PHYSICO-CHEMICAL AND SHELF-LIFE BETWEEN BAKED AND EXTRUDED PET FOODS

by

MICHAEL GIBSON

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Approved by:

Major Professor
Sajid Alavi

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Abstract

The U.S. pet food market was approximately worth $22 billion in 2013. Further growth is predicted at a pace faster than most major human food product categories. More than 60% of pet food products are processed using extrusion, and a significant proportion is produced using baking. However, research is lacking on fundamental process and product differences between extrusion and baking. The current study focuses on this aspect and also in-depth characterization of process and product quality. Three iso-nutritional diets were formulated for dry expanded dog food using 0%, 7% and 15% fresh meat inclusion. Major variations between diets were inclusion rates of mechanically deboned chicken, cereal grains, and poultry fat. Each diet was processed with a single screw extruder using various thermal and/or mechanical energy inputs (obtained by varying pre-conditioner stem injection and/or extruder screw speeds). Diets were also processed by baking using a 30 foot experimental oven at 425°F, although the fresh meat inclusion was at 0%, 10% and 20% levels. Proximate analysis of products was conducted. Products were also characterized for physico-chemical properties such as bulk density, piece density, expansion ratio, degree of gelatinization and textural attributes. As fresh meat inclusion increased (0–15%), expansion ratio (4.1–3.5) decreased irrespective of extrusion treatment. Expansion was not evident in the baked kibbles, and bulk and piece densities were up to 56% higher for baked versus extruded kibbles. Textural analysis of extruded kibbles revealed serrated force-deformation response, typical of cellular products, with peak hardness of 2.9–1.5 kgf. On the other hand, baked products had a ‘smooth’ force-deformation response with higher peak hardness than extruded products (up to 3 kgf). Microbial counts for baked products were higher than extruded products, and rancidity profiles as obtained from gas chromatography also had marked differences. The extrusion process was characterized by detailed mass and energy

balance analyses, and compared with baking that lacks mechanical energy input. Results from this study provide a useful bench-mark for dry expanded pet food product quality and commonly used processing technologies.

Table of Contents

List of Figures

List of Tables

List of Equations

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Chapter 1 - Baked and Extruded Pet Food: Mini Literature Review

1.1. Introduction

According to the American Pet Products Association, Inc., as of 2013 there were approximately 396 million pets in the United States; 178.9 million cats and dogs. The U.S. spent $55.72 billion in 2013 on pets last year which includes $21.57 billion spent on pet food. There has been continuous growth in the pet food sector over the past 20 years and it is estimated pet food sales will reach $22.62 in 2014. The most common type of dry pet food production is via extrusion, which accounts for 80% of total. The other major types of dry pet food production are baking and pelleting.

In human foods, baked and extruded products are generally a complimentary addition or a supplementation to a daily diet, e.g. breads, cookies, and corn puffs. Recent research regarding baking and extrusion has focused on the fortification of additional, healthier ingredients to specific products. Pet foods are unique compared to other products which are processed using baking and extrusion in that they are a complete food matrix. Complete pet foods include the pet's total nutritional requirements (protein, lipids, carbohydrates, etc.) for each meal or for an entire day. The primary goals of pet foods are to provide a nutritionally adequate diet for the consumer's pet, to promote longevity, and prevent pet disease. For cats and dogs, guidelines for minimal and maximum nutritional requirements are posted by the National Research Council and labeling regulations are set forth by the Association of American Feed Control Officials.

Although the pet food industry produces large volumes of pet food and revenue, there has been little published research regarding the interactions of multiple pet food ingredients during processing. There is also a severe lack of published research categorizing the type of energy used during processing which could affect the final product characteristics (such as kibble density, starch gelatinization, and other physic-chemical attributes.)

1.2. Processing

Baking of a pet food is a batch type processing technique. All ingredients (cereal flours, meat/meat by-products, vitamins, etc.) are mixed together. After mixing, the pet food mash is brought up to around 35% moisture on a wet basis with the addition of water and the material is mixed/kneaded into a short dough or undeveloped dough. In commercial operations, the dough is then sheeted and/ or cut and formed into desired shapes. In industry settings these set of operations are done using a rotary molder. During rotary molding, the pet food dough is pressed into a die having the desired shape and depth. The shaped kibbles pass through a tunnel oven with temperature settings ranging from 175-230˚C for 10-20 minutes or until the kibbles have reached a shelf stable moisture. The kibbles are then allowed to cool to room temperature and packaged. The baking process uses only thermal energy to cook the kibble, kill harmful bacteria and drive off excess moisture to make the final product shelf stable. Each pet food may use slightly different techniques and/or parameters to achieve a desired final product.

Dry pet food extrusion is a continuous processing technique where all cooking and kibble forming takes place in the extruder. Traditionally, all dry ingredients are mixed together, with the exception of water and fresh meat or lipids if the formulation calls for such ingredients. The dry mix is then added into the live bin of the extruder and is conveyed into the preconditioner. In the preconditioner water and steam are added to begin softening and raising the temperature of the pet food mash. If the formulation calls for fresh meat addition or additional lipid sources, then these ingredients are traditionally pumped in the preconditioner where they are mixed together with the pet food mash. The resulting mash from the preconditioner is then conveyed to the extruder barrel. In the extruder barrel, material is conveyed through multiple screw elements designed for a medium shear process. (Riaz, 2000) As the material conveys forward in the extruder barrel pressure and temperature increase to cook the starches and kill harmful bacteria. At the end of the extruder a die is placed to create back-pressure and form the product as it expands the pet food mash into the desired shape. As it exits the die a knife assembly cuts the exiting food to the desired length. Post extrusion, kibbles are conveyed to a dryer where excess moisture is driven off to achieve a shelf stable moisture with temperatures around 105 ˚C and retention times between 10-15 minutes. Many process parameters, e.g. steam and water addition, extruder screw speed, die configuration, dryer temperature and retention times, etc., can be changed to adjust for formulation differences.

Baking and extruding can both be further processed where the final kibbles are coated with fat and palatants to increase the palatability.

Due to baking, it is a much lower throughput process when compared to extrusion processing. It is obvious that there are fundamental differences between the extrusion and baking process for making dry pet food kibbles.

1.3. Effects on Proximates

For both processing styles, there are some commonalities. The cooking of starch is also known as starch gelatinization. It is an endothermic reaction that leads to leads cessation of crystalline structure, absorption of water, swelling in size and accessibility to digestive enzymes such as amylase. When heat processing a starch with baking or extrusion, gelatinization will increase as well as increase the digestibility of starch (Hernot et al. 2008, Murray et al. 1999, and Wootton & Chaudhry 1980). Cooking of proteins within a pet food matrix can denature proteins and yield higher digestibility (Hendriks W. H. & Sritharan K. 2002). But in extreme heat processing cases, protein digestibility can be decreased due to over cooking (Hendriks et al. 1999). In general, cooking of a pet food increases the digestibility of the protein, by slight denaturization, and starch, by starch gelatinization.

Gelatinization is not the only starch-related phenomenon which has been observed during heating of pet food formulations. Another thermal transformation that occurs is amylose-lipid complexation, which is often observed during the cooking of starch in presence of a lipid. The formation and extent of amylose-lipid complexation is a function of heat, moisture content, type of starch, type of lipid, and also the degree of gelatinization (Eliasson 1994 & Pilli et al. 2011). Because of the multiple starch sources, lipid sources in a pet food formulation, and high degree of starch gelatinization, amylose-lipid complexation could easily. When the complexes are formed, the amount of free or unbound fat in the pet food matrix is decreased. With less free fat available for oxidation, amylose-lipid complexation has been shown to the extend shelf-life of the product. A higher degree of complexation also slows the digestion of starch (Muoki et al. 2011). Thus this phenomenon has potential benefits in the context of pet food.

While the understanding of what happens to the macromolecules of a food and its impact has been studied to great extent, process characterization or what happens during processing at different conditions and the impact has not been explored in depth for baked or extruded pet food.

1.4. Effects on Physical

While there have been studies researching how specific ingredients impact texture and other physical aspects via baking or extrusion, there is very scant or no research on process characterization of a pet food and the relation to physical attributes (Carvalho et al. (2010), Cheng et al. (2007), Kim et al. (2012), Laguna et al. (2011), and Zucco et al. (2010)). For example, Laguna et al. (2011) found by changing the ratio of fat to a fat replacement in a baked biscuit the textural attributes change significantly; such as breaking strength and hardness. Although baking biscuits from a short dough is a similar process to that producing pet food, biscuits lack the multitude of ingredients that a pet food contains. Also, multiple cooking parameters were not utilized to observe the impact on the studied textural attributes.

One study in which a process characterization of extrusion was explored was Garg and Singh (2010); wherein, they optimized a soy-rice blend by manipulating the extruder screw RPM, moisture content of mash, and formulation ratios to achieve the best acceptable physical and textural attributes.

1.5. Conclusion

The first section of workwas to study the effects of varying fresh meat inclusions. The goal was to observe the physio-chemical differences, such as kibble density and starch gelatinization. Another goal was to observe the impact of physio-chemical attributes on the textural differences between baking and extruding as well as the effects of differing fresh meat inclusions. The tertiary goal was to observe effect of fresh meat inclusions on processing conditions.

The second section of this study was to manipulate and apply different energies to the pet food. These differing energies will then be characterized and quantified. Processed pet foods would be subjected to physico-chemical, shelf life, and vitamin analysis trials.

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Chapter 2 - Physico-Chemical Differences Between Extruded and Baked Pet Foods

2.1. Introduction

According to the American Pet Products Association, Inc. pet food sales in 2012 were $20.64 billion. The pet food industry is expected to grow to $21.26 billion by 2013. The pet food industry has seen steady growth over the past 15 years. In the industry of dry pet food production, over 60% of all the products are extruded (Riaz (2010). The other types of dry pet food processing include baking and pelting.

Baked and extruded products are generally a complimentary addition or a supplementation to a consumer's diet, e.g. corn puffs and cookies. Recent research regarding baking and extrusion has focused on the fortification of additional, healthier nutrients to specific products. Pet foods are unique compared to other products which are processed using baking and extrusion in that they are a complete food matrix. Complete pet food matrices include the pet's total nutritional requirements for each meal or for an entire day. The primary goals of pet foods are to provide a nutritionally adequate diet for the consumer's pet, to promote longevity, and prevent pet disease.

There has been little research completed on the processing of extruded pet foods and no research completed on the baking of pet foods. This research focused on the physical and textural attributes of a dog food kibble that had been processed by either baking or extrusion. The interaction of the different components of the complex matrix of the dog food, e.g. fresh meat addition and extrinsic fat addition, were also observed in this research.

2.2. Materials and Methods

2.2.1. Diet Formulation

Three base diets were formulated using a computer program, Concept5 (Creative Formulation Concepts, LLC., Annapolis, MD), such that the final diets would be iso-nutritional based on carbohydrate (46-48% dry basis or db NFE), lipid (12% db crude fat) , protein (22-23% db crude protein) and sodium (0.31-0.32% db) content after mixing with varying levels of mechanically deboned chicken or fresh meat (and chicken fat for balancing the lipid content). The base diets and ingredients as percentages are listed in Table 1.

All major and minor dry ingredients were procured from Lortscher Agri Service, Inc. (Bern, KS), except for ground (1.5-2.0mm screen) mechanically deboned frozen chicken (C J Foods, Bern, KS) and chicken fat (American Dehydrated Foods, Springfield, MO).

2.2.2. Grinding

Corn and wheat grains were ground using a Fitz mill (Model D, Fitzpatrick Company, Elmhurst, IL) equipped with a 1532-0040 Fitz mill screen. The screen had a round hole opening of 1016 microns. Brewers rice was obtained pre-ground using the same screen size. Post mixing, all major and minor ingredients were ground through the same milling system. The post mix milling was conducted to achieve greater uniformity of particle size distribution in the base recipes and ensuring all ingredients (macros and micros) had particle size lower than 1016 microns.

2.2.3. Mixing

For the dry mix, major and minor ingredients were mix together in a ribbon mixer (Wenger Manufacturing, Sabetha, KS). Major ingredients were weighed and mixed for three minutes. Minor ingredients were weighed, added into the mixer, and mixed for an additional two minutes.

2.2.4. Baking

All batches were mixed using a planetary mixer (HL800 Hobart, Troy, Ohio). The final mixes comprised of the base diets (Table 1), mechanically deboned chicken added at 0%, 10% and 20% level (final mix basis), and chicken fat (with 5000ppm liquid antioxidant) at 7.35, 5.90 and 4.03% (final mix basis), for the control, medium fresh meat and high fresh meat treatments, respectively. Water was added to help achieve proper dough quality. The final dough moistures were 33-34% on a wet basis (wb). Dough from each treatment was passed through a rotary molder (RM14B81, Weidenmiller Company, Itasca, IL) to obtain kibbles having the shape of a frustum of a cone.

The kibbles were passed through an experimental 30 foot, 3 zone tunnel oven (APV, Charlotte, North Carolina**)** with temperature zones set at 425°F for baking. Retention time of the kibbles in the tunnel oven was set at 7 minutes to achieve final product moisture of below 10% wb. A heat lamp (Digital Moisture Balance, CSC Scientific Company Inc., Fairfax, VA) was used as a rapid test to determine if products had reached proper moisture. The control product had final moisture above 10% wb and was dried further using a rack oven (Model 626, Revent, Inc., Somerset, NJ) at 170°F for 30 minutes to remove the excess moisture. After drying, the kibbles were allowed to cool to room temperature and samples collected for further analysis.

2.2.5. Extrusion

Diets were processed using a pilot-scale X-20 single screw extruder (Wenger Manufacturing Inc., Sabetha, KS). The extruder screw diameter was 82.1 mm and L:D (length : diameter) ratio 8:1. The extruder barrel set up and screw configuration are shown in Figure 1.

The barrel had six heads that were divided into three heating zones with set temperatures of 60, 75 and 90^{o}C from feed to discharge end. The screw configuration was designed for a compression ratio of less than 0.5 from feed to discharge, with a single flighted full pitch screw at the feed throat and gradual transition to a double flighted half pitch conical screw element at the discharge end. Shear locks were placed between each screw element with increase in size from small to large. One circular insert-type die of 4.7 mm diameter was used, along with a die face-cutting knife arrangement with 6 blades. The knife speed was kept constant at 1660 rpm. Prior to the extruder, the diets were conditioned with steam and water using a differential diameter cylinder (DDC) pre-conditioner (Wenger Manufacturing Inc., Sabetha, KS) to achieve downspout temperatures in the range of 85-89^{o}C. Pre-conditioner shaft speed was kept constant at 400 rpm for each treatment. A volumetric feeding system with feeder screw speed control was used to deliver the diets to the preconditioner. Prior to the experiment, the feeding system was calibrated to obtain feed rates.

The feeder screw speed was varied between 13.9 – 17.0 rpm to achieve a dry recipe feed rate of approximately 170, 155 and 140 kg/hr, respectively, for treatments containing no fresh meat (control), medium level of fresh meat and high level of fresh meat. The ground and frozen mechanically deboned chicken was thawed at room temperature to obtain a fresh meat slurry (65.9% wb moisture), which was pumped into the middle section of the preconditioner using a Waukesha Cherry-Burrell sanitary pump (serial number D043674SS; SPX, Charlotte, NC) at rates of approximately 0, 12 and 26 kg/hr for the control, medium and high fresh meat treatments, respectively. Chicken fat was pumped into the preconditioner discharge section using a Seepex pump (Pressure Stage 12; Range, MO) at rates of approximately 9.6, 7.4 and 4.1 kg/hr, respectively, for these three treatments. Both pumps were calibrated to the required set points prior to extrusion. The flow rates for dry recipe, fresh meat slurry and chicken fat were adjusted as above in order to achieve the same target carbohydrate, protein and lipid content as described earlier, while varying the fresh meat content.

Preconditioner steam injection rate was kept constant at 16 kg/hr, while preconditioner water injection rate was 23, 9 and 0 kg/hr and extruder water injection was 5, 5 and 0 kg/hr, respectively, for the three treatments. Water injection rates were adjusted as above in order to account for the moisture in the fresh meat slurry while keeping the in-barrel moisture content in the range of 25-27% wb.

Each of three diets (control, medium fresh meat and high fresh meat) were processed at two extruder screw speeds, 353 and 453 rpm in order to achieve different processing histories as characterized by mechanical energy and residence time. This resulted in an overall 3x2 factorial experimental design with 3 diets and 2 screw speeds. The die pressure varied between 450-600 psi depending on the treatment. Extruded diets were pneumatically conveyed to a double pass, gas-fired 4800 series pilot-scale dryer/ cooler system (Wenger Manufacturing, Sabetha, KS). The dryer set points were temperature 104.4^{o}C and retention of 5 min each at the top dryer belt, bottom dryer belt and cooler belt. Product samples were collected at the end of the cooler for various analyses. Specific mechanical energy (SME) input during the extrusion process was calculated using the following standard equation (Karkle *et al.* 2012),

Equation 2.1 Specific Mechanical Energy (SME)

$$SME\,(kJ\;kg^{-1}) = \frac{\left(\frac{\tau - \tau_0}{100}\right) \times \frac{N}{N_r} \times P_r}{\dot{m}} \qquad (1)$$

where, τ is operational torque (%); τ_0 is the no load torque (24%); N is the extruder screw speed; N_r is the rated screw speed (508 rpm); P_r is the rated motor power (37.29 kW); and $\dot{m}$ is the total throughput.

2.2.6. Proximate Analysis

AOAC official methods were followed for ash (942.05), crude fat (920.39 & 954.02), crude protein (990.03), and moisture (930.15). Crude fiber was determined by the Ankom method for each product. All of the proximate analysis results were set to a 100% dry matter basis for comparison. To determine carbohydrate concentration or nitrogen free extract (NFE) for each product the conservation of mass equation 2.2 was applied. Proximate analysis results are displayed in table2. 3. Samples were ground and placed into a water activity meter (CX-2, Decagon Devices, Pullman, WA). Results for water activities (aw) are displayed in table 2.3.

Equation 2.2 Nitrogen Free Extract (NFE)

$$\mathbf{100\% - (\mathit{Ash} + \mathit{Crude\ fat} + \mathit{Crude\ Protien} + \mathit{Crude\ Fiber} + \mathit{Moisture}) = \mathit{NFE}}$$

2.2.8. Differential Scanning Calorimetry (DSC)

A DSC (Q200, TA Instruments Waters- LLC, New Castle, DE) was used to determine the degree of starch gelatinization. To determine the gelatinization of a product, the raw product and the processed product must be tested. Each diet and product was put into solution of two parts water to one part dry matter. 25-40 milligrams of the solution was placed into a stainless steel high volume pan and closed with a lid that had an O-ring insertion. The DSC run parameters were to equilibrate at 10˚C and ramp up to 140 ˚C at 10 ˚C /minute for each test pan. Each product was run in duplicate. Integration of the endothermic curves provided by the DSC was completed using Universal Analysis 2000 software (version 4.7A, TA Instruments Waters-LLC, New

Castle, DE). The degree of gelatinization was calculated by comparing the differences between endothermic heat of the raw formulation, ΔH_{raw}, and the endothermic heat of the kibble, ΔH_{kibble}, for each treatment. Formula for the degree of gelatinization using DSC is $(\Delta H_{raw} - \Delta H_{kibble})/\Delta H_{raw} * 100$. DSC testing identified amylose-lipid compounds, these results were quantified using the same integration technique.

2.2.9. Glucoamylase Testing

To determine total starch of both baked and extruded dog food kibbles a method developed by Wenger Manufacturing (Sabetha, KS). This method was also used to confirm the starch cook or starch gelatinization that the DSC testing provided. Each baked and extruded sample was ground. Two 0.500g samples were weighed out for this procedure. These two samples went through different procedures. The first 0.500g sample was subjected to a chemical solubilization. To chemically solubilize the pet food sample, the sample was subjected to a sodium hydroxide simmer for 20 minutes then hydrochloric acid was added to balance the pH. After chemical solubilization, both samples (one non solubilized and one solubilized) were put through an enzymatic digestion procedure. Post enzymatic digestion glucose levels were measure with a glucose anylazer (YSI 2300, YSI Incorporated, Yellow Springs, OH)

2.2.10. Specific Length

The lengths (l) of five kibbles from each extrusion treatment were measured by calipers. The same five kibbles were then weighed (m) on a laboratory scale. Specific length is determined by dividing the length of the kibble by the mass of the kibble displayed in equation 2.3. Specific length results for the extruded kibbles are displayed in table 2.4.

Equation 2.3 Specific Length Equation for Extruded Products

$$\frac{l}{m} = Specific\ Length$$

2.2.11. Bulk Density & Expansion Ratio

A liter cup was tarred on a scale. Bulk densities for each product were measure by over-filling a liter cup. The cup was leveled to remove excess product. The cup was then weighed to determine the bulk densities in g/L. Bulk density results are displayed in table 4.

Expansion ratios were calculated to determine the expansion after the dog food kibbles were released from the die of the extruder and the rotary moulder for the baked products. The diameters of five pieces from each extruded product were measured using calipers. The diameters of the extruded products (k) were divided by the extruder die diameter dimension (d), displayed in equation 2.3.

The baked products were shaped as frustums of a cone. To measure the expansion ratios, total surface area of the kibble was divided by the total surface area of the die. Surface area for a frustum of a cone is displayed in equation 2.4 where r_1 is the bottom radius, l_1 is the total length of the frustum's side if the kibble would be completed to a cone, r_2 is the top radius, l_2 is the length of the side of the frustum, r_3 is the bottom of the die radius, l_3 is the total length of the frustum's side if the die would be completed to a cone, r_4 is the top radius of the die, and l_4 is the total height of the die. Results are displayed in Table 2.3 for baked and extruded products.

Equation 2.4 Expansion Ratio for Baked Kibbles

$$\frac{k^2}{d^2} = Expansion\ Ratio$$

Equation 2.5 Expansion Ratio for Extruded Kibbles

$$\frac{\pi r_1 l_1 - \pi r_2 l_2 + \pi r_1^2 + \pi r_2^2}{\pi r_3 l_3 - \pi r_4 l_4 + \pi r_3^2 + \pi r_4^2} = Expansion\,Ratio$$

2.2.12. Piece Density

Piece Density is used to determine the amount of solid material in the kibbles volume. For extruded products length (L) and diameter were measured using calipers. To determine volume the diameter was divided by two to reach the radius(r). Each extruded kibble was then weighed to determine the mass (m). Equation 2.5 lists the equation used for the extruded piece density. Baked kibbles' top and bottom diameters, and length (h_2) were measured using calipers. Because the baked kibble is a frustum of a cone, the total height was determined (h_1). Diameters were divided by two to determine the bottom (r_1) and top (r_2) radii. Volumes were calculated using these dimensions. Each baked kibble was then weighed to determine the mass (m). The equation for piece density of baked kibbles is listed in Equation 2.6. Piece density results are listed in Table 2.3 for baked and extruded products.

Equation 2.6 Piece Density for Extruded Kibbles

$$\frac{m}{(\pi r^2) * L} = Piece\ Density$$

Equation 2.7 Piece Density for Baked Kibbles

$$\frac{m}{\left(\frac{1}{3}\pi r_1 h_1\right) - \left(\frac{1}{3}\pi r_2 h_2\right)} = Piece\ Density$$

2.2.13. Texture Analysis

To determine the peak fracture force, total number of fractures, number of spatial ruptures, crispiness, and area beneath the texture curve (toughness) of the kibbles produced, a texture analyzer (TA-XT2, Stable Micro Systems Ltd., Godalming, Surrey, UK) was used. One kibble at a time was subjected to this test and 20 (replicates) kibbles from each product were tested. A 38 mm circular probe crushed each kibble at 2mm/second and stopped once 50% strain was achieved. Crispiness is a calculation which utilizes total area under the texture analysis curve

(AUC) and the total number of peaks (TNP). Crispiness is a measure of how much force is required to create one fracture. The formula for crispiness =(AUC/TNP). The number of spatial ruptures is a calculation which uses TNP and compression distance to reach 50% strain (D). The number of spatial ruptures is a calculation which shows how many ruptures or peaks there are in a given distance. The formula for number of spatial ruptures = (TNP/D). Results for each treatment are displayed in table 4. Representative texture analysis curves for baked, 353 RPM, and 453 RPM pet food kibbles are displayed in figures 2, 3, and 4 respectively.

2.2.14. Statistical Analysis

Statistics were calculated with assistance from the Kansas State University Statistics Department, using the computer program SAS 9.3(SAS Institute Inc., Cary, NC). A total of five contrast comparisons were used. The first contrast was the combination of all meat levels compared across the 353 RPM and 453 RPM, e.g. 0%+10%+20% fresh meat inclusion at 353 RPM. The second contrast was the combination of a meat inclusion rate (0% fresh meat at 353 RPM + 0% fresh meat at 453 RPM) compared versus the 10% and 20% fresh meat inclusion rates. Another contrast considered was all fresh meat levels added together compared between treatments, baked, 353 RPM, and 453 RPM. The fourth contrast applied was the addition of one fresh meat inclusion versus the others at 353 RPM and 453 RPM. The final contrast was the different meat levels within the baked treatments.

2.3. Results & Discussion

2.3.1. Proximate analysis

To ensure all products were similar crude fat, crude fiber, crude protein, ash, and gross energy content results were calculated to a 100% dry matter basis for each product. There was a discrepancy in the crude fat content between the baked and extruded products. The crude fat for the six extruded products were 6% lower than the baked products.

During extrusion, lipids form complexes with amylose. In order to achieve accurate results for crude fat when lipid-amylose complexes occur, AOAC method 954.02 must be used. AOAC method 920.39, an ether extraction method, was initially used to determine the crude fat. This method should not be used for baked or expanded products specified by the method guidelines. AOAC method 954.02 uses acid hydrolysis to hydrolyze bonds between the lipid and amylose. The extruded samples yielded 49.57-55.51% higher crude fat results when using acid hydrolysis compared to the ether extraction method.

The variation between the crude protein, crude fiber, and ash contents between the extruded and baked kibbles for each diet formulation was attributed to the pumps used during extrusion. The chicken fat and fresh meat pumps were calibrated to set points that would allow the best fit for the formulation needs.

Final moistures for the extruded kibbles displayed a decreasing trend from 0-20% fresh meat inclusion for both extruder screw speeds when exposed to the same drying conditions. The 353 RPM had final moistures of 5.88%, 4.58%, and 3.64% W.B. from 0-20% fresh meat inclusion respectively. 453 RPM had final moistures of 5.13%, 3.99%, and 3.86% W.B. from 0-20% fresh meat inclusion respectively. The baked products showed a similar trend of decreasing moisture content from 0-20% fresh meat inclusion of 5.14%, 8.27%, 6.26% W.B. The 0% formulation was subject to extra cook in the rack oven. Prior to the rack oven, the moisture of the 0% fresh meat diet was 11.12% W.B (determined by heat lamp).

The decreases in moisture from the low to high meat inclusion suggest the chicken by product meal has a higher water holding capacity than the mechanically deboned chicken due to the higher moistures in the final products. The mechanically deboned chicken's inherent moisture did not appear to be trapped in the matrix of the fresh meat. The amount of energy required to dry the final kibble to meet specific final moisture could be costly in a diet that lacks fresh meat. Water activities also displayed a decreasing trend from 0-20% fresh meat inclusion and showed a strong correlation with the final moisture content of the kibbles. The 353 RPM treatment from 0-20% fresh meat inclusion's a_w were 0.261, 0.161, and 0.100. The 453 RPM treatment from 0-20% fresh meat inclusion's a_w were 0.212, 0.122, and 0.118. The baked kibbles from 0-20% fresh meat inclusions a_w were 0.164, 0.483, and 0.281.

The final moisture of the 0% fresh meat formulation was similar, but the a_w was slightly higher in the extrusion process compared to the baking process.

2.3.2. Specific Mechanical Energy (SME)

SME results were inconclusive for the trial. The baked kibbles were not subject to any mechanical energy for the cooking process. For the low screw speed (353 RPM) from 0% fresh meat inclusion to 20% fresh meat inclusion the SME was 77.911, 111.301, and 80.796 kJ/kg. The high screw speed (453 RPM) from 0-20% fresh meat inclusion was 84.833, 88.873, and 72.714 kJ/kg. The two highest SME's were achieved at the 10% fresh meat inclusion. The 353 RPM and 453 RPM 10% fresh meat diet were the first to be run through the extruder. Improper sequencing of the diets may have led to the higher SME because the extruder was not completely warm.

The 20% fresh meat inclusion had a higher crude fat of 1.87%. The difference in fat is attributed to pump inconsistence, but fat is a lubricant inside the extruder barrel. With less fat, the friction will become greater. This in turn causes a higher SME, which was apparent between the two extruder screw RPMs.

The 0% fresh meat formulation did not appear to be subjected to sequencing issues and did not vary significantly in crude fat content. At the higher screw RPM (453) there was greater motor load, therefore higher SME.

2.3.3. DSC

For all extruded kibbles, the DSC results showed complete or 100% gelatinization. The extruded kibbles did not display a peak within the gelatinization temperatures of the endothermic curve. These results show there was enough mechanical and thermal energy provided by the extruder to completely cook all the starch in the each diet at both extruder screw RPM's. The baked kibbles from 0-20% had starch gelatinization of 45.46%, 32.39%, and 38.02% respectively. The 0% fresh meat diet had the highest starch gelatinization attributed to the extra cook period in the rack oven that was not subject to the 10% and 20% formulations.

The amylose-lipid complexes were discovered only in the extruded products. For the 353 RPM treatment from 0%-20% fresh meat inclusion the enthalpy of the complex was 3.675, 2.569, and 4.042 J/g. For the 453 RPM treatment from 0%-20% fresh meat inclusion the enthalpy of the complex was 3.612, 2.541, and 1.722 J/g. There is no correlation for the 353 RPM treatment comparing the level of fresh meat addition and the enthalpy of amylose-lipid complex. But, there is a very strong correlation of -0.997 for the 453 RPM treatment.

The difference in complexed fat, between the two crude fat methods, remains constant for each extrusion treatment between 5% and 6%. DSC only shows amylose-lipid complexes and the enthalpy of the complexes decrease with increasing fresh meat addition. If the amylose-lipid complexes are quantified by DSC at a decreasing rate from 0-20% fresh meat and there is a constant amount of complexed fat, there may be protein-lipid complexes formed under the proper extrusion condtions which was suggested by Tran et. al.(8). The DSC would not be able to identify protein-lipid complexes.

Also, the dry mix for each meat inclusion was post ground after mixing to ensure even particle size (≤1016 microns). The vendor specification for the mechanically deboned chicken's particle size was 1.5-2.0 mm. The difference in particle size may not have allowed the two streams to combine well in the extruder decreasing the amount of particle surface area to be complexed.

2.3.4. Glucoamylase testing

The results for the extruded samples of glucoamylase test were slightly lower than the DSC results. Extruded samples ranged from 98.35%- 93.18%. The highest level of gelatinization for the 353 RPM treatment was found in the 0% FM inclusion (98.35%) diet and the lowest level for the same extruder screw speed was found in the 20% FM inclusion diet (93.18%). With an increase of fresh meat in the 353 extruder RPM seemed to have an effect on gelatinization. The 453 RPM treatment did not have the same type of trend. The highest level of gelatinization for the 453 RPM treatment was found in the 10% FM inclusion (98.35%) diet and the lowest level for the same extruder screw speed was found in the 0% and 20% FM inclusion diet (93.50%). The baked dog foods were higher than DSC results. The highest level of gelatinization according to the glucoamylase test was found in the 0 % FM inclusion diet (57.00%) and the lowest level of gelatinization was found in the 20% FM inclusion diet (55.39%).

2.3.5. Bulk Density, Expansion Ratio, & Specific Length

When packaging pet food, bulk densities are used to help determine the bag size. Bulk densities vary on how the material packs into a certain area and the individual piece densities. Bulk densities for the extrusion treatments were significantly lower than the baked treatments. (significance) The bulk densities for the baked treatments from 0-20% were 569, 571, and 583.5

g/L respectively. The 0% fresh meat inclusion had the lowest bulk density. The 10% fresh meat was not much larger than (significance) than the 0% formulation. The 20% fresh meat diet was diets with fresh meat added had a higher bulk density. The bulk densities of the baked kibbles increased with increasing meat inclusion. The addition of fresh meat appears to have a higher level of compaction in the rotory moulder causing a higher bulk density in the final kibble.

The 353 RPM treatment had a decreasing bulk density from 0-20% fresh meat inclusion, 357, 332, and 326 g/L. The 453 RPM treatment also displayed the same decreasing trend from 0-20% fresh meat inclusion, bulk densities were 323, 316, 305 g/L respectively.

The expansion ratios of the baked and extruded kibbles there were notable differences. The baked products from 0%-20% fresh meat inclusion had an expansion of 0.96, 1.00, and 0.96, respectively. An expansion ratio of 1 reflects the kibble retaining the same dimensions of the die. The baked kibbles at 0% and 20% fresh meat inclusion shrank by 4%. Diet formulation and the baking process did not affect the expansion of the final baked kibble.

For kibbles extruded at 353 RPM expansion ratio decreased from 0%-20% fresh meat inclusion; 4.15, 3.79, 3.54 respectively. The 353 RPM treatment had a strong correlation of -0.9945. When fresh meat enters the system, it replaces the chicken by-product meal to maintain the same protein content of the other formulations. The addition fresh meat did not have the same structure/expansion forming ability of the 0% fresh meat formulation. The difference in particle size discussed earlier may have been the cause of the lower expansion in the kibbles with mechanically deboned chicken addition.

Expansion ratios of the kibbles extruded at 453 RPM were 3.78, 2.73, and 3.69 from 0-20% fresh meat inclusion. The higher screw RPM decreased the retention time of pet food mash in the extruder. With a short retention time the extruder was not able to create a dough forming area which also decreased the amount of thermal energy applied to the pet food mash when inside the extruder. The decreased time and thermal energy lowered the expansion ratio of the 453 RPM treatment compared to the 353 RPM treatment.

2.3.6. Piece Density

The extruded kibbles had a lower piece density than the baked products. The piece density for the 353 RPM treatment from 0% fresh meat inclusion to 20% fresh meat inclusion was 0.4693, 0.5014, and 0.4986 kg/m^3. The piece density for the 453 RPM treatment from 0% fresh meat inclusion to 20% fresh meat inclusion was 0.5547, 0.7033, and 0.4665 kg/m^3. The mechanical energy provided by the shear and pressure from the extruder created a pet food kibble with air pockets due to expansion.

The baked products were not exposed to the shear and pressure of the extruder. This resulted in a denser kibble with a piece density of 0.9541, 0.9405, and 0.9706 kg/m^3 from 0-20% fresh meat inclusion. Because thermal energy was the only energy input into the baked products, there was little to no nucleation and expansion. Without cell structure like the extruded products, the baked kibbles had more material in the given kibble volume.

2.3.7. Texture Analysis

The average peak crushing force of the baked kibbles from low meat to high meat inclusion 2.744, 3.436, and 2.973 kg. The average number of fractures for the baked dog food from 0-20% fresh meat inclusion was 102.35, 89.95, and 96.4 and the area under the texture curve was 2.347, 3.725, 3.942 joules (J). The calculated number of spatial ruptures from 0-20% fresh meat addition for the baked kibbles was 5.692, 5.002, and 5.361 peaks/mm. Crispiness from 0-20% fresh meat addition for the baked kibbles was 0.0232, 0.0419, and 0.0413 kg*mm. The toughness of the kibbles increased with increasing fresh meat inclusion. The textural curves display a smooth and constant amount of force applied throughout the test.

The average peak crushing force of the 353 RPM treatment 0-20% fresh meat inclusion was 3.013, 2.218, and 1.582 kg. The average number of fractures for the 353 RPM kibbles from 0-20% fresh meat inclusion was 108.2, 117.0, and 117.9 and the toughness was 2.932, 2.893, and 2.454 J. The calculated number of spatial ruptures from 0-20% fresh meat addition for the baked kibbles was 6.017, 6.506, 6.556 peaks/mm. Crispiness from 0-20% fresh meat addition for the baked kibbles was 0.0274, 0.0249, and 0.0212 kg*mm.

The average peak crushing force of the 453 RPM treatment 0-20% fresh meat inclusion was 2.497, 2.148, and 1.950 kg. The average number of fractures for the 453 RPM kibbles from 0-20% fresh meat inclusion was 118.3, 120.35, 117.85 and the area under the texture curve was 2.388, 2.620, and 2.727 J. The calculated number of spatial ruptures from 0-20% fresh meat addition for the baked kibbles was 6.578, 6.693, 6.554 peaks/mm. Crispiness from 0-20% fresh meat addition for the baked kibbles was 0.0210, 0.0223, and 0.0231 kg*mm.

