ABSTRACT

Title of Thesis: RELATING SPORE POPULATION OF ACID PRODUCING THERMOPHILIC ORGANISMS AND ARTIFICIALLY INOCULATED *BACILLUS STEAROTHERMOPHILUS* TO PROCESSING VARIABLES DURING EXTRUSION OF A STANDARDIZED FEED FORMULATION

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Salmonella and other pathogenic organisms that infect poultry and other livestock can originate from feed or the environment. Technologies to control *Salmonella* infection in poultry are important measures taken to reduce turkey and poultry production losses. A study was designed to determine extrusion conditions that optimized microbial inactivation in feed using the Extru-tech E325 single-screw extruder. *Bacillus stearothermophilus* was selected as the indicator organism after no viable cells of *Salmonella typhimurium* were detected when 28.5 % moisture content mash feed (wet basis) was extruded at 83 ^{o}C extruder barrel exit temperature with a 7 second feed retention time in the extruder barrel. Spores of *B. stearothermophilus* inoculated into a standardized feed formulation consisting of 60% corn meal, 30 % soybean meal,

and 10% animal protein blend, by mass, respectively, was used to investigate the effect of 3 extrusion variables on microbial inactivation. The 3 variables were extruder barrel exit temperature (T), mash feed moisture content (Mc), and mean retention time of feed in the extruder barrel (Rt). A rotatable central composite statistical design was used with 3 factors and five levels. The quadratic response surface model fit to acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* spore inactivation data was used to predict extrusion conditions that maximized inactivation. The response surface indicated a stationary point within the design region that was a saddle at T = 81 ^{o}C, Mc = 27.6 % wet basis (wb) and Rt = 8 s resulting in 0.170 log cycles of acid producing, thermophilic organisms and *B. stearothermophilus* spore inactivation. An estimated ridge of maximum inactivation showed a maximum of 1.03 log cycles at T = 110 ^{o}C, Mc = 24.5 % wb and Rt = 11 s. Because the least severe extrusion conditions (T = 83 ^{o}C, Mc = 28.5 % wb and Rt = 7 s) completely inactivated *S. typhimurium* in the standardized feed, it was speculated that all *S. typhimurium* cells would be inactivated at any set of extruder conditions within the central composite design region.

RELATING SPORE POPULATION OF ACID PRODUCING THERMOPHILIC ORGANISMS AND ARTIFICIALLY INOCULATED *BACILLUS STEAROTHERMOPHILUS* TO PROCESSING VARIABLES DURING EXTRUSION OF A STANDARDIZED FEED FORMULATION

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Dissertation submitted to the Faculty of the Graduate School
of the University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2003

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ACKNOWLEDGEMENTS

The completion of this dissertation has been made possible by family, friends, and associates who have kindly and freely given of their time and effort to support me in a multitude of ways throughout the period of this study. I would like to take this opportunity to thank those who have made it possible:

Dr. Lewis Carr, Extension Agricultural Engineer / Instructor, Department of Biological Resources Engineering, for his invaluable encouragement and timely guidance during this study.

Dr. Fredrick Wheaton, Professor, Chairman, Department of Biological Resources Engineering, for providing funds, incessant encouragement, guidance and encouragement during this study.

Dr. David Wagner, Division of Veterinary Medicine, U. S. Food and Drug Administration, for arranging access to the feed extrusion and related facilities at U.S. Food and Drug Administration laboratories in Laurel, Maryland, and guidance throughout this study.

Dr. Larry Douglass, Professor, Department of Animal and Avian Sciences, for providing statistical guidance during this study.

Dr. Sam Joseph, Professor, Department of Cell Biology and Molecular Genetics, for guidance and encouragement during the study.

Dr. Paul Schreuders, Assistant Professor, Department of Biological Resources Engineering, for guidance and encouragement throughout this study.

Dr. Yang Tao, Department of Biological Resources Engineering, for guidance and encouragement during this study.

To Mr. Neil Schibblehut, Division of Veterinary Medicine, U. S. Food and Drug Administration, for assisting with operation of and ensuring the smooth running of feed processing milling, mixing and extrusion equipment, respectively.

To my father, Mr. Elijah Okelo, my mother Mrs. Pamela Okelo and all my siblings for their invaluable support in all ways throughout this study.

To my wife, Andy and son Ethan, for their love, support and encouragements throughout this study.

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A_d	=	area across which diffusion of a substance occurs, m^2
A_h	=	area normal to x direction through which heat flows, m^2
a_w	=	water activity, a measure of water available for bacterial activity(p / p_o), dimensionless
B	=	Arrhenius equation constant, dimensionless
CFU	=	colony forming units, colonies / 20 g feed
CFU_D	=	desired spore concentration in final inoculated feed mash, colonies / 20 g feed
CFU_{FI}	=	spore density in feed inoculum, colonies / 20 g feed
CFU_{MF}	=	spore density in mash feed, colonies / 20 g feed
dc	=	change in concentration of substance, kg / m^3
d_{gw}	=	geometric mean diameter of particles by mass, microns ($1x10^{-6}$ m)
d_i	=	nominal sieve aperture size of the i^{th} sieve, microns ($1x10^{-6}$ m)
dT	=	temperature drop between heat source, barrel wall, and spore location, ^oC
dx	=	change in linear distance in the direction of mass transfer of substance, m
DTA	=	dextrose tryptone agar
D	=	diffusivity of diffusing substance, m^2 / s
D_x	=	decimal reduction time of an organism at a temperature x, minutes

Ea	=	activation energy (enthalpy of inactivation of bacterial spores), kJ / kg
GMD	=	geometric mean particle diameter, microns ($1x10^{-6}$ m)
Hz	=	Hertz (1 Hz = 1 revolution / second or 2∏ radians / second), radians / second
IU/lb	=	International Unit per pound. IU is a unit used to measure the biological activity (that is, the effect) of many vitamins and drugs (1 IU is 0.0003 mg for vitamin A, 0.05 mg for vitamin C, 0.000 025 mg for vitamin D, and 2/3 milligram for (natural) vitamin E).
k	=	rate constant of inactivation of bacterial spores, per unit of time
k_c	=	thermal conductivity of feed material, W / m ^oC
L	=	liter
m	=	replication at the center of the central composite statistical design, dimensionless
$\dot{m}$	=	mass flow rate of diffusing substance, kg / s
M_{AF}	=	mass of ambient feed, before addition of water, kg
Mc	=	moisture content, wet basis, of feed prior to extrusion, %
Mc_{AF}	=	moisture content of ambient feed, before addition of water, wet basis, %
Mc_D	=	targeted moisture content of feed, %
M_{MF}	=	mass of mash feed, kg
M_W	=	mass of water added to a 10 kg feed batch, kg
M_{wet}	=	mass of feed sample before oven-drying, g
M_{dry}	=	mass of feed sample after oven-drying, g
M_{FI}	=	mass of feed inoculum, kg

N_v	=	total number of independent variable combinations, required in a rotatable central composite statistical design, dimensionless
n	=	number of independent variables, dimensionless
p	=	vapor pressure of a food product, Pascal
p_o	=	vapor pressure of pure water, Pascal
PSD	=	particle size distribution, microns ($1x10^{-6}$ m)
q_x	=	rate of heat flow in x direction, W
R	=	ideal gas constant (8.314), kJ / kg ^oC,
$R_{targeted}$	=	density of targeted organism recovered from feed sample, CFU / g feed
R_{total}	=	density of all organisms recovered from feed sample using a specified enumeration media, CFU / g feed
R^2	=	R–squared, coefficient of determination, dimensionless
Rt	=	retention time of feed in the extruder barrel, s
RTD	=	retention time distribution
RSREG	=	response surface regression
SE	=	standard error of the quantity in question, units of quantity measured
$SD_{observed}$	=	spore density as observed by the specified enumeration method, CFU / g feed
$SD_{predicted}$	=	predicted spore density as based on amount of artificially inoculated spores, CFU / g feed
S_{gw}	=	geometric standard deviation of particle diameter, d_i, by mass, microns ($1x10^{-6}$ m)
Slog	=	geometric standard deviation of log-normal distribution by mass, dimensionless
S_{sen}	=	sensitivity of a specified spore enumeration method, %

S_{spe}	=	specificity of a bacterial spore enumeration method,%
T	=	extruder barrel exit temperature, ^{o}C
T_K	=	extruder barrel exit temperature, K
T_{aw}	=	temperature in a_w meter sample chamber, ^{o}C
W_i	=	mass on i^{th} sieve, g
x	=	linear distance in the direction of heat flow, m
z	=	thermal resistance constant for bacterial spores, ^{o}C
α	=	value of the axial coded factor in a central composite design, dimensionless

1. INTRODUCTION

Under certain conditions of ambient temperature and humidity, contamination of agricultural commodities with microorganisms is unavoidable. Decontamination techniques must then be considered for such products. Molds and bacteria such as *Salmonella*, *Bacillus spp., Listeria* and pathogenic *Escherichia coli* often contaminate grain, feed ingredients and animal feed. These microorganisms are transmitted to livestock through consumption of contaminated feedstuffs (Wyatt, 1995) and ultimately affect the human food supply (Cox et al., 1986).

Some of *Salmonellae* cause diseases that have adverse financial implications on the poultry industry; poultry and other livestock are considered important sources of *Salmonellae* species that cause human Salmonellosis (Ekperigin et al., 1991). In a study to investigate the feasibility of producing and maintaining *Salmonella*-free turkeys, Pomeroy et al. (1989) reported that contaminated feed might have been the source of *Salmonella* contamination in the turkey breeding stock that had been *Salmonella*-free for over three years. The United States Advisory Committee on Salmonella reported that production of *Salmonella*-free feed is dependent on determination of optimum combinations of temperature, heating time, and moisture content of the feed that will kill *Salmonellae* contained in the feed. Providing *Salmonella*-free feed is considered an essential part of efforts to control *Salmonella* in poultry (Liu et al., 1969).

Pomeroy et al. (1989) reported that measures to control production losses related to *Salmonella* infections in turkey operations in the United States cost about $10 million yearly. Contaminated feed, eggs and the environment are the main sources of *Salmonella* infection in poultry (Ekperigin et al., 1991). As few as one colony-forming unit of *Salmonella* per gram of feed is all that is required to initiate infection in poultry feed. In order to eliminate *Salmonella* from poultry flocks, it necessary to raise poultry on *Salmonella*-free feed (Schleifer et al., 1984; Liu et al., 1969). Lieu et al. (1969) further observed that optimum combinations of temperature, heating time and feed moisture content required to kill the *Salmonellae* in feed during processing may be defined, but there were no equipment that can achieve the desired temperatures under practical conditions. Combinations of extrusion or pelleting conditions used in the manufacture of feed vary widely and are often not carefully controlled; the moisture content of feed required to produce a desired pellet quality varies with the feed formulation (Levine, 1992).

It is recognized that excessive heat application can reduce the nutritional value of feed and drugs as well as increase the cost of heating, adding moisture, subsequent cooling, and drying. The heat energy input for manufacture of feed should be minimized as long as it produces a nutritious and pathogen-free product.

2. LITERATURE REVIEW

2.1 Effect of Thermal Processing on Feed Microorganisms

Ekperigin et al. (1991) examined raw and processed poultry mash feed for *Salmonella* and other enteric organisms as a means of evalulating the efficiency of a new pelleting process in destroying microorganisms. The new pelleting process, thoroughly mixed poultry mash with steam and other hot gases in a vertical conditioner before pelleting. The hot gases were generated by direct combustion in an evaporator. *Salmonella* was not isolated from pelleted mash feed in any of the samples examined. In assessing the dissemination of *Salmonella* from hatchery to the broiler farm and from the broiler farm to the processing plant, Bhatia and McNabb (1980) reported that properly pelleted feed did not seem to be an important source of infection. In a survey of the Dutch feed industry, Veldman et al. (1995) reported that mash feeds were more (21 %) frequently contaminated than pelleted feeds (1.4%). Of the feed ingredients examined, they found fishmeal to be the most frequently contaminated (31 %) followed by corn grits at 27 %. They also showed that thermotrophic *Enterobacteriaceae* were useful markers of the rate of contamination of feedstuff with *Salmonella* as well as the efficiency of decontamination by pelletization. In studies to assess the feasibility of producing *Salmonella*-free turkeys, Pomeroy et al. (1989) reported that when pelleted feed with no animal protein products except fish solubles was given to flocks of turkeys over a period of about 11 years, no *Salmonellae* were isolated from the feed on the farm.

Likimani et al. (1990) observed that extrusion cooking could be used as a decontamination process. It also destroyed microorganisms in products that were rehydrated before consumption such as reconstituted drinks.

Chemical composition as well as the particle size of a feed formulation may affect the thermal susceptibility of feed microorganisms (Lui et al., 1969). The authors further observed that the virulence of *Salmonellae* surviving thermal processing that reduces their population by several logs might be greatly decreased.

In conducting studies to examine the destruction of liquid or freeze-dried spore suspensions of *B. stearothermophilus* during extrusion processing of a starch-protein-sucrose biscuit mix using a twin screw extruder, Bouveresse et al. (1982) and Van de Velde et al. (1984) observed 5 to 8 $\log_{10}$ reductions in spore populations at temperatures between 150 and 180 ^{o}C. In studies directed at examining the potential for bacterial spore injury during extrusion cooking, Likimani et al. (1990) extruded an 18 % moisture corn and soybean (70 and 30%, w / w) mix inoculated with viable spores of *B. globigii*. They used a single screw extruder with the barrel temperature in zone 1 of the extruder maintained at 80 ^{o}C while varying the temperature in zone 2 from 100 to 120 and 140 ^{o} C, mass temperatures. The extruder was operated at two mass residence times of 25.5 and 11.2 s. The authors reported injury to *B. globigii* at 100 ^{o} C as indicated by the reduced recovery of viable spores by the minimum growth requirement medium-1. They observed extensive spore destruction at 120 and 140 ^{o} C with no detectable spore injury; similar numbers of spores were detected with all

culture media. Further, Likimani et al. (1990) concluded that extrusion cooking under those conditions was an adequate heat activation treatment and that additional heat shocking might have served only to damage the spores. Recovery of injured spores required the inclusion of additional nutrients beyond those found in the minimum requirement medium. In addition, heat shocking for spore activation after extrusion was unnecessary before enumeration.

2.2 *Bacillus* Species

Spores are the dormant form of bacteria that allow them to survive unfavorable environmental and nutritional conditions (Leboffe and Pierce, 1996). Bacterial spores may be located in the middle of the cell, at the end of the cell, or between the end and middle of the cell; they are resistant to heat and chemicals. Leboffe and Pierce (1996) also noted that *Bacillus* and *Clostridium* are among the few genera of bacteria that produce spores.

2.2.1 Cell Wall Structure

All vegetative cells of spore-forming bacteria are Gram-positive (Foster, 1994). The cytoplasmic membrane of a vegetative cell is surrounded by a 20-50 nm thick cell wall composed of 40 % or more peptidoglycan (Shockman and Barrett, 1983). A mature

dormant endospore is surrounded by a thick cell wall consisting of an inner and outer layer of peptidoglycan namely the primordial and spore cortex, respectively (Figure 2.1) (Foster, 1994). In the primordial cell wall, most muramic acid residues in the peptidoglycan are substituted with a peptide side chain. In contrast, approximately 50% of the muramic acid residues in the peptidoglycan of the spore cortex are present as muramic acid delta-lactam (Warth, 1978; Foster, 1994).

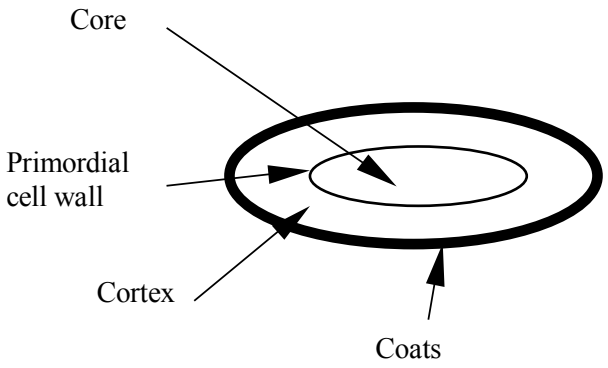

Figure 2.1. An illustration of a mature dormant spore of *Bacillus* species.

2.2.2 Mechanisms of Resistance to Heat Damage

Spores are much more resistant than their vegetative cell counterparts to a variety of environmental stresses, such as heat, chemicals and radiation (Setlow, 1994). Optimal growth of spore formers, spore protoplast water content, specific mineral content, temperature of sporulation and cortex size are key parameters that can be used to predict heat resistance of spore formers (Gerhardt and Marquis, 1989). Warth (1978a)

observed that generally, thermophiles produce more heat resistant spores than mesophiles, which produce more heat resistant spores than psychrophiles. Further, spores with less water in the protoplast are more heat resistant than those with higher water content (Beaman and Gerhardt, 1986). Beaman and Gerhardt also observed that sporulation at higher temperatures within an organism's normal growth range resulted in spores with more dehydrated protoplasts and greater heat resistance. In addition, mineralization enhances heat resistance in part by increasing protoplast dehydration as well as by other protective mechanisms against dry heat damage (Marquis et al., 1994). Warth (1985) estimated that spore internal water activity (a_w) of 0.7 was required to achieve the degree of thermal stabilization of enzymes found in spores. It is generally accepted that the presence and size of the spore cortex significantly influence heat resistance of spores (Marquis et al., 1994). These authors also found that the major functions of the cortex are to maintain osmotic dehydration of the spore protoplast during its formation and to resist water movements into the protoplast later during spore mineralization.

2.2.3 *Bacillus* Species in Feed Commodities

B. stearothermophilus has been used as an indicator organism of bacterial inactivation during extrusion cooking (Bouveresse et al., 1982 and Van de Velde et al., 1984). Spores of *B. stearothermophilus* have been used as sterilization indicators since they are among the most heat-resistant spores encountered in foods (van de Velde et al.,

1984). Traditionally, *B. stearothermophilus* and *Clostridium sporogenes* spores have been used in inoculated experimental pack studies to asses the effectiveness of a sterilization process (Ocio et al., 1996).

2.2.4 *Bacillus stearothermophilus* in Food Spoilage

B. stearothermophilus is the organism commonly implicated in thermophilic flat sour spoilage of low-acid canned foods (AOAC, 1995; Ayres et al., 1980). *Bacillus* species commonly ferment carbohydrates producing short-chain fatty acids that give rise to a sour taste however they do not produce enough gas, if any, to cause the flat ends of the can to bulge outwards under pressure hence the "flat sour" description (Walker and Wolf, 1971). Olson and Sorrells (1992) observed that as long as holding temperatures were not allowed to rise above 43 ^{o}C for canned low-acid foods with pH no lower than 5.3, thermophilic flat sour spoilage is not common. The authors further noted that exposure of canned low-acid food products containing viable spores of thermophilic organisms to temperatures above 43 ^{o}C for long periods of time may result in flat sour spoilage.

Heat-induced thermal resistance may contribute to errors when estimating time for a sterilization process in canning (Etoa and Michiels, 1988). It is for this reason that come-up time is minimized to reduce the potential for heat-induced thermal resistance. Come-up time is the time required to raise product temperature from its ambient state to the desired sterilization temperature during a sterilization process in canning. Mafart and Leguerinel (1997) reported that while calculations used in thermal processing in the food industry are based on the exponential death rate of heated spores, spores that are not activated during the thermal process may be sub lethally injured and unable to grow on a medium under conditions such as unfavorable

incubation temperatures. The injured spore population ought to be accounted for in the calculations. The authors further noted that of the factors that influence cell repair after thermal injury, culture medium and incubation temperature have the most impact. These two factors influence both the ratio of cell recovery and estimated thermal resistance values. Moreover one disadvantage of conventional survival models applied in canned food industries is that heat resistance estimates are made by recovering heat-activated surviving spores or cells at optimal incubation conditions (Mafart, 2000). At non-optimal incubation temperatures or in acid recovery media supplemented with salt, heat resistance is often underestimated.

2.2.5 Thermal Resistance of *Bacillus* Spores

Heat resistance of microorganisms is often quantified in decimal reduction time (D_x) values (Russel, 1982; Gerhardt and Marquis, 1989; Setlow, 1992). Decimal reduction time is defined as the time required to reduce cell or spore population at a constant temperature of X ^{o}C to 10% of its original value. It is the time required to kill 90% of a cell or spore population at a specified temperature. The z value is defined as the temperature increase required to cause a tenfold reduction in D_x value for that organism (Teixeira, 1992). In general, the D_x values of spores at a temperature of (X + 40) ^{o}C are approximately comparable to those of their vegetative cell counterparts at a temperature of X ^{o}C (Setlow, 1994). *Bacillus stearothermophilus* spores have exceptionally high resistance to heat with D_{120C} values ranging between 4.0 and 5.0

minutes and a z-value of between 7.8 and 12.2 ^{o}C (Olson and Sorrells, 1992). For this reason it is normal to find commercially sterile low-acid canned foods containing spores of *B. stearothermophilus.* D_{120C} means decimal reduction time of an organism at 120 ^{o}C. Proper cooling after thermal processing and maintenance of appropriate storage temperatures are used to prevent flat sour spoilage. Segner et al. (1963) demonstrated that *B. stearothermophilus* spores showed the same thermal resistance in 3:1 milk concentrate as when they were suspended in phosphate buffer. Frankline et al. (1959) however found that *B. stearothermophilus* spores were more heat resistant when suspended in water than in milk. Mayou and Jezeski (1977) observed that spores of *B. stearothermophilus* showed lower thermal resistance when suspended in 10 % reconstituted milk than in 0.01 M phosphate buffer, pH 6.5. They further observed that increasing the pH of phosphate buffer from 6.5 to 7.2 had little effect on spore resistance, while the same increase in pH of reconstituted 10 % milk resulted in a marked increase in the apparent heat resistance of *B. stearothermophilus* spores. Mayou and Jezeski (1977) noted that spores harvested from microbiological media may show different thermal resistance than spores of the same strain sporulated in a natural environment.

Lefebvre and Antipa (1982) observed that the transformation of bacterial spores from a dormant and heat-resistant state into a thermolabile and metabolically active cell involves two stages namely activation and germination. They noted that the spore loses its fundamental heat resistance first, then other changes take place that lead to germination. In their review of work by several investigators, Etoa and Michiels

(1988) concluded that for *B. stearothermophilus* spores, germination and loss of heat resistance occurred best after heat-shock treatment at 100 or 110 ^{o}C. The authors reported a 28.5 % increase in the heat resistance of spores in distilled water and a 62% increase in heat resistance of spores in nutrient medium within the first 60 minutes of incubation at 100 ^{o}C. While it is recognized that at sublethal temperatures some spores enter heat-induced dormancy rather than germinate (Rossignol and Vary, 1979). Etoa and Michiels (1988) observed that *B. stearothermophilus* spores acquired supplemental heat resistance that they described as "heat-induced resistance." They concluded that the longer the spores were held at sublethal temperatures, the greater might be the increase in spore heat resistance. It is generally recognized that, in addition to temperature, factors such as pH and water activity of the heating medium greatly influence the survival of heated cells or spores (Mafart, 2000).

In general, acidification depresses the thermal resistance of spores, the degree of the effect being dependent on the microorganism (Martinez et al., 1997). In studies that mostly involved vegetative cells suspended in oil, many investigators have indicated that these microorganisms had higher thermal resistance than those suspended in water (Rodrigo et al., 1999). In studies to explore the effects of two vegetable oils on various spores of *Bacillus* and *Clostridium* spp., Molin and Snygg (1967) found that thermal resistance increased more in *Bacillus subtillis* and *Clostridium botulinum* type E than in the other spores studied. Thermal resistance increases depended on the type of lipid used. In a similar study, Ababouch and Busta (1987) observed that spores of *Bacillus* and *Clostridium* species were more heat-resistant in olive oil than in the other

vegetable oils studied despite the water activity of olive oil being higher than that of the other vegetable oils. Rodrigo et al., (1999) observed that *B. stearothermophilus* spores showed reduced thermal resistance in food at 115, 118, 121 and 125 ^{o}C, respectively. Setlow (1994) summarized the D_{65C} values of *B. subtilis* as being less than 15s for vegetative cells but 105 hours for wild-type spores. The same author reported D_{85C} and D_{95C} values of 320 and 14 minutes, respectively, for *B. subtilis*.

2.2.6 Sources of *Bacillus* Spores

Spores of *B. stearothermophilus* are found in soil, spices, sugar, flour, and other ingredients (Stumbo, 1973). Bacterial spores gain entry into canneries through these media. Within the canneries, spore numbers may increase where appropriate propagation conditions exist. The author also noted that methods of spore contamination control in a food production plant include proper sanitation and use of food ingredients with acceptable spore population levels.

2.2.7 Injury of Microbial Cells or Spores

Injured microorganisms are important in food processing and preservation because they may repair their damage, multiply, and present a potential to cause spoilage and health hazards (Likimani et al., 1990). These authors further noted in their reviews that

food processing treatments such as heating, cooling, irradiation and use of chemicals, when applied at sub lethal levels, can cause injury to microbial spores or vegetative cells. Likimani et al. (1990) also observed that few studies have been done on the destruction of bacterial spores during extrusion cooking. Adams (1978) demonstrated that injured or damaged microbial entities express the injury through their inability to grow under conditions normally suitable for the proliferation of uninjured organisms; injured organisms might need more complex or different cultural and nutritional requirements for growth (Hurst, 1984).

2.2.8 Sporulation Media

Supplementation of culture media with divalent cations affects the heat resistance of bacterial spores. Russell (1982) reviewed the work of several investigators and concluded that with the exception of high concentrations (close to 0.1 %), inclusion of manganese sulphate in culture media for the production of *B. stearothermophilus* spores does not have a major effect on the heat resistance of the spores. Chemically defined media can be used to produce *B. stearothermophilus* spores with reproducible heat resistance (de Guzman et al., 1972). Mayou and Jezeski (1977) produced spores of *B. stearothermophilus* by inoculating plates of nutrient agar supplemented with 40 ppm manganese sulphate with vegetative cells of the organism and incubating at 55 ^{o}C for 72 hours.

2.2.9 Water Activity (a_w) and Bacterial Spore Properties

The water activity (a_w) of a food product or solution is the ratio of water vapor pressure of the food or solution (p) to that of pure water (p_o) at the same temperature given by Raoult's law (Hallstrom, 1992) (Equation 2.1).

$$a_w = p / p_o \quad (2.1)$$

Water activity (a_w) has a direct impact on microbiological safety of food. Salting, an ancient way of preserving foods, works by reducing a_w of the food commodity. Microorganisms typically grow best between values of 0.995 and 0.980 with most microbes ceasing growth at a_w below 0.9000 (Singh and Heldman, 2001a). The investigators further noted that a_w influences the storage stability of foods since some food spoilage processes are regulated by water. Murrell and Scott (1966) demonstrated that the spores of *B. stearothermophilus* increase in thermal resistance as water activity (a_w) decreases, with the maximum resistance occurring at an a_w of 0.2. The authors further postulated that bacterial spores are most heat-resistant when nearly, but not completely, dry because water hydrates some component(s) of the spore to produce marked stabilization against the effects of elevated temperatures. Proteins or complexes containing proteins are involved in the stabilization.

2.2.10 Thermal Activation of Bacterial Spores

Heat activation of mould spores was discovered when red moulds appeared in large numbers on carbonized trees after forest fires (Keynan and Evenchik, 1969). Heat was first used to activate bacterial spores in 1919 (US patent number 138, 978, Weizmann, C. 1919). Russel (1982) noted that different species of sporeformers varied in their temperature requirements for activation with *B. stearothermophilus* and other thermophilic and thermotolerant bacteria requiring from 105 - 115 ^{o}C for optimal activation. Segner et al. (1963) demonstrated that prolonged heating of spores of *B. stearothermophilus* at 100 ^{o}C failed to give the desired heat-shock effect. They concluded that heating from 100 to 120 ^{o}C for 1 minute produced the activation treatment desired resulting in a tenfold increase in the viable spore count of *B. stearothermophilus* compared with counts after heat-shock treatments at 100 ^{o}C or lower temperatures.

2.2.11 Recovery of *B. stearothermophilus* Spores

After a heat-shock treatment of samples at 100 ^{o}C for 20 minutes, Mayou and Jezeski (1977) used dextrose tryptone agar as the recovery medium to enumerate spores of *B. stearothermophilus* in studies to determine the effect of using milk as the heating menstruum on apparent heat resistance of the organism. Olson and Sorrels (1992) observed that optimum recovery of heated *B. stearothermophilus* spores occured best at 45 to 50 ^{o}C, in neutral media with distilled water as the diluent and heating medium, respectively. These authors recommended dextrose tryptone agar as the recovery media.

2.3 *Salmonellae* Species

2.3.1 Salmonellosis in Humans

Salmonella infection in humans often occurs through consumption of poultry-meat products contaminated with *Salmonella*. The prevalence of *Salmonella* in poultry products is frequently traceable to the farm level (Rose et al., 1999). It is estimated that the reported cases of *Salmonella* infections worldwide represent between 1 and 10 % of the real incidence of the disease in humans (Oosterom, 1991). Human salmonellosis is a serious problem all around the world; pigs and poultry, and to a lesser degree cattle and sheep, are the major sources of *Salmonella*. From

epidemiological studies, Oosterom (1991) further reported that fecal excretion by human carriers, domesticated and wild animals carriers, and the disposal of slaughter offal, sludge, slurry and manure all contribute to the spread of *Salmonella* in the environment. Environmental sources of *Salmonella* in turn contaminate surface waters from which birds, rodents and insect may further transmit the organism to animal feed. In a study to determine the mode of transmission of *S. enteritidis* in patients, Muhlenberg (1992) established that contaminated feed was the primary source of contamination in livestock; subsequent consumption of contaminated food of animal origin by humans resulted in salmonellosis.

2.3.2 Sources of *Salmonella* Contamination in Feeds

Smeltzer et al. (1980) evaluated the quality of feeds with respect to *Salmonella* contamination and found 44 out of the100 stock feeds examined were positive. They found significantly greater total aerobic bacteria, coliforms, fungi and *Salmonella* count in mash feeds than in crumbles and pellets. *Salmonella* counts ranged from 1.2 CFU / 100 g to greater than 147 CFU / 100 g feed. Kohler (1993) conducted a study to evaluate the environmental sources of *Salmonella*. The author detected *Salmonella* in soil samples contaminated with bird feces, and demonstrated that recontamination from the environment was the major source of *Salmonella* in feed plants. In studies to determine the persistence of *S. enteritidis* in poultry production operations, Davies and Wray (1996) established that the organism persisted for at least

one year in an empty trial house in which naturally infected broiler breeder birds had previously been housed. They also reported that the organism was frequently found surviving outside poultry houses in small litter pockets and fan dust at plants that had previously been cleansed and disinfected. Artificially contaminated *S. enteritidis* appeared to persist in bird droppings, floor and fan dust, and in feed in feed troughs for at least 26 months. In surveys of the Dutch poultry feed industry, Veldman et. al. (1995) found that 10 % of the 360 samples taken were contaminated with *Salmonella*. They found twenty-eight serotypes in all; the serotypes isolated most frequently were not the same as those encountered in poultry flocks. Environmental samples were taken from fecal material from pig pens, building doors, windows, floors, ventilation units, dust and farm accessories to identify possible sources of contamination and to study the distribution of Salmonella within herds of pigs in Quebec, Canada (Letellier et al., 1999). Most of the samples were positive. Flies and rodents sampled within the farm precincts were also positive for *Salmonella*. In a study to asses the association of management factors, environmental prevalence, and general hygiene condition in the chicken house to *Salmonella* contamination of broilers at harvest, Rose et al. (1999) found that *Salmonella* contamination of day-old chicks was significantly related to *Salmonella* contamination of the flock at the end of the production period. Of the flocks surveyed, 70 % of those contaminated with *Salmonella* also showed at least one contaminated environmental sample. Rose et al. (1999) further determined that the risk for *Salmonella* contamination was increased when feed trucks were parked near the entrance of the change room and when feed mash rather than small pellets was given to day old-chicks.

Ekperigin et al. (1991) investigated the effects of a pelleting process on the microbial load of poultry feed. Five to 10 % of samples tested positive for *Salmonella*. In a study to evaluate the feasibility of producing Salmonella-free turkeys, Pomeroy et al. (1989) reported that contaminated feed was suspected to be the source of *Salmonella* in the flocks of turkeys found to have *Salmonella*.

2.3.3 Control of *Salmonellae* Infection

The spread of *Salmonellae* during the slaughtering process of broiler chickens is difficult to control. For this reason, control measures to reduce the prevalence of the organism before slaughtering is a recommended practice (Rose et al., 1999). Ekperigin and Nagaraja (1998) observed that control of *Salmonella* contamination involved action to prevent establishment of the organism in the food animal or its immediate neutralization if it did establish itself; commercial vaccines were effectively used to control *Salmonella* infection under some circumstances although they were of limited value in controlling all *Salmonella*. The authors described prevention of *Salmonella* infection as involving keeping the organism away from the host animal through the knowledge of its epidemiology or the diseases it caused. They suggested that producing *Salmonella*-free food animals, husbandry practices that precluded environmental Salmonella as well as the use of pelleting techniques for producing *Salmonella*-free feeds, would be effective ways to prevent *Salmonella* infection in humans. Further, animal housing to keep rodents, wild birds, and insects

away from the immediate environment of food animal production facilities would be preventive measures against Salmonella infection in humans.

2.3.4 Thermal Death Time of *Salmonella*

Pomeroy et al. (1989) used commercially available, naturally contaminated dry meat and bone meals with modified thermal death time tubes to determine that *Salmonellae* in the meals were not destroyed when a temperature of 77 ^{o}C was used for a period of 15 minutes. At 82 ^{o}C, *Salmonellae* was consistently destroyed after 7 minutes. In a third meal with a higher fat content and relatively high level of *Salmonella* contamination, 91 ^{o}C for 7 minutes was required for consistent destruction of the bacteria although 88 ^{o}C was sometimes sufficient. They found that when a relatively heat resistant strain of *S. senftenberg* was inoculated in sterile meal, lower temperatures were sufficient to destroy the added *Salmonellae*. They concluded that heat tolerance studies on *Salmonellae* using naturally contaminated meals rather than artificially contaminated meals would be more representative of the true thermal resistance of the organisms. In a separate heat tolerance study using naturally contaminated meals and a pugmill, Pomeroy et al. (1989) found that samples were negative for *Salmonellae* when they were heated to 70 ^{o}C or above over a period of 50 minutes. Raising the temperature in a pugmill up to 82 ^{o} C over a period of 63 minutes resulted in consistent destruction of *Salmonellae.* They also concluded that

the severity of the heat treatment required to destroy *Salmonellae* in naturally contaminated meals varies considerably for different meals.

2.3.5 Heat Resistance of *Salmonella*

Liu et al. (1969) determined that the heat resistance of *Salmonellae*, like that of most microorganisms, is much greater in products containing less than 12 % moisture than at higher moisture levels. They found that the D_x value of *S. seftenberg* 775W in simulated-naturally-contaminated meat and bone meal at 60 ^{o} C decreased at a decreasing rate as the feed moisture content increased. D_x value of an organism is its decimal reduction time at a temperature of T. It is the time in minutes required to reduce the organism's viable population density to 10% of its original value by thermal means at a temperature of T. It has also been reported that the thermal resistance of *Salmonellae* is higher in natural than in artificially contaminated products (Rasmussen et al., 1964; Liu et al., 1969). Liu et al. (1969) found that the minimum temperature of the conditioner required to kill all the *Salmonellae* in the feed during commercial feed pelleting was 88 ^{o} C.

2.4 Feed Inoculation with Bacterial Cells or Spores

In order to prepare inocula, Flowers et al. (1987) ground freeze-dried pellets of bacterial cells by using a mortar and pestle and then mixed the cells into samples of

dry powdered, granulated or semi-solid test products to form the seed inocula. Liu et al. (1969) prepared inocula for artificial contamination of feed in thermal death time studies by adding 24-hour Trypticase Soy Broth (TSB) culture of the test organism to quantities of feed stabilized at various moisture contents in mason jars that were refrigerated for about 1 week in order to stabilize the cell population. They mixed the inoculum for periods of about 2 minutes each day to secure an even distribution of the cells during the one week refrigeration. Ten grams of the feed inoculum was added to 90 grams of the test material in a mason jar and mixed for 4 minutes. This contaminated feed was then added to 50 pounds of chick starter feed at room temperature and mixed mechanically for half an hour to give approximately 10^3 cells per gram of feed.

2.5 Extrusion Cooking Technology

Food extrusion is the operation that shapes a dough-like food material by forcing it through a specifically designed restriction called a die (Riaz, 2000); food ingredients are forced to flow, under one or various conditions of mixing, heating and shear, through a die that forms and/or expands the ingredients as the extrudate exits the extruder (Riaz, 2001). Food extruders consist of turning screws that convey food material through a stationary barrel. The barrel and screw are typically segmented. The screw consists of a splined shaft with a keyway onto which several flight sections, and shearlocks/steamlocks, respectively, are slipped and locked in place (Riaz, 2001).

During extrusion cooking of cereal grains and protein blends, moistened, granular or flour-like materials are converted into a dough; starchy components gelatinize resulting in high moisture uptake and an increase in dough viscosity. Hydrated protein constituents may influence elasticity and the gas-holding capacity of the dough (Rokey and Huber, 1994). Temperatures as high as 200 ^{o}C can be achieved during the extrusion cooking process. The residence time of feed at such elevated temperatures is kept short, 5 to 10 seconds, to maximize the benefits of heating on digestibility, inactivation of anti-nutritional factors, and pasteurization, respectively, while minimizing nutritional destruction.

Extrusion cooking has been practiced for nearly 50 years. The food extruder has been developed into a high-temperature-short-time equipment used to process a variety of food ingredients into finished food commodities from its initial role of mixing and forming pasta and ready-to eat cereals (Harper, 1984). Further, Harper observed that more research is needed to give users and manufactures of extruders recommendations on measurement of key extrusion variables. Of primary interest are the screws and barrel wear over time, increase of throughput by increasing slip at the root of the screw, measurement of pertinent extrusion parameters such as product and barrel temperatures and the accuracy of the measurements, and pressure measurement behind the die. The use of computers to monitor and adjust extrusion conditions needs to be explored. Harper (1984) lamented the lack of detailed knowledge despite a considerable body of literature on the impact of extrusion on food nutritional value. He suggested the kinetics of microorganism destruction and aflotoxin inactivation

during extrusion cooking as possible research topics for the future. He reported that little is known about the effect of extrusion on the fiber fractions of cereals and how their biological activity may be altered.

A single screw extruder operates like a high viscosity pump. The plasticized food product moistens the inner wall of the barrel but due to the grooving, no slip conditions apply around the inner wall of the barrel (van Zuilichem et al., 1997). The moving surface drags the product with a low mechanical efficiency longitudinally along the barrel, the larger part of the power being dissipated in the form of heat. In twin-screw extruders with intermeshing screws, the product is constrained and physically prevented from rotating with the screw (Frame, 1994). Here the frictional force between the product and the barrel wall is less important than in single screw extruders.

High temperature short time expanders and extruders are equipment in which feed that is heated to high temperatures in a high-pressure zone is subjected to a rapid decrease in pressure on exit from the die. This sudden decrease in pressure ruptures cell structures and organelles (Plavnik and Sklan, 1995). An expander is a high temperature-short-time thermal processing device that is similar in its operation to an extruder. Expanders typically are designed to increase feed pellet durability (Fairfield, 1994). It is constructed similar to an extruder, with a thick walled barrel, heavy screw and attachments for steam addition. It is equipped with a conical discharge valve at the discharge end, which provides an adjustable annular gap, distinguishing it from the

extruder. Exposure to elevated temperatures and sudden pressure changes, respectively, during processing using expanders or extruders alter the structure, texture and specific gravity of the feed (Armstrong, 1993).

2.6 Residence Time Distribution in Extruders

The time that feed mash spends in the extruder barrel is the extruder retention time, a measure of the duration of exposure of feed mash and indigenous microorganisms to processing conditions of heat, steam and mechanical shear. The rotation of the screw relative to the barrel wall creates cross channel flow which mixes material in the channel between screw flights as it is dragged down the barrel (Frame, 1994). Because of longitudinal and axial mixing, flow of material within the extruder barrel cannot be represented as simple plug-flow. Retention time may be better represented as a residence time distribution (RTD) function (van Zuilichem et al., 1997). RTD is a mathematical expression that describes the dwell time of mash feed components within the extruder with respect to time. RTD of feed in an extruder is one of the important operating conditions that affect mixing and chemical properties of feed during extrusion processing (Peng et al., 1994). RTD in an extruder is a useful way of determining optimal processing conditions for mixing, cooking, and shearing reactions during the process (Ganjyal and Hanna, 2002). Fichtali and van de Voot (1989) observed that from the knowledge of the RTD function one can estimate the residence of mass flow as well as other variables such as the degree of mixing and the average

total strain exerted on the mass during its transition. Retention time of feed in the extruder barrel was used in this research.

2.7 Summary from Literature Review

1. Agricultural commodities are found in environments that make their contamination by bacteria and molds inevitable. Decontamination techniques are often necessary to eliminate such contamination.
2. Contaminated eggs and the environment are the major sources of *Salmonella* infection that result in significant production losses in poultry operations. Poultry as well as other livestock are considered important sources of *Salmonella* species that cause human salmonellosis.
3. While feed processing conditions that could kill *Salmonella* may be defined, control of these conditions in extrusion and pelleting equipment is difficult.
4. Whereas mash feed are frequently contaminated with *Salmonella,* pelleted feed often test negative for *Salmonella*. Animal protein components of feed formulations are believed to be the main source of *Salmonella* in mash feed.
5. *B. globuli* spores survived extrusion processing at 100 ^{o}C for 25.5 s. No spores were detected at 140 ^{o}C.
6. Processing at under sub-lethal temperature and time conditions imparts heat-induced thermal resistance to organisms. Come up time during thermal processing of food is minimized to prevent this phenomenon.
7. *B. stearothermophilus* naturally reside in soil, and on raw food commodities. Their spores are among the most heat-resistant spores encountered in foods. They have been used in inoculated food packs to assess the effectiveness of sterilization processes.

8. *B. stearothermophilus* is not a human pathogen but is commonly implicated in thermophilic flat sour spoilage of canned foods. Storage of canned food products below 43 ^{o}C typically reduces the risk of such spoilage.
9. Decimal reduction, D_x, values at 120 ^{o}C of up to 5 minutes, and z values between 7.8 to 12.2 ^{o}C have been reported for *B. stearothermophilus* spores. For this reason it is common to find commercially sterile foods containing *B. stearothermophilus* spores.
10. Germination and loss of heat resistance of *B. stearothermophilus* spores occurs best after a heat shocking treatment at 100 – 110 ^{o}C.

