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Salmonella Inactivation During Extrusion of an Oat Flour Model Food

Nathan M. Anderson
U.S. Food and Drug Administration, nathan.anderson@fda.hhs.gov

Susanne E. Keller
U.S. Food and Drug Administration

Niharika Mishra
U.S. Food and Drug Administration

Shannon Pickens
Illinois Institute of Technology

Dana Gradl
U.S. Food and Drug Administration

See next page for additional authors

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Abstract: Little research exists on *Salmonella* inactivation during extrusion processing, yet many outbreaks associated with low water activity foods since 2006 were linked to extruded foods. The aim of this research was to study *Salmonella* inactivation during extrusion of a model cereal product. Oat flour was inoculated with *Salmonella* enterica serovar Agona, an outbreak strain isolated from puffed cereals, and processed using a single-screw extruder at a feed rate of 75 kg/h and a screw speed of 500 rpm. Extrudate samples were collected from the barrel outlet in sterile bags and immediately cooled in an ice–water bath. Populations were determined using standard plate count methods or a modified most probable number when populations were low. Reductions in population were determined and analyzed using a general linear model. The regression model obtained for the response surface tested was Log $(N_R/N_O) = 20.50 + 0.82T - 141.16a_w - 0.0039T^2 + 87.91a_w^2$ ($R^2 = 0.69$). The model showed significant ($p < 0.05$) linear and quadratic effects of a_w and temperature and enabled an assessment of critical control parameters. Reductions of 0.67 ± 0.14 to 7.34 ± 0.02 log CFU/g were observed over ranges of a_w (0.72 to 0.96) and temperature (65 to 100 °C) tested. Processing conditions above 82 °C and 0.89 a_w achieved on average greater than a 5-log reduction of *Salmonella*. Results indicate that extrusion is an effective means for reducing *Salmonella* as most processes commonly employed to produce cereals and other low water activity foods exceed these parameters. Thus, contamination of an extruded food product would most likely occur postprocessing as a result of environmental contamination or through the addition of coatings and flavorings.

Keywords: cereal, extrusion, flour, low water activity, *Salmonella*, thermal inactivation

Practical Application: This study investigated inactivation of *Salmonella* enterica serovar Agona during extrusion processing of an oat flour model food over a wide range of water activity (0.72 to 0.96) and temperature (65 to 100 °C). Results of this study indicate that extrusion is an effective means for reducing bacterial pathogens and may be used by industry when establishing critical limits for extrusion processes.

Introduction

Among the outbreaks of salmonellosis associated with low water activity (a_w) foods in the United States in recent years, several were associated with dry dog food and cereal products (CDC 2016). Extruded pet foods, snacks, and treats remain among the most frequently recalled low a_w products due to contamination with microbial pathogens (FDA 2016). These foods are produced by extrusion processing, a continuous high-temperature, short-time process (HTST) that performs several unit operations including mixing, shearing, cooking, and forming (Grasso and others 2014). The food and feed industry uses extrusion extensively to produce snack foods, cereals, pet foods, and pet treats. Although extrusion processing provides thermal treatment to the product, it was designed mainly to achieve quality outcomes, not necessarily safety outcomes with respect to the destruction of foodborne pathogens. However, it is now being implemented to achieve food safety outcomes as a preventive control to meet new food safety regulations for human and animal food (FDA 2015a, b). Yet, little research exists on microbial inactivation during this operation. Under typical circumstances, where product moisture content (14% to 28%) and extrusion temperatures are high (>100 °C), *Salmonella* would not be expected to survive the thermal extrusion process. However, the possibility that *Salmonella* may survive the extrusion process, as evidenced by both outbreaks and recalls, is troubling. Since thermal resistance of *Salmonella* in foods increases with decreasing product a_w, much longer times and higher temperatures may be required during thermal extrusion in order to significantly reduce or eliminate pathogens (Podolak and others 2010).

