EFFECT OF MEAT INGREDIENTS AND STORAGE TIME ON GERMINATION AND OUTGROWTH OF *Clostridium perfringens* SPORES IN HAM

Mauricio Redondo-Solano[1], Carol Valenzuela-Martínez[1], Vijay K. Juneja[2], Dennis E. Burson[3] and Harshavardhan Thippareddi[1]

[1] Applied Food Safety Laboratory, Department of Food Science and Technology, University of Nebraska, Lincoln, United States

[2] Residue Chemistry and Predictive Microbiology Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Wyndmoor, PA, United States

[3] Department of Animal Science, University of Nebraska, Lincoln, United States

Abstract – The effect of meat ingredients (NaCl, $NaNO_2$ and sodium erythorbate) and refrigerated storage prior to heat treatment on *C. perfringens* spore germination and outgrowth in ham during abusive cooling was evaluated. Ham formulation contained $NaNO_2$ (0 or 100 ppm), NaCl (1 or 2%), sodium erythorbate (0 or 547 ppm) and *Opti.Form* PD4 (0, 1.5 or 2.5 %). Portions of ham (10 g) were vacuum-packaged and stored under refrigeration (5C) for either 3 or 24 h. Ham was inoculated with a *C. perfringens* spore cocktail (2.5 log spores/g), vacuum-packaged, heat treated (75C, 20 min) and exponentially cooled from 54.4C to 4.4C within 15 or 21 h. In samples stored for 3 h containing $NaNO_2$ (100 ppm) and NaCl (1%), *C. perfringens* population of 1.22 log CFU/g was observed subsequent to 21 h of cooling. Longer storage of the same ham formulation (24 h) resulted in *C. perfringens* populations of 4.71 log CFU/g subsequent to 21 h of cooling. Incorporation of *Opti.Form* PD4 (2.5%) inhibited *C. perfringens* spore germination and outgrowth regardless of the storage time. Organic acid salts may provide an additional measure of safety in reduced NaCl and $NaNO_2$ meat products stored for longer periods of time subsequent to preparation.

Key Words – NaCl, $NaNO_2$, organic acid salts

- INTRODUCTION

Clostridium perfringens causes more than 966,000 illnesses and 439 hospitalizations in the United States annually [1]. Consumers demand for reduced NaCl and $NaNO_2$ meat products due to health concerns of hypertension and cancer, respectively. Reduction of NaCl and/or $NaNO_2$ can pose a challenge for meat processors as these ingredients contribute to microbial safety of meat products. Sodium chloride (NaCl) and sodium nitrite ($NaNO_2$) have been used in meat formulations to minimize the risk of *C. perfringens* spore germination and outgrowth during abusive cooling [2]. The effect of changes in meat formulation on *C. perfringens* outgrowth must be evaluated as affected by intrinsic (other ingredients) or extrinsic (storage time) factors during processing. The objective of this study was to evaluate the effect of meat ingredients ($NaNO_2$, sodium erythorbate and organic acids salts) and refrigerated storage prior to heat treatment (cooking), on the germination and outgrowth of *C. perfringens* spores during abusive cooling of ham.

- MATERIALS AND METHODS

In the first experiment, the effect of incorporation of organic acids salts and storage time of ham

(prior to heat treatment) containing reduced NaCl and/or $NaNO_2$ levels was evaluated. Meat was prepared by mixing 500 g of fresh ground pork (*Gluteus medius*) with a brine solution in a bowl mixer (Model K5SSWH, Kitchen Aid, Troy, OH) for 6 min at low speed to obtain desired concentrations of NaCl (1.0 or 2.0% wt/wt), $NaNO_2$ (0 or 100 ppm, Heller Seasonings and Ingredients, Bedford Park, IL), sugar (0.5% wt/wt), sodium polyphosphate (0.3% wt/wt, Brifisol®512, BK Giulini Corporation, Simi Valley, CA) and sodium erythorbate (547 ppm), calculated as percentage of meat block. Appropriate amount of *Opti.Form* PD4 (0, 1.5 or 2.5% of a mixture of potasium lactate and sodium diacetate; PURAC, Lincolnshire, IL) was added to the product requiring the antimicrobial. Ten-gram portions of the meat of each treatment were weighed into vacuum pouches (Nylon; 3-mil standard barrier; water vapor transmission rate of 10 g/liter/m^2/24 h at 37.8°C and 100% relative humidity; oxygen transmission rate of 3,000 cm^3/liter/m^2/24 h at 23°C and 1 atm [101.29 kPa], Prime Source, Kansas City, MO), vacuum sealed at 12 mbar (1.2 kPa) using a vacuum-packaging machine (A300/H, Multivac, Wolfertschwenden, Germany) and stored at 5°C for either 3 or 24 h. After storage, samples were inoculated with a three-strain *C. perfringens* spore cocktail (2.5 log spores/g) and vacuum packaged.

