956 ± 0.002	48.91 ± 0.88	31.02	1.58	20.07 ± 1.88	2.46 ± 0.17	27.1 ± 1.7
5	5.2	5.1	0.925 ± 0.003	38.86 ± 1.49	37.38	1.04	23.76 ± 3.23	2.95 ± 0.07	27.1 ± 1.7
6	5.2	5.1	0.876 ± 0.003	27.71 ± 1.22	44.06	0.63	28.23 ± 1.14	3.23 ± 0.22	27.1 ± 1.7
7	4.8	4.7	0.960 ± 0.004	50.16 ± 2.13	28.38	1.77	21.46 ± 0.78	2.33 ± 0.28	24.7 ± 1.6
8	4.8	4.7	0.926 ± 0.006	38.72 ± 1.35	36.95	1.05	24.33 ± 2.61	2.82 ± 0.22	24.7 ± 1.6
9	4.8	4.7	0.883 ± 0.004	31.32 ± 5.69	41.81	0.67	26.87 ± 3.33	3.18 ± 0.19	24.7 ± 1.6

^a Target (generated in experiment design) and actual pH achieved for the various treatments. Indirect pH by using Orion 8104 combination pH probe and Accumet pH meter, 10 g of meat to 90 ml of distilled water.

^b Measured using a Decagon AquaLab 4TE water activity meter.

^c Vacuum oven method, 5 h, 100°C; Association of Official Analytical Chemists, method 950.46 (3).

^d Protein was determined by subtraction (100 – % fat – % moisture).

^e MPR, moisture:protein ratio calculated by dividing % moisture by % protein × 100.

^f Crude fat method, Soxhlet ether extraction; Association of Official Analytical Chemists, method 991.36 (3).

^g Measured as Cl⁻ using Mettler Toledo DL-22 Food and Beverage analyzer, silver nitrate titration.

^h Residual nitrite measured using colorimetric method and reported in ppm (mg/kg); Association of Official Analytical Chemists, method 973.31 (3).

quality by creating overly dry products substantially different from what consumers would expect for these products (13). As such, applying a hurdle approach utilizing a combination of pH and a_w has been proposed as a viable solution to achieve shelf stability, without negatively impacting important quality and economic attributes.

Recognizing the pH and a_w relationship impacting shelf stability, many meat and poultry manufacturers have implemented critical food safety parameters based upon shelf stability criteria provided in USDA-FSIS training guidance materials (14) and originating from a book chapter (9), where three options for shelf stability for pH-reduced products are outlined. These safe harbor parameters state products can be considered shelf stable if their composition has a pH < 5.0 alone, a_w < 0.91 alone, or a combination of pH < 5.2 and a_w < 0.95 (9). Which parameter (a_w versus pH) has a greater impact in contributing toward shelf stability, however, is not clearly understood. The USDA-FSIS document suggests that a_w is likely the more important intrinsic factor for controlling pathogens in semidry sausages (14); however, the American Meat Institute Foundation suggests that pH has greater relative impact in controlling pathogen growth (1).

Recent studies support using a combination of the pH and a_w approach to control *S. aureus* growth. Borneman et al. collected compositional (including pH and a_w) and *S. aureus* growth data on 34 RTE products to develop *S. aureus* growth equations for predicting shelf stability (6). The equations were created based on pH and a_w or pH and percentage of water phase salt (%WPS). Although the results of this study provided scientific insight toward shelf stability, the scope of the study was focused on generating a predictive model but did not validate specific pH and a_w combinations; thus, the study's practical impact is limited.

Today, debate remains over whether various combinations of the intrinsic values of pH and a_w truly provide shelf stability and what those critical combinations might be. The pH and a_w criteria for shelf stability from USDA training materials has been questioned, prompting concern about the source and scientific validity of these critical parameters and the appropriateness of their use. Therefore, the objective of this study was to identify combinations of pH and a_w that provide shelf stability for pH-reduced, RTE meat products, specifically for the control of *S. aureus*. A secondary objective was to gain insight regarding which factor, pH or a_w , has a greater contribution toward shelf stability.

