51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

EFFECTS OF OZONATION ON STRENGTH AND COLOR OF NATURAL HOG CASING

Hakan Benli[a]*, Brian S. Hafley[a], Jimmy T. Keeton[a], Gary R. Acuff[a]

[a]*Department of Animal Science, Texas A&M University, College Station, TX 77840, USA*

Key Words: natural casings, pork, ozone

Introduction

Natural casings from lamb, pork, and beef are used throughout the U. S. sausage industry and are derived from the gastrointestinal tracts of the respective species above (Bakker, Houben, Koolmees, Bindrich, & Sprehe, 1999; Madhwaraj, Nair, Nair, Kadkol, & Baliga, 1980; Pearson & Gillett, 1999). After removal, tracts are washed, scraped, and treated to remove soluble components. Anatomical structures, such as the esophagus, stomach, small and large intestines, appendix, and rectum are separated, and the appropriate portions cleaned, salted, and graded according to size and condition (Pearson & Gillett, 1999).

Many factors influence the quality and properties of natural casings such as age of the animal, breed, type of fodder consumed, and other factors related to the animals themselves or the conditions in which they were raised. To be useful, natural casings must be sufficiently strong enough to withstand the pressure exerted on them during filling, stuffing and processing (Bakker *et al.*, 1999; Ockerman and Hansen, 2000).

Natural casings, by their nature, are contaminated with bacteria (10^4 to 10^7CFU/gram) (Gabis and Silliker, 1974; Bakker *et al*., 1999; Ockerman and Hansen, 2000) and may contain viruses within the interstitial portions of the casing. Ozone has been shown to be antimicrobial and antiviral in low ozone demand media, but its efficacy may be affected by the presence of readily available organic matter (Kim, Yousef, & Khadre, 2003). The United States Food and Drug Administration recently amended the food additive regulations to allow the use of gaseous and/or aqueous ozone as an antimicrobial treatment for foods including meat and poultry (CFR, 2003).

The purpose of this study was to initially determine the effects of ozonation on casing quality and ultimately evaluate its potential for inactivation of bacterial and viral pathogens while preserving the integrity of the casings.

Objective

The specific objective of this study was to: Determine the quality and integrity of natural hog casings exposed to ozonated water at ≈7 mg/L for up to four hours.

Methodology

Salted natural hog casings were obtained from a commercial supplier (DeWied International, Inc., San Antonio, TX) and held at ≈4ºC. An Envirowash® Ozone Generator (Pure-O-Tech) was connected to a potable water supply line and used to produce up to 10 mg/L ozonated water. A stainless steel coil submerged in a 55-gallon plastic container filled with iced water served to chill recirculated, ozonated water to a constant temperature range. Previous, trials had shown that chilled water retained more ozone and that a constant ozone level could be achieved in a temperature range of 14.2º-15.9ºC.

After preliminary studies to establish consistent operating parameters, three consecutive sections, 20.3 cm in length, from 30 individual hog casings (38/42 mm dia) were removed and randomly assigned to 0, 2 or 4 h ozonation at ≈7 mg/L ozone and a water temperature of 14.2° – 15.9°C. Each casing section was assigned to a specific ozonation time and identified by attaching different colored plastic clips to the sample. A number was also attached to each sample to identify the casing strand (1 – 30) from which it was taken. The 2 and 4 h treatments were submerged under plastic grating in a 25 L plastic tub filled with recirculating ozonated water. Control samples (0 h ozonation), were placed in a container filled with distilled water and stored in a cooler (≤ 4ºC). At the end of each ozonation period, designated samples were removed from the tub, placed in a container of distilled water and stored at ≤4°C. pH, temperature and ozone level of the water were monitored at 0, 2 and 4 h. All samples were analyzed the following day for bursting strength, and L*a*b* color space values. Maximum rupture force values of casing segments from the same set were collected on three of five replications. The entire experiment was replicated 5 times.

pH Measurement

pH measurements were taken using an Orion™ (model 720A, Orion Research, Inc., Beverly, MA) pre-calibrated pH meter fitted with a combination electrode.