Residence time and temperature of the extrusion process may be controlled by altering feed rates, screw speeds, and barrel temperature during processing. These physical parameters are often explored, but extrusion studies describing microbial inactivation reported in the literature often lack comprehensive data with respect to varying moisture content (4% to 18%) of extruded foods and feed examined (Quéguiner and others 1989; Likimani and others 1990; Ukuku and others 2012). In the few studies that did examine both the effect of moisture content and temperature

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on microbial reduction, intermediate to high moisture products (21% to 31%) were used (Okelo and others 2006; Bianchini and others 2012).As *Salmonella* has greater thermal resistance at low a_w, inactivation of *Salmonella* by thermal treatment may become difficult as moisture content decreases (Goepfert and others 1970; Quéguiner and others 1989). Thus, it would be beneficial to explore the efficacy of extrusion at lower a_w levels.

Therefore, the goals of this research were to evaluate the efficacy of thermal extrusion as an inactivation step for *Salmonella* in a model low a_w food over a wide range of moisture contents and extrusion temperatures, and to identify fail-safe conditions for *Salmonella* inactivation during thermal extrusion processing.

Materials and Methods

Bacterial culture preparation

Salmonella enterica serovar Agona (strain 447967; originally isolated from puffed rice cereal) was obtained from the FDA Arkansas Regional Laboratory (Jefferson, Alaska, U.S.A.) and stored as a stock culture at refrigeration temperatures on trypticase soy agar supplemented with 0.6% yeast extract (TSAYE) (BD Difco, Sparks, Md., U.S.A.). The culture was transferred to a fresh TSAYE plate on a monthly basis. The $D_{80°C}$ value for this isolate is 29.7 min (Hildebrandt and others 2016). For each inoculum, a single isolated colony was transferred from the stock plate to 10 mL of tryptic soy broth supplemented with 0.6% yeast extract (TSBYE) (BD Difco) and incubated for 24 h at 37 °C. The culture (0.5 mL) was transferred to TSAYE plates, spread using a sterile L-shaped spreader, and then incubated for 24 h at 37 °C. The cells were harvested by pipetting 3 mL of buffered peptone water (BPW) (Remel, Lenexa, Kans., U.S.A.) onto the plate, which was then scraped gently using a sterile L-shaped spreader. The resultant slurry was then pipetted into a sterile Falcon tube (BD, Franklin Lakes, N.J., U.S.A.). Each set of 5 plates harvested yielded approximately 11 mL of cells at approximately 11 log CFU/mL. The harvested cells were serially diluted in BPW, plated on TSAYE, and incubated for 24 h at 37 °C for enumeration.

Preparation of inoculated flour

Oat flour (ConAgra Foods, Omaha, Nebr., U.S.A.), which has an indigenous fat content of approximately 8.5%, was selected for use as a simple model food system that fills a gap in the literature with respect to extrusion of low a_w foods. Oat flour (1 kg) was aseptically transferred to a sterile mixing bowl that was placed in a mixer (Model N50A, Hobart, Troy, Ohio, U.S.A.) inside of a biosafety level 2 (BSL-2) cabinet (SterilGard III Advance SG603, The Baker Co., Sanford, Maine, U.S.A.). Prepared inoculum (10 mL) was loaded into a sterile syringe (Cole Parmer Instrument Co., Vernon Hills, Ill., U.S.A.) that was attached using sterile silicone tubing to a liquid atomizer (Model CV24, Sonics and Materials, Inc., Newtown, Conn., U.S.A.). The inoculum was atomized (660 Hz) onto the flour, which was then mixed for 30 min to homogeneously distribute the cells. The inoculated flour was stored in airtight buckets for 3 d at 25 °C for moisture equilibration. From each batch of inoculated flour, ten 1-g samples were aseptically taken from random locations to evaluate homogeneity. Each 1-g sample was weighed on a balance (Model E1B120, Ohaus Corp., Parsippany, N.J.) and was mixed with 99 mL BPW and stomached for 30 s. Samples were enumerated on TSAYE as indicated previously. Sampling was reduced to five 1-g samples per week after the stability of the batches and the consistency of the inoculation procedure were established.