3. OBJECTIVES

The objectives of this study were to:

1) Determine the effect of extruder barrel exit temperature, mean retention time of mash feed in the extruder barrel, and mash feed moisture content on acid producing, thermophilic organisms and artificially inoculated *B. stearothermophilus* spore survival.
2) Develop a mathematical model using a central composite statistical design and the response surface method, and from the model develop response surface plots.
3) Estimate optimum extrusion process conditions that maximize acid producing, thermophilic organisms and *B. stearothermophilus* spore inactivation within the range of parameter settings used in the study.

4. EQUIPMENT

4.1 Feed Milling and Mixing

Figure 4.1 shows the Roskamp roller mill (Model DP900-12, SN 435386, California Pellet Mill Co., Waterloo, Iowa) used to grind corn and soybean. Once ground, feed was conveyed in a U-trough conveyor illustrated in Figure 4.2, to the ribbon mixer (Model number TR63-796, Hayes and Stolz Industrial Manufacturing Company, Fortworth, Texas) (Figures 4.3a and 4.3b), where a predetermined amount of pre-ground, pre-bagged animal protein blend was manually added. Mixed feed was conveyed by a U-trough conveyor and collected in storage barrels equipped with rubber O-rings on the lids to prevent moisture exchange between dry feed and environmental air when the barrels were sealed. Figure 4.4 illustrates the sequence of feed milling and mixing processes. Figure 4.5 shows storage barrels used to store feed during the studies.

4.2 Weighing Device for Manual Mash Feed Moisture Control

Predetermined amounts of tap water required in dry feed to obtain desired mash feed moisture content, and batches of dry feed (10 kg), respectively, were weighed using the Weigh-Tronix scale (Weigh-Tronix scale - indicator, model number W1-125, Toledo base, model number 2155, Fairmont, Minnesota).

Figure 4.1. A roller mill (green) used to prepare the standardized feed formulation in the studies (Roskamp, Model DP900-12, SN 435386, California Pellet Mill Company. Waterloo, Iowa).

Figure 4.2. A section of the U-trough drag conveyor, paddle and chain guide used in the studies.

Figure 4.3a. A side view of the ribbon mixer (gray) used to mix corn meal, soybean meal and animal protein blend, respectively, to form the standardized feed formulation used in the studies.

Figure 4.3b. A top view of the ribbon mixer used in the studies showing the ribbons

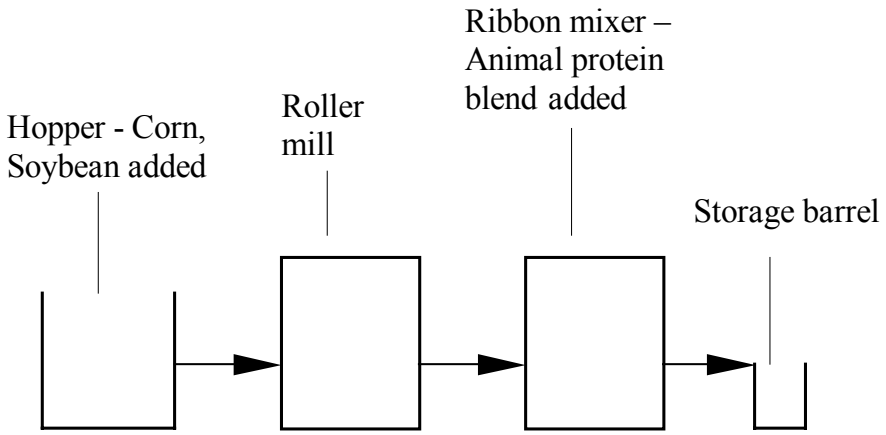

Figure 4.4. An illustration of feed milling and mixing process used in the studies. Arrows indicate direction of feed conveyance.

Figure 4.5 Storage of standardized feed formulation in sealed barrels. Barrel lids were equipped with O-rings to prevent moisture exchange between feed and environmental air.

The weighing scale consisted of a weighing platform connected to a liquid crystal display indicator by a cord as shown in Figure 4.6. The weighing unit's accuracy was ± 0.05 kg.

Figure 4.6. A Weigh-Tronix scale showing the loading ramps on either side of the weighing platform and a 10-kg batch of feed in a mixing container placed on the weighing platform (Weigh -Tronix scale indicator: Model Number W1-125, Toledo base: Model Number 2155. Fairmont, Minnesota).

4.3 Vortex Mixer

A variable speed, touch-activated vortex mixer equipped with a start/stop switch was used to thoroughly mix spore and cell suspensions in water through a vortex mixing

action during the serial dilution and inocula preparation procedures. A flat head provided for tube or flask mixing.

4.4 Refrigerator

A refrigerator was used to maintain the temperature of dry feed inoculum of *B. stearothermophilus* spores between 0 and 4 ± 2 ^{o}C until used.

4.5 Autoclave

An autoclave (Eagle Series, Model 2321) was used to sterilize culture media and glassware for use during aseptic techniques of the microbial methods. A sterilizing temperature of 121 ^{o}C for 15 minutes was used.

4.6 Weighing Balance

Portable bench top balances sensitive to 0.1 g with a 2 kg capacity were used to weigh media during culture media preparation and feed samples during microbial and particle size distribution analyses, respectively (PG-S Electronic Scale, Model 5002-SDR, ; Model PM34-K, Mettler Instruments, Stown, New Jersey).

4.7 Colony Counter

The Darkfield Quebec Colony Counter was used to manually count bacteria colonies quickly and accurately. The Darkfield Quebec colony counter provided even, glare-free illumination. It was equipped with an annular reflector to spread light from a light bulb uniformly over the entire culture plate, a lens to magnify bacterial colonies and an adjustable focusing rod that allowed rotation of the lens for easy access to culture plates and a white-ruled counting plate. Contrasted against the darkfield background, colonies were bright and readily distinguishable from other structures in the culture media.

4.8 Biohazard Safety Cabinet

Microbial analysis of acid producing, thermophilic organisms and *B. stearothermophilus* spores and *S. typhimurium* cells were carried out in a biohazard safety cabinet class II that provided a work area that was sterile (Model 240, Contamination Control Incorporated, Lansdale, Pennsylvania). The safety cabinet consisted of a lamina flow hood, which filtered air and then passed it over an enclosed work surface. A barrier of filtered air also protected the operator from any infectious agents during the procedures.

4.9 Direct Microscopic Spore Count and Cell Enumeration Equipment

The Petroff-Hausser counting chamber (Hausser scientific, Blue Bell, Pennsylvania) was used to estimate the spore count in the initial *B. stearothermophilus* suspension that was used to prepare the feed inoculum. It consisted of a grid of etched squares of a given area covered with a glass cover slip positioned at a fixed height above the etched surface (Splittstoesser, 1992; Smith, 2000). Each small square (Figure 4.7) in the counting chamber grid of the Petroff-Hausser chamber had an area of 0.0025 mm^2 and the chamber or well above each small square was 0.1 mm deep, giving a volume above a small square of 2.5 x 10^{-4} mm^3 or 2.5 x 10^{-7} mL. The Petroff-Hausser counting chamber -was placed under the objective of the light microscope (Model CHT 800064, Olympus Optical Company Limited, Japan).

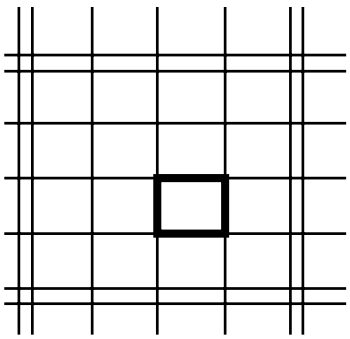

Figure 4.7. Illustration of a portion of the Petroff-Hausser counting chamber grid with a small square highlighted as a bold square.

4.10 Incubation Equipment

A steam cabinet (Figure 4.8) was used for heat shocking acid producing, thermophilic organisms and *B. stearothermophilus* spores in feed samples. Pour plated samples of acid producing, thermophilic organisms and *B. stearothermophilus* spores in feed and spread plated samples of *S. typhimurium* cells in feed, respectively, were incubated in an incubator set to a suitable temperature (heat shocked spores of acid producing, thermophilic organisms and *Bacillus stearothermophilus* at 60 - 65 ^{o}C or *Salmonella typhimurium* at 37 ^{o}C VIP Imperial II CO_2 incubator with dual chambers, dual control. Lab-line Instruments Incorporated, Melrose Park, Illinois).

Figure 4.8. A custom made steam cabinet used for heat shocking acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores at 100 ^{o}C for 15 minutes. A gate valve used to control the steam flow rate is shown on the insulated steam line to the right of the steam cabinet.

4.11 Determination of Feed Particle Size Distribution

A nest of sieves and the sieve shaker shown in (serial number 8-200-3834. Endecotts Octagon 200, London, England) were used to analyze the particle size distribution of the standardized feed formulation.

4.12 Paddle Mixer and Mixing Container

A Hobart paddle mixer (Model H600, Hobart Corporation, Troy, Ohio) was used to mix dry feed with feed inoculum and tap water, respectively, for mash feed moisture control (Figure 4.9).

Figure 4.9 Mash feed moisture content control. Mixing feed with predetermined amounts of water using a Hobart paddle mixer (Model H600, Hobart corporation, Troy, Ohio).

4.13 Feed Moisture Content Determination

4.13.1 Vacuum Oven

A vacuum oven (Fisher scientific, Model 285A) was used to dry mash feed samples and extrudate samples, respectively, for moisture content determination.

4.13.2 Dessicator

Dry feed samples were transferred to a dessicator containing indicating drierite (anhydrous Calcium sulfate impregnated with Cobalt chloride) for storage. Figure 4.10 shows the dessicator used in the studies.

Figure 4.10. A custom made desiccator used to hold dry feed samples during reweighing after oven drying. Aluminum feed sample trays and partially hydrated (pink drierite are shown inside the dessicator.

4.14 Feed Water Activity Measurement

An Aqualab water activity (a_w) meter (Model CX-2, Decagon Devices Inc., Pullman, Washington) was used to measure the amount of free, unbound or active water present in feed samples. This device determines water activity from the relative humidity of the air surrounding the sample when the air and the sample are at equilibrium. The sample is loaded in an enclosed space where the equilibrium occurs.

4.15 Extru-tech E325 Single-Screw Extruder

Extru-tech E325 (Extru-tech, Sabetha, Kasnsas) single-screw extruder was used in the studies. It is a single-screw cooking extruder developed for the purpose of research and development. The E325 cooking extruder has been scaled down proportionately from extru-Tech's larger production units in order to provide data that are representative of actual production conditions (Extru-Tech, Sabetha, Kansas). The extruder consists of the following components: 1) a feed delivery system, 2) a tempering or preconditioning system, 3) extruder barrel and 4) the die and knife configurations. Feed was delivered to the continuous agitation bin by an auger system (Figure 4.11). Preconditioning involving addition of moisture and steam in the preconditioner was not performed in this study. Three temperature probes (Table A 1, Appendix A) for measuring barrel temperature in different cooking zones were installed on the extruder barrelheads as shown in Figure 4.12. The knife assembly was not used in this study to enhance operator safety.

4.15.1 Feed Delivery System

Consistent and uniform delivery of feed ingredients to the extruder is necessary to ensure efficient operation of the extruder. Feed mash (batches of 10 kg) was held in the continuous agitation feed bin. The feeders consisted of a rotating shaft with radially attached paddles to agitate the feed blend, and a variable feed auger to convey the feed blend to the preconditioner (Figure 4.11). The auger was operated at one speed throughout the study. The operating speed was determined experimentally as described in procedures under "Feed Delivery Rate". Feed mash was conveyed to the preconditioner through an auger conveyor system capable of providing uniform flow at any desired extrusion rate.

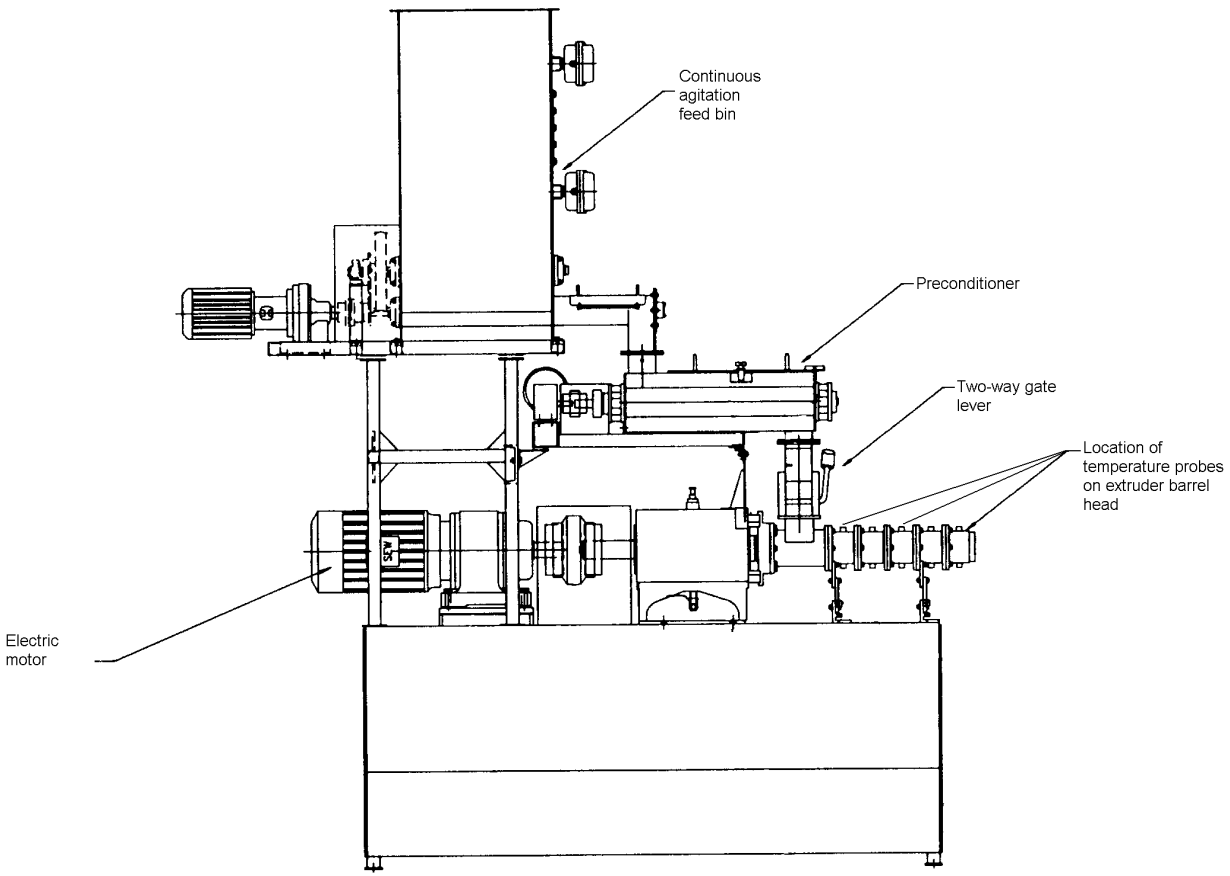

Figure 4.11 Diagram of Extru–tech E325 (Extru – tech, Sabetha, Kansas) single screw extruder and its major accessories.

4.15.2 Extruder Preconditioner

In the preconditioner cylinder (Figure 4.11), mash feed is continuously mixed and heated. No liquid blends were added at the preconditioner in this study. Typically, the intense mixing of the feed blend coupled with the ability to vary the retention time during the preconditioning phase allows the moisture levels to be maintained at an optimum (Hauck et al., 1994). The retention time of the preconditioner was not varied in this study. Atmospheric conditioning chambers (e.g. the preconditioning chamber) usually provide up to 240 seconds retention during which time the feed blend is heated and individual particles absorb moisture. The same authors further observed that preconditioning process enhances flavor development and improves the final feed texture.

4.15.3 Extruder Barrel Components

The extruder assembly consisted of the barrel and screw configurations for a single screw cooking extruder (Figure 4.12). Extruder heads make up the housing and provide the wall of the flow channel, the extruder screw configuration propels the extrudate from the inlet to the die, the steam locks disrupt the extrudate flow increasing mixing and the conversion of mechanical energy into heat, while the extruder die provides resistance to flow at the exit end of the barrel and shapes the final product of the extrusion process (Hauck et al., 1994). In the

feeding zone, the low-density discrete particles of feed exiting the preconditioner are transported into the extruder barrel.

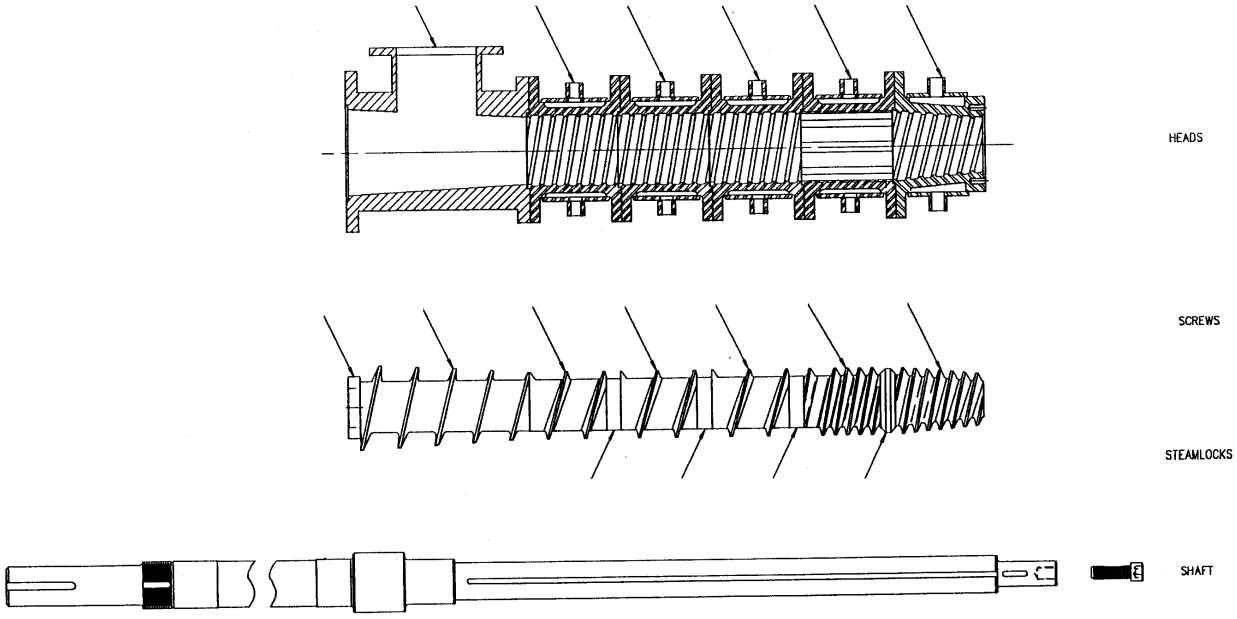

Figure 4.12. Diagram of Extru – tech E325 (Extru – tech, Sabetha, Kansas) single screw extruder showing from top to bottom the extruder barrel, screw and shaft, respectively. Top drawing: Arrows from left to right show the feeding zone, barrel heads 1 - 4 and exit temperature probe locations, respectively. Center drawing: arrows on the top side of the screw illustrate the location of the screws while arrows on the lower side indicate the location of steam locks.

This material is then propelled further into the interior of the kneading zone where the material is compressed to increase the degree of fill of the flow channels. Steam injected into the barrel jacket further raises the temperature of feed. Particles of the feed material begin to agglomerate in this zone. In the final cooking zone, temperature and pressure increase rapidly resulting in the desired final product texture, density, color and functional properties.

4.15.4 Die and Knife

The design of the die affects expansion, uniformity and appearance of the final product. A custom made die consisting of a 2.2 cm diameter central hole drilled in a 6 mm thick steel plate was used throughout the study (Figure B 1 in Appendix B). The knife is driven by a variable speed drive motor and is secured close to the face of the die. The knife assembly was not used in this study for safety reasons. Figure 4.13 shows a dimensioned diagram of the entire extruder assembly including the die and knife assembly just beyond the extruder barrel.

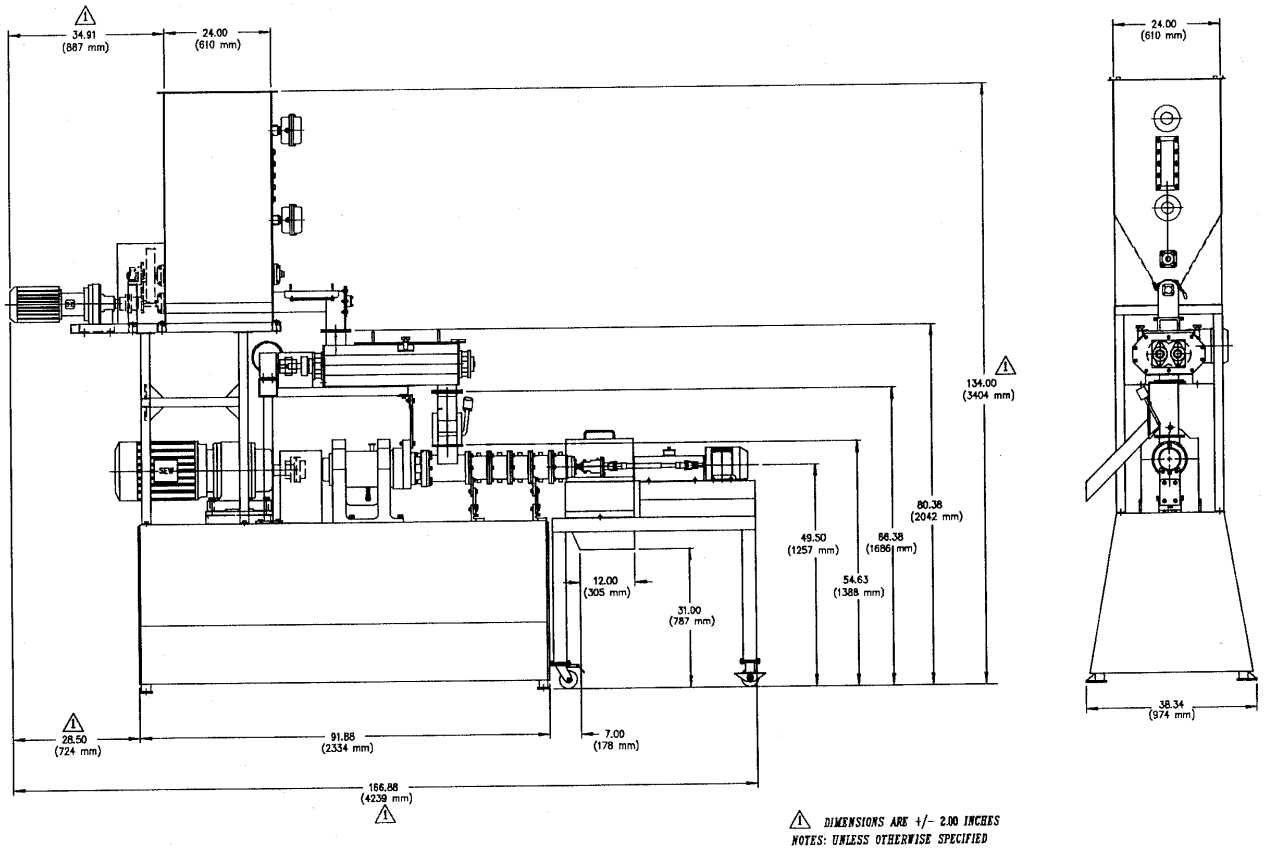

Figure 4.13. A dimensioned assembly of Extru–tech E325 single screw extruder (Extru-tech, Sabetha, Kansas).

5. PROCEDURES

A single screw extruder (Extru-tech E325, Extru-tech, Sabetha, Kansas) was used to process a standardized feed formulation artificially inoculated with an indicator organism during the studies. It was necessary to obtain an estimate of workable extruder operating conditions and the ranges of its selected independent variables namely extruder barrel exit temperature (T), mash feed moisture content (Mc), and retention time of feed in the extruder barrel (Rt). It was also necessary to select and prepare an appropriate inoculum of indicator organism in an appropriate form for inoculating test feed samples. Once the indicator organism was selected, it was desirable to establish cultural methodology for its recovery and enumeration in the test feed samples.

5.1 Feed Milling

Corn and soybean, respectively, were conveyed by an auger and ground using a roller mill (Roskamp Roller Mill, Model DP900-12, SN 435386, California Pellet Mill Co., Waterloo, Iowa) as described in the equipment section under “Feed Milling and Mixing”.

5.2 Preliminary Studies

5.2.1 Feed Delivery Rate

Mash feed was delivered through the continuous agitation feed bin with its delivery auger running at a predetermined constant speed. To estimate the feeding rate, mash feed flow was diverted from the feeding zone into a bucket for a known duration of time (1 minute). Feed mash collected in a bucket for the time duration was weighed using a weighing balance (Weigh-Tronix scale - indicator, model number W1-125, Toledo base, model number 2155, Fairmont, Minnesota). The feeding rate estimation was repeated 3 more times. An average feeding rate that produced an adequate throughput of extrudate without stalling the extruder was determined.

5.2.2 Determination of Mash Feed Moisture Content Range for Extrusion

It was desirable to estimate a workable range of mash feed moisture content to use in the extrusion studies. The minimum mash feed moisture content that could be extruded without causing the extruder to stall was determined by extruding feed containing 16.5, 20.5, and 24.5 % moisture, wet basis. The maximum range of mash feed moisture was determined by extruding mash feed containing 26.5, 28.5, 30.5 and 32.5 % moisture , wet basis. The maximum mash feed moisture content was reached when extrudate appeared too soft and uncooked.

5.2.3 Estimation of Retention Time of Feed in the Extruder Barrel

Feed retention time in the extruder barrel was estimated experimentally according to the following procedure. The extruder barrel speed indicator was calibrated in Hertz; one Hertz was one revolution / second or 2∏ radians / second.

1. The feed bin delivery auger was operated at a preset constant rate of 360 rpm (6 Hz) while the extruder barrel speed was varied between 1500 rpm (25 Hz) and 5400 rpm (90 Hz).
2. With the extruder barrel speed running at 25 Hz, a scoop of mash feed was manually added to the extruder barrel feeding zone to fill the extruder barrel flow channel.

3. Once the extruder barrel flow channel was filled, a separate scoop of mash feed was added to the extruder barrel feeding zone.

4. The time duration lapsed between addition of mash feed to the extruder barrel feeding zone and the emergence of extrudate at the extruder barrel exit was measured with a stop watch. This procedure was repeated two more times.

5. The extruder barrel speed was then increased to 1800 (30), 3000 (50), 3600 (60), 4200 (70), 4800 (80), and 5400 (90) rpm (Hz), respectively, while repeating step 4 of these procedures for each of the extruder barrel speeds.

6. The time and extruder speed were recorded. Steps 1 through six were repeated two more times for a total of three replications.

5.3 Inoculum Preparation

5.3.1 Propagation Media for *S. typhimurium*

Nutrient broth (Difco, Sparks, Maryland) was used to propagate *S. typhimurium.* Ingredients of nutrient broth:

Beef extract	3.0 g
Peptone	5.0 g

Eight grams of premixed dry ingredients were weighed and 1 L of deionized water added to dissolve the ingredients. Nutrient broth was then autoclaved in a screw-cap bottle for 15 minutes at 121 ^{o}C.

5.3.2 Dry Chalk Inoculum of *Salmonella typhimurium*

Salmonella was selected as a representative pathogenic organism commonly encountered in feed commodities (Rose et al., 1999). Dry chalk inoculum was prepared by submerging blocks of chalk (Triangle A & E Incorporated, Oklahoma City, Oklahoma) for 12 hours in nutrient broth culture of *S. typhimurium*, drying the

chalk blocks in an incubator set to 37 ^{o}C to their original dry weight and then pulverizing the dried chalk using a motor and pestle to obtain a powdered inoculum.

5.3.3 Dry Feed Inoculum of *Salmonella typhimurium*

One kg of dry feed was inoculated with 15 mL of nutrient broth culture of *S. typhimurium* previously incubated at 37 ^{o}C for 24 hours. Inoculated dry feed was shaken vigorously by hand in 30 cm arcs for 5 minutes in a sterile stomacher bag. The shaking was repeated two more times to secure a homogeneous dry feed inoculum.

5.3.4 Enumeration of *Salmonella typhimurium* in Dry Chalk Inoculum

Enumeration of viable bacterial cells was performed using the approved methods of the American Association of Cereal Chemists (method 42-40, AACC, 1995a) procedures. The initial 10^{-1} dilution was prepared by weighing out 10 g of dry chalk inoculum in a sterile sampling container and adding nutrient broth to make 100 g of suspension. To make the 10^{-2} dilution, 1 mL of the 10^{-1} dilution was transferred to a test tube containing 9 mL of nutrient broth. Subsequent ten fold dilutions were obtained similarly by transferring 1 mL of a dilution to dilution blanks consisting of 9 mL of nutrient broth in test tubes. One hundred micro liters of appropriate dilutions were plated on McConkey agar (Difco, Becton Dickinson and Company, Sparks,

Maryland) plates with 100 ppm nalidixic acid. Each batch of McConkey agar plates prepared was tested for its selectivity using the test organism and a negative control (a strain of *S. typhimurium* susceptible to nalidixic acid).

5.3.5 *Bacillus stearothermophilus*

An organism that would not be completely inactivated by extrusion conditions expected using the Extru-Tech E325 cooking extruder was sought. A mesophile (*S. typhimurium*) and a thermophile (*B. stearothermophilus*) were used. Other indigenous acid producing thermophiles in the feed were enumerated as well. *B. stearothermophilus* 12980 (American Type Culture Collection (ATCC), Rockville, Maryland) was selected for the extrusion studies since spores of *B. stearothermophilus* are among the most heat-resistant spores (van de Velde et al., 1984). *B. stearothermophilus* spores have been used in inoculated experimental pack studies to asses the effectiveness of a sterilization process (Ocio et al., 1996).

5.4 Propagation and Harvesting of *Bacillus stearothermophilus* Spores

A vial containing the dehydrated pellet of *B. stearothermophilus* 12980 (ATCC, Rockville, Maryland) was disinfected with alcohol-dampened gauze. From a test tube containing 6 mL of nutrient broth, 1 mL of nutrient broth (Difco, Becton Dickinson and Company, Sparks, Maryland) was aseptically pipetted and used to rehydrate the pellet. The rehydrated pellet was aseptically transferred back into the test tube

containing nutrient broth and the mixture vortexed to thoroughly mix the material. Using a sterile loop, plates of trypticase soy agar (TSA) were streaked with this suspension. The plates were inverted and incubated at 55 ^{o}C for 24 hours.

B. stearothermophilus 12980 spores were propagated on plates of TSA (Difco Manual, 1998) supplemented with $MnSO_4$ (100 mgL^{-1}) by aseptically inoculating the plates with pure isolates of *B. stearothermophilus* 12980, the plates were incubated at 55 ^{o}C for 5 days, and then further incubated at room temperature for an extra 5 days on the bench top. Once the plates were transferred from the 55 ^{o}C incubator, they were sealed with parafilm to minimize drying of the media. Growth on each TSA plate was scraped from the agar surface with a sterile loop and aseptically transferred into a centrifuge tube containing 10 mL of sterile water. The mixture was vortexed thoroughly to obtain a suspension of *B. stearothermophilus* 12980 spores.

5.5 Initial Liquid Inoculum of *B. stearothermophilus* 12980 Spores

A suspension of *B. stearothermophilus* 12980 spores was prepared by aseptically transferring growth of *B. stearothermophilus* on one agar plate into 5 mL of deionized water in a centrifuge tube and vortexing thoroughly (5 to 10 seconds) to mix using a vortex mixer. An estimate of spore concentration in this suspension was made using the direct microscopic count.

5.5.1 Direct Microscopic Count of *Bacillus stearothermophilus*12980

The following procedure was followed.

1. The sample was diluted so that the concentration of bacterial spores would equal 5 to 15 spores in each small square in the grid of the counting chamber.
2. The suspension was added to the counting chamber using a pipette.
3. The spores were allowed to settle for about 5 minutes.
4. The counting chamber was placed on the stage of the light microscope.

A sufficient number of squares to give a total count of about 600 spores were counted for greatest accuracy. Manual counts were made at 630 X magnification. Spore density in the suspension was obtained using Equation 5.1.

$$\text{Original spore density} = \text{SPSQ} / (v_{ssq} \times \text{df}) \quad (5.1)$$

Where:

SPSQ = average number of spores per small square

v_{ssq} = volume above a small square, 2.5×10^{-7} mL

df = dilution factor, dimensionless

The dilution factor was obtained by using Equation 5.2.

$$df = v_1 / v_2 \quad (5.2)$$

Where:

v_1 = volume of sample to be diluted, mL

v_2 = combined volume of sample and diluent, mL

5.5.2 Limits of Detection of Spores by Direct Microscopic Count

The lower and upper limits of the number of spores in a sample that could be detected by the direct microscopic count method using the Petroff-Hausser counting chamber were determined as follows (Equations 5.3 and 5.4). The lower and upper limits were based on the minimum and maximum, respectively, number of spores per small square that could be counted accurately. The volume of each small square was 2.5×10^{-7} mL

$$\text{Lower limit} = df \times 2 \times 10^7 \text{ spores / mL} \quad (5.3)$$

$$\text{Upper limit} = df \times 6 \times 10^7 \text{ spores / mL} \quad (5.4)$$

Where:

df = dilution factor of sample of spores

5.6 preparation of Dry Feed Inoculum of *B. stearothermophilus* 12980

To prepare feed inoculum , 10 mL of *B. stearothermophilus* ATCC 12980 spore suspension containing (3 ± 0.02)x 10^6 spores / 20 mL was pipetted and aseptically added to 1 kg of the standardized feed formulation in a container with a lid. The lid was replaced and its contents vigorously shaken manually for 5 minutes in a 30 cm arc. Feed inoculum was then refrigerated at 4 ^oC until used.

5.7 *B. stearothermophilus* Spore Enumeration Media

The American Association of Cereal Chemists (AACC) method specifies dextrose tryptone agar for use in isolating thermophilic organisms from food products (Method 42 – 40, AACC, 1995a). In "flat sour" spoilage, bacterial growth lowers the pH of canned food products by 0.3 – 0.5 while the ends of the can remain flat as a result of low or no gas production. Dextrose tryptone agar can also be used to isolate thermophilic flat sour spore formers such as *B. stearothermophilus* (Difco Manual, 1998).

Formula per liter for dextrose tryptone agar:

Bacto tryptone	10 g
Bacto dextrose	5 g
Bacto agar	15 g
Bacto brom cresol purple	0.04 g
Final pH 6.7 ± 0.2 at 25 ^oC	

Dextrose tryptone agar contains tryptone, which provides carbon, and nitrogen for the general growth requirements of the bacterial cells. Dextrose is the carbohydrate source while brom cresol purple is the pH indicator. A change in color of the medium from purple to yellow indicates dextrose fermentation (Figure 5.1). Thirty grams of dextrose tryptone agar was suspended in 1.0 L of deionized water and then heated, while agitating with a magnetic stirrer, to a boil. The agar was allowed to boil for one minute and then it was autoclaved for 15 minutes at 121 ^{o}C. The agar was used in the pour plating technique for *B. stearothermophilus* recovery and enumeration.

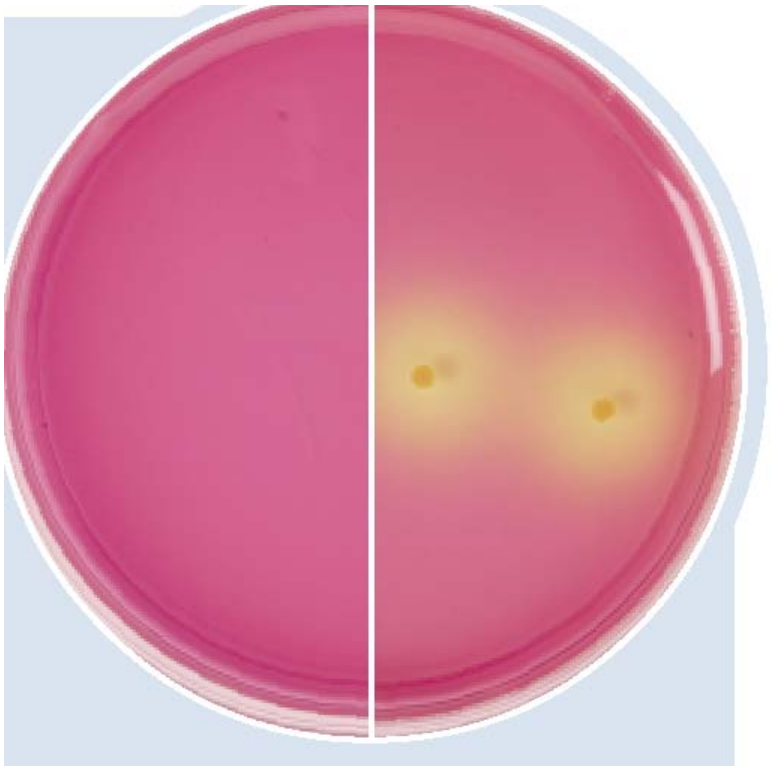

Figure 5.1. A plate of dextrose tryptone agar illustrating the changes that occur after incubation at 55 ^{o}C for 36 – 48 hours. The left hand side of the plate was not inoculated. The right hand side of the plate was inoculated with *Bacillus* coagulans (Difco, 1995).

5.8 *Salmonella typhimurium* Enumeration Media

McConkey agar (Difco, 1998) supplemented with 100 ppm of $MnSO_4$ (Manganese sulfate) was used for the recovery and enumeration of *S. typhimurium* in feed (method 42-25A, AACC, 1995b).

Formula per liter for McConkey agar was as follows:

Polypeptone	3.0 g
Peptone	17.0 g
Lactose	10.0 g
Bile salts No. 3	1.5 g
$NaCl_2$	5.0 g
Neutral red	0.03 g
Agar	13.5 g

Dry ingredients of McConkey agar were weighed and dissolved in 1 L deionized water. The agar was heated to a boil while being agitated with a magnetic stirrer. Next, the agar was autoclaved for 15 minutes at 121 ^{o}C, cooled to 45 – 50 ^{o}C and poured into 15x100 mm petri dishes. Poured plates were allowed to dry covered for at least 2 hours. Final plate pH was 7.1± 0.1. A 10% stock solution of nalidixic acid (Lot number 95H5009, Sigma Chemical Company, St. Louis, Montana) was prepared by adding 0.5 g nalidixic acid into a 250 mL beaker and then adding deionized water to make up 5 g of solution. One mL of filter sterilized 10% stock solution was added to 1 L of McConkey agar to produce the desired McConkey with 100 ppm nalidixid acid agar.

5.9 Feed Inoculation with Dry Feed Inoculum

5.9.1 Serial Dilution Technique

Dilutions of feed suspension were selected for pour plating to yield plates containing 25 to 250 colonies. Serial dilutions were used to dilute cultures containing large numbers of organisms, for example 3.0×10^6 CFU per mL of broth to a manageable number, such as 30 organisms per mL, without the use of a tremendous amount of diluent. The original dilution was diluted in progressive steps as shown in the top row of Figure 5.2 (Smith, 2000.)

5.9.2 Enumeration of *Salmonella typhimurium* in Control and Inoculated Feed

The following procedure was used to enumerate *Salmonella typhimurium* (method 42-25A, AACC, 1995b).

1. Using aseptic techniques, 25 g of control or inoculated feed sample was weighed in a sterile 250-mL sampling container placed on a weighing scale (± 0.01g).
2. Deionized water was added to make up 250 g of suspension. The sampling container lid was tightly replaced and the suspension shaken vigorously in 30 cm arcs for 2 minutes by hand to make the 10^{-1} dilution.

3. Serial dilutions were prepared from the 10^{-1} dilution prepared in step 2 above by transferring 1mL of the suspension into a test tube containing 9 mL of deionized water. Subsequent serial dilutions were prepared similarly.
4. 100 micro liters of appropriate dilutions were plated in duplicates on McConkey agar supplemented with 100 ppm nalidixic acid.
5. Inoculated plates were incubated for 24 hours at 37 ^{o}C.
6. Colonies on plates were counted after 24 hours of incubation using the Darkfield Quebec colony counter (Model 3330. Darkfield Quebeck, Buffalo, New York).

5.9.3 Decimal Dilutions of *B. stearothermophilus* Spores in Initial Liquid Inoculum

Dilution blanks consisting of 9 mL deionized water were prepared in test tubes. One mL of the initial suspension was aseptically transferred to the first dilution blank to make the 10^{-1} dilution. After thoroughly vortexing the 10^{-1} dilution, 1 mL was transferred to the next dilution blank using a separate sterile pipette tip to make the 10^{-2} dilution. Subsequent decimal dilutions were prepared using the serial dilution technique, transferring 1 mL quantities into dilution blanks by using a separate sterile pipette tip for each dilution (Figure 5.3).