Actual (Spectrophotometric) Ozone Concentration

Actual ozone concentration was determined by the spectrophotometric method of Shechter, (1973). The method involved oxidation of a buffered iodide solution and measurement of the triiodide ion liberated by ozone. A stock iodine solution 0.01 N (1 mL = 240 µg of ozone) was diluted with a neutral potassium iodide reagent to obtain a 4.17×10^{-5} N (1 mL = 1 µg ozone) solution of iodine. This solution was serially diluted and used to prepare a standard curve with neutral potassium iodide reagent as the reference. Ozonated water samples (5 mL) were combined with 45 mL neutral potassium iodide buffered reagent held for 30 min in a cool and dark place and the absorbance was read at 352 nm against a standard curve.

Bursting Strength

Bursting strength was assessed using a modification of the method described by Bakker *et al.* (1999). Bursting strength was defined as the pressure required for rupturing a casing as it filled with water at a constant flow rate of ≈1800 mL/min. Each casing segment (≈ 20.3 cm) was threaded onto a plastic pipe (15 cm length, 19.05 mm o.d. and 12.70 mm i.d.) with seven sets of six holes (3.18 mm dia.) or 42 water outlets equally spaced around the pipe ≈2 cm apart (Figure 1). After clamping each end of the casing to the pipe, one side of the pipe was connected to a distillated water source and the other side connected to a pressure transducer. The casing was then uniformly filled with water at a constant flow rate until the casing burst. The bursting strength data was collected at 20 points/second using a computer connected to the transducer.

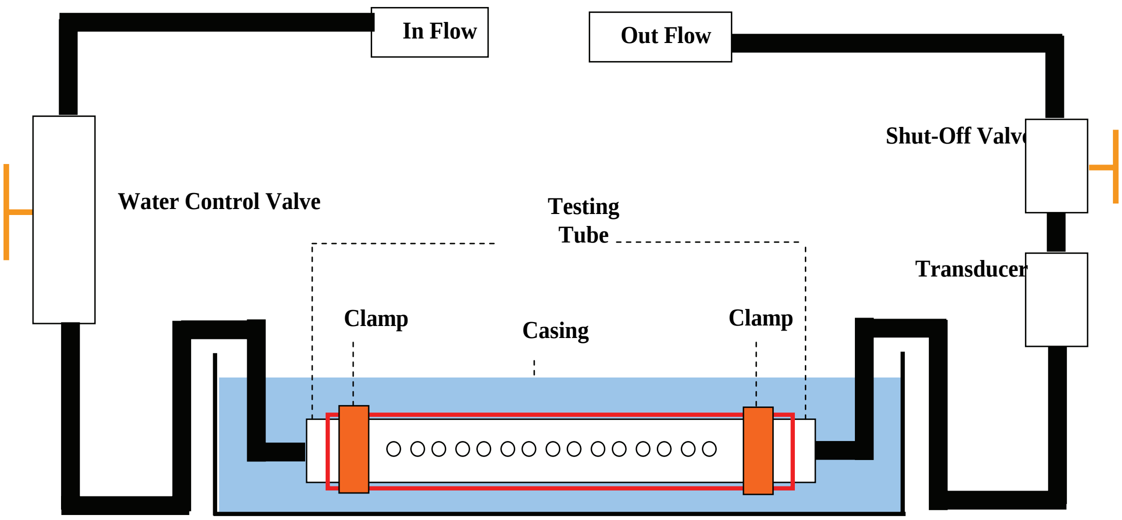

Figure 1: Bursting strength measuring device.