For the extruded treatments, the average peak crushing force decreased when fresh meat increased. This is due to the particle size difference between the dry mix and the fresh meat. The ability for particles to melt together to form a homogeneous pet food matrix is limited because of the increased surface area of a smaller particle. Toughness decreased with increasing meat inclusion, which can be attributed to the same particle size reasoning. The average number of fractures increased with increasing fresh meat addition. Considering the number of spatial ruptures and the crispiness, the more meat addition there is a greater number of weak cells. Because there are greater number of weak cells with increasing meat addition, there is less material in the given kibble area resulting in higher specific length and lower piece densities.

From 0-20% fresh meat addition for the extruded treatments, there is very little difference between the toughness even with a higher peak crushing force. With increasing fresh meat addition the texture curves flatten out over the duration of the test. This shows the mechanically deboned chicken adds a pliable characteristic the extruded kibbles.

Comparing the baked kibbles texture analysis against the extruded kibbles, the average number of fractures is lower. The crispiness of the baked kibbles is higher than the extruded kibbles and has fewer spatial ruptures. This means the baked products are significantly denser than the extruded kibbles which are supported by the higher bulk density and the higher piece densities of the baked kibbles.

The texture curves (Figures 2, 3, and 4) the extruded kibbles curve are serrated or jagged displaying the difference in number of fractures whereas the baked kibbles' curve displays a lack of cell structure with a smooth curve. The average crushing force of the baked kibbles is higher than the extruded kibbles. The extruded curves force applied decreases immediately after the peak force is reached whereas the baked curves have a more evenly distributed force curve after the peak force is established.

2.4. Conclusion

Bulk and piece densities of the kibbles are directly related to the expansion due to the extrusion process. The extruded kibbles have a cell structure which leads to higher number of total fractures, lower crispiness values, and higher number of spatial ruptures. Due to extrusion's thermal and mechanical input energies, complete gelatinization is achieved and is able to create amylose-lipid complexes. The differences between the extruded treatments (353 and 453 RPMs) are attributed to the residence time in the extruder. With a lower residence time at the higher extruder screw RPM the pet food material has less contact with the extruder screw, the extruder barrel, and pet food mash. All of these variables will decrease the amount of thermal energy and mechanical energy.

Baked kibbles lack the mechanical energy, which is present in the extrusion process, and depends solely on thermal energy to cook the product. The baked products did not achieve complete gelatinization, but do not form amylose-lipid complexes. Baked kibbles were denser than the extruded products which lead to higher peak crushing forces and higher toughness. The baked kibbles lacked cell structure and had higher densities than the extruded kibbles.

Variations of fresh meat inclusion affected the final extruded densities, expansion ratios, and peak crushing force more significantly than the baked products. The differences are attributed to differences in particle sizes between the fresh meat and the dry mixes.

More explorative studies need to be completed on shelf life characteristics such as lipid oxidation and microbial growth between the two processes. Exploration in the differences of vitamin retention, protein cook, amino acid availability, and costs between the two processes would be beneficial to the pet food industry.

2.5. References

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Table 2.1 Base Diets for Baking and Extrusion

Fresh Meat Level	Control[2]	Medium	High
Macro Ingredients			
Brewers Rice	20.9	22.4	22.9
Corn	20.9	22.4	22.9
Wheat	20.9	22.4	22.9
Beet Pulp	4.32	4.76	5.27
Chicken By-Product Meal	26.7	21.0	18.2
Corn Gluten Meal, 75% protein	3.24	3.57	3.95
Micro Ingredients			
Calcium Carbonate	0.81	0.89	0.99
Potassium Chloride	0.38	0.44	0.46
Salt	0.43	0.48	0.53
Dicalcium Phosphate	0.89	1.26	1.38
Choline Chloride	0.22	0.24	0.26
Natural AOX, Dry[3]	0.04	0.04	0.04
Trace Mineral Premix	0.11	0.12	0.13
Vitamin Premix	0.16	0.18	0.20

[1]Base diets were mixed with varying levels of mechanically deboned chicken (or fresh meat) and chicken fat
[2]Control diet did not contain any fresh meat
[3]Dry antioxidant (Naturox™); in addition, chicken fat was mixed with liquid antioxidant (Naturox™) at 5000 ppm.

Table 2.2 Chemical Composition Results for Pet foods

Process	**353 RPM**			**453 RPM**			**Baked**		
Fresh Meat	0%	10%	20%	0%	10%	20%	0%	10%	20%
Crude Protein, %	25.27	23.44	23.45	25.45	23.56	23.08	26.10	23.73	23.62
Ether Crude Fat, %	5.15	5.16	4.74	5.20	4.73	5.62	10.67	10.41	11.18
AH Crude Fat, %	11.15	10.23	9.76	11.25	10.63	11.63	10.69	10.53	11.33
Crude Fiber, %	2.93	2.56	2.74	2.91	2.84	2.73	2.67	2.45	2.43
Ash, %	6.51	6.01	6.66	6.68	6.36	6.85	6.33	6.52	6.44
NFE, %	59.97	62.83	62.41	60.23	62.44	61.72	54.28	56.89	56.33
Gross Energy, cal/g	4787	4731	4581	4650	4905	4676	4881	5092	4887

Chemical composition results for pet foods extruded at 353and 453 RPM as well as baked.

*Proximate results are calculated on 100% dry matter basis.

Table 2.3Measurements of Pet Foods Post Processing

Process	353 RPM			453 RPM			Baked		
Fresh Meat	0%	10%	20%	0%	10%	20%	0%	10%	20%
SME(kJ/kg)	77.911	111.301	80.796	84.833	88.873	72.714	N/A	N/A	N/A
Bulk Density(g/L)	357	332	326	323	316	305	569	571	583.5
Piece Density(kg/m^3)	0.4693	0.5014	0.4986	0.5547	0.7033	0.4665	0.9541	0.9405	0.9706
Specific Length(mm/g)	29.91	29.97	32.86	28.01	30.21	33.50	N/A	N/A	N/A
Expansion Ratio	4.15	3.79	3.54	3.78	2.73	3.69	0.96	1	0.96
Final Moisture (% W.B.)	5.88	4.58	3.64	5.13	3.99	3.86	5.14	8.27	6.26
Water Activity (a_w)	0.261	0.161	0.1	0.212	0.122	0.118	0.164	0.483	0.281
Avg. Number of Factures	108.2	117.0	117.9	118.3	120.35	117.85	102.35	89.95	96.4
Avg. Peak Crushing Force(kg)	3.013	2.218	1.582	2.497	2.148	1.950	2.744	3.436	2.973
Avg. Area Under Curve (J)	2.932	2.893	2.454	2.388	2.620	2.727	2.347	3.725	3.942
Number of Spatial Ruptures (mm)	6.017	6.506	6.556	6.578	6.693	6.554	5.692	5.002	5.361
Crispiness (kg*mm)	0.0274	0.0249	0.0212	0.0210	0.0223	0.0231	0.0232	0.0419	0.0413
DSC Starch Gelatinization (%)	100	100	100	100	100	100	45.46	32.39	38.02
Glucoamylase Gelatinization(%)	98.35	94.72	93.18	93.50	98.35	93.50	57.00	56.95	55.39
Amylose-Lipid Complex (J/g)	3.675	2.569	4.042	3.612	2.541	1.722	0	0	0

Measurements of pet foods extruded at 353and 453 RPM as well as baked.

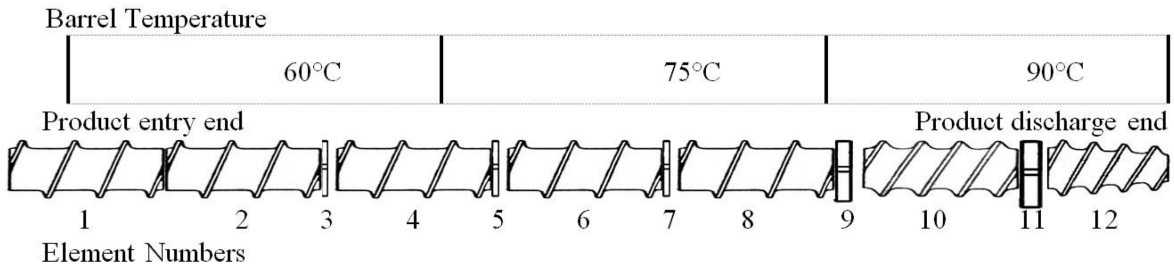

Figure 2.1 Schematic Showing Pilot Scale Single Screw Extruder Profile and Barrel

Extruder screw elements numbers with screw types. 1-2 =single flight screws; 3=small steamlock; 4=single flight screw; 5=small steamlock; 6=single flight screw; 7=small steamlock; 8=single flight screw; 9=medium steamlock; 10=half pitch, double flight screw; 11=large steamlock; and 12=half pitch, double flight cone.

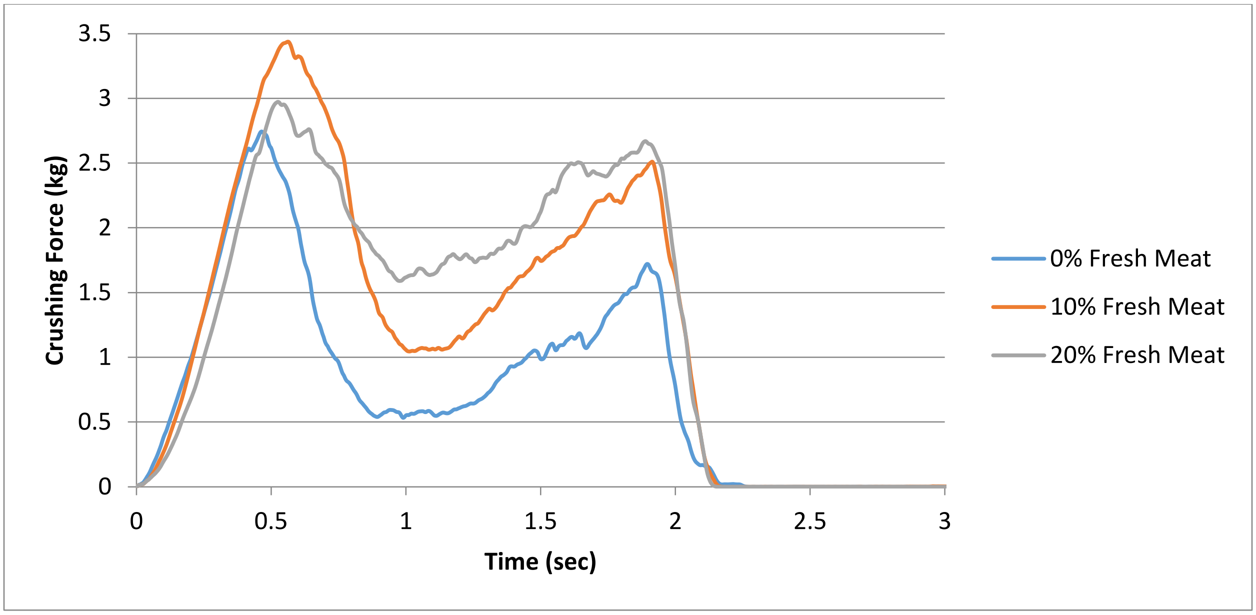

Figure 2.2 Baked Texture Analysis

Representative curves of texture analysis for baked kibbles at 0, 10, and 20% fresh meat inclusion.

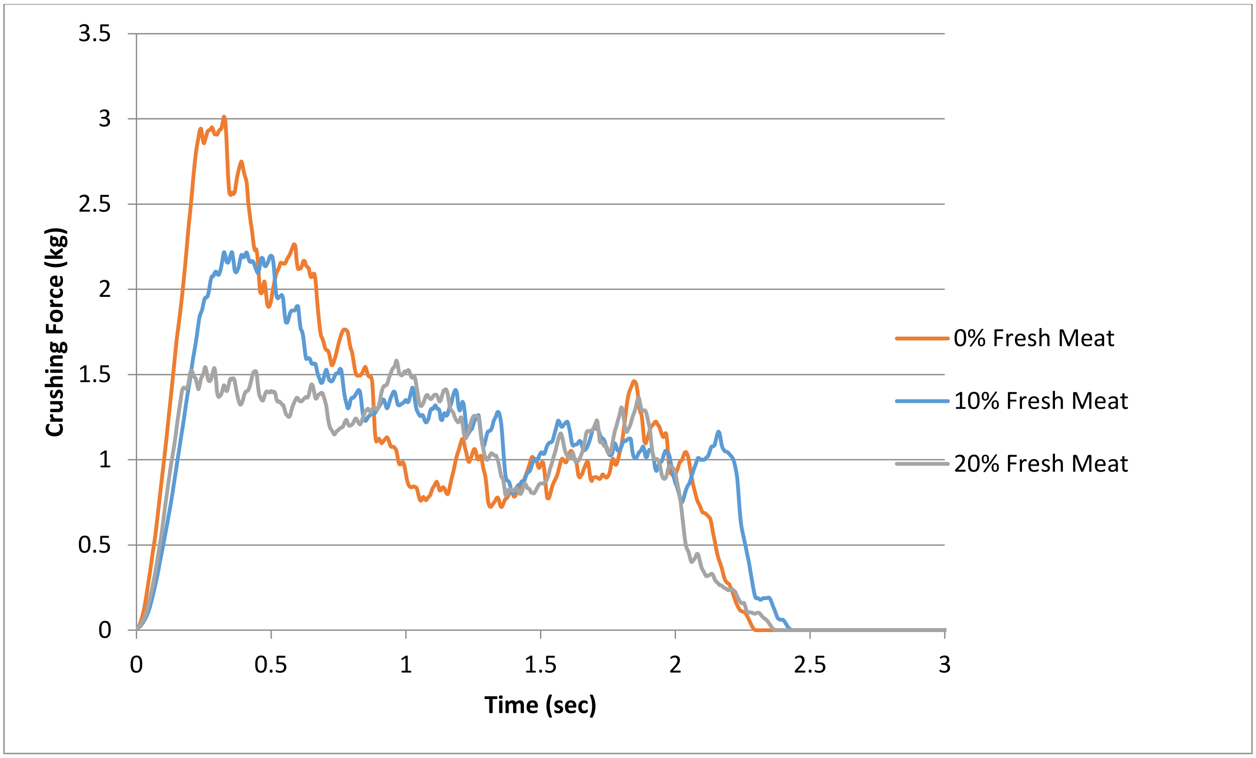

Figure 2.3 353 RPM Extruded Kibble Texture Analysis

Representative curves of texture analysis for extruded RPM 353 kibbles at 0, 10, and 20% fresh meat inclusion.

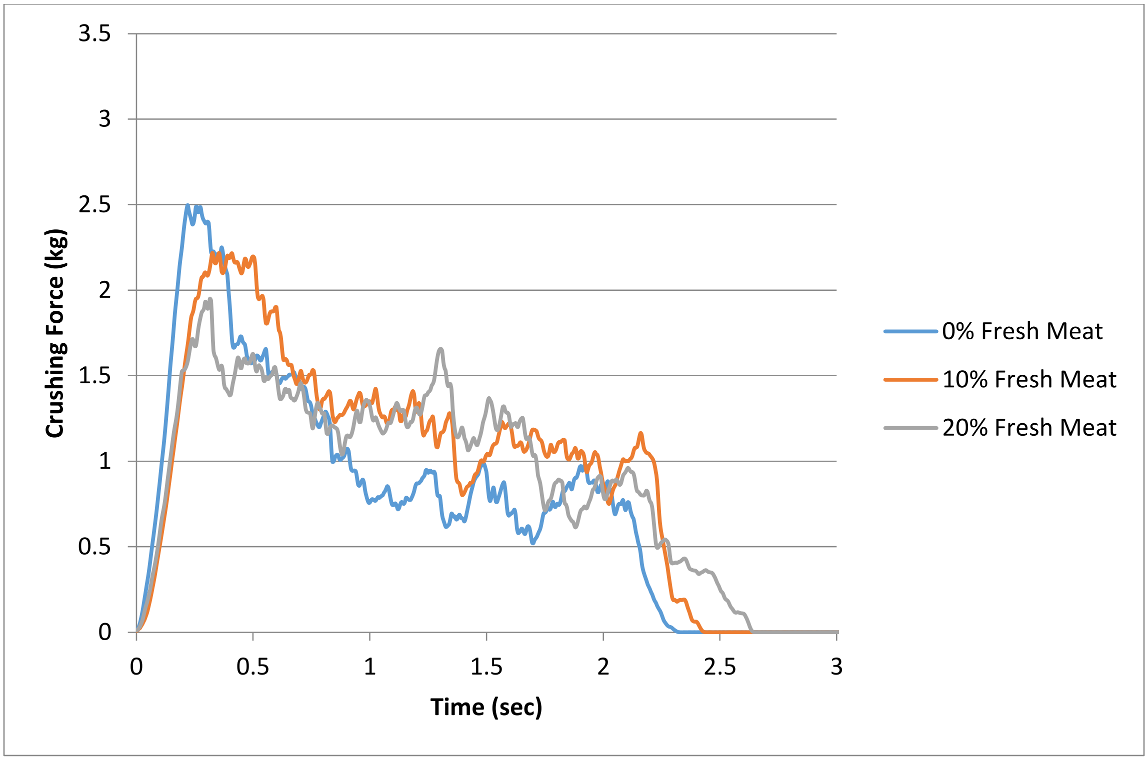

Figure 2.4 453 RPM Extruded Kibbles Texture Analysis

Representative curves of texture analysis for extruded RPM 453 kibbles at 0, 10, and 20% fresh meat inclusion.

Chapter 3 - Process Characterization of Extruded Pet Food and Differences in Shelf Life With Baked Pet Food

3.1. Introduction

According to the American Pet Products Association, Inc., as of 2013 there are approximately 396 million pets in the United States; 178.9 million cats and dogs. The U.S. spent $55.72 billion in 2013 on pets last year which includes $21.57 billion spent on pet food. There has been continuous growth in the pet food sector over the past 20 years and it is estimated pet food sales would reach $22.62 in 2014. The most common type of dry pet food production is via extrusion, which accounts for 80% of total production. The other major types of dry pet food production is baking and pelleting.

In human foods, baked and extruded products are generally a complimentary addition or a supplementation to a daily diet, e.g. breads, cookies, and corn puffs. Recent research regarding baking and extrusion has focused on the fortification of additional, healthier ingredients to specific products. Pet foods are unique compared to other products which are processed using baking and extrusion in that they are a complete food matrix. Complete pet foods include the pet's total nutritional requirements (protein, lipids, carbohydrates, etc.) for each meal or for an entire day. The primary goals of pet foods are to provide a nutritionally adequate diet for the consumer's pet, to promote longevity, and prevent pet disease. For cats and dogs, guidelines for minimal and maximum nutritional requirements are posted by the National Research Council and labeling regulations are set forth by the Association of American Feed Control Officials.

Extrusion is a complex process that involves a combination of continuous thermal and mechanical treatment of starch and proteins resulting in cooking and expansion of the product.

There is no research available in literature that focuses on in-depth process characterization of extrusion for manufacture of dry expanded pet food products.

Thermal treatment of proteins, either baking or extrusion, promotes the destruction of amino acids as well as Maillard reactions. Cooking of protein increases digestibility and over cooking can decrease digestibility. Lysine is generally limiting amino acid in a diet and is most likely to be denatured during processing. Research regarding the implications of protein cook, with lysine as digestibility indicator, of a pet food has been studied through extrusion on a few occasions (Rutherfurd et al., 1997 and Lankhorst et al, 2007), but there is no research in the field of baking of a pet food or a product with a meat or meat by-product as an ingredient.

Thermal processing of lipids can form amylose lipid complexations (ALC). Eliasson (1994) addressed the components which could yield higher ALC. These components are multiple lipid sources, multiple starch sources, high levels of heat, and high levels of gelatinization. ALC have been shown to increase shelf life. Excessive thermal processing of lipids can cause oxidation of lipids (Lin et al. 1998).

Extrusion has been shown to reduce and kill bacteria in animal feeds under most operating conditions (Okela et al., 2009). Baking on the other had has shown reduction and kill of bacteria (Thorsen 2006, Nigatu & Gashe, 1998) in common baked goods, but there is no literature on animal feeds or pet foods.

This study explored the effects of varying thermal energies on baked and extruded pet foods and the effects on lysine availability, shelf-life characteristics, and thermal characterization of the two processes. The expectations of this study are to manipulate and quantify energy inputs for both processes, shelf-life is expected to increase with higher levels of ALC, and lysine availability is expected to decrease with increasing amounts of thermal energy inputs.

3.2. Materials and Methods

3.2.1. Diet Formulation

Two adult maintenance dog food diets were formulated to be iso-nutritional based on carbohydrate, lipid, protein, and sodium content. Major variations within the diets were fresh meat inclusion (0% and 20%), chicken fat, and chicken by-product meal (Table 3.1). Dry ingredients were procured from Lortscher Agri Service, Inc. (Bern, KS, USA). Mechanically deboned chicken was acquired from C J Foods (Bern, KS, USA). Chicken fat was procured from American Dehydrated Foods (Springfield, MO, USA).

3.2.2. Grinding and Mixing

Whole grains (corn and wheat) were ground using a Fitz mill (Model D, Fitzpatrick Company, Elmhurst, IL, USA) equipped with a 1532-0040 screen with a round hole opening of 1.02 mm. Dry ingredients (Table 3.1) were mixed together in a double-ribbon horizontal mixer (Wenger Manufacturing, Sabetha, KS, USA). Major ingredients (brewer's rice, corn, wheat, beet pulp, chicken by-product meal, and corn gluten meal) were mixed for three minutes then minor ingredients (calcium carbonate, potassium chloride, sodium chloride, dicalcium phosphate, choline chloride, dry antioxidant, trace mineral and vitamin premixes) were added into the mixer for an additional two minutes. Post mixing, the entire batch was ground through the same milling system in order to achieve a more uniform particle size for the entirety of dry ingredients (major and minor ingredients).

3.2.3. Processing

Two cooking methods – extrusion or baking - were used to manufacture the pet food samples. For extruded samples three thermal energy input levels (Low; LE, Medium; ME, and High; HE) and 0 or 20% meat inclusion were used. Two types of meat addition were used during this trail as well; traditional (use of pumps; T) and non-traditional (no pumps; N). This yielded ten extruded pet food treatments: 0TLE, 20TLE, 0TME, 0NME, 20TME, 20NME, 0THE, 0NHE, 20THE and 20NHE. For baked samples four processing times (5 min, 7 min, 9 min, and 11 min) were used with 0 and 20% meat inclusion resulting in 8 baked samples (0B5, 0B7, 0B9, 0B11, 20B5, 20B7, 20B9, and 20B11). The extrusion and baking processes are described in detail below.

3.2.4. Extrusion

Diets were processed using a pilot-scale X-20 single screw extruder (Wenger Manufacturing Inc., Sabetha, KS). The extruder screw diameter was 82.1 mm and L:D (length : diameter) ratio 8:1. The extruder barrel set up and screw configuration are shown in Figure 3.1. The barrel had six heads that were divided into three heating zones with set temperatures of 60, 75 and 90^{o}C from feed to discharge end. The screw configuration was designed for a compression ratio of less than 0.5 from feed to discharge, with a single flighted full pitch screw at the feed throat and gradual transition to a double flighted half pitch conical screw element at the discharge end. Shear locks were placed between each screw element with increase in size from small to large. One circular insert-type die of 4.7 mm diameter was used, along with a die face-cutting knife arrangement with 6 blades. The knife speed was kept constant at 1660 rpm. Prior to the extruder, the diets were conditioned with steam and water using a differential diameter cylinder (DDC) pre-conditioner (Wenger Manufacturing Inc., Sabetha, KS) to achieve downspout temperatures in the range of 85-89^{o}C. Pre-conditioner shaft speed was kept constant at 400 rpm for each treatment. A volumetric feeding system with feeder screw speed control was used to deliver the diets to the preconditioner. Prior to the experiment, the feeding system was calibrated to obtain feed rates.

Processing conditions, e.g., extruder screw RPM (350 for High, 425 RPM for Medium, and 500 RPM for Low thermal energy treatments) and preconditioner steam input (8kg/h, 12 kg/h and 16 kg/h, respetively) were varied to achieve different thermal:mechanical ratios. The feeder screw speed was varied to achieve the appropriate dry recipe feed rate for treatments containing no fresh meat (control) and high level of fresh meat. The ground and frozen mechanically deboned chicken was thawed at room temperature to obtain a fresh meat slurry (65.9% wb moisture), which was pumped into the middle section of the preconditioner using a Waukesha Cherry-Burrell sanitary pump (serial number D043674SS; SPX, Charlotte, NC) at varying rates for the control and high fresh meat treatments. Chicken fat was pumped into the preconditioner discharge section using a Seepex pump (Pressure Stage 12; Range, MO) at adjusted rates for these two treatments. Both pumps were calibrated to the required set points prior to extrusion. The flow rates for dry recipe, fresh meat slurry and chicken fat were adjusted as above in order to achieve the same target carbohydrate, protein and lipid content as described earlier, while varying the fresh meat content.

Preconditioner steam injection rate was kept constant at 16 kg/hr, while preconditioner water injection rate and extruder water injection were adjusted in order to account for the moisture in the fresh meat slurry while keeping the in-barrel moisture content in the range of 27% wb.

Each of three diets (control, medium fresh meat and high fresh meat) were processed at two extruder screw speeds, 353 and 453 rpm in order to achieve different processing histories as characterized by mechanical energy and residence time. This resulted in an overall 3x2 factorial experimental design with 3 diets and 2 screw speeds. The die pressure varied between 450-600 psi depending on the treatment. Extruded diets were pneumatically conveyed to a double pass, gas-fired 4800 series pilot-scale dryer/ cooler system (Wenger Manufacturing, Sabetha, KS). The dryer set points were temperature 104.4°C and retention of 5 min each at the top dryer belt, bottom dryer belt and cooler belt. Product samples were collected at the end of the cooler for various analyses. Specific mechanical energy (SME) input during the extrusion process was calculated using the following standard equation (Karkle *et al.* 2012),

$$SME\,(kJ\ kg^{-1}) = \frac{\left(\frac{\tau - \tau_0}{100}\right) \times \frac{N}{N_r} \times P_r}{\dot{m}} \tag{1}$$

where, τ is operational torque (%); τ_0 is the no load torque (24%); N is the extruder screw speed; N_r is the rated screw speed (508 rpm); P_r is the rated motor power (37.29 kW); and $\dot{m}$ is the total throughput.

In another experiment, chicken fat and mechanically deboned chicken were added to the dry mix in a double-ribbon horizontal mixer (Wenger Manufacturing, Sabetha, KS, USA) for 3 mins and this composite formulation was fed to the extrusion system rather than pumping the meat and fat into the extruder.

3.2.5. Baking

The baked treatments were mixed together in a planetary mixer (HL800 Hobart, Troy, OH, USA) at the American Institute of Baking (Manhattan, KS, USA). The mixes were comprised of the dry mix described above, mechanically deboned chicken, chicken fat and water. The final dough moisture levels were targeted to be 33-34% on a wet basis (w.b.). The dough was passed through a rotary molder (RM14B81, Weidenmiller Company, Itasca, IL, USA) to form frustum-shaped kibbles.

The rotary molded kibbles were placed into a rack oven (Model 626, Revent, Inc., Somerset, NJ) preheated to 220°C and baked for 5 min, 7 min, 9 min, or 11 min. Post baking, kibbles were placed in a drying oven at 50°C for 5 hours to drive off excess moisture. To confirm the dog food achieved the final target moisture level below 10% w.b., moisture content was measured following AOAC method 930.15.

3.2.6. Chemical Composition

3.3.6.1 Proximate Analysis

Samples collected were sent to Missouri University's Analytical Lab (Columbia, MO). AOAC official methods were followed for ash (942.05), crude fat (920.39 & 954.02), crude fiber (978.10), crude protein (990.03), and moisture (930.15) to determine each product's proximate analysis. All of the proximate analysis results were set to a 100% dry matter basis for comparison. To determine carbohydrate concentration or nitrogen free extract (NFE) for each product, equation 3.1 was applied considering the conservation of mass. Lipid complexation due to ALC were quantified by the comparing the two AOAC methods to determine crude fat. Ether extracted fat, AOAC 920.39, will quantify all lipids which have not been complexed into ALC due to processing. AOAC 954.02, acid hydrolysis for crude fat, completely solubilizes the sample and in doing so will break down all chemical bonds between amylose and lipids which yields the total amount of fat in the sample. Equation 3.2 calculates the percent of complexed lipids (CL) due to ALC created from processing. Proximate analysis results are displayed in Table 3.2 for extruded products and Table 3.3 for baked products.

3.3.6.2 Vitamin Analysis

Pet food samples were sent to Research Products Company (Salina, KS, US) to be analyzed for vitamin and mineral content. Vitamin B_1 was analyzed following AACC 88-80, B_2 was analyzed following AACC 86-70, Vitamin B_3 was analyzed following AOAC 43.051. Vitamins A, D, and E were tested following an HPLC procedure. Finally Iron content of the pet food was analyzed following AACC method 40-41B. All results are listed in Table 3.4 along with the expected values for each formulation.

3.3.6.3 Amino Acid Analysis

Lysine Samples were sent to Missouri University's Analytical Lab (Columbia, MO) for a complete Amino Acid profile was run following AOAC 982.30 E(a,b,c), chp. 45.3.05, 2006. Samples were also tested for available lysine was tested following AOAC Official Method 975.44. All results were set to 100% D.M. for comparison. To determine the degradation of lysine Equation 3.3 was applied to the results. All amino acid results and lysine degradation are listed in table 3.5.

Equation 3.1 Nitrogen Free Extract Equation

$$100\% - (\boldsymbol{Ash} + \boldsymbol{Crude\ fat} + \boldsymbol{Crude\ Protien} + \boldsymbol{Crude\ Fiber} + \boldsymbol{Moisture}) = \boldsymbol{NFE}$$

Equation 3.2 ALC and Percent of Complexed Fat Equation

$$\left(1 - \frac{\boldsymbol{Ether\ Extracted\ Crude\ Fat}}{\boldsymbol{Acid\ Hyrolysis\ Crude\ Fat}}\right) * 100 = \boldsymbol{Complexed\ Fat}(\%)$$

Equation 3.3 Percentage of Lysine Degradation

$$\frac{\boldsymbol{Available\ Lysine}}{\boldsymbol{Total\ Lysine}} * 100 = \boldsymbol{Lysine\ Degredation}(\%)$$

3.2.7. Specific Mechanical Energy (SME)

SME is calculated to determine the amount of mechanical energy or shear that is applied to the product during the extrusion process. SME was calculated using the extruder RPM (N), rated extruder RPM (N_r), loaded torque (τ), no load torque (τ_0), the motor's rated power in kW (P_r), and the mass flow rate in kg/s ($\dot{m}$). These processing parameters can be located on the run sheets located at the end of this report. SME was calculated for all test runs using Equation 3.4. The results are listed in Table 3.2.

Equation 3.4 Specific Mechanical Energy

$$SME\,(kJ/kg) = \frac{\left(\frac{\tau - \tau_0}{100}\right) \times \frac{N}{N_r} \times P_r}{\dot{m}}$$

3.2.8. Mass and Energy Balance

For mass balance in the extrusion preconditoner and barrel, the basic thermodynamic principle of conservation of mass was employed as described below:

$$\sum m_{in} = \sum m_{out}$$

where $\sum m_{in}$ = summation of all mass flows into the preconditoner or extruder, $\sum m_{out}$ = summation of all mass flows out of the preconditoner or extruder. Mass balance was carried out for both the overall mass flow and also moisture to calculate the raw material flow rate into the preconditioner (as delivered by the feeder screw speed) and also the steam loss at the preconditoner and steam flash off at the extruder die.

For energy balance in the extrusion preconditoner and barrel, the basic thermodynamic principle of conservation of energy was employed as described below:

$$\sum Q_{in} = \sum Q_{out} + \sum \Delta h_{reaction}$$

Where $\sum Q_{in}$ = summation of all energy flows into the preconditoner or extruder, $\sum Q_{out}$ = summation of all energy flows out of the preconditoner or extruder and $\sum \Delta h_{reaction}$ = energy absorbed by any reactions occurring during the process (typically starch gelatinization and protein denaturation, in the case of extrusion). Energy balance was carried out to calculate the heat loss by convection and/or conduction from the preconditioner surface and extruder barrel.

The energy supplied by steam injection to the preconditioner was used to calculate the specific thermal net energy input after adjusting for the energy lost due to steam loss and base energy of water at room temperature. The specific thermal and specific mechanical energy were expressed in the form of percentage of the total specific energy.

3.2.9. Baking Energy

Baking thermal energy was measured indirectly by quantifying the amount of energy taken to evaporate water from the kibble during processing following the phase changes of water. To determine the amount of water evaporated, initial moisture was subtracted from the final moisture (pre- post drying). Calculations to determine the amount of energy applied to the kibble are displayed in equation 3.5 and equation 3.6. Equation 3.5 is the amount of heat to bring 1 g of water from room temperature to boiling where q is the amount of heat, m is the mass of water, ΔT is the change of temperature, and c is the heat capacity of water. Equation 3.6 is the amount of energy in joules to bring 1 gram of water to vaporization where q is the energy, ΔH is the heat of vaporization, and n is the number of moles of water. Results are displayed in Fig.(???).

Equation 3.5 Enthalpy for Heating of Water

$$m\Delta Tc = q$$

Equation 3.6 Enthalpy for Vaporization of Water

$$\Delta Hn = q$$

3.2.10. Expansion Ratio

Expansion ratios were calculated to determine the expansion after the dog food kibbles were released from the die of the extruder and the rotary moulder for the baked products. The diameters of five pieces from each extruded product were measured using calipers. The diameters of the extruded products (k) were divided by the extruder die diameter dimension (d), displayed in equation 3.7.

The baked products were shaped as frustums of a cone. To measure the expansion ratios, total surface area of the kibble was divided by the total surface area of the die. Surface area for a frustum of a cone is displayed in equation 3.8 where r_1 is the bottom radius, l_1 is the total length of the frustum's side if the kibble would be completed to a cone, r_2 is the top radius, l_2 is the length of the side of the frustum, r_3 is the bottom of the die radius, l_3 is the total length of the frustum's side if the die would be completed to a cone, r_4 is the top radius of the die, and l_4 is the total height of the die. Results are displayed in Table 3.2 for extruded products and Table 3.3 for baked products.

Equation 3.7 Expansion Ratio for Extruded Kibbles

$$\frac{k^2}{d^2} = Expansion\ Ratio$$

Equation 3.8 Expansion Ratio for Baked Kibbles

$$\frac{\pi r_1 l_1 - \pi r_2 l_2 + \pi r_1^2 + \pi r_2^2}{\pi r_3 l_3 - \pi r_4 l_4 + \pi r_3^2 + \pi r_4^2} = Expansion\ Ratio$$

3.2.11. Piece Density

Piece Density is used to determine the amount of solid material in the kibbles volume. For extruded products length (L) and diameter were measured using calipers. To determine volume the diameter was divided by two to reach the radius(r). Each extruded kibble was then weighed to determine the mass (m). Equation 3.9 lists the equation used for the extruded piece density. Baked kibbles' top and bottom diameters, and length (h_2) were measured using calipers. Because the baked kibble is a frustum of a cone, the total height was determined (h_1). Diameters were divided by two to determine the bottom (r_1) and top (r_2) radii. Volumes were calculated using these dimensions. Each baked kibble was then weighed to determine the mass (m). The equation for piece density of baked kibbles is listed in Equation 3.10. Piece density results are listed in Table 3.2 for extruded products and Table 3.3 for baked products.

Equation 3.9 Piece Density for Extruded Kibbles

$$\frac{\boldsymbol{m}}{(\boldsymbol{\pi r^2}) * \boldsymbol{L}} = \boldsymbol{Piece\ Density}$$

Equation 3.10 Piece density for Baked Kibbles

$$\frac{\boldsymbol{m}}{\left(\frac{1}{3}\boldsymbol{\pi r_1 h_1}\right) - \left(\frac{1}{3}\boldsymbol{\pi r_2 h_2}\right)} = \boldsymbol{Piece\ Density}$$

3.2.12. Differential Scanning Calorimetry (DSC)

A DSC (Q200, TA Instruments Waters- LLC, New Castle, DE) was used to determine the degree of starch gelatinization. To determine the gelatinization of a product, the raw product and the processed product must be tested. Each diet and product was put into solution of two parts water to one part dry matter. 25-40 milligrams of the solution was placed into a stainless steel high volume pan and closed with a lid that has O-ring insertion. The DSC run parameters were equilibrate at 10˚ C, ramp up to 140 ˚C at 10 ˚C /minute, the DSC chamber then cooled down to 10˚ C, then rescanned by 10˚ C up to 140˚ C. Each product was run in duplicate. Integration of the endothermic curves provided by the DSC was completed using Universal Analysis 2000 software (version 4.7A, TA Instruments Waters-LLC, New Castle, DE). The degree of gelatinization was calculated by comparing the differences between endothermic heat of the raw formulation, $\Delta H_{raw\ formulation}$, and the endothermic heat of the kibble, ΔH_{kibble}, for each processing technique. Formula for the degree of gelatinization is shown in equation 3.11. During the DSC run integration of the amylose-lipid compound were also identified. Results are displayed in Table 3.2 for extruded products and Table 3.3 for baked products.