MATERIALS AND METHODS

Study design. The experimental design for this study was a 3 × 3 factorial with pH (5.6, 5.2, and 4.8) and a_w (0.96, 0.92, and 0.88) as main factors, resulting in nine total treatments. The factor levels were selected to span the ranges of pH and a_w combinations that would likely be found in the pH-reduced, snack sausage category, as well as to align with currently used USDA-FSIS training guidance material parameters (14, 15). The treatment combinations investigated, including both target and actual factor levels for pH, are found in Table 1. For all physiochemical data, means and standard deviations were generated using the AVERAGE and SD of Excel (Microsoft Corporation, Redmond, WA). For microbiological data, analysis of variance for repeated measures was performed by using the MIXED procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). Compound symmetry was used as the covariance structure. Means were separated using Tukey's method at a 5% level of significance. The experiment was replicated two times.

Snack sausage production. RTE beef and pork snack sausages were manufactured at the University of Wisconsin–Madison Meat Science and Muscle Biology Laboratory by using typical snack sausage formulations and meat processing methods. The meat block for the snack sausages was comprised of 50% beef

clods (~80% lean) and 50% pork trimmings (42% lean). The nonmeat ingredients used low levels of functional ingredients and no spices or flavorings to minimize any impact these ingredients would have on bacterial growth. Furthermore, the pH was adjusted by direct acidification with citric acid rather than fermentation that could contribute lactic acid and competition with the starter culture. As such, the formulation was designed to represent a "worst-case scenario." Nonmeat ingredients (calculated on a meat-block basis) included 2.23% salt, 0.75% dextrose, 547 ppm of sodium erythorbate, 156 ppm of sodium nitrite, and encapsulated citric acid. Small, pilot experiments determined that concentrations of 0.3, 0.5, and 1.2% encapsulated citric acid were required to achieve target product pH values of 5.6, 5.2, and 4.8, respectively.

Three batches (15 kg) were produced for this experiment to represent each different pH group. The beef and pork trimmings were coarsely ground (model 4732, Hobart Corp., Troy, OH) separately by using a 12.7-mm plate and reground by using a 3.2-mm plate. Finely ground beef and pork were briefly mixed individually for uniformity and separated into 7.5-kg lots. The ground beef was transferred to a mixer (model 100DA, Leland Detroit Manufacturing Company, Detroit, MI) and mixed with 2.23% salt for 2 min. The ground pork was then added along with all other nonmeat ingredients and the prescribed encapsulated citric acid level (0.3, 0.5, or 1.2), and the batter was mixed for an additional 3 min. Each of the three batches was stuffed into 30-mm-diameter cellulose casings (Viscofan USA Inc., Montgomery, AL) by using a rotary vane vacuum stuffer (Handtmann VF 608 Plus Vacuum Filler, Handtmann CNC Technologies, Inc., Buffalo Grove, IL) into approximately 75-cm-long pieces and hung on a smokehouse rack. Thermal processing was accomplished by using a single-truck thermal processing oven (model 450 MiniSmoker, Alkar Engineering Corp., Lodi, WI) and a common snack stick smokehouse schedule, modified for a slightly larger diameter to reach an internal temperature of 71.1°C. After thermal processing, the starting a_w of each of the three batches was measured, and the snack sausages were immediately transferred to a climate-controlled drying chamber (model Climatic/MM 200, Eller LTD, South Tyrol, Italy) to achieve treatment target a_w levels of 0.96, 0.92, and 0.88. To accomplish this, the climate-controlled drying chamber was initially set at an ambient relative humidity of 80% (24 h) and slowly lowered to 77%, 71%, and finally 63% over a 10-day period to remove moisture from the sausages at a rate that prevented mold growth or case hardening. The a_w of each batch was measured at least every 24 h to monitor progress toward achieving the prescribed treatment a_w level. Drying continued for each snack sausage pH batch until all three a_w levels reached the nine experimental treatments. As the specific treatment a_w was attained during the drying process, that treatment, representing one-third of the entire pH batch was removed from the environmental drying chamber, vacuum packaged in bulk, and stored at 3 to 4°C until transportation to the University of Wisconsin–Madison Food Research Institute for inoculation.