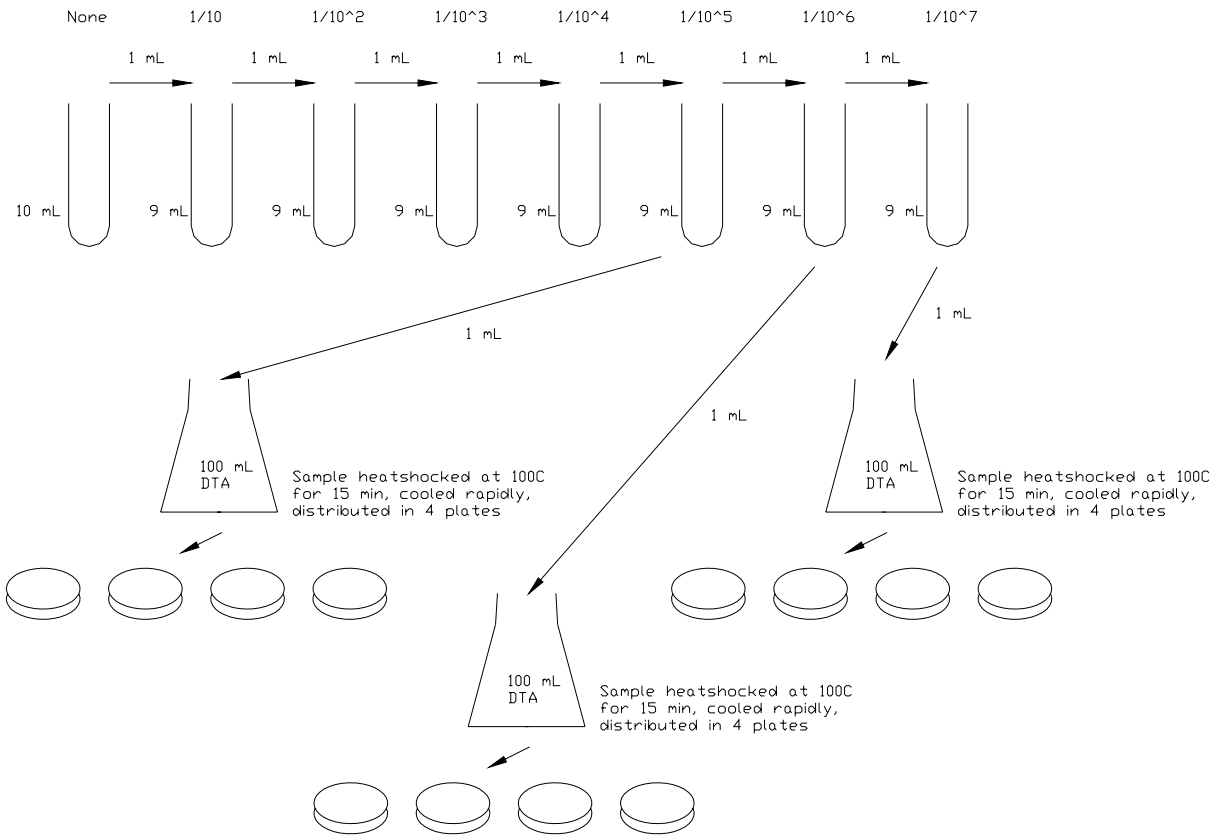

Figure 5.2. Preparation of dilutions from an initial suspension of *Bacillus stearothermophilus* spores in distilled water.

While the spore sample was agitated 1 mL samples of appropriate dilutions were transferred to 250-mL Erlenmeyer flask containing 100 mL sterile dextrose tryptone agar at a temperature of between 50 and 60 ^{o}C. Samples were then heat shocked at 100 ^{o}C for 15 minutes. After heat shocking, samples were cooled rapidly in a cold water bath to 50 - 60 ^{o}C. Samples were then equally distributed into 4 sterile Petri plates under a laminar flow biohazard hood (model 240, serial number 19795B, Contamination). Four plates were used instead of the 5 specified in method 42 – 40 procedures of the AACC (AACC, 1995a) because the 100 mL samples could not completely cover the bottom of 5 plates.

5.9.4 Enumeration Bacterial Spores in Control and Inoculated Feed

The AACC method 42-40 procedures were used for spore enumeration (AACC, 1995a). Twenty grams of each feed sample was weighed in a sterile sampling container set on a portable bench top weighing scale. Deionized water was then added till the scale read 100 g. The container lid was tightly replaced and the suspension vigorously shaken by hand for 10 seconds in a 30 cm arch to obtain a uniform suspension of feed in water. Serial dilutions were prepared from this initial 1:5 dilution by aseptically transferring 10 mL quantities of suspension into 90 mL dilution blanks consisting of deionized water in 250-mL sterile sampling containers with lids (Figure 5.3).

While the suspension was agitated, 20 mL of the feed suspension was pipetted using a large bore pipette into a 250-mL Erlenmeyer flask containing 100 mL sterile dextrose tryptone agar at a temperature of between 50 and 60 ^{o}C. The flask was placed in the steam cabinet and heat shocked at 100 ^{o}C for 15 minutes. A thermometer was set in a 250-mL flask containing 100 mL dextrose tryptone agar as a temperature control. After heat shocking, samples were cooled rapidly in a cold water bath to 50 - 60 ^{o}C after which the entire mixture was equally distributed into 5 sterile Petri plates under a laminar flow biohazard hood (model 240, serial number 19795B, Contamination

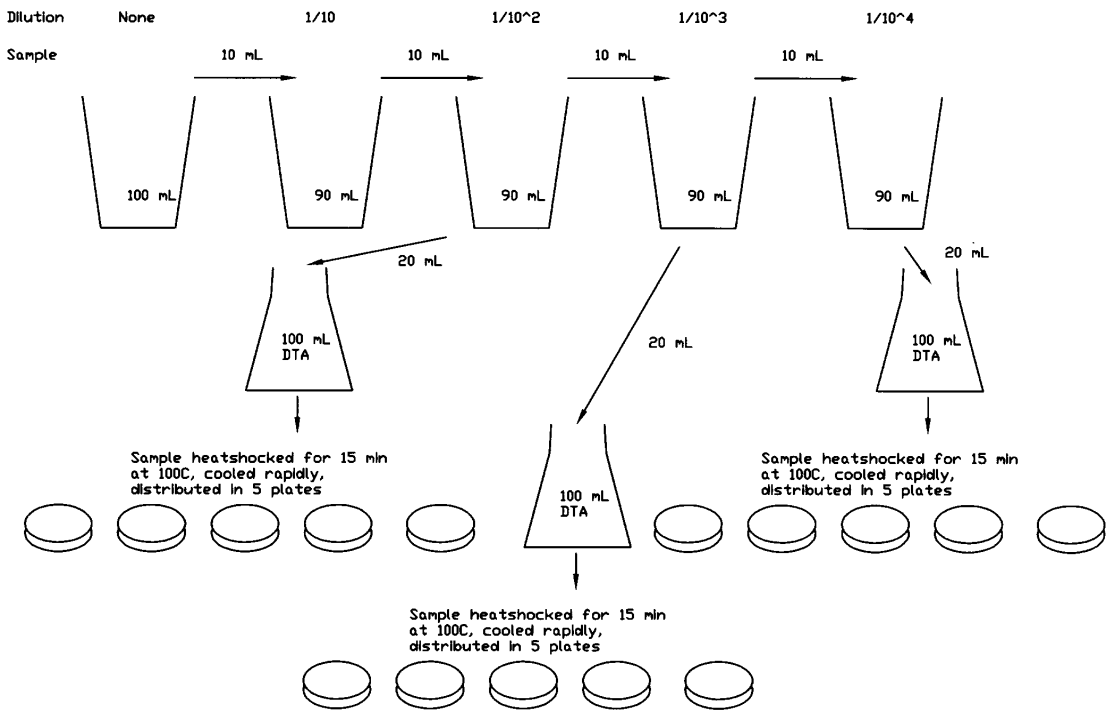

Figure 5.3. Preparation of dilutions from an initial 1:5 (20 g feed: 100 g feed and water) of feed in deionized water. DTA = dextrose tryptone agar.

Control Incorporated, Lansdale, Pennsylvania). A similar temperature control flask was exposed to the same heating and cooling conditions as the test sample to monitor the heat shocking and cooling steps of the procedures. Plates were allowed to dry partially covered for 45 minutes to 1 hour at room temperature under the laminar flow biohazard hood then incubated at 60 - 65 ^{o}C for 24 hours (VIP Imperial II CO_2 incubator with dual chambers, dual control. Lab-line Instruments Incorporated, Melrose Park, Illinois). Colonies were counted between 16 and 24 hours using a colony counter (Model 3330, Darkfield Quebeck, Buffalo, New York).

5.10 Determination of Spore Density by Cultural Method

Spore density of *B. stearothermophilus* in feed was determined using Equation 6.

Spore density = spore count x df (5.5)

Where:

spore density	=	spore count / 20 g feed
spore count	=	sum of spore counts in 5 plates poured from mixture of 100 mL dextrose tryptone agar and 20 mL suspension of a selected dilution
df	=	dilution factor (5×10^{3}), an initial suspension of 20 g inoculated feed was suspended in 80 g deionized water, 3 decimal dilutions of 10^{-1} each were serially made from the initial suspension

5.11 Limits of Detection of Spores in Feed by Cultural Methods

The lower limit of detection was based upon the lowest number of bacterial colonies per plate (25 CFU) that could be counted accurately (Smith, 2000). As an example, suppose that for a 20 g feed sample there were 25 CFU on each of the five plates poured. The total number of CFU would be 125 CFU per 20 g of feed. This may be rounded of to 1.2 x 10^2 CFU / 20 g of feed (2 significant figures). Similarly, the upper limit of detection was based on the greatest number of bacterial colonies per plate (250 CFU) that could be counted accurately (Smith, 2000). For a 20 g feed sample, if 250 CFU were counted on each of the five plates poured, the total would be 1250 CFU / 20 g of feed. This rounds off to 1.2 x 10^3 CFU / 20 g of feed. The lower and upper limits of detection of spore in feed by cultural methods were estimated as follows (Equations 5.6 and 5.7).

Lower limit of detection = df x 1.2 x 10^2, spores / 20 g feed (5.6)

Upper limit of detection = df x 1.2 x 10^3, spores / 20 g feed (5.7)

Where:

Lower and upper limits of detection = least and greatest, respectively, detectable number of spores per 20 g of feed

df = dilution factor (5 x 10^3), based on an initial suspension of 20 g of inoculated feed suspended in 80 g deionized water then 3 serial dilutions of 10^{-1} each of this

5.12 Sensitivity and Specificity of Spore Enumeration Method

Spores of *B. stearothermophilus* were artificially added to mash feed containing indigenous acid producing, thermophilic microorganisms in order to elevate the total

feed spore density (CFU / g of feed) such that a detectable level of spores were left in the feed after extrusion cooking under the conditions used in this study. Sensitivity of the method used to recover and enumerate bacterial spores in the test feed can be expressed as the ratio of the spore density (CFU / g feed) as determined by the method to the predicted spore density (CFU / g feed) based upon the amount of artificially inoculated spores (Equation 5.8). It measures the ability of the method to detect the targeted organism in the feed sample.

$$S_{sen} = (SD_{observed} / SD_{predicted}) \times 100\% \quad (5.8)$$

Where:

S_{sen} = sensitivity, %

$SD_{observed}$ = spore density as observed by enumeration method, CFU / g feed

$SD_{predicted}$ = predicted spore density as based on amount of artificially inoculated spores, CFU / g feed

Specificity measures the ability of the enumeration media to correctly recover the targeted organism using a specified enumeration media. It can be expressed as the ratio of the density of the targeted organisms recovered to the total number (targeted + any other) of organisms recovered from the feed sample using the enumeration media (Equation 5.9).

$$S_{spe} = (R_{targeted} / R_{total}) \times 100\ \% \quad (5.9)$$

Where:

S_{spe} = specificity of enumeration method,%

$R_{targeted}$	=	density of targeted organism recovered from feed sample, CFU / g feed
R_{total}	=	density of all organisms recovered from feed sample using a specified enumeration media, CFU / g feed

Spores of all indigenous acid producing,, thermophilic organisms present in the feed and artificially inoculated *B. stearothermophilus* were recovered using method 42 – 40 of the AACC procedures (AACC, 1995a). Because the bacterial spores used in this research were a mixed culture, sensitivity and specificity, respectively, of the spore enumeration method was not determined.

5.13 Standardized Feed Formulation

A standardized feed formulation consisting of 60 % corn meal, 30 % soybean meal, and 10% animal protein blend was prepared at the site of the experiment. Corn meal (Cooperative Milling, Gettysburg, Pennsylvania) was prepared using a roller mill (serial number 435386, Roskamp roller mill, Waterloo, Iowa). Soybean meal (Cooperative milling, Gettysburg, Pennsylvania) and animal protein blend (AKEYS Incorporated, Lewisburg, Ohio) were obtained from commercial suppliers of animal feed products. Tables C 1, C 2 and C 3, respectively, in Appendix C contain specifications of the standardized feed formulation by constituent. Feed formulation was stored at ambient temperature in sealed barrels until used.

5.14 Determination of Particle Size Distribution

5.14.1 Method of Sieving

One large batch of the standardized feed formulation (2000 pounds) was prepared and used throughout the studies. Representative samples were taken from this batch of feed by sampling from different but equally spaced locations in a horizontal plane and different depths in randomly selected storage barrels. This particle size distribution analysis was included to describe the standardized feed formulation used in the studies. The American Society of Agricultural Engineers (ASAE) method ASAE S319.3 was used in sieving feed samples as follows (ASAE Standards, 2002).

1. A nest of 8 test sieves was arranged in order of descending sieve opening (US sieve numbers 4, 10, 18, 20, 35, 60, 100 and 200).
2. 100 g of feed sample was placed on the top sieve of the nest of sieves and shaken using the Octagon 200 test sieve shaker (serial number 8-200-3834. Endecotts Octagon 200, London, England) until the mass of material on any one sieve reached an end-point (15 minutes).
3. Mass of material on each screen was determined using a weighing scale (serial number N54689, model PM34-K, Mettler Instruments, Stown New Jerser) and recorded.

5.14.2 Data Analysis

This analysis was completed in order to describe the standardized feed formulation used in the studies. Particle size was presented as cumulative distributions (Table D 1 in Appendix D). Geometric mean diameter, geometric standard deviation of log normal distribution by mass, and geometric standard deviation of particle diameter by mass were determined based on derivations by Pfost and Headley (1976) (Equations 5.10, 5.11, and 5.12).

$$d_{gw} = \log^{-1}[\ \sum(W_i \log d_i) / \sum W_i\ ] \quad (5.10)$$

$$S_{log} = [\ \sum W_i(\log d_i - \log d_{gw})^2 / \sum W_i\ ]^{0.5} \quad (5.11)$$

$$S_{gw} = 10^{Slog} \quad (5.12)$$

Where:

d_i = nominal sieve aperture size of the ith sieve, microns

d_{gw} = geometric mean diameter of particles by mass, microns

Slog = geometric standard deviation of log-normal distribution by mass in ten-based logarithm, dimensionless

S_{gw} = geometric standard deviation of particle d_i by mass, microns

W_i = mass on i^{th} sieve, g

$\log^{-1}(x)$= 10^x, x being a variable

5.15 Feed Mash Moisture Content Control

To obtain feed of the desired moisture content, a predetermined amount of tap water was added to 10 kg of feed mash and the two mixed for 10 minutes using a paddle mixer (Model H600, Hobart corporation, Troy, Ohio)(Figure 4.9). A mixing container was tared on a weighing balance (Weigh-Tronix scale – indicator model number W1-125, base model number 2155, Weigh-Tronix incorporated, Fairmont, Minnesota); 10 kg of dry feed and a previously determined amount of water were then added. The amount of tap water, M_W, required in 10 kg (M_{AF}) of dry feed was determined using Equation 5.13. This method of feed moisture regulation provided for a more precise control of the feed moisture content than the regulation of feed moisture content by addition of water and steam at the extruder barrel. Two replications of moisture content determinations were made of the following targeted mash feed moisture content: 26%, 28%, 30%, 32%, and 34 %, respectively, wet basis.

$$M_W = M_{AF} (Mc_D - Mc_{AF}) / (100 - Mc_D) \quad (5.13)$$

Where:

M_W = mass of water added, kg

M_{AF} = mass of ambient feed, before addition of water, kg

Mc_{AF} = moisture content of ambient feed, before addition of water, wb

Mc_D = targeted moisture content of feed, % wb

5.16 Mash Feed Moisture Content Measurement

Following mixing of 10 kg – batches of ambient feed with predetermined amounts of water, two 100 g - feed - samples were extracted from the surface and the center of the 10 kg - batches of mash feed for moisture content analysis. The batches of mash feed were not inoculated with *B. stearothermophilus* spores. The approved methods of the American Association of Cereal Chemists (Method 44 - 01, AACC, 1995c) were used in this measurement. Samples were prepared and analyzed as follows:

1. Two grams of well-mixed mash feed were accurately weighed in a covered dry dish using an electronic scale, (PG 5002-S Delta range, model number 5002-SDR, serial number 1116313872, Mettler Toledo, Columbus, Ohio). With caps loosened, samples were heated at 98 – 100 ^{o}C for about 5 hours to a constant dry weight in a partial vacuum equivalent to 30 mmHg using the Isotemp Vacuum Oven (Fisher scientific, Model number 285A). Dry air was admitted into the oven to return oven chamber to atmospheric pressure before removing samples.
2. Samples were transferred to a desiccator (Figure 4.10) to cool to room temperature
3. Mass of samples were observed and recorded soon after they reached room temperature.
4. Moisture content was determined using Equation 5.14 (AACC, 1995c).

$$\text{Percent moisture (\%)} = 100 \times (M_{wet} - M_{dry}) / M_{wet} \quad (5.14)$$

Where:

M_{wet} = mass of feed sample before drying, g

M_{dry} = mass of feed sample after drying, g

5.17 Feed Wetting and Drying Experiment

An experiment was conducted where about 5 g samples of feed were dried, rewetted to achieve the 24.5 to 32.5 % (wet basis) range of feed moisture covered in this research and then dried again to see if all the rewetting water was recovered. Amounts of water added to the 5 g feed samples were estimated using Equation 5.13. Drying was accomplished using an Isotemp® oven (Model 655F, Fisher Scientific) similar to the one described under Section 4.13.1 ("Vacuum oven"); an aluminum tray containing a layer of indicating drierite (anhydrous calcium sulfate impregnated with cobalt chloride) was placed in the oven to control oven air humidity. The following procedure was used.

1. Approximately 5.5 g samples of the standardized feed formulation were weighed in 15 separate aluminum weighing pans using an electronic scale (PG 5002-S Delta range, model 5002-SDR, Mettler Toledo, Columbus, Ohio) . Weighing pans were labeled with symbols indicating a predicted moisture level and a replicate number (3 replicates each for moisture levels

24.5 %, 26.5 %, 28.5 %, 30.5% and 32.5% wet basis, respectively). Weight data of each sample was recorded.

2. Samples were then dried at 98 – 100 ^{o}C for 5 hours.
3. Dried samples were transferred to a desicator (Fisher Scientific) similar to the one described under Section 4.13.2 ("Desiccator") and allowed to cool for 10 minutes.
4. To each sample, a predetermined amount of water corresponding to the predicted moisture level was added, 3 replicates for each moisture level as indicated in 1) above. Rewetted feed samples were then dried at a temperature of 98 – 100 ^{o}C for 5 hours in the Isotemp® oven (Model 655F, Fisher Scientific). The amount of water in grams added to each sample was recorded.
5. Redried feed samples were reweighed and the weight of each one recorded.

5.18 Mash Feed Water Activity (a_w) Measurement

A water activity meter (model CX-2, Decagon devices incorporated, Pullman, Washington) was used to measure a_w of the mash feed. Mash feed samples used in this determination were collected as described in procedures under "Mash Feed Moisture Content Measurement". A 2 g sample of mash feed was placed in a sample tray and inserted into the reading chamber of the water activity meter. Values of a_w were observed directly on the meter's liquid crystal display after a 1 to 15 minute

equilibration depending on the sample. The meter sounded (intermittent beeps) when equilibration was complete.

5.19 Mash Feed Inoculation with *B. stearothermophilus* Spores

An amount of feed inoculum required in 10 kg of feed mash that would give approximately 2.0 x 10^7 spores / 20 g of feed was determined using Equation 5.15.

$$M_{FI} = M_{MF} (CFU_D - CFU_{MF}) / (CFU_{FI} - CFU_D) \quad (5.15)$$

Where:

M_{FI} = mass of feed inoculum, kg

CFU = colony forming unit

CFU_D = desired spore concentration in final inoculated feed mash CFU / g feed

CFU_{MF} = spore concentration in mash feed, CFU / g feed

CFU_{FI} = spore concentration in feed inoculum, CFU / g feed

M_{MF} = mass of mash feed, kg

5.20 Experimental Design

5.20.1 Central Composite Design

Some of the desirable characteristics of experimental designs for response surface estimation include: 1) the ability to estimate experimental error variance, and 2) allow for a test of lack of fit to the model. In addition, designs should efficiently estimate the model coefficients and predict responses (Kuehl, 2000). Central composite designs are 2^n factorial treatment designs with 2n additional treatment combinations referred to as the axial points along the coordinate axes of the coded factor levels. Equation 5.16 (Kuehl, 2000) gives the number of experimental units required for the central composite design with n independent variables. Central composite designs are more economical in terms of experimental units and enable the estimation of quadratic response equations (Kuehl, 2000). Table 5.1 summarizes all the combinations (treatments) of independent extrusion variables required for the central composite design used in these studies.

$$N_v = 2^n + 2n + m \qquad (5.16)$$

Where;

N_v = total number of independent variable combinations required in a rotatable central composite design

n = number of independent variables,

m = replication at the center of the central composite design

A response surface model was used because the experimental variables were 1) continuous, and 2) used to derive levels of extrusion independent variables that optimized spore inactivation, that is, a maxima, or a series of independent variable combinations that produced maximum spore inactivation.

Table 5.1. A list of feed extrusion independent variables. Treatments were combinations of feed extrusion independent variables. Treatments were arbitrarily assigned numbers 1 through 15.

Treatment	Extruder Barrel Exit Temperature, T °F (°C)	Moisture Content of Mash Feed, Mc (% wb)	Retention Time of Feed in Extruder Barrel, Rt (s)
1	170 (77)	28.5	7
2	182 (83)	26.5	5
3	182 (83)	26.5	9
4	182 (83)	30.5	5
5	182 (83)	30.5	9
6	200 (93)	24.5	7
7	200 (93)	28.5	3
8	200 (93)	28.5	7
8	200 (93)	28.5	7
8	200 (93)	28.5	7
8	200 93)	28.5	7
8	200 (93)	28.5	7
8	200 (93)	28.5	7
9	200 (93)	28.5	11
10	200 (93)	32.5	7
11	217 (103)	26.5	5
12	217 (103)	26.5	9
13	217 (103)	30.5	5
14	217 (103)	30.5	9
15	230 (110)	28.5	7

Five levels of each one of the 3 variables coded as –α, -1, 0, 1, and α, respectively, were chosen on the basis of preliminary test results that were used to determine the possible ranges of each of the variables (Table 5.2). The value of $\alpha = (2^n)^{1/4}$, the axial point, was selected to obtain a rotatable, spherical central composite design, where n is the number of variables. Further, 6 runs of the design center point were selected for each replicate to obtain uniform precision of the spore inactivation at all points equidistant from the center of the central composite design (Kuehl, 2000). Table 5.3 summarizes a complete set of runs for one replication of the experiment in coded units. A spherical design region was desirable because the extruder could not be operated at one or more of the extremes of the design region, and it was strongly suspected that the response would express a simple maxima or minima within the study variable ranges.

Table 5.2. Coded Levels of Extrusion Cooking Variables (Cochran And Cox, 1957).

Variable	Coded level	-1.682	-1	0	1	1.682
Rt[a], s		3	4.62	7	9.38	11
T[b], °C		77	83	93	103	110
Mc[c],% wb		24.5	26.5	28.5	30.5	32.5

[a]Rt retention time, [b]T = extruder barrel exit temperature, [c]Mc = mash feed moisture content

5.20.2 Split-Plot Design

Extruder barrel exit temperature was hard to vary during the experiment and was therefore used to restrict the randomization order by grouping sets of runs together that had the same extruder barrel exit temperature or runs were grouped such that extruder barrel exit temperature of a set of runs was in an ascending order. Because feed samples taken from not more than four runs could be appropriately analyzed for *B. stearothermophilus* spore density in one experiment day, runs were grouped into sets of four runs per day. Table G 1 in Appendix H lists the order of experimental runs during the studies.

The experiment consisted of extruding a standardized feed inoculated with B. *stearothermophilus* spores to determine spore inactivation achieved at various extruder settings. The three feed extrusion variables considered were: 1) moisture content of feed mash (Mc), 2) extruder barrel exit temperature (T), and 3) retention time of feed in the extruder barrel (Rt). To prepare feed for the experiment, each of four batches of ambient moisture feed (10 kg) were mixed with specified amounts of moisture corresponding to four runs of an experiment day using a paddle mixer. Keeping extruder barrel exit temperature at a specified level, four runs were performed involving levels of the other two factors, Mc and Rt.

Table 5.3. The Central composite design showing a complete set of experimental units (treatments) in coded levels for three variables (Cochran and Cox, 1957). Treatments were arbitrarily identified with numbers 1 through 15. Key: Rt = retention time of feed in extruder barrel (s), T = temperature at extruder barrel exit (^{o}C), Mc = mash feed moisture content, % wet basis.

Treatment	T, ^{o}C	Mc, % wb	Rt, s
1	77	28.5	7
2	83	26.5	5
3	83	26.5	9
4	83	30.5	5
5	83	30.5	9
6	93	24.5	7
7	93	28.5	3
8	93	28.5	7
8	93	28.5	7
8	93	28.5	7
8	93	28.5	7
8	93	28.5	7
8	93	28.5	7
9	93	28.5	11
10	93	32.5	7
11	103	26.5	5
12	103	26.5	9
13	103	30.5	5
14	103	30.5	9
15	110	28.5	7

Treatment	T, ^{o}C	Mc, % wb	Rt, s
1	77	28.5	7
2	83	26.5	5
3	83	26.5	9
4	83	30.5	5
5	83	30.5	9
6	93	24.5	7
7	93	28.5	3
8	93	28.5	7

8	93	28.5	7
8	93	28.5	7
8	93	28.5	7
8	93	28.5	7
8	93	28.5	7
9	93	28.5	11
10	93	32.5	7
11	103	26.5	5
12	103	26.5	9
13	103	30.5	5
14	103	30.5	9
15	110	28.5	7

The object of the experiment was to build a response surface model that describes the effect of the extrusion cooking process on B. *stearothermophilus* spores in feed. The selected model consisted of linear terms, quadratic terms, and cross product terms of the three factors.

5.21 Statistical Procedures

Normality tests were performed on residuals of Log (N_o/N), where N_o and N are the initial and final spore count in CFU / 20 g feed, respectively. Once the normality requirements were met, a response surface model was obtained since factors involved in the experiment were: 1) continuous, and 2) would be used to derive an important feature, namely levels of variables that optimized the response (stationary point). Data was fit to a regression curve for the inactivation of *B. stearothermophilus* spores. Response surface analysis was used to estimate the model coefficients, perform a lack of fit test and to obtain the stationary point and it's estimated value using the response

surface regression (RSREG) procedure of SAS (Freund and Littell, 2000). The mean day within replicate effect for each set of four runs performed on each experiment day was obtained using the "mixed procedure" of SAS (Littell et al., 1996). Each dependent observation was then adjusted for day within replicate variation before performing the RSREG procedure on the data set. The "ridge max" option in RSREG procedure was included to generate the ridge of maximum response, which is, the series of extrusion treatments that maximized spore inactivation. The analysis was based on a second-degree polynomial with three explanatory factors.

6. RESULTS AND DISCUSSION

6.1 Particle Size Distribution

Particulate material is normally described by its mean particle size and particle size distribution (PSD). Figure 6.1shows the cumulative particle size distribution of the feed. The analysis of PSD was completed to describe the standardized feed formulation used in the studies. The standardized feed formulation showed a log normal distribution (Figure 6.1). Therefore PSD was expressed as a geometric mean diameter (GMD) and the geometric standard deviation (Table D 1 in Appendix D). The geometric mean particle diameter was 648 microns with a geometric standard deviation of 1.89 microns. Particle size affects the rate of heat and moisture flows into the interior of the feed particle during extrusion cooking (Bouvier, 2001). Fourier' second law deals with the rate of heat flow while Fick's second law governs the mass flux per unit area, respectively, into the interior of the feed particle as shown in Equation 6.1. As an example, thermal diffusivity for starch materials at ambient temperature is about 10^{-7} m^2 / s while the water diffusivity (D) is nearly 10^{-9} m^2 / s (Bouvier, 2001). Consider a feed particle in the standardized feed formulation at moisture content of 28 % wet basis. Assuming the particle density of 1500 kg / m^3, for starch (Singh and Heldman, 2001e), and a dry particle interior, we get a moisture concentration at the surface of 368.4 kg / m^3. From Equation 6.1(Singh and Heldman, 2001b), the mass flow rate per unit area, $\dot{m} / A_d$, can be calculated as follows:

$(\dot{m} / A) = (1 \times 10^{-9} m^2 / s) \times (368.4 kg / m^3) / (648 \times 10^{-6} m / 2)$ giving a flux of 0.00114 kg / s m^2 of water into the interior of the particle. This information may be useful in estimating the time necessary to humidify the particles homogeneously, that is hydration time, during preconditioning as part of extrusion cooking.

$$\dot{m} = - D A (dc / dx) \qquad (6.1)$$

Where:

$\dot{m}$ = mass flow rate of diffusing substance, kg / s

D = diffusivity of diffusing substance, m^2 / s

A_d = area across which diffusion of a substance occurs, m^2

dc = change in concentration of diffusing substance, kg / m^3

dx = change in linear distance in the direction of mass transfer of diffusing substance, m

6.2 Manual Control of Feed Moisture Content Prior to Extrusion

Manual feed moisture content control by addition of predetermined amounts of water to 10 kg samples of mash feed was found to be adequate as indicated by a slope of the calibration curve which was 0.95 and R^2 value equal to 0.98 (predicted versus observed feed moisture content) (Figure 6.2). Table E 1.1 in Appendix E contains the mean moisture content data used to plot Figure 6.2. Feed moisture content was calculated using equation 5.12. The same procedure of manual feed moisture content control was employed for every experimental run. Observed feed moisture content were on average lower than predicted feed moisture content by 1.5%. Values of observed feed moisture content were adjusted for that difference.

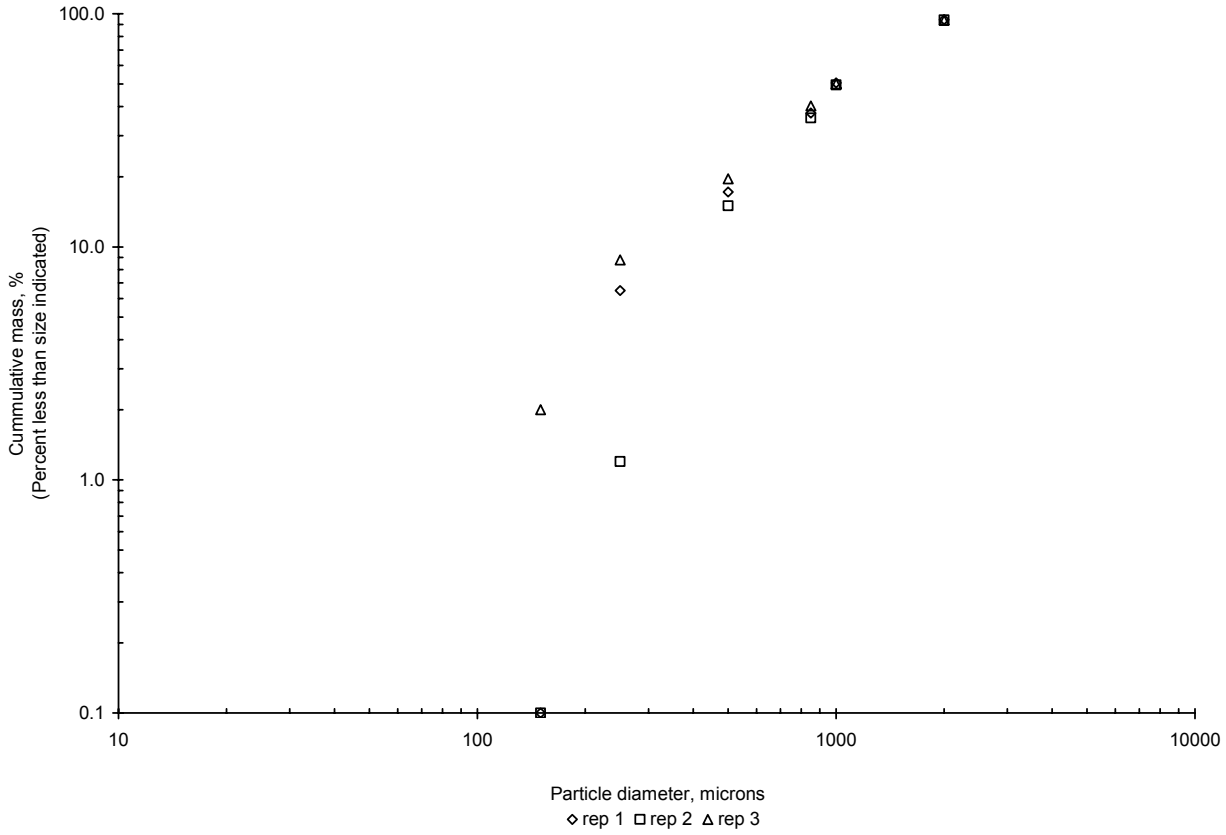

Figure 6.1 Particle size distribution curve of the standardized feed prior to extrusion (60% corn meal, 30% soybean meal and 10% animal protein blend, by weight) prior to extrusion. The data points represent three replicates (rep 1 – rep 3) plotted on logarithmic scales.

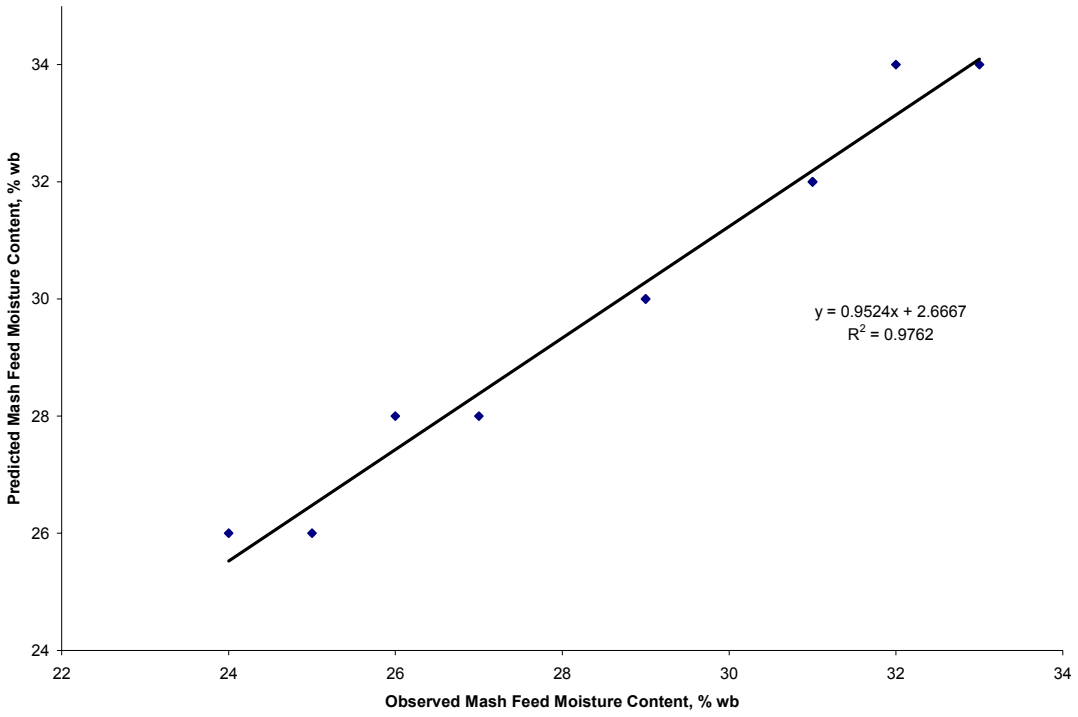

Figure 6.2. A calibration curve for mash feed moisture content estimation. Two replicates were used in this determination. wb = wet basis.

6.3 Mean Treatment Moisture Content and a_w of Feed Before and After Extrusion

No statistical analysis was completed on feed a_w because it was not controlled for in the studies. Feed a_w values of the 15 treatments were between 0.830 and 0.959. Although no trend in a_w of feed across treatments was observed, it was speculated that the thermal resistance of acid producing thermophilic organisms and *B. stearothermophilus* spores in the feed would be less than the maximal value as predicted for *B. stearothermophilus* by Murrell and Scott (1966) at an a_w value of 0.2000. The moisture content of feed before and after extrusion was compared for

each of the 15 extrusion conditions tested during the studies. Figure 6.3 shows a bar chart of these results. Raw data used in the moisture content determination is contained in Tables E 1.1 and E 1.2 in Appendix E. Observed mash feed moisture content was estimated by subtracting 1.5 % from predicted mash feed moisture content values, 1.5% being the mean difference between predicted and observed values obtained during calibration (Figure 6.2). All observed mash feed moisture contents based on this adjustment was used in the response surface model analysis. In most of the extrusion treatments, the moisture content of extrudate was lower than that of the mash feed prior to extrusion. No pattern in moisture reduction relative to Mc, T or Rt was observed. This reduction in moisture content was attributed to evaporation of moisture as a result of an interaction between retention time of feed in the extruder barrel and the high temperatures within the extruder barrel experienced by feed during extrusion. Moisture content measurement data of ambient feed in storage barrels are contained in Table E 1.3 and Figure E 1, respectively, in Appendix E. Moisture content of ambient feed did not change in storage during the 8-week study period.

Table E 1.4 and Figure E 2 show the results of the feed drying and rewetting experiment. All the rewetting water was recovered from each sample tested in the range of the feed moisture content tested (Table E 1.4 and Figure E 2). It was concluded that the standardized feed did not retain any of the water added to the samples during drying. The method used to estimate observed mash feed moisture content was not influenced by water binding by feed particles.

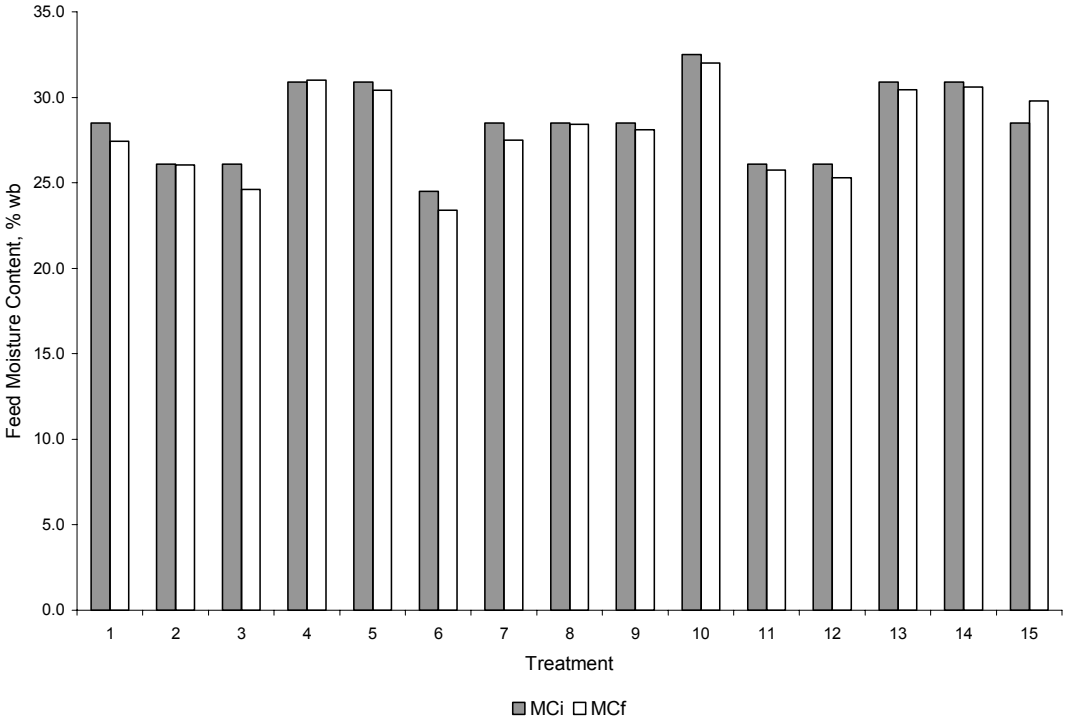

Figure 6.3 A bar chart showing mean observed moisture content of feed before extrusion (Mc_i) and observed moisture content of extrudate (Mc_f) under different extrusion treatments. Treatments are predetermined combinations of extruder barrel exit temperature, feed moisture content and retention time of feed in the extruder barrel, respectively. Table 5.1 relates treatment numbers to the set of treatment variables.

6.4 Feed Retention Time in Extruder Barrel

A calibration curve was developed and used to select extruder barrel speeds in Hertz that produced desired retention times of feed in the extruder barrel, respectively. Figure 6.4 shows calibration results. Extruder barrel speed was displayed in Hertz (equivalent to revolutions per second). The calibration curve was used to determine

extruder barrel speed in Hertz corresponding to desired retention time of feed in the extruder barrel in seconds. Raw data for the calibration is summarized in Table E 2 in Appendix E.

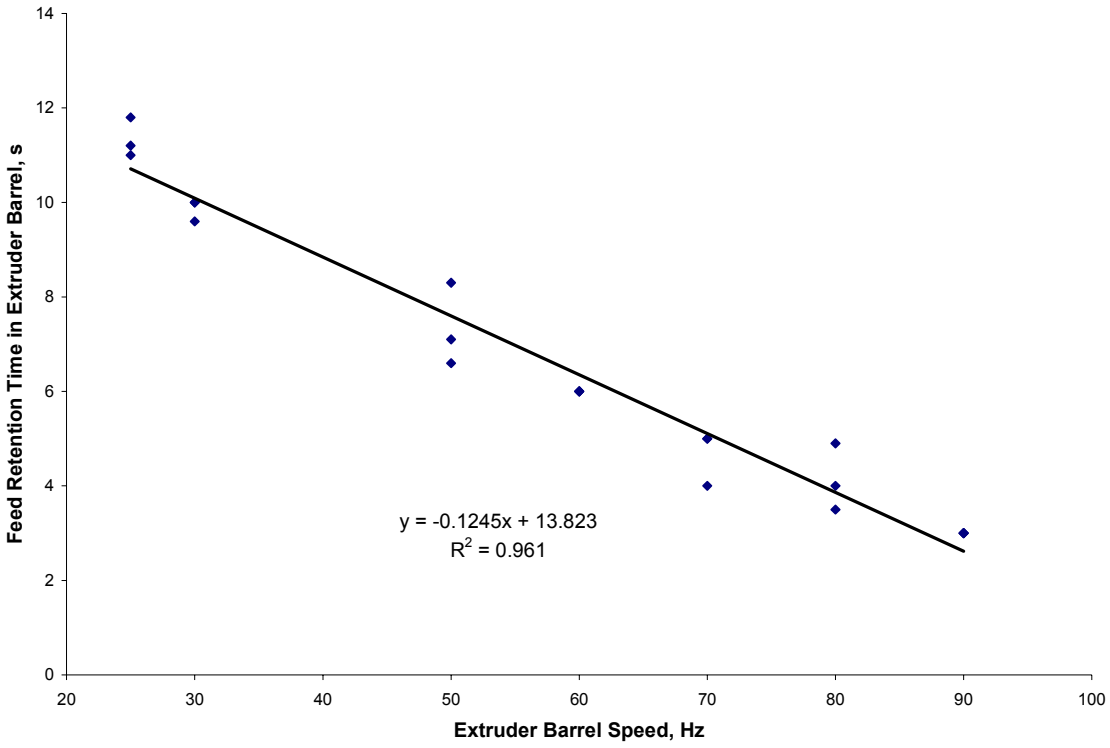

Figure 6.4. A calibration curve used for estimating extruder barrel speed in Hertz (equivalent to revolutions per second) required to produce desired feed retention time in the extruder barrel in seconds, respectively. Three replicates were used.