Maximum Rupture Force

A "pull – apart" testing device, similar to that described by Bakker *et al.*(1999) was developed to measure the maximum radial rupture force along a longitudinal section of casing. The device consisted of a solid aluminum bar (2.5 cm dia.; 15.5 cm length) split lengthwise (axially) into two equal halves. The ends of each bar half could then be attached to support brackets affixed to a Materials Testing System (MTS) instrument. For each measurement, a segment of casing (≈ 20.3 cm) was threaded onto the joined bar and the ends attached to upper and lower support brackets. The upper bracket was fixed while the lower bracket was attached to a 20 Kip Axial Torsion Load Frame set in the tension mode. A Sensotec 45.36 kg (100 lbs) load cell applied a 22.68 kg (50 lbs) full scale force to the lower bar at a cross-head speed of 20.32 mm/min. The force at rupture was recorded in kg.

*L*a*b* Color Space Values*

L*a*b* color space values were obtained by reflectance using a Minolta Colorimeter (model CR-200, Minolta Co., Ramsey, N.J., U.S.A) calibrated to a white plate standard surface (C $Y = 93.24$, $x = 0.3137$, y = 0.3196) at channel 00. Two readings were taken from each end of a folded piece of casing (38/42 mm) exposed to ≈7 mg/L ozone for 0, 2 and 4 h. The results were expressed as positive or negative L* (lightness), a* (redness) and b* (yellowness) values.

Statistical Analysis

Mechanical characteristics and the color values of the casings were analyzed as a randomized block split-plot design using the Proc GLM procedure of SAS version 9.0 (SAS Institute, Cary, NC, 2002). The model was tested to determine if differences could be attributed to ozonation times or their interactions. Replications (5) were used as the random effect (blocking factor). When differences between treatment effects were significant ($p \leq 0.05$), a multiple comparison of means was performed using the least significant differences (LSD) method.

Results & Discussion

Preliminary Studies

From preliminary studies, it was determined that chilling the re-circulated water to ≈15.7ºC or lower was required to maintain a steady-state level of ozone (≈7 mg/L) over a 12 h period. After 4 h of ozonation (≈7 mg/L), it was observed that a significant decrease in the bursting strength of the hog casings (38/42 mm) occurred and that they were easily ruptured. Thus, the maximum ozonation time for 38/42 mm casings was determined to be limited to ≤4 h to prevent excessive weakening or deterioration. It was also observed that casings within a hank or even segments within a casing were quite variable in bursting strength due to the inherent nature of the raw material.

Process Conditions

Mean values of temperature, pH and ozone concentrations of ozonated water are presented in Table 1. Temperature values ranged from 14.2º to 15.9 ºC during processing. pH values of ozonated water remained relatively constant after 4 h ozonation. Ozonator readings ranged from 6.4–6.6 mg/L, while the actual (spectrophotometric) ozone concentration of ozonated water varied from 6.8-7.5 mg/L.

Table 1: Mean values of process conditions for ozonation of natural hog casings from 0 to 4 h.

Treatments (h)	Temp. ºC	pH	Ozone Concentration (Ozonator) mg/L	Actual (Spectrophotometric) Ozone Conc. mg/L
0	14.5	8.14	6.6	6.9
2	14.2	8.15	6.4	7.5
4	15.9	8.12	6.5	6.8

Bursting Strength

Analysis of variance p values for bursting strength and L*a*b* color space values of natural hog casings (38/42 mm) exposed to ≈7 mg/L ozone for 0, 2 and 4 h are presented in Table 2. There was no significant interaction effect between casing and ozone treatment nor were there significant differences among casing segments for these attributes.

Table 2: p-values of analysis of variance (ANOVA) for bursting strength, maximum rupture force and color space values of natural hog casings (38/42 mm) treated with ≈7 mg/L ozone for 0, 2 or 4 h.