Each 1-kg batch of inoculated oat flour was divided into two 500-g batches and each portion was added to 5.5 kg of uninoculated flour. Approximately 1.5 g of Red 40 pellets (Sensient Technologies, Milwaukee, Wis., U.S.A.) were added to each 6-kg batch to aid in detection of the inoculated flour during processing. The 6-kg batch was mixed in a large mixer (Model HL200, Hobart) inside of a walk-in BSL-2 cabinet (Model S125.636, Nuaire, Plymouth, Minn., U.S.A.) for 30 min and stored for 7 d in an airtight plastic bucket at ambient temperature (23 ± 2 °C) until utilized for experimentation. On the day of experimentation (day 7), in a BSL-3 pilot plant (Inst. for Food Safety and Health/Illinois Inst. of Technology, Bedford Park, Ill.), the two 6-kg batches produced from 1 inoculum were combined as a single 12-kg batch in a plastic bucket, lidded and tumbled to combine. To evaluate homogeneity in the final inoculated batches, five 1-g samples were removed from random locations and weighed into sterile Whirl-Pak bags. Each 1-g sample was enumerated as described earlier. Sampling was reduced to three 1-g samples per batch each week after the stability of the batches and the consistency of the method was established.

Experimental design

Worst-case process criteria such as low temperature (<100 °C), low a_w, low pressure, low shear, and a single-screw extruder were targeted in thermal extrusion processing experiments. A central-composite response surface design (CCRSD) was used to determine 9 target extrusion treatment conditions to be tested over target ranges for water activity (a_w 0.72 to 0.96; 14% to 28% moisture content) and temperature (60 to 100 °C). The response surface design was developed based on previously published values of thermal resistance of *Salmonella* at similar a_w levels. As shown in Figure 1, 2 replications of the factorial points (open circles), 2 replications of the axial points along the coordinate axes of the factor levels (gray circles), and 5 replications of the central point of the 2-level factorial design (solid black circle) were planned to improve the precision of the experiment. Three treatment conditions of similar temperature (for example, the axial and center points along the horizontal centerline) were, on any given day of extrusion trials, evaluated in decreasing order of a_w to minimize carryover effects. Replications of the treatment conditions were conducted on different days with different inoculum.

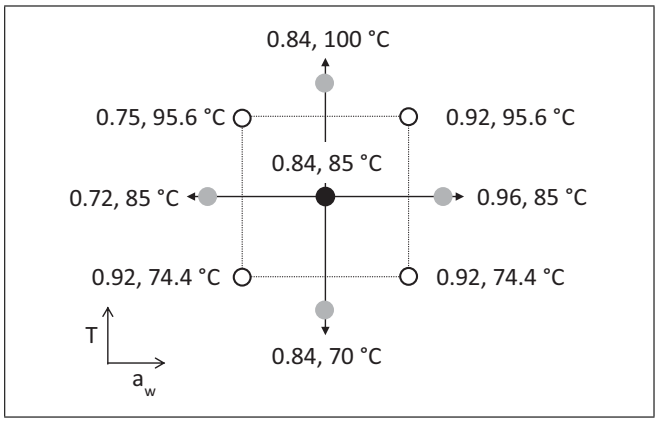

Figure 1–Central composite response surface experimental design for temperature and water activity treatment conditions targeted during extrusion trials. Open circles (○) indicate factorial points, shaded gray circles (●) represent axial points, and the solid black circle (●) represents the central point.