6.5 Feed Delivery Rate

The feed delivery auger of the live feed bin was set to run at 6 Hz for all treatments during the studies. Throughput of extrudate at this speed (94 ton/hour) was adequate and did not cause the extruder to stall (Table 6.1).

Table 6.1. Mash feed delivery rate. SE = standard error of the mean.

Trial	Mass of Feed Collected (kg)	Time Elapsed (s)	Feed Delivery Rate	
			kg/s	ton /hour
1	1.5	60	0.025	90
2	1.6	60	0.027	96
3	1.6	60	0.027	96
Mean Feed Delivery Rate			0.026	94
Standard Error			0.00096	3.5

6.6 Dry Inoculum Preparation Studies

6.6.1 Recovery of *Salmonella typhimurium* from Feed

The nalidixic acid resistant strain of *S. typhimurium* and a negative control (nalidixic acid susceptible strain of *S. typhimurium*) were inoculated on McConkey agar supplemented with 100 ppm nalidixic acid and brain heart infusion agar, respectively. Results of these studies are shown in Table 6.2. These results indicate that the use of McConkey agar with 100 ppm nalidixic acid to selectively isolate the test organism in feed samples was effective since the growth of the nalidixic acid susceptible strain of *S. typhimurium* was inhibited by the media while the test organism was successfully isolated.

Table 6.2 . Growth results of the test organism (nalidixic acid resistant *S. typhimurium*) and a control on selective and non-selective media, respectively. "+" = growth. "-" = no growth.

	McConkey agar supplemented with 100 ppm nalidixic acid	Brain heart infusion agar
Test organism, nalidixic acid resistant *S. typhimurium*	+	+
Negative control, smooth variant of *S.typhimurium* DT104	-	+

The test organism was recovered from feed samples inoculated with dry chalk inoculum of nalidixic acid resistant *S. typhimurium* but not from uninoculated feed samples, respectively, prior to extrusion. No detectable cells of the test organism were recovered from the extrudate when feed with 24.5 % (wet basis) moisture was extruded for 3 seconds at the lowest extruder barrel temperature of 82 ^{o}C. In addition, no detectable cells of the test organism were recovered under more severe extrusion conditions tested as shown in Table 6.3. It was concluded that *S. typhimurium* was not an appropriate organism for exploring extrusion conditions that optimized bacterial inactivation over the permissible ranges of the Extru-Tech E325 extruder. In seeking an organism with greater thermal resistance than *S. typhimurium, B. stearothermophilus* was selected.

Table 6.3. Summary of *Salmonella typhimurium* population densities in feed inoculated with dry chalk inoculum before and after extrusion cooking, respectively. Treatments were arbitrarily assigned to letters A_o through A_4 for identification. T = extruder barrel exit temperature, Mc = feed moisture content, wb = wet basis, Rt = retention time, CFU = colony forming units.

Treatment	T, ^oC	Mc, % wb	Rt, s	Sample Description	CFU / 20g feed	
					Mean	SE
A_0	*	24.5	*	Uninoculated feed	0	0
A_1	*	24.5	*	Inoculated feed	5.2E+04	1.9E+04
A_2	82	24.5	3	Inoculated feed	0	0
A_3	103	24.5	3	Inoculated feed	0	0
A_4	103	24.5	4	Inoculated feed	0	0

* Missing data since feed was not extruded. Two temperature settings in the mid section of the 77 to 110 ^oC range of extruder barrel exit temperatures were selected in order to rapidly assess whether *S. typhimurium* cells would withstand extrusion conditions over the whole range of temperatures.

Dry feed inocula of *S. typhimuri*um showed higher densities (Table 6.4) of the test organism prior to extrusion than dry chalk inoculum indicating that use of dry feed inoculum to elevate bacterial loads in feed would be more appropriate than dry chalk inoculum in these studies. No detectable cells of the test organism were recovered from extrudate following extrusion under conditions summarized in Table 6.4. Tables F 1.1 and F 1.2 in Appendix F show the complete *S. typhimurium* inoculum data obtained during the studies. This result was consistent with results obtained previously in which feed inoculated with dry chalk inoculum of *S. typhimurium* and extruded showed no detectable cells of the test organism.

Table 6.4 Summary of *Salmonella typhimurium* population densities before and after extrusion cooking of feed inoculated with dry feed inoculum of *Salmonella typhimurium.* T = extruder barrel exit temperature, Mc = feed moisture content, wb = wet basis, Rt = retention time, CFU = colony forming units.

					Density before extrusion, CFU / 20 g of feed		Density after extrusion, CFU / 20 g of feed	
Treatment	T, ^{o}C	Mc, % wb	Rt, s	Sample	Sample	Sample Mean	SE	CFU / 20 g feed
A	83	28.5	7	1	5.6E+08	5.6E+08	0.0E+00	0
A	83	28.5	7	2	5.6E+08			
B	88	28.5	7	1	5.2E+08	4.6E+08	9.2E+07	0
B	88	28.5	7	2	3.9E+08			
C	103	28.5	7	1	4.4E+08	5.3E+08	1.2E+08	0
C	103	28.5	7	2	6.1E+08			

SE = standard error of the mean CFU / 20 g of feed

6.6.2 Recovery of *Bacillus stearothermophilus* Spores from Feed

Results of the spore count in dry feed inoculum of *B. stearothermophilus* are shown in Table 6.5. Spore densities in the range of $2.0 \times 10^6 - 3.0 \times 10^6$ per 20 g of feed were obtained. Spores of acid producing, thermophilic organisms and *B. stearothermophilus* were recovered from dry feed inoculum after doubling the duration of heat shocking to increase thermal stress on spores beyond those achieved during the first 15 minutes of heat heating. Similarly, it was expected that the Extru-Tech E325 single-screw extruder would provide thermal stress to bacterial spores beyond that achieved by heat shocking at 100 ^{o}C for 15 minutes. A summary of recovery of spores from feed using

deionized or tap water to manually control mash feed moisture content is presented in Table F 2.1 in Appendix F. Complete spore enumeration data in the dry feed inoculum are contained in Table F 2.2 in Appendix F. It was concluded that *B. stearothermophilus* was a more suitable test organism than *S. typhimurium* for investigating the optimum feed extrusion conditions that maximized spore inactivation using the Extru-Tech E325.

6.6.3 Direct Microscopic Spore Count

A direct microscopic spore count of *B. stearothermophilus* in dry feed inoculum was made using the Petroff-Hausser counting chamber (Hausser Scientific, Blue Bell, Pensylvania). A mean density of $(3.6 \pm 0.05) \times 10^6$ CFU / 20 g feed was obtained. The spore count of *B. stearothermophilus* in feed obtained by the direct microscopic count using the Petroff-Hausser counting chamber (Table F 3 in Appendix F) was higher than that obtained by the cultural methods using dextrose tryptone agar as the pour plating media (Table 6.5). This was to be expected because whereas the cultural method recovered only the viable spores, the direct microscopic method of spore enumeration represented all spores in the sample, that is, viable and culturable as well as viable but non-culturable spores. The difference in spore densities obtained by the two methods might also be attributed to inherent random errors of the two methods.

Table 6.5. Summary of *B. stearothermophilus* spore densities in dry feed inoculum. CFU = colony forming units. SE = standard error of the mean.

Sample Description	Replicate	CFU / 20g feed	Mean	SE
Control feed	1	1.1E+04	1.0E+04	2.5E+02
Control feed	2	1.0E+04		
Feed inoculum	1	2.3E+06	2.3E+06	1.4E+04
Feed inoculum	2	2.3E+06		
Inoculated feed*	1	2.8E+06	2.9E+06	2.8E+04
Inoculated feed*	2	2.9E+06		

*Sample heat shocked twice (15 min. x 2 = 30 min.) at 100 ^{o}C to subject *B. stearothermophilus* and other acid producing, thermiphilic spores in the feed to extra thermal stress expected in the Extru-Tech E325 singles-screw extruder.

6.6.4 Stability of *B. stearothermophilus* Spores in Refrigerated Dry Feed Inoculum

Figure 6.5 shows the spore densities of *B. stearothermophilus* in refrigerated dry feed inoculum over a period of 7 weeks. It was concluded that spore densities remained in the desired region of 1.0×10^6 CFU / 20 g of feed during the studies (8 weeks).

6.6.5 Inoculation of Mash Feed with *B. stearothermophilus* Dry Feed Inoculum

Estimation of dry feed inoculum of *B. stearothermophilus* required in 10 kg of mash feed to produce approximately 2.0×10^7 CFU / 20 g feed was based on the following estimates. The spore density in mash feed, CFU_{MF}, was estimated to be 1.0×10^4 CFU / g feed and spore density in dry feed inoculum, CFU_{FI}, equal to 1.0×10^7 CFU / g feed (Table 6.5). Equation 5.13 was then used to estimate the dosage required.

6.6.6 Use of Deionized Versus Tap Water to Modify Feed Moisture Content

The results of *B. stearothermophilus* spore densities in the standardized feed treated with deionized or tap water are presented in Figure 6.6 and Table F 2.1 in Appendix F. It was concluded that the use of tap water to manually modify the moisture content of the standardized test feed did not significantly affect the spore count of the artificially inoculated spores in the feed.

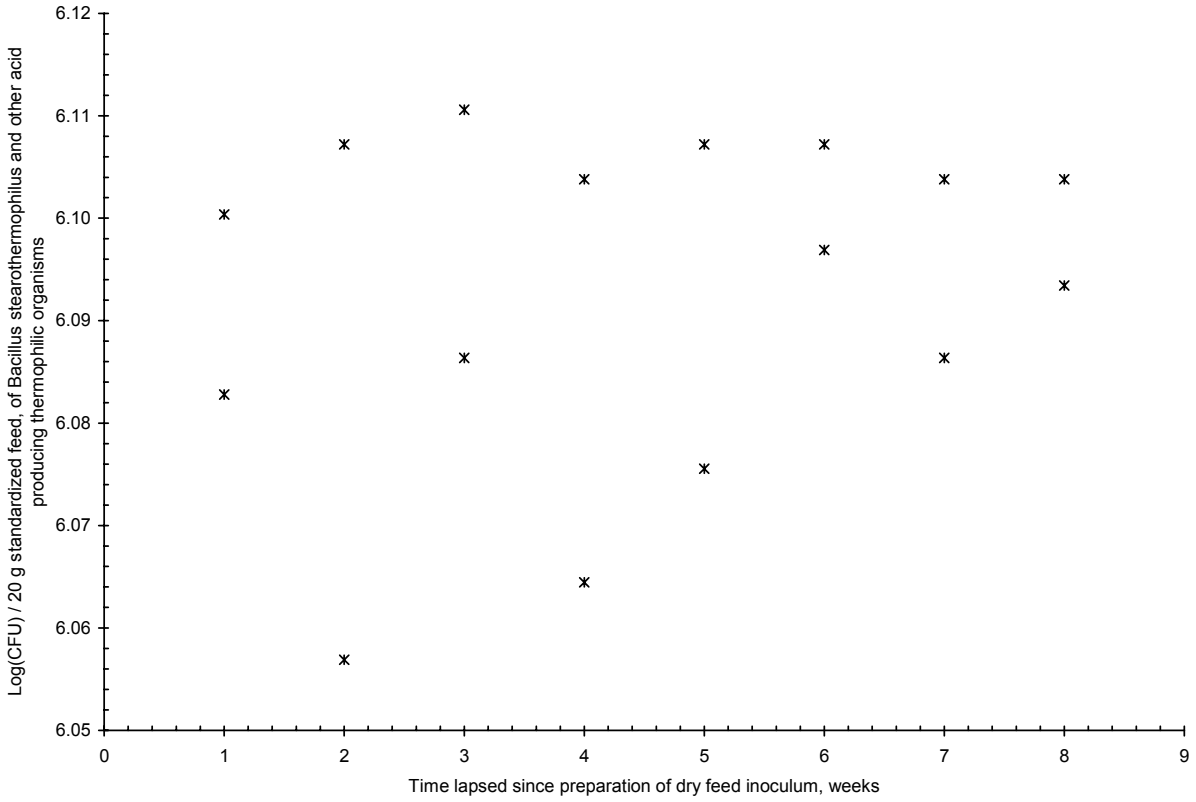

Figure 6.5. Spore count of *Bacillus stearothermophilus* and other acid producing,, thermophilic organisms in dry feed inoculum over a two-month storage period. Spore count was determined for duplicate 20 g inoculum samples by a cultural method using dextrose tryptone agar as the pour plating media.

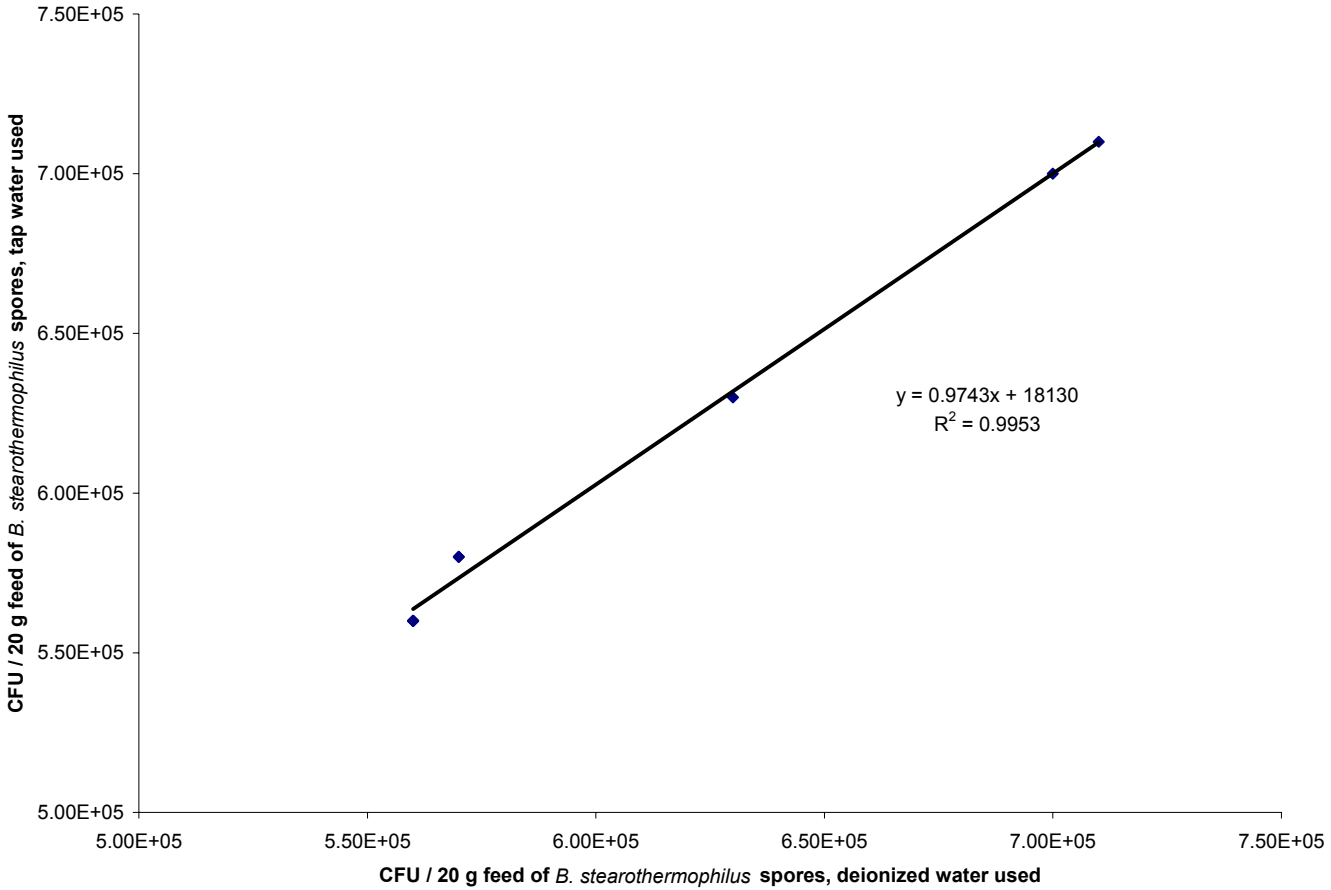

Figure 6.6. Comparison of spore densities of artificially inoculated *Bacillus stearothermophilus* spores in feed whose moisture content was modified by adding predetermined amounts of deionized or tap water, respectively.

6.7 Feed Extrusion Studies

Figure 6.7 shows the mean spore inactivation of acid producing, thermophilic organisms and *B. stearothermophilus* in feed for each of the combinations of extrusion variables. Of the 15 treatments tested during the studies, treatments 9 and 12 showed the highest percent inactivation of 65 and 64, respectively. Treatments 3 and 10 showed the least percent inactivation of 25 and 15, respectively. Table 6.6 summarizes the means of spore inactivation of 15 different extrusion conditions expressed as log reduction and as a percentage of initial spore densities in feed inoculated with *B. stearothermophilus*. In addition, the appearances of extrudates obtained from the 15 different treatments tested are shown in Figures H 1 – H 15 in Appendix H. It was noted that the greatest spore inactivation occurred under the following conditions: 93 – 103 ^{o}C extruder barrel exit temperature, 26.5 – 28.5 % moisture content and 7 – 10 s retention time of feed in the extruder barrel. No pattern in appearance of extrudates was observed across treatments.

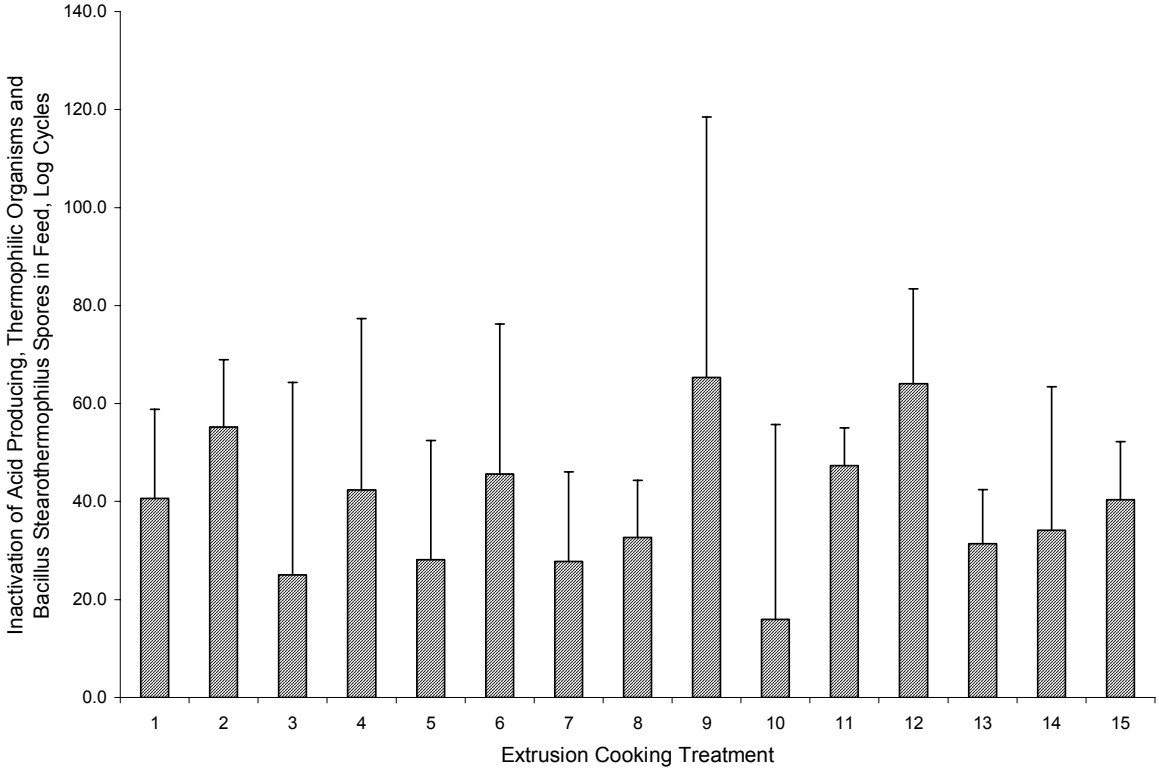

Figure 6.7. A bar chart showing mean spore inactivation of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* under different extrusion cooking conditions. Treatments are predetermined combinations of extruder barrel exit temperature, mash feed moisture content and retention time of feed in the extruder barrel, respectively (Table 5.1).

Table 6.6. A summary of the mean spore inactivation of acid producing,, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* under different extrusion cooking conditions. No = CFU / 20 g feed prior to extrusion, N = CFU / 20 g feed after extrusion. CFU = colony forming units. Means were not tested for significant differences because a continuous response surface was preferred for showing extrusion conditions that maximized spore bacterial inactivation.

	Extrusion Cooking Variables			Reduction in Spore Population of acid producing, thermophilic organisms and *Bacillus stearothermophilus*			
Treatment	Extruder Barrel Exit Temperature, ^{o}C	Feed Moisture Content, % wet basis	Retention Time of Feed in Extruder Barrel, s	Log (N_o/N)		Percent	
				Mean	SE	Mean	SE, %
1	77	28.5	7	0.23	0.087	40.7	18.2
2	83	26.5	5	0.35	0.064	55.2	13.8
3	83	26.5	9	0.13	0.216	25.1	39.3
4	83	30.5	5	0.24	0.187	42.3	35.0
5	83	30.5	9	0.14	0.121	28.1	24.3
6	93	24.5	7	0.26	0.158	45.6	30.6
7	93	28.5	3	0.14	0.088	27.8	18.3
8	93	28.5	7	0.17	0.054	32.6	11.7
9	93	28.5	11	0.46	0.330	65.3	53.2
10	93	32.5	7	0.08	0.221	15.9	39.8
11	103	26.5	5	0.28	0.035	47.3	7.7
12	103	26.5	9	0.44	0.093	64.1	19.4
13	103	30.5	5	0.16	0.051	31.4	11.0
14	103	30.5	9	0.18	0.151	34.1	29.4
15	110	28.5	7	0.22	0.055	40.3	11.9

6.8 Response Surface Model for Bacterial Spore Inactivation

A response surface model analysis with uncoded units was used to predict Log (N_o/N) for *B. stearothermophilus* spores inoculated into a standardized feed formulation and subjected to different conditions of extruder barrel exit temperature, mash feed moisture content and retention time of feed in the extruder barrel, respectively. Data obtained using central composite design in the studies would not allow three-way terms such as T by Mc by Rt. The model was of the form shown in Equation 6.2.

$$Y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_4 X_1^2 + \alpha_5 X_2^2 + \alpha_6 X_3^2 + \alpha_7 X_1 X_2 + \alpha_8 X_1 X_3 + \alpha_9 X_2 X_3 \quad (6.2)$$

Where:

Y	=	Log (N_o/N), dimensionless
N_o	=	CFU / 20 g feed prior to extrusion, colonies per 20 g feed
N	=	CFU / 20 g feed after extrusion, colonies per 20 g feed
CFU	=	colony forming units, colonies
α_0	=	constant term, dimensionless
$\alpha_{1...9}$	=	coefficients, units are the inverses of the units of variables corresponding to respective coefficient
X_1	=	extruder barrel exit temperature, ^oC,
X_2	=	mash feed moisture content, % wet basis,
X_3	=	retention time of feed in extruder barrel, s

Gibson et al. (1987) observed that empirical models are primarily concerned with practical consequences and are used to describe data under prevailing experimental conditions. The authors further noted that while such models were straight forward and frequently took the form of polynomial equations, they were 1) non-linear, 2) valid only over the range of variables covered in the data, 3) often had no theoretical basis, and 4) had numerous parameters with no biophysical meaning. The final response surface model for the inactivation of *B. stearothermophilus* spores during extrusion using the Extru-Tech E325 extruder is presented in Equation 6.3.

$$\begin{aligned} \text{Log } (N_o/N) = \; & 0.0702 - 0.00958T + 0.143Mc - 0.377Rt \\ & + 0.000307T^2 - 0.00236TMc + 0.00101Mc^2 \\ & + 0.00319TRt - 0.000929RtMc \\ & + 0.00918Rt^2 \end{aligned} \qquad (6.3)$$

Standard Error: 0.0226 log cycles at center of central Composite statistical design to 0.0511 log cycles at a radius of 1.0 coded units

Where:

N_o = CFU/ 20 g of feed before extrusion cooking,

N = CFU/ 20 g of feed after extrusion cooking,

T = Extruder barrel exit temperature, ^oC,

Mc = Moisture content of feed before extrusion cooking, % wet basis,

Rt = Retention time of feed in the extruder barrel, s

Model parameter estimates and their standard errors are contained in Table 6.7. The order of experimental runs is presented in Table G 1 in Appendix G. The Shapiro-Wilk test is a test for the null hypothesis that the input data values are a random sample from a normal distribution. In this test of normality of the data, it is only necessary to examine the probability associated with the test statistic. A Shapiro-Wilk statistic of 0.977 ($p = 0.316$, $\alpha = 0.05$) was obtained on the distribution of residuals indicating that the normality assumption was satisfied (Table G 2.1, Appendix G). The quartile data on residuals shown in Table G 2.2 in Appendix G indicated that the minimum and maximum were approximately symmetrical about the median value of the residuals. Figure G 1.1 in Appendix G also shows that residuals were on the average equal for all treatments. The stem and leaf and box plot shown in Figure G 1.2 indicates that there were no significant outliers. The plot of the standardized residuals verses standard deviations of observed residuals adequately coincided with that of the normally distributed population of residuals with the same standard deviation as the observed residuals (Figure G 2.3, Appendix G) as shown by the "*" and "+" in the plot. Overall, results of the normality assumption tests performed on residuals showed that residuals were normally distributed with a mean of zero.

Linear, quadratic and cross product components of the model, respectively, were significant; the overall model was significant indicating that inactivation acid producing, thermophilic organisms and of *B. stearothermophilus* spores in log (N_o/N)

due to extrusion was adequately explained by the extrusion variables (T, Mc and Rt) ($p < 0.0001$ at $\alpha = 0.05$, Table G 2.3 in Appendix G). Table G 2.4 summarizes the results for the analysis of contribution of independent extrusion cooking variables to the overall response surface model. All three independent variables were significant. Rt was most significant. A lack-of-fit test is a statistical test of the hypothesis that the model is sufficient, often indicating whether any important variables are missing or misspecified in the functional part of the model and may be helpful if the plots leave any doubt. The non-significant lack of fit test on the model showed that the quadratic model was adequate ($p = 0.136$ at $\alpha = 0.05$, Table G 2.5 in Appendix G).

Appendix I contains a summary of data set used to generate the response surface model. Standard errors of spore inactivation ranged between 0.0226 and 0.0511 log cycles at the center and surface of spherical shell with coded radius of 1.682 of the central composite statistical design, respectively. Control of temperature along the extruder barrel might improve the model prediction capacity. Whereas the extruder barrel exit temperature could be controlled consistently, temperatures of the preceding barrelheads were hard to control. A more precise method of measuring retention time might also improve the model prediction capacity. More variables, such as pH, salt and fat contents of mash feed, respectively, in addition to the three considered during the studies might be required to completely explain the response of *B. stearothermophilus* to extrusion.

The only stationary point obtained within the range of Extru-Tech E325 extruder variables was a saddle point. Table 6.8 shows the coordinates of the saddle point. A saddle point does not have any significant value from the stand point of feed extrusion. A maxima within the range of the Extru-Tech E325 extruder variables would indicate the point of maximal spore inactivation.

Table 6.7. Estimates of response surface model parameters for *Bacillus stearothermophilus* inactivation during extrusion cooking in a single screw extruder (Extru-tech E325, Extru-tech, Sabetha, Kansas). wb = wet basis. SE = standard error.

Estimates of Coefficients	Estimate	SE
Intercept	0.0703	4.012
T[a]	-0.00958	0.04064
Mc[b]	0.143	0.1847
Rt[c]	-0.377	0.1717
T*T	0.000307	0.000153
Mc*T	-0.00236	0.000982
Mc*Mc	0.00101	0.002747
Rt*T	0.00319	0.000982
Rt*Mc	-0.000929	0.004912
Rt*Rt	0.009181	0.002747

[a]T = extruder barrel exit temperature (^{o}C), [b]Mc = mash feed moisture content (% wb), [c]Rt = retention time of feed in extruder barrel (s).

Table 6.8. Stationary point. Stationary point was a saddle point. Predicted response value at the saddle point was 0.170 logs.

Variable	Estimated value at stationary point, (saddle point)
T[a], °C	81
Mc[b], % wb	28
Rt[c], s	8

[a]T = extruder barrel exit temperature (°C), [b]Mc = mash feed moisture content (% wb), [c]Rt = retention time of feed in extruder barrel (s).

Predicted log reduction at the saddle point was 0.170. An estimated ridge of maximum inactivation of spores was generated to show the desirable region of extrusion conditions that maximized spore inactivation of the test organism (Table 6.9). Experimental treatments that showed the greatest inactivation of *B. stearothermophilus* spores (treatments 9 and 12 in Table 6.6) fell within the estimated ridge of maximum response. Treatments that showed the least inactivation of test organism spores were located outside the estimated ridge of maximum response.

Table 6.9 Estimated ridge of maximum inactivation of acid producing,, thermophilic and *B. stearothermophilus* spores. SE = standard error, T = extruder barrel exit temperature, Mc = mash feed moisture content, Rt = retention time of feed in extruder barrel.

Coded Radius	Estimated Spore Inactivation, $Log_{10}(N_o/N)$	SE	T, ^oC	Mc, % wb	Rt, s
0.0	0.166	0.0226	93.5	28.5	7.0
0.1	0.183	0.0225	94.2	28.2	7.3
0.2	0.204	0.0224	95.1	28.1	7.5
0.3	0.229	0.0224	96.0	27.9	7.8
0.4	0.259	0.0.228	97.0	27.7	8.1
0.5	0.294	0.0242	98.1	27.6	8.4
0.6	0.333	0.0268	99.1	27.5	8.7
0.7	0.376	0.0307	100.1	27.4	9.0
0.8	0.425	0.0362	101.1	27.2	9.3
0.9	0.478	0.0430	102.2	27.1	9.6
1.0	0.536	0.0511	103.2	27.0	9.9
1.682	1.030	-	110.0	24.5	11.0

6.9 Constant Extruder Barrel Exit Temperature

It was predicted that at constant extruder barrel exit temperature (T) in the 77 to 83 ^{o}C range, increasingly greater spore inactivation would occur if mash feed with greater moisture content were extruded for shorter (3 to 6 s) feed retention times in the extruder barrel (Figures 6.8 and 6.9). The color bar indicates spore inactivation values of the response surface corresponding to the range of colors. In a review on heat activation of bacterial spores, Russel (1982) observed that thermal death curves of *B. stearothermophilus* showed three phases : 1) a sharp initial rise in viable count, due to heat activation of dormant spores, followed by 2) a slow rate of death which gradually increased to, 3) the logarithmic death phase at a maximal rate. It was speculated that some spores might have exhibited the rise in viable count described above (1) during the entire range of Rt values (3 to 11 s) when extruding feed in the 77 to 83 ^{o}C range of T values. This might explain the reduced inactivation of bacterial spores at longer retention times (in the 2 to 8 s range) in the extruder barrel. In moving from constant T of 77 to 83 ^{o}C, the high Rt (11 s) and low Mc (24.5 % wb) corner of the response surface shifted upwards by 48 % while the rest of the corners shifted downwards indicating greater inactivation efficiency at the higher temperatures. The average slopes of the surface remained negative with respect to Rt and positive with respect to Mc, respectively. This result was expected because thermal inactivation of bacterial spores is primarily influenced by temperature at which the process is carried out (Likimani et al., 1990).

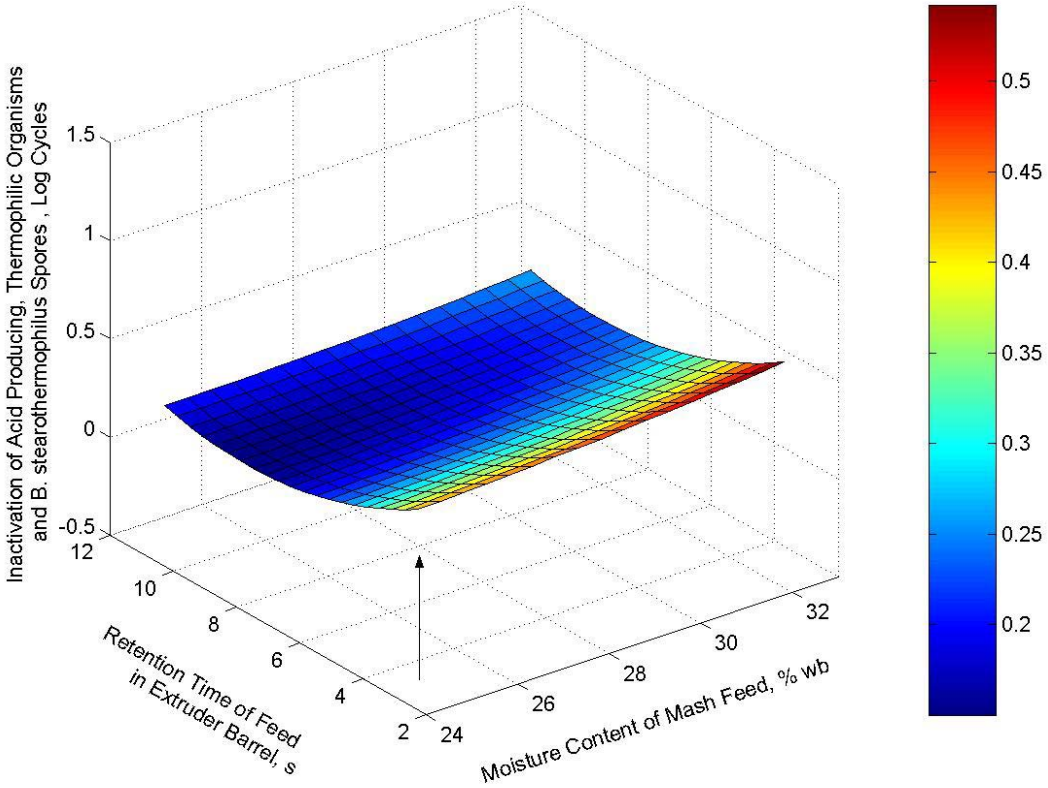

Figure 6.8. Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing,, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 77 ^oC extruder barrel exit temperature. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking. The color bar indicates spore inactivation values of the response surface corresponding to the range of colors.

At the higher constant extrusion temperatures in the 77 to 83 ^oC range however, the average slope of the response surface was positive but was reduced with respect to mash feed moisture content (Mc) as observed in moving from Figure 6.8 to Figure 6.9. This reduction in slope might be attributed to the diminishing benefit of higher moisture content of feed beyond a threshold value thought to be between 26.5 and 28.5 %, wet basis. The curvature of the response surface along the Rt axis in Figures

6.8 and 6.9 was significant (quadratic effect, $p = 0.007$ at $\alpha = 0.05$, Table G 2.1 in Appendix G).

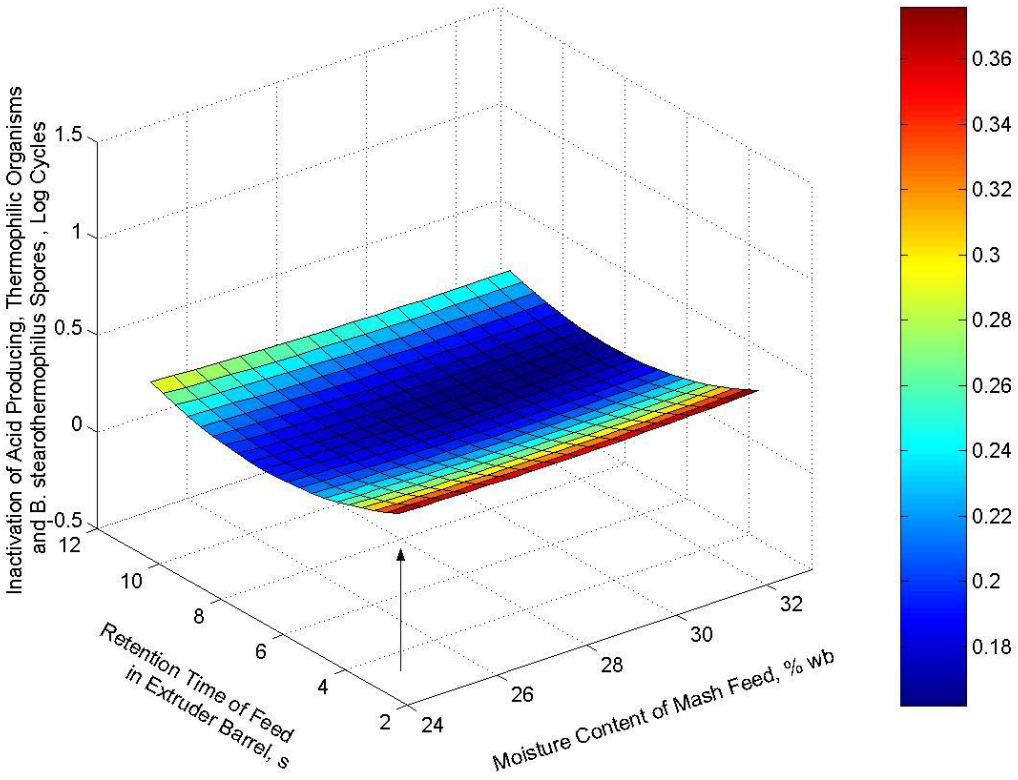

Figure 6.9. Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing,, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 83 ^{o}C extruder barrel exit temperature. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking.

It was predicted that mash feed with greater moisture content had greater thermal conductivities than mash feed with lower feed moisture content (Figure 6.10). At lower steam and heat flow rates corresponding to the 77 – 83 ^{o}C range of extruder barrel exit temperature, the temperature experienced by spores at equilibrium conditions was greatly influenced by the thermal conductivity of the feed.

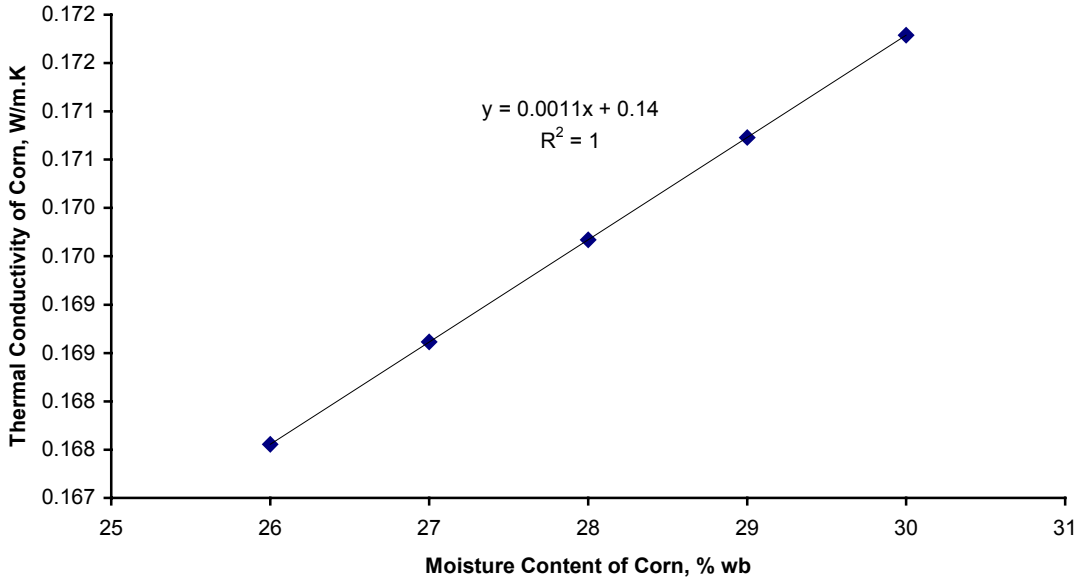

Figure 6.10. Predicted variation of thermal conductivity of corn with moisture content. Corn was selected for simplicity and because it constituted the largest proportion of the feed formulation. The plot is based on linear interpolation of thermal conductivity data for corn at 0.91 and 30.2 % wet basis, respectively (Singh and Heldman, 2001f).

Spores of the test organism in feed with greater moisture content were thus exposed to increasingly higher temperatures (Figure 6.11) than spores in feed with lower moisture

content due to higher heat transfer rates achieved at greater thermal conductivity values (Equation 6.4). Within the extruder barrel, lower temperature drops between the inner wall and the location of a bacterial spore imply that the spore at that location would experience temperatures closer to that of the extruder barrel wall, that is, higher temperatures.

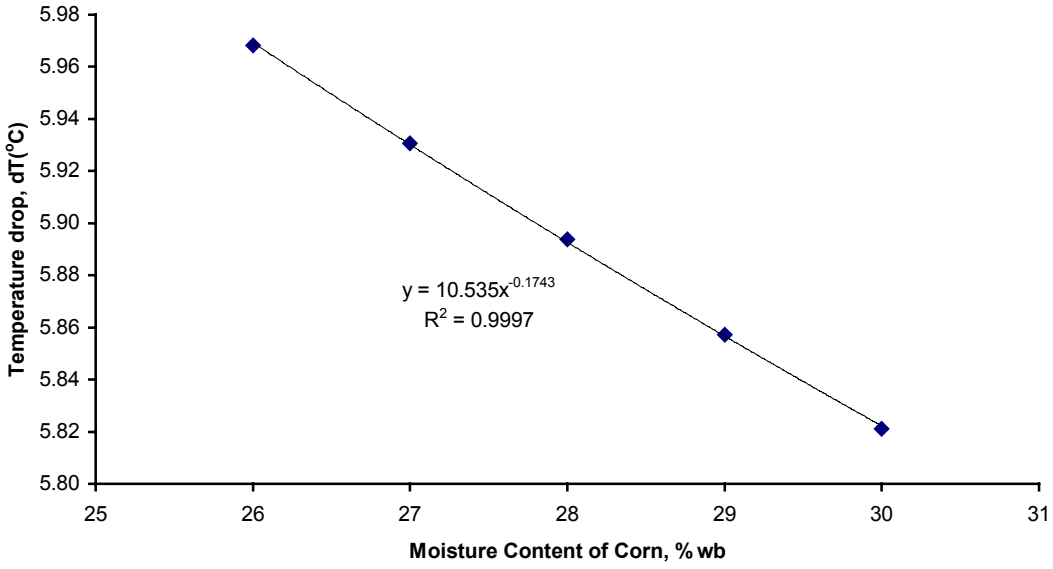

Figure 6.11. A graph illustrating variation of temperature drop, dT, between extruder barrel wall and a spore in the feed formulation located at a distance, x, from the extruder barrel wall. Predicted dT was based on setting the value of $q_x.(dx/A)$ in Equation 6.4 to 1 for simplicity.