Equation 3.11 Degree of Gelatinization

$$\frac{\Delta Hraw\ formulation - \Delta Hkibble}{\Delta Hraw\ formulation} * 100 = Degree\ of\ Gelatinization$$

3.2.13. Glucoamylase Testing

Degree of starch gelatinization of both baked and extruded dog food kibbles was determined by a modified glucoamylase enzymatic method developed Wenger Manufacturing (Sabetha, KS). This method determines the starch gelatinization by quantifying the amount of glucose in a given sample. Each baked and extruded sample was ground. Two 0.500g samples were weighed into 100 ml volumetric flasks and 25 mls of distilled water was added to each flask. The first flask was subjected to a chemical solubilization. 10 mls of 2N sodium hydroxide(Sigma Aldrich, St. Louis, MO, USA) was added to the sample, mixed, then placed on a heating element, and was allowed to simmer for 20 minutes. Post simmering, 10 mls of 2N hydrochloric acid (Sigma

Aldrich, St. Louis, MO, USA) was added and allowed to cool to less than 50° C. Each flask (one non-solubilized and one solubilized) were then subjected to the enzymatic digestion procedure. 10mls of 1N acetate buffer with a pH of 4.2 was added to each flask. Each flask then had 5 mls of an Optidex enzyme solution (45 mls of Optidex L-300 [Dupont Industrial Biosciences, Rochester, NY] 0.1% of ethlenediaminetetraacetic acid [Sigma Aldrich, St. Louis, MO, USA], diluted to volume with 150 mls of distilled water) added. The flasks were then placed into a 40° C water bath for 70 mins. Immediately after 70 mins of incubation, 5 mls of 25% trichloroacetic acid was added to each flask to stop hydrolysis, and then filled to volume with distilled water. Post enzymatic digestion glucose levels were measure with a glucose anylazer (YSI 2300, YSI Incorporated, Yellow Springs, OH). Results are displayed in Table 3.2 for extruded products and Table 3.3 for baked products.

3.2.14. Mold and bacteria

Mold and Bacterial counts were estimated using Dichloran-glycerol (DG-18) agar base (Oxoid, Basinkgstoke, Hampshire, England) and plate count agar (PCA) (Beckton, Dickinson and Company, Sparks, MD). Each of the media prepared was for a capacity of 750 ml distilled water to which 23.62 grams of DG-18 and 17.62 grams of PCA were added and placed on a 103 heater cum shaker (Thermolyne, Dubuque, IA) for 5 minutes with a magnetic stirrer placed inside each of the prepared media for homogenous mixing. The media was placed in an autoclave high pressure steam sterilizer (Yamato Scientific America Inc. Orangeburg, NY) set to 121°C and a pressure of 29.0 psi. After 2 hours, both mediums were removed from the autoclave and were placed in the stirrer for 10 minutes. Thereafter to the DG-18 media, 75 mg of Chloramphenicol (Genlantis,San Diego, CA) dissolved in 0.5 ml of ethyl alcohol (Decon Labs, King of Prussia, PA) was mixed and stirred again for 5 minutes. Both mediums were then poured into 100 x

15mm petri plates (Fisher Scientific, Pittsburg, PA) and were inverted after two hours after solidification. Thereafter, inoculums consisting of 100 ml distilled water for all samples were prepared by dissolving 0.1% (1gm to 1 liter distilled water) of peptone (Beckton, Dickinson and Company, Sparks, MD) and were placed in the autoclave at 121°C and removed after 2 hours. 9ml test tube samples were also prepared separately and autoclaved along with the inoculums. The blanks/inoculums were then treated with 10 grams of each pet food sample, and contents were transferred into sterilized bags and placed in a stomacher (Seaward Medical Ltd, OGN, London, UK) for 120 seconds for a homogenous mixture. Serial dilutions (10^{-2} to 10^{-3}) were made and 0.1mL aliquots were inoculated in duplicates onto the culture media and evenly spread using inseminated glass rods in a controlled environment chamber for all replicates. Results are listed in Table 3.2 for extruded products and Table 3.3 for baked products. And results are displayed in Figures 3.9a-3.9f.

3.2.15. Salmonella

Samples tested for salmonella were tested in Kansas State University's Grain Science Microbial Lab (Manhattan, KS, US). FDA BAM method for meats and meat by-product meals were followed to test for the presence salmonella. Results are displayed in Table 3.2 for extruded products and Table 3.3 for baked products.

3.2.16. Shelf-life analysis

Shelf-life study was set-up for accelerated shelf-life test (ASLT) at 55°C and 70% relative humidity (~12 weeks). ASLT set points were based on the well-known Q10 factor (Ragnarsson and Labuza 1977). The Q value is a temperature quotient and reflects the change in reaction rate for every 10°C rise in temperature expressed mathematically as Q10 (based on the assumption that for every 10C, deterioration factor is 2). In this study, 18 months of real time shelf-life of pet foods at 25°C equates to approximately 18 months (12 weeks) in ASLT at 55°C with a deteriorative Q10 reaction of 4 is calculated as shown in equation 3.12:

Equation 3.12 Q10 Reaction of 4

$$Q_{10} = \frac{\theta_{S(T1)}}{\theta_{S(T+10)}}$$

whereΔ = temperature difference , θs(T1) = shelf-life at 25°C (72 weeks);θs(T + 10) = shelf-life solved for 12 weeks at 55°C.

Table 3.6 illustrates estimated ASLT time point equivalents to its real time duration. The design consisted comparative study of chosen five treatments; 20TLE, 20THE, 0THE, 0B7, and 20B7. Ball glass jars (Broomfield, CL, USA) measuring 3x4.5x10.2 inches in diameter, height and width respectively was used as a storage material for the study. The top-lids of the ball jars were replaced by a common packaging material which was consisted of a basic four ply paper bag; 1 ply 50pound natural kraft, ½ mL high density poly ethylene(HDPE), 1 ply 50 pound natural kraft, and 1 ply 50 pound bleached white kraft.

The canning jars were sanitized using 70% v/v alcohol and 90 grams of each pet food were filled under a sanitized controlled environment chamber to avoid any microbial contamination at the time of packing and storage. The study was conducted in a temperature and humidity controlled chambers (BIOCOLD Environmental Inc, Fenton, MO, USA) with compartment space dimensions of 11.4 x 8.10 x 8.9 ft in length, height and width respectively. Each chamber was sanitized and dried using a germicidal detergent (Sunflo Max 128, Kansas Correctional Industries, Lansing, KS, USA). The chambers were checked for tightly fitting doors, roof leaks, holes in walls, hard-packed floors to avoid any burrowing of rodents, and other potential risk aspects for any microbial intervention. The temperature and relative humidity logs were constantly recorded using circular recording charts from sensors inside the chambers (Honeywell, MN, USA) and every one hour data was verified with HOBO data loggers (onset, Bourne, MA, USA) that were placed in chamber beside the temperature and RH sensors.

3.2.17. Volatile Compounds Measurement

3.2.17.1. Extraction Procedure of Volatile Aroma Compounds

The extraction method chosen for studying the aroma profile and, more specifically, the secondary oxidation products in the dry dog foods was headspace-solid phase microextraction (HS-SPME) as described by Koppel et al. [13]. The samples were ground in pestle & mortar, then a 0.5 gram samples was weighed into a 10 ml screw-cap vial with a polytetrafluoroethylene / silicone septa. Exactly 0.48 ml distilled water was added to the ground sample in the vial. To this an internal standard consisting of 0.02 ml 1,3-dichlorobenzene (98%, Sigma Aldrich, St. Louis, MO, USA) dissolved in hexane (mixture of isomers, optima grade, Fisher Scientific; Pittsburgh, PA, USA), with final concentration in the sample of 0.2 mg/kg was added. The vials were equilibrated for 10 min at 40 °C in the autosampler (Pal system, model CombiPal, CTC Analytics, Zwingen, Switzerland) and agitated at 250 rpm. After the equilibration, a 50/30 µm divinylbenzene / carboxen / polydimethylsiloxane fiber was exposed to the sample headspace for 30 min at 40 °C. The fiber method was chosen for its high capacity of trapping volatile compounds in food products (Ceva-Antunes, P.M.N).

After sampling, the analytes were desorbed from the SPME fiber coating prior in the GC injection port at 270 °C for 3 min in splitless mode.

3.2.17.2. Chromatographic Analyses

The isolation, tentative identification, and semi-quantification of the volatile compounds were performed on a gas chromatograph (Varian GC CP3800; Varian Inc., Walnut Creek, CA, USA), coupled with a Varian mass spectrometer (MS) detector (Saturn 2000). The GC-MS system was equipped with an RTX-5MS (Crossbond® 5% diphenyl/95% dimethyl polysiloxane) column (Restek, U.S., Bellefonte, PA, USA; 30 m × 0.25 mm × 0.25 μm film thickness). The initial temperature of the column was 40 °C held for 4 min; the temperature was then increased by 5 °C per min to 260 °C, and held at this temperature for 7 min. All samples were analyzed in triplicates. The quantities of volatile compounds were calculated against the internal standard peaks.

Most of the compounds were identified using 2 different analytical methods: (1) mass spectra (>80%) and (2) Kovats indices (NIST/EPA/NIH Mass Spectral Library, Version 2.0, 2005). Identification was considered tentative when it was based on only mass spectral data. The retention times for a C7-C40 saturated alkane mix (Supelco Analytical, Bellefonte, PA, USA) was used to determine experimental Kovats indices for the volatile compounds detected. Results for the secondary oxidation product hexanal are displayed in Figure 3.10 b and total aldehydes are displayed in Figure 3.10b. Appendix 1a-1h display all other secondary oxidation products identified.

Due to length and time of analysis for testing each of the samples, 5 products were chosen and subject to this trial. These treatments were 0B7, 20B7, 0THE, 20LTE, and 20THE.

3.3. Results & Discussion

3.3.1.1 Proximate Analysis

For all treatments proximate analysis results were similar in regards to the crude protein, crude fiber, ash, NFE, and acid hydrolysis fat. Results for ether extracted fat testing for the extruded treatments were lower in comparison to the baked treatments. Crude fat for extruded treatments from low to high thermal mechanical energy decreased from 7.97-3.54% respectively.

CL percentage increased with increasing of thermal mechanical ratios. The lowest amount of CL was found in 0TLE and 20TLE at 29.06% and 30.82%. Highest levels of CL were observed in the 0TME and 0THE; 55.35% and 63.05% respectively. The traditional versus the non-traditional extrusion yielded a slight difference, in that the traditional extrusion showed up to 6.28 % higher level of CL in 0THE and 0NHE. In between the two formulations, the 0 % Fresh Meat formulation showed a slightly higher level of CL. The observation was made during this study with increasing thermal energy, a greater amount of ALC and CL were created. According to Eliasson(1994), with increased heat, increased gelatinization, multiple starch and lipid sources, the ability to create ALC are increased.

Baked treatments displayed some CL, but to a much lower degree. The highest level of CL was observed in the 0B5 treatment at 20.11%. There did not seem to be any affect due to formulation (0 or 20% Fresh Meat) or retention time (5-11 mins) on the degree of CL in the baked treatments. Moisture of the baked treatments, before drying down to a shelf stable level, showed a strong decreasing trend with an increase of baking time for both formulations.

3.3.1.2 Vitamin Analysis

Vitamin Analysis proved inconclusive. Calculated levels of vitamins tested were far from actual measured values. Testing raw materials as well as final products could allow for better results.

There may also be some negative implications due to methods used for vitamin testing and/or fat soluble vitamins solubilized in ALC.

3.3.1.3 Amino Acids

Lysine degradation did not have any remarkable changes for any of the baked or extruded treatments tested. Lysine degradation percentages ranged from 92- 94. These finding reaffirms the results Lankhorst's(2007) and Rutherfurd and Moughan (1997) findings which are the level of processing did not change the amount of lysine degradation.

3.3.2. Extrusion Process Characterization

Specific thermal energy (STE) of treatments varied from 66-282 kJ/kg (Figure 2.2) and STE constituted 28-69% of the total energy input (Figure 2.4). In general, STE increased as thermal intensity (preconditioner steam addition) increased from low to high. Higher STE was observed for treatments with pumped fresh meat as compared to no fresh meat (Figure 2.2a) and also 6-11^{o}C higher preconditioner discharge temperature. Lower throughputs in the case of former were responsible for this difference. Measurements of mass flow rate of extruder discharge suggested that treatments with 20% fresh meat pumped into the preconditioner had flow rates 20-46 kg/hr lower than corresponding treatments with no fresh meat added. Calculated dry recipe flow rates for treatments with 20% pumped fresh meat were also 21-40kg/hr lower than corresponding treatments with no fresh meat added, although dry recipe rates were consistent with the feeder screw speed. Similarly higher STE and up to 4^{o}C higher preconditioner discharge temperature was observed for treatments with fresh meat and/or fat premixed as compared to corresponding treatments without any premixing. Extruder discharge was 24-33kg/hr lower for the former, calculated recipe flow rates were lower than expected based on the feeder screw speed and preconditioner discharge moistures were up to 4% points higher, thus indicating poorer feed delivery possibly due to fat and fresh meat in the recipe.

3.3.3. Expansion Ratio, Piece Density, and Specific Length

All baked treatments displayed shrinkage or after cooking a kibble smaller than the rotary moulded die. Shrinkage is represented by an expansion ratio below 1.0 and the baked treatments ranged from 0.65-0.72. There were no discernible expansion ratio differences between formulation and amount of energy applied to the baked kibble.

Low thermal mechanical ratio treatments did have considerably lower expansion ratios (1.60-1.82) in comparison to the medium and high treatments (4.02-4.33). Little differences were observed between 0TME, 0THE, 20TME, and 20THE (ER<.31).

Non-traditional extrusion had a negative effect on the expansion compared to traditional extrusion. The greatest difference between the two methods of extrusion was observed between 20THE and 20NHE with expansion ratios of 4.22 and 3.42 respectively.

Piece density for 0TLE was 0.857 kg/m^3 and 0.937 kg/m^3 for 20TLE. Piece densities for medium and high thermal mechanical ratios and 0-20% fresh meat were similar and ranged from 0.335-0.376 kg/m^3.

A higher expansion ratio represents more radial expansion, which translates to a lower piece density because more material is spread out over a larger volume. A lower expansion ratio means there is less radial expansion and leads to a higher piece density, meaning a smaller volume with the same amount of material. The baked products nearly doubled the piece density of the extrudates ranging from 1.41-1.92 kg/m^3, but there is no correlation to the formulation or the amount of energy applied to the kibbles.

Specific length did not vary for this experiment due to a constant through put. This measurement is unique to extrusion and was not calculated for the baked treatments.

3.3.4. DSC

All extruded treatments using DSC were completely cooked or 100% gelatinized, including the low thermal mechanical ratio treatments. Before rescanning ALC were identified. Post rescanning the ALC curves became more evident. Quantification of ALC was performed but the results were identical. Due to the nature of DSC there is a potential of creating or increasing the amount of ALC in a given substrate. Especially in excess water, high heat, multiple completely gelatinized starch sources, and multiple lipid sources.

Baked pet foods ranged from (-) 3.65% - 25.58% starch gelatinization. ALC were not identified until after rescanning.

Many of DSC curves contained considerable noise, even after re-running samples multiple times. Pet food is comprised of a multitude of ingredients, in this studies case 16 to 17, each of which has independent enthalpies. With a 20 mg sample size with uniform grind of dry matter, each DSC thermogram has the potential to contain different ratios of the 17 ingredients. This could have caused the inaccuracy in between duplicates and final results.

3.3.5. Glucoamylase Test

Results from the Wenger method of glucoamylase gelatinization yielded only 20NME and 20NHE achieving complete gelatinization. The lowest levels of gelatinization were observed in the low thermal mechanical ratio treatments due to the low levels of steam during processing; 0TLE (78.3%) and 20TLE (87.92%). There were negligible differences between the medium and high thermal treatments ranging from 93.08%-95.81%. There were greater differences between the traditional and non-traditional types of extrusion ranging from 0.88% (0TME and 0NME) to 4.67% (20THE and 20NHE). There is a possibility in which the non-traditional pet food mash had a longer retention time in the preconditioner which allowed the material better mixing and hydration of the steam.

Baked kibble gelatinization ranged from 49.77 % -58.96% for the 0% fresh meat formulation and 55.15%-58.06% for the 20% fresh meat formulation. 20% Fresh meat baked kibbles achieve a 3% greater average than that of the 0% formulation. An increase in retention time, for both formulations, showed no trend of increased retention time in the oven/ applied thermal energy.

3.3.6. Aerobic Plate Count

Plate counts decreased significantly from raw formulation to processed kibble. Initial APC counts for 0% fresh meat raw formulation were 151600 and 72000 for 20 % fresh meat raw formulation. At the low thermal mechanical energy input extruded treatments the APC decreased from 151600 to 2900 for 0 % fresh meat and 72000 to 850 for the 20% fresh meat formulation; about a 98% decrease of viable bacteria. The medium and high thermal mechanical treatments decreased to 50-350. There were negligible differences between traditional and non-traditional extrusion and not one extrusion treatment was 100% sterilized due to processing.

Baking treatments also were substantially decreased but not to the same level of extrusion. There were no decreasing or increasing trends with increased retention time in the oven. Baked treatments decreased viable bacteria from 90% (20B9) to 97% (0B7).

The combination of energies (thermal and mechanical) applied to the extruded kibbles appeared to be a major factor in the decrease of APC. Thermal energy alone, e.g. baking, significantly decreased the APC but not to the same effect of extrusion.

3.3.7. Salmonella

Native salmonella was identified in both 0% fresh meat and 20% fresh meat raw formulations. All thermal mechanical ratio extrusion treatments, including traditional and non-traditional, tested negative for salmonella. In the case of this study, the combination of low steam and high mechanical shear or high steam and low mechanical shear, extrusion processing was able to kill the bacteria.

0B5 retained salmonella after thermal processing and was the only baked treatment to do so. Therefore a cooking time 7 mins and above would be sufficient to eradicate salmonella in the 0% fresh meat formulation.

3.3.8. Shelf-Life and Oxidation

Before processing, the 0% raw formulation contained 136 ppb of total aldehydes which accounted for 29% of the total volatiles identified. The 20% fresh meat formulation contained 82 ppb of total aldehydes which accounted for around 19% of the total volatiles identified. Day 0 or the day of processing, the 0% baked product increased to 160 ppb for the total aldehydes and the 20% baked increased significantly to 286 ppb. For each baked treatment subjected to the ASL, according to the total aldehyde numbers, there was little to no oxidation up to the accelerated point of 14 months. Post 14 months, the total aldehyde numbers began to slowly decrease until the end of the trial. One possibility for the lack of oxidation could be the lack of porosity or cell structure in the baked kibbles.

20TLE and 20THE had total aldehyde results of 286 ppb and 243 ppb, respectively. Initial processing did not show any ill effects whether it was a low or high energy process. As ASL progressed, 20THE peaked in ASL month 14 at 1196 ppb and then decreased until ASL month 18. 20TLE increased steadily until ASL month 16 and peaked at 5798 ppb and then decreased in the final month of testing to 523 ppb of total aldehydes. Because of the ALC differences in the low and high thermal mechanical energy processing, 20TLE oxidized to a much higher level than the 20THE. Initially the thought of having a much higher thermal process would cause excess oxidation was not true in the case of this study.

0THE had a total aldehyde level of 141 ppb immediately after processing. The formulation without fresh meat was affected to a lesser degree in the high thermal mechanical energy process. 0THE total aldehyde numbers rose steadily throughout the shelf life process until it finally peaked in ASL of 18 months at 637 ppb.

3.4. Conclusion

With increasing amounts of total energy input into the extrusion system there was an increase in starch gelatinization percentage, a lower level of piece density, and a larger kibble expansion ratio. There was an observed decrease in the amount of APC and salmonella with an increase of total energy input into the extrusion system. Shelf-life, secondary oxidation products, deteriorated more rapidly with a low energy extrusion when compared to a high level of energy input.

The baked products displayed no differences in expansion, piece density or gelatinization with an increase of baking time or energy input. APC was reduced in the baked products but not as significantly as the extruded products.

More explorative studies need to be completed to determine the digestibility and availability of macronutrients and micronutrients in a pet food when subjected to differing cooking methods such as baking and extrusion as well as varying energy inputs.

3.5. References

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Table 3.1 Base Diets for Baking and Extrusion

Ingredients, %	0% Fresh Meat	20% Fresh Meat
Mechanically Deboned Chicken	0.00	20.00
Chicken Fat	5.32	2.34
Chicken By-Product Meal	20.94	10.91
Brewers Rice	21.21	18.84
Corn	21.21	18.84
Wheat	21.21	18.84
Beet Pulp	4.00	4.00
Corn Gluten Meal, 75%	3.00	3.00
Calcium Carbonate	0.75	0.75
Potassium Chloride	0.49	0.42
Sodium Chloride	0.46	0.43
Dicalcium Phosphate	0.87	1.12
Choline Chloride	0.20	0.20
Natural antioxidant, Dry	0.07	0.07
Natural antioxidant, Liquid	0.02	0.01
Trace Mineral Premix	0.10	0.10
Vitamin Premix	0.15	0.15

Table 3.2 Chemical and Physical Measurements of Extruded Pet Foods

	0TLE	0TME	0THE	20TLE	20TME	20THE
SME (kJ/kg)	172.9	137.07	137.6	186.82	152.83	161.51
Specific Length (mm/g)	38.20^{A}	40.04^{A}	38.33^{A}	38.69^{A}	39.60^{A}	38.42^{A}
Piece Density (kg/m^3)	0.857^{A}	0.335^{B}	0.374^{B}	0.937^{A}	0.363^{B}	0.356^{B}
Expansion Ratio	1.82^{A}	4.33^{B}	4.06^{B}	1.60^{A}	4.02^{B}	4.22^{B}
DSC Gelatinization (%)	100	100	100	100	100	100
Glucose Gelate (%)	78.8^{A}	95.9^{BC}	93.6^{BC}	88.8^{C}	98.5^{B}	93.6^{BC}
Lactose Gelate (%)	33.7^{AB}	38.4^{A}	30.8^{B}	36.6^{A}	36.2^{AB}	35.0^{AB}
Glucose (mg/dL)	19.55^{AB}	19.70^{AB}	21.95^{A}	18.45^{B}	19.80^{AB}	21.10^{AB}
Lactose (mg/dL)	0.207^{A}	0.198^{A}	0.206^{A}	0.221^{A}	0.220^{A}	0.219^{A}

Chemical and physical measurements of extruded pet foods with 0% and 20% fresh meat at low (LE), medium (ME), and high (HE) total energy input into preconditioner and extruder.

Table 3.3 Proximate Analysis Results of Extruded Pet Foods

	0TLE	0TME	0THE	20TLE	20TME	20THE
Crude Protein (%)	21.69	21.78	20.69	21.25	20.52	20.66
Moisture (%)	6.67	6.1	6.21	6.25	5.83	6.15
Crude Fat (%)	7.64	4.09	3.54	7.97	4.33	4.19
Crude Fiber (%)	2.15	3.07	2.24	2.15	2.67	2.32
Ash (%)	10.74	6.73	6.5	6.54	6.77	6.46
Acid Hydrolysis Fat (%)	10.77	10.05	9.58	11.52	10.06	8.99
NFE (%)	47.98	52.27	54.78	52.29	54.15	55.42
Complexed Fat (%)	29.06	59.3	63.05	30.82	56.96	53.39

Proximate analysis results of extruded pet foods with 0% and 20% fresh meat at low (LE), medium (ME), and high (HE) total energy input into preconditioner and extruder.

Table 3.4 Microbial Results of Extruded Pet Foods

	0TLE	0TME	0THE	20TLE	20TME	20THE
APC						
Day 1	700^{AC}	50^{C}	0^{C}	450^{C}	250^{C}	100^{C}
Day 2	2450^{AB}	50^{C}	0^{C}	700^{AC}	300^{C}	100^{C}
Day 3	2900^{B}	100^{C}	50^{C}	850^{AC}	350^{C}	150^{C}
Salmonella (+ or -)	-	-	-	-	-	-

Microbial results of extruded pet foods with 0% and 20% fresh meat at low (LE), medium (ME), and high (HE) total energy input into preconditioner and extruder.

Table 3.5 Chemical and Physical Measurements of Extruded Pet Foods

	0TME	0NME	0THE	0NHE	20TME	20NME	20THE	20NHE
SME (kJ/kg)	137.07	130.82	137.6	118.99	152.83	138.77	161.51	125.96
Specific Length (mm/g)	40.04^{AB}	37.43^{A}	38.34^{A}	39.26^{AB}	39.60^{AB}	42.82^{BC}	38.42^{A}	44.75^{C}
Piece Density (kg/m^3)	0.335^{A}	0.405^{A}	0.374^{A}	0.422^{A}	0.363^{A}	0.412^{A}	0.356^{A}	0.379^{A}
Expansion Ratio	4.33^{A}	3.82^{ABC}	4.06^{ABC}	3.50^{BC}	4.02^{ABC}	3.45^{C}	4.22^{AB}	3.42^{C}
DSC Gelatinization (%)	100	100	100	100	100	100	100	100
Glucose Gelate (%)	95.9^{AB}	94.8^{AB}	93.6^{B}	95.1^{AB}	98.5^{AB}	100^{A}	93.6^{B}	100^{A}
Lactose Gelate (%)	38.4^{A}	35.6^{AB}	30.8^{B}	37.1^{AB}	36.2^{AB}	34.4^{AB}	35.0^{AB}	31.7^{AB}
Glucose (mg/dL)	19.70^{A}	20.15^{AB}	21.95^{C}	19.40^{A}	19.80^{A}	20.25^{AB}	21.10^{BC}	19.75^{A}
Lactose (mg/dL)	0.198^{A}	0.217^{A}	0.206^{B}	0.196^{A}	0.220^{A}	0.274^{B}	0.219^{A}	0.270^{B}

Chemical and physical measurements of extruded pet foods with 0% and 20% fresh meat at medium (ME), and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping.

Table 3.6 Proximate Analysis of Extruded Pet Foods

	0TME	0NME	0THE	0NHE	20TME	20NME	20THE	20NHE
Crude Protein (%)	21.78	22.08	20.69	22.17	20.52	21.29	20.66	21.02
Moisture (%)	6.1	6.06	6.21	5.88	5.83	5.77	6.15	6.15
Crude Fat (%)	4.09	4.97	3.54	4.53	4.33	5.29	4.19	5.54
Crude Fiber (%)	3.07	2.03	2.24	2.36	2.67	2.31	2.32	2.57
Ash (%)	6.73	6.59	6.5	6.74	6.77	7.08	6.46	6.37
Acid Hydrolysis Fat (%)	10.05	11.13	9.58	10.48	10.06	12.17	8.99	11.22
NFE (%)	52.27	52.11	54.78	52.37	54.15	51.38	55.42	52.67
Complexed Fat (%)	59.3	55.35	63.05	56.77	56.96	56.53	53.39	50.62

Proximate analysis results of extruded pet foods with 0% and 20% fresh meat at medium (ME), and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping.

Table 3.7 Microbial Analysis Results of Extruded Pet Foods

	0TME	0NME	0THE	0NHE	20TME	20NME	20THE	20NHE
APC								
Day 1	50^{A}	0^{A}	0^{A}	50^{A}	250^{A}	0^{A}	100^{A}	300^{A}
Day 2	50^{A}	50^{A}	0^{A}	100^{A}	300^{A}	50^{A}	100^{A}	300^{A}
Day 3	100^{A}	50^{A}	50^{A}	100^{A}	350^{A}	100^{A}	150^{A}	300^{A}
Salmonella (+ or -)	-	-	-	-	-	-	-	-

Microbial analysis results of extruded pet foods with 0% and 20% fresh meat at medium (ME), and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping.

Table 3.8 Chemical and Physical Measurements of Baked Pet Foods

	0B5	0B7	0B9	0B11	20B5	20B7	20B9	20B11
PD	1.545^{AC}	1.924^{B}	1.414^{C}	1.705^{AB}	1.736^{AB}	1.844^{B}	1.439^{C}	1.779^{AB}
ER	0.68^{AB}	0.65^{C}	0.72^{A}	0.69^{AB}	0.67^{BC}	0.65^{C}	0.69^{AB}	0.66^{BC}
Final Processing Moisture (%)	20.71	17.48	15.85	10.27	21.91	19.55	16.65	12.05
DSC Gelatinization (%)	13.28	25.58	13.02	16.71	24.23	-2.68	-3.65	5.08
Glucose Gelate	51.3^{AC}	49.1^{A}	59.2^{D}	53.7^{ABC}	54.5^{BCD}	58.0^{BD}	54.9^{BCD}	57.9^{BD}
Lactose Gelate	16.8^{A}	18.9^{AB}	23.7^{A}	17.6^{A}	16.7^{A}	19.7^{AB}	18.7^{AB}	21.2^{AB}
Glucose	19.55^{A}	20.10^{A}	19.20^{A}	19.10^{A}	18.80^{A}	19.40^{A}	19.15^{A}	19.70^{A}
Lactose	0.333^{ABC}	0.284^{BC}	0.273^{C}	0.276^{C}	0.369^{A}	0.360^{A}	0.332^{ABC}	0.353^{AB}

Chemical and physical measurements of baked pet foods with 0% (0) and 20% (20) fresh meat at 5 (B5), 7 (B7), 9 (B9), and 11 (B11) minutes.

Table 3.9 Proximate Analysis Results of Baked Pet Foods

	0B5	0B11	20B5	20B11
Crude Protein (%)	22.2	22.36	21.17	21.58
Post Drying Moisture (%)	6.56	4.7	6.91	4.23
Crude Fat (%)	8.66	8.86	9.46	9.65
Crude Fiber (%)	2.11	2.42	2.44	7.86
Ash (%)	7	7.3	6.47	6.75
Acid Hydrolysis Fat (%)	10.84	10.78	11.59	11.67
NFE (%)	51.29	52.44	51.42	47.91
Complexed Fat (%)	20.11	17.81	18.38	17.31

Proximate analysis results of baked pet foods with 0% (0) and 20% (20) fresh meat at 5 (B5), 7 (B7), 9 (B9), and 11 (B11) minutes.

Table 3.10 Microbial Analysis Results of Baked Pet Foods

	0B5	0B7	0B9	0B11	20B5	20B7	20B9	20B11
APC								
Day 1	3450^{CDEF}	1200^{F}	2650^{CDEF}	2900^{CDEF}	2350^{DEF}	1050^{F}	2650^{CDEF}	1000^{F}
Day 2	5200^{ABCDE}	2400^{DEF}	4400^{ABCDE}	4450^{ABCDE}	3550^{BCDEF}	2100^{EF}	4850^{ABCDE}	2200^{DEF}
Day 3	6750^{A}	3350^{CDEF}	5650^{ABC}	5300^{ABCD}	4500^{ABCDE}	2750^{CDEF}	6650^{AB}	2550^{CDEF}
Salmonella (+ or -)	+	-	-	-	-	-	-	-

Microbial analysis results of baked pet foods with 0% (0) and 20% (20) fresh meat at 5 (B5), 7 (B7), 9 (B9), and 11 (B11) minutes.

Table 3.11 Vitamin Analysis Results of Extruded Pet Foods

	B1(Thiamin)	B2(Riboflavin)	B3(Niacin)	Fe(Iron)	B9(Folic Acid)	Vit A	Vit D	Vit E
	mg/kg	*mg/kg*	*mg/kg*	*mg/kg*	*mg/kg*	*iu/kg*	*iu/kg*	*iu/kg*
Expected 0%	20.02	8.52	118.76	334.05	1.17	17211	1131	126.77
0B11	16.20	8.05	48.08	78.87	2.20	46586	BDL	BDL
0TLE	16.57	8.95	45.89	81.95	1.79	63282	BDL	BDL
0THE	17.45	11.37	49.80	84.04	2.05	44638	BDL	BDL
Expected 20%	22.91	8.81	142.97	353.46	1.26	20024	1302	145.77
20B11	19.56	11.09	53.50	76.78	2.33	68290	BDL	BDL
20TLE	21.12	10.49	50.94	81.51	2.10	48403	BDL	BDL
20THE	21.21	10.67	56.21	85.21	2.75	57582	BDL	BDL

Vitamin analysis results of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 11 minutes (B11).

Table 3.12 Complete Amino Acid Profiles of Baked and Extruded Pet Foods

	0LTE	0THE	20LTE	20THE	0B5	0B11	20B5	20B11
Taurine	0.12	0.12	0.11	0.12	0.12	0.13	0.12	0.11
Hydroxyproline	0.48	0.48	0.37	0.38	0.51	0.47	0.38	0.38
Aspartic Acid	1.76	1.77	1.66	1.65	1.74	1.73	1.70	1.67
Threonine	0.85	0.86	0.80	0.80	0.83	0.84	0.82	0.80
Serine	1.08	1.10	0.96	0.96	1.04	1.08	0.97	0.96
Glutamic Acid	3.41	3.45	3.34	3.33	3.41	3.44	3.43	3.35
Proline	1.65	1.68	1.50	1.48	1.69	1.65	1.48	1.49
Lanthionine	0.14	0.15	0.13	0.13	0.14	0.15	0.13	0.13
Glycine	1.70	1.71	1.43	1.43	1.78	1.73	1.47	1.45
Alanine	1.44	1.45	1.33	1.33	1.44	1.43	1.39	1.38
Cysteine	0.45	0.45	0.39	0.39	0.43	0.45	0.39	0.38
Valine	1.18	1.20	1.09	1.09	1.17	1.19	1.11	1.10
Methionine	0.41	0.42	0.42	0.40	0.42	0.41	0.42	0.42
Isoleucine	0.90	0.91	0.85	0.85	0.89	0.88	0.88	0.87
Leucine	1.96	2.00	1.89	1.88	1.94	1.93	1.94	1.93
Tyrosine	0.71	0.73	0.70	0.70	0.73	0.71	0.72	0.71
Phenylalanine	1.03	1.05	0.97	0.97	1.03	1.02	0.99	0.98
Hydroxylysine	0.08	0.09	0.07	0.07	0.09	0.08	0.08	0.07
Ornithine	0.02	0.03	0.03	0.03	0.02	0.04	0.03	0.03
Lysine	1.08	1.10	1.09	1.08	1.08	1.05	1.12	1.07
Histidine	0.49	0.49	0.50	0.50	0.50	0.49	0.50	0.49
Arginine	1.40	1.41	1.29	1.29	1.43	1.40	1.31	1.28
Tryptophan	0.21	0.21	0.21	0.22	0.19	0.21	0.21	0.21
Total	22.54	22.83	21.15	21.09	22.62	22.51	21.57	21.26
Available Lysine	1.02	1.01	1.02	1.01	1.00	0.97	1.05	0.99
Lysine Ratio	0.94	0.92	0.94	0.94	0.92	0.92	0.94	0.93

Complete amino acid profiles with available lysine and lysine ratio measurements of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 5 (B5) and 11 (B11) minutes.