Extruder setup

A single-screw extruder (Model X85, Wenger Manufacturing, Inc., Sebetha, Kans., U.S.A.) was installed in the BSL-3 pilot plant mentioned previously (Figure 2). The system utilized was comprised of several components. A live bin feed hopper metered the oat flour into the preconditioner at a set weight-basis rate of 75 kg/h, and a variable speed peristaltic pump delivered ambient temperature water to the preconditioner near the inlet. The preconditioner served as a continuous mixer and operated at ambient temperature, that is, no heat, hot water, or steam was added. The preconditioner fed the moistened oat flour into the inlet of the extruder barrel. The extruder had a screw diameter of 85 mm (3.3 inch), a capacity of 75 to 800 kg/h with a variable screw speed up to 600 rpm. The extruder barrel was divided into 5 sections, each of which had a linear groove pattern parallel to the direction of product flow. In the first 4 sections, a single-flight conveying screw was used; the barrel jacket was not heated and no steam or water was added. In the last section before the outlet, a double-flight screw with a uniform shaft diameter was used and the barrel steam jacket was used to preheat the extruder and to maintain the target temperature. A conical-shaped die head assembly (Figure 2) was fitted to the extruder outlet. A manual screw was used to move the die head in or out, which resulted in extrudate of thickness that varied from 3 to 40 mm, to achieve the desired target extrudate temperature. The die head was also equipped with a resistive thermal device (RTD) probe (Model RBF2853MB38Z-00-9HP23-F3J012-9, Pyromation, Fort Wayne, Ind., U.S.A.) to measure temperature of the extrudate at the die face. The feed rate and extruder speed were held constant at 75 kg/h and 500 rpm, respectively, and monitored for all trials. At the beginning of each trial, appropriate steady-state conditions were first established by running uninoculated flour through the system. Residence time of the product inside the barrel was measured by dropping Red 40 colored dough balls ($n = 4$) into the inlet of the extruder barrel and recording the time required for red color to appear.

The relationship between moisture content of oat flour and a_w was determined to facilitate rapid measurement during extrusion trials. Moisture content was measured with Moisture Analyzer (Model MB 45, Ohaus Corp.) and a_w was measured with a Dew Point Water Activity Meter (AquaLab Model 4TEV, Decagon Devices, Pullman, Wash., U.S.A.). At the preconditioner, flour moisture content was initially adjusted to the target value and measured using a moisture analyzer prior extrusion experiments. Based on the measurement, flour moisture content was readjusted, as needed. After achieving the desired steady-state treatment condition per the experimental design, the uninoculated flour in the live bin feed hopper was allowed to empty to a minimal amount (approximately 1 to 2 kg). Then, a 12-kg batch of inoculated flour was added to the feed hopper and run through the extruder. When the red dye present in the inoculated flour became visible at the die face, sampling commenced. Ten extrudate samples (approximately 500 g each) were collected into sterile Whirl-Pak bags, manually pulverized and cooled immediately in an ice–water bath. Moisture content and a_w of flour and extrudate samples were measured in the lab post-processing as described below.

Enumeration of *Salmonella*

Inoculated oat flour was examined for populations of *Salmonella* during and after extrusion. From each collected sample, 10 g was removed and added to a Whirl-Pak bag with 90 mL of BPW. When high populations of *Salmonella* were anticipated, that is, above background microflora levels (2.63 ± 0.58 log CFU/g), appropriate serial dilutions were made with BPW and 0.1 mL was spread plated in duplicate onto both TSAYE and xylose lysine deoxycholate agar (XLD, BD Difco). Plates were incubated for 24 h at 37 °C. When total colony counts on TSAYE and XLD did not coincide, which indicated the presence of either injured cells or other microorganisms, colonies on TSAYE were picked and cultured on XLD for confirmation. Plates showing growth of black colonies typical of *Salmonella* on XLD were considered positive for *Salmonella*. Representative colonies from XLD were later confirmed as *Salmonella* using a *Salmonella* O Antiserum Poly A test (BD Difco).

When low populations of *Salmonella* were anticipated, a modified 5-tube most probable number (MPN) method (Blodgett 2010) was used to determine *Salmonella* populations. Serial dilutions ($n \geq 3$) were inoculated into test tubes containing 10 mL TSBYE (limit of detection 0.018 MPN/g). Tubes were incubated for 24 h at 37 °C. Following incubation, tubes showing growth were streaked onto XLD agar and incubated at 37 °C for 24 h.