The rate of conductive heat flow through feed material may be modeled as follows (Equation 6.4) (Singh and Heldman, 2001c).

$$q_x = -k_c A_h (dT/dx) \quad (6.4)$$

Where:

q_x = rate of heat flow in x direction, Watts,

k_c = thermal conductivity of feed material, W/m.^oC,

A_h = area normal to x direction through which heat flows, m^2,

dT = temperature drop between heat source, extruder barrel wall and spore location, ^oC

dx = thickness of feed material between heat source and spore location in the direction of heat flow, m

The temperature gradient dT/dx is negative since temperature decreases as the distance from the source of heat increases. Supposing that, the major source of heat during extrusion was steam in the extruder barrel jacket, the following may be deduced from Equation 6.4. Assuming equilibrium conditions, a bacterial spore at a distance, x, from the inner wall of the extruder barrel would be subjected to higher temperatures (lower dT values) if feed material had higher thermal conductivity, all other variables (q_x, dx and A) being the same. Higher thermal conductivities would maximize time of exposure of spores to the equilibrium temperature.

In the 93 – 110 ^oC range of T, the response surface model indicated that spore inactivation decreased as mash feed moisture content increased. Conversely, greater spore inactivation was achieved at higher Rt. Figures 6.12 to 6.14 show response surfaces of bacterial spore inactivation due to extrusion at constant T values of 93, 103 and 110 ^oC, respectively. In moving from T = 93 ^oC to T = 110 ^oC the response surface generally shifted upwards, the high Rt edge showing greater increment than

the low Rt edge. It was speculated that at the higher T range, evaporation of moisture from feed at the extruder barrel exit (observed as explosive puffing) might have lowered average moisture content of feed and through that decreased the average thermal conductivity of feed during extrusion. Bacterial spores in feed with reduced thermal conductivity thus experienced lower equilibrium temperatures in the extruder barrel than expected.

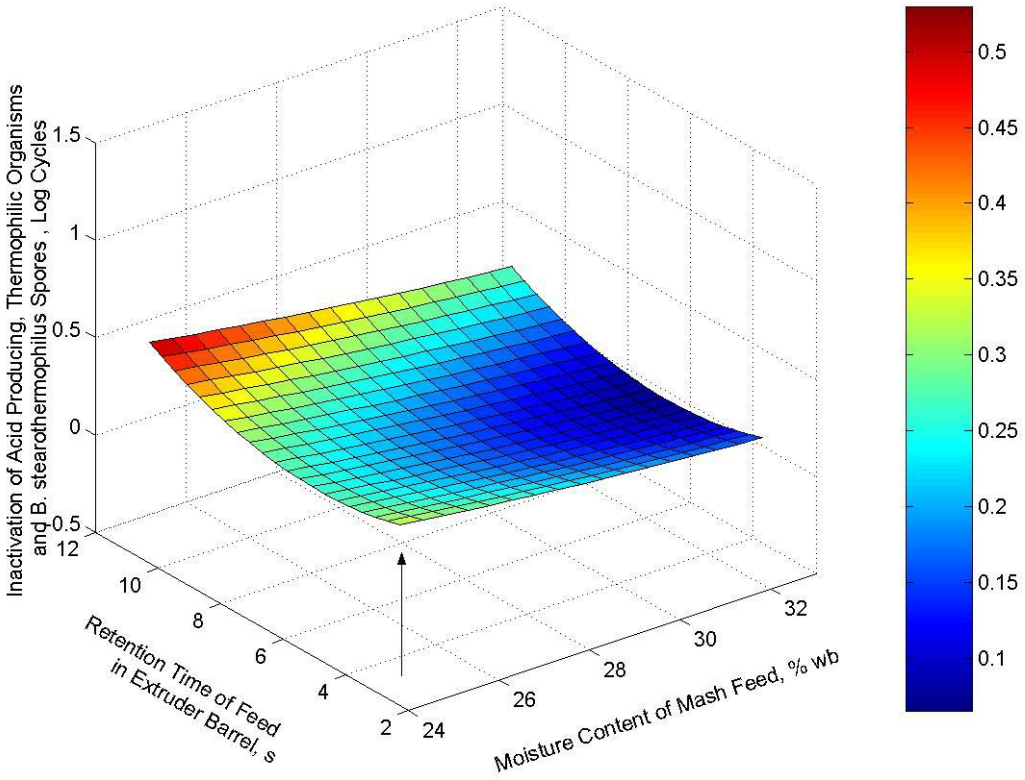

Figure 6.12. Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing,, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 93 ^oC extruder barrel exit temperature. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking.

As the moisture content of mash feed increased, it took more heat to raise the temperature of a fixed volume of feed due to increased mass per unit volume and to supply the sensible heat requirement of the higher moisture feed. In addition to the reduction in thermal conductivity of feed due to evaporative moisture loss from feed, absorption of heat to supply the required latent heat of vaporization reduced the total amount of heat available for spore inactivation. Lowered thermal conductivity of the feed and absorption of latent heat of vaporization by evaporating moisture at higher constant extrusion temperatures in the range of 93 – 110 ^{o}C might account for the negative slope along the Mc axis of the response surfaces (Figures 6.12 – 6.14). Inactivation of bacterial spores was greater at higher Rt values.

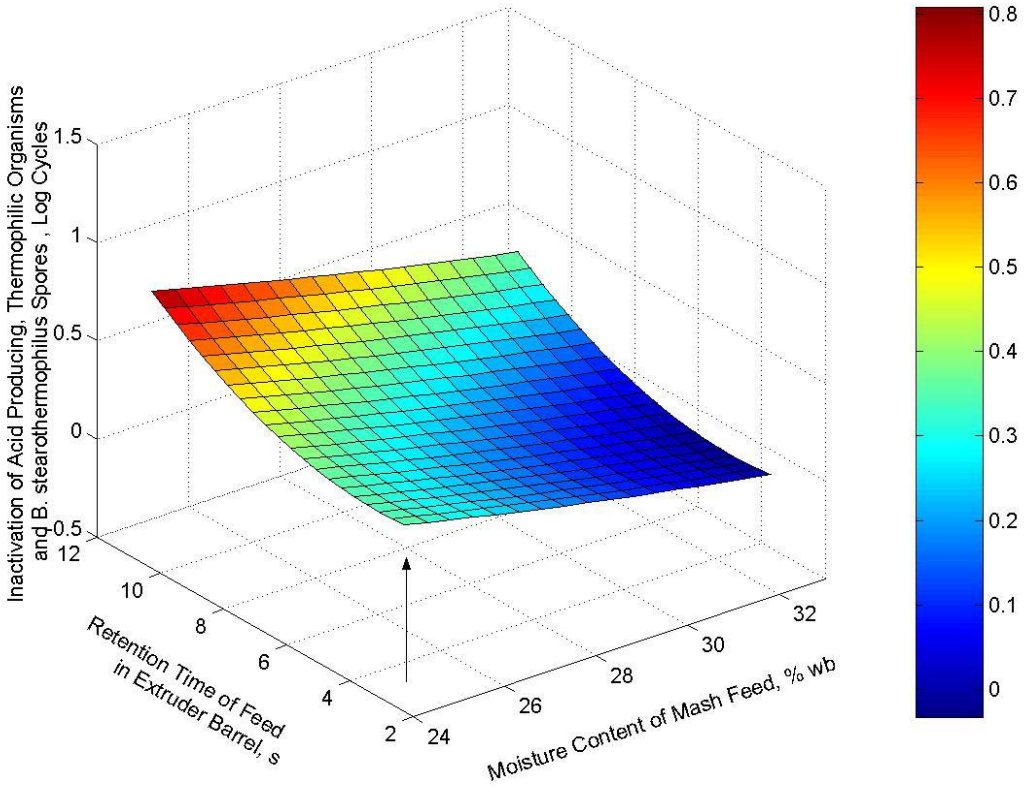

Figure 6.13. Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing,, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 103 ^{o}C extruder barrel exit temperature. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking.

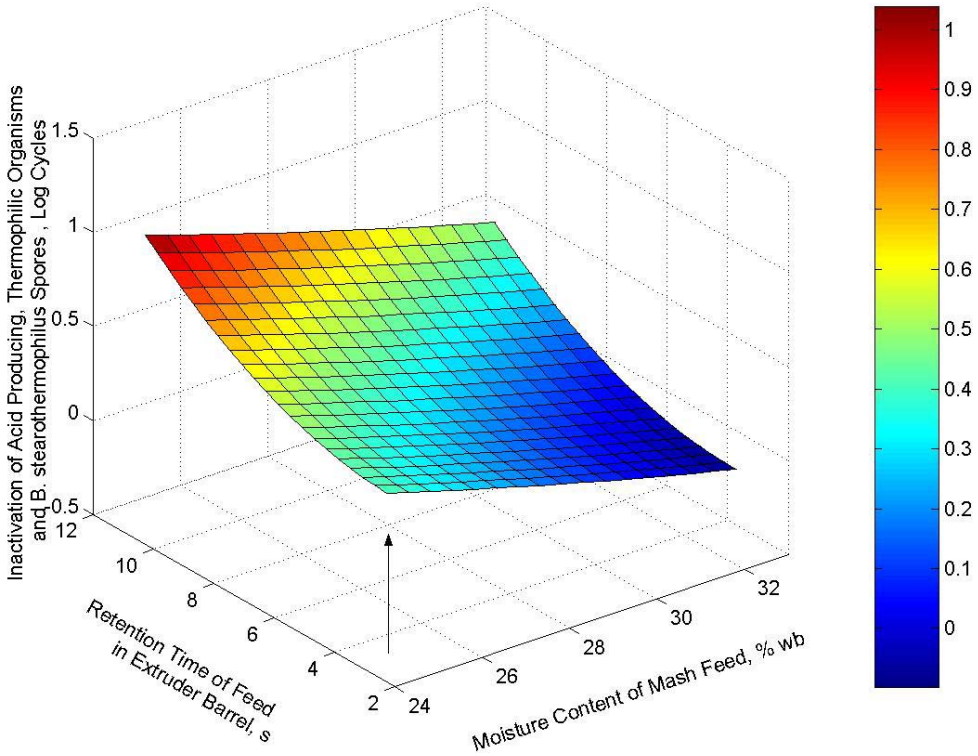

Figure 6.14. Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 110 ^oC extruder barrel exit temperature. The arrow points to the corner of the surface nearest the reference axis. N_o , N = spore count per 20 g feed before and after extrusion cooking.

In moving from constant T values of 93 to 103 and then to 110 ^oC during extrusion, the response surface shifted upwards indicating increasingly greater spore inactivation at higher extrusion temperatures. The slope of the response surface became more positive with respect to Rt but more negative with respect to Mc, respectively.

It was speculated that evaporation of moisture from feed at higher constant extrusion temperatures might have reduced water activity (Tables E 1.1 and E 1.2 in Appendix E) of feed and stabilized spores making them more resistant to thermal inactivation. In studies to elucidate the role of dehydration on heat stability of organisms and proteins, Warth (1985) observed that when bound water starts to be removed, at $a_w < 0.93$, stability greatly increases.

6.10 Constant Mash Feed Moisture Content

The model predicted that at constant Mc between 24.5 and 26.5 % wb, increasingly greater spore inactivation would occur at higher T and Rt values, respectively, in the upper half of the T range (Figures 6.15 and 6.16). In Figures 6.15 and 6.16, the average slopes of the response surface with respect to Rt and T were both positive. In moving from constant Mc of 24.5 to 26.5 % the average slopes decreased but remained positive with respect to Rt and T, respectively. At higher constant Mc the low T edge of the response surface shifted upwards while the high T edge shifted downward indicating lower spore inactivation at the higher moisture levels. At the lower range of Mc tested, spore inactivation was predominantly influenced by T and Rt of extrusion. Greater inactivation occurred when extrusion was performed at higher T for longer Rt in the upper half of the T range. In a review of the application of moist heat for sterilization, Russel (1982) noted that during ultra high temperature

treatments (130 – 150 ^{o}C), only the heating and holding times contributed to the overall sporicidal effect on bacterial spores.

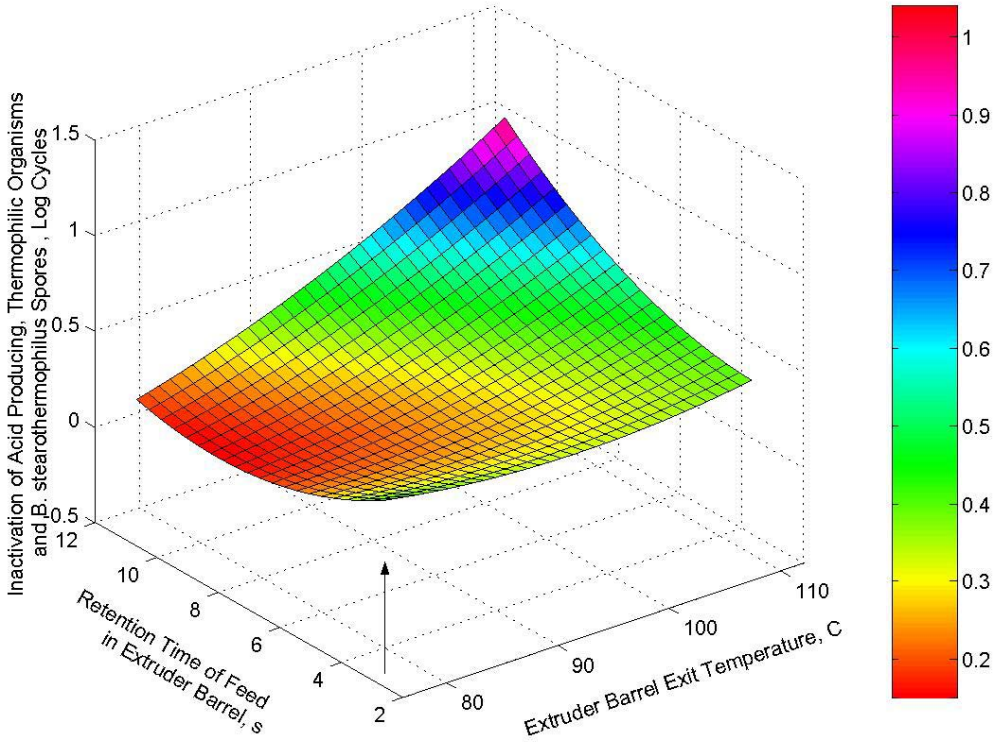

Figure 6.15. Response surface plot showing spore inactivation (Log (No/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 24.5% mash feed moisture content. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking.

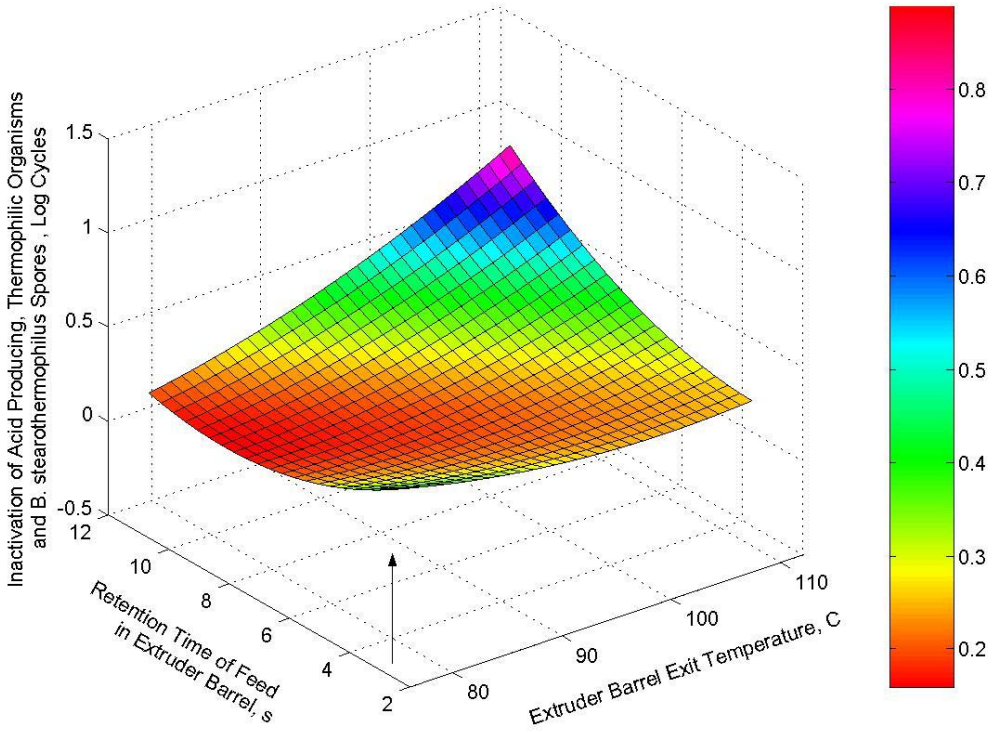

Figure 6.16. Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 26.5% mash feed moisture content. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking.

The lethality of the extrusion process increased rapidly with increases in both T and Rt. At higher constant Mc values (28.5 to 32.5 % in Figures 6.17 to 6.19), the average slope of the response surface remained positive with respect to Rt, in the upper half of the T range. In the 3 to 7 s range of Rt, the average slope of the response surface with respect to T was negative and became increasingly more negative at higher Mc.

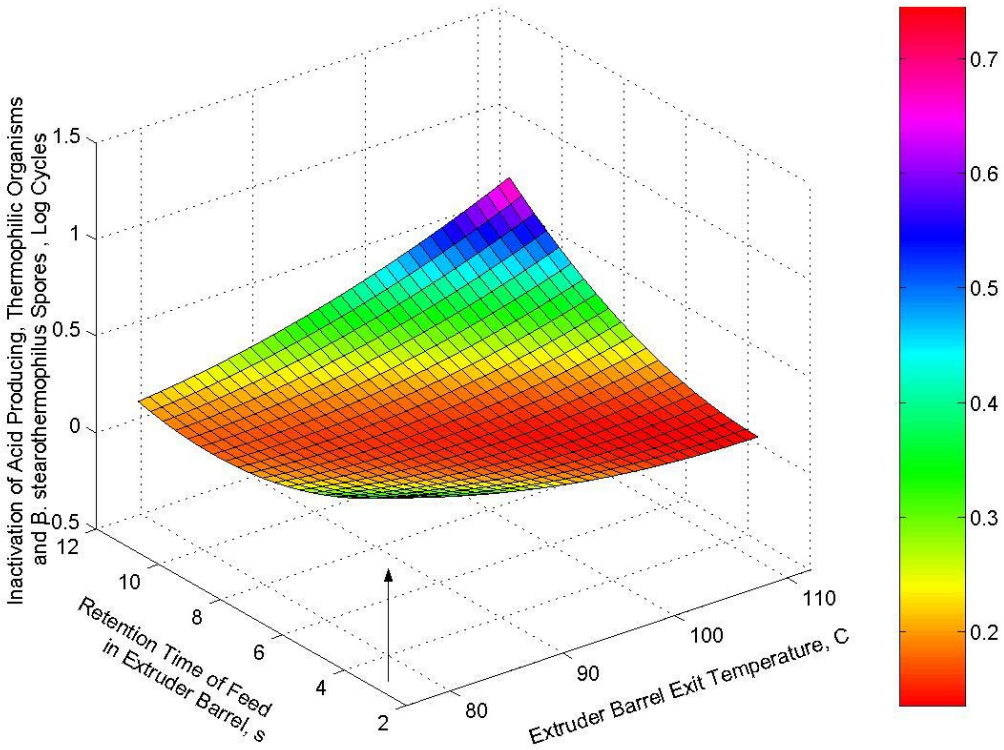

Figure 6.17. Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 28.5% mash feed moisture content. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking, respectively.

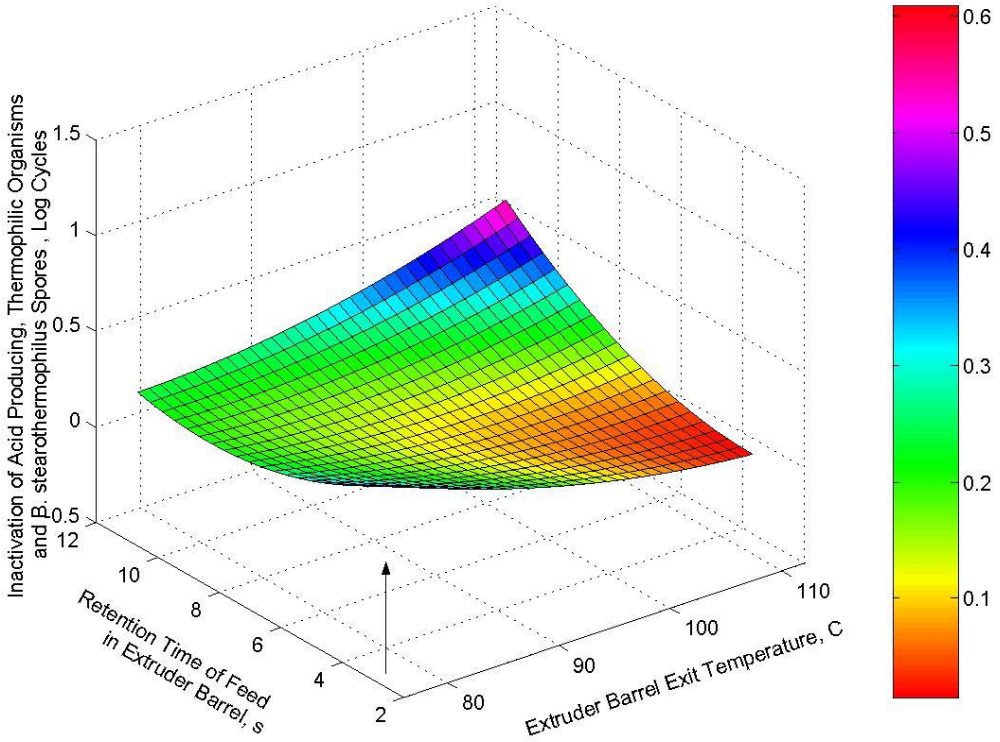

Figure 6.18. Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 30.5% mash feed moisture content. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking, respectively.

The low T edge of the response surface also continued to shift upwards while the high T edge shifted downward as higher Mc was selected. It was speculated that at higher constant Mc, evaporation of moisture from feed might have been a predominant factor in reducing available heat for spore inactivation as well as increasing the thermal resistance of spores due to lowered feed a_w values during extrusion.

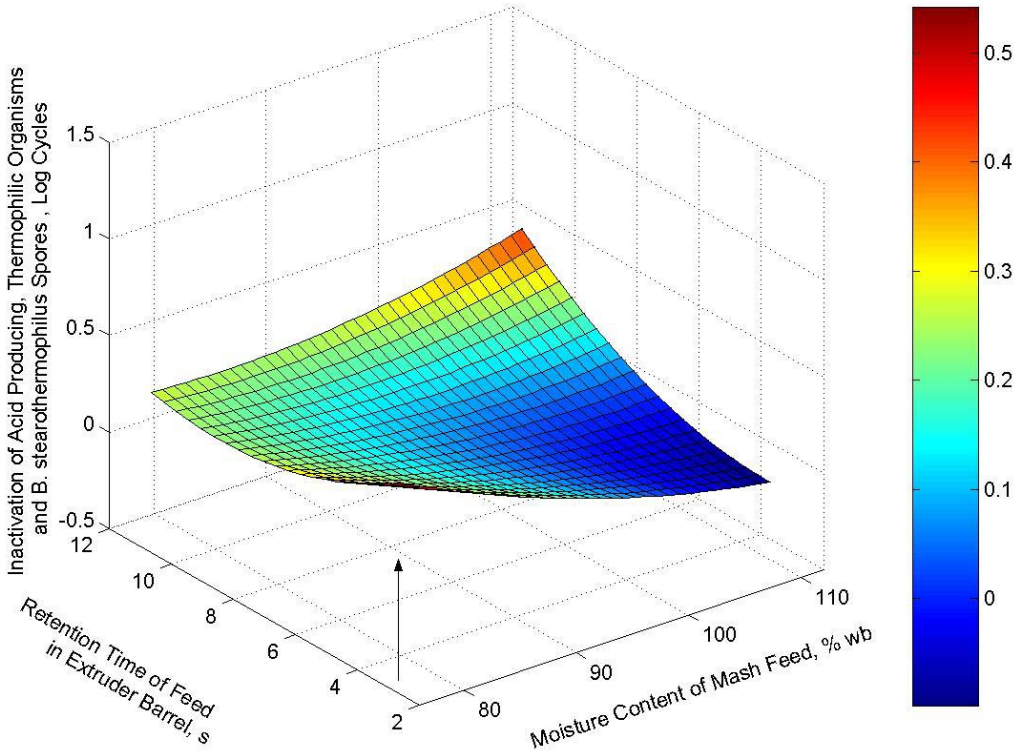

Figure 6.19. Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 32.5% mash feed moisture content. The arrow points to the corner of the surface nearest the reference axis. N_o , N = spore count per 20 g feed before and after extrusion cooking, respectively.

6.11 Constant Retention Time of Feed in the Extruder Barrel

Figures 6.20 to 6.24 show the response surface of *B. stearothermophilus* inactivation at constant Rt values of 3, 5, 7, 9 and 11, respectively. In moving progressively from 3 to 11s constant Rt values, greater spore inactivation was achieved at higher Rt values indicated by the tilt of the entire response surface about an axis near the 93 ^{o}C axis.

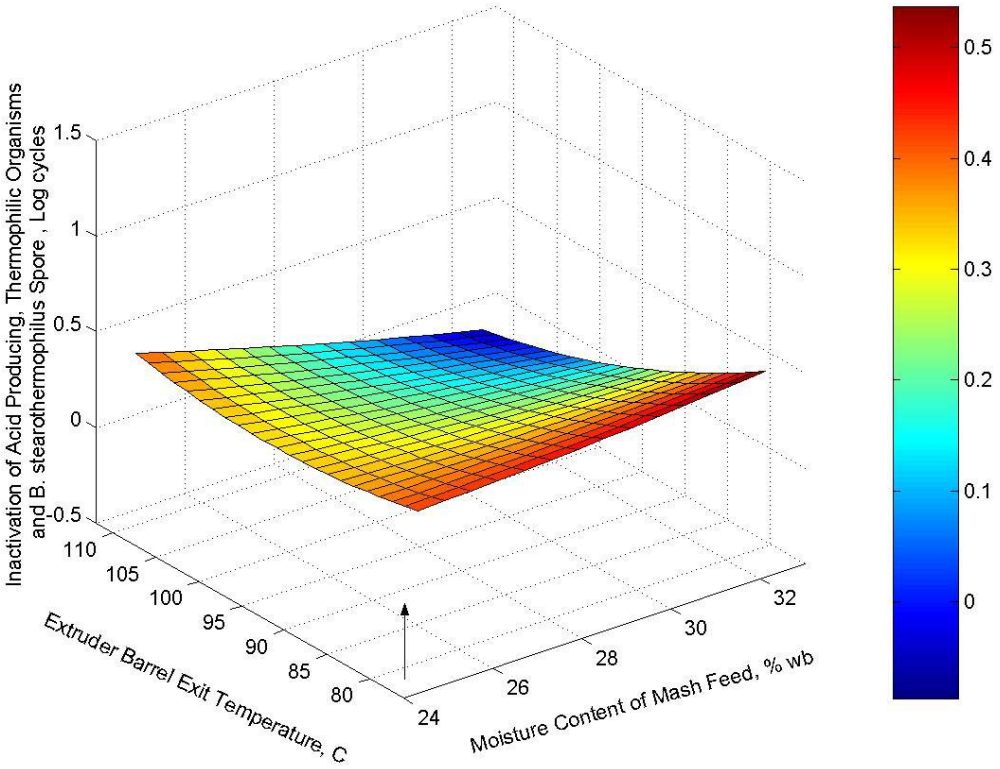

Figure 6.20 Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 3 s feed retention time in the extruder barrel. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking, respectively.

Both T and Mc affected spore inactivation. Greater inactivation occurred at higher T values but in the lower range of Mc values or the lower range of T values but the higher range of Mc values. It was speculated that in these ranges of T and Rt values, respectively, evaporative moisture losses were not predominant.

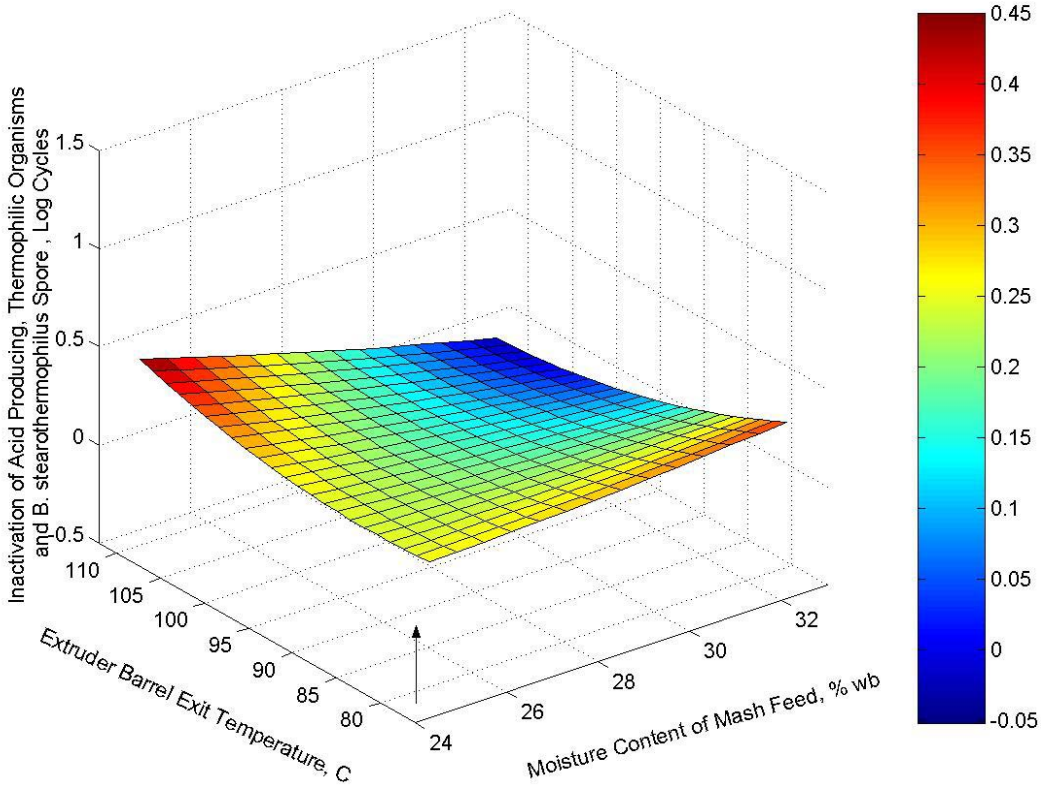

Figure 6.21 Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 5 s feed retention time in the extruder barrel. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking, respectively.

When conditions that increased evaporative moisture loss prevailed, heat available for thermal inactivation of spores was reduced. This, combined with an increase in thermal resistance of spores at the reduced feed a_w, might have contributed to the lower inactivation of spores under those conditions. The slope of the response surface with respect to T rapidly increased as Rt increased but did not appear to change much with respect to Mc as Rt increased. Higher evaporative moisture losses at higher T and Mc values might have contributed to the lower spore inactivation observed in the response surfaces.

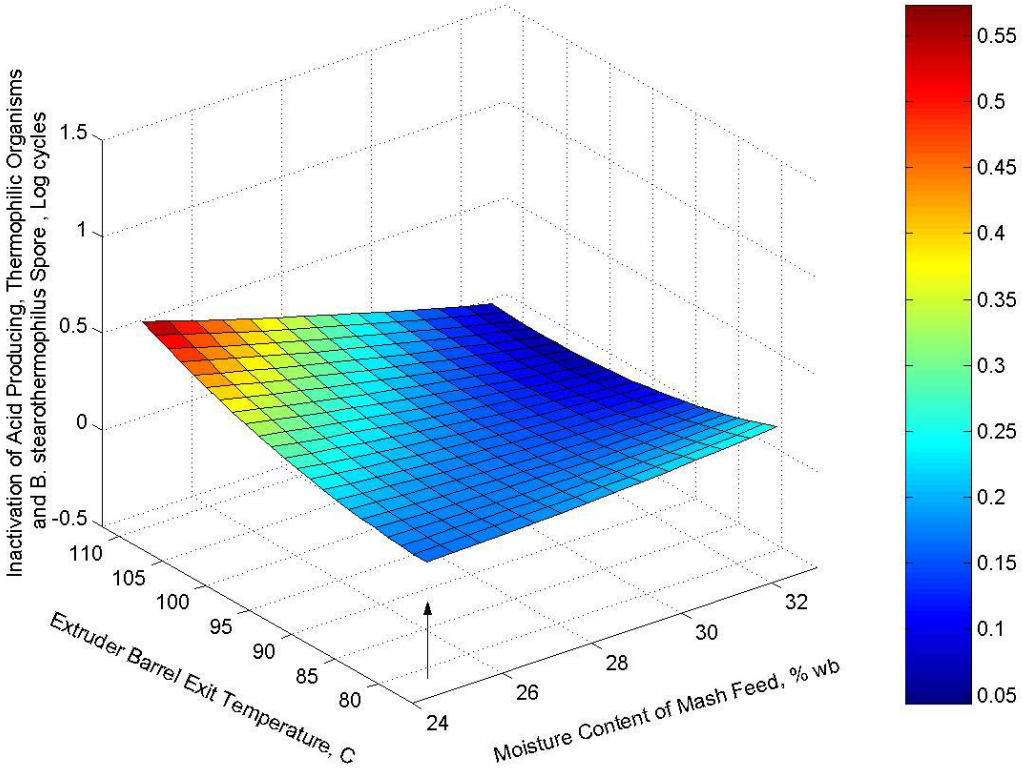

Figure 6.22 Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 7 s feed retention time in the extruder barrel. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking, respectively.

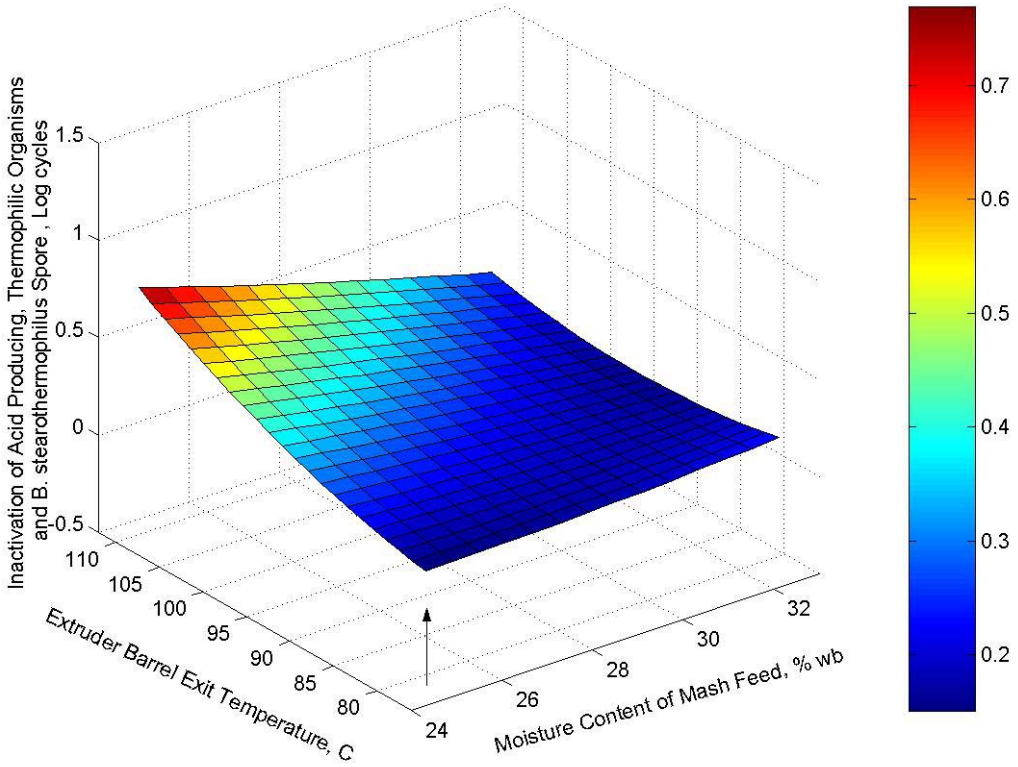

Figure 6.23 Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 9 s feed retention time in the extruder barrel. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking, respectively

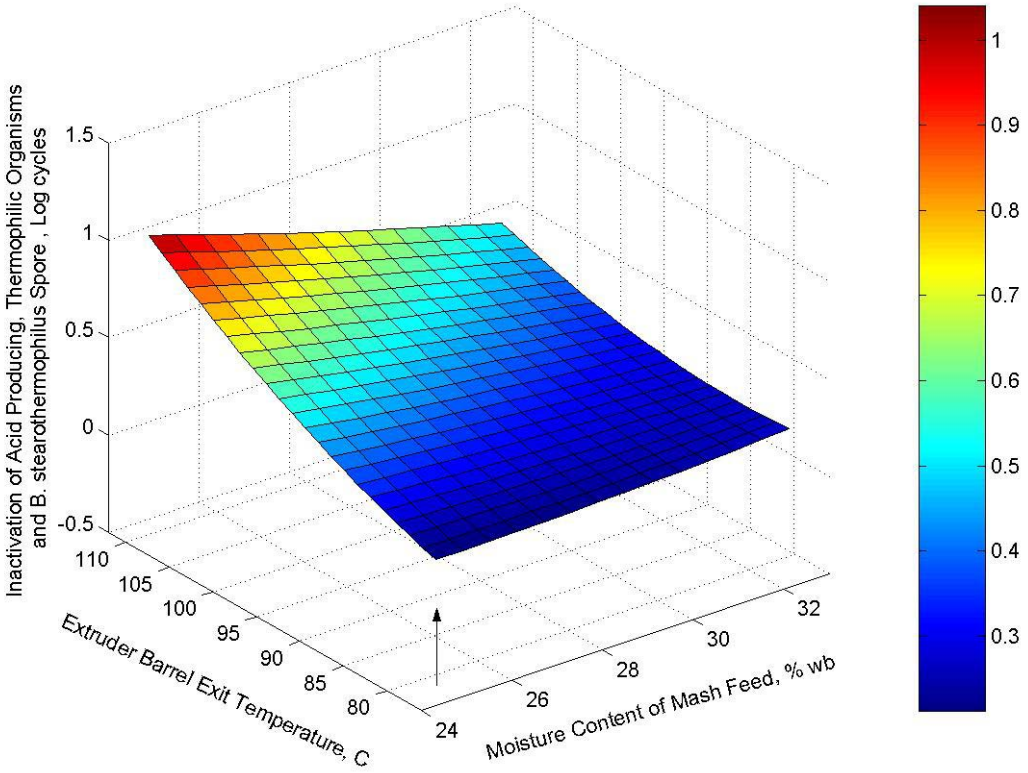

Figure 6.24 Response surface plot showing spore inactivation (Log (N_o/N)) of acid producing, thermophilic organisms and artificially inoculated *Bacillus stearothermophilus* at 11 s feed retention time in the extruder barrel. The arrow points to the corner of the surface nearest the reference axis. N_o, N = spore count per 20 g feed before and after extrusion cooking, respectively

6.12 Response Surface Model Validation

The mean values of observed log reduction of *B. stearothermophilus* spores to inactivation due to extrusion were compared with predicted response values at all the levels of T, Mc and Rt tested. The correlation procedure (PROC CORR) was used to obtain a Pearson's correlation coefficient of 0.545 (SAS Release 8.2, 2001). This

indicates that the fitted response surface model explained 55 % of the *B. stearothermophilus* spore inactivation observed during the studies. The remaining 45 % would possibly be explained by other extrusion variables not controlled in these studies such as, control of temperature of some extruder barrel heads, pH of mash feed, and salt and fat contents of mash feed, respectively.

6.13 Secondary Models

Primary models describe changes in bacterial numbers as a function of time under specified environmental and cultural conditions. Models that describe the responses of one or more parameters of a primary model changing as a result of changes in environmental (such as pH, a_w or temperature) or cultural conditions are considered secondary models (McDonald and Sun, 1999).

During thermal processing, the rate of inactivation of microbial populations is considered to follow first order kinetics represented by Equation 6.5 (Juneja et al., 2001).

$$N = N_o e^{-kt} \qquad (6.5)$$

Equation 6.5 may be rearranged as shown in Equation 6.6, a form that allows linearization using logarithm to base ten.

$$N = N_o 10^{-t/Dx} \quad (6.6)$$

Where:

N = microbial population at time t, CFU/ unit of mass or volume

N_o = initial microbial population, CFU/ unit of mass or volume

t = duration of time, units of time

CFU = colony forming units

k = death rate of the microbial population or rate constant of inactivation of bacterial population, min

The death rate, k, of a microbial population may be related to its decimal reduction time, D_x, by Equation 6.7.

$$k = 2.303 / D_x \quad (6.7)$$

Where:

D_x = decimal reduction time at a specified temperature, units of time

A regression model of k as a function of extruder barrel exit temperature, T, and feed moisture content prior to extrusion, Mc, was obtained (Equation 6.8). Table 6.10 summarizes the model parameters and their standard errors for k.

$$k = 0.332 + 0.00017T - 0.00901\ Mc, \quad (6.8)$$

Table G 2.6 indicates that the model significantly explained the variation of k as a function of T and Mc ($p = 0.040$ at $\alpha = 0.05$).

Table 6.10. Estimated model coefficients for the death rate, k, as a function of T and Mc. T = extruder barrel exit temperature (^{o}C), Mc = mash feed moisture content, wet basis, SE = standard error of the variable/parameter estimate.