Product analysis. Samples of each of the nine treatments were characterized for finished product pH, a_w , %WPS, proximate moisture, MPR, proximate fat, and residual nitrite levels. The pH was measured for duplicate samples of each treatment (Accumet Basic pH meter and Orion 8104 combination electrode, Thermo Fisher Scientific, Waltham MA) in slurries produced by homogenizing 10 g of sample in 90 ml of deionized water (Stomacher 400, A. J. Seward, London, UK). The a_w (Decagon AquaLab 4TE water activity meter, Pullman, WA), percentage of salt (measured as percentage of Cl^- , AgNO_3 potentiometric titration, Mettler

DL22 food and beverage analyzer, Columbus, OH), and moisture (5 h, 100°C, vacuum oven method, Association of Official Analytical Chemists 950.46) (3) were analyzed in triplicate for each treatment. The crude fat was analyzed after drying in duplicate for each treatment (3). The protein was calculated by subtraction, and %WPS was calculated using the equation $\%WPS = (\% \text{ salt})/(\% \text{ salt} + \% \text{ moisture})$. Triplicate samples were analyzed for residual nitrite concentrations in the cooked product before the drying process for the three batches consisting of different pH values (Association of Official Analytical Chemists colorimetric method 973.31) (3).

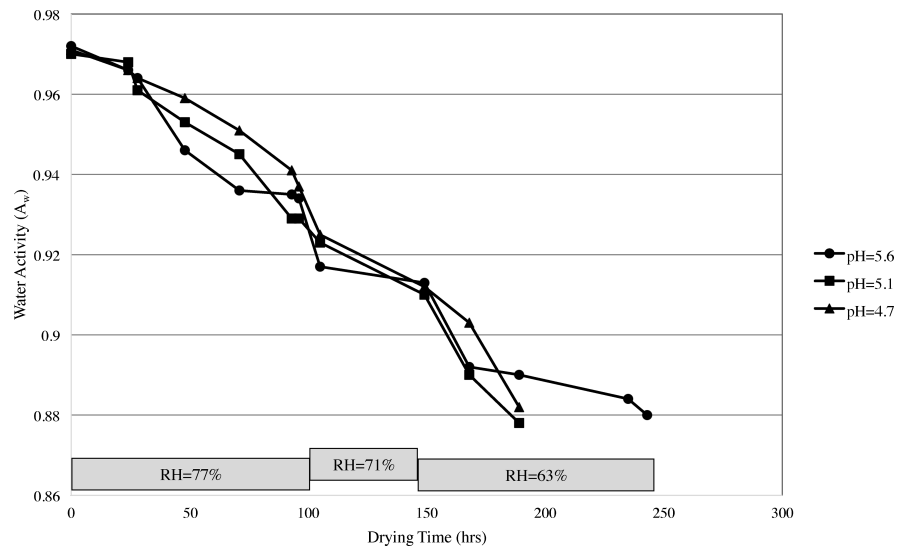
Inoculum preparation. A three-strain cocktail of *S. aureus* from the Food Research Institute stock culture collection (Madison, WI) was made using S6 (enterotoxins A and B), 196E (enterotoxins A and D, associated with a 1940 outbreak in ham), and 952 (enterotoxin A, associated with an outbreak in whipped butter) strains. Each strain was individually grown in 10 ml of Trypticase soy broth (TSB; BBL, BD, Sparks, MD) and shaken in a 37°C incubator for 24 h. Each culture was streaked onto Baird-Parker with egg yolk tellurite enrichment (Difco, BD, Sparks, MD) and Trypticase soy agar (BBL, BD) to verify purity of the strains. From each of the three cultures, 100 μl was transferred to a fresh 10-ml TSB tube. The streaked plates and fresh TSB cultures were incubated at 37°C for 18 to 22 h to achieve early stationary phase.

The TSB cultures were centrifuged at $2,500 \times g$ for 20 min. The supernatant was discarded, and the pellet was suspended in 4.5 ml of 0.1% buffered peptone water (pH 7.2). A mixture was formulated to produce a target inoculum of 10^3 CFU/g with equal contribution from each of the three strains. Populations of each strain and the mixture were verified by plating on Baird-Parker with egg yolk tellurite enrichment.

Inoculation and plating. The snack sausages were aseptically removed from the cellulose casing, cut into 25-g samples, and split longitudinally using sterile tools to expose the inner sausage surface. The 25-g samples were inoculated on the cut surface with 50 μl of the inoculum mixture to reach a target of 3 log CFU/g of *S. aureus*. Half of the inoculated samples for each of the nine treatments was vacuum packaged (3-mil high barrier pouches; oxygen transmission rate, 50 to 70 cm^3/m^2 , 24 h at 25°C, and 60% relative humidity; water transmission rate, 6 to 7.5 g/m^2 , 24 h at 25°C, and 90% relative humidity; UltraSource, Kansas City, MO), while the other half was placed into Whirl-Pak bags (Nasco, Fort Atkinson, WI), representing anaerobic and aerobic conditions, respectively. Uninoculated samples of each treatment were similarly packaged. All samples were stored at 21°C for 4 weeks.