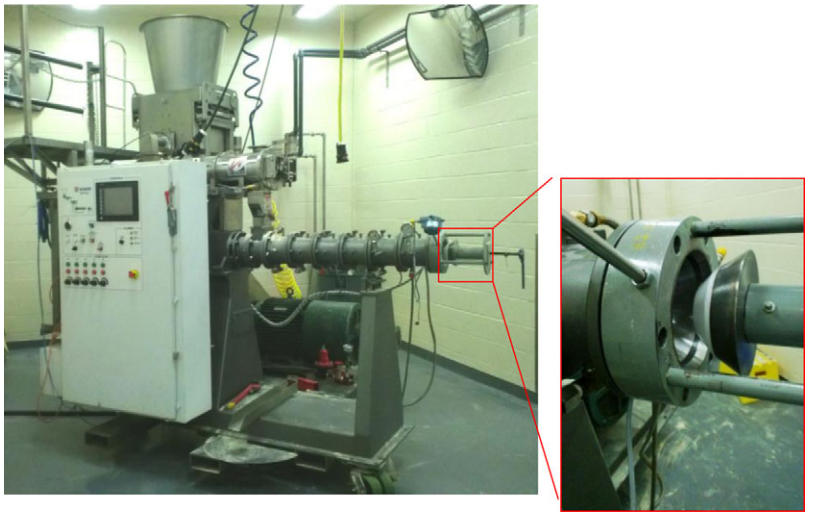

Figure 2–Wenger X85 single screw extruder and adjustable, conical shaped die face.

Table 1–Product and process parameters recorded during extrusion trials and *Salmonella* population reduction obtained at test conditions.

Target		Measured		Die opening		Reduction
T (°C)	a_w	T (°C)	a_w	(mm)	Replicates	(log CFU/g)
65.0	0.72	65.3	0.70	30	1	0.67 ± 0.14
74.4	0.72	74.6	0.72	28	1	4.20 ± 0.63
85.0	0.72	84.8	0.68	26	2	6.26 ± 1.15[a]
74.4	0.75	74.4	0.78	29	2	3.84 ± 0.72
95.6	0.75	95.4	0.75	12	1	5.00 ± 1.76[a]
70.0	0.84	69.6	0.84	29	2	1.95 ± 0.75
85.0	0.84	84.8	0.82	18	5	4.67 ± 1.15
100.0	0.84	101.1	0.81	6	1	7.24 ± 0.02[a]
74.4	0.92	74.5	0.90	12	2	3.33 ± 1.21
95.6	0.92	95.8	0.90	4	1	7.34 ± 0.02[a]
95.6	0.92	88.8	0.92	3	1	5.36 ± 1.46[a]
85.0	0.96	84.1	0.95	7	2	7.05 ± 0.36[a]

[a]Denotes log reduction values plated on TSAYE, nonasterisk values enumerated with MPN.

Typical black colonies on XLD were presumed to be *Salmonella*. MPN tubes showing both growth and subsequently identified as containing *Salmonella* via black colonies on XLD were considered positive and enumerated as MPN/g. Representative colonies from XLD were later confirmed as *Salmonella* using a *Salmonella* O Antiserum Poly A test (BD Difco).

Statistical analysis

A 2nd-order regression model was fitted to the log reduction data to describe the effect of extrusion process variables (a_w and temperature) on inactivation of *Salmonella*. The model included the linear and quadratic terms of the independent variables. Statistical analysis with a general linear model (GLM) was completed using SAS 9.2 (SAS Inst. Inc., Cary, N.C., U.S.A.).