Variable / Parameter	Estimate	SE
Intercept	0.332	0.126
T	0.00017	0.000759
Mc	-0.00901	0.00348

A plot of the predicted D_x values versus Mc (Figure 6.25) for acid producing, thermophilic organisms and *B. stearothermophilus* may be used to select the retention time of feed in the extruder barrel required to produce a desired level of spore inactivation when extruding feed of known moisture content at a given setting of extruder barrel exit temperature. As an example, suppose we wished to extrude feed with moisture content of 26.5 % wb at an extruder barrel exit temperature setting of 110 ^{o}C. These conditions indicate an estimated decimal reduction time of 0.4 minutes (Figure 6.25). In order to achieve 1 decimal reduction of viable spores we would require a retention time of feed in the extruder barrel of 0.4 minutes. Figure 6.25 indicates that spores of acid producing, thermophilic organisms and *B. stearothermophilus* in feed with higher moisture content required longer retention times in the extruder barrel at a given setting of extruder barrel exit temperature to achieve the same level of spore inactivation. This phenomenon may be explained by the absorption of latent heat of vaporization by free moisture in the feed matrix during extrusion cooking. Moisture evaporation might have had a net cooling effect thereby reducing the severity of the thermal inactivation process on bacterial spores.

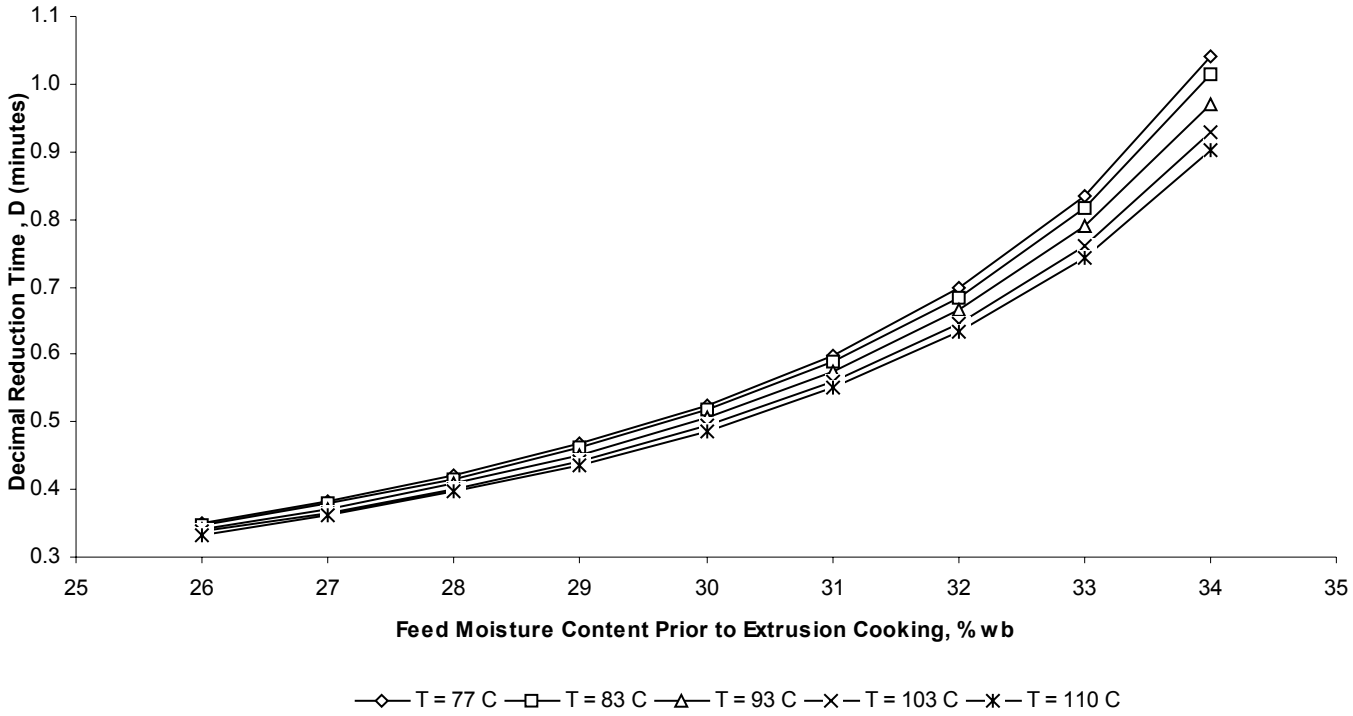

Figure 6.25. Variation of predicted decimal reduction time of acid producing, thermophilic organisms and *Bacillus stearothermophilus* with moisture content of feed. Curves in the plot were obtained by combining Equations 6.7 and 6.8 to give $D_x = 2.303 / (0.332 + 0.00017T - 0.00901\ Mc)$.

The Arrhenius equation (Equation 6.9) can be used to estimate the influence of temperature on the rate constant of bacterial inactivation (Singh and Heldman, 2001d). Sing and Heldman also showed that Equation 6.9 may be used to estimate the enthalpy of inactivation of bacterial spores or cells at a temperature of T when the thermal resistance constant, z, is known.

$$k = Be^{-Ea/(RTk)} \quad (6.9)$$

Where:

k = rate constant of inactivation, per unit of time

B = function constant

E_a = activation energy, kJ/kg

R = ideal gas constant (8.314), kJ/kg. ^oC

T_k = extruder barrel exit temperature, K

It can be shown that enthalpy of inactivation of bacterial spores or cells can be calculated as a function of temperature T and thermal resistance z, respectively (Equation 6.10) (Singh and Heldman, 2001d):

$$E_a = 19.15T^2 / z \quad (6.10)$$

Where:

T = temperature (of extruder barrel exit, in this application), ^oC

z = thermal resistance constant for the spores, ^oC

The average thermal resistance of acid producing, thermophilic organisms and *B. stearothermophilus* spores, z, was estimated using Equation 6.11 (Singh and Heldman, 2001).

$$z = 10 / \log Q_{10} \quad (6.11)$$

Where:

Q_{10} = the effect of temperature on the inactivation rate, in increasing from temperature 1 to temperature 2 (a 10 ^{o}C rise), dimensionless

Q_{10} values for bacterial spores in the feed at various moisture contents were estimated using Equation 6.12. The values of the inactivation constant (k) were obtained using the empirical model of k at the given feed moisture contents and temperature values, respectively, obtained earlier (Equation 6.8).

$$Q_{10} = k_2 / k_1 \quad (6.12)$$

Where:

k_1 = rate constant of inactivation at temperature 1, per unit of time

k_2 = rate constant of inactivation at temperature 2, per unit of time

Extrusion managers could use Figure 6.26 to predict thermal energy requirements of the extrusion process when extruding feed at given moisture content at a specific extruder barrel temperature that would inactivate *B. stearothermophilus* spores. This information would be useful, within the tested range, in selecting extruder barrel heating rates that would supply the required thermal energy to achieve desired levels

of bacterial inactivation during extrusion. As an example, suppose we desired to inactivate acid producing, thermophilic organisms and *B. stearothermophilus* spores in feed at 30 % (wet basis) extruded with the extruder barrel exit temperature set to100 ^{o}C. From Figure 6.26, we follow the 100 ^{o}C axis to the 30 % curve and then read off the heating requirement on the activation energy axis (175 kJ/kg). A Steam flow rate in the extruder barrel jacket that delivered at least 175 kJ/kg of feed would be required. Heating rates lower than that would not be adequate for inactivation of the bacterial spores in the feed matrix.

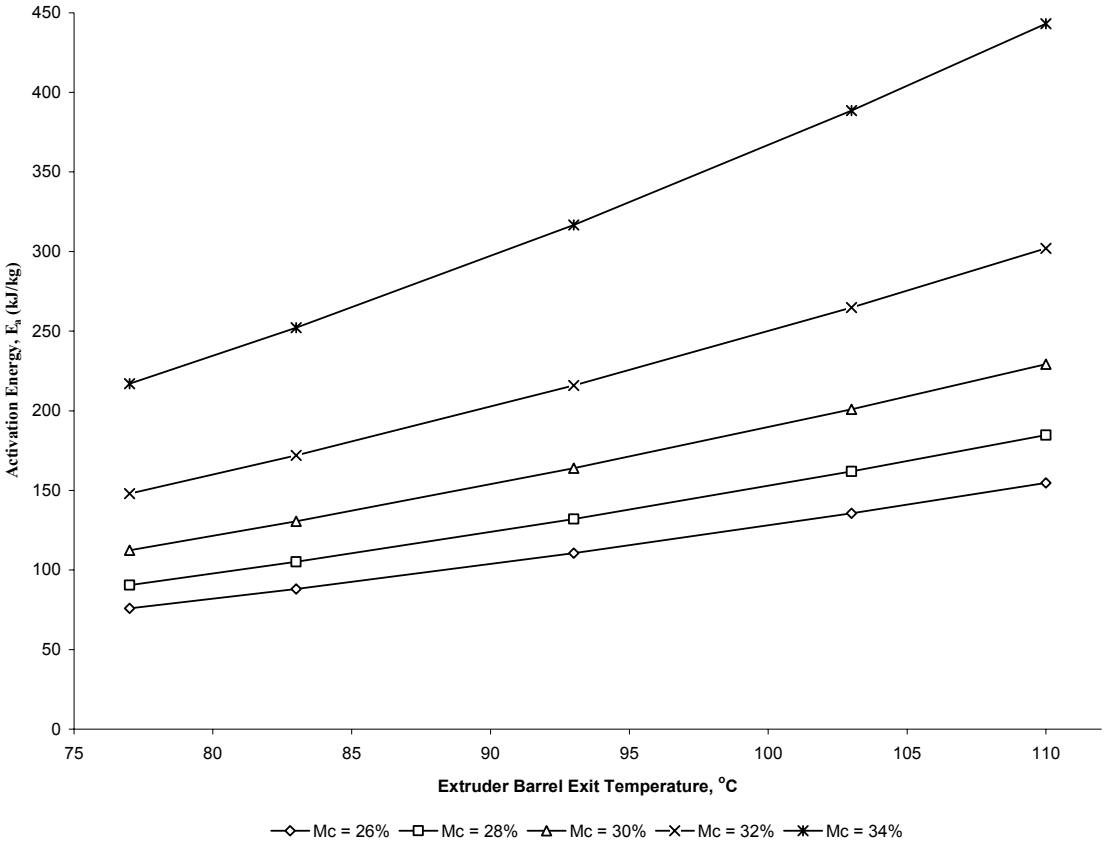

Figure 6.26. Variation of predicted activation energy (E_a) with extruder barrel exit temperature (T) at various feed moisture content (Mc), wet basis. The plots were obtained by combining Equation 6.10, 6.11 and 6.12.

7. CONCLUSIONS

1. A quadratic response surface model was fit to data on acid producing, thermophilic organisms and *B. stearothermophilus* spore inactivation obtained from the feed extrusion cooking studies. The stationary point within the central composite design region was a saddle point. A ridge of maximum response was therefore estimated in order to obtain extruder settings within the measured range that optimized *B. stearothermophilus* spore inactivation. The response surface model predicted that the maximum reduction in acid producing, thermophilic organisms and *B. stearothermophilus* of 1.03 log cycles would be obtained when the E325 Extru-Tech single-screw extruder was operated at T = 110 ^{o}C, Mc = 24.5 % wb and Rt = 11 s, respectively (coded radius of 1.682 in the central composite statistical design for good data).
2. No *S. typhimurium* cells were recovered in the standardized feed after extrusion of mash feed of 28.5 % wet basis moisture content at 83 ^{o}C for 7 s. Up to one log cycle reduction in acid producing, thermophilic organisms and *B. stearothermophilus* spore populations in the standardized feed formulation was achieved during extrusion using the E325 Extru-Tech single screw extruder.
3. In general, thermal processing is designed to eliminate mesophilic organisms and not thermophilic organisms such as *B. stearothermophilus* (Meng and Schaffner, 1997). It was predicted that most pathogenic organisms in feed would be inactivated by extrusion cooking using the E325 Extru-Tech single screw extruder through selecting

extruder conditions in the response surface within the measured range that maximized acid producing, thermophilic organisms and *B. stearothermophilus* spore reduction.

4. Spores of *Bacillus stearothermophilus* were more suitable indicator organisms than cells of *Salmonella typhimurium* in a standardized feed formulation for studying the effects of extrusion on bacterial inactivation.
5. Refrigerated, dry feed inoculum of *Bacillus stearothermophilus* adequately remained stable in the region of 2.0×10^7 CFU / 20 g of feed for 2 months.

8. SUGGESTIONS FOR FURTHER STUDY

1. Improve the extruder barrel temperature control by providing independent steam lines for each extruder barrel head.
2. Increase the number of temperature probes along the extruder barrel.
3. Inclusion of pressure transducers to monitor pressure applied on the feed due to the extrusion process.
4. Use of bacterial spore count techniques that take into account viable but non-culturable spores in feed.
5. Include in the experimental design other extrusion variables such as mash feed pH, salt and fat contents, residence time distribution of feed in the extruder barrel and pressure exerted on the feed during extrusion.
6. Include in the experimental design other measurable continuous response variables such as energy consumption of the extruder during extrusion, physical and nutritional properties of the extrudate.
7. Use of the extruder die and knife assembly to form distinct pellets upon extrusion, in contrast with the continuous pellets obtained in these studies. Use a pellet drier to dry the feed pellets. Measure the pellet durability index (DI) and use DI as a response variable in the analysis of the effect of extrusion variables on physical properties of the product (feed pellet).
8. Effect of extrusion cooking on aflatoxins, molds and other important organisms.

9. APPENDICES

9.1 APPENDIX A

Description of Extruder Barrel Temperature Probes

Table A 1. Description of extruder barrel temperature probes.

Location of temperature probe on extruder barrel	Temperature probe type	Model	Manufacturer
Feeding zone	Bimetal dial type	52-2185	H. O. Trerice company, Oak Park, Michigan
Extruder barrel zone number 1	Bimetal dial type	CR3007E	Trend instruments incorporated, Kenner, Louisiana
Extruder barrel zone number 4	Bimetal dial type	CR3007E	Trend instruments incorporated, Kenner, Louisiana
Extruder barrel exit	Type T thermocouple (copper/constantan wire)		Extru-Tech Incorporated, Sabetha, Kansas

9.2 APPENDIX B

Experimental Extruder Die Drawings

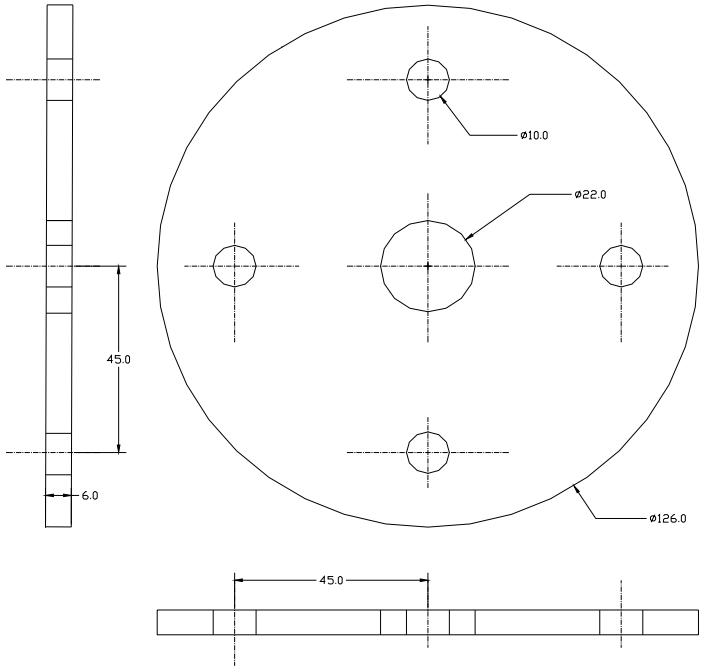

Figure B 1. Dimensioned drawings of plan, front and side elevation views of extruder barrel die used in the studies. The die was fabricated using a steel plate. Dimensions are in mm.

9.3 APPENDIX C

Specifications of constituents of the standardized feed formulation

Table C 1. Animal feed ingredients specification for corn meal.

Supplier:	Cooperative milling, Gettysburg, Pennsylvania.
Product:	Whole corn (yellow)
Packaging:	22.68 kg (50 lb) bags
Product number:	SSC-24-911500

Table C 2. Animal feed ingredients specifications for soy bean meal.

Supplier:	Cooperative milling, Gettysburg, Pennsylvania	
Product:	Plant protein products, soy bean meal HI PRO	
Packaging:	22.68 kg (50 lb) bags	
Product number:	CM-24-924900	
Guaranteed analysis		Percent
Crude protein	(min)	47.50
Crude fat	(min)	0.70
Crude fiber	(min)	3.60

Table C 3. Animal feed ingredients specification for animal protein blend.

Supplier:	AKEY Incorporated, Lewisburg, Ohio	
Product:	Animal protein blend, premium layer premix	
Product number:	45338-5002	

Guaranteed analysis

Selenium (Se)	(min)	120 ppm
Zinc (Zn)	(min)	2.00 %
Vitamin A	(min)	1600000 IU/lb
Vitamin D_3		600 000 IU/lb
Vitamin E		2000 IU/lb

Ingredients

Vitamin A, acetate in gelatin, vitamin D_3 supplement vitamin E supplement, riboflavin supplement, d-calcium pantothenate, niacin supplement, vitamin B_{12} supplement, menadione sodium bisulfite complex, choline chloride, folic acid, manganous oxide, ferrous sulfate, copper sulfate, basic copper chloride, zinc oxide, sodium selenite, ethylene diamine, dihydroiodide, calcium carbonate, mineral oil and roughage products.

9.4 APPENDIX D

Feed Particle Size Distribution

Table D 1. Particle size distribution of a standardized feed formulation consisting of 60 % corn meal, 30 % soybean meal and 10 % animal protein blend. d_{gw} = geometric mean diameter of feed particles by mass, S_{log} = geometric standard deviation of log-normal distribution by mass in ten-based logarithm. W_i = mass of feed material on i^{th} sieve. S_{gw} = geometric standard deviation of feed particle diameter by mass.

US Screen No	d, microns	Mass of feed on sieve, (W_i, g) Trial 1	2	3	4	5	Mean W_i, g	P, %	Percent finer by weight	$logd_i$	$W_i logd_i$	$(logd_i - logd_{gw})$	$W_i(logd_i - logd_{gw})^2$
4	4760	0	0	0.0	0.0	0.0	0.0	0.0	100.0	3.68	0.00	0.86	0.000
10	2000	3.8	4	3.4	3.5	3.1	3.6	3.6	96.4	3.30	11.75	0.48	0.833
18	1000	43.9	44.8	43.8	43.7	42.5	43.7	44.7	51.6	3.00	131.22	0.18	1.458
20	850	13	13.8	9.8	10.2	10.4	11.4	11.7	39.9	2.93	33.51	0.11	0.143
35	500	20.3	20.7	20.7	20.9	21.3	20.8	21.3	18.7	2.70	56.08	-0.12	0.292
60	250	10.7	13.8	10.8	12.3	16.0	12.7	13.0	5.7	2.40	30.50	-0.42	2.238
100	150	6.4	1.1	6.8	6.8	3.6	4.9	5.1	0.6	2.18	10.75	-0.64	2.032
200	75	0.1	0.1	1.9	0.3	0.5	0.6	0.6	0.0	1.88	1.09	-0.94	0.515
Pan	0	0	0	0.1	0.0	0.0	0.0	0.0					
Summation							97.8	100.0			274.91		7.511

d_{gw}, microns	648
s_{log}	0.277
s_{gw}, microns	1.89

9.5 APPENDIX E

Feed Moisture Content and a_w Data, Estimation of Retention Time of Feed in the Extruder Barrel for E325 Extru-Tech Extruder, Observed Moisture Content of Stored Feed and Results of Feed Drying and Rewetting Experiment, and Linear Interpolation of Thermal Conductivity Data for Corn

Table E 1.1. Feed moisture content measurement. Predicted and observed moisture content of feed mash before extrusion, % wet basis. Mc = moisture content. a_w = Observed water activity. T_{aw} = temperature in a_w meter sample chamber.

Predicted MC (wb, %)	Sample	Mass of Weighing dish + Wet feed (g)	Dry feed + Weighing dish (g)	Weighing dish (g)	Dry feed (g)	Observed Mc (wb, %)	Mean observed Mc (wb, %)	a_w	T_{aw} (^oC)
26	1	4.58	4.09	2.57	1.52	24	25	0.907	22.3
26	2	4.61	4.11	2.60	1.51	25		0.922	23.0
28	1	4.62	4.09	2.62	1.47	27	26	0.931	22.6
28	2	4.56	4.04	2.56	1.48	26		0.934	22.8
30	1	4.58	4.00	2.56	1.44	29	29	0.944	22.8
30	2	4.62	4.04	2.61	1.43	29		0.946	22.9
32	1	4.61	3.99	2.61	1.38	31	31	0.959	22.8
32	2	4.57	3.96	2.57	1.39	31		0.953	23.2
34	1	4.56	3.92	2.56	1.36	32	32	0.959	23.0
34	2	4.63	3.98	2.63	1.35	33		0.956	23.2

Table E 1.2. Observed moisture content of feed after extrusion and feed water activity at specified extrusion conditions represented by treatments (moisture content of mash feed, extruder barrel exit temperature and retention time of feed in the extruder barrel). Mc = moisture content. a_w = Observed water activity. T_{aw} = temperature in a_w meter sample chamber.

Treatment	Sample	Mass of Weighing dish + Wet feed (g)	Mass of Dry feed + Weighing dish (g)	Mass of Weighing dish (g)	Mass of Dry feed (g)	Observed Mc (wb, %)	Mean observed Mc (wb, %)	a_w	T_{aw} (^oC)
1	1	4.58	4.02	2.58	1.44	28	27	0.939	23.7
1	2	4.59	4.05	2.58	1.47	27		0.939	23.7
2	1	4.60	4.09	2.58	1.51	25	26	0.942	24.4
2	2	4.65	4.11	2.64	1.47	27		0.925	24.1
3	1	4.63	4.16	2.62	1.54	23	25	0.917	24.0
3	2	4.62	4.10	2.61	1.49	26		0.924	23.9
4	1	4.62	4.01	2.62	1.39	31	31	0.947	23.8
4	2	4.58	3.95	2.58	1.37	32		0.952	23.8
5	1	4.59	3.97	2.59	1.38	31	30	0.947	23.9
5	2	4.62	4.02	2.61	1.41	30		0.850	23.8
6	1	4.64	4.17	2.63	1.54	23	23	0.915	24.0
6	2	4.58	4.11	2.57	1.54	23		0.912	24.1
7	1	4.58	4.04	2.58	1.46	27	28	0.939	23.9
7	2	4.60	4.04	2.60	1.44	28		0.930	23.9
8	1	4.58	4.00	2.57	1.43	29	28	0.938	24.1
8	2	4.6	4.04	2.6	1.44	28		0.936	24.0
9	1	4.6	4.01	2.58	1.43	29	28	0.923	24.0
9	2	4.6	4.06	2.6	1.46	27		0.929	24.1
10	1	4.63	3.98	2.6	1.38	32	32	0.953	24.0
10	2	4.56	3.92	2.56	1.36	32		0.956	24.1
11	1	4.55	4.02	2.55	1.47	27	26	0.941	24.1
11	2	4.62	4.12	2.62	1.5	25		0.931	24.2
12	1	4.51	4.02	2.5	1.52	24	25	0.916	24.2
12	2	4.59	4.06	2.57	1.49	26		0.795	23.9
13	1	4.63	4.01	2.62	1.39	31	30	0.951	24.1
13	2	4.68	4.07	2.65	1.42	30		0.832	23.9
14	1	4.59	3.98	2.58	1.4	30	31	0.954	24.5
14	2	4.56	3.94	2.55	1.39	31		0.95	24.1
15	1	4.59	4	2.57	1.43	29	30	0.943	24.3
15	2	4.57	3.96	2.56	1.4	30		0.830	24.2

Table E 1.3. Observed moisture content of stored feed over the 8-week study period. Feed was stored in sealed storage barrels. Mc = moisture content, % wet basis (wb).

Week	Sample	Weighing Dish + Wet Feed (g)	Dry Feed + Weighing Dish (g)	Weighing Dish (g)	Dry Feed (g)	Observed Mc (wb, %)
1	1	4.58	4.32	2.58	1.74	13.0
1	2	4.57	4.32	2.57	1.75	12.5
1	3	4.59	4.33	2.59	1.74	13.0
2	1	4.58	4.32	2.58	1.74	13.0
2	2	4.60	4.34	2.6	1.74	13.0
2	3	4.62	4.36	2.62	1.74	13.0
3	1	4.60	4.35	2.6	1.75	12.5
3	2	4.61	4.35	2.61	1.74	13.0
3	3	4.59	4.34	2.59	1.75	12.5
4	1	4.61	4.35	2.61	1.74	13.0
4	2	4.58	4.32	2.58	1.74	13.0
4	3	4.61	4.36	2.61	1.75	12.5
5	1	4.59	4.33	2.59	1.74	13.0
5	2	4.61	4.34	2.61	1.73	13.5
5	3	4.59	4.33	2.59	1.74	13.0
6	1	4.59	4.34	2.59	1.75	12.5
6	2	4.58	4.32	2.58	1.74	13.0
6	3	4.59	4.32	2.59	1.73	13.5
7	1	4.59	4.33	2.59	1.74	13.0
7	2	4.61	4.35	2.61	1.74	13.0
7	3	4.59	4.33	2.59	1.74	13.0
8	1	4.59	4.34	2.59	1.75	12.5
8	2	4.61	4.35	2.61	1.74	13.0
8	3	4.61	4.35	2.6	1.75	12.9

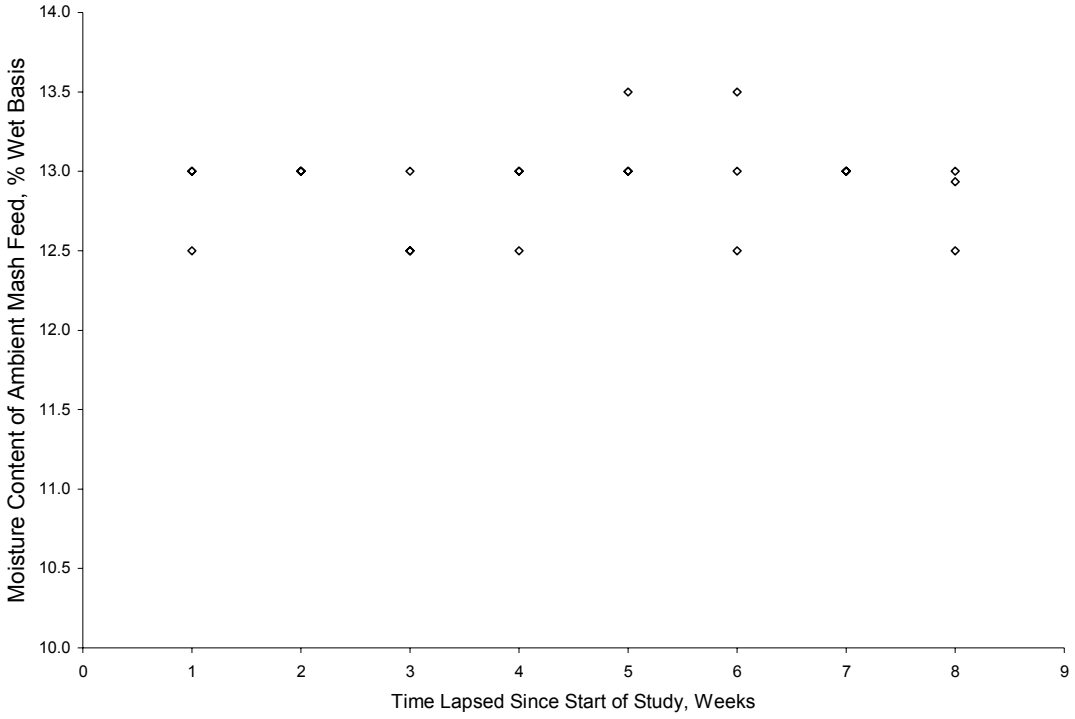

Figure E 1. Moisture content of ambient feed (% wet basis) in sealed storage barrels over the 8-week study period.

Table E 1.4. Results of the feed drying and rewetting experiment. Feed samples were dried, rewetted within the range of mash feed moisture content covered in this research and redried to verify that all the rewetting water was recovered during drying. Mc = feed moisture content (%, wb), Amb Feed = moisture content of feed in storage barrel (%, wb), Rep = replicate, wb = wet basis (%).

Predicted Mc (%, wb)	Rep	Wet Feed + Weighing Dish (g)	Dry Feed + Weighing Dish (g)	Weighing Dish (g)	Wet Feed (g)	Dry Feed (g)	Initial Feed Mc (%, wb)	Rewetting Water (g)	Rewetted Feed + Weighing Dish (g)	Redried Feed + Weighing Dish (g)	Recovered Water (g)	Rewetted Feed Mc, (%, wb)
26	1	6.470	5.85	0.990	5.48	4.86	11.3	1.880	7.73	5.820	1.91	28.3
26	2	6.440	5.83	0.990	5.45	4.84	11.2	1.750	7.58	5.790	1.79	27.2
26	3	6.440	5.83	0.980	5.46	4.85	11.2	1.890	7.72	5.800	1.92	28.5
28	1	6.430	5.83	0.990	5.44	4.84	11.0	1.260	7.09	5.780	1.31	21.5
28	2	6.420	5.82	0.980	5.44	4.84	11.0	2.120	7.94	5.780	2.16	31.0
28	3	6.430	5.83	0.980	5.45	4.85	11.0	2.140	7.97	5.790	2.18	31.2
30	1	6.360	5.77	0.980	5.38	4.79	11.0	2.330	8.10	5.740	2.36	33.1
30	2	6.440	5.83	0.990	5.45	4.84	11.2	2.100	7.93	5.800	2.13	30.7
30	3	6.480	5.88	0.990	5.49	4.89	10.9	2.660	8.54	5.830	2.71	35.9
32	1	6.440	5.83	0.990	5.45	4.84	11.2	2.460	8.29	5.790	2.50	34.2
32	2	6.430	5.83	0.990	5.44	4.84	11.0	2.590	8.42	5.790	2.63	35.4
32	3	6.490	5.87	0.980	5.51	4.89	11.3	2.590	8.46	5.850	2.61	34.9
34	1	6.120	5.54	0.990	5.13	4.55	11.3	2.530	8.07	5.500	2.57	36.3
34	2	6.470	5.86	0.980	5.49	4.88	11.1	2.560	8.42	5.830	2.59	34.8
34	3	6.450	5.85	0.990	5.46	4.86	11.0	2.620	8.47	5.820	2.65	35.4
Amb Feed	1	3.000	2.79	1.000	2.00	1.79	10.5	0.000	2.79	2.780	0.01	0.6
Amb Feed	2	3.000	2.78	0.980	2.02	1.80	10.9	0.000	2.78	2.780	0.00	0.0
Amb Feed	3	3.000	2.78	0.980	2.02	1.80	10.9	0.000	2.78	2.770	0.01	0.6

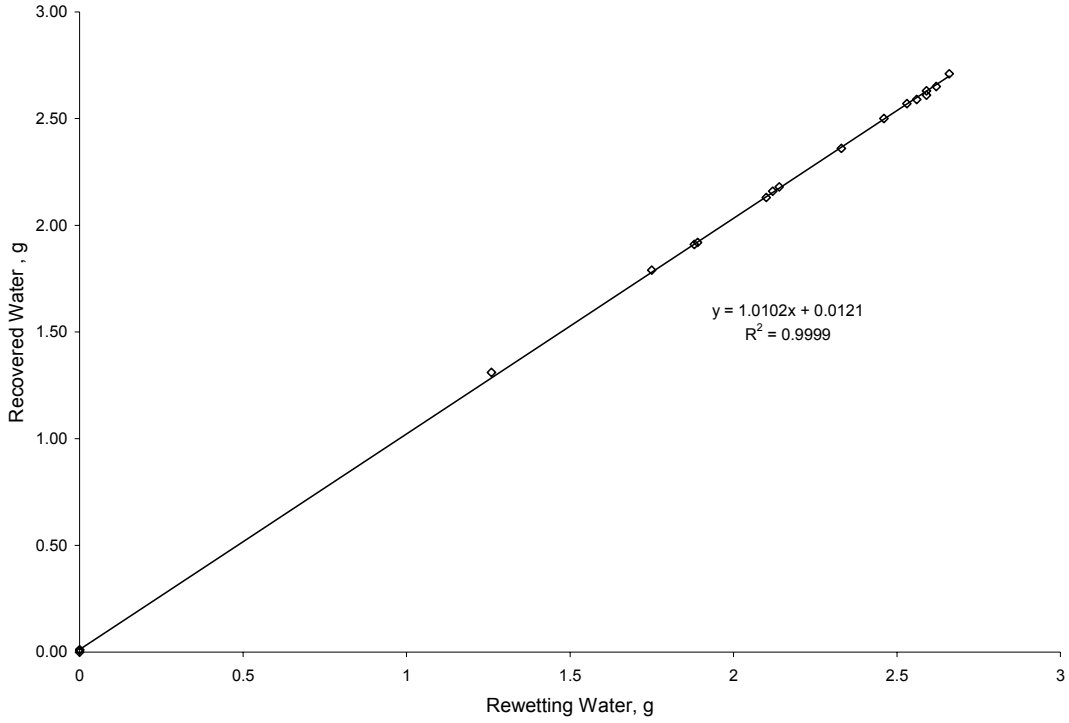

Figure E 2. A graphical representation of the feed drying and rewetting experiment. Feed samples were dried, rewetted and dried again to verify that all rewetting water was recovered by drying. Data from 3 replicates of the 5 levels of feed moisture content (24.5, 26.5, 28.5, 30.5 and 32.5 %, wet basis) were used in this plot.

Table E 2. Estimation of retention time (s) of feed in the extruder barrel at different barrel speeds (Hertz, equivalent to revolutions per second).

Replicate	Extruder barrel speed, Hz	Retention time, s
1	25	11.8
2	25	11.0
3	25	11.2
1	30	10.0
2	30	9.6
3	30	10.0
1	50	8.3
2	50	6.6
3	50	7.1
1	60	6.0
2	60	6.0
3	60	6.0
1	70	5.0
2	70	5.0
3	70	4.0
1	80	4.9
2	80	3.5
3	80	4.0
1	90	3.0
2	90	3.0
3	90	3.0

Table E 3. Summary of linear interpolation of thermal conductivity data for corn at 0.91 and 30.2 % wet basis moisture content at 8 - 52 ^{o}C. Temperature drops associated with thermal conductivity values are presented based on setting $[q_x.(dx/A_h)] = 1$, for simplicity. Rearranging Equation 6.4 yields $dT = -[q_x (dx / A_h)] / k_c$.

Corn, yellow dust			
Moisture Content, % wet basis	Temperature, ^{o}C	Thermal conductivity, k (W/m.K)	Temperature drop, dT (^{o}C)
0.91	8 - 52	0.141	
30.2	8 - 52	0.172	
Source: Sing and Heldman (2001)			
Interpolation			
26	8 - 52	0.168	5.97
27	8 - 52	0.169	5.93
28	8 - 52	0.170	5.89
29	8 - 52	0.171	5.86
30	8 - 52	0.172	5.82

9.6 APPENDIX F

Dry Feed Inoculum Preparation Studies (*Salmonella typhimurium* in Chalk and in Feed, *Bacillus stearothermophilus* Spores in Dry Feed)

Table F1.1. Summary of *S. typhimurium* population densities in dry chalk inoculum, inoculated feed and extruded feed inoculated with dry chalk inoculum prior to extrusion. "*" indicates feed not extruded. "control" refers to uninoculated feed, "inoculated" refers to feed inoculated with dry chalk inoculum.

Treatment	Extruder Variables			Sample Description	Rep	Sample	Serial Dilution Plated, (d)	Volume plated, mL (v)	Plate count CFU	CFU / 20g of feed (20*CFU/d/v)	
	T, °C	Mc, % wb	Rt, s							Mean	SE
A_0	*	26	*	Control	1	1	0.10	0.1	0	0.00E+00	0	0
A_0	*	26	*	Control	1	2	0.10	0.1	0	0.00E+00		
A_0	*	26	*	Control	2	1	0.10	0.1	0	0.00E+00		
A_0	*	26	*	Control	2	2	0.10	0.1	0	0.00E+00		
A_1	*	26	*	Inoculated	1	1	0.10	0.1	29	5.80E+04	5.2E+04	1.9E+04
A_1	*	26	*	Inoculated	1	2	0.10	0.1	28	5.60E+04		
A_1	*	26	*	Inoculated	2	1	0.10	0.1	34	6.80E+04		
A_1	*	26	*	Inoculated	2	2	0.10	0.1	12	2.40E+04		
A_2	82	26	3	Inoculated	1	1	0.10	0.1	0	0.00E+00	0	0
A_2	82	26	3	Inoculated	1	2	0.10	0.1	0	0.00E+00		
A_2	82	26	3	Inoculated	2	1	0.10	0.1	0	0.00E+00		
A_2	82	26	3	Inoculated	2	2	0.10	0.1	0	0.00E+00		

Table F1.1. (‘cont.).

Treatment	Extruder Variables T, °C	Mc, % wb	Rt, s	Sample Description	Rep	Sample	Serial Dilution Plated, (d)	Volume plated, mL (v)	Plate count CFU	CFU / 20g of feed (20*CFU/d/v) Mean	SE
A_3	103	26	3	Inoculated	1	1	0.10	0.1	0	0.00E+00	
A_3	103	26	3	Inoculated	1	2	0.10	0.1	0	0.00E+00	
A_3	103	26	3	Inoculated	2	1	0.10	0.1	0	0.00E+00	0	0
A_3	103	26	3	Inoculated	2	2	0.10	0.1	0	0.00E+00	
A_4	103	26	4	Inoculated	1	1	0.10	0.1	0	0.00E+00	
A_4	103	26	4	Inoculated	1	2	0.10	0.1	0	0.00E+00	
A_4	103	26	4	Inoculated	2	1	0.10	0.1	0	0.00E+00	0	0
A_4	103	26	4	Inoculated	2	2	0.10	0.1	0	0.00E+00	

Table F 1.2. Summary of *S. typhimurium* population densities in feed inoculated with dry feed inoculum before and after extrusion, respectively. Initial dilution of feed suspension was 0.2. Treatments were arbitrarily assigned letters A, B, and C, respectively, for identification. Trt = treatment.

Trt	Extruder barrel head temperature, °C				Mc, % wb	Rt, s	Rep	Sample	Volume plated, mL	Decimal dilution of initial dilution plated	Before extrusion		After extrusion		Spore inactivation	
	Barrel section temperature, °C										CFU, per plate	CFU / 20 g of feed, (N_o)	CFU, per plate	CFU/ 20 g of feed, (N)	Log_{10} (N_o/N)	{100*(No-N)/No}%
	Entrance	1	4	Exit												
A	41	68	54	83	30	7	1	1	0.1	1.0E-04	56	5.6E+08	0	0.0E+00	7.45	100
A	41	60	52	83	30	7	1	2	0.1	1.0E-04	56	5.6E+08	0	0.0E+00	7.45	100
B	41	60	52	88	30	7	1	1	0.1	1.0E-04	52	5.2E+08	0	0.0E+00	7.41	100
B	41	63	54	88	30	7	1	2	0.1	1.0E-04	39	3.9E+08	0	0.0E+00	7.29	100
C	40	66	52	103	30	7	1	1	0.1	1.0E-04	44	4.4E+08	0	0.0E+00	7.34	100
C	40	66	52	103	30	7	1	2	0.1	1.0E-04	61	6.1E+08	0	0.0E+00	7.48	100

Table F 2.1. Comparisons of *Bacillus stearothermophilus* spore population densities in artificially inoculated feed mixed with deionized and tap water, respectively.

Sample	Plate Number	Initial dilution 20 g : 100 g	Decimal dilution of initial dilution poured	Deionized water used to modify moisture content of ambient feed			Tap water used to modify moisture content of ambient feed			N_1/N_2)
				Spore count per plate	Spores in 5 plates	CFU / 20 g feed (N_1)	Spore count per plate	Spores in 5 plates	CFU / 20 g feed (N_2)	
1	1	0.20	1.00E-02	306	1426	7.1E+05	290	1415	7.1E+05	1.01
1	2	0.20	1.00E-02	291			299			
1	3	0.20	1.00E-02	303			298			
1	4	0.20	1.00E-02	278			278			
1	5	0.20	1.00E-02	248			250			
2	1	0.20	1.00E-02	300	1400	7.0E+05	298	1398	7.0E+05	1.00
2	2	0.20	1.00E-02	280			281			
2	3	0.20	1.00E-02	250			260			
2	4	0.20	1.00E-02	293			280			
2	5	0.20	1.00E-02	277			279			
1	1	0.20	1.00E-02	200	1144	5.7E+05	205	1155	5.8E+05	0.99
1	2	0.20	1.00E-02	250			252			
1	3	0.20	1.00E-02	225			230			
1	4	0.20	1.00E-02	221			223			
1	5	0.20	1.00E-02	248			245			
2	1	0.20	1.00E-02	215	1119	5.6E+05	210	1114	5.6E+05	1.00
2	2	0.20	1.00E-02	250			261			
2	3	0.20	1.00E-02	213			200			
2	4	0.20	1.00E-02	223			220			
2	5	0.20	1.00E-02	218			223			

Table F 2.1. (‘cont.)

Sample	Plate Number	Decimal dilution of initial dilution poured	Deionized water used to modify moisture content of ambient feed			Tap water used to modify moisture content of ambient feed			N_1/N_2)
			Spore count per plate	Spores in 5 plates	CFU / 20 g feed (N_1)	Spore count per plate	Spores in 5 plates	CFU / 20 g feed (N_2)	
1	1	1.00E-02	203	1124	5.6E+05	200	1123	5.6E+05	1.00
1	2	1.00E-02	247			250			
1	3	1.00E-02	218			215			
1	4	1.00E-02	230			228			
1	5	1.00E-02	226			230			
2	1	1.00E-02	214	1119	5.6E+05	210	1120	5.6E+05	1.00
2	2	1.00E-02	253			259			
2	3	1.00E-02	214			220			
2	4	1.00E-02	221			215			
2	5	1.00E-02	217			216			
1	1	1.00E-02	208	1149	5.7E+05	210	1156	5.8E+05	0.99
1	2	1.00E-02	241			238			
1	3	1.00E-02	246			248			
1	4	1.00E-02	224			225			
1	5	1.00E-02	230			235			
2	1	1.00E-02	125	1250	6.3E+05	130	1250	6.3E+05	1.00
2	2	1.00E-02	237			230			
2	3	1.00E-02	271			265			
2	4	1.00E-02	340			345			
2	5	1.00E-02	277			280			

Table F 2.2. Summary of acid producing, thermophilic organisms and *B. stearothermophilus* population densities in dry feed inoculum, by the cultural method. Samples of dry feed inoculum were heated for 30 minutes to increase thermal stress on spores beyond that provided by the standard 15 minute heat shocking treatment. Similarly, extrusion cooking was expected to achieve thermal stress on spores beyond the heat shocking treatment.