Triplicate inoculated samples were analyzed for growth at time days 0, 7, 14, and 28. In addition, triplicate samples were also removed and placed at 4°C on day 3 to be enumerated as necessary if growth was observed at the day 7 sampling point. At each time point, the 25-g samples were macerated and homogenized with 25 ml of Butterfield's phosphate buffer. Serial dilutions were prepared using 0.1% peptone water blanks, and 0.1 ml of the appropriate dilutions was plated on Baird-Parker with egg yolk tellurite enrichment. Duplicate uninoculated samples of each treatment were analyzed to detect any competitive microflora on all-purpose Tween (Difco, BD) agar, supplemented with 0.004% bromocresol purple, at day 0 and week 4. The plates were incubated for 48 h at 37°C before enumeration. The log CFU per gram was calculated for each 25-g sample, and the average value was determined for each meat treatment. If no colonies were observed on the lowest dilution, populations were recorded as <1.0 log CFU/g. Two replications of this study were carried out, and the

FIGURE 1. The change in a_w over time during drying of three pH groups (5.6, 5.1, and 4.7) for snack sausage treatment ($n = 9$) generation. RH, relative humidity set points during the drying process.



data was averaged ($n = 6$ for inoculated treatments; $n = 4$ for uninoculated treatments).

RESULTS AND DISCUSSION

Treatment generation and product analysis. For the high pH (5.6) group, the target pH was successfully met; however, for the medium (5.2) and low (4.8) pH groups, the actual pH obtained was slightly lower (0.1 pH unit) than expected. As such, the actual values of pH 5.6, 5.1, and 4.7 were used for data analysis (Table 1). All a_w target treatment levels (0.96, 0.92, and 0.88) were successfully achieved. Drying data revealed each of the three pH groups had similar drying patterns; however, the high pH group took longer to dry to the final a_w 0.88 than the medium and low pH groups (Fig. 1). Immediately after cooking, all treatments had a starting a_w of approximately 0.97. After 28 h in the environmental drying chamber, all of the pH groups reached the first a_w target of 0.96 and were removed to establish treatments 1, 4, and 7. The second a_w target of 0.92 was achieved at approximately 105 h for all pH groups to create treatments 2, 5, and 8. Reaching the final a_w of 0.88, however, resulted in significant time variation among the pH groups. The pH groups having a pH of 5.1 and 4.7 reached the treatment target a_w of 0.88 at approximately 189 h to establish treatments 6 and 9, while it took 243 h for the pH group of 5.6 to reach that same a_w (treatment 3). The least acidic treatment retained water for a longer time causing the drying process to be extended. The differences in drying were not unexpected as the pH groups closer to the isoelectric point of salted meat (pH = 4.7–5.0) would result in a decreased water-binding ability of the protein and subsequently a more rapid moisture loss due to a lower ionic strength present (13).

The physicochemical properties and related characterization for each treatment are reported in Table 1. All of the target a_w levels were reached for each treatment; however, as already mentioned, the target pH values were not reached for the medium and low pH treatments. The pH differences between the test and calibration samples and the actual treatments can be attributed to differences in buffering

capacity from variation existing with two raw material sources used for the experiments. As expected, the proximate moisture and MPR decreased, as treatment a_w level requirements decreased. The proximate protein, fat, and %WPS values also increased, as the a_w treatment parameters decreased, due to concentration with loss of moisture. Lower residual nitrite levels corresponded to pH reduction. Previous studies have shown that depletion of nitrite concentration in cured meats is commonly observed if a reduction in the pH of the meat system occurs, creating a slightly acidic environment that favors nitrite-to-nitrous oxide conversion and results in lower residual nitrite concentrations (7). The use of encapsulated citric acid, which reduces the pH of the meat system during the manufacturing process, explains the depletion of nitrite observed in the treatments between the different pH groups.

Aerobic environment. Results for changes in *S. aureus* populations during aerobic storage are shown in Table 2. In general, interpretation of the data obtained from samples stored under aerobic conditions was complicated by mold growth. Although no competitive microflora was observed when plated on all-purpose Tween at time 0, mold grew on most of the samples. At day 7, treatments 1, 2, and 4 displayed some mold growth, and all samples displayed significant mold growth by day 14. As a result, sampling was discontinued after 14 days due to mold spoilage, and only day 7 data were reported.