In the second experiment, the effect of $NaNO_2$, sodium erythorbate, storage time and packaging atmosphere during preparation (aerobic or anaerobic) on *C. perfringens* spore germination and outgrowth subsequent to abusive cooling was evaluated. Meat was prepared as described above to obtain desired concentrations of NaCl (2.0% wt/wt), $NaNO_2$ (0 or 100 ppm), sugar (0.5% wt/wt), sodium polyphosphate (0.3% wt/wt) and sodium erythorbate (0 or 547 ppm). Meat for this experiment was not vacuum-packaged before preparing the samples. Half of the samples were prepared inside an anaerobic chamber (Bactron IV, ShelLab, Cornelius, OR) and sealed using a Rival® Vacuum Sealer (Model FSFGSL0150-015, Jarden Corporation, Rye, NY). The meat was exposed to the anaerobic chamber environment (5% H_2, 5% CO_2, and 90% N_2) for 15 min before preparation to create reducing conditions in the samples. The second group of samples (pouches) was vacuum-packaged outside the anaerobic chamber (aerobic environment) as described previously. Samples were stored at 5°C for either 3 or 24 h before heat treatment. After storage, samples were inoculated as described previously.

The inoculated samples were vacuum-packaged and submerged in a water bath (Isotemp 3013H, Fisher Scientific, Bridgewater, NJ) set to 75°C (167°F) for 20 min to simulate cooking and to activate the *C. perfringens* spores. After heat treatment, one bag per treatment was chilled immediately in an ice water bath, and the *C. perfringens* population was determined. The second bag was transferred to a refrigerated bath with water circulation capabilities (RTE 740, Thermo Neslab, Portsmouth, NH) set to 54.4°C, allowed to equilibrate to this temperature for 10 min and chilled from 54.4 to 7.2°C exponentially within 15 or 21 h. After heat treatment and cooling, the contents of each bag were aseptically transferred to a filter stomacher bag (BagFilter, Spiral Biotech, Norwood, MA). Twenty ml of 0.1% peptone water (PW, Difco, Becton Dickinson, Sparks, MD) was added, and the contents were stomached for 1 min in a laboratory blender (NEUTEC, Albuquerque, NM). Ten-fold serial dilutions were prepared in PW and appropriate dilutions were either pour plated or spiral plated on tryptose sulfite cycloserine agar (TSC; Oxoid, Ltd., Basingstoke, UK) without egg yolk. After solidification of the agar, plates were incubated at 35°C for 18 h in an anaerobic chamber. Typical *C. perfringens* colonies were enumerated and the counts were expressed as log CFU/g of meat. Three independent replications were performed and data (log CFU/g) were compared using analysis of variance of the General Linear Model procedure of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Fisher's least significant difference (LSD; = 0.05) was used to separate the means.

- RESULTS AND DISCUSSION

Cooling of ham (no $NaNO_2$ or *Opti.Form* PD4 and stored for 3 h prior to heat treatment) within 21 h resulted in *C. perfringens* populations of 7.46 and 6.55 log CFU/g in formulations containing NaCl at 1 and 2% concentration, respectively (Fig. 1). Incorporation of $NaNO_2$ and *Opti.Form* PD4 into the ham formulation inhibited *C. perfringens* spore germination and outgrowth during abusive cooling (<1.0 log CFU/g), even in ham containing reduced NaCl (1%).