Sample	Plate number	Initial suspension, 20 g feed: 100 g suspension	Dilution of initial suspension poured	CFU / plate	Total CFU in 5 plates	CFU / 20 g feed
Control feed	1	0.20	1.00E-01	40	211	1.06E+04
Control feed	2	0.20	1.00E-01	50		
Control feed	3	0.20	1.00E-01	40		
Control feed	4	0.20	1.00E-01	46		
Control feed	5	0.20	1.00E-01	35		
Control feed	1	0.20	1.00E-01	44	204	1.02E+04
Control feed	2	0.20	1.00E-01	44		
Control feed	3	0.20	1.00E-01	45		
Control feed	4	0.20	1.00E-01	37		
Control feed	5	0.20	1.00E-01	34		
Feed inoculum	1	0.20	1.00E-03	109	468	2.34E+06
Feed inoculum	2	0.20	1.00E-03	88		
Feed inoculum	3	0.20	1.00E-03	80		
Feed inoculum	4	0.20	1.00E-03	96		
Feed inoculum	5	0.20	1.00E-03	95		
Feed inoculum	1	0.20	1.00E-03	86	464	2.32E+06
Feed inoculum	2	0.20	1.00E-03	108		
Feed inoculum	3	0.20	1.00E-03	98		
Feed inoculum	4	0.20	1.00E-03	78		
Feed inoculum	5	0.20	1.00E-03	94		
Feed inoculum*	1	0.20	1.00E-03	99	565	2.83E+06
Feed inoculum*	2	0.20	1.00E-03	116		
Feed inoculum*	3	0.20	1.00E-03	96		
Feed inoculum*	4	0.20	1.00E-03	120		
Feed inoculum*	5	0.20	1.00E-03	134		
Feed inoculum*	1	0.20	1.00E-03	100	573	2.87E+06
Feed inoculum*	2	0.20	1.00E-03	115		
Feed inoculum*	3	0.20	1.00E-03	100		
Feed inoculum*	4	0.20	1.00E-03	136		
Feed inoculum*	5	0.20	1.00E-03	122		

* Heat shocked for 30 minutes at 100 ^{o}C, twice the standard duration

Table F 3. Direct microscopic count results of *Bacillus stearothermophilus* spores in dry feed inoculuum obtained using a Petroff-Hausser counting chamber.

	Spore Count Per Small Square		
Observation	Replicate		
	1	2	3
1	8	9	8
2	6	7	7
3	7	9	9
4	7	8	9
5	9	9	10
6	10	12	7
7	12	10	9
8	9	11	7
9	7	7	9
10	8	8	11
11	9	8	9
12	8	7	7
13	7	10	8
14	9	11	9
15	9	9	7
16	8	7	9
17	10	6	10
18	12	8	14
19	11	9	12
20	9	12	7
21	8	10	9
22	7	14	8
23	13	9	9
24	14	8	8
	Spore Count, Spores / 20 mL		SE
	3.6.E+06		5.4.E+04

Table F 4. *Bacillus stearothermophilus* spore count in dry feed inoculum over a seven-week storage period by the cultural method.

Week	Sample	Plate number	Initial dilution, 20 g : 100 g	Decimal dilution of initial dilution poured	CFU / plate	Total CFU in 5 plates	CFU / 20 g feed
1	1	1	0.20	1.00E-03	43		
1	1	2	0.20	1.00E-03	45		
1	1	3	0.20	1.00E-03	46	241	1.21E+06
1	1	4	0.20	1.00E-03	50		
1	1	5	0.20	1.00E-03	57		
1	2	1	0.20	1.00E-03	47		
1	2	2	0.20	1.00E-03	52		
1	2	3	0.20	1.00E-03	61	252	1.26E+06
1	2	4	0.20	1.00E-03	45		
1	2	5	0.20	1.00E-03	47		
2	1	1	0.20	1.00E-03	40		
2	1	2	0.20	1.00E-03	46		
2	1	3	0.20	1.00E-03	45	228	1.14E+06
2	1	4	0.20	1.00E-03	49		
2	1	5	0.20	1.00E-03	48		
2	2	1	0.20	1.00E-03	49		
2	2	2	0.20	1.00E-03	50		
2	2	3	0.20	1.00E-03	63	256	1.28E+06
2	2	4	0.20	1.00E-03	46		
2	2	5	0.20	1.00E-03	48		
3	1	1	0.20	1.00E-03	44		
3	1	2	0.20	1.00E-03	46		
3	1	3	0.20	1.00E-03	44	244	1.22E+06
3	1	4	0.20	1.00E-03	52		
3	1	5	0.20	1.00E-03	58		
3	2	1	0.20	1.00E-03	48		
3	2	2	0.20	1.00E-03	52		
3	2	3	0.20	1.00E-03	65	257	1.29E+06
3	2	4	0.20	1.00E-03	44		
3	2	5	0.20	1.00E-03	48		
4	1	1	0.20	1.00E-03	43		
4	1	2	0.20	1.00E-03	44		
4	1	3	0.20	1.00E-03	48	231	1.16E+06
4	1	4	0.20	1.00E-03	48		
4	1	5	0.20	1.00E-03	48		
4	2	1	0.20	1.00E-03	48		
4	2	2	0.20	1.00E-03	50		
4	2	3	0.20	1.00E-03	62	254	1.27E+06
4	2	4	0.20	1.00E-03	46		
4	2	5	0.20	1.00E-03	48		

Table F 4. (‘cont.’)

Week	Sample	Plate number	Initial dilution, 20 g : 100 g	Decimal dilution of initial dilution poured	CFU / plate	Total CFU in 5 plates	CFU / 20 g feed
5	1	1	0.20	1.00E-03	44		
5	1	2	0.20	1.00E-03	46		
5	1	3	0.20	1.00E-03	48	238	1.19E+06
5	1	4	0.20	1.00E-03	51		
5	1	5	0.20	1.00E-03	49		
5	2	1	0.20	1.00E-03	48		
5	2	2	0.20	1.00E-03	51		
5	2	3	0.20	1.00E-03	63	256	1.28E+06
5	2	4	0.20	1.00E-03	46		
5	2	5	0.20	1.00E-03	48		
6	1	1	0.20	1.00E-03	44		
6	1	2	0.20	1.00E-03	46		
6	1	3	0.20	1.00E-03	48	249	1.25E+06
6	1	4	0.20	1.00E-03	53		
6	1	5	0.20	1.00E-03	58		
6	2	1	0.20	1.00E-03	49		
6	2	2	0.20	1.00E-03	50		
6	2	3	0.20	1.00E-03	63	256	1.28E+06
6	2	4	0.20	1.00E-03	46		
6	2	5	0.20	1.00E-03	48		
7	1	1	0.20	1.00E-03	46		
7	1	2	0.20	1.00E-03	44		
7	1	3	0.20	1.00E-03	46	244	1.22E+06
7	1	4	0.20	1.00E-03	52		
7	1	5	0.20	1.00E-03	56		
7	2	1	0.20	1.00E-03	46		
7	2	2	0.20	1.00E-03	50		
7	2	3	0.20	1.00E-03	63	253	1.27E+06
7	2	4	0.20	1.00E-03	46		
7	2	5	0.20	1.00E-03	48		
8	1	1	0.20	1.00E-03	46		
8	1	2	0.20	1.00E-03	44		
8	1	3	0.20	1.00E-03	48	247	1.24E+06
8	1	4	0.20	1.00E-03	53		
8	1	5	0.20	1.00E-03	56		
8	2	1	0.20	1.00E-03	47		
8	2	2	0.20	1.00E-03	56		
8	2	3	0.20	1.00E-03	60	254	1.27E+06
8	2	4	0.20	1.00E-03	46		
8	2	5	0.20	1.00E-03	45		

9.7 APPENDIX G

Randomization of Feed Extrusion Treatments,
Results of Test for Normality of Distribution of Residuals and ANOVA Tables for the Response Surface and Secondary Models

Table G 1. Order of extrusion experimental runs. Runs were grouped in a way that simplified and minimized the need to change extruder barrel temperature. T = Extruder barrel exit temperature, Mc = moisture content of feed mash, and Rt = retention time of feed in the extruder barrel.

Replicate	Day	Treatment	Extruder Variables			
			T, °F	T, °C	Mc, % wb	Rt, s
1	1	5	182	83	30.5	9
1	1	7	200	93	28.5	3
1	1	8	200	93	28.5	7
1	1	6	200	93	24.5	7
1	2	3	182	83	26.5	9
1	2	12	218	103	26.5	9
1	2	14	218	103	30.5	9
1	2	15	230	110	28.5	7
1	3	2	182	83	26.5	5
1	3	4	182	83	30.5	5
1	3	13	218	103	30.5	5
1	3	1	170	77	28.5	7
1	4	8	200	93	28.5	7
1	4	9	200	93	28.5	11
1	4	10	200	93	32.5	7
1	4	11	218	103	26.5	5
1	5	8	200	93	28.5	7
1	5	8	200	93	28.5	7
1	5	8	200	93	28.5	7
1	5	8	200	93	28.5	7
2	1	8	200	93	28.5	7
2	1	8	200	93	28.5	7
2	1	2	182	83	26.5	5
2	1	3	182	83	26.5	9
2	2	8	200	93	28.5	7
2	2	9	200	93	28.5	11
2	2	10	200	93	32.5	7
2	2	15	230	110	28.5	7
2	3	8	200	93	28.5	7
2	3	8	200	93	28.5	7
2	3	13	218	103	30.5	5
2	3	14	218	103	30.5	9
2	4	4	182	83	30.5	5
2	4	5	182	83	30.5	9
2	4	11	217	103	26.5	5
2	4	12	217	103	26.5	9
2	5	1	170	77	28.5	7

Extruder Variables

Replicate	Day	Treatment	T, °F	T, °C	Mc, % wb	Rt, s
1	1	5	182	83	30.5	9
1	1	7	200	93	28.5	3
1	1	8	200	93	28.5	7
1	1	6	200	93	24.5	7
1	2	3	182	83	26.5	9
1	2	12	218	103	26.5	9
1	2	14	218	103	30.5	9
1	2	15	230	110	28.5	7
1	3	2	182	83	26.5	5
1	3	4	182	83	30.5	5
1	3	13	218	103	30.5	5
1	3	1	170	77	28.5	7
1	4	8	200	93	28.5	7
1	4	9	200	93	28.5	11
1	4	10	200	93	32.5	7
1	4	11	218	103	26.5	5
1	5	8	200	93	28.5	7
1	5	8	200	93	28.5	7
1	5	8	200	93	28.5	7
1	5	8	200	93	28.5	7
2	1	8	200	93	28.5	7
2	1	8	200	93	28.5	7
2	1	2	182	83	26.5	5
2	1	3	182	83	26.5	9
2	2	8	200	93	28.5	7
2	2	9	200	93	28.5	11
2	2	10	200	93	32.5	7
2	2	15	230	110	28.5	7
2	3	8	200	93	28.5	7
2	3	8	200	93	28.5	7
2	3	13	218	103	30.5	5
2	3	14	218	103	30.5	9
2	4	4	182	83	30.5	5
2	4	5	182	83	30.5	9
2	4	11	217	103	26.5	5
2	4	12	217	103	26.5	9
2	5	1	170	77	28.5	7

Table G 1. (‘cont.).

Replicate	Day	Treatment	Extruder Variables			
			T, ^{o}F	T, ^{o}C	Mc, % wb	Rt, s
2	5	6	200	93	24.5	7
2	5	7	200	93	28.5	3
2	5	8	200	93	28.5	7
3	1	9	200	93	28.5	11
3	1	10	200	93	32.5	7
3	1	13	217	103	30.5	5
3	1	14	217	103	30.5	9
3	2	8	200	93	28.5	7
3	2	8	200	93	28.5	7
3	2	8	200	93	28.5	7
3	2	8	200	93	28.5	7
3	3	8	200	93	28.5	7
3	3	8	200	93	28.5	7
3	3	15	230	110	28.5	7
3	3	1	170	77	28.5	7
3	4	11	217	103	26.5	5
3	4	12	217	103	26.5	9
3	4	4	182	83	30.5	5
3	4	5	182	83	30.5	9
3	5	7	200	93	28.5	3
3	5	6	200	93	24.5	7
3	5	2	182	83	26.5	5
3	5	3	182	83	26.5	9

Table G 2.1. Normality statistics of the distribution of residuals.

Test	Statistic	Criterion	p Value
Shapiro-Wilk	0.977	Pr < W	0.316

Table G 2.2. Univariate results of normality assumption testing on residuals. Quartile information on residuals. Variable tested was residuals. Max = maximum, Min = minimum, Q1, Q3 are 25% and 75% quartiles, respectively.

Quartile	Estimate
100% Max	0.2171
99%	0.2171
95%	0.1251
90%	0.0876
75% Q3	0.0491
50% Median	0.0043
25% Q1	-0.0417
10%	-0.1102
5%	-0.1239
1%	-0.2699
0% Min	-0.2698

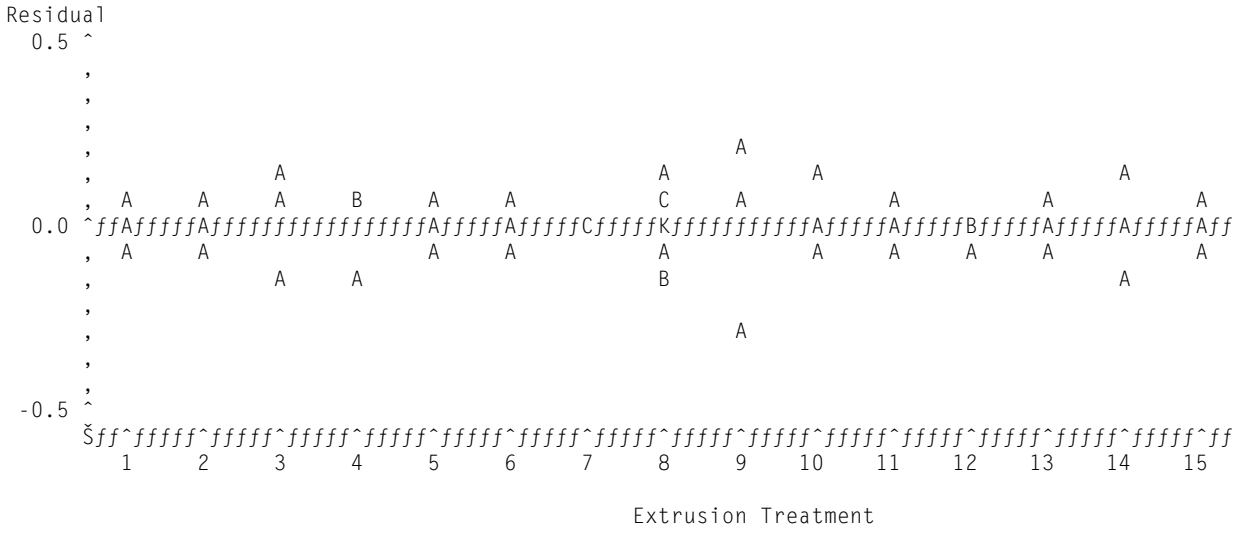

Figure G 1.1. Plot of residuals versus treatment. Treatment = predetermined combinations of extrusion conditions of feed moisture content, extruder barrel temperature and retention time of feed in the extruder barrel. Key: A = 1 observation, B = 2 observations, etc.

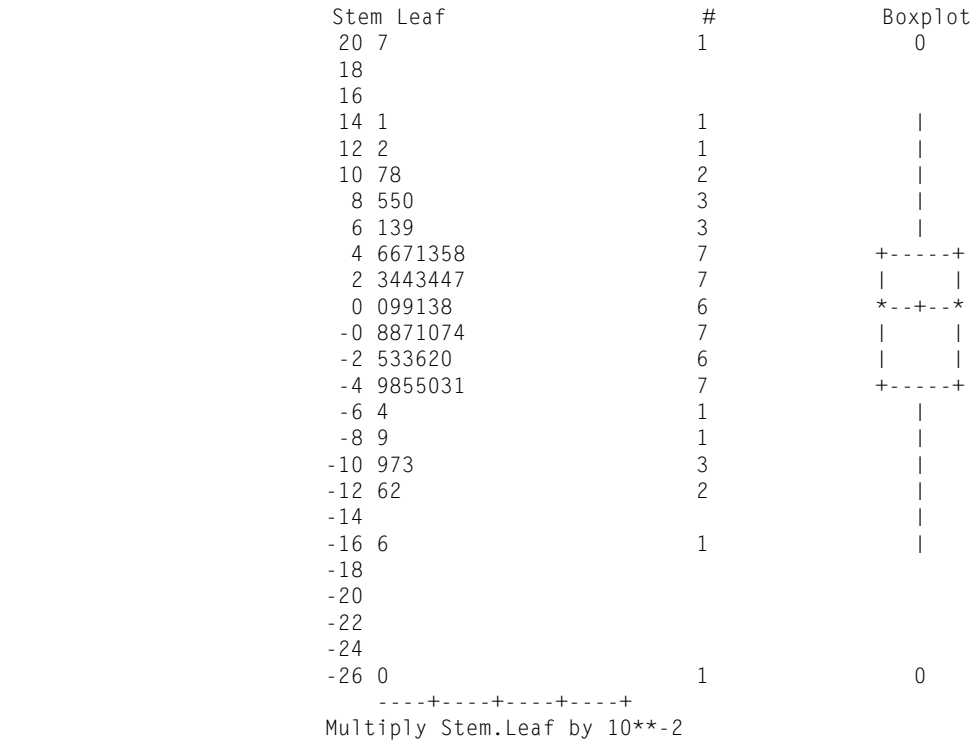

Figure G 1.2. Test for normality of distribution of residuals. Stem and leaf and box plot.

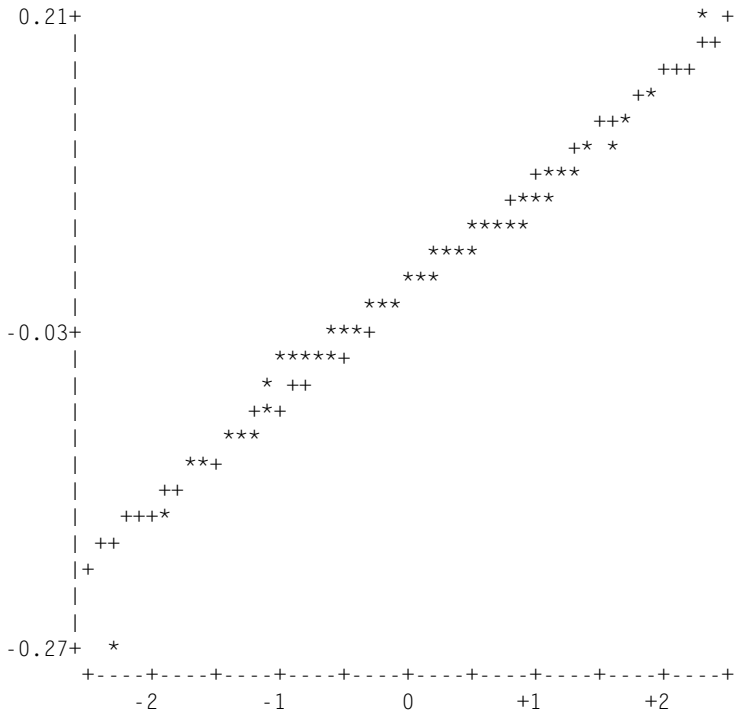

Figure G 2.3. Normal probability plot (residuals versus standard deviation). "+" indicate location of a normally distributed population of residuals with the same standard deviation as that of the observed residuals indicated by "*".

Table G 2.3. ANOVA table for the response surface model for acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore inactivation during feed extrusion.

Regression	DF	MS	R-square	F	p
Linear	3	0.083	0.253	9.03	< 0.001
Quadratic	3	0.042	0.127	4.52	0.007
Cross product	3	0.051	0.153	5.46	0.003
Total model	9	0.059	0.533	6.34	< 0.001

Table G 2.4. ANOVA table for the contribution of independent variables to the overal response surface model for acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore inactivation during feed extrusion.

Variable	DF	MS	F	p
T	4	0.055	5.94	0.0005
Mc	4	0.046	4.96	0.0019
Rt	4	0.073	7.91	< 0.0001

Table G 2.5. ANOVA table for lack of fit test for the response surface model.

Residual	DF	MS	F	p
Lack of fit	5	0.015	1.78	0.136
Pure error	45	0.009		
Total error	50	0.009		

Table G 2.6. ANOVA table for the secondary model, k.

Source	DF	MS	F	P
Model	2	0.0015	3.41	0.040
Error	57	0.00044		
Corrected total	59			

9.8 APPENDIX H

Appearance of Extrudates Under 15 Different Extru-Tech E325 extruder Settings Tested

Figure H 1. Extrudate obtained with Extru-Tech E325 extruder operating at 77 ^{o}C extruder barrel exit temperature, 28.5% wet basis mash feed moisture content and 7 s retention time of feed in the extruder barrel (Treatment 1). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 40.7±18.2 %.

Figure H 2. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 83 ^{o}C extruder barrel exit temperature, 26.5% wet basis mash feed moisture content and 5 s retention time of feed in the extruder barrel (Treatment 2). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 55.2±13.8 %.

Figure H 3. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 83 ^{o}C extruder barrel exit temperature, 26.5% wet basis mash feed moisture content and 9 s retention time of feed in the extruder barrel (Treatment 3). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 25.1±39.3 %.

Figure H 4. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 83 ^{o}C extruder barrel exit temperature, 30.5% wet basis mash feed moisture content and 5 s retention time of feed in the extruder barrel (Treatment 4). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 42.3±35.0 %.

Figure H 5. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 83 ^{o}C extruder barrel exit temperature, 30.5% wet basis mash feed moisture content and 9 s retention time of feed in the extruder barrel (Treatment 5). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 28.1±24.3 %.

Figure H 6. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 93 ^{o}C extruder barrel exit temperature, 24.5% wet basis mash feed moisture content and 7 s retention time of feed in the extruder barrel (Treatment 6). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 45.6±30.6.1%.

Figure H 7. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 93 ^{o}C extruder barrel exit temperature, 28.5% wet basis mash feed moisture content and 3 s retention time of feed in the extruder barrel (Treatment 7). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 27.8±18.3 %.

Figure H 8. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 93 ^{o}C extruder barrel exit temperature, 28.5% wet basis mash feed moisture content and 7 s retention time of feed in the extruder barrel (Treatment 8). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 32.6±11.7 %.

Figure H 9. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 93 ^{o}C extruder barrel exit temperature, 28.5% wet basis mash feed moisture content and 11 s retention time of feed in the extruder barrel (Treatment 9). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 65.3±53.2 %.

Figure H 10. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 93 ^{o}C extruder barrel exit temperature, 32.5% wet basis mash feed moisture content and 7 s retention time of feed in the extruder barrel (Treatment 10). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 15.9±39.8 %.

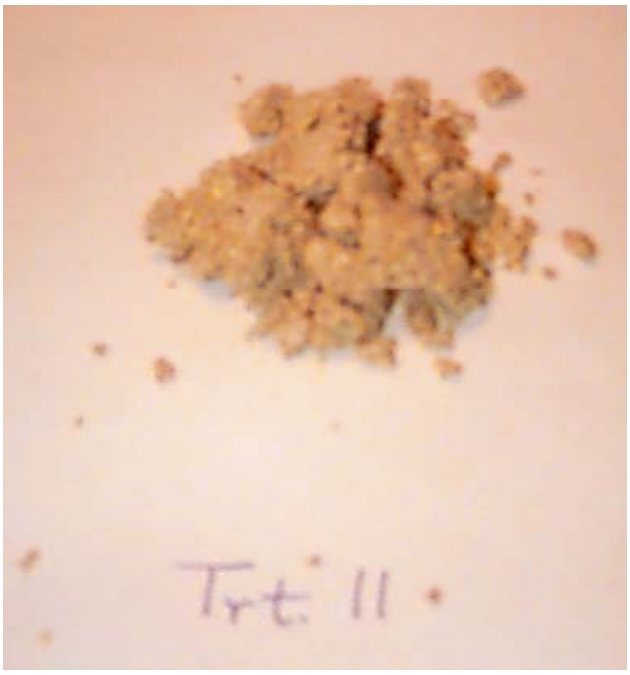

Figure H 11. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 103 ^{o}C extruder barrel exit temperature, 26.5% wet basis mash feed moisture content and 5 s retention time of feed in the extruder barrel (Treatment 11). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 47.3±7.7 %.

Figure H 12. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 103 ^{o}C extruder barrel exit temperature, 26.5% wet basis mash feed moisture content and 9 s retention time of feed in the extruder barrel (Treatment 12). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 64.1±19.4 %.

Figure H 13. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 103 ^{o}C extruder barrel exit temperature, 30.5% wet basis mash feed moisture content and 5 s retention time of feed in the extruder barrel (Treatment 13). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 31.4±11.0 %.

Figure H 14. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 103 ^{o}C extruder barrel exit temperature, 30.5% wet basis mash feed moisture content and 9 s retention time of feed in the extruder barrel (Treatment 14). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 34.1±29.4 %.

Figure H 15. Appearance of extrudate obtained with Extru-ech E325 extruder operating at 110 ^{o}C extruder barrel exit temperature, 28.5% wet basis mash feed moisture content and 7 s retention time of feed in the extruder barrel (Treatment 15). Inactivation of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spores was 40.3±11.9 %.

9.9 APPENDIX I

Raw data: Temperature Across Extruder Barrel (^{o}C) and Bacterial Spore Estimates, Before and After Extrusion (CFU / 20 g feed), respectively.

Table I 1.1. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep1, day 1. Rep = replicate.

		Extruder barrel head temperature, °C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
1	5	20	27	83	82	1	1	1	0.20	1.00E-02	306	1426	7.13E+05	208	1164	5.82E+05	8.82E-02
1	5	20	27	83	82	1	1	2	0.20	1.00E-02	291			225			
1	5	20	27	83	82	1	1	3	0.20	1.00E-02	303			226			
1	5	20	27	83	82	1	1	4	0.20	1.00E-02	278			230			
1	5	20	27	83	82	1	1	5	0.20	1.00E-02	248			275			
1	5	20	28	84	84	1	2	1	0.20	1.00E-02	300	1400	7.00E+05	188	1022	5.11E+05	1.37E-01
1	5	20	28	84	84	1	2	2	0.20	1.00E-02	280			201			
1	5	20	28	84	84	1	2	3	0.20	1.00E-02	250			203			
1	5	20	28	84	84	1	2	4	0.20	1.00E-02	293			230			
1	5	20	28	84	84	1	2	5	0.20	1.00E-02	277			200			
1	7	20	38	60	93	1	1	1	0.20	1.00E-02	200	1144	5.72E+05	183	1021	5.11E+05	4.94E-02
1	7	20	38	60	93	1	1	2	0.20	1.00E-02	250			182			
1	7	20	38	60	93	1	1	3	0.20	1.00E-02	225			199			
1	7	20	38	60	93	1	1	4	0.20	1.00E-02	221			244			
1	7	20	38	60	93	1	1	5	0.20	1.00E-02	248			213			
1	7	20	38	60	93	1	2	1	0.20	1.00E-02	215	1119	5.60E+05	167	1042	5.21E+05	3.10E-02
1	7	20	38	60	93	1	2	2	0.20	1.00E-02	250			225			
1	7	20	38	60	93	1	2	3	0.20	1.00E-02	213			211			
1	7	20	38	60	93	1	2	4	0.20	1.00E-02	223			219			
1	7	20	38	60	93	1	2	5	0.20	1.00E-02	218			220			

Table I 1.1. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
1	8	20	38	56	93	1	1	1	0.20	1.00E-02	203	1124	5.62E+05	151	886	4.43E+05	1.03E-01
1	8	20	38	56	93	1	1	2	0.20	1.00E-02	247			147			
1	8	20	38	56	93	1	1	3	0.20	1.00E-02	218			194			
1	8	20	38	56	93	1	1	4	0.20	1.00E-02	230			208			
1	8	20	38	56	93	1	1	5	0.20	1.00E-02	226			186			
1	8	20	38	56	93	1	2	1	0.20	1.00E-02	214	1119	5.60E+05	186	1061	5.31E+05	2.31E-02
1	8	20	38	56	93	1	2	2	0.20	1.00E-02	253			195			
1	8	20	38	56	93	1	2	3	0.20	1.00E-02	214			218			
1	8	20	38	56	93	1	2	4	0.20	1.00E-02	221			239			
1	8	20	38	56	93	1	2	5	0.20	1.00E-02	217			223			
1	6	20	32	52	93	1	1	1	0.20	1.00E-02	208	1149	5.75E+05	242	999	5.00E+05	6.08E-02
1	6	20	32	52	93	1	1	2	0.20	1.00E-02	241			194			
1	6	20	32	52	93	1	1	3	0.20	1.00E-02	246			170			
1	6	20	32	52	93	1	1	4	0.20	1.00E-02	224			195			
1	6	20	32	52	93	1	1	5	0.20	1.00E-02	230			198			
1	6	20	32	49	94	1	2	1	0.20	1.00E-02	125	1250	6.25E+05	150	986	4.93E+05	1.03E-01
1	6	20	32	49	94	1	2	2	0.20	1.00E-02	237			212			
1	6	20	32	49	94	1	2	3	0.20	1.00E-02	271			196			
1	6	20	32	49	94	1	2	4	0.20	1.00E-02	340			268			
1	6	20	32	49	94	1	2	5	0.20	1.00E-02	277			160			

Table I 1.2. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep1, day 2. Rep = replicate.

		Extruder barrel head temperature, °C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
2	3	18	38	60	83	1	1	1	0.20	1.00E-02	61	698	3.49E+05	253	1171	5.86E+05	-2.25E-01
2	3	18	38	60	83	1	1	2	0.20	1.00E-02	161			298			
2	3	18	38	60	83	1	1	3	0.20	1.00E-02	140			152			
2	3	18	38	60	83	1	1	4	0.20	1.00E-02	159			168			
2	3	18	38	60	83	1	1	5	0.20	1.00E-02	177			300			
2	3	18	38	52	83	1	2	1	0.20	1.00E-02	246	1202	6.01E+05	320	1260	6.30E+05	-2.05E-02
2	3	18	38	52	83	1	2	2	0.20	1.00E-02	243			262			
2	3	18	38	52	83	1	2	3	0.20	1.00E-02	34			243			
2	3	18	38	52	83	1	2	4	0.20	1.00E-02	297			219			
2	3	18	38	52	83	1	2	5	0.20	1.00E-02	382			216			
2	14	18	35	82	103	1	1	1	0.20	1.00E-02	122	838	4.19E+05	176	518	2.59E+05	2.09E-01
2	14	18	35	82	103	1	1	2	0.20	1.00E-02	215			114			
2	14	18	35	82	103	1	1	3	0.20	1.00E-02	274			98			
2	14	18	35	82	103	1	1	4	0.20	1.00E-02	120			67			
2	14	18	35	82	103	1	1	5	0.20	1.00E-02	107			63			
2	14	18	38	91	103	1	2	1	0.20	1.00E-02	149	1052	5.26E+05	74	513	2.57E+05	3.12E-01
2	14	18	38	91	103	1	2	2	0.20	1.00E-02	195			98			
2	14	18	38	91	103	1	2	3	0.20	1.00E-02	236			133			
2	14	18	38	91	103	1	2	4	0.20	1.00E-02	211			120			
2	14	18	38	91	103	1	2	5	0.20	1.00E-02	261			88			

Table I 1.2. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
2	12	18	41	96	103	1	1	1	0.20	1.00E-02	207	1383	6.92E+05	41	416	2.08E+05	5.22E-01
2	12	18	41	96	103	1	1	2	0.20	1.00E-02	288			39			
2	12	18	41	96	103	1	1	3	0.20	1.00E-02	315			128			
2	12	18	41	96	103	1	1	4	0.20	1.00E-02	353			106			
2	12	18	41	96	103	1	1	5	0.20	1.00E-02	220			102			
2	12	18	41	71	103	1	2	1	0.20	1.00E-02	329	1479	7.40E+05	179	605	3.03E+05	3.88E-01
2	12	18	41	71	103	1	2	2	0.20	1.00E-02	283			133			
2	12	18	41	71	103	1	2	3	0.20	1.00E-02	239			124			
2	12	18	41	71	103	1	2	4	0.20	1.00E-02	274			86			
2	12	18	41	71	103	1	2	5	0.20	1.00E-02	354			83			
2	15	18	38	99	106	1	1	1	0.20	1.00E-02	182	1167	5.84E+05	88	619	3.10E+05	2.75E-01
2	15	18	38	99	106	1	1	2	0.20	1.00E-02	242			85			
2	15	18	38	99	106	1	1	3	0.20	1.00E-02	264			134			
2	15	18	38	99	106	1	1	4	0.20	1.00E-02	261			130			
2	15	18	38	99	106	1	1	5	0.20	1.00E-02	218			182			
2	15	18	38	93	106	1	2	1	0.20	1.00E-02	236	1272	6.36E+05	149	1143	5.72E+05	4.64E-02
2	15	18	38	93	106	1	2	2	0.20	1.00E-02	312			178			
2	15	18	38	93	106	1	2	3	0.20	1.00E-02	289			336			
2	15	18	38	93	106	1	2	4	0.20	1.00E-02	206			180			
2	15	18	38	93	106	1	2	5	0.20	1.00E-02	229			300			

Table I 1.3. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep1, day 3. Rep = replicate.

		Extruder barrel head temperature, °C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
3	2	19	41	71	83	1	1	1	0.20	1.00E-02	164	1169	5.85E+05	134	629	3.15E+05	2.69E-01
3	2	19	41	71	83	1	1	2	0.20	1.00E-02	319			128			
3	2	19	41	71	83	1	1	3	0.20	1.00E-02	257			166			
3	2	19	41	71	83	1	1	4	0.20	1.00E-02	216			124			
3	2	19	41	71	83	1	1	5	0.20	1.00E-02	213			77			
3	2	19	41	74	83	1	2	1	0.20	1.00E-02	372	1413	7.07E+05	174	741	3.71E+05	2.80E-01
3	2	19	41	74	83	1	2	2	0.20	1.00E-02	288			163			
3	2	19	41	74	83	1	2	3	0.20	1.00E-02	270			146			
3	2	19	41	74	83	1	2	4	0.20	1.00E-02	263			120			
3	2	19	41	74	83	1	2	5	0.20	1.00E-02	220			138			
3	4	19	43	74	81	1	1	1	0.20	1.00E-02	300	1074	5.37E+05	79	464	2.32E+05	3.64E-01
3	4	19	43	74	81	1	1	2	0.20	1.00E-02	268			88			
3	4	19	43	74	81	1	1	3	0.20	1.00E-02	187			77			
3	4	19	43	74	81	1	1	4	0.20	1.00E-02	169			144			
3	4	19	43	74	81	1	1	5	0.20	1.00E-02	150			76			
3	4	19	43	74	83	1	2	1	0.20	1.00E-02	161	969	4.85E+05	66	403	2.02E+05	3.81E-01
3	4	19	43	74	83	1	2	2	0.20	1.00E-02	107			68			
3	4	19	43	74	83	1	2	3	0.20	1.00E-02	203			69			
3	4	19	43	74	83	1	2	4	0.20	1.00E-02	271			139			
3	4	19	43	74	83	1	2	5	0.20	1.00E-02	227			61			

Table I 1.3. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
3	13	19	43	77	102	1	1	1	0.20	1.00E-02	221	908	4.54E+05	67	478	2.39E+05	2.79E-01
3	13	19	43	77	102	1	1	2	0.20	1.00E-02	228			70			
3	13	19	43	77	102	1	1	3	0.20	1.00E-02	165			104			
3	13	19	43	77	102	1	1	4	0.20	1.00E-02	163			116			
3	13	19	43	77	102	1	1	5	0.20	1.00E-02	131			121			
3	13	19	43	77	102	1	2	1	0.20	1.00E-02	210	786	3.93E+05	147	540	2.70E+05	1.63E-01
3	13	19	43	77	102	1	2	2	0.20	1.00E-02	219			122			
3	13	19	43	77	102	1	2	3	0.20	1.00E-02	132			104			
3	13	19	43	77	102	1	2	4	0.20	1.00E-02	113			95			
3	13	19	43	77	102	1	2	5	0.20	1.00E-02	112			72			
3	1	19	41	79	79	1	1	1	0.20	1.00E-02	247	955	4.78E+05	69	500	2.50E+05	2.81E-01
3	1	19	41	79	79	1	1	2	0.20	1.00E-02	204			101			
3	1	19	41	79	79	1	1	3	0.20	1.00E-02	194			100			
3	1	19	41	79	79	1	1	4	0.20	1.00E-02	176			101			
3	1	19	41	79	79	1	1	5	0.20	1.00E-02	134			129			
3	1	19	41	79	77	1	2	1	0.20	1.00E-02	292	1103	5.52E+05	64	467	2.34E+05	3.73E-01
3	1	19	41	79	77	1	2	2	0.20	1.00E-02	225			111			
3	1	19	41	79	77	1	2	3	0.20	1.00E-02	232			99			
3	1	19	41	79	77	1	2	4	0.20	1.00E-02	156			105			
3	1	19	41	79	77	1	2	5	0.20	1.00E-02	198			88			

Table I 1.4. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep1, day 4. Rep = replicate.

		Extruder barrel head temperature, ^{o}C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
4	8	22	28	54	93	1	1	1	0.20	1.00E-02	108	653	3.27E+05	61	381	1.91E+05	2.34E-01
4	8	22	28	54	93	1	1	2	0.20	1.00E-02	157			94			
4	8	22	28	54	93	1	1	3	0.20	1.00E-02	110			74			
4	8	22	28	54	93	1	1	4	0.20	1.00E-02	132			92			
4	8	22	28	54	93	1	1	5	0.20	1.00E-02	146			60			
4	8	22	28	52	93	1	2	1	0.20	1.00E-02	52	542	2.71E+05	89	458	2.29E+05	7.31E-02
4	8	22	28	52	93	1	2	2	0.20	1.00E-02	125			103			
4	8	22	28	52	93	1	2	3	0.20	1.00E-02	125			99			
4	8	22	28	52	93	1	2	4	0.20	1.00E-02	136			101			
4	8	22	28	52	93	1	2	5	0.20	1.00E-02	104			66			
4	9	20	27	49	93	1	1	1	0.20	1.00E-02	189	724	3.62E+05	37	221	1.11E+05	5.15E-01
4	9	20	27	49	93	1	1	2	0.20	1.00E-02	133			30			
4	9	20	27	49	93	1	1	3	0.20	1.00E-02	158			55			
4	9	20	27	49	93	1	1	4	0.20	1.00E-02	105			50			
4	9	20	27	49	93	1	1	5	0.20	1.00E-02	139			49			
4	9	20	27	46	93	1	2	1	0.20	1.00E-02	127	715	3.58E+05	17	60	3.00E+04	1.08E+00
4	9	20	27	46	93	1	2	2	0.20	1.00E-02	153			24			
4	9	20	27	46	93	1	2	3	0.20	1.00E-02	135			3			
4	9	20	27	46	93	1	2	4	0.20	1.00E-02	140			9			
4	9	20	27	46	93	1	2	5	0.20	1.00E-02	160			7			

Table I 1.4. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
4	10	20	27	43	93	1	1	1	0.20	1.00E-02	117	719	3.60E+05	59	359	1.80E+05	3.02E-01
4	10	20	27	43	93	1	1	2	0.20	1.00E-02	140			48			
4	10	20	27	43	93	1	1	3	0.20	1.00E-02	136			102			
4	10	20	27	43	93	1	1	4	0.20	1.00E-02	187			100			
4	10	20	27	43	93	1	1	5	0.20	1.00E-02	139			50			
4	10	20	27	43	93	1	2	1	0.20	1.00E-02	141	824	4.12E+05	70	368	1.84E+05	3.50E-01
4	10	20	27	43	93	1	2	2	0.20	1.00E-02	143			61			
4	10	20	27	43	93	1	2	3	0.20	1.00E-02	150			44			
4	10	20	27	43	93	1	2	4	0.20	1.00E-02	190			105			
4	10	20	27	43	93	1	2	5	0.20	1.00E-02	200			88			
4	11	19	24	43	103	1	1	1	0.20	1.00E-02	93	768	3.84E+05	83	393	1.97E+05	2.91E-01
4	11	19	24	43	103	1	1	2	0.20	1.00E-02	121			64			
4	11	19	24	43	103	1	1	3	0.20	1.00E-02	147			63			
4	11	19	24	43	103	1	1	4	0.20	1.00E-02	192			125			
4	11	19	24	43	103	1	1	5	0.20	1.00E-02	215			58			
4	11	19	24	43	103	1	2	1	0.20	1.00E-02	107	816	4.08E+05	72	382	1.91E+05	3.30E-01
4	11	19	24	43	103	1	2	2	0.20	1.00E-02	123			71			
4	11	19	24	43	103	1	2	3	0.20	1.00E-02	170			89			
4	11	19	24	43	103	1	2	4	0.20	1.00E-02	194			105			
4	11	19	24	43	103	1	2	5	0.20	1.00E-02	222			45			

Table I 1.5. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep1, day 5. Rep = replicate.