Results and Discussion

Homogeneity and stability of inoculum

In total, 21 extrusion trials were conducted. A separate 12-kg batch of inoculated flour was prepared for each trial. The background microflora of the oat flour was determined to be 2.63 ± 0.58 log CFU/g. The average *Salmonella* population of the initial cultures (n = 21) was 10.81 ± 0.19 log CFU/mL. The average *Salmonella* population of the 1-kg inoculated flour batches was 8.44 ± 0.21 log CFU/g. A representative data set collected to evaluate homogeneity and stability of inoculated flour is shown in Figure 3. *Salmonella* populations were considered to be homogenous if the standard deviation for the *Salmonella* levels was ≤0.3 log CFU/g. Because the extruder was running 75 kg of material per hour,12 kg of inoculated flour was needed in order to provide adequate run time (approximately 10 min) with inoculated material for obtaining samples. The larger 12-kg batches of inoculated flour had *Salmonella* populations of 7.00 ± 0.16 and 6.48 ± 0.20 log CFU/g at days 7 and 15, respectively. Extrusion trials were carried out on day 15 after preparation of the inoculated flour. An average of a 0.68 log CFU/g drop was observed between the day of preparation of inoculated flour and the day of use. This initial drop and subsequent *Salmonella* survival is consistent with observations made in other low a_w foods (Keller and others 2013; Blessington and others 2014; Gradl and others 2015)

Extrusion of inoculated oat flour

Extrusion treatment conditions, the respective number of replications, and *Salmonella* reductions achieved are given in Table 1. At the beginning of each trial, appropriate steady-state conditions (75 kg/h, 500 rpm, and the target temperature and moisture content) were first established by running uninoculated flour through the system. Target moisture content was determined from the moisture sorption isotherm shown in Figure 4 and per the a_w level in experimental design. Pressure inside the extruder barrel was observed to be 0 kPa for all trials, which indicated that back pressure in the barrel was minimized. The residence time of the product inside the barrel was 11.3 s. The actual a_w and temperatures documented during extrusion are given in Table 1. Extrudate temperature and a_w at the die confirmed that target treatment conditions were achieved. Reaching the highest temperatures (95.6 and 100 °C) proved difficult, and therefore it was only possible to run 1 replicate at these temperatures. When attempting to replicate the trial at a_w 0.92 and 95.6 °C, the temperature only reached 88.8 °C (see Table 1), but these data were still included in the analysis. During actual extrusion trials, the GLM output from preliminary analysis suggested that additional testing was necessary to better define *Salmonella* inactivation at the extremes of the response surface. Thus, a_w and temperature combinations of a_w

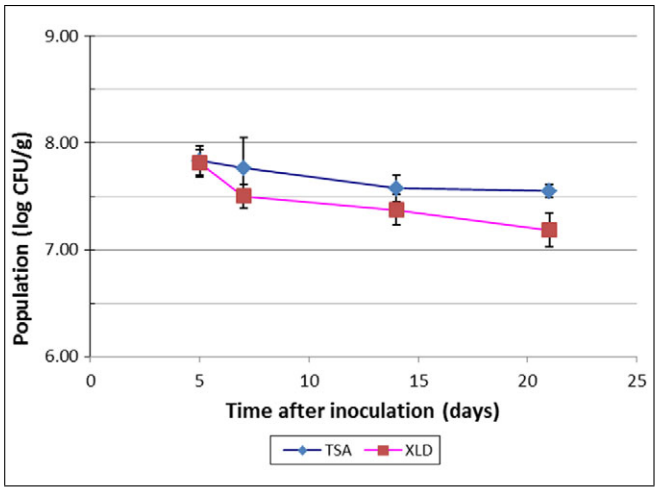

Figure 3–Homogeneity and stability over time of *Salmonella* Agona inoculated in 1 kg of oat flour. Error bars expressed as standard deviation.

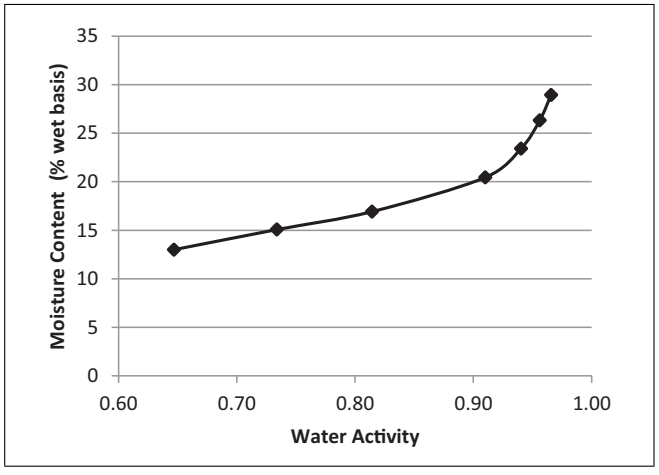

Figure 4–Moisture sorption isotherm for oat flour at 25 °C.