At inoculation, all of the treatments started with similar levels of *S. aureus*, 3.60 ± 0.13 log CFU/g. After 7 days of storage at 21°C, populations of *S. aureus* increased an average 2.8, 0.9, and 0.4 log in treatments 1, 2, and 4, respectively (Table 2). In contrast, populations of *S. aureus* decreased 1 to 2 log in treatments 3 and 5 through 9. At day 14 (data not shown), *S. aureus* populations increased 4.4 and 1.0 log in treatments 1 and 2, respectively, from day 0, while the remaining treatments were <1.0 log CFU/g or moldy. Although mold growth confounded the study, the growth observed in treatments 1 and 2 within the first 14 days of storage supports previous findings in which

TABLE 2. Population changes of *S. aureus* for snack sausage samples stored at 21 °C in anaerobic and aerobic conditions on days 0, 3, 7, 14, and 28

Treatment ^e	pH	a _w	Population (log CFU/g)						
			Day 0 ^b	Aerobic ^c			Anaerobic ^d		
				Day 7	Day 3	Day 7	Day 14	Day 28	
1	5.6	0.96	3.64 ± 0.09 a A ^{ef}	6.36 ± 0.59	4.79 ± 0.85	5.50 ± 0.34 b A	6.13 ± 0.86 bc A	6.95 ± 0.16 c A	
2	5.6	0.92	3.68 ± 0.05 a A	4.49 ± 1.64	4.30 ± 0.84	4.18 ± 1.14 a AB	3.60 ± 0.80 a B	3.35 ± 0.42 a B	
3	5.6	0.88	3.61 ± 0.14 ab A	2.79 ± 0.98	4.26 ± 0.85	4.10 ± 0.72 b BC	2.98 ± 1.06 a BC	3.41 ± 0.88 ab B	
4	5.1	0.96	3.72 ± 0.09 b A	4.15 ± 2.73	NA ^g	3.20 ± 0.09 ab BCDE	3.23 ± 0.78 ab BC	2.75 ± 0.86 a B	
5	5.1	0.92	3.67 ± 0.07 b A	2.48 ± 1.31	NA	2.92 ± 1.01 ab CDEF	2.83 ± 0.50 ab BC	2.51 ± 0.87 a B	
6	5.1	0.88	3.56 ± 0.10 b A	1.15 ± 0.37	NA	3.36 ± 0.94 ab BCD	2.76 ± 1.10 ab BC	2.53 ± 1.66 a B	
7	4.7	0.96	3.52 ± 0.17 c A	1.14 ± 0.34	NA	2.09 ± 0.83 b EFG	1.18 ± 0.40 a D	<1 ^h a c	
8	4.7	0.92	3.50 ± 0.05 b A	1.57 ± 0.96	NA	1.65 ± 0.54 a G	1.28 ± 0.44 a D	<1 a c	
9	4.7	0.88	3.49 ± 0.15 c A	1.62 ± 0.58	NA	1.90 ± 1.24 b FG	0.90 ± 0.00 a D	<1 a c	

^a Snack sausage treatments tested, with varying combinations of pH (4.7, 5.1, and 5.6) and water activity (a_w; 0.88, 0.92, and 0.96).

^b Day 0, initial inoculation.

^c Samples stored in Whirl-Pak bags for 7 days.

^d Samples stored in vacuum bags for up to 28 days. Any samples in which populations of *S. aureus* increased at day 7 were enumerated for *S. aureus* at day 3.

^e Means followed by the same uppercase letters in the same row within each time period are not significantly different ($P \geq 0.05$).

^f Means followed by the same lowercase letters in the same column within each treatment are not significantly different ($P \geq 0.05$).

^g NA, microbial populations were not measured at this testing interval.

^h Less than detectable limit by direct plating.

combinations of a pH of 5.6 and a_w between 0.96 to 0.92 can result in conditions that support the growth of *S. aureus* when stored at room temperature in an aerobic environment (9).

The pH of the uninoculated treatments increased 0.6 to 1 units for nearly all treatments. A previous study demonstrated that mold growth increases the pH of the meat product due to the metabolizing of protein and release of ammonia and other basic molecules within the sausage (10). In addition, the increase in pH provided a more optimal environment for *S. aureus* growth, especially observed in treatments 1 and 2.

Anaerobic environment. Populations of background microflora in uninoculated samples on all-purpose Tween were typically less than the detectable limit by direct plating (<1 log CFU/g) at day 0 and day 28, while sample pH remained stable throughout the study (data not shown). In addition, no visible mold growth was observed throughout the storage period.