Table 3.13 Accelerated Shelf Life Vs Real time Points Applying the Q-10 Principals

ASLT (Weeks)	Real Time (Months)
0	0
2	3
4	6
6	9
8	12
9.33	14
10.66	16
12	18

Table 3.14 Real Time Hexanal Shelf Life Results

		Product				
		0B7	0THE	20B7	20TLE	20THE
Time	*Initial (0)*	98.06^{Aa}	96.23^{Aa}	162.74^{Ab}	225.34^{Aa}	182.45^{Aa}
	1 Month	45.92^{Aa}	44.57^{Aa}	60.69^{Ab}	96.99^{Aa}	114.64^{Aa}
	3 Month	108.31^{Aa}	65.16^{Aa}	170.14^{Ab}	109.81^{Aa}	111.61^{Aa}
	6 Month	256.35^{Ba}	126.51^{Ba}	635.84^{Aa}	208.53^{Ba}	215.08^{Ba}

Gas Chromatography shelf life results for secondary oxidation product Hexanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 6 months of real time.
Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table 3.15 Accelerated Time Hexanal Shelf Life Results

Hexanal		**Product**				
		0B7	*0THE*	*20B7*	*20LTE*	*20THE*
Time	*Initial (0)*	98.06^{Aa}	96.23^{Aa}	162.74^{Aa}	225.34^{Ac}	182.45^{Aa}
	1 Month	189.15^{Aa}	117.87^{Aa}	133.12^{Aa}	296.06^{Ac}	175.26^{Aa}
	3 Month	6.06^{Aa}	146.74^{Aa}	79.82^{Aa}	217.12^{Ac}	189.64^{Aa}
	6 Month	107.12^{Aa}	184.87^{Aa}	158.30^{Aa}	251.08^{Ac}	296.42^{Aa}
	9 Month	49.16^{Aa}	189.93^{Aa}	125.99^{Aa}	217.15^{Ac}	271.20^{Aa}
	12 Month	75.69^{Ba}	189.63^{ABa}	125.71^{ABa}	327.63^{Ac}	181.48^{ABa}
	14 Month	65.81^{Ca}	121.09^{BCa}	72.26^{Ca}	681.08^{Ab}	365.31^{Ba}
	16 Month	35.20^{Ba}	187.08^{Ba}	90.86^{Ba}	1249.01^{Aa}	207.49^{Ba}
	18 Month	27.82^{Aa}	190.37^{Aa}	54.92^{Aa}	88.10^{Ac}	140.07^{Aa}

Gas Chromatography shelf life results for secondary oxidation product Hexanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 18 months of accelerated time.
Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

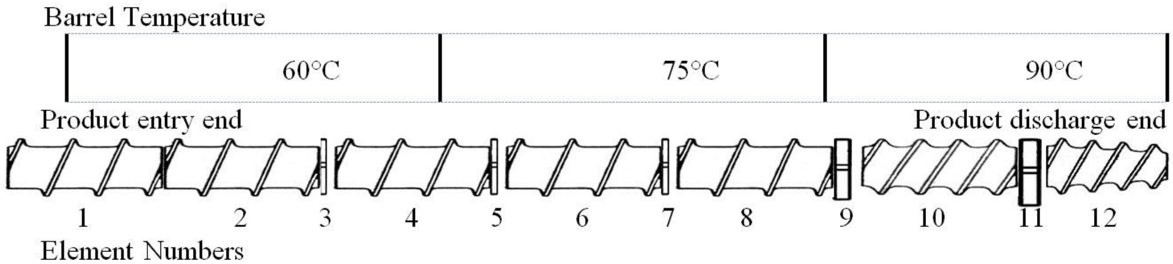

Figure 3.1 Schematic Showing Pilot Scale Single Screw Extruder Profile and Barrel

Extruder screw elements numbers with screw types. 1-2 =single flight screws; 3=small steamlock; 4=single flight screw; 5=small steamlock; 6=single flight screw; 7=small steamlock; 8=single flight screw; 9=medium steamlock; 10=half pitch, double flight screw; 11=large steamlock; and 12=half pitch, double flight cone.

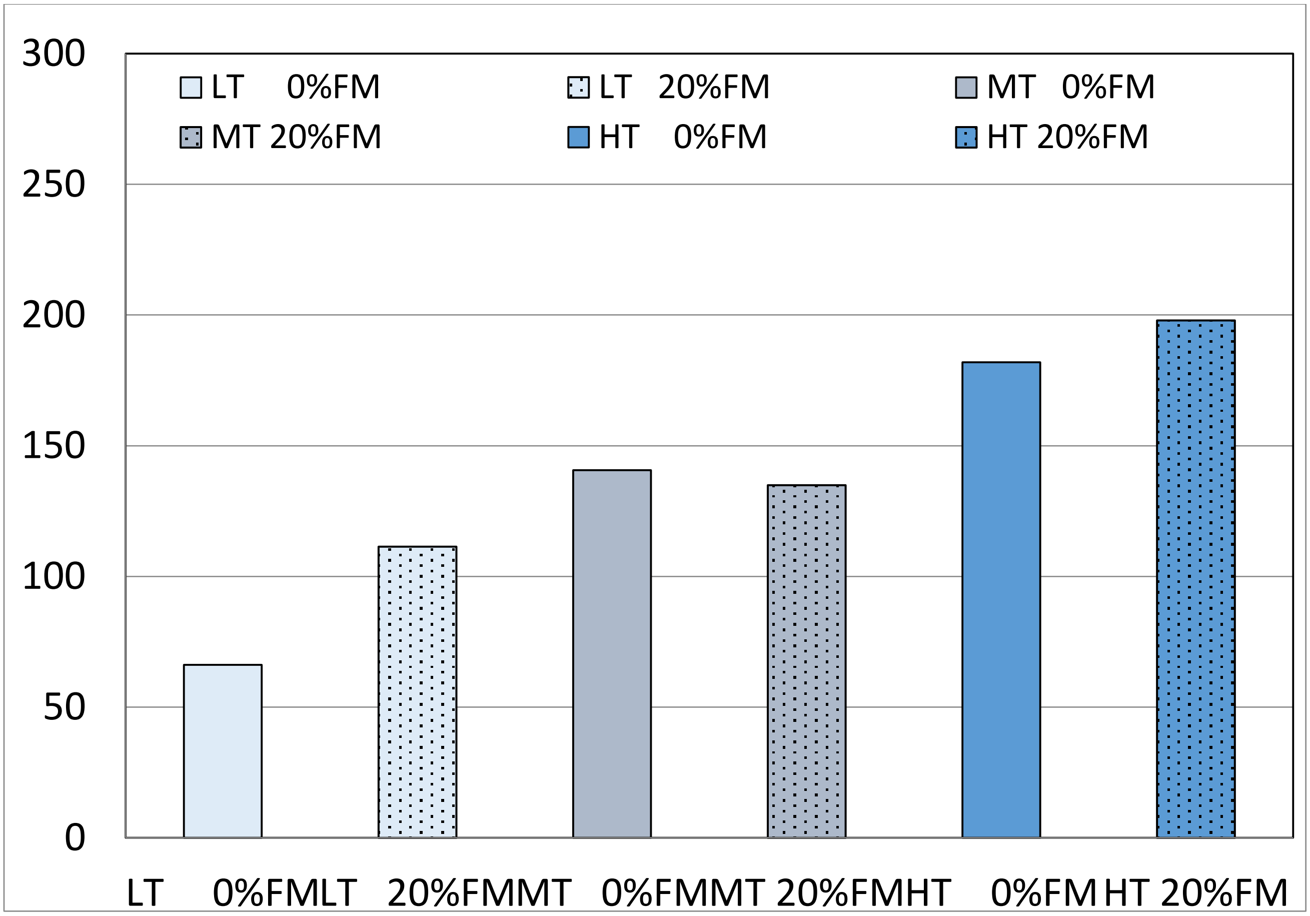

Figure 3.2 Specific Thermal Energy For Extruded Pet Foods

Specific thermal energy or STE (kJ/kg) for treatments with low (LT), medium (MT)and high (HT) thermal intensity and 0 and 20% fresh meat pumped.

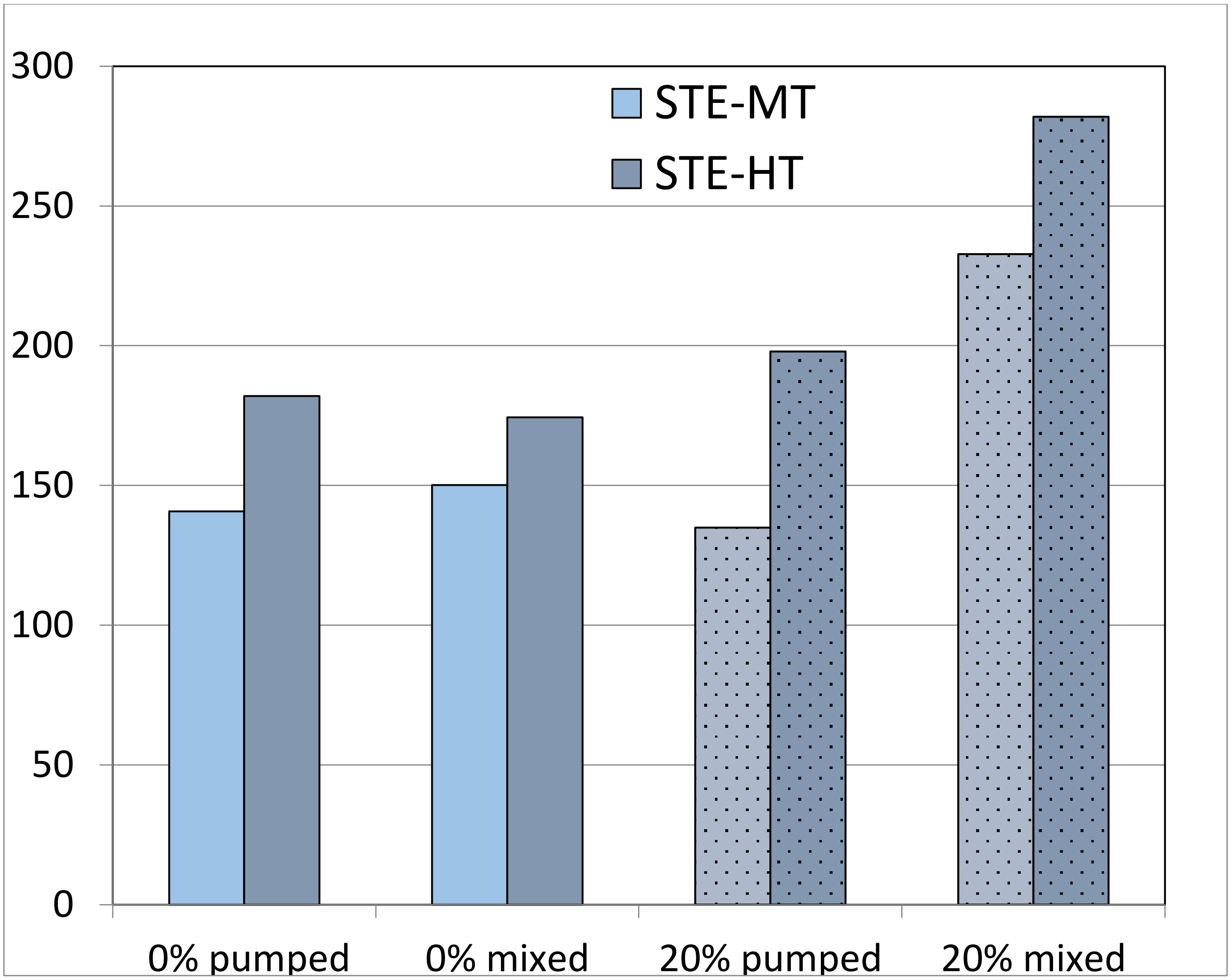

Figure 3.3 Specfic Thermal Energy For Extruded Pet Foods

Specific thermal energy or STE (kJ/kg) for treatments with 0% and 20% fresh meat, obtained by meat and/ or fat premixed versus pumped into the preconditioner.

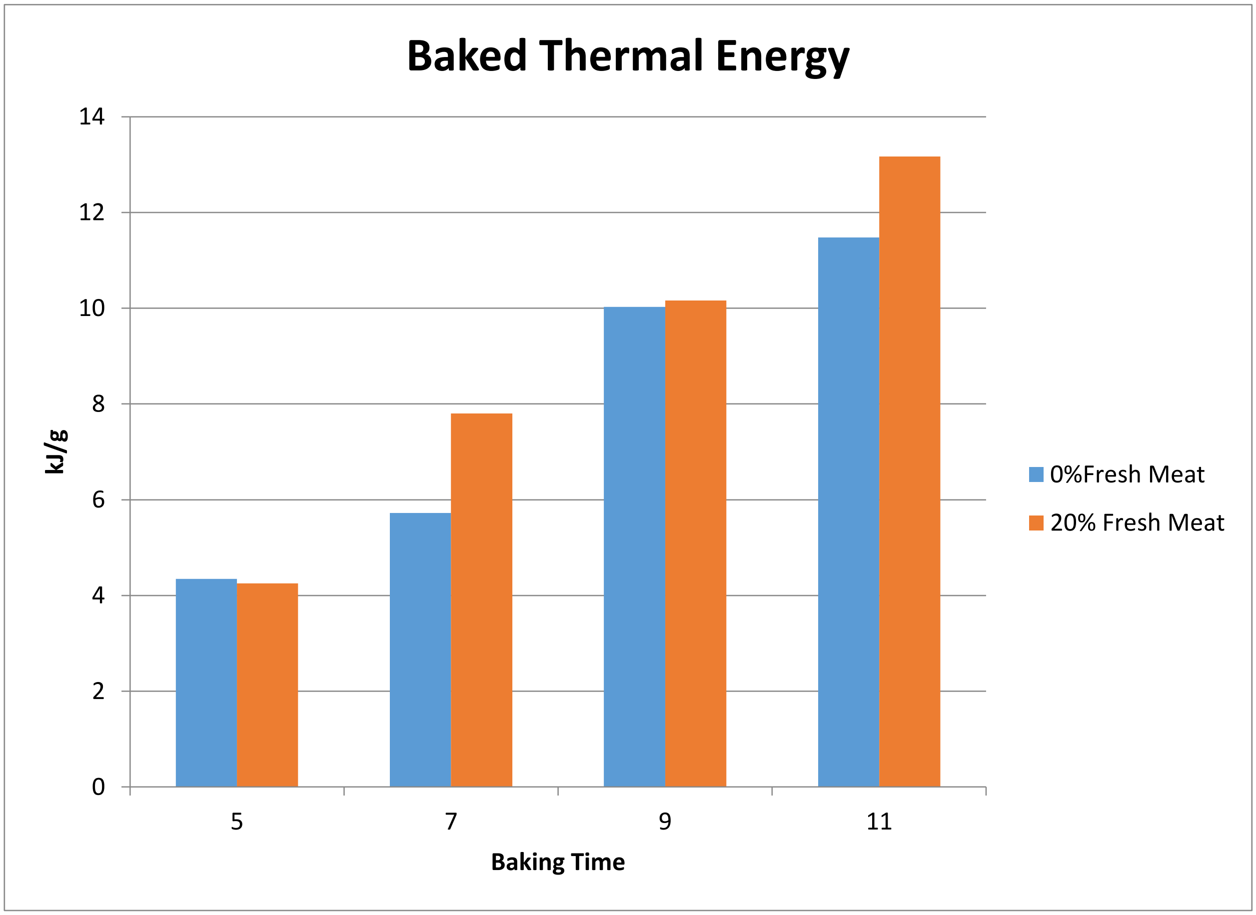

Figure 3.4 Specific Thermal Energy for Baked Pet Foods

Specific thermal energy or STE (kJ/kg) for baked treatments with 0% and 20% fresh

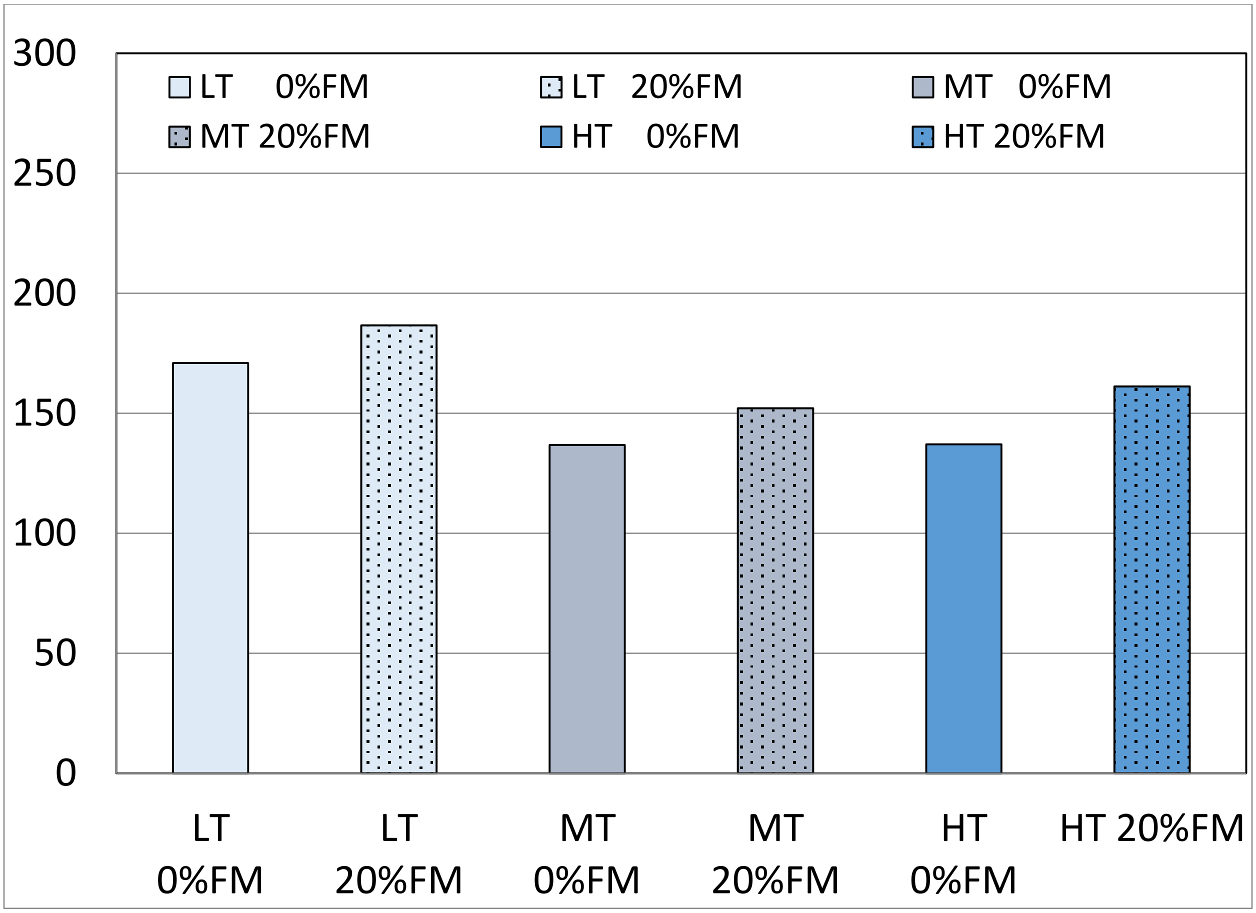

Figure 3.5 Specific Mechanical Energy of Extruded Pet Foods

Specific mechanical energy or SME (kJ/kg) for treatments with low (LT), medium (MT)and high (HT) thermal intensity and 0 and 20% fresh meat pumped.

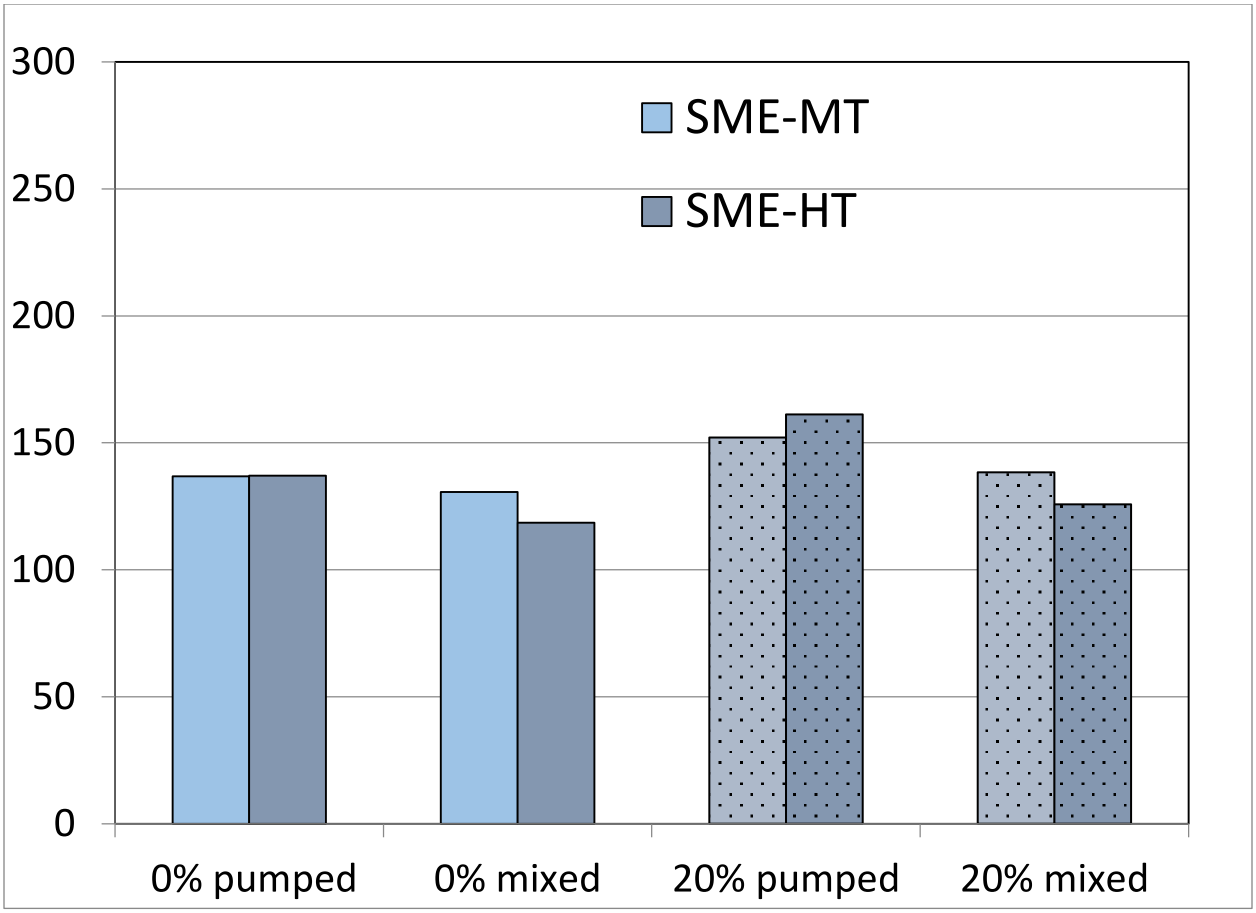

Figure 3.6 Specific Mechanical Energy Measurements of Extruded Pet Foods

Specific mechanical energy or SME (kJ/kg) for treatments with 0% and 20% fresh meat, obtained by meat and/ or fat premixed versus pumped into the preconditioner.

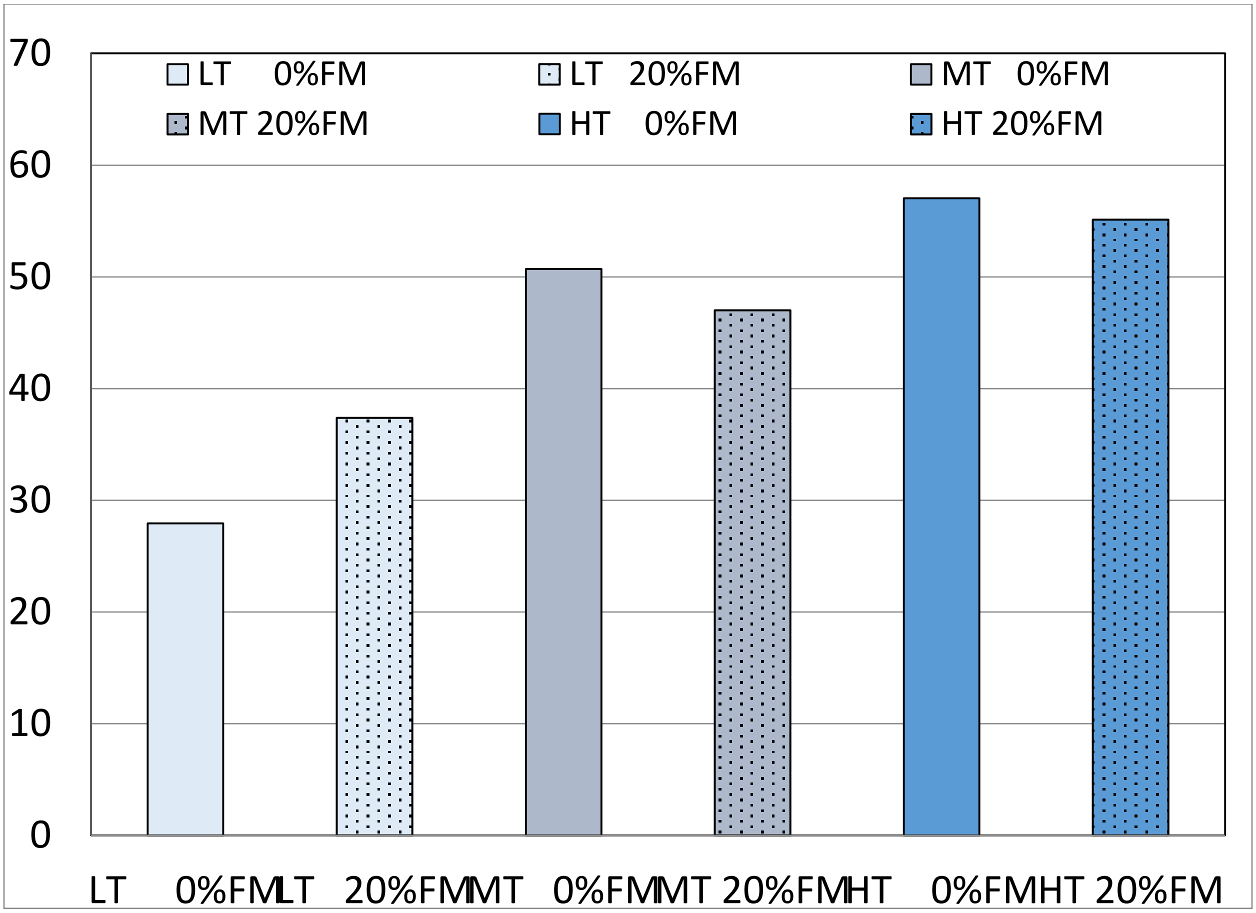

Figure 3.7 Percent of Specific Thermal Energy of Extruded Pet Foods

STE% for treatments with low (LT), medium (MT)and high (HT) thermal intensity and 0 and 20% fresh meat pumped.

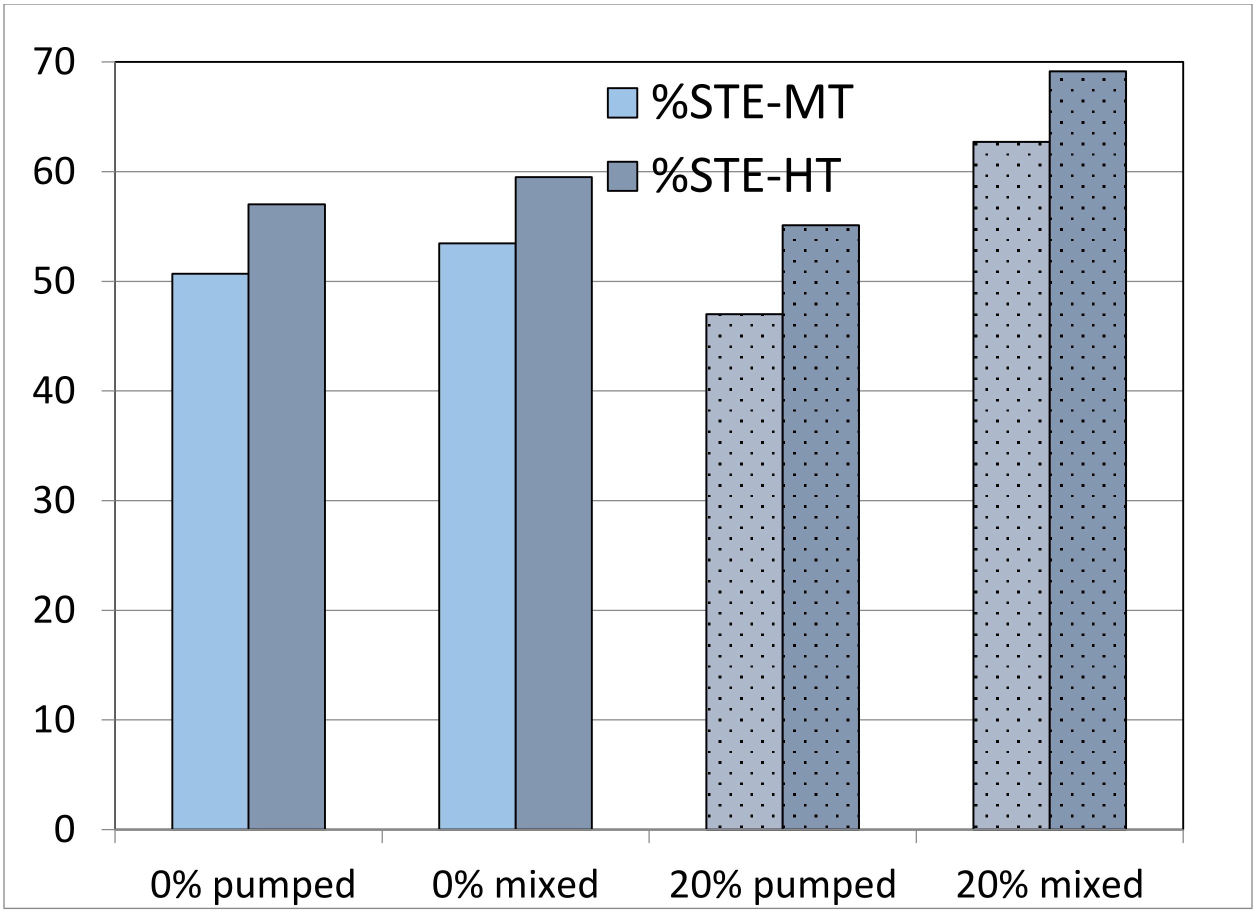

Figure 3.8 Percent of Specific Thermal Energy of Extruded Pet Foods

STE% for treatments with 0% and 20% fresh meat, obtained by meat and/ or fat premixed versus pumped into the preconditioner.

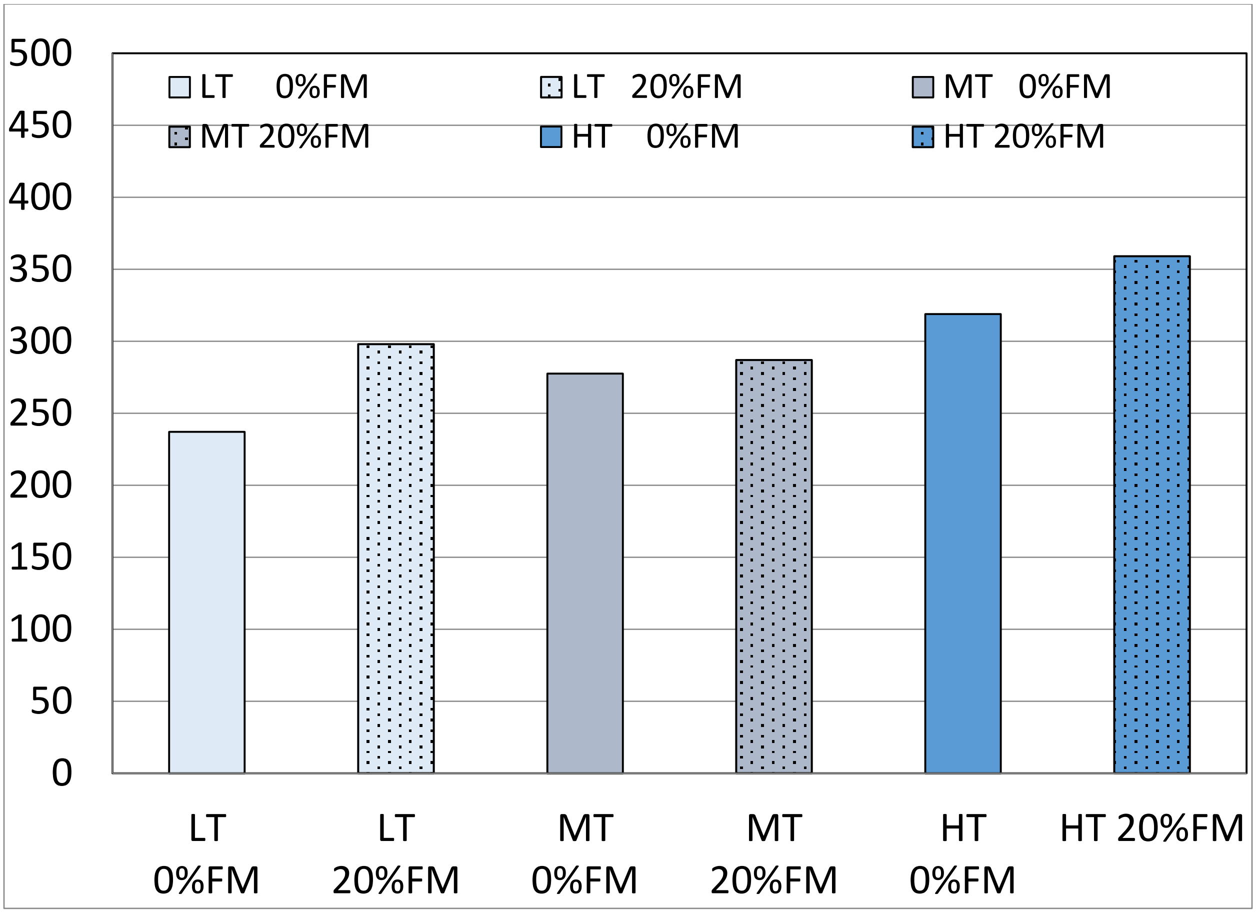

Figure 3.9 Total Specific Energy of Extruded Pet Foods

Total specific energy (STE+SME) for treatments with low (LT), medium (MT)and high (HT) thermal intensity and 0 and 20% fresh meat pumped.

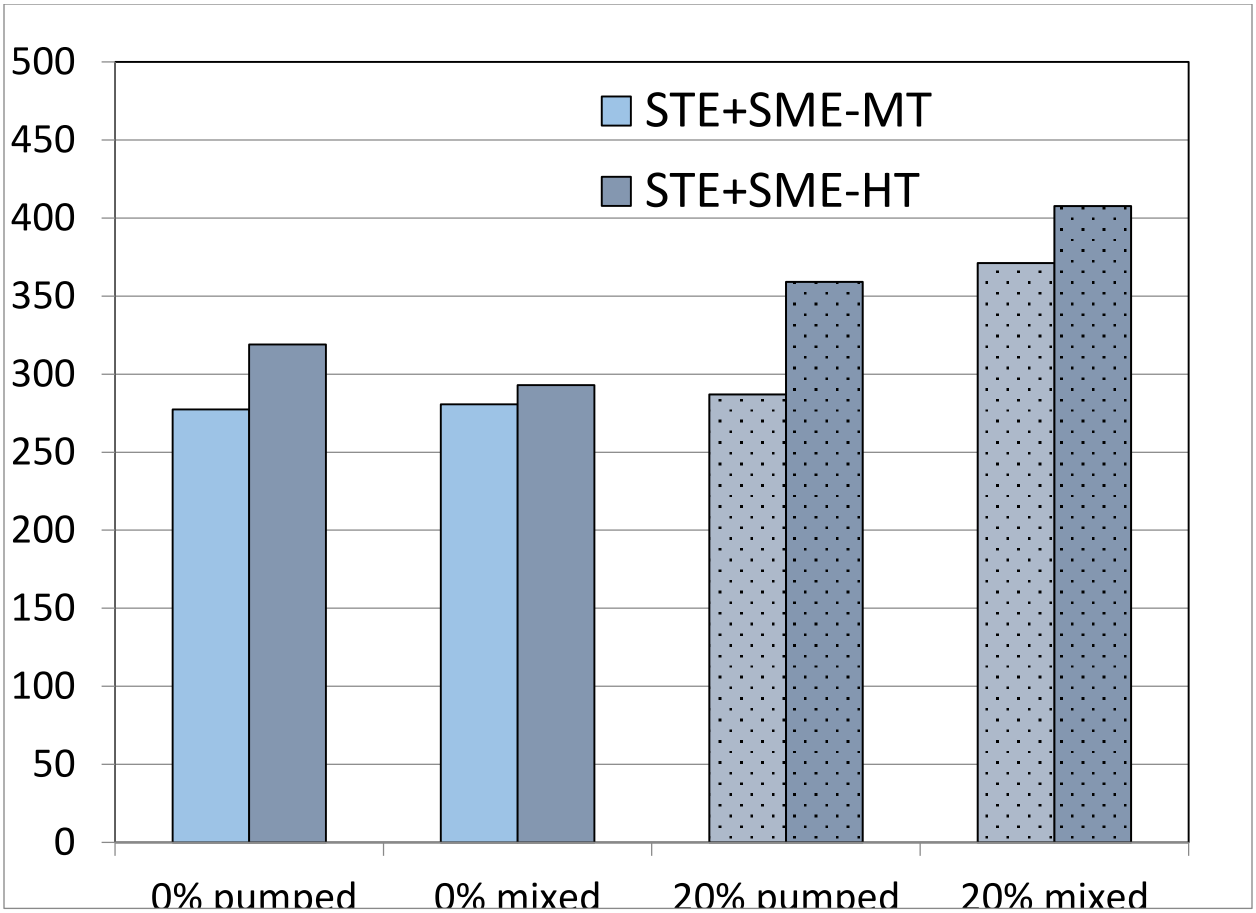

Figure 3.10 Total Specific Energy of Extruded Pet Foods

Total specific energy (STE+SME) for treatments with 0% and 20% fresh meat, obtained by meat and/ or fat premixed versus pumped into the preconditioner.