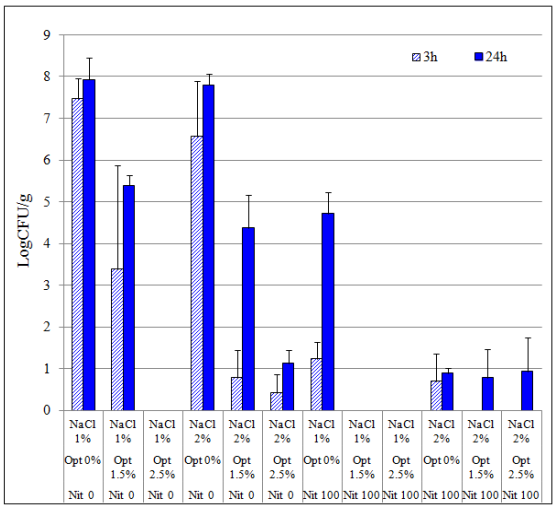

Figure 1. Mean *Clostridium perfringens* populations (log CFU/g) after cooling exponentially from 54.4C to 7.2C in 21 h in ham containing various concentrations of NaCl, $NaNO_2$ and *Opti.Form* PD4. Samples were prepared and heat treated (75C for 20 min) within 3 h or 24 h after preparation.

Jackson and others [3] reported that $NaNO_2$ (156 ppm) in combination *Opti.Form* PD4 (2.5%) and NaCl (2%) inhibited germination and outgrowth of *C. perfringens* spores in ham and frankfurters during storage for 10 days at 22C. Antimicrobial activity of lactates has been attributed to the reduction in water activity of the product [4]. Inhibitory activity of diacetate (sodium acetate and acetic acid) is related to its high pKa (4.76). The concentration of the undissociated form of the acid is high at the normal pH values in meat (*ca*. 5.4-5.5) [5]; therefore it can pass through microbial membranes and interfere with metabolic activity. In the present study, increasing the $NaNO_2$ concentration (from 0 ppm to 100 ppm) resulted in greater antimicrobial efficacy of organic acid salts. The additive effect of $NaNO_2$ and organic acid salts on the inhibition of *C. perfringens* spore germination and outgrowth allows for a further reduction in the NaCl and $NaNO_2$ content of cured products.

Holding the ham for 24 h prior to heat treatment followed by cooling within 21 h resulted in greater *C. perfringens* populations ($p \leq 0.05$) compared to ham stored for 3 h (Fig. 1). Incorporation of $NaNO_2$ and *Opti.form* PD4 in combination resulted in inhibition of *C. perfringens* outgrowth (21 h). *C. perfringens* populations of 5.39 and 4.37 log CFU/g were observed subsequent to cooling (21 h) in ham (no $NaNO_2$) formulated with 1.5% *Opti.Form* PD4, containing 1 and 2% of NaCl, respectively. In ham formulated with 1% of NaCl and 100 ppm of $NaNO_2$, *C. perfringens* population of 4.71 log CFU/g was observed. The antimicrobial activity of $NaNO_2$ and *Opti.Form* PD4 against *C. perfringens* was reduced in ham stored for a longer period of time (24 h).

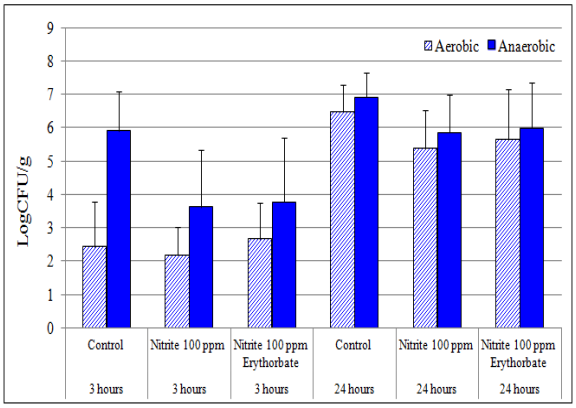

Figure 2. Mean *Clostridium perfringens* populations (log CFU/g) after cooling exponentially from 54.4C to 7.2C in 15 h in ham containing various concentrations of $NaNO_2$ and sodium erythorbate. Samples were processed either aerobically (outside the anaerobic chamber) or anaerobically (inside the anaerobic chamber) and heat treated (75C for 20) within 3 h or 24 h after preparation.

Abusive cooling (15 h) of ham (no $NaNO_2$) prepared under anaerobic conditions and stored for 3 h prior thermal treatment resulted in a *C. perfringens* population of 5.91 log CFU/g (Fig. 2). Preparation of the non cured ham under aerobic environment resulted in final *C. perfringens* populations of 2.43 log CFU/g subsequent to cooling (15 h). Similar differences in *C. perfringens* spore germination and outgrowth were observed in ham containing $NaNO_2$. Cooling of ham prepared under aerobic environment within 3 h showed lower ($p \leq 0.05$) *C. perfringens* spore germination and outgrowth compared to the product that was vacuum-packaged and stored under refrigeration for 24 h. Preparation of ham under anaerobic conditions enhanced ($p \leq 0.05$) *C. perfringens* spore germination and outgrowth during subsequent abusive cooling with similar *C. perfringens* populations in ham ($p > 0.05$) stored for either 3 or 24 h. Incorporation of sodium erythorbate (557 ppm) into the meat formulation reduced the inhibitory effect of the aerobic meat preparation on *C. perfringens* spore germination and outgrowth.