Treatments	Bursting Strength	Maximum Rupture Force	L*	a*	b*
Casing segments	0.3640	0.0128	0.6677	0.7437	0.5927
Ozone Treatment (h)	<0.0001	0.0127	0.0009	<0.0001	<0.0001
Casing * Treatment	0.6425	0.5718	0.6220	0.6469	0.6855

Mean bursting strength values of hog casings for three ozone exposure times are shown in Table 3 and Figure 2. As ozone treatment time increased ($p \leq 0.05$), the strength of the casing decreased. After 2 and 4 h of ozonation, casings became progressively weaker with the 4 h treatment being more detrimental than the 2 h treatment. It can be concluded that casings exposed to ≈7 mg/L ozone for 2 or 4 h became progressively weaker, compared to non-ozonated casings. These results suggest that ozonation of hog casings (38/42 mm) be limited to not more than 2 h. Further testing by actually stuffing ozone treated casings with sausage will determine the extent of damage caused by 2 and 4 h ozonation times.

Table 3: Mean bursting strength values of natural hog casings (38/42 mm) treated with ≈7 mg/L ozone for 0, 2 or 4 h.

Ozone Treatments (h)	Bursting Strength (kPa)	Standard Deviation (s.d.)
0	16.55[a]	± 4.00
2	14.48[b]	± 3.86
4	13.51[c]	± 4.41

[a,b,c] Different superscript letters in the same column indicate that means are different ($p \leq 0.05$) (LSD).

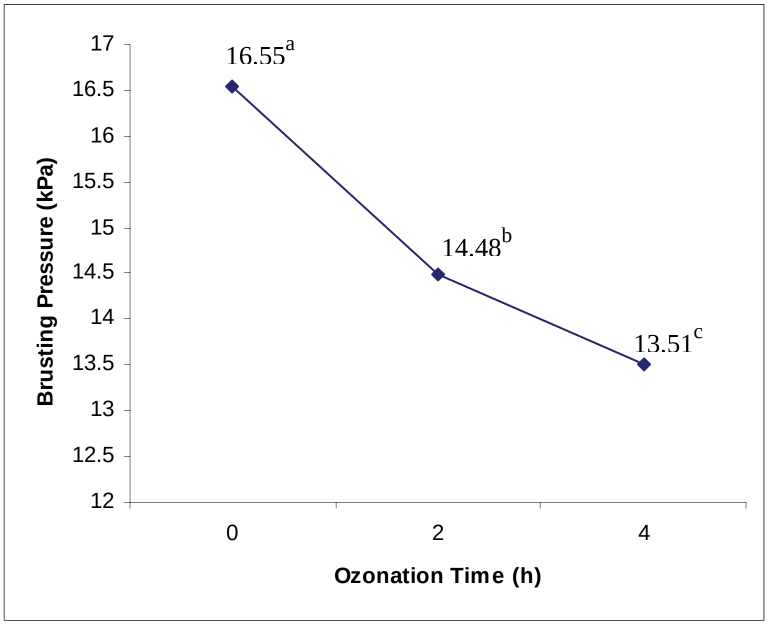

Figure 2: Mean bursting strength (kPa) values of natural hog casings (38/42 mm) treated with ≈7 mg/L ozone for 0, 2 and 4 h.

Maximum Rupture Force

Maximum rupture force was designed to give an indication of a casing's capacity to resist the internal forces applied during filling of a casing. Maximum rupture force values are presented in Table 4. There were no significant interaction effect between casing and ozone treatment. Casing and ozonation treatment effect were significant ($p \leq 0.05$) for maximum rupture force of the casings. Ozonation treatment caused a decline in maximum rupture force values. A significant decrease ($p \leq 0.05$) in maximum rupture force was noted between 0 and 4 h suggesting that ozonation time be limited to ≤ 2 h. This conclusion is supported by the observed decline in bursting strength as shown in Table 2 and Figure 2.

Table 4. Mean maximum rupture force values of natural hog casings (38/42 mm) treated with ≈7 mg/L ozone for 0, 2 or 4 h.

Ozone Treatments (h)	Maximum Rupture Force (kg)	Standard Deviation (s.d.)
0	9.18[a]	±1.94
2	8.95[ab]	±2.07
4	8.66[b]	±2.05

[a,b,c]Different superscript letters in the same column indicate that means are different ($p \leq 0.05$) (LSD).