0.72, 65 °C and a_w 0.75, 74.4 °C were also tested. The trial at a_w 0.72, 65 °C served as a control run as there was virtually no back pressure at the die face, and thus the extrudate leaving the extruder barrel was essentially untreated flour. Consequently, this treatment condition resulted in negligible inactivation of *Salmonella* (that is, levels posttreatment was approximately equal to the population levels of the inoculated flour).

Reductions of 0.67 ± 0.14 log CFU/g at a_w 0.72, 65 °C and 7.34 ± 0.02 log CFU/g at a_w 0.96, 100 °C were demonstrated (Table 1). The a_w and temperature treatment combinations that resulted in greater than 5-log reductions were a_w 0.72, 85.0 °C; a_w 0.75, 95.6 °C; a_w 0.84, 100 °C; a_w 0.92, 88.8 °C; a_w 0.92, 95.6 °C; and a_w 0.96, 85.0 °C. With the exception of the treatment conditions of a_w 0.72, 65 °C and a_w 0.84, 70 °C, plate counts for all other treatment conditions were below the limit of detection (50 CFU/g) even on nonselective media (TSAYE). Furthermore, when treatment conditions allowed the use of plate media, populations determined on XLD were always much lower than those determined on TSAYE. Subsequent testing of colonies on TSAYE suggested that the *Salmonella* that failed to grow on XLD, but grew on TSAYE, were injured cells. Therefore, the log reduction values for all the treatment conditions except the aforementioned 2 conditions were determined by the modified MPN, which involved the use of TSBYE as enrichment and general growth, followed by identification of that growth as *Salmonella* on XLD.

Statistical analysis

The regression model consisted of linear and quadratic terms of the 2 factors (Eq. (1))

$$\log\left(\frac{N_R}{N_0}\right) = 20.50 + 0.82T - 141.16a_w - 0.004T^2 + 87.92a_{w^2} \quad (1)$$

where N_0 represents the initial population, N_R corresponds to the population after extrusion, T is the temperature and a_w is the water activity. The response surface curve of the reduction data is given in Figure 5. Though the model showed significant ($p < 0.05$) linear and quadratic effects of a_w and temperature (Table 2), Figure 5 shows that temperature has a pronounced effect on inactivation of *Salmonella* during extrusion. For example, at a_w 0.84, the average reductions obtained at 70, 85, and 100 °C were 1.95, 4.67, and 7.24 log CFU/g, respectively; however, at a constant temperature with increasing a_w, a decreasing trend in *Salmonella* reduction was not observed. At 85 °C, the average reductions achieved at a_w 0.72, 0.84, and 0.96 were 6.26, 4.67, and 7.05 log CFU/g, respectively. The higher reduction observed at a_w 0.84 compared with 0.72 is unexpected and there is no clear explanation for this result. At processing conditions above 82 °C and a_w of 0.89 (19% moisture content, Figure 4), an average 5-log reduction was achieved (Figure 4). These findings are consistent with the findings of others. Crane and others (1972) demonstrated that thermal extrusion completely eliminated *Salmonella* from feed at 25% to 35 % moisture content when temperatures were above 93.3 °C. Similarly, Okelo and others (2006) reported complete inactivation of *Salmonella* Typhimurium in feed containing 28.5% moisture that was extruded at 83 °C and 7 s residence time. Similar results were also observed by Himathongkham and others (1996) and Bianchini and others (2012) for inactivation of the pathogen, *Salmonella* Enteritidis, and *Enterococcus faecium*, a common surrogate test organism for *Salmonella,* during extrusion of feed. However, Bianchini and others (2014) reported that the *Salmonella* population in a balanced carbohydrate–protein meal typical of an extruded pet food formula at 28% moisture was reduced by 5 log CFU/g at just 60.6 °C, which conflicts with the findings of our study and a study by Walsh and Funke (1975) that demonstrated a 1-log reduction of *Staphylococcus aureus* during low-temperature extrusion (35 to 55 °C) of spaghetti at an initial moisture content of 31.5%. Our results also differ with those obtained by Ukuku and others (2012) who demonstrated that corn meal and whey protein isolate extruded at 55 °C with a_w of 0.98 to 0.99 (35.5% to 42.1% moisture content) resulted in greater than 5-log and 4-log reductions of *Escherichia coli*, respectively, and Quéguiner and others (1989) who found that temperatures over 130 °C were necessary to achieve a greater than 4-log reduction of *Streptococcus thermophiles* in whey powder having a very low initial moisture content (4% to 5%). Nevertheless, it is apparent that thermal extrusion processing conditions typically employed by industry using twin-screw extruders (14% to 28% moisture, >100 °C, high shear, and high pressure) effectively reduce *Salmonella*. However, the appropriate target reduction is difficult to pinpoint as the risk profile of extruded products is not well known and may vary with product. Dry pet foods derived from rendered animal protein meals appear to have the worst-case initial bioburden of extruded products. Prevalence of *Salmonella* in these products may be assumed to be 100% and levels may exceed 10^3 CFU/g (Franco 2005; Lambertini and others 2016).