		Extruder barrel head temperature, °C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
5	8	26	132	66	93	1	1	1	0.20	1.00E-02	167	805	4.03E+05	88	474	2.37E+05	2.30E-01
5	8	26	132	66	93	1	1	2	0.20	1.00E-02	174			84			
5	8	26	132	66	93	1	1	3	0.20	1.00E-02	125			98			
5	8	26	132	66	93	1	1	4	0.20	1.00E-02	124			108			
5	8	26	132	66	93	1	1	5	0.20	1.00E-02	215			96			
5	8	26	132	63	93	1	2	1	0.20	1.00E-02	165	801	4.01E+05	89	479	2.40E+05	2.23E-01
5	8	26	132	63	93	1	2	2	0.20	1.00E-02	177			86			
5	8	26	132	63	93	1	2	3	0.20	1.00E-02	123			100			
5	8	26	132	63	93	1	2	4	0.20	1.00E-02	126			110			
5	8	26	132	63	93	1	2	5	0.20	1.00E-02	210			94			
5	8	26	132	63	93	1	1	1	0.20	1.00E-02	119	908	4.54E+05	85	585	2.93E+05	1.91E-01
5	8	26	132	63	93	1	1	2	0.20	1.00E-02	148			89			
5	8	26	132	63	93	1	1	3	0.20	1.00E-02	231			132			
5	8	26	132	63	93	1	1	4	0.20	1.00E-02	240			136			
5	8	26	132	63	93	1	1	5	0.20	1.00E-02	170			143			
5	8	26	132	63	95	1	2	1	0.20	1.00E-02	109	886	4.43E+05	86	583	2.92E+05	1.82E-01
5	8	26	132	63	95	1	2	2	0.20	1.00E-02	138			92			
5	8	26	132	63	95	1	2	3	0.20	1.00E-02	240			130			
5	8	26	132	63	95	1	2	4	0.20	1.00E-02	230			135			
5	8	26	132	63	95	1	2	5	0.20	1.00E-02	169			140			

Table I 1.5. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
5	8	24	132	60	93	1	1	1	0.20	1.00E-02	112	1002	5.01E+05	75	519	2.60E+05	2.86E-01
5	8	24	132	60	93	1	1	2	0.20	1.00E-02	174			98			
5	8	24	132	60	93	1	1	3	0.20	1.00E-02	239			126			
5	8	24	132	60	93	1	1	4	0.20	1.00E-02	249			112			
5	8	24	132	60	93	1	1	5	0.20	1.00E-02	228			108			
5	8	24	132	60	92	1	2	1	0.20	1.00E-02	109	993	4.97E+05	76	524	2.62E+05	2.78E-01
5	8	24	132	60	92	1	2	2	0.20	1.00E-02	170			90			
5	8	24	132	60	92	1	2	3	0.20	1.00E-02	240			130			
5	8	24	132	60	92	1	2	4	0.20	1.00E-02	250			118			
5	8	24	132	60	92	1	2	5	0.20	1.00E-02	224			110			
5	8	24	132	60	95	1	1	1	0.20	1.00E-02	103	1150	5.75E+05	58	446	2.23E+05	4.11E-01
5	8	24	132	60	95	1	1	2	0.20	1.00E-02	200			91			
5	8	24	132	60	95	1	1	3	0.20	1.00E-02	251			90			
5	8	24	132	60	95	1	1	4	0.20	1.00E-02	236			99			
5	8	24	132	60	95	1	1	5	0.20	1.00E-02	200			108			
5	8	24	132	60	93	1	2	1	0.20	1.00E-02	160	787	3.94E+05	82	471	2.36E+05	2.23E-01
5	8	24	132	60	93	1	2	2	0.20	1.00E-02	165			92			
5	8	24	132	60	93	1	2	3	0.20	1.00E-02	109			114			
5	8	24	132	60	93	1	2	4	0.20	1.00E-02	263			85			
5	8	24	132	60	93	1	2	5	0.20	1.00E-02	250			98			

Table I 2.1. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 2, day 1. Rep = replicate.

		Extruder barrel head temperature, ^{o}C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
1	8	24	43	43	93	2	1	1	0.20	1.00E-02	76	613	3.07E+05	81	434	2.17E+05	1.50E-01
1	8	24	43	43	93	2	1	2	0.20	1.00E-02	87			57			
1	8	24	43	43	93	2	1	3	0.20	1.00E-02	142			87			
1	8	24	43	43	93	2	1	4	0.20	1.00E-02	132			99			
1	8	24	43	43	93	2	1	5	0.20	1.00E-02	176			110			
1	8	24	43	43	93	2	2	1	0.20	1.00E-02	66	539	2.70E+05	88	504	2.52E+05	2.92E-02
1	8	24	43	43	93	2	2	2	0.20	1.00E-02	100			105			
1	8	24	43	43	93	2	2	3	0.20	1.00E-02	106			86			
1	8	24	43	43	93	2	2	4	0.20	1.00E-02	130			130			
1	8	24	43	43	93	2	2	5	0.20	1.00E-02	137			95			
1	8	24	43	43	93	2	1	1	0.20	1.00E-02	79	713	3.57E+05	52	352	1.76E+05	3.07E-01
1	8	24	43	43	93	2	1	2	0.20	1.00E-02	132			71			
1	8	24	43	43	93	2	1	3	0.20	1.00E-02	142			59			
1	8	24	43	43	93	2	1	4	0.20	1.00E-02	166			83			
1	8	24	43	43	93	2	1	5	0.20	1.00E-02	194			87			
1	8	24	43	43	93	2	2	1	0.20	1.00E-02	76	661	3.31E+05	90	457	2.29E+05	1.60E-01
1	8	24	43	43	93	2	2	2	0.20	1.00E-02	95			95			
1	8	24	43	43	93	2	2	3	0.20	1.00E-02	93			87			
1	8	24	43	43	93	2	2	4	0.20	1.00E-02	170			94			
1	8	24	43	43	93	2	2	5	0.20	1.00E-02	227			91			

Table I 2.1. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
1	2	26	77	77	83	2	1	1	0.20	1.00E-02	77	707	3.54E+05	43	264	1.32E+05	4.28E-01
1	2	26	77	77	83	2	1	2	0.20	1.00E-02	98			66			
1	2	26	77	77	83	2	1	3	0.20	1.00E-02	155			55			
1	2	26	77	77	83	2	1	4	0.20	1.00E-02	186			49			
1	2	26	77	77	83	2	1	5	0.20	1.00E-02	191			51			
1	2	26	68	71	84	2	2	1	0.20	1.00E-02	110	803	4.02E+05	73	350	1.75E+05	3.61E-01
1	2	26	68	71	84	2	2	2	0.20	1.00E-02	116			67			
1	2	26	68	71	84	2	2	3	0.20	1.00E-02	178			65			
1	2	26	68	71	84	2	2	4	0.20	1.00E-02	178			77			
1	2	26	68	71	84	2	2	5	0.20	1.00E-02	221			68			
1	3	26	60	63	83	2	1	1	0.20	1.00E-02	80	720	3.60E+05	110	496	2.48E+05	1.62E-01
1	3	26	60	63	83	2	1	2	0.20	1.00E-02	104			83			
1	3	26	60	63	83	2	1	3	0.20	1.00E-02	138			110			
1	3	26	60	63	83	2	1	4	0.20	1.00E-02	197			105			
1	3	26	60	63	83	2	1	5	0.20	1.00E-02	201			88			
1	3	26	54	57	84	2	2	1	0.20	1.00E-02	89	852	4.26E+05	50	350	1.75E+05	3.86E-01
1	3	26	54	57	84	2	2	2	0.20	1.00E-02	96			73			
1	3	26	54	57	84	2	2	3	0.20	1.00E-02	191			85			
1	3	26	54	57	84	2	2	4	0.20	1.00E-02	256			70			
1	3	26	54	57	84	2	2	5	0.20	1.00E-02	220			72			

Table I 2.2. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 2, day 2. Rep = replicate.

		Extruder barrel head temperature, ^{o}C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
2	8	24	110	52	93	2	1	1	0.20	1.00E-02	56	492	2.46E+05	67	277	1.39E+05	2.49E-01
2	8	24	110	52	93	2	1	2	0.20	1.00E-02	83			56			
2	8	24	110	52	93	2	1	3	0.20	1.00E-02	80			62			
2	8	24	110	52	93	2	1	4	0.20	1.00E-02	118			48			
2	8	24	110	52	93	2	1	5	0.20	1.00E-02	155			44			
2	8	24	110	52	93	2	2	1	0.20	1.00E-02	55	478	2.39E+05	60	268	1.34E+05	2.51E-01
2	8	24	110	52	93	2	2	2	0.20	1.00E-02	81			66			
2	8	24	110	52	93	2	2	3	0.20	1.00E-02	76			46			
2	8	24	110	52	93	2	2	4	0.20	1.00E-02	116			43			
2	8	24	110	52	93	2	2	5	0.20	1.00E-02	150			53			
2	9	24	113	52	94	2	1	1	0.20	1.00E-02	64	454	2.27E+05	39	254	1.27E+05	2.52E-01
2	9	24	113	52	94	2	1	2	0.20	1.00E-02	62			45			
2	9	24	113	52	94	2	1	3	0.20	1.00E-02	128			67			
2	9	24	113	52	94	2	1	4	0.20	1.00E-02	99			62			
2	9	24	113	52	94	2	1	5	0.20	1.00E-02	101			41			
2	9	24	116	52	94	2	2	1	0.20	1.00E-02	63	432	2.16E+05	72	411	2.06E+05	2.16E-02
2	9	24	116	52	94	2	2	2	0.20	1.00E-02	68			85			
2	9	24	116	52	94	2	2	3	0.20	1.00E-02	114			99			
2	9	24	116	52	94	2	2	4	0.20	1.00E-02	85			80			
2	9	24	116	52	94	2	2	5	0.20	1.00E-02	102			75			

Table I 2.2. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
2	10	24	116	43	93	2	1	1	0.20	1.00E-02	99	569	2.85E+05	124	632	3.16E+05	-4.56E-02
2	10	24	116	43	93	2	1	2	0.20	1.00E-02	100			143			
2	10	24	116	43	93	2	1	3	0.20	1.00E-02	125			141			
2	10	24	116	43	93	2	1	4	0.20	1.00E-02	122			105			
2	10	24	116	43	93	2	1	5	0.20	1.00E-02	123			119			
2	10	24	116	43	93	2	2	1	0.20	1.00E-02	90	601	3.01E+05	100	568	2.84E+05	2.45E-02
2	10	24	116	43	93	2	2	2	0.20	1.00E-02	110			109			
2	10	24	116	43	93	2	2	3	0.20	1.00E-02	128			126			
2	10	24	116	43	93	2	2	4	0.20	1.00E-02	137			110			
2	10	24	116	43	93	2	2	5	0.20	1.00E-02	136			123			
2	15	24	107	57	110	2	1	1	0.20	1.00E-02	116	692	3.46E+05	96	532	2.66E+05	1.14E-01
2	15	24	107	57	110	2	1	2	0.20	1.00E-02	129			110			
2	15	24	107	57	110	2	1	3	0.20	1.00E-02	147			113			
2	15	24	107	57	110	2	1	4	0.20	1.00E-02	136			118			
2	15	24	107	57	110	2	1	5	0.20	1.00E-02	164			95			
2	15	24	88	66	109	2	2	1	0.20	1.00E-02	131	773	3.87E+05	64	306	1.53E+05	4.02E-01
2	15	24	88	66	109	2	2	2	0.20	1.00E-02	151			66			
2	15	24	88	66	109	2	2	3	0.20	1.00E-02	148			71			
2	15	24	88	66	109	2	2	4	0.20	1.00E-02	171			59			
2	15	24	88	66	109	2	2	5	0.20	1.00E-02	172			46			

Table I 2.3. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 2, day 3. Rep = replicate.

		Extruder barrel head temperature, ^{o}C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
3	8	33	96	79	94	2	1	1	0.20	1.00E-02	75	476	2.38E+05	51	280	1.40E+05	2.30E-01
3	8	33	96	79	94	2	1	2	0.20	1.00E-02	88			48			
3	8	33	96	79	94	2	1	3	0.20	1.00E-02	106			44			
3	8	33	96	79	94	2	1	4	0.20	1.00E-02	105			68			
3	8	33	96	79	94	2	1	5	0.20	1.00E-02	102			69			
3	8	33	91	79	94	2	2	1	0.20	1.00E-02	76	483	2.42E+05	36	209	1.05E+05	3.64E-01
3	8	33	91	79	94	2	2	2	0.20	1.00E-02	89			27			
3	8	33	91	79	94	2	2	3	0.20	1.00E-02	100			60			
3	8	33	91	79	94	2	2	4	0.20	1.00E-02	110			41			
3	8	33	91	79	94	2	2	5	0.20	1.00E-02	108			45			
3	8	31	79	77	94	2	1	1	0.20	1.00E-02	73	481	2.41E+05	46	293	1.47E+05	2.15E-01
3	8	31	79	77	94	2	1	2	0.20	1.00E-02	90			56			
3	8	31	79	77	94	2	1	3	0.20	1.00E-02	104			60			
3	8	31	79	77	94	2	1	4	0.20	1.00E-02	108			65			
3	8	31	79	77	94	2	1	5	0.20	1.00E-02	106			66			
3	8	31	74	77	93	2	2	1	0.20	1.00E-02	74	482	2.41E+05	39	255	1.28E+05	2.77E-01
3	8	31	74	77	93	2	2	2	0.20	1.00E-02	91			44			
3	8	31	74	77	93	2	2	3	0.20	1.00E-02	103			59			
3	8	31	74	77	93	2	2	4	0.20	1.00E-02	112			50			
3	8	31	74	77	93	2	2	5	0.20	1.00E-02	102			63			

Table I 2.3. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
3	13	26	49	71	103	2	1	1	0.20	1.00E-02	75	421	2.11E+05	56	313	1.57E+05	1.29E-01
3	13	26	49	71	103	2	1	2	0.20	1.00E-02	74			52			
3	13	26	49	71	103	2	1	3	0.20	1.00E-02	75			72			
3	13	26	49	71	103	2	1	4	0.20	1.00E-02	100			63			
3	13	26	49	71	103	2	1	5	0.20	1.00E-02	97			70			
3	13	26	49	71	103	2	2	1	0.20	1.00E-02	71	418	2.09E+05	47	289	1.45E+05	1.60E-01
3	13	26	49	71	103	2	2	2	0.20	1.00E-02	73			60			
3	13	26	49	71	103	2	2	3	0.20	1.00E-02	71			61			
3	13	26	49	71	103	2	2	4	0.20	1.00E-02	103			56			
3	13	26	49	71	103	2	2	5	0.20	1.00E-02	100			65			
3	14	26	46	74	102	2	1	1	0.20	1.00E-02	70	421	2.11E+05	28	225	1.13E+05	2.72E-01
3	14	26	46	74	102	2	1	2	0.20	1.00E-02	78			44			
3	14	26	46	74	102	2	1	3	0.20	1.00E-02	73			50			
3	14	26	46	74	102	2	1	4	0.20	1.00E-02	101			50			
3	14	26	46	74	102	2	1	5	0.20	1.00E-02	99			53			
3	14	26	46	74	103	2	2	1	0.20	1.00E-02	70	421	2.11E+05	35	221	1.11E+05	2.80E-01
3	14	26	46	74	103	2	2	2	0.20	1.00E-02	76			54			
3	14	26	46	74	103	2	2	3	0.20	1.00E-02	70			53			
3	14	26	46	74	103	2	2	4	0.20	1.00E-02	102			36			
3	14	26	46	74	103	2	2	5	0.20	1.00E-02	103			43			

Table I 2.4. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 2, day 4. Rep = replicate.

		Extruder barrel head temperature, ^{o}C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
4	4	21	82	54	83	2	1	1	0.20	1.00E-02	132	599	3.00E+05	76	292	1.46E+05	3.12E-01
4	4	21	82	54	83	2	1	2	0.20	1.00E-02	149			66			
4	4	21	82	54	83	2	1	3	0.20	1.00E-02	129			65			
4	4	21	82	54	83	2	1	4	0.20	1.00E-02	94			58			
4	4	21	82	54	83	2	1	5	0.20	1.00E-02	95			27			
4	4	21	82	54	83	2	2	1	0.20	1.00E-02	130	595	2.98E+05	57	281	1.41E+05	3.26E-01
4	4	21	82	54	83	2	2	2	0.20	1.00E-02	150			49			
4	4	21	82	54	83	2	2	3	0.20	1.00E-02	128			63			
4	4	21	82	54	83	2	2	4	0.20	1.00E-02	93			57			
4	4	21	82	54	83	2	2	5	0.20	1.00E-02	94			55			
4	5	21	82	54	84	2	1	1	0.20	1.00E-02	130	587	2.94E+05	63	306	1.53E+05	2.83E-01
4	5	21	82	54	84	2	1	2	0.20	1.00E-02	150			47			
4	5	21	82	54	84	2	1	3	0.20	1.00E-02	123			67			
4	5	21	82	54	84	2	1	4	0.20	1.00E-02	93			65			
4	5	21	82	54	84	2	1	5	0.20	1.00E-02	91			64			
4	5	21	82	54	84	2	2	1	0.20	1.00E-02	129	600	3.00E+05	53	322	1.61E+05	2.70E-01
4	5	21	82	54	84	2	2	2	0.20	1.00E-02	149			66			
4	5	21	82	54	84	2	2	3	0.20	1.00E-02	131			75			
4	5	21	82	54	84	2	2	4	0.20	1.00E-02	96			61			
4	5	21	82	54	84	2	2	5	0.20	1.00E-02	95			67			

Table I 2.4. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
4	11	21	85	54	104	2	1	1	0.20	1.00E-02	173	679	3.40E+05	58	315	1.58E+05	3.34E-01
4	11	21	85	54	104	2	1	2	0.20	1.00E-02	142			77			
4	11	21	85	54	104	2	1	3	0.20	1.00E-02	135			67			
4	11	21	85	54	104	2	1	4	0.20	1.00E-02	91			63			
4	11	21	85	54	104	2	1	5	0.20	1.00E-02	138			50			
4	11	21	85	54	104	2	2	1	0.20	1.00E-02	170	680	3.40E+05	86	396	1.98E+05	2.35E-01
4	11	21	85	54	104	2	2	2	0.20	1.00E-02	145			92			
4	11	21	85	54	104	2	2	3	0.20	1.00E-02	140			79			
4	11	21	85	54	104	2	2	4	0.20	1.00E-02	90			73			
4	11	21	85	54	104	2	2	5	0.20	1.00E-02	135			66			
4	12	21	85	57	103	2	1	1	0.20	1.00E-02	170	683	3.42E+05	56	210	1.05E+05	5.12E-01
4	12	21	85	57	103	2	1	2	0.20	1.00E-02	140			43			
4	12	21	85	57	103	2	1	3	0.20	1.00E-02	140			41			
4	12	21	85	57	103	2	1	4	0.20	1.00E-02	93			40			
4	12	21	85	57	103	2	1	5	0.20	1.00E-02	140			30			
4	12	21	85	57	103	2	2	1	0.20	1.00E-02	168	677	3.39E+05	46	190	9.50E+04	5.52E-01
4	12	21	85	57	103	2	2	2	0.20	1.00E-02	146			47			
4	12	21	85	57	103	2	2	3	0.20	1.00E-02	140			33			
4	12	21	85	57	103	2	2	4	0.20	1.00E-02	92			22			
4	12	21	85	57	103	2	2	5	0.20	1.00E-02	131			42			

Table I 2.5. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 2, day 5. Rep = replicate.

		Extruder barrel head temperature, ^{o}C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
5	1	23	52	66	77	2	1	1	0.20	1.00E-02	70	585	2.93E+05	64	304	1.52E+05	2.84E-01
5	1	23	52	66	77	2	1	2	0.20	1.00E-02	70			61			
5	1	23	52	66	77	2	1	3	0.20	1.00E-02	123			58			
5	1	23	52	66	77	2	1	4	0.20	1.00E-02	155			45			
5	1	23	52	66	77	2	1	5	0.20	1.00E-02	167			76			
5	1	23	52	66	77	2	2	1	0.20	1.00E-02	71	596	2.98E+05	98	499	2.50E+05	7.71E-02
5	1	23	52	66	77	2	2	2	0.20	1.00E-02	73			109			
5	1	23	52	66	77	2	2	3	0.20	1.00E-02	128			114			
5	1	23	52	66	77	2	2	4	0.20	1.00E-02	170			100			
5	1	23	52	66	77	2	2	5	0.20	1.00E-02	154			78			
5	6	21	38	49	93	2	1	1	0.20	1.00E-02	121	707	3.54E+05	60	297	1.49E+05	3.77E-01
5	6	21	38	49	93	2	1	2	0.20	1.00E-02	137			51			
5	6	21	38	49	93	2	1	3	0.20	1.00E-02	150			42			
5	6	21	38	49	93	2	1	4	0.20	1.00E-02	158			54			
5	6	21	38	49	93	2	1	5	0.20	1.00E-02	141			90			
5	6	21	38	49	93	2	2	1	0.20	1.00E-02	140	712	3.56E+05	48	319	1.60E+05	3.49E-01
5	6	21	38	49	93	2	2	2	0.20	1.00E-02	123			46			
5	6	21	38	49	93	2	2	3	0.20	1.00E-02	161			79			
5	6	21	38	49	93	2	2	4	0.20	1.00E-02	148			76			
5	6	21	38	49	93	2	2	5	0.20	1.00E-02	140			70			

Table I 2.5. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
5	7	21	43	49	93	2	1	1	0.20	1.00E-02	70	583	2.92E+05	71	439	2.20E+05	1.23E-01
5	7	21	43	49	93	2	1	2	0.20	1.00E-02	69			80			
5	7	21	43	49	93	2	1	3	0.20	1.00E-02	120			99			
5	7	21	43	49	93	2	1	4	0.20	1.00E-02	156			83			
5	7	21	43	49	93	2	1	5	0.20	1.00E-02	168			106			
5	7	21	43	49	93	2	2	1	0.20	1.00E-02	68	591	2.96E+05	66	321	1.61E+05	2.65E-01
5	7	21	43	49	93	2	2	2	0.20	1.00E-02	71			70			
5	7	21	43	49	93	2	2	3	0.20	1.00E-02	124			62			
5	7	21	43	49	93	2	2	4	0.20	1.00E-02	158			61			
5	7	21	43	49	93	2	2	5	0.20	1.00E-02	170			62			
5	8	21	43	52	93	2	1	1	0.20	1.00E-02	69	584	2.92E+05	73	426	2.13E+05	1.37E-01
5	8	21	43	52	93	2	1	2	0.20	1.00E-02	70			90			
5	8	21	43	52	93	2	1	3	0.20	1.00E-02	122			78			
5	8	21	43	52	93	2	1	4	0.20	1.00E-02	154			76			
5	8	21	43	52	93	2	1	5	0.20	1.00E-02	169			109			
5	8	21	43	52	94	2	2	1	0.20	1.00E-02	71	590	2.95E+05	75	411	2.06E+05	1.57E-01
5	8	21	43	52	94	2	2	2	0.20	1.00E-02	70			82			
5	8	21	43	52	94	2	2	3	0.20	1.00E-02	121			85			
5	8	21	43	52	94	2	2	4	0.20	1.00E-02	160			81			
5	8	21	43	52	94	2	2	5	0.20	1.00E-02	168			88			

Table I 3.1. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 3, day 1. Rep = replicate.

		Extruder barrel head temperature, °C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
1	9	39	102	79	94	3	1	1	0.20	1.00E-02	118	632	3.16E+05	33	189	9.45E+04	5.24E-01
1	9	39	102	79	94	3	1	2	0.20	1.00E-02	113			40			
1	9	39	102	79	94	3	1	3	0.20	1.00E-02	118			38			
1	9	39	102	79	94	3	1	4	0.20	1.00E-02	138			38			
1	9	39	102	79	94	3	1	5	0.20	1.00E-02	145			40			
1	9	39	93	74	93	3	2	1	0.20	1.00E-02	108	601	3.01E+05	60	256	1.28E+05	3.71E-01
1	9	39	93	74	93	3	2	2	0.20	1.00E-02	100			42			
1	9	39	93	74	93	3	2	3	0.20	1.00E-02	110			55			
1	9	39	93	74	93	3	2	4	0.20	1.00E-02	140			41			
1	9	39	93	74	93	3	2	5	0.20	1.00E-02	143			58			
1	10	33	71	63	92	3	1	1	0.20	1.00E-02	57	324	1.62E+05	108	406	2.03E+05	-9.80E-02
1	10	33	71	63	92	3	1	2	0.20	1.00E-02	53			82			
1	10	33	71	63	92	3	1	3	0.20	1.00E-02	61			72			
1	10	33	71	63	92	3	1	4	0.20	1.00E-02	79			58			
1	10	33	71	63	92	3	1	5	0.20	1.00E-02	74			86			
1	10	32	68	60	94	3	2	1	0.20	1.00E-02	60	330	1.65E+05	92	396	1.98E+05	-7.92E-02
1	10	32	68	60	94	3	2	2	0.20	1.00E-02	55			80			
1	10	32	68	60	94	3	2	3	0.20	1.00E-02	64			80			
1	10	32	68	60	94	3	2	4	0.20	1.00E-02	81			57			
1	10	32	68	60	94	3	2	5	0.20	1.00E-02	70			87			

Table I 3.1. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
1	13	27	41	43	103	3	1	1	0.20	1.00E-02	71	453	2.27E+05	75	379	1.90E+05	7.75E-02
1	13	27	41	43	103	3	1	2	0.20	1.00E-02	65			84			
1	13	27	41	43	103	3	1	3	0.20	1.00E-02	83			68			
1	13	27	41	43	103	3	1	4	0.20	1.00E-02	103			82			
1	13	27	41	43	103	3	1	5	0.20	1.00E-02	131			70			
1	13	27	41	43	103	3	2	1	0.20	1.00E-02	73	470	2.35E+05	90	316	1.58E+05	1.72E-01
1	13	27	41	43	103	3	2	2	0.20	1.00E-02	68			68			
1	13	27	41	43	103	3	2	3	0.20	1.00E-02	81			69			
1	13	27	41	43	103	3	2	4	0.20	1.00E-02	108			41			
1	13	27	41	43	103	3	2	5	0.20	1.00E-02	140			48			
1	14	27	63	46	103	3	1	1	0.20	1.00E-02	73	463	2.32E+05	65	451	2.26E+05	1.14E-02
1	14	27	63	46	103	3	1	2	0.20	1.00E-02	68			93			
1	14	27	63	46	103	3	1	3	0.20	1.00E-02	100			84			
1	14	27	63	46	103	3	1	4	0.20	1.00E-02	136			91			
1	14	27	63	46	103	3	1	5	0.20	1.00E-02	86			118			
1	14	27	63	46	103	3	2	1	0.20	1.00E-02	75	473	2.37E+05	65	470	2.35E+05	2.76E-03
1	14	27	63	46	103	3	2	2	0.20	1.00E-02	70			108			
1	14	27	63	46	103	3	2	3	0.20	1.00E-02	138			96			
1	14	27	63	46	103	3	2	4	0.20	1.00E-02	102			101			
1	14	27	63	46	103	3	2	5	0.20	1.00E-02	88			100			

Table I 3.2. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 3, day 2. Rep = replicate.

		Extruder barrel head temperature, ^{o}C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
2	8	23	93	74	93	3	1	1	0.20	1.00E-02	68	414	2.07E+05	65	307	1.54E+05	1.30E-01
2	8	23	93	74	93	3	1	2	0.20	1.00E-02	88			53			
2	8	23	93	74	93	3	1	3	0.20	1.00E-02	96			69			
2	8	23	93	74	93	3	1	4	0.20	1.00E-02	81			66			
2	8	23	93	74	93	3	1	5	0.20	1.00E-02	81			54			
2	8	23	116	77	93	3	2	1	0.20	1.00E-02	70	414	2.07E+05	66	320	1.60E+05	1.12E-01
2	8	23	116	77	93	3	2	2	0.20	1.00E-02	86			58			
2	8	23	116	77	93	3	2	3	0.20	1.00E-02	90			70			
2	8	23	116	77	93	3	2	4	0.20	1.00E-02	88			68			
2	8	23	116	77	93	3	2	5	0.20	1.00E-02	80			58			
2	8	22	116	71	95	3	1	1	0.20	1.00E-02	80	420	2.10E+05	85	317	1.59E+05	1.22E-01
2	8	22	116	71	95	3	1	2	0.20	1.00E-02	88			47			
2	8	22	116	71	95	3	1	3	0.20	1.00E-02	86			71			
2	8	22	116	71	95	3	1	4	0.20	1.00E-02	93			52			
2	8	22	116	71	95	3	1	5	0.20	1.00E-02	73			62			
2	8	22	116	71	92	3	2	1	0.20	1.00E-02	81	427	2.14E+05	81	319	1.60E+05	1.27E-01
2	8	22	116	71	92	3	2	2	0.20	1.00E-02	89			50			
2	8	22	116	71	92	3	2	3	0.20	1.00E-02	90			70			
2	8	22	116	71	92	3	2	4	0.20	1.00E-02	92			53			
2	8	22	116	71	92	3	2	5	0.20	1.00E-02	75			65			

Table I 3.2. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
2	8	22	107	66	92	3	1	1	0.20	1.00E-02	81	410	2.05E+05	58	323	1.62E+05	1.04E-01
2	8	22	107	66	92	3	1	2	0.20	1.00E-02	84			70			
2	8	22	107	66	92	3	1	3	0.20	1.00E-02	90			63			
2	8	22	107	66	92	3	1	4	0.20	1.00E-02	69			63			
2	8	22	107	66	92	3	1	5	0.20	1.00E-02	86			69			
2	8	22	99	66	93	3	2	1	0.20	1.00E-02	79	419	2.10E+05	59	324	1.62E+05	1.12E-01
2	8	22	99	66	93	3	2	2	0.20	1.00E-02	90			69			
2	8	22	99	66	93	3	2	3	0.20	1.00E-02	88			65			
2	8	22	99	66	93	3	2	4	0.20	1.00E-02	90			68			
2	8	22	99	66	93	3	2	5	0.20	1.00E-02	72			63			
2	8	22	96	63	93	3	1	1	0.20	1.00E-02	81	406	2.03E+05	72	327	1.64E+05	9.40E-02
2	8	22	96	63	93	3	1	2	0.20	1.00E-02	83			65			
2	8	22	96	63	93	3	1	3	0.20	1.00E-02	89			55			
2	8	22	96	63	93	3	1	4	0.20	1.00E-02	70			68			
2	8	22	96	63	93	3	1	5	0.20	1.00E-02	83			67			
2	8	22	85	60	93	3	2	1	0.20	1.00E-02	80	423	2.12E+05	66	329	1.65E+05	1.09E-01
2	8	22	85	60	93	3	2	2	0.20	1.00E-02	89			67			
2	8	22	85	60	93	3	2	3	0.20	1.00E-02	86			58			
2	8	22	85	60	93	3	2	4	0.20	1.00E-02	93			70			
2	8	22	85	60	93	3	2	5	0.20	1.00E-02	75			68			

Table I 3.3 Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 3, day 3. Rep = replicate.

		Extruder barrel head temperature, ^{o}C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
3	8	28	99	49	77	3	1	1	0.20	1.00E-02	68	587	2.94E+05	70	428	2.14E+05	1.37E-01
3	8	28	99	49	77	3	1	2	0.20	1.00E-02	69			93			
3	8	28	99	49	77	3	1	3	0.20	1.00E-02	128			80			
3	8	28	99	49	77	3	1	4	0.20	1.00E-02	150			110			
3	8	28	99	49	77	3	1	5	0.20	1.00E-02	172			75			
3	8	27	102	49	77	3	2	1	0.20	1.00E-02	68	501	2.51E+05	70	406	2.03E+05	9.13E-02
3	8	27	102	49	77	3	2	2	0.20	1.00E-02	73			84			
3	8	27	102	49	77	3	2	3	0.20	1.00E-02	75			87			
3	8	27	102	49	77	3	2	4	0.20	1.00E-02	120			80			
3	8	27	102	49	77	3	2	5	0.20	1.00E-02	165			85			
3	8	24	110	46	93	3	1	1	0.20	1.00E-02	60	524	2.62E+05	69	418	2.09E+05	9.82E-02
3	8	24	110	46	93	3	1	2	0.20	1.00E-02	75			68			
3	8	24	110	46	93	3	1	3	0.20	1.00E-02	70			78			
3	8	24	110	46	93	3	1	4	0.20	1.00E-02	151			95			
3	8	24	110	46	93	3	1	5	0.20	1.00E-02	168			108			
3	8	24	113	46	93	3	2	1	0.20	1.00E-02	70	510	2.55E+05	86	411	2.06E+05	9.37E-02
3	8	24	113	46	93	3	2	2	0.20	1.00E-02	130			89			
3	8	24	113	46	93	3	2	3	0.20	1.00E-02	165			80			
3	8	24	113	46	93	3	2	4	0.20	1.00E-02	75			86			

Table I 3.3. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
3	8	24	113	46	93	3	2	5	0.20	1.00E-02	70			70			
3	15	24	116	46	93	3	1	1	0.20	1.00E-02	120	686	3.43E+05	98	539	2.70E+05	1.05E-01
3	15	24	116	46	93	3	1	2	0.20	1.00E-02	130			113			
3	15	24	116	46	93	3	1	3	0.20	1.00E-02	145			120			
3	15	24	116	46	93	3	1	4	0.20	1.00E-02	131			110			
3	15	24	116	46	93	3	1	5	0.20	1.00E-02	160			98			
3	15	24	113	46	93	3	2	1	0.20	1.00E-02	128	778	3.89E+05	60	308	1.54E+05	4.02E-01
3	15	24	113	46	93	3	2	2	0.20	1.00E-02	150			68			
3	15	24	113	46	93	3	2	3	0.20	1.00E-02	150			70			
3	15	24	113	46	93	3	2	4	0.20	1.00E-02	170			60			
3	15	24	113	46	93	3	2	5	0.20	1.00E-02	180			50			
3	1	24	82	52	105	3	1	1	0.20	1.00E-02	68	582	2.91E+05	60	310	1.55E+05	2.74E-01
3	1	24	82	52	105	3	1	2	0.20	1.00E-02	69			63			
3	1	24	82	52	105	3	1	3	0.20	1.00E-02	125			61			
3	1	24	82	52	105	3	1	4	0.20	1.00E-02	150			78			
3	1	24	82	52	105	3	1	5	0.20	1.00E-02	170			48			
3	1	24	82	52	105	3	2	1	0.20	1.00E-02	70	595	2.98E+05	100	507	2.54E+05	6.95E-02
3	1	24	82	52	105	3	2	2	0.20	1.00E-02	75			110			
3	1	24	82	52	105	3	2	3	0.20	1.00E-02	130			115			
3	1	24	82	52	105	3	2	4	0.20	1.00E-02	170			79			
3	1	24	82	52	105	3	2	5	0.20	1.00E-02	150			103			

Table I 3.4. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 3, day 4. Rep = replicate.

		Extruder barrel head temperature, °C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
4	11	24	107	52	103	3	1	1	0.20	1.00E-02	81	583	2.92E+05	54	361	1.81E+05	2.08E-01
4	11	24	107	52	103	3	1	2	0.20	1.00E-02	126			68			
4	11	24	107	52	103	3	1	3	0.20	1.00E-02	144			90			
4	11	24	107	52	103	3	1	4	0.20	1.00E-02	124			85			
4	11	24	107	52	103	3	1	5	0.20	1.00E-02	108			64			
4	11	24	107	52	102	3	2	1	0.20	1.00E-02	100	588	2.94E+05	68	313	1.57E+05	2.74E-01
4	11	24	107	52	102	3	2	2	0.20	1.00E-02	90			58			
4	11	24	107	52	102	3	2	3	0.20	1.00E-02	140			71			
4	11	24	107	52	102	3	2	4	0.20	1.00E-02	130			74			
4	11	24	107	52	102	3	2	5	0.20	1.00E-02	128			42			
4	12	26	102	49	103	3	1	1	0.20	1.00E-02	83	591	2.96E+05	35	310	1.55E+05	2.80E-01
4	12	26	102	49	103	3	1	2	0.20	1.00E-02	130			48			
4	12	26	102	49	103	3	1	3	0.20	1.00E-02	140			62			
4	12	26	102	49	103	3	1	4	0.20	1.00E-02	128			76			
4	12	26	102	49	103	3	1	5	0.20	1.00E-02	110			89			
4	12	26	102	49	102	3	2	1	0.20	1.00E-02	99	584	2.92E+05	30	226	1.13E+05	4.12E-01
4	12	26	102	49	102	3	2	2	0.20	1.00E-02	89			48			
4	12	26	102	49	102	3	2	3	0.20	1.00E-02	128			49			
4	12	26	102	49	102	3	2	4	0.20	1.00E-02	138			44			
4	12	26	102	49	102	3	2	5	0.20	1.00E-02	130			55			

Table I 3.4. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
4	4	27	27	49	84	3	1	1	0.20	1.00E-02	61	483	2.42E+05	78	463	2.32E+05	1.84E-02
4	4	27	27	49	84	3	1	2	0.20	1.00E-02	65			103			
4	4	27	27	49	84	3	1	3	0.20	1.00E-02	118			99			
4	4	27	27	49	84	3	1	4	0.20	1.00E-02	129			91			
4	4	27	27	49	84	3	1	5	0.20	1.00E-02	110			92			
4	4	27	27	49	83	3	2	1	0.20	1.00E-02	64	498	2.49E+05	79	464	2.32E+05	3.07E-02
4	4	27	27	49	83	3	2	2	0.20	1.00E-02	68			105			
4	4	27	27	49	83	3	2	3	0.20	1.00E-02	120			87			
4	4	27	27	49	83	3	2	4	0.20	1.00E-02	131			95			
4	4	27	27	49	83	3	2	5	0.20	1.00E-02	115			98			
4	5	27	27	46	84	3	1	1	0.20	1.00E-02	63	488	2.44E+05	78	491	2.46E+05	-2.66E-03
4	5	27	27	46	84	3	1	2	0.20	1.00E-02	66			87			
4	5	27	27	46	84	3	1	3	0.20	1.00E-02	120			102			
4	5	27	27	46	84	3	1	4	0.20	1.00E-02	131			100			
4	5	27	27	46	84	3	1	5	0.20	1.00E-02	108			124			
4	5	27	27	49	83	3	2	1	0.20	1.00E-02	69	503	2.52E+05	83	414	2.07E+05	8.46E-02
4	5	27	27	49	83	3	2	2	0.20	1.00E-02	68			96			
4	5	27	27	49	83	3	2	3	0.20	1.00E-02	123			77			
4	5	27	27	49	83	3	2	4	0.20	1.00E-02	140			69			
4	5	27	27	49	83	3	2	5	0.20	1.00E-02	103			89			

Table I 3.5. Temperature of extruder barrel zones and estimates of acid producing, thermophilic organisms and *Bacillus stearothermophilus* spore count in feed before and after extrusion, respectively; Rep 3, day 5. Rep = replicate.

		Extruder barrel head temperature, ^{o}C									Before extrusion			After extrusion			
Day	Trt	Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit	Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	Log (N_o/N)
5	7	23	129	52	94	3	1	1	0.20	1.00E-02	69	580	2.90E+05	70	441	2.21E+05	1.19E-01
5	7	23	129	52	94	3	1	2	0.20	1.00E-02	70			81			
5	7	23	129	52	94	3	1	3	0.20	1.00E-02	121			100			
5	7	23	129	52	94	3	1	4	0.20	1.00E-02	150			85			
5	7	23	129	52	94	3	1	5	0.20	1.00E-02	170			105			
5	7	23	124	49	93	3	2	1	0.20	1.00E-02	68	601	3.01E+05	70	329	1.65E+05	2.62E-01
5	7	23	124	49	93	3	2	2	0.20	1.00E-02	73			71			
5	7	23	124	49	93	3	2	3	0.20	1.00E-02	130			60			
5	7	23	124	49	93	3	2	4	0.20	1.00E-02	160			63			
5	7	23	124	49	93	3	2	5	0.20	1.00E-02	170			65			
5	6	24	129	49	93	3	1	1	0.20	1.00E-02	120	705	3.53E+05	61	309	1.55E+05	3.58E-01
5	6	24	129	49	93	3	1	2	0.20	1.00E-02	140			50			
5	6	24	129	49	93	3	1	3	0.20	1.00E-02	150			45			
5	6	24	129	49	93	3	1	4	0.20	1.00E-02	155			55			
5	6	24	129	49	93	3	1	5	0.20	1.00E-02	140			98			
5	6	24	129	49	93	3	2	1	0.20	1.00E-02	138	714	3.57E+05	50	327	1.64E+05	3.39E-01
5	6	24	129	49	93	3	2	2	0.20	1.00E-02	150			40			
5	6	24	129	49	93	3	2	3	0.20	1.00E-02	158			80			
5	6	24	129	49	93	3	2	4	0.20	1.00E-02	130			81			
5	6	24	129	49	93	3	2	5	0.20	1.00E-02	138			76			

Table I 3.5. (‘cont.).

Day	Trt	Extruder barrel head temperature, °C				Rep	Sample	Plate	Initial dilution (20 g: 100 g)	Decimal dilution of initial dilution poured	Before extrusion			After extrusion			Log (N_o/N)
		Extruder barrel feeding zone	Extruder barrel head number 1	Extruder barrel head number 4	Extruder barrel exit						Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of original feed suspension (N_o)	Spore count, per plate	Total number of spores on 5 plates	Spore count, per 20 mL of initial feed suspension (N)	
5	2	24	127	43	83	3	1	1	0.20	1.00E-02	78	703	3.52E+05	45	276	1.38E+05	4.06E-01
5	2	24	127	43	83	3	1	2	0.20	1.00E-02	100			68			
5	2	24	127	43	83	3	1	3	0.20	1.00E-02	150			60			
5	2	24	127	43	83	3	1	4	0.20	1.00E-02	180			50			
5	2	24	127	43	83	3	1	5	0.20	1.00E-02	195			53			
5	2	24	127	43	83	3	2	1	0.20	1.00E-02	108	753	3.77E+05	75	338	1.69E+05	3.48E-01
5	2	24	127	43	83	3	2	2	0.20	1.00E-02	218			60			
5	2	24	127	43	83	3	2	3	0.20	1.00E-02	239			60			
5	2	24	127	43	83	3	2	4	0.20	1.00E-02	98			73			
5	2	24	127	43	83	3	2	5	0.20	1.00E-02	90			70			
5	3	24	127	43	84	3	1	1	0.20	1.00E-02	81	724	3.62E+05	109	509	2.55E+05	1.53E-01
5	3	24	127	43	84	3	1	2	0.20	1.00E-02	100			86			
5	3	24	127	43	84	3	1	3	0.20	1.00E-02	140			113			
5	3	24	127	43	84	3	1	4	0.20	1.00E-02	200			92			
5	3	24	127	43	84	3	1	5	0.20	1.00E-02	203			109			
5	3	24	129	46	83	3	2	1	0.20	1.00E-02	93	695	3.48E+05	48	351	1.76E+05	2.97E-01
5	3	24	129	46	83	3	2	2	0.20	1.00E-02	102			75			
5	3	24	129	46	83	3	2	3	0.20	1.00E-02	195			86			
5	3	24	129	46	83	3	2	4	0.20	1.00E-02	215			69			
5	3	24	129	46	83	3	2	5	0.20	1.00E-02	90			73			

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