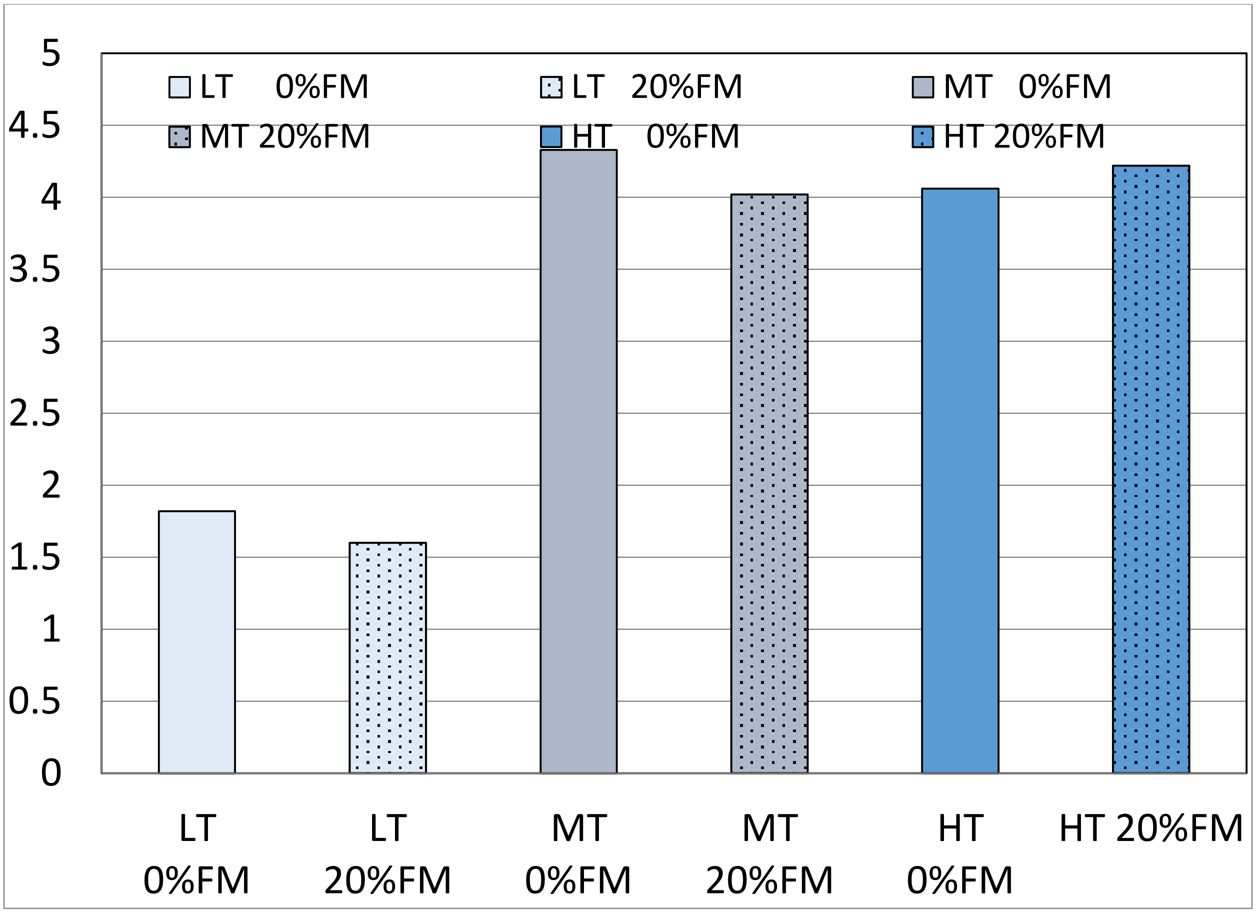

Figure 3.11 Expansion Ratio of Extruded Pet Foods
Radial expansion ratio for treatments with low (LT), medium (MT) and high (HT) thermal intensity and 0 and 20% fresh meat pumped.

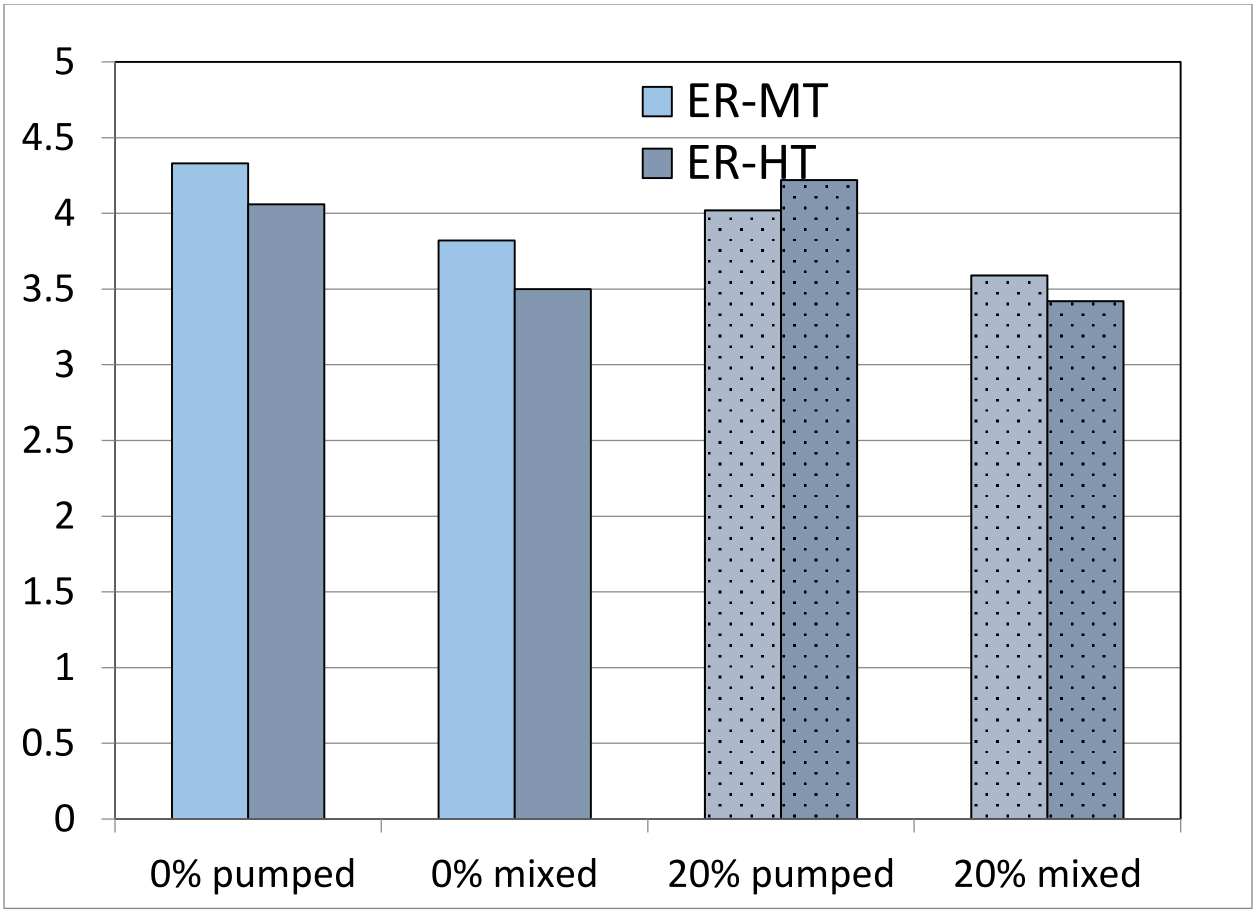

Figure 3.12 Expansion Ratio of Extruded Pet Foods

Radial expansion ratio for treatments with 0% and 20% fresh meat, obtained by meat and/ or fat premixed versus pumped into the preconditioner.

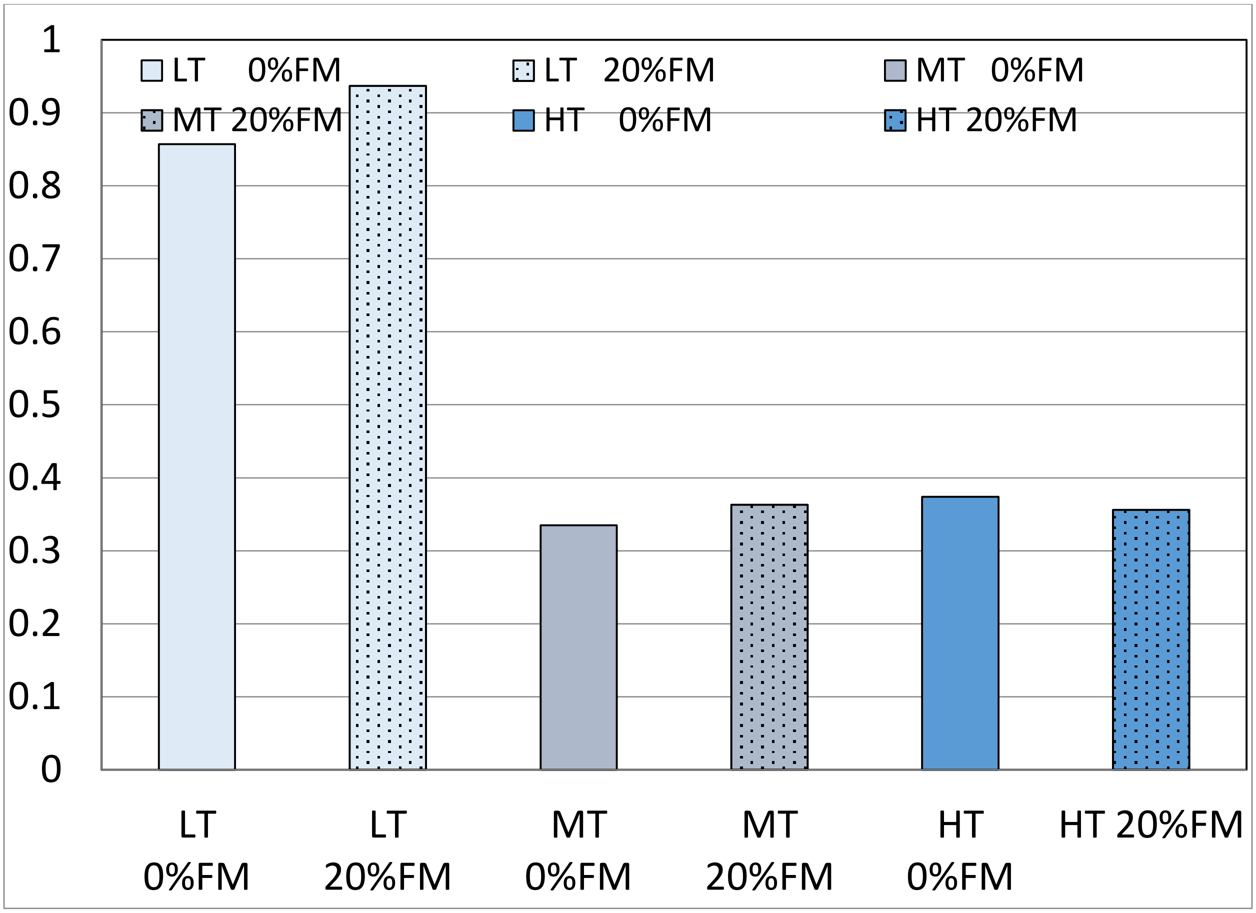

Figure 3.13 Piece Density of Extruded Pet Foods

Piece density (g/cm^3) for treatments with low (LT), medium (MT)and high (HT) thermal intensity and 0 and 20% fresh meat pumped.

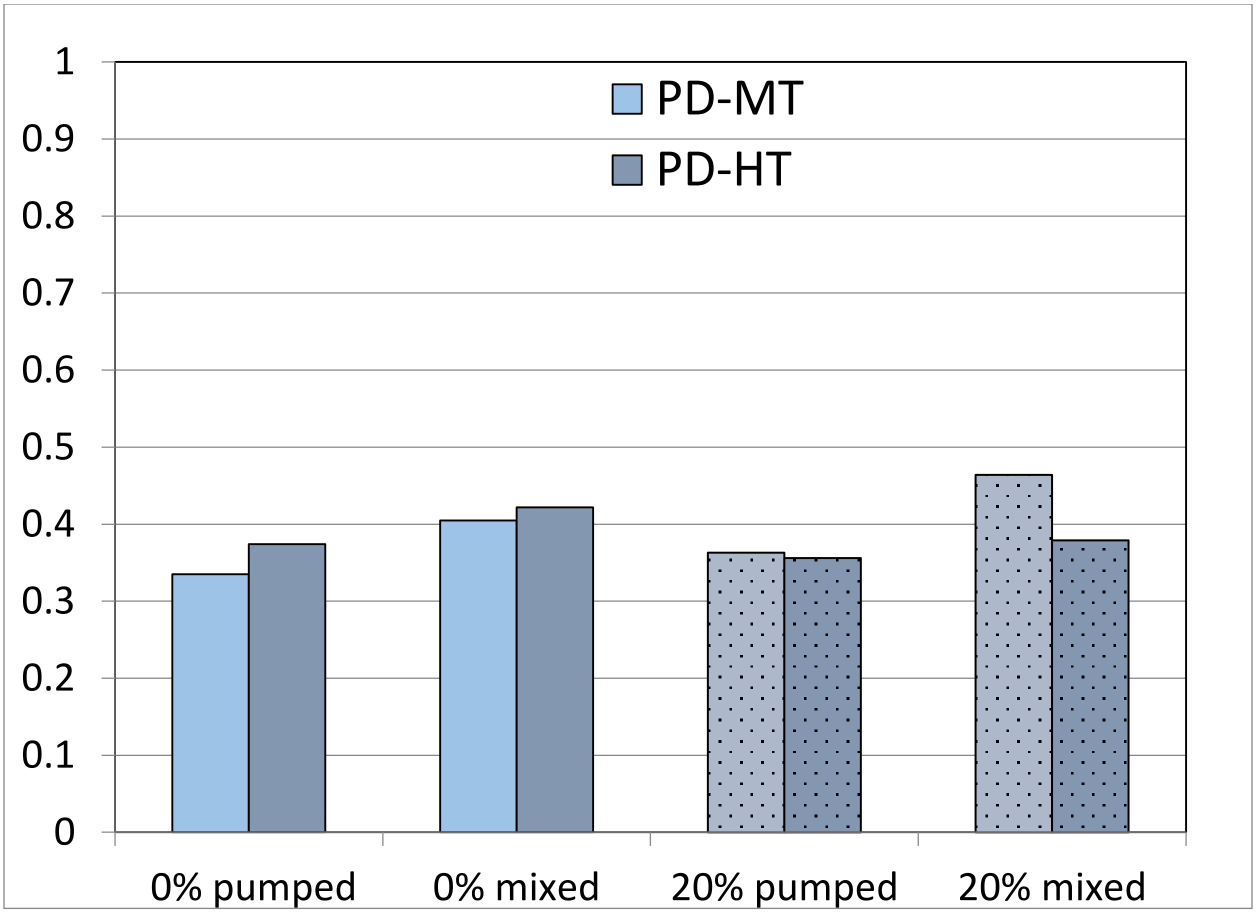

Figure 3.14 Piece Density of Extruded Pet Foods

Piece density (g/cm^3) for treatments with 0% and 20% fresh meat, obtained by meat and/ or fat premixed versus pumped into the preconditioner.

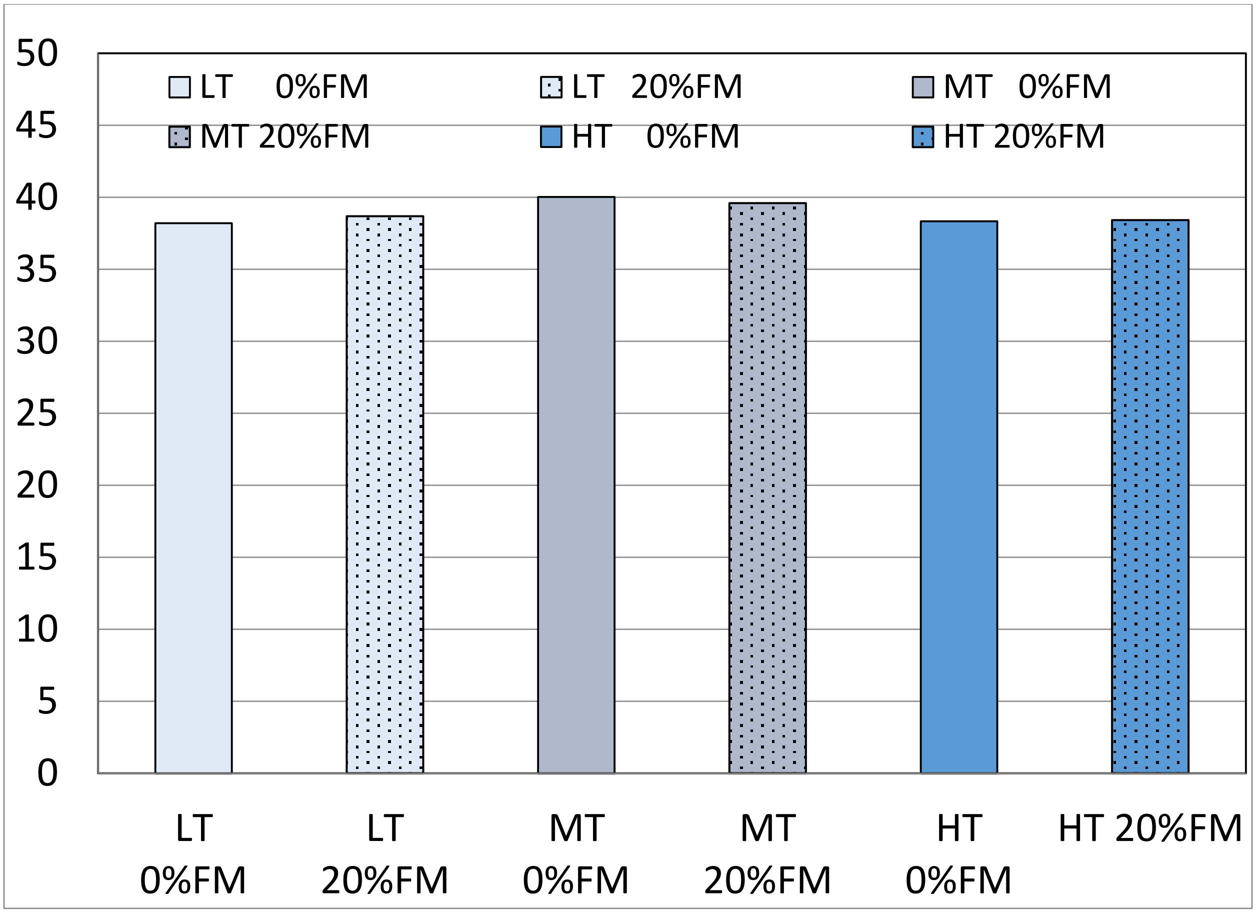

Figure 3.15 Specific Length of Extruded Pet Foods

Specific length (mm/g) for treatments with low (LT), medium (MT), and high (HT) thermal intensity and 0 and 20% fresh meat pumped.

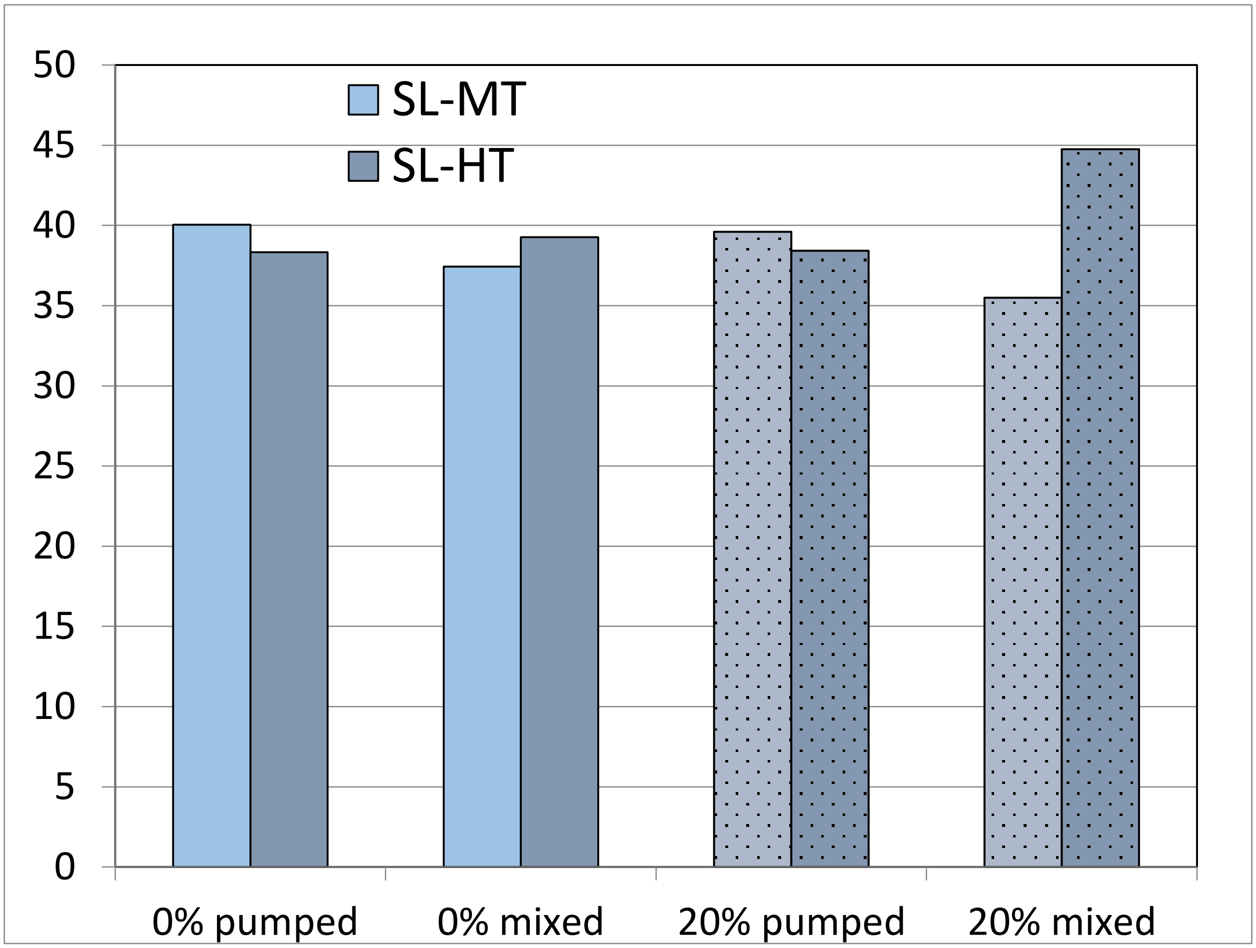

Figure 3.16 Specific Length of Extruded Pet Foods

Specific length (mm/g) for treatments with 0% and 20% fresh meat, obtained by meat and/ or fat premixed versus pumped into the preconditioner.

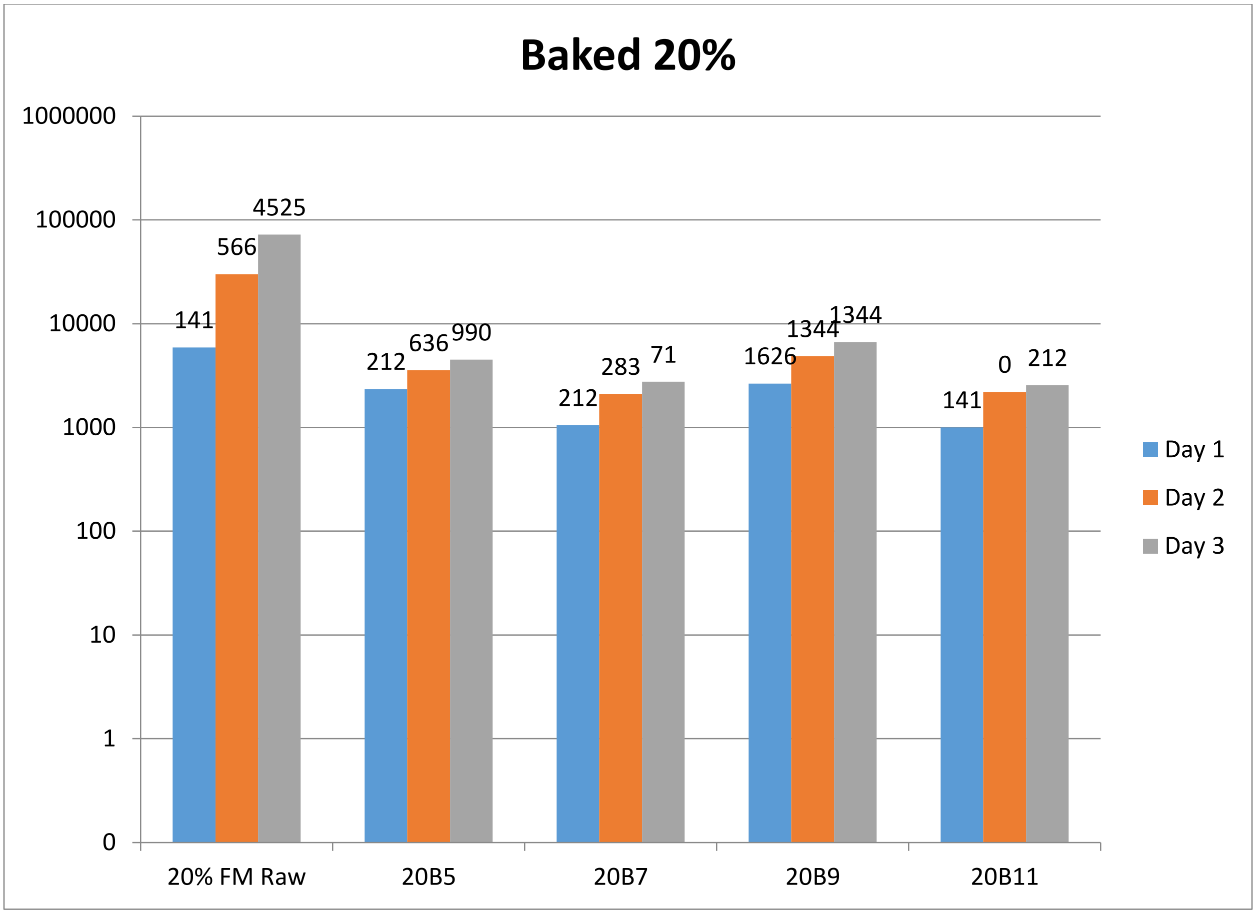

Figure 3.17 Aerobic Plate Count Results for 20% FM Baked Pet Foods

APC results for 20% fresh meat formulations of Raw and Baked products,5, 7, 9, and 11 mins. Standard deviation for each treatment is displayed above the data point.

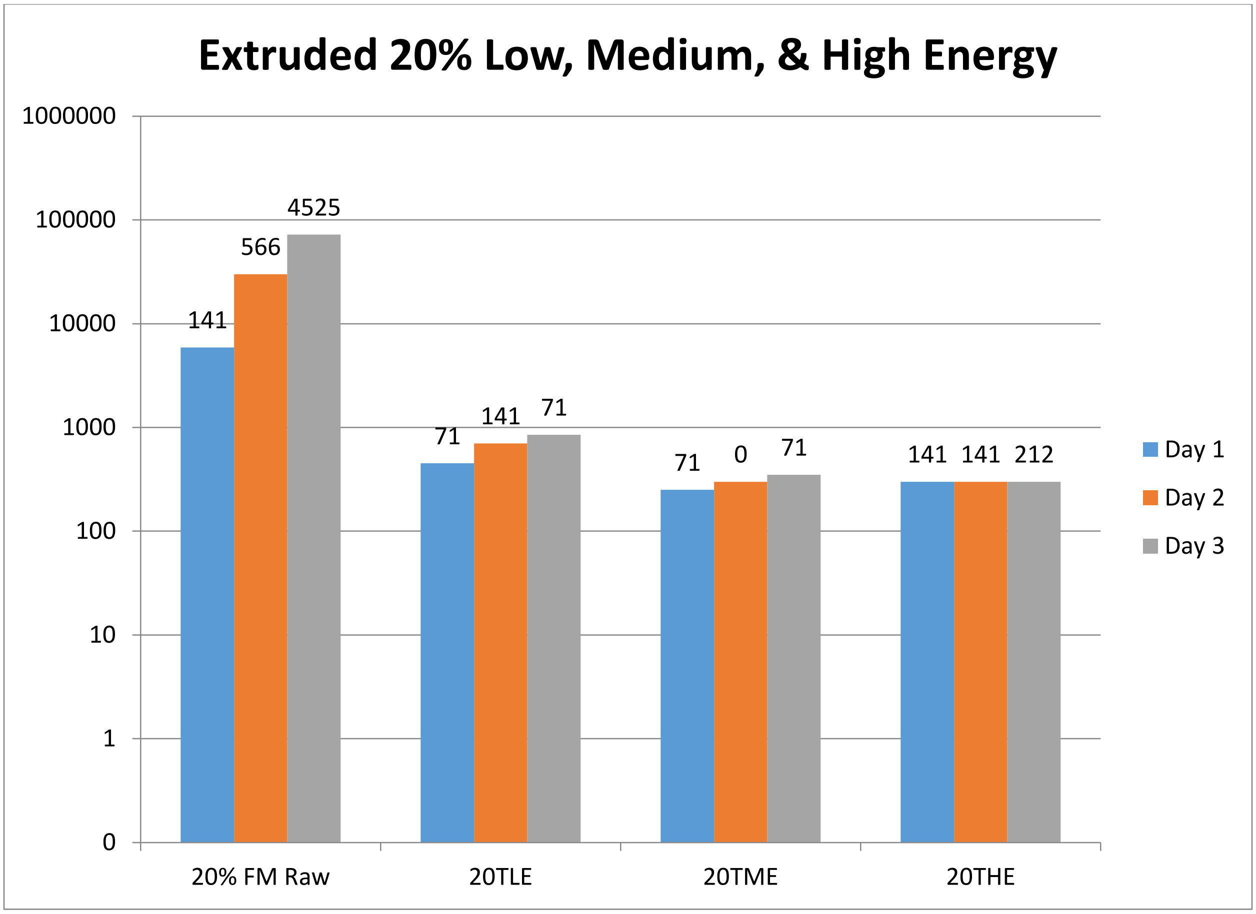

Figure 3.18 Aerobic Plate Count Results for 20% FM Extruded Pet Foods

APC results for 20% fresh meat formulations of Raw and Extruded products, low, medium, and high energy.

Standard deviation for each treatment is displayed above the data point.

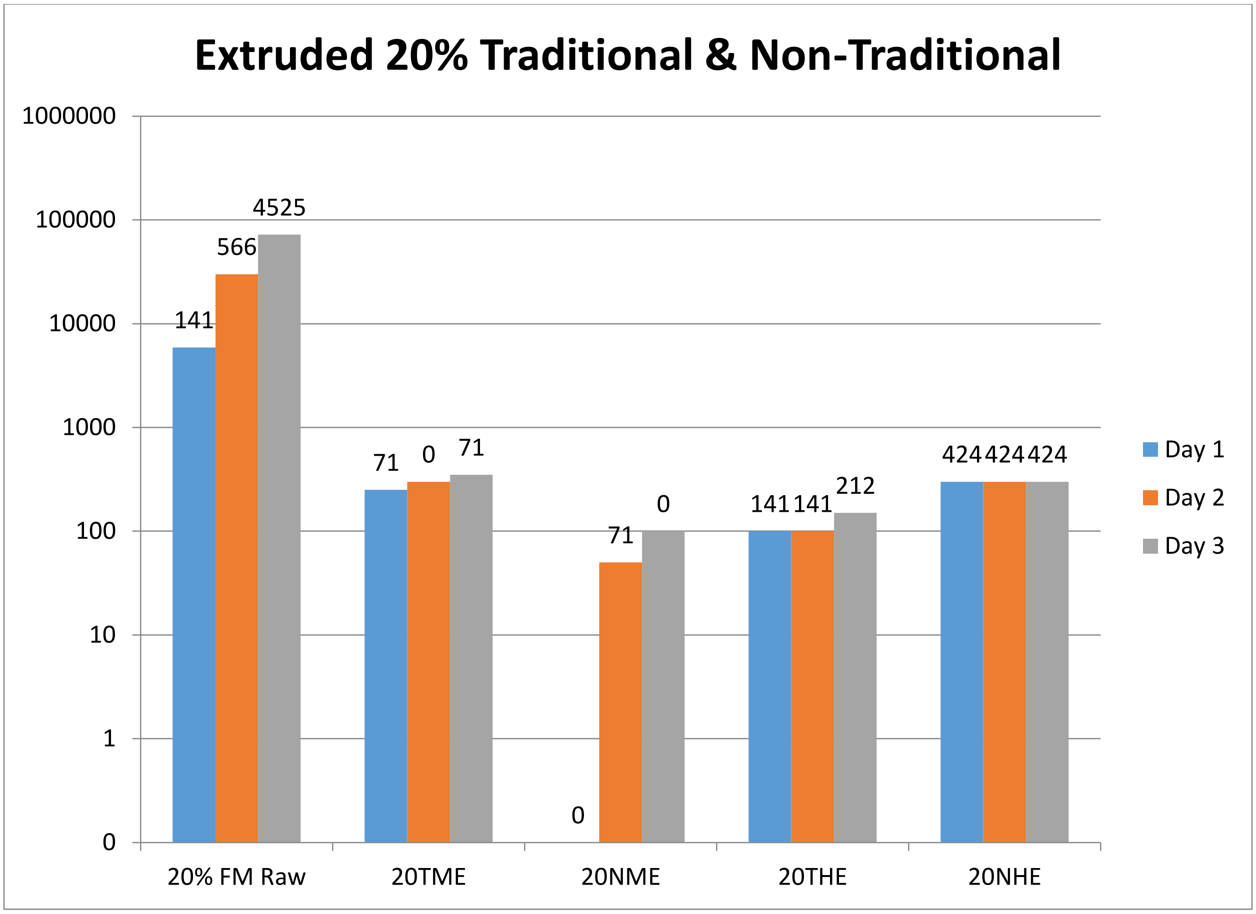

Figure 3.19 Aerobic Plate Count Results for 20% FM Extruded Pet Foods

APC results for 20% fresh meat formulations of Raw and Extruded products, traditional and non-traditional.

Standard deviation for each treatment is displayed above the data point.

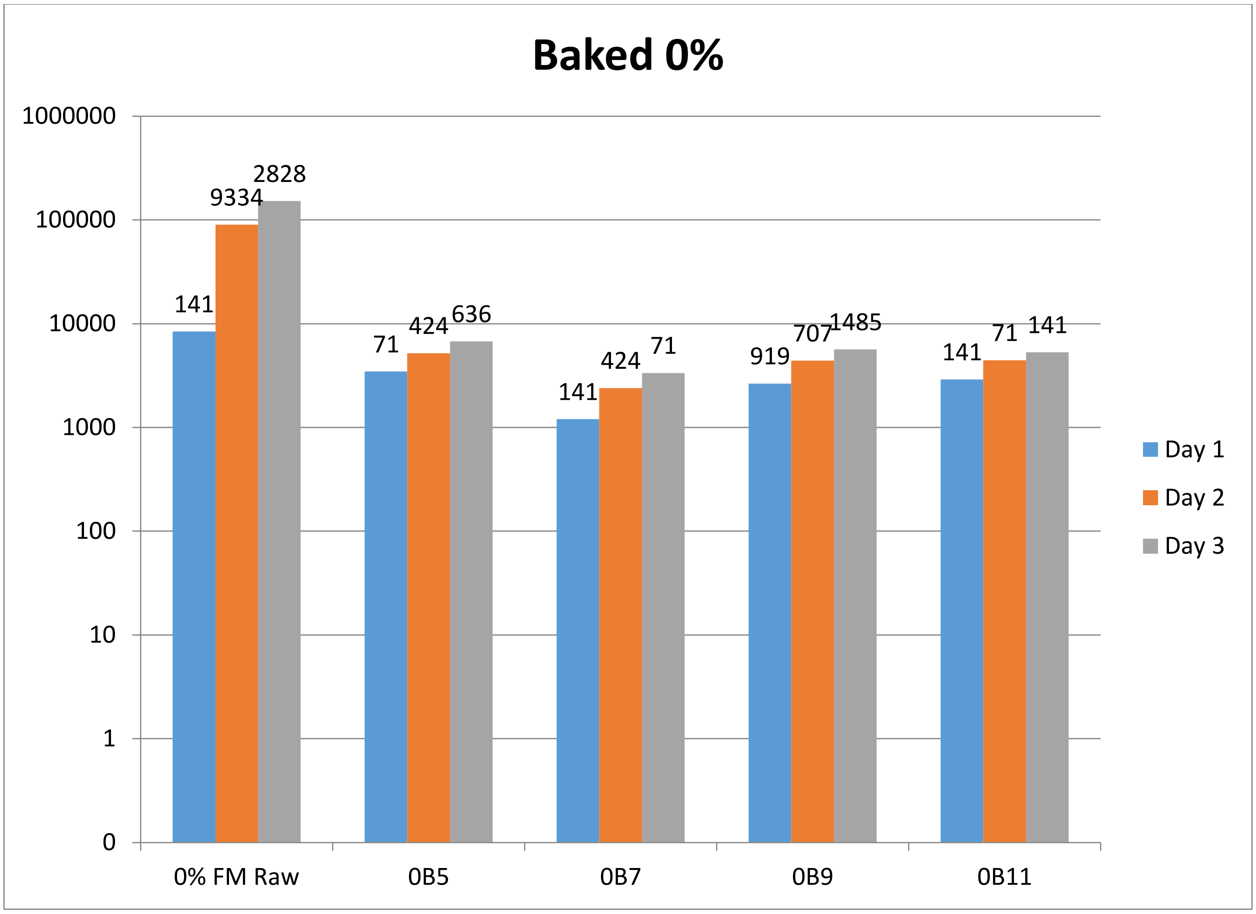

Figure 3.20 Aerobic Plate Count Results for 0% FM of Baked Pet Foods

APC results for 0% fresh meat formulations of Raw and Baked products,5, 7, 9, and 11 mins. Standard deviation for each treatment is displayed above the data point.