Oxygen may be incorporated into the processed meat during grinding, chopping and other similar meat processing unit operations. Residual oxygen in the meat could be reduced by the inherent reducing power of meat and/or the reducing activity of ingredients (reducing agents) such as erythorbate subsequent to vacuum-packaging. The presence of residual oxygen in the meat has been reported previously to cause photochemical degradation (discoloration) of vacuum-packaged ham within the first 24 h of display in illuminated cabinets [6]. Oxygen concentration in vacuum-packaged products was shown to be a more relevant factor for *C. perfringens* inhibition than the redox potential [7]. It is possible that the reduction in oxygen concentration either during storage of the ham for 24 h under vacuum or preclusion of oxygen during anaerobic preparation conditions may be responsible for higher *C. perfrigens* spore germination and outgrowth during abusive cooling. Additionally, myoglobin in the meat can initiate oxidative reactions due to the presence of residual oxygen, leading to accumulation of reactive oxygen species (ROS) [8]. It is possible that the ROS may cause toxicity to *C. perfringens* spores on the initial stages after meat preparation and vacuum-packaging and inhibit spore germination and outgrowth during subsequent abusive cooling.

- CONCLUSION

Incorporation of antimicrobials such as organic acid salts in the ham formulations can allow further reductions in sodium chloride or sodium nitrite without compromising the microbial safety of the product. The antimicrobial effect of nitrites and organic acid salts on *C. perfringens* spore germination and outgrowth was more evident in hams stored under

refrigeration for 24 h prior to heat treatment and abusive cooling. Meat product formulation and preparation procedures (such as length of refrigerated storage) can affect *C. perfringens* spore germination and outgrowth prior to preparation and processing. These conditions should be replicated while validating procedures of processed meat manufacture to ensure microbial safety. Inhibition of *C. perfringens* spore germination and outgrowth due to holding of product under refrigeration can lead to inconsistencies in the conclusions regarding the efficacy of antimicrobials in meat products.

REFERENCES

- Scallan E., Hoekstra R., Angulo F., Tauxe R., Widdowson M., Roy S., Jones J. & Griffin P. (2011). Foodborne Illness acquired in the United States-Major Pathogens. Emerg. Infect. Dis. 17: 7-14.
- Amezquita A., Weller C. L., Wang L, Thippareddi H. & Burson D. (2005). Development of an integrated model for heat transfer and dynamic growth of *Clostridium perfringens* during the cooling of cooked boneless ham. Int. J. Food Microbiol. 101: 123-144.
- Jackson A., Kulchaiyawat C., Sullivan G. A., Sebranek J. G. & Dickson J. (2011). Use of natural ingredients to control growth of *Clostridium perfringens* in naturally cooked frankfurters and hams. J. Food Prot. 74: 417-424.
- Apostolidis P., Kwon Y. I. & Shetty K. (2008). Inhibition of *Listeria monocytogenes* by oregano, cranberry and sodium lactate combination in broth and cooked ground beef systems and likely mode of action through proline metabolism. Int J Food Microbiol. 128: 317-324.
- Vasseur C., Baverel L., Hebraud M. & Labadie J. (1999). Effect of osmotic, alkaline, acid or thermal stresses on the growth and inhibition of *Listeria monocytogenes*. J. Appl Microbiol. 86: 469-476.
- Andersen H. J., Bertelsen G., Ohlen A. & Skibsted H. (1990). Modified packaging as protection against photodegradation of the colour of pasteurized sliced ham. Meat Sci. 28: 77-83.
- Pearson C. B. & Walker H. W. (1976). Effect of oxidation-reduction potential upon growth and sporulation of *Clostridium perfringens*. J. Milk Food Technol. 39: 421-425.
- Lund M. N., Heinonen M., Baron C. P. & Estevez M. (2011). Protein oxidation in muscle foods: A review. Mol. Nutr. Food Res. 55: 83-95.