Table 2–Statistical analysis of extrusion temperature and a_w parameters.

Parameter	Estimate	Standard error	*t* Value	Pr > |*t*|
Intercept	20.50	14.90	1.38	0.1720
Temperature	0.82	0.20	4.17	<0.0001
a_w	−141.16	28.15	−5.02	<0.0001
Temperature^2	−0.004	0.001	−3.38	0.0010
$a_w{}^2$	87.92	17.32	5.08	<.0001

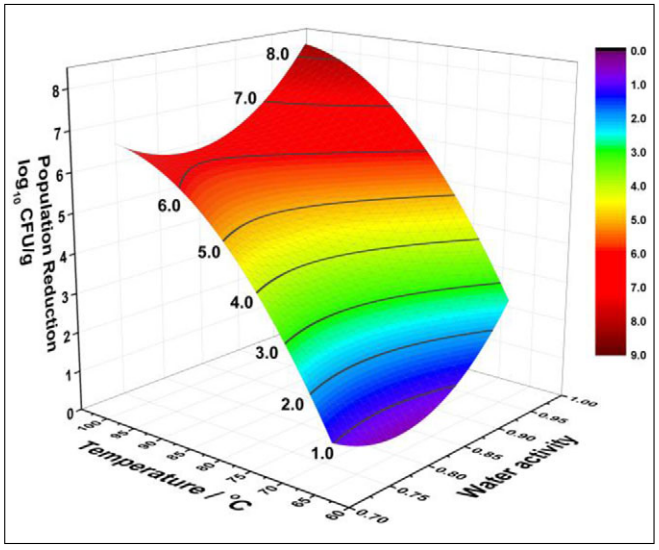

Figure 5–Response surface curve for survival of *Salmonella* Agona during extrusion as a function of temperature and a_w.

Conclusion

The current study examined inactivation of *Salmonella* in a wide range of initial product moisture levels (14% to 28%, 0.72 to 0.96

a_w) and extrusion temperatures (<100 °C) and fulfilled a need for information on *Salmonella* inactivation during extrusion processing at low temperatures. The result of the extrusion study and model indicate that extrusion is an effective method to reduce *Salmonella* under extrusion processing conditions currently employed in industry (14% to 28% moisture, >100 °C). Because worst-case and often failure prone conditions were tested, this model also defines validated processing criteria that provide adequate levels of treatment and margins of safety. Consequently, *Salmonella* contamination in extruded foods is most likely the result of improper processing, processing failures, or postprocessing contamination.

Acknowledgments

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