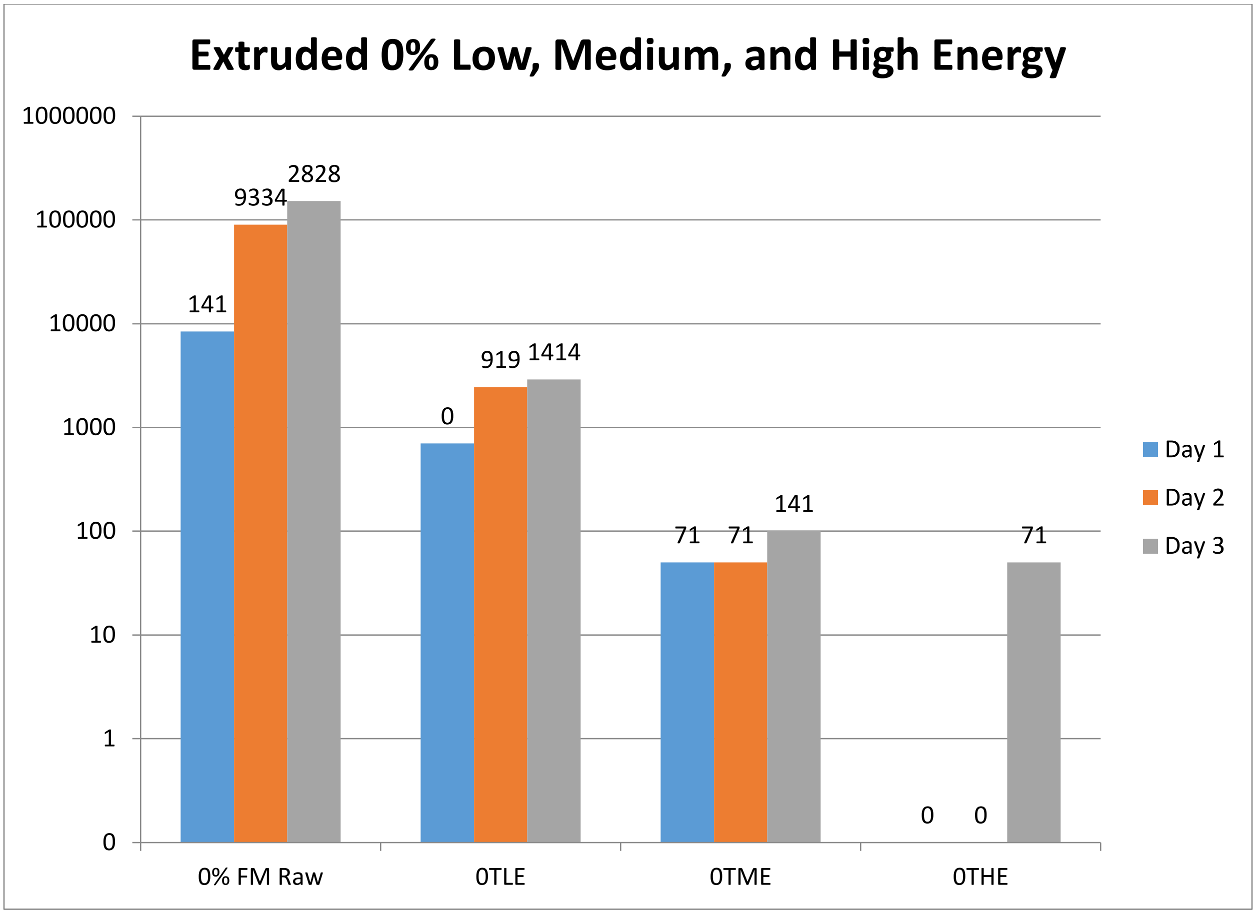

Figure 3.21 Aerobic Plate Count Results for 0% FM Extruded Pet Foods

APC results for 0% fresh meat formulations of Raw and Extruded products,low, medium and high energy.

Standard deviation for each treatment is displayed above the data point.

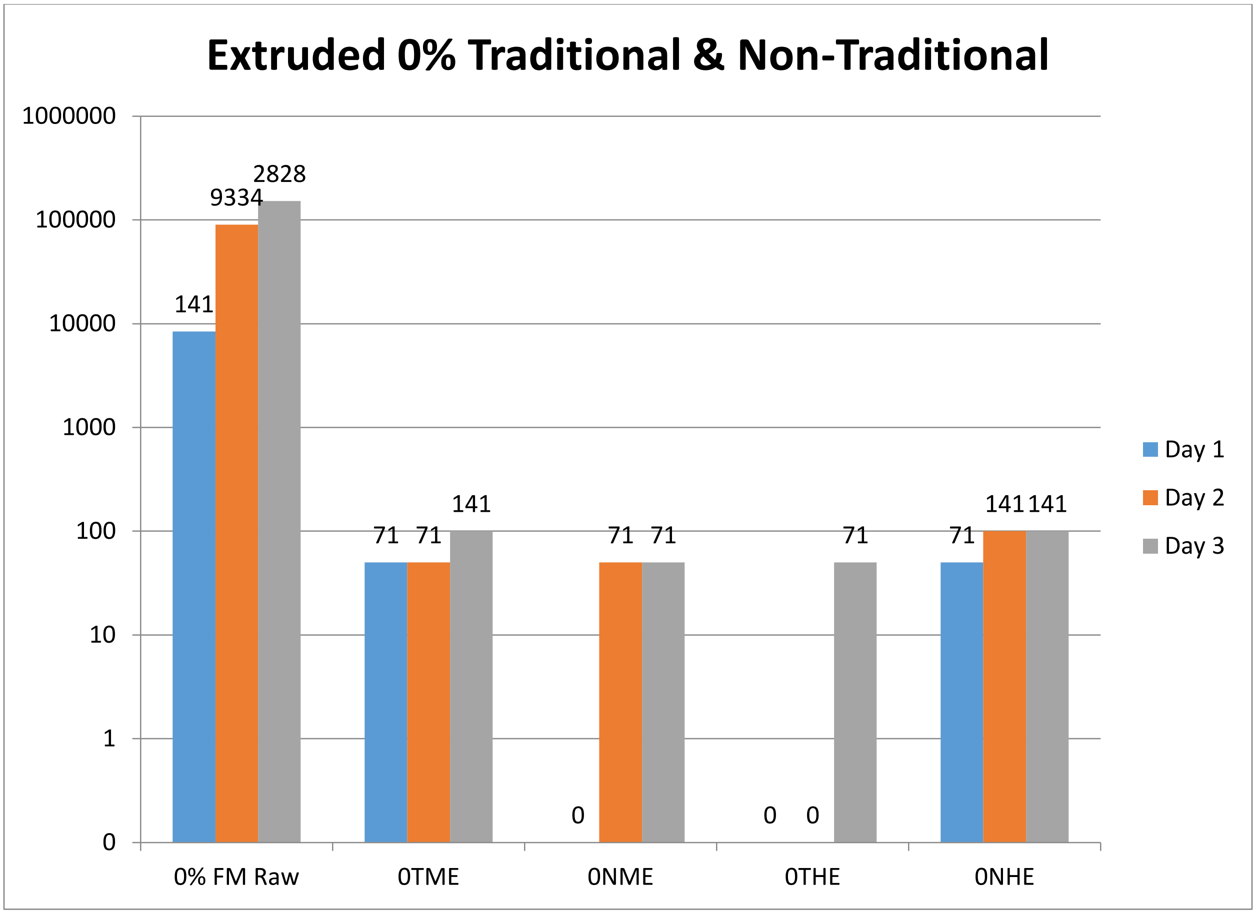

Figure 3.22 Aerobic Plate Count Results for 0% FM Extruded Pet Foods

APC results for 0% fresh meat formulations of Raw and Extruded products, traditional and non-traditional.

Standard deviation for each treatment is displayed above the data point.

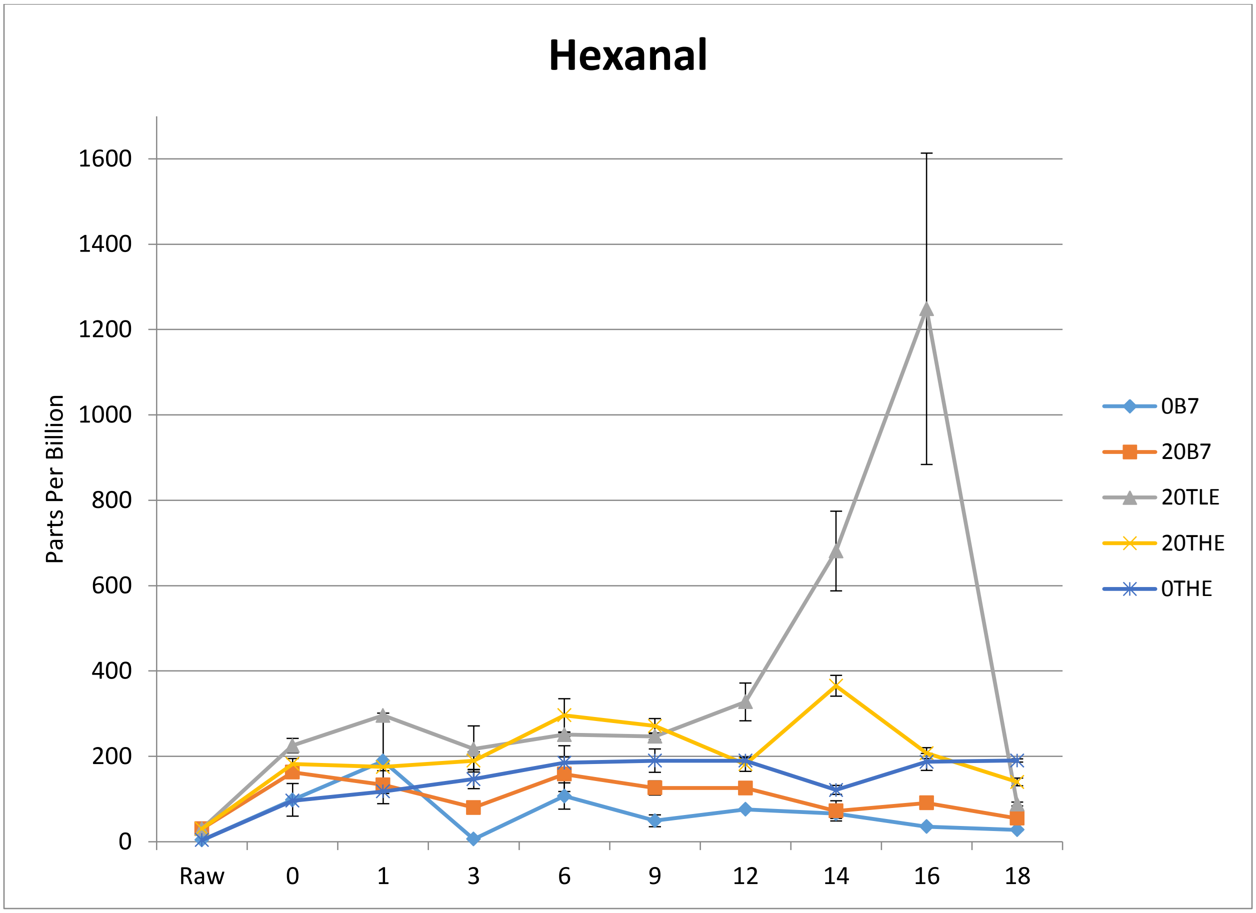

Figure 3.23 Accelerated Time Shelf Life Results for Hexanal

Gas Chromatography shelf life results for secondary oxidation product Hexenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time. Standard deviation(+/-) for each treatment is displayed on the data point.

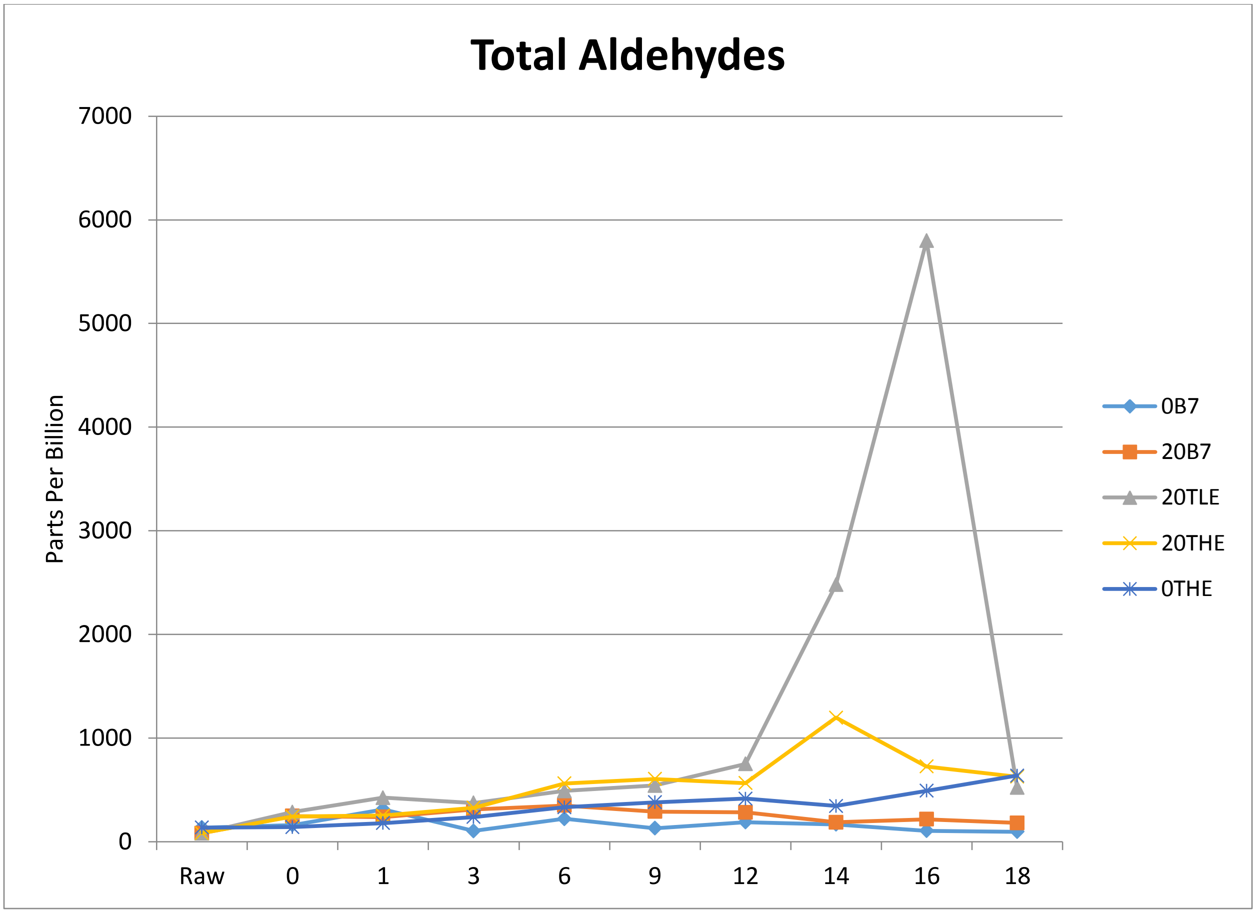

Figure 3.24 Accelerated Time Shelf Life Results for Total Aldehydes

Gas Chromatography shelf life results for Total Aldehydes of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time.

Appendix A - Additional Accelerated Shelf Life Tables with Statistics

Table A-1. Gas Chromatography shelf life results for secondary oxidation product 2-Hexenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 18 months of accelerated time.

2-Hexenal		**Product**				
		0B7	*0THE*	*20B7*	*20LTE*	*20THE*
Time	*Initial (0)*	3.08^{Aa}	1.53^{Aa}	1.90^{Ab}	1.33^{Aa}	1.11^{Aa}
	1 Month	8.29^{Aa}	0.00^{Aa}	11.93^{Ab}	2.30^{Aa}	0.00^{Aa}
	3 Month	59.59^{ABa}	0^{Ba}	146.42^{Aa}	0^{Ba}	0^{Ba}
	6 Month	0^{Aa}	0^{Aa}	0.70^{Ab}	1.25^{Aa}	0.59^{Aa}
	9 Month	0^{Aa}	0.87^{Aa}	0.84^{Ab}	0.77^{Aa}	0.94^{Aa}
	12 Month	0^{Aa}	0^{Aa}	0^{Ab}	0^{Aa}	0^{Aa}
	14 Month	0^{Aa}	0^{Aa}	0^{Ab}	0^{Aa}	0^{Aa}
	16 Month	0^{Aa}	0^{Aa}	0^{Ab}	0^{Aa}	0^{Aa}
	18 Month	0^{Aa}	0^{Aa}	0^{Ab}	0^{Aa}	0^{Aa}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).

Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table A-2. Gas Chromatography shelf life results for secondary oxidation product Heptanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 18 months of accelerated time.

Heptanal		**Product**				
		0B7	*0THE*	*20B7*	*20LTE*	*20THE*
Time	*Initial (0)*	11.28^{Aa}	9.15^{Aa}	16.98^{Ab}	9.76^{Ac}	12.14^{Ab}
	1 Month	24.80^{Aa}	12.55^{Aa}	20.01^{Aa}	26.61^{Ac}	17.57^{Aab}
	3 Month	3.41^{Aa}	20.09^{Aa}	9.14^{Aa}	28.34^{Ac}	29.24^{Aab}
	6 Month	20.25^{Aa}	30.88^{Aa}	33.33^{Aa}	46.38^{Ac}	53.14^{Aab}
	9 Month	12.83^{Aa}	35.88^{Aa}	29.34^{Aa}	30.46^{Ac}	55.60^{Aab}
	12 Month	17.61^{Aa}	40.98^{Aa}	30.12^{Aa}	56.79^{Ac}	51.97^{Aab}
	14 Month	14.429^{Ca}	36.49^{BCa}	17.65^{BCa}	201.27^{Ab}	99.98^{Ba}
	16 Month	9.44^{Ba}	44.64^{Ba}	21.76^{Ba}	506.63^{Ba}	61.90^{Bab}
	18 Month	8.31^{Aa}	53.180^{Aa}	16.49^{Aa}	43.31^{Ac}	48.84^{Aab}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table A-3. Gas Chromatography shelf life results for secondary oxidation product Heptanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 18 months of accelerated time.

2-Heptanal		**Product**				
		0B7	*0THE*	*20B7*	*20LTE*	*20THE*
Time	*Initial (0)*	12.94^{Aa}	3.06^{Aa}	12.20^{Aa}	6.27^{Ac}	4.41^{Aa}
	1 Month	10.39^{Aa}	4.12^{Aa}	10.54^{Aa}	8.31^{Ac}	4.65^{Aa}
	3 Month	3.22^{Aa}	5.39^{Aa}	7.38^{Aa}	9.74^{Ac}	7.30^{Aa}
	6 Month	6.44^{Aa}	6.85^{Aa}	10.76^{Aa}	8.92^{Ac}	10.39^{Aa}
	9 Month	6.34^{Aa}	7.91^{Aa}	12.63^{Aa}	11.94^{Ac}	10.94^{Aa}
	12 Month	7.63^{Aa}	7.06^{Aa}	11.81^{Aa}	18.49^{Abc}	9.57^{Aa}
	14 Month	4.09^{Ba}	6.18^{Ba}	4.79^{Ba}	37.86^{Abc}	16.46^{Ba}
	16 Month	4.06^{Ba}	7.42^{Ba}	8.36^{Ba}	47.62^{Aab}	9.47^{Ba}
	18 Month	2.62^{Aa}	8.65^{Aa}	4.48^{Aa}	7.12^{Ac}	6.45^{Aa}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table A-4. Gas Chromatography shelf life results for secondary oxidation product Benzaldehyde of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 18 months of accelerated time.

Benzaldehyde		**Product**				
		0B7	*0THE*	*20B7*	*20LTE*	*20THE*
Time	*Initial (0)*	11.38^{Aa}	12.92^{Ab}	16.82^{Aa}	14.78^{Ac}	14.54^{Ad}
	1 Month	38.17^{Aa}	20.67^{Ab}	30.64^{Aa}	35.68^{Ac}	21.07^{Ad}
	3 Month	6.30^{Aa}	27.03^{Ab}	23.98^{Aa}	46.23^{Ac}	35.04^{Acd}
	6 Month	42.68^{Aa}	38.92^{Aab}	60.58^{Aa}	52.53^{Ac}	57.59^{Abcd}
	9 Month	26.05^{Aa}	52.32^{Aab}	54.41^{Aa}	47.97^{Ac}	68.98^{Abcd}
	12 Month	52.93^{Aa}	54.31^{Aab}	62.19^{Aa}	100.43^{Ac}	113.88^{Abc}
	14 Month	45.63^{Aa}	42.07^{Cab}	45.01^{Ca}	369.94^{Ab}	226.32^{Ba}
	16 Month	37.55^{Aa}	68.82^{Bab}	46.87^{Ba}	886.57^{Aa}	127.66^{Bbc}
	18 Month	37.67^{Aa}	127.58^{Ab}	60.65^{Aa}	101.73^{Ac}	114.14^{Abc}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).

Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table A-5. Gas Chromatography shelf life results for secondary oxidation product Octanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 18 months of accelerated time.

Octanal		**Product**				
		0B7	*0THE*	*20B7*	*20LTE*	*20THE*
	Initial (0)	7.79^{Aa}	7.23^{Aa}	11.52^{Aa}	9.40^{Ac}	10.97^{Ab}
	1 Month	14.96^{Aa}	10.21^{Aa}	13.15^{Aa}	23.82^{Ac}	15.15^{Ab}
	3 Month	11.40^{Aa}	18.20^{Aa}	18.19^{Aa}	32.03^{Ac}	31.60^{Ab}
	6 Month	18.73^{Aa}	33.18^{Aa}	36.80^{Aa}	58.19^{Ac}	66.90^{Aab}
Time	*9 Month*	14.05^{Aa}	42.74^{Aa}	34.09^{Aa}	57.24^{Ac}	83.77^{Aab}
	12 Month	15.75^{Aa}	54.65^{Aa}	30.57^{Aa}	91.86^{Ac}	83.17^{Aab}
	14 Month	16.61^{Ca}	58.52^{BCa}	19.79^{Ca}	421.41^{Ab}	192.23^{Ba}
	16 Month	7.78^{Ba}	74.27^{Ba}	21.66^{Ba}	1117.70^{Aa}	124.80^{Bab}
	18 Month	7.68^{Aa}	101.16^{Aa}	18.81^{Aa}	101.06^{Ac}	115.40^{Aab}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table A-6. Gas Chromatography shelf life results for secondary oxidation product 2-Octenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 18 months of accelerated time.

2-Octenal		**Product**				
		0B7	*0THE*	*20B7*	*20LTE*	*20THE*
Time	*Initial (0)*	5.03^{Aa}	2.35^{Aa}	6.28^{Aa}	4.27^{Ad}	3.47^{Ab}
	1 Month	5.74^{Aa}	2.22^{Aa}	5.71^{Aa}	5.10^{Acd}	2.52^{Ab}
	3 Month	1.96^{Aa}	3.00^{Aa}	4.51^{Aa}	6.94^{Acd}	4.47^{Aab}
	6 Month	4.18^{Aa}	6.23^{Aa}	8.40^{Aa}	12.06^{Acd}	11.84^{Aab}
	9 Month	2.07^{Aa}	9.62^{Aa}	8.90^{Aa}	18.29^{Acd}	18.75^{Aab}
	12 Month	2.91^{Ba}	9.93^{ABa}	7.21^{ABa}	37.97^{Ac}	22.15^{ABab}
	14 Month	2.11^{Ca}	9.57^{BCa}	3.43^{Ca}	116.71^{Ab}	37.39^{Ba}
	16 Month	0^{Ba}	13.89^{Ba}	3.67^{Ba}	249.34^{Aa}	26.09^{Bab}
	18 Month	1.47^{Aa}	18.87^{Aa}	3.21^{Aa}	24.06^{Acd}	25.05^{Aab}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).

Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table A-7. Gas Chromatography shelf life results for secondary oxidation product Nonanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 18 months of accelerated time.

Nonanal		**Product**				
		0B7	*0THE*	*20B7*	*20LTE*	*20THE*
Time	*Initial (0)*	13.49^{Aa}	8.43^{Aa}	17.89^{Aa}	14.36^{Ac}	13.58^{Ab}
	1 Month	20.15^{Aa}	11.35^{Aa}	13.21^{Aa}	26.59^{Ac}	15.30^{Ab}
	3 Month	12.05^{Aa}	17.69^{Aa}	21.28^{Aa}	32.65^{Ac}	30.01^{Ab}
	6 Month	21.28^{Aa}	30.95^{Aa}	39.60^{Aa}	58.20^{Ac}	64.21^{Ab}
	9 Month	18.91^{Aa}	40.61^{Aa}	22.82^{Aa}	73.25^{Ac}	92.65^{Ab}
	12 Month	16.14^{Aa}	56.54^{Aa}	15.40^{Aa}	108.91^{Ac}	98.99^{Aab}
	14 Month	18.30^{Ca}	67.95^{Ca}	23.79^{Ca}	613.50^{Ab}	248.74^{Ba}
	16 Month	10.68^{Ba}	90.88^{Ba}	23.76^{Ba}	1654.87^{Aa}	161.81^{Bab}
	18 Month	10.59^{Aa}	131.56^{Aa}	22.10^{Aa}	150.85^{Ac}	169.79^{Aab}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).

Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table A-8. Gas Chromatography shelf life results for secondary oxidation product Nonanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 18 months of accelerated time.

2-Nonenal		**Product**				
		0B7	*0THE*	*20B7*	*20LTE*	*20THE*
Time	*Initial (0)*	1.39^{Aa}	0.25^{Aa}	1.48^{Aa}	0.84^{Ac}	0.73^{Abc}
	1 Month	0.35^{Aa}	0^{Aa}	0.34^{Aa}	0.97^{Ac}	0^{Ac}
	3 Month	0^{Aa}	0^{Aa}	0.46^{Aa}	0.83^{Ac}	0.32^{Ac}
	6 Month	0^{Aa}	0.86^{Aa}	1.31^{Aa}	1.43^{Ac}	1.72^{Abc}
	9 Month	0.45^{Aa}	1.04^{Aa}	1.51^{Aa}	2.88^{Ac}	2.81^{Abc}
	12 Month	0^{Ba}	2.23^{ABa}	0^{Ba}	8.00^{Ac}	4.26^{ABabc}
	14 Month	0^{Ca}	2.69^{Ca}	0^{Ca}	39.24^{Ab}	10.39^{Ba}
	16 Month	0^{Ba}	3.63^{Ba}	0^{Ba}	86.36^{Aa}	6.69^{Babc}
	18 Month	0^{Ba}	5.99^{ABa}	0.91^{ABa}	7.44^{ABc}	7.94^{Aab}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).

Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Appendix B - Accelerated Shelf Life Figures

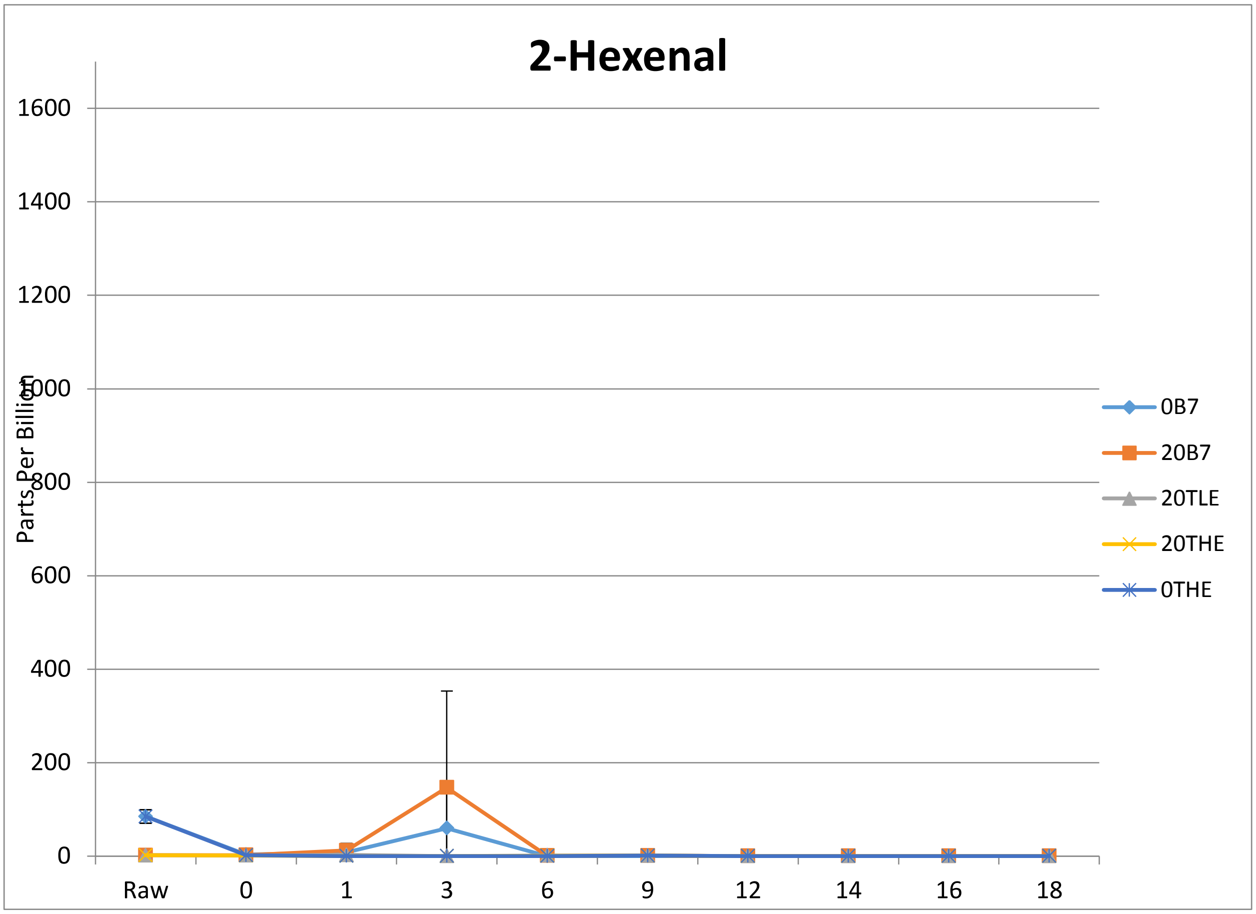

Figure B-1. Gas Chromatography shelf life results for secondary oxidation product 2-Hexenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time.

Standard deviation(+/-) for each treatment is displayed on the data point.

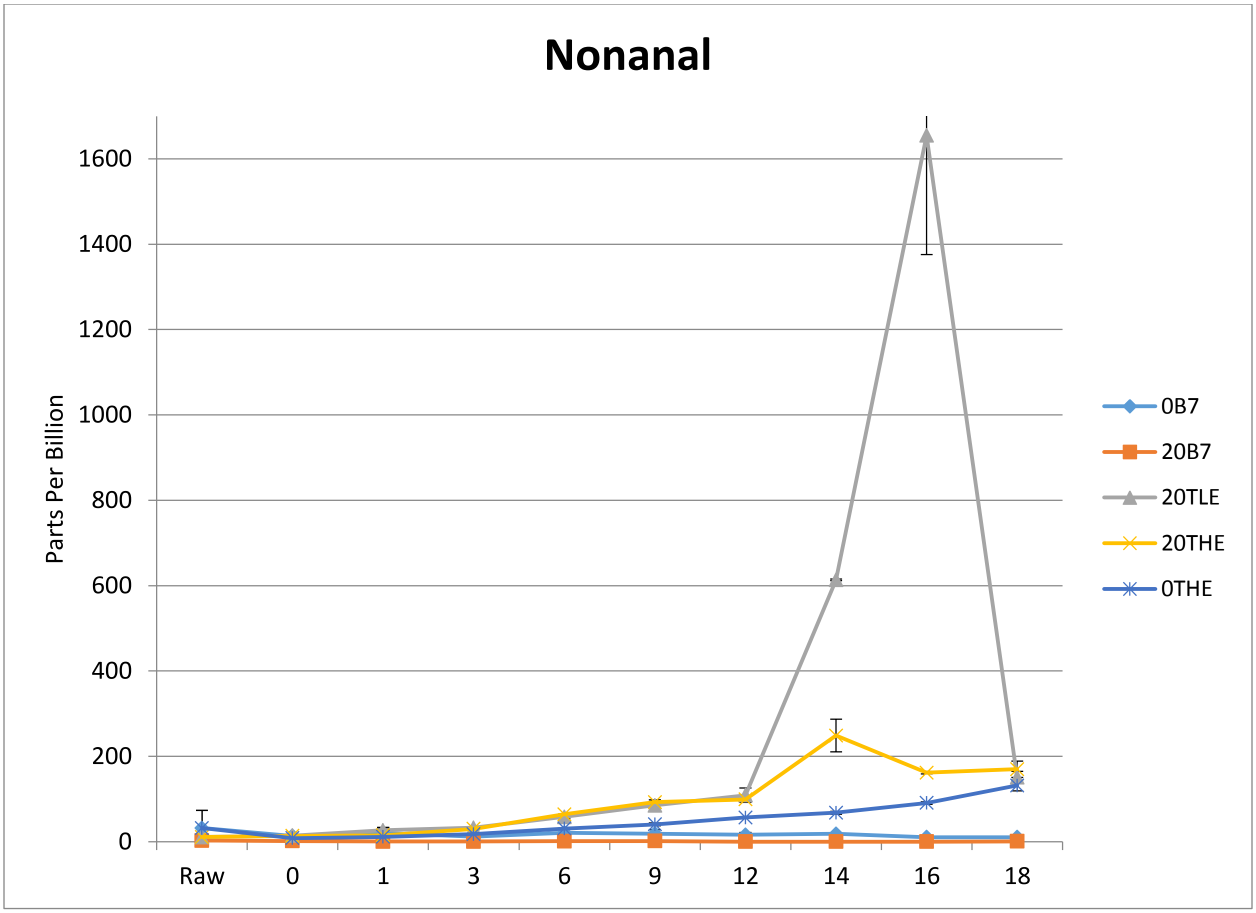

Figure B-2. Gas Chromatography shelf life results for secondary oxidation product Nonanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time.

Standard deviation(+/-) for each treatment is displayed on the data point.