*L*a*b* Color Space Values*

Ozone treatment affected ($p \leq 0.05$) all L*a*b* color space values of the casings (Table 5). L*a*b* values decreased slightly due to ozone treatments, but the magnitude of these differences were small. A decrease in L* value signifies a slight decrease in lightness of the casing or a slight darkening with exposure to ozone. a* values for redness decreased (became negative) indicating that the casing became less red while declining b* values indicated that casings became less yellow. Corresponding declines in redness and yellowness with ozonation make the casings actually appear lighter, because these components make up the actual hue or color (red, green, blue, etc.) of the casings. The ozone treated casings actually appeared less red and less yellow or in effect slightly lighter.

Table 5: Means and standard deviations of L*a*b* color space values of natural hog casings (38/42 mm) treated with ≈7 mg/L ozone for 0, 2 and 4 h.

	Color Space Values (Minolta)[1]					
Ozone Treatment (h)	L*	s.d. (L*)	a*	s.d. (a*)	b*	s.d. (b*)
0	80.27[a]	±2.96	0.62[a]	±1.18	6.75[a]	±1.52
2	79.69[b]	±2.52	-0.38[b]	±0.84	5.71[b]	±1.49
4	79.83[b]	±2.90	-0.66[c]	±0.75	5.73[b]	±1.63

[1]L* = Lightness (0 = black, 100 = white), a*= redness, – a* = greenness, b* = yellowness, – b* = blueness.

[a,b,c] Different superscript letters in the same column indicate that means are different ($p \leq 0.05$) (LSD).

Conclusions

The bursting strength of natural hog casings ozonated for 0 to 4 h at ≈7 mg/L decreased over time and the casings became progressively weaker. In addition, 4 h of ozonation was noted to be more detrimental than the 2 h treatment. The maximum rupture force also decreased with ozonation time, however, the 2 h treatment was not different from the control. L*a*b* color space values of ozone treatments were slightly lower than the control, but the magnitude of these differences were small. Although there was a slight decrease in L* value (slightly darker), ozonation made the casings actually appear lighter due to declines in the *a** and *b** values.

Results from the mechanical tests performed suggests that ozonation of hog casings (38/42 mm) weakens the casing and that treatment to be limited to less than 2 h at ≈7 mg/L in chilled (< 18°C) ozonated water. Further confirmatory pilot plant filling tests must be conducted to determine if the 2 and 4 h ozone treatments are sufficiently detrimental to cause premature rupture of hog casings.

References

Bakker, W.A.M., Houben, J.H., Koolmees, P.A., Bindrich, U., Sprehe, L. (1999). Effect of initial mild curing, with additives, of hog and sheep sausage casings on their microbial quality and mechanical properties after storage at difference temperatures. Meat Science 51(2), 163–174.

CFR. (2003). Title 21. Ch I – Food and Drug Administration, Department of Health and Human Services. Part 173 – Secondary direct food additives permitted in food for human consumption. Subpart D – Specific Usage Additives. Section 173.368 – Ozone. Code of Federal Regulations. The U.S. Government Printing Office, Washington, DC.

Gabis, D.A., Silliker, J.H. (1974). Salmonella in natural animal casings. Applied Microbiology 27, 66–71.

Kim J.G., Yousef, A.E., Khadre, M.A. (2003). Ozone and its current and future application in the food industry. Advances in Food and Nutrition Research 45, 167–218.

Madhwaraj, S., Nair, K.K.S., Nair P.R., Kadkol, S.B., Baliga, B.R. (1980). Preservation of salted casings. Journal of Food Science and Technology 17, 273–275.

Ockerman, H.W., Hansen, C.L. (2000). Animal By-Product Processing and Utilization. Lancaster, PA: Technomic Publishing Co.

Pearson, A.M., Gillett, T.A. (1999). Processed Meats. Gaithersburg, MD: Aspen Publishers.

Shechter, H. (1973). Spectrophotometric method for determination of ozone in aqueous solutions. Water Research 7, 729–739.