Results for changes in *S. aureus* populations on snack sausages during anaerobic storage are found in Table 2. Treatments 4 through 9 (pH values 5.1 or 4.7 and a_w ≤ 0.96) inhibited the growth of *S. aureus* throughout the 28-day study. At day 7, there was a 0.2- to 0.8-log CFU/g decrease in population for treatments 4 to 6 (pH 5.1 and a_w 0.96; pH 5.1 and a_w 0.92; and pH 5.1 and a_w 0.88). *S. aureus* levels continued to decrease throughout the 28-day sampling period and provided an overall decrease of approximately 1.0 log from the inoculation level of 3.65 ± 0.11 log CFU/g. Treatment 4 (pH 5.1 and a_w 0.96) was included in this study to mimic currently used stability criteria (pH of 5.2 and a_w < 0.95), prescribed by Leistner and Rodel (9). Although the intrinsic parameters were not exactly reproduced (pH 0.1 and

a_w +0.01), conditions were sufficiently similar to validate the control of *S. aureus*. Borneman et al. (6) performed similar *S. aureus*, 28-day growth studies on two products with the same intrinsic factors as those in treatments 5 and 6 (pH 5.1 and a_w 0.88; and pH 5.1 and a_w 0.92) and observed the same no-growth outcome as found in this study. Our treatments revealed about 1.0 log CFU/g decrease in *S. aureus* after 28 days, confirming that these conditions do not support growth of this pathogen. Reducing the pH to 4.7 in combination with a_w ≤ 0.96 (treatments 7 to 9, with a_w 0.96, 0.92, and 0.88, respectively) had an even greater effect on the population of *S. aureus* during the storage period. By day 7, populations decreased an average 1.6 log, while these three treatments and populations continued to decrease such that <1 log CFU/g of *S. aureus* was observed at day 28.

In contrast, treatment 1 sausage, adjusted to a pH 5.6 and a_w 0.96, supported a 1.1-log increase of *S. aureus* within 3 days. By day 7, the bacterial population had reached 5.5 ± 0.34 log CFU/g, and these populations could support sufficient enterotoxin production to cause human illness. Growth continued through day 28 to 6.95 ± 0.16 log CFU/g. Although growth was not unexpected, these results confirm that a meat product having both a high pH and a_w offers little control of pathogenic bacterial growth if stored in nonrefrigerated conditions, even under anaerobic conditions.

Populations of *S. aureus* increased slightly (~0.6 log) in treatments 2 (pH 5.6 and a_w 0.92) and 3 (pH 5.6 and a_w 0.88) at day 3 and day 7, but no additional growth was observed at the latter time points. These data suggest that the combination of pH 5.6 and a_w of 0.92 or 0.88 were marginal in their ability to inhibit growth of *S. aureus* and, as such, may not qualify as shelf stable, even though the population

of *S. aureus* at the 28-day storage period was not greater than inoculated levels. Further exploration of these treatments would be necessary to confirm their adequacy to be shelf stable and identify the boundary for growth and no growth, based on pH and a_w . However, shelf stability criteria provided by Leistner and Rodel (9) state a product with $a_w < 0.91$ would also be deemed shelf stable. The authors imply the product that implements this single critical limit would be pH reduced, thus creating multiple hurdle protection provided by an increase in environmental acidity. However, what that pH would need to be is not defined, suggesting any pH reduction normally associated with a fermented sausage would be sufficient.

This study confirmed the effects that both pH and a_w have on shelf stability of RTE meat products. Although, collectively, the two intrinsic factors have been shown to effectively control the growth of *S. aureus*, at the pH and a_w levels tested in this study, the research data suggest that pH may have a greater influence on shelf stability than a_w . None of the three high pH (5.6) treatments resulted in shelf stability; yet, the highest a_w (0.96) investigated was found to result in shelf stability when the pH was reduced to the medium (5.1) test level.

This challenge study has provided evidence of pH and a_w combinations that will result in a shelf-stable product. This study demonstrated that products having a pH ≤ 5.1 and $a_w \leq 0.96$ result in physiochemical product conditions that inhibit the growth of *S. aureus*, resulting in shelf stability and the ability to safely store products in nonrefrigerated environments. For future research, it would be beneficial to narrow the pH and a_w ranges near those of treatments 1 through 3 to obtain a better understanding of the shelf stability of products with these combinations.

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