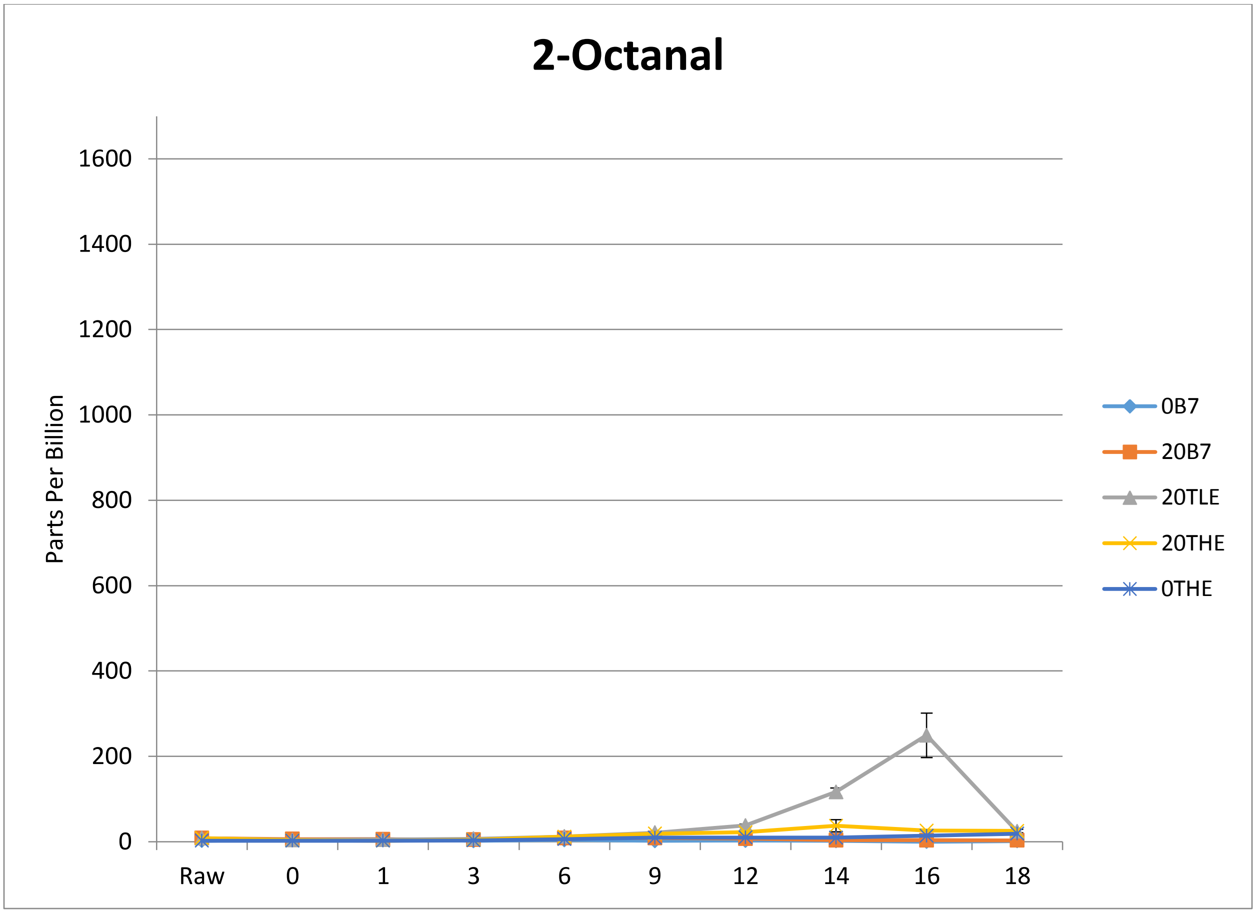

Figure B-3. Gas Chromatography shelf life results for secondary oxidation product 2-Octanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time.

Standard deviation(+/-) for each treatment is displayed on the data point.

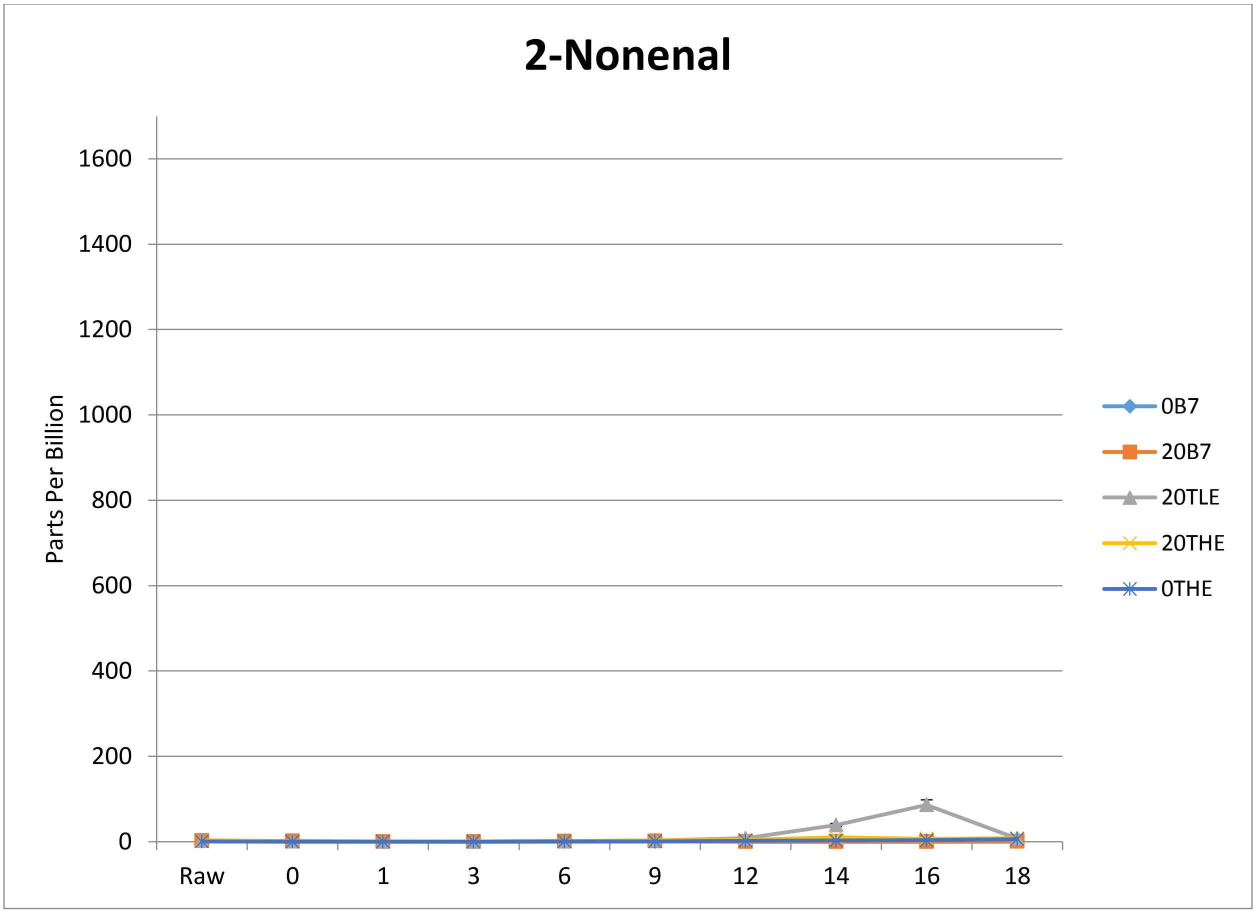

Figure B-4. Gas Chromatography shelf life results for secondary oxidation product 2-Nonenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time.

Standard deviation(+/-) for each treatment is displayed on the data point.

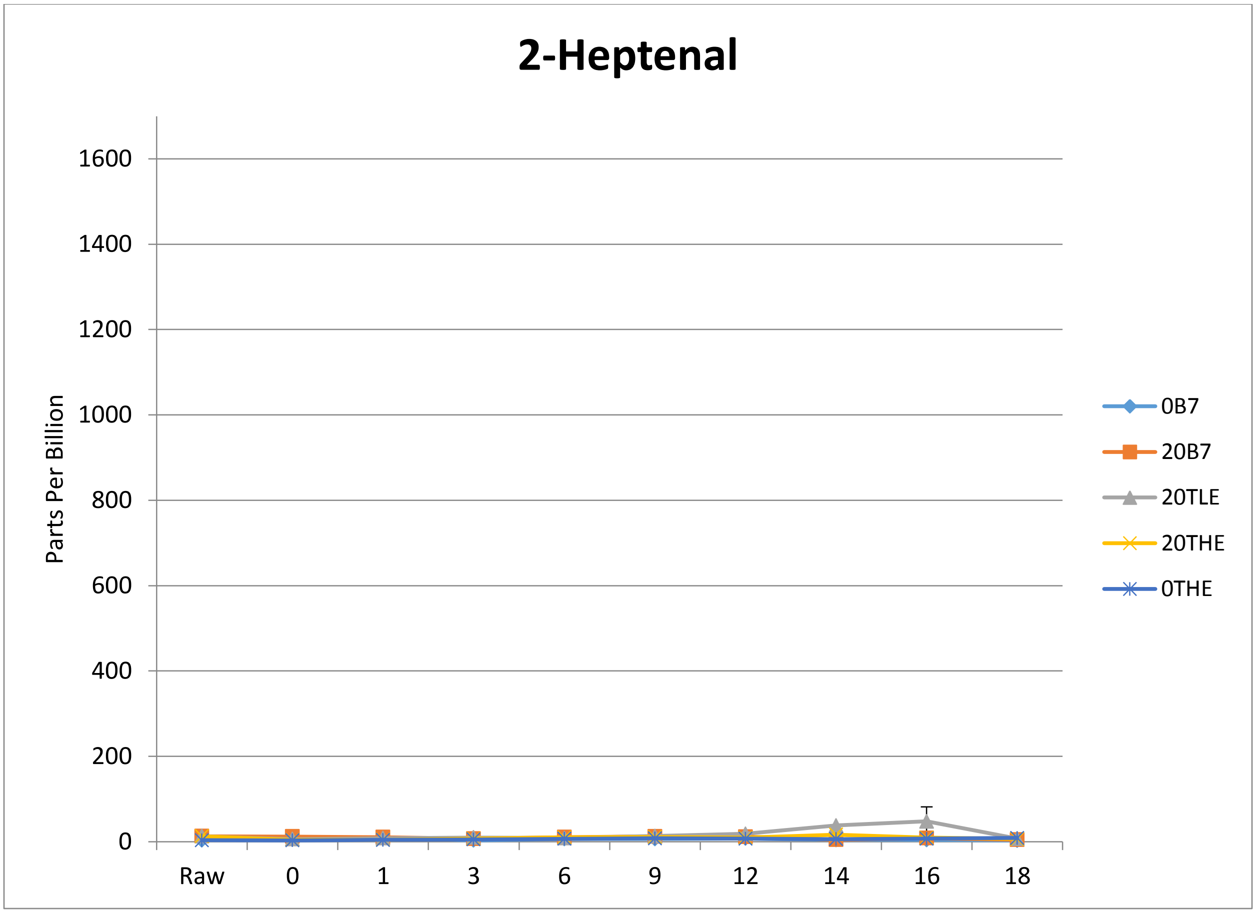

Figure B-5. Gas Chromatography shelf life results for secondary oxidation product 2-Heptenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time.

Standard deviation(+/-) for each treatment is displayed on the data point.

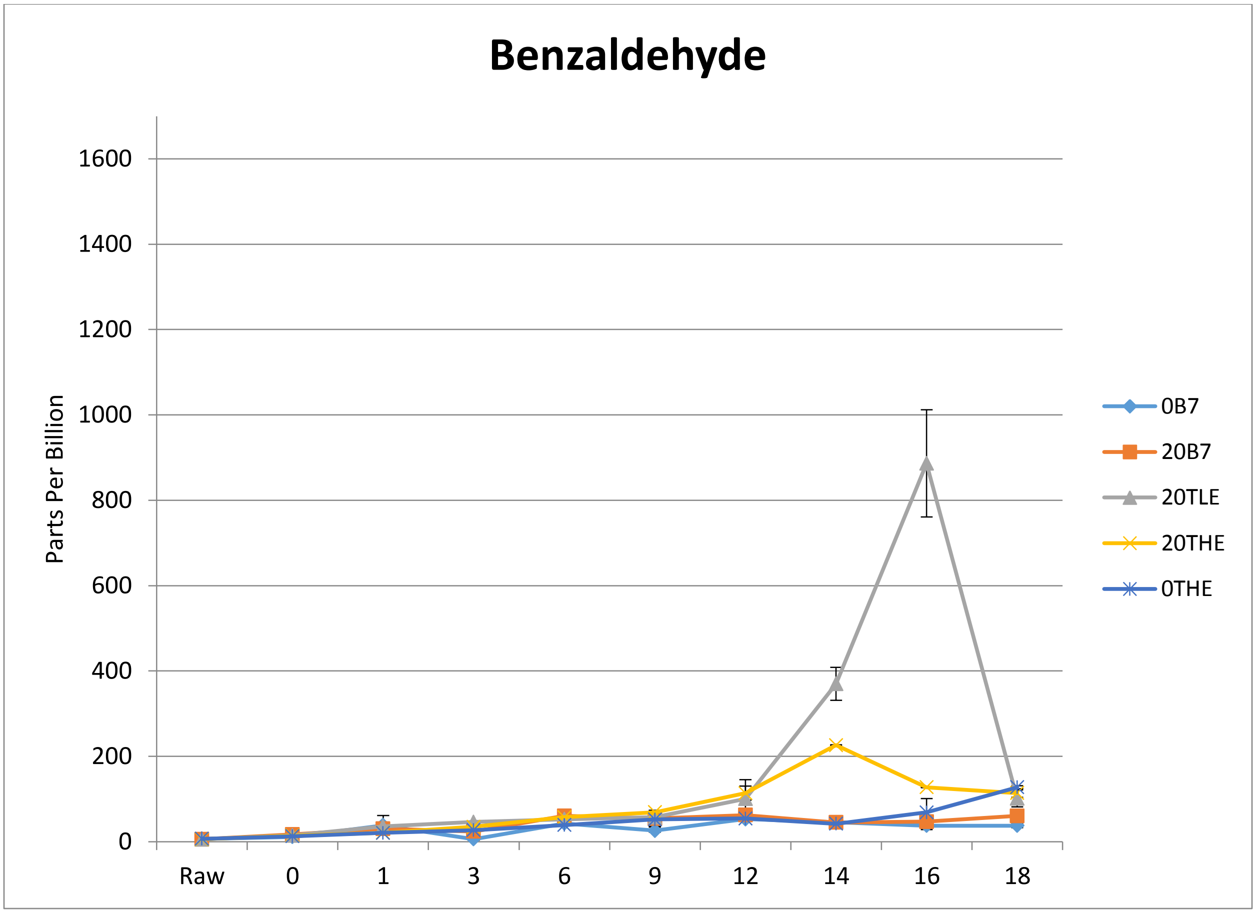

Figure B-6. Gas Chromatography shelf life results for secondary oxidation product Benzaldehyde of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time.

Standard deviation(+/-) for each treatment is displayed on the data point.

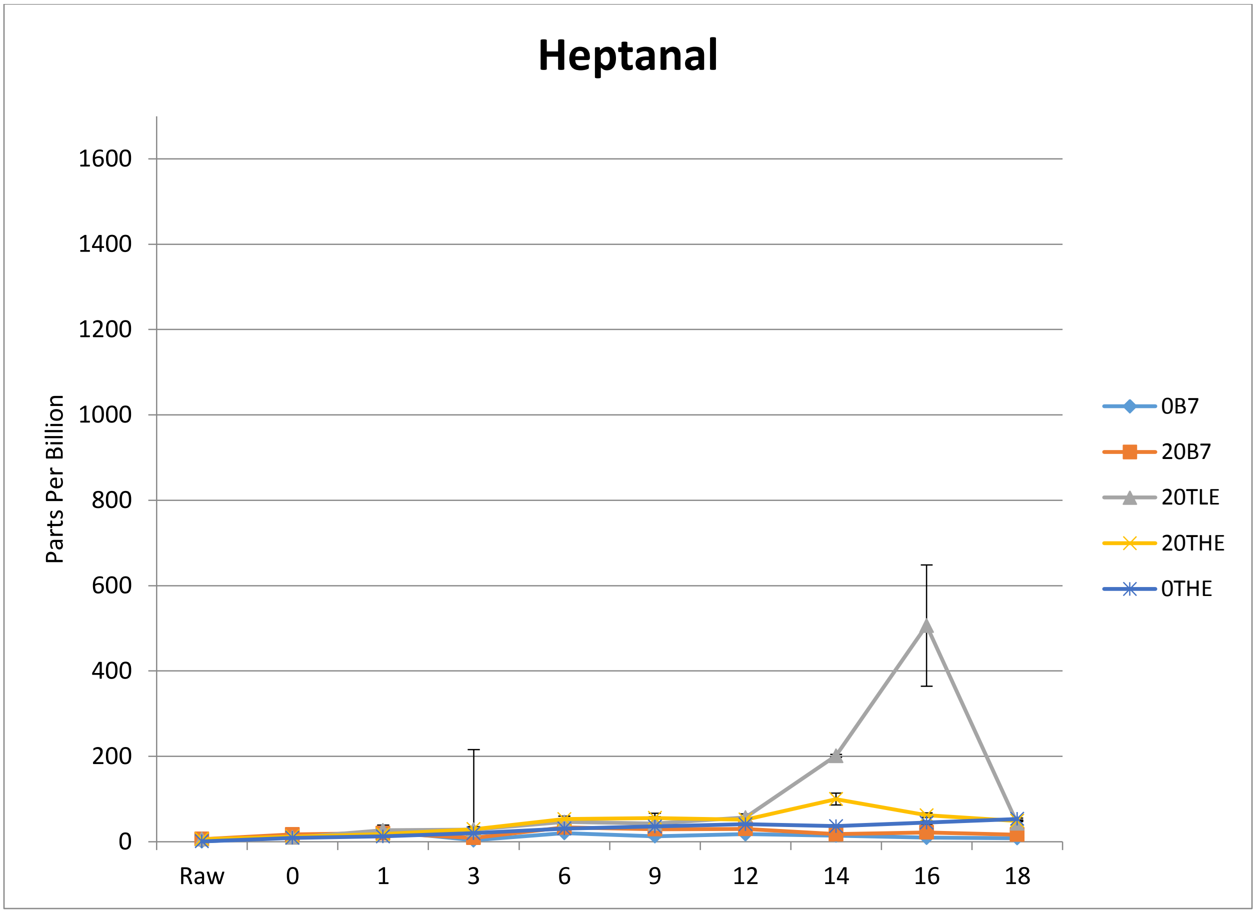

Figure B-7. Gas Chromatography shelf life results for secondary oxidation product Heptanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time.

Standard deviation(+/-) for each treatment is displayed on the data point.

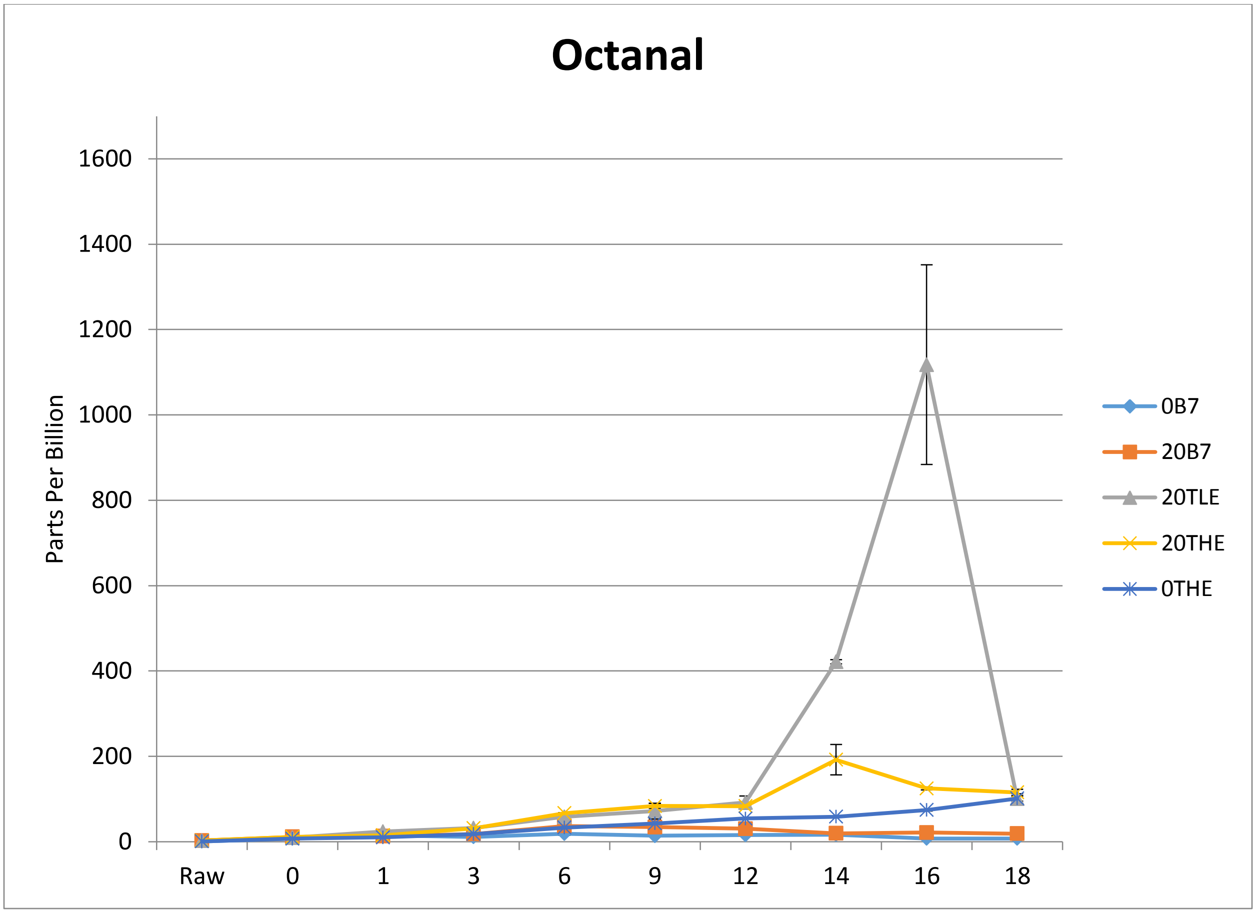

Figure B-8. Gas Chromatography shelf life results for secondary oxidation product Octanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 18 months accelerated time.

Standard deviation(+/-) for each treatment is displayed on the data point.

Appendix C - Real-Time Shelf Life Tables with Statistics

Table C-1. Gas Chromatography shelf life results for secondary oxidation product 2-Hexenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 6 months of real time.

2-Hexenal		**Product**				
		0B7	0THE	20B7	20TLE	20THE
Time	*Initial (0)*	3.08^{Aa}	1.53^{Aa}	1.90^{Aab}	1.33^{Aa}	1.11^{Aa}
	1 Month	0.52^{Ab}	0^{Aa}	0.67^{Ab}	0^{Aa}	0^{Aa}
	3 Month	0^{Aa}	0^{Aa}	1.38^{Ab}	0^{Aa}	0^{Aa}
	6 Month	0.43^{Bb}	0^{Ba}	4.10^{Aa}	0^{Ba}	0^{Ba}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table C-2. Gas Chromatography shelf life results for secondary oxidation product Heptanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 6 months of real time.

Heptanal		**Product**				
		0B7	0THE	20B7	20TLE	20THE
Time	*Initial (0)*	11.28^{Ab}	9.15^{Aa}	16.98^{Ab}	9.76^{Aa}	12.14^{Aa}
	1 Month	5.70^{Aa}	4.26^{Aa}	6.78^{Ab}	1.82^{Aa}	10.35^{Aa}
	3 Month	18.62^{Aa}	2.82^{Aa}	22.09^{Ab}	6.84^{Aa}	9.56^{Aa}
	6 Month	44.09^{Ba}	12.04^{Ca}	79.38^{Aa}	17.52^{Ca}	24.88^{BCa}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table C-3. Gas Chromatography shelf life results for secondary oxidation product 2-Heptanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 6 months of real time.

2-Heptanal		**Product**				
		0B7	0THE	20B7	20TLE	20THE
Time	*Initial (0)*	8.97^{Ab}	3.06^{Aa}	12.20^{Ab}	6.27^{Aa}	4.41^{Aa}
	1 Month	10.44^{Ab}	4.24^{Aa}	16.34^{Ab}	7.30^{Aa}	6.75^{Aa}
	3 Month	19.59^{ABab}	4.23^{Ba}	28.91^{Aab}	10.17^{ABa}	4.33^{Ba}
	6 Month	35.98^{Aa}	8.18^{Ba}	44.88^{Aa}	17.97^{Ba}	10.24^{Ba}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table C-4. Gas Chromatography shelf life results for secondary oxidation product Benzaldehyde of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 6 months of real time.

Benzaldehyde		**Product**				
		0B7	0THE	20B7	20TLE	20THE
Time	*Initial (0)*	11.38^Aa^	12.92^Aa^	16.82^Ab^	14.78^Aa^	14.54^Aa^
	1 Month	12.11^Aa^	9.15^Aa^	20.54^Ab^	13.12^Aa^	16.28^Aa^
	3 Month	28.93^Aa^	11.16^Aa^	55.65^Ab^	26.99^Aa^	16.75^Aa^
	6 Month	44.20^Ba^	22.65^Ba^	101.67^Aa^	44.92^Ba^	32.92^Ba^

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table C-5. Gas Chromatography shelf life results for secondary oxidation product Benzaldehyde of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 6 months of real time.

Octanal		**Product**				
		0B7	0THE	20B7	20TLE	20THE
Time	*Initial (0)*	7.79^{Aa}	7.23^{Aa}	11.52^{Aab}	9.40^{Aab}	10.97^{Ab}
	1 Month	6.89^{Aa}	5.36^{Aa}	7.48^{Aab}	7.36^{Ab}	13.35^{Ab}
	3 Month	11.98^{Aa}	7.14^{Aa}	21.08^{Aa}	10.37^{Aab}	12.74^{Ab}
	6 Month	20.64^{ABa}	18.13^{BCa}	0^{Cb}	30.92^{ABa}	39.70^{Aa}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table C-6. Gas Chromatography shelf life results for secondary oxidation product 2-Octenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 6 months of real time.

2-Octenal		**Product**				
		0B7	0THE	20B7	20TLE	20THE
Time	*Initial (0)*	5.03^{Aa}	2.35^{Aa}	6.28^{Ab}	4.27^{Aa}	3.47^{Aa}
	1 Month	2.95^{Aa}	1.48^{Aa}	4.51^{Ab}	3.76^{Aa}	3.13^{Aa}
	3 Month	5.83^{Aa}	2.25^{Aa}	8.48^{Ab}	4.55^{Aa}	2.89^{Aa}
	6 Month	9.81^{Ba}	5.59^{Ba}	45.28^{Aa}	11.40^{Ba}	9.11^{Ba}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table C-7. Gas Chromatography shelf life results for secondary oxidation product Nonanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 6 months of real time.

Nonanal		**Product**				
		0B7	0THE	20B7	20TLE	20THE
Time	*Initial (0)*	13.49^{Aa}	8.43^{Aa}	17.89^{Ab}	14.36^{Aa}	13.58^{Aa}
	1 Month	8.49^{Aa}	6.74^{Aa}	9.13^{Ab}	10.23^{Aa}	14.91^{Aa}
	3 Month	15.11^{Aa}	7.48^{Aa}	28.12^{Ab}	13.77^{Aa}	13.59^{Aa}
	6 Month	39.51^{Ba}	16.24^{Ba}	173.26^{Aa}	31.57^{Ba}	40.09^{Ba}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Table C-8. Gas Chromatography shelf life results for secondary oxidation product 2-Nonenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from initial to 6 months of real time.

2-Nonenal		**Product**				
		0B7	0THE	20B7	20TLE	20THE
Time	*Initial (0)*	1.39^{Ab}	0.25^{Aa}	1.48^{Ab}	0.84^{Ab}	0.73^{Ab}
	1 Month	0.44^{Ab}	0^{Aa}	0.54^{Ab}	0.27^{Ab}	0.33^{Ab}
	3 Month	0.90^{Ab}	0^{Aa}	1.01^{Ab}	11.09^{Ab}	0.46^{Ab}
	6 Month	33.35^{Ba}	3.97^{Ba}	35.00^{Aa}	11.09^{Bc}	7.46^{Ba}

Capital letters represent groupings of Simple Effect Comparisons sliced by Time (A being group with largest value).
Lowercase letters represent groupings of Simple Effect Comparisons sliced by Product(a being group with largest value).

Appendix D - Accelerated Shelf Life Figures

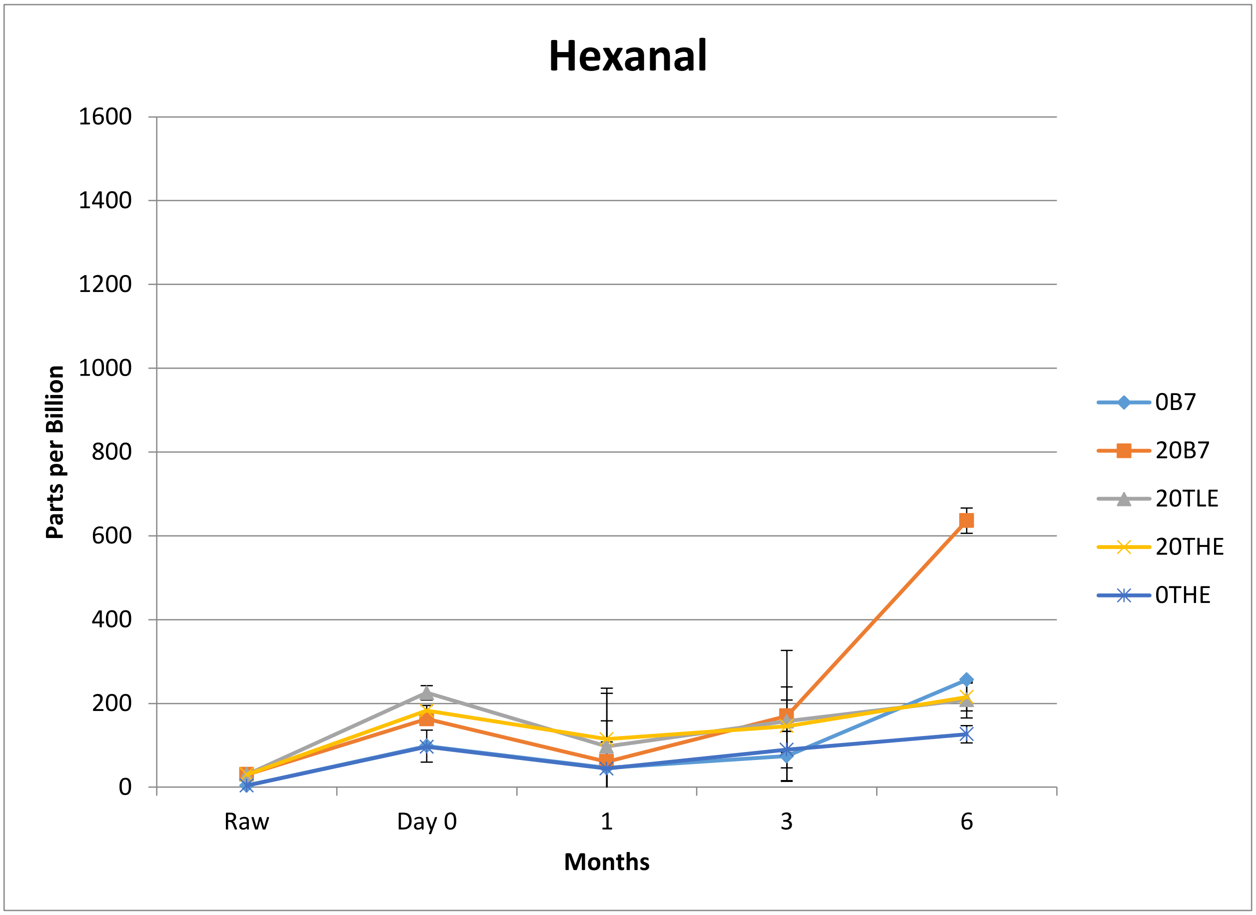

Figure D-1. Gas Chromatography shelf life results for secondary oxidation product Hexanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Standard deviation(+/-) for each treatment is displayed on the data point.

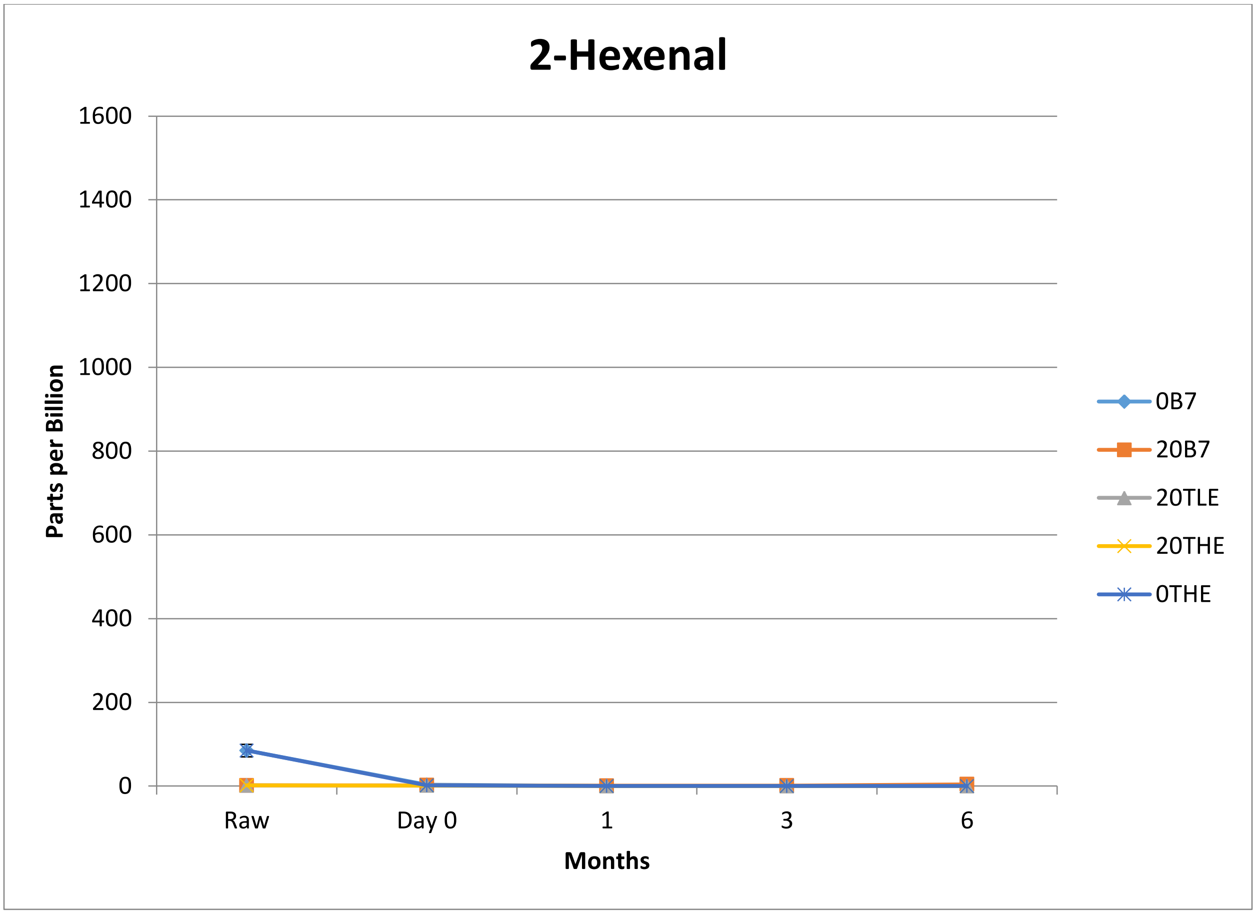

Figure D-2. Gas Chromatography shelf life results for secondary oxidation product 2-Hexenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Standard deviation(+/-) for each treatment is displayed on the data point.

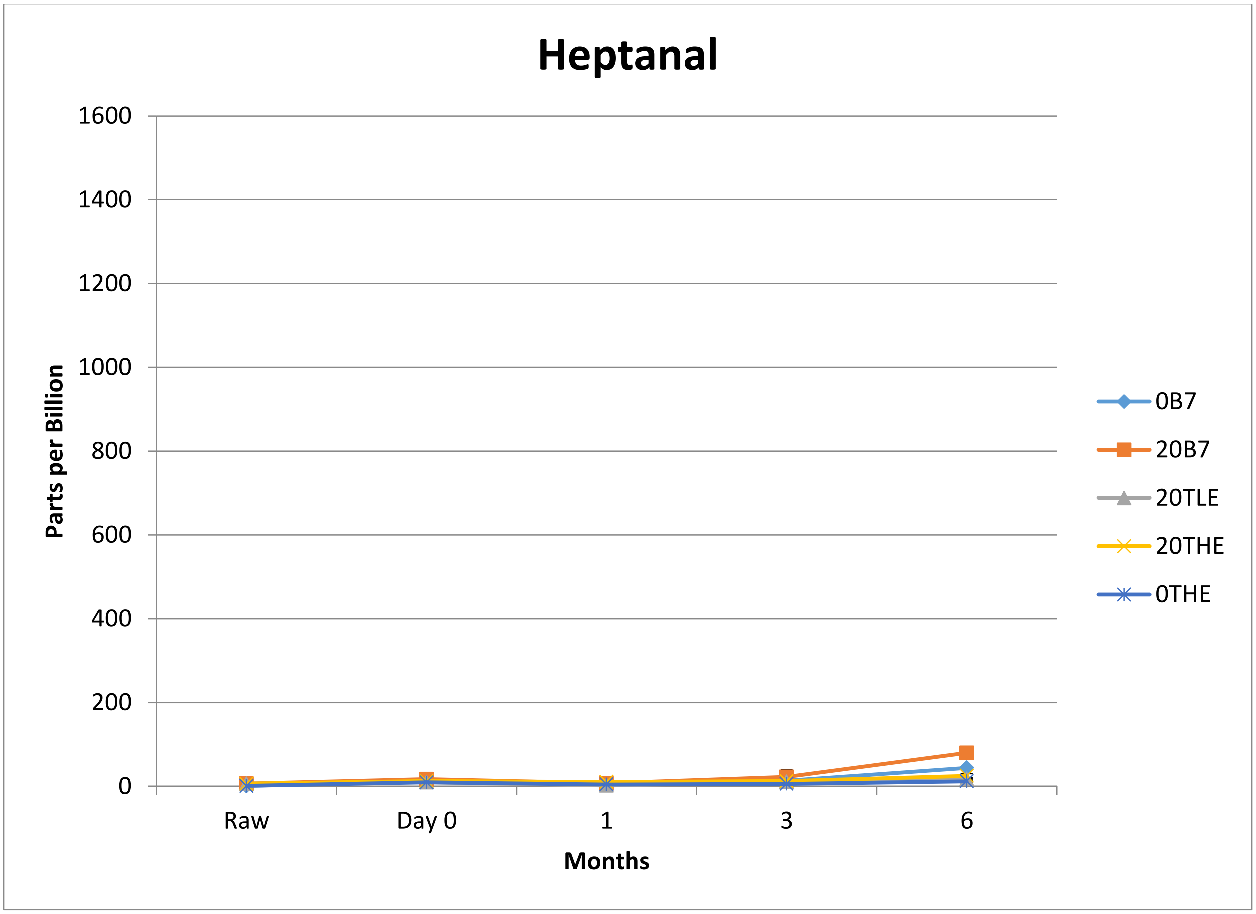

Figure D-3. Gas Chromatography shelf life results for secondary oxidation product Heptanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Standard deviation(+/-) for each treatment is displayed on the data point.

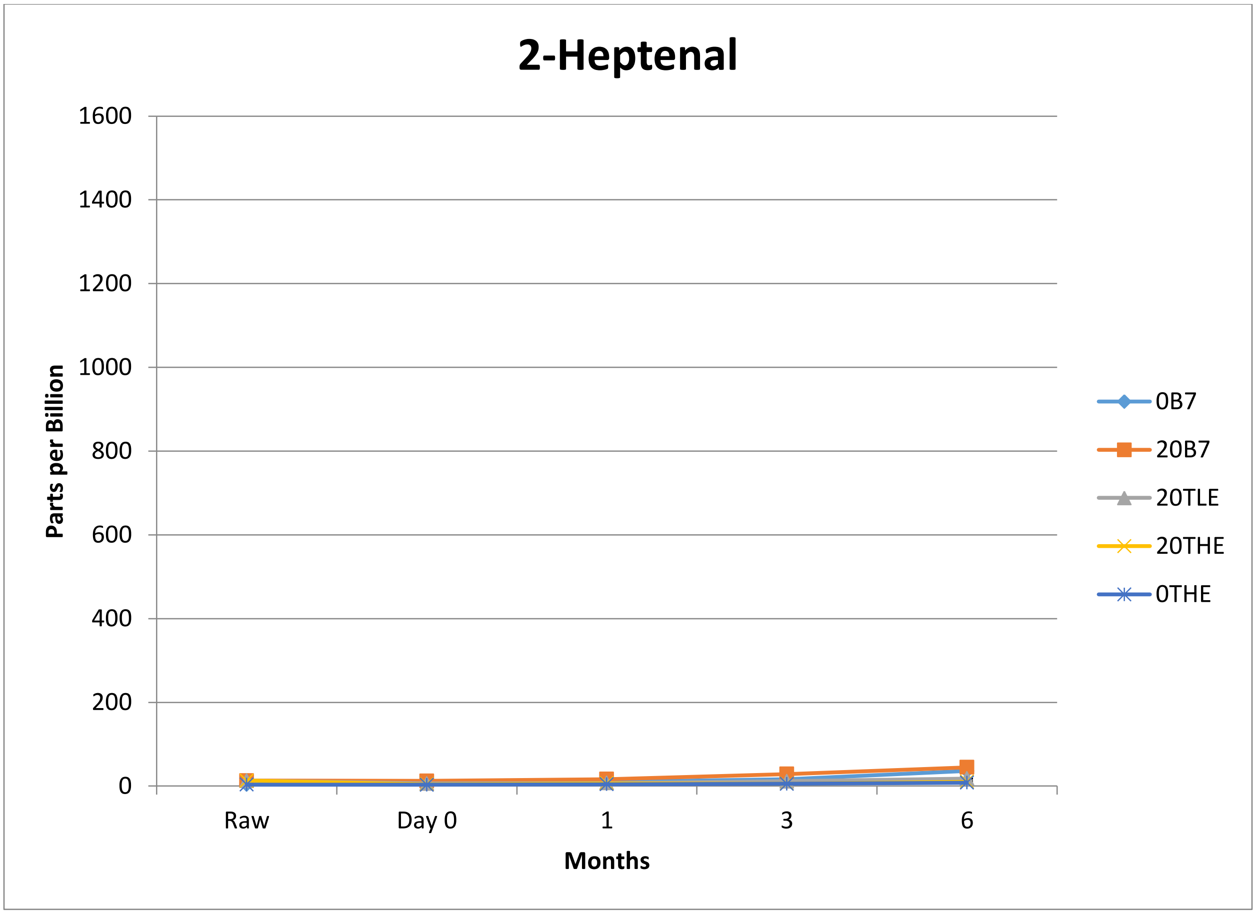

Figure D-4. Gas Chromatography shelf life results for secondary oxidation product 2-Heptenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Standard deviation(+/-) for each treatment is displayed on the data point.

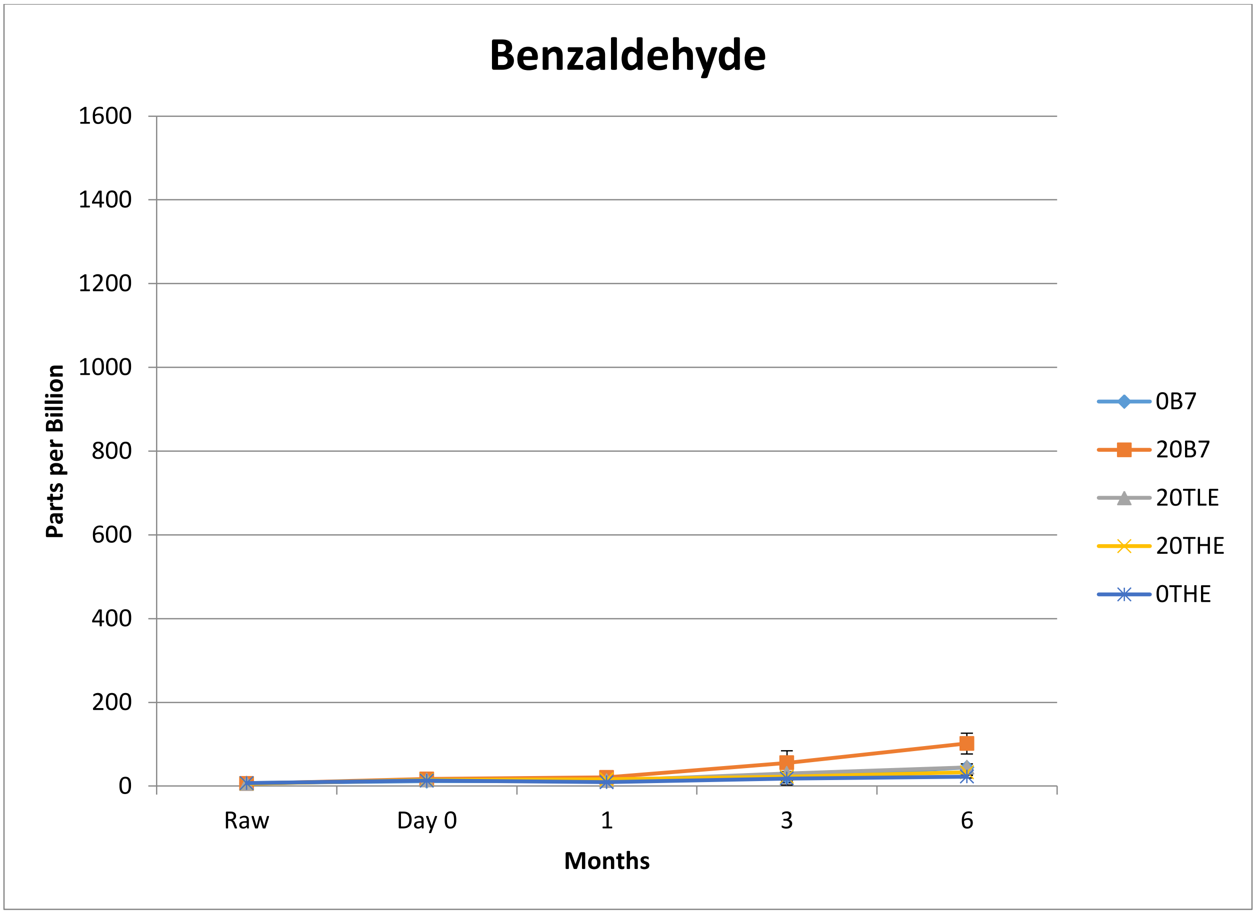

Figure D-5. Gas Chromatography shelf life results for secondary oxidation product Benzaldehyde of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Standard deviation(+/-) for each treatment is displayed on the data point.

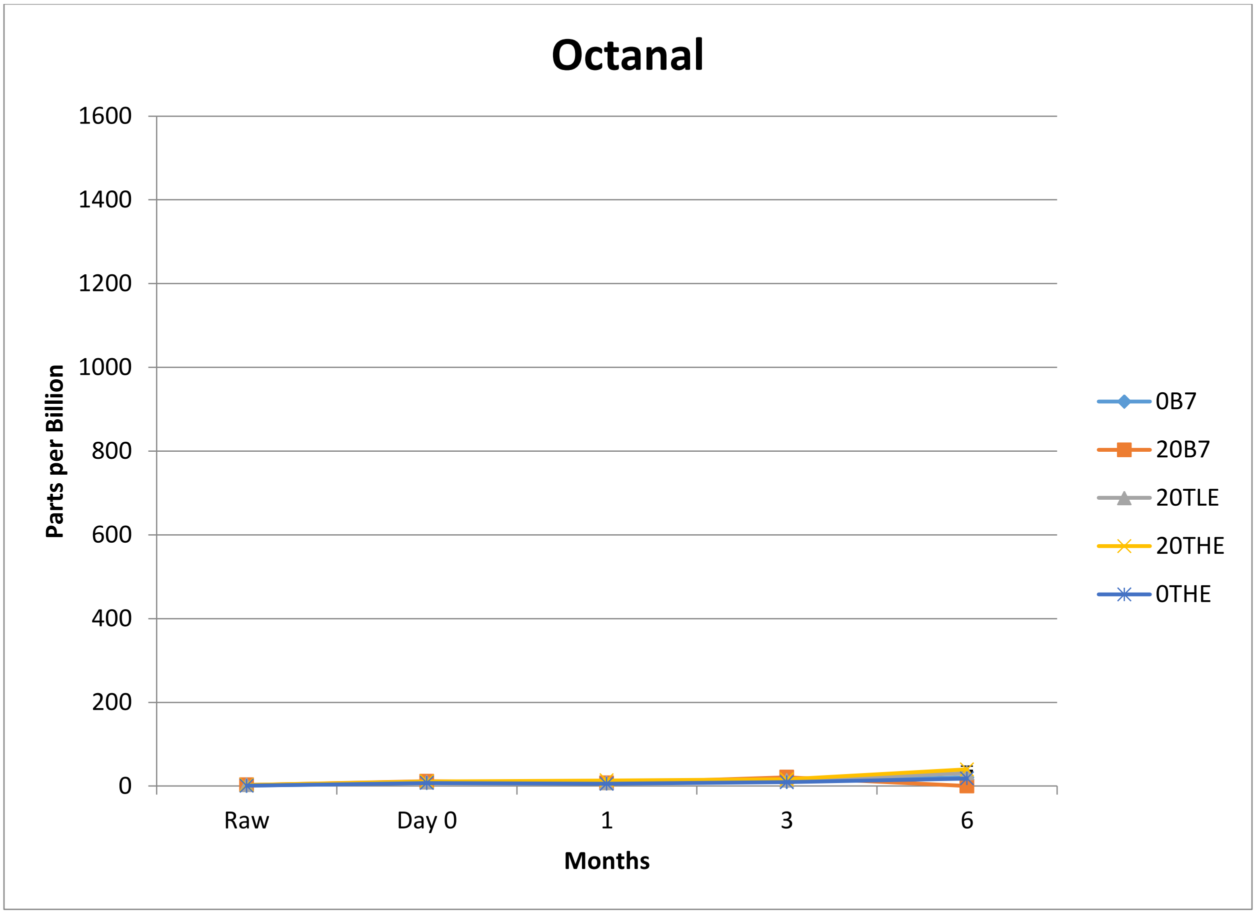

Figure D-6. Gas Chromatography shelf life results for secondary oxidation product Octanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Standard deviation(+/-) for each treatment is displayed on the data point.

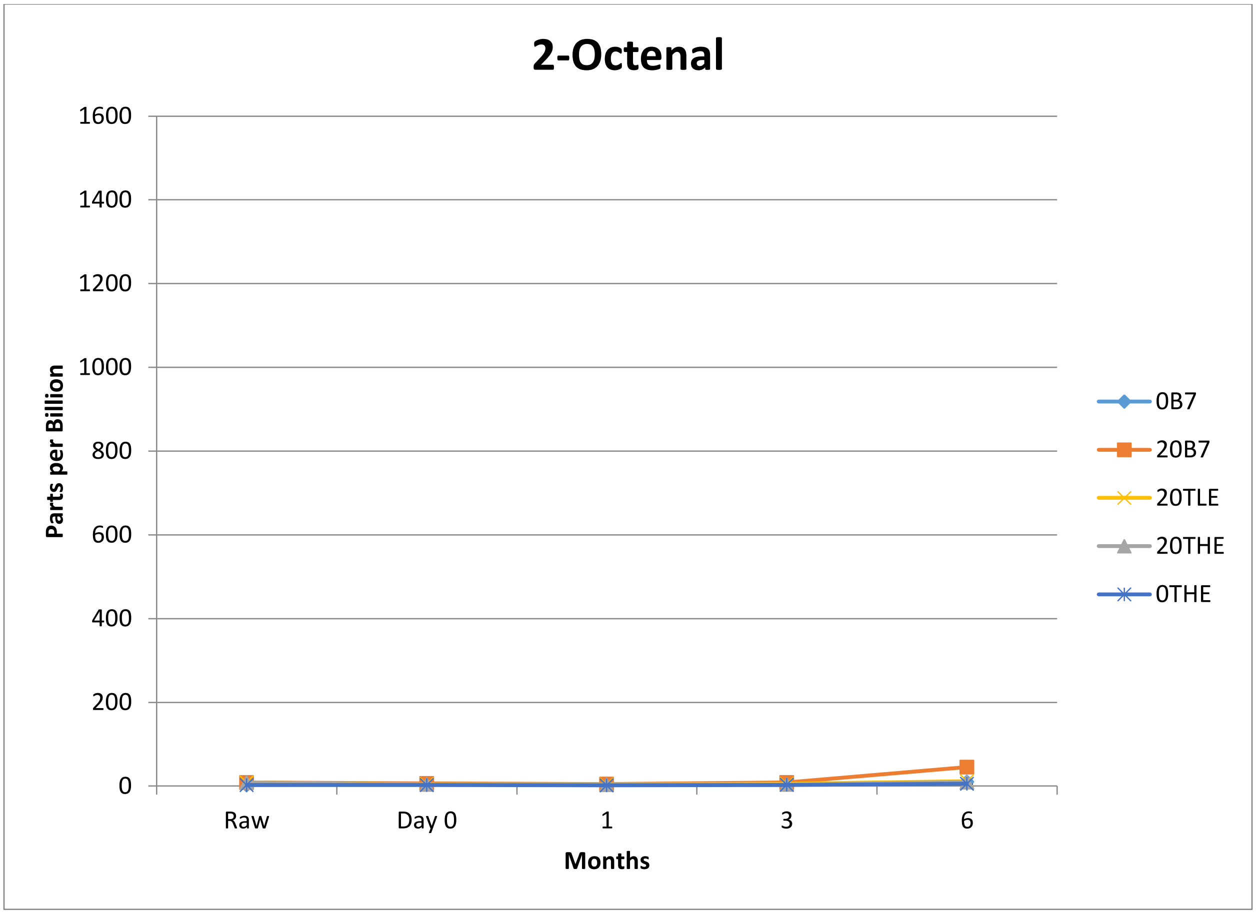

Figure D-7. Gas Chromatography shelf life results for secondary oxidation product 2-Octenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Standard deviation(+/-) for each treatment is displayed on the data point.

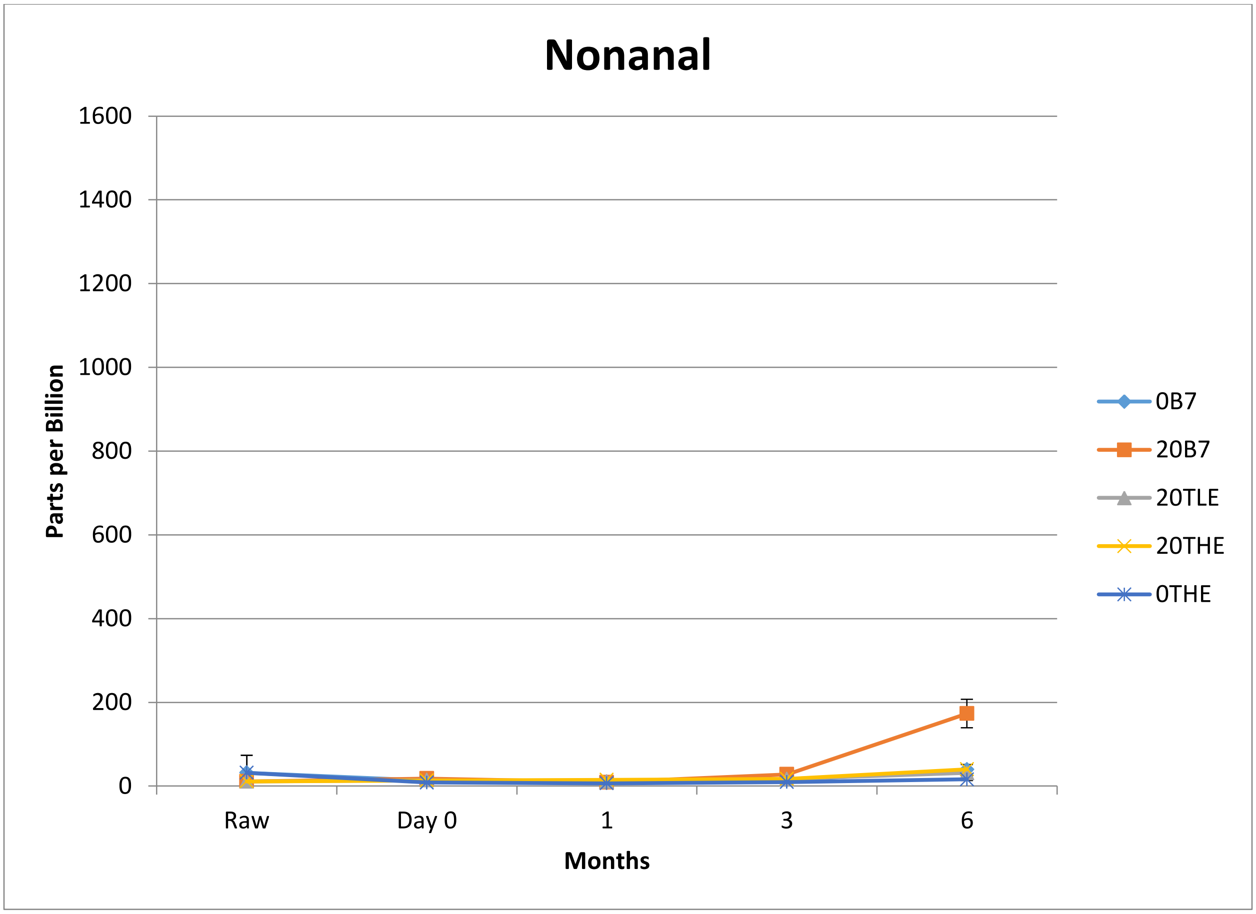

Figure D-8. Gas Chromatography shelf life results for secondary oxidation product Nonanal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Standard deviation(+/-) for each treatment is displayed on the data point.

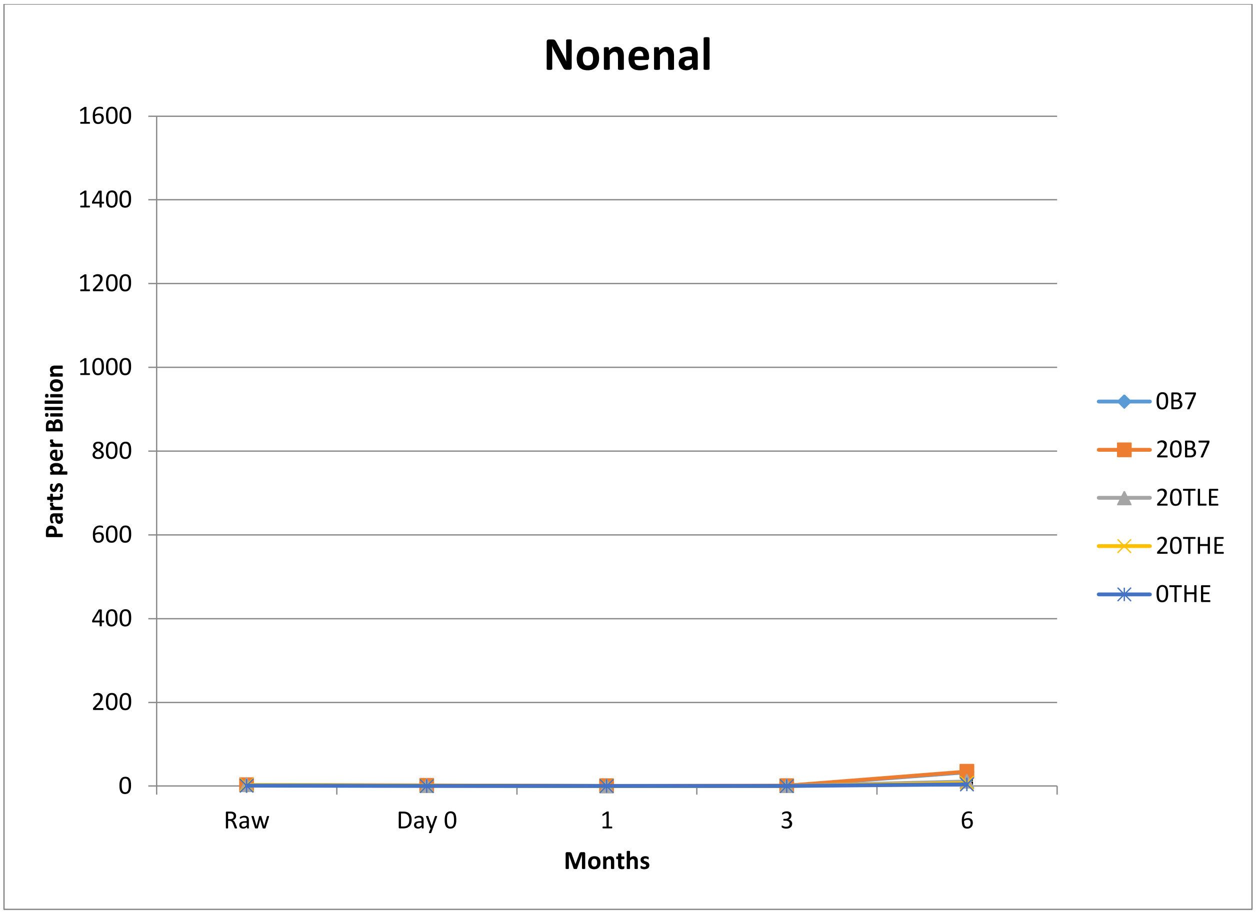

Figure D-9. Gas Chromatography shelf life results for secondary oxidation product Nonenal of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Standard deviation(+/-) for each treatment is displayed on the data point.

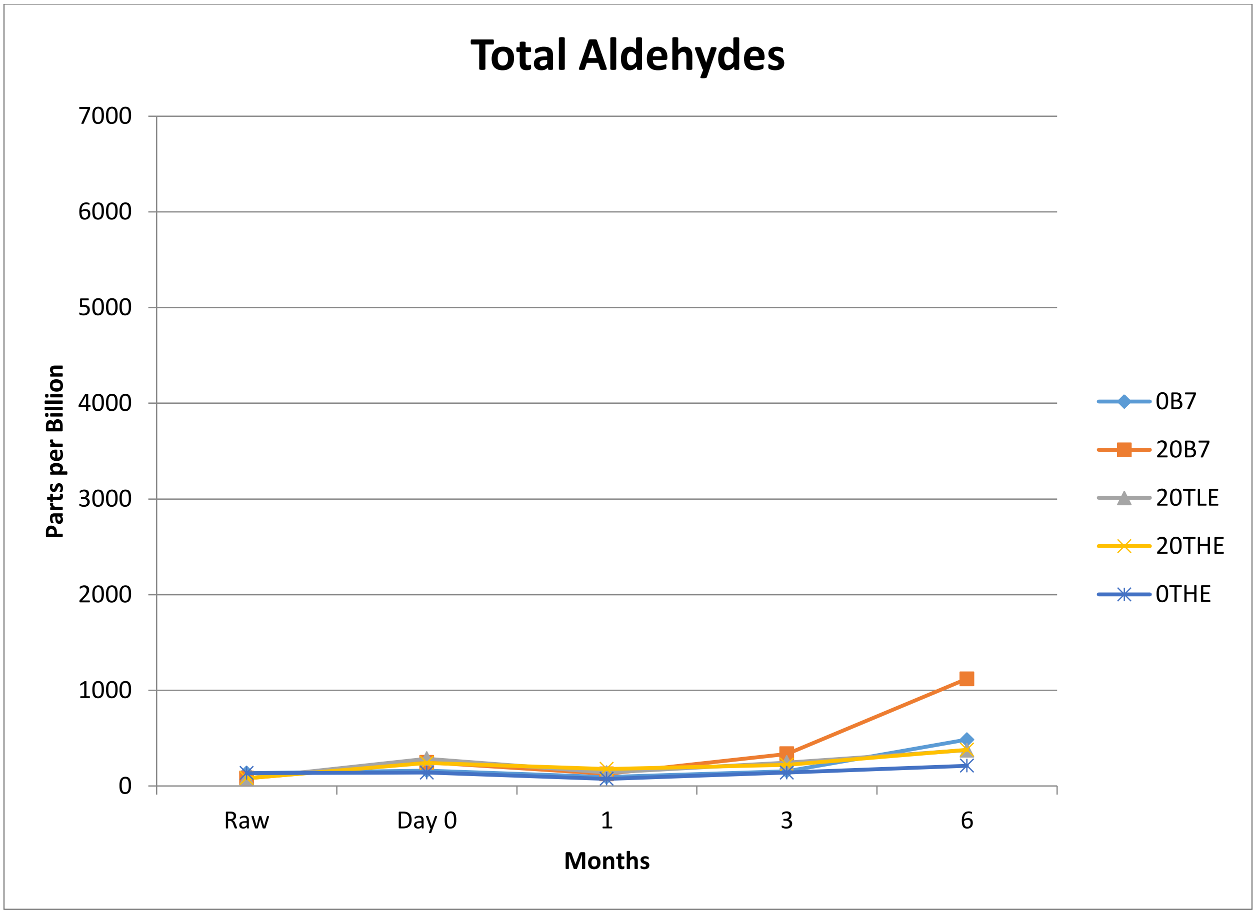

Figure D10. Gas Chromatography shelf life results for secondary oxidation product Total Aldehydes of extruded pet foods at low (LE) and high (HE) total energy input into preconditioner and extruder as well as baked for 7 minutes (B7) in parts per billion from raw to 6 months real time.

Appendix E - APPENDIX Additional Chemical and Physical Characteristics Comparisons with Statistics

Table E-1. Least mean square comparison of chemical and physical characteristics of extruded pet foods formulated with 0% and 20% fresh meat at low (LE), medium (ME), and high (HE) total energy input into preconditioner and extruder; wherein fresh meat levels are combined.

	LE	ME	HE
Specific Length (mm/g)	38.45^{A}	39.82^{A}	38.38^{A}
Piece Density (kg/m^3)	0.897^{A}	0.349^{B}	0.365^{B}
Expansion Ratio	1.70^{A}	4.18^{B}	4.14^{B}
Glucose Gelate (%)	83.8^{A}	97.2^{B}	93.6^{B}
Lactose Gelate (%)	35.1^{A}	37.3^{AB}	32.9^{B}
Glucose (mg/dL)	19.0^{A}	19.75^{AB}	21.53^{B}
Lactose (mg/dL)	0.214^{A}	0.209^{A}	0.213^{A}

Table E-2. Least mean square comparison of chemical and physical characteristics of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein pumping styles are combined.

	0%		20%	
	ME	HE	ME	HE
Specific Length (mm/g)	38.73^{A}	38.80^{A}	41.21^{AB}	41.58^{B}
Piece Density (kg/m^3)	0.370^{A}	0.398^{A}	0.388^{A}	0.367^{A}
Expansion Ratio	4.08^{A}	3.78^{A}	3.73^{A}	3.82^{A}
Glucose Gelate (%)	95.4^{A}	94.4^{A}	99.5^{B}	96.9^{AB}
Lactose Gelate (%)	37.0^{A}	34.0^{A}	37.0^{A}	33.3^{A}
Glucose (mg/dL)	19.93^{A}	20.68^{B}	20.03^{AB}	20.43^{AB}
Lactose (mg/dL)	0.207^{A}	0.201^{A}	0.247^{B}	0.244^{B}

Table E-3. Least mean square comparison of chemical and physical characteristics of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein energy levels are combined.

	0%		20%	
	T	N	T	N
Specific Length (mm/g)	39.19^{A}	38.34^{A}	39.00^{A}	43.79^{B}
Piece Density (kg/m^3)	0.355^{A}	0.413^{B}	0.360^{AB}	0.395^{AB}
Expansion Ratio	4.20^{A}	3.66^{B}	4.12^{A}	3.43^{B}
Glucose Gelate (%)	94.8^{A}	95.0^{A}	96.1^{A}	100^{B}
Lactose Gelate (%)	34.6^{A}	36.3^{A}	35.6^{A}	33.1^{A}
Glucose (mg/dL)	20.83^{A}	19.78^{B}	20.45^{AB}	20.00^{B}
Lactose (mg/dL)	0.202^{A}	0.206^{A}	0.220^{A}	0.272^{B}

Table E-4. Least mean square comparison of chemical and physical characteristics of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein fresh meat levels are combined.

	ME		HE	
	T	N	T	N
Specific Length (mm/g)	39.82^{AB}	40.12^{AB}	38.38^{B}	42.01^{A}
Piece Density (kg/m^3)	0.349^{A}	0.408^{B}	0.365^{AB}	0.400^{AB}
Expansion Ratio	4.18^{A}	3.63^{B}	4.14^{A}	3.46^{B}
Glucose Gelate (%)	97.2^{AB}	97.6^{A}	93.6^{B}	97.7^{A}
Lactose Gelate (%)	37.3^{A}	35.0^{AB}	32.9^{B}	34.4^{AB}
Glucose (mg/dL)	19.75^{A}	20.2^{A}	21.53^{B}	19.58^{A}
Lactose (mg/dL)	0.209^{A}	0.245^{B}	0.213^{A}	0.233^{B}

Table E-5. Least mean square comparison of chemical and physical characteristics of baked pet foods formulated with 0% and 20% fresh meat at baked at 5, 7, 9, and 11 minutes; wherein fresh meat levels are combined.

	5	7	9	11
Piece Density (kg/m^3)	1.640^{A}	1.884^{B}	1.427^{C}	1.742^{A}
Expansion Ratio	0.68^{A}	0.65^{B}	0.71^{C}	0.684^{A}
Glucose Gelate (%)	52.9^{A}	53.6^{A}	57.0^{B}	55.8^{AB}
Lactose Gelate (%)	16.8^{A}	19.3^{AB}	21.2^{A}	19.4^{AB}
Glucose (mg/dL)	19.18^{A}	19.75^{A}	19.18^{A}	19.40^{A}
Lactose (mg/dL)	0.351^{A}	0.322^{AB}	0.302^{B}	0.314^{AB}

Appendix F - Additional Aerobic Plate Count Data Comparisons with Statistics

Table F-1. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at low (LE), medium (ME), and high (HE) total energy input into preconditioner and extruder; wherein fresh meat levels and energy input levels are combined.

Day	1	2	3
	258^{A}	600^{B}	733^{B}

Table F-2. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at low (LE), medium (ME), and high (HE) total energy input into preconditioner and extruder; wherein count days are combined.

0%			20%		
LE	ME	HE	LE	ME	HE
2016^{A}	66^{B}	16^{B}	666^{AB}	300^{B}	116^{B}

Table F-3. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at low (LE), medium (ME), and high (HE) total energy input into preconditioner and extruder; wherein energy input levels are combined.

	0%			20%		
DAY	1	2	3	1	2	3
	250^{A}	833^{B}	1017^{B}	267^{AB}	367^{AB}	450^{AB}

Table F-4. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at low (LE), medium (ME), and high (HE) total energy input into preconditioner and extruder; wherein fresh meat levels are combined.

	LE			ME			HE		
DAY	1	2	3	1	2	3	1	2	3
	575^{A}	1575^{B}	1875^{B}	150^{A}	175^{A}	225^{A}	50^{A}	50^{A}	100^{A}

Table F-5. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein fresh meat levels, input energy levels, and pumping styles are combined.

Day	1	2	3
	94^{A}	119^{AB}	150^{B}

Table F-6. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein count days are combined.

0%		20%	
T	N	T	N
42^{A}	58^{A}	208^{A}	175^{A}

Table F-7. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein fresh meat levels and count days are combined.

ME		HE	
T	N	T	N
183^{A}	42^{A}	66^{A}	192^{A}

Table F-8. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein count days are combined.

0%				20%			
ME		HE		ME		HE	
T	N	T	N	T	N	T	N
67^{A}	33^{A}	17^{A}	83^{A}	300^{A}	50^{A}	117^{A}	300^{A}

Table F-9. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein count days and pumping styles are combined.

0%		20%	
ME	HE	ME	HE
50^{A}	50^{A}	175^{A}	208^{A}

Table F-10. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein fresh meat levels and count days are combined.

ME		HE	
T	N	T	N
183^{A}	42^{A}	67^{A}	192^{A}

Table F-11. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein fresh meat levels and pumping styles are combined.

0%			20%		
1	2	3	1	2	3
25^A	50^A	75^A	163^A	188^A	225^A

Table F-12. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of extruded pet foods formulated with 0% and 20% fresh meat at medium (ME) and high (HE) total energy input into preconditioner and extruder as well as traditional (T) and non-traditional (N) pumping; wherein energy input levels and fresh meat levels are combined.

T			N		
1	2	3	1	2	3
100^{A}	113^{A}	163^{A}	88^{A}	125^{A}	138^{A}

Table F-13. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of baked pet foods formulated with 0% and 20% fresh meat at baked for 5, 7, 9, and 11 minutes; wherein fresh meat levels and plate count days are combined.

TIME	5	7	9	11
	4300^{A}	2142^{B}	4475^{A}	3067^{AB}

Table F-14. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of baked pet foods formulated with 0% and 20% fresh meat at baked for 5, 7, 9, and 11 minutes; wherein fresh meat levels and retention times are combined.

DAY	1	2	3
	2156^{A}	3644^{B}	4688^{C}

Table F-15. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of baked pet foods formulated with 0% and 20% fresh meat at baked for 5, 7, 9, and 11 minutes; wherein plate count days are combined.

0%				20%			
5	7	9	11	5	7	9	11
5133^{A}	2317^{BC}	4233^{ABC}	4217^{ABC}	3467^{ABC}	1967^{BC}	4717^{AB}	1917^{C}

Table F-16. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of baked pet foods formulated with 0% and 20% fresh meat at baked for 5, 7, 9, and 11 minutes; wherein retention times are combined.

0%			20%		
1	2	3	1	2	3
2550^{A}	4113^{BD}	5263^{D}	1763^{A}	3175^{BC}	4113^{BD}

Table F-17. Least mean square comparison of Aerobic Plate Count (counted at day 1, day 2, and day 3) of baked pet foods formulated with 0% and 20% fresh meat at baked for 5, 7, 9, and 11 minutes; wherein fresh meat levels are combined.

5			7			9			11		
1	2	3	1	2	3	1	2	3	1	2	3
2900^{CDEFG}	4375^{ABCD}	5625^{AB}	1125^{G}	2250^{EFG}	3050^{CDEF}	2650^{DEFG}	4625^{ABC}	6150^{A}	1950^{FG}	3325^{CDE}	3925^{BCDE}