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Project Title: The impact of rendered protein meal level of oxidation on shelf life and acceptability in extruded pet foods.

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INDUSTRY SUMMARY

1. Introduction: The pet food industry is a $23 billion industry in the US that utilizes an estimated 8.5 million metric tonnes of raw materials. The author estimates that nearly 35% of that is derived from rendered ingredients - much of that from protein meals.

   Lipids as bulk oil or protein meals can oxidize. Products from this oxidation can result in the loss of essential vitamins, fatty acids, reduce acceptance of the food, and even produce harmful elements that compromise animal health (Turek et al., 2001), alter the functionality of some ingredients, and make the food less marketable (Addis, 1986).

   Pet food distributors and retailers expect the food to have a 1 year shelf life and more recently have been demanding 18 and 24 months. Aside from issues with logistics, warehouse management, and handling losses, the single largest factor to retaining product freshness and integrity is supposedly oxidation. The prevailing logic would suggest that fresh (unoxidized non-rancid) ingredients are essential to long shelf-life. Or conversely that oxidized ingredients accelerate product degradation and acceptability. Attempts to manage this have involved use of antioxidant preservatives, removal of oxygen, high barrier packaging, and rigorous testing for oxidation (mostly Peroxides).

   The other major challenge with these assumptions are that the notion of what is “acceptable” to the pet or owner has not been defined. In essence what constitutes shelf-life itself is ambiguous. The most common measure of oxidation for ingredients and finished goods has been the peroxide value. Whether this measure in ingredients has relevance to shelf-life of the finished food or not, and whether it is reset by the food processing has not been addressed.

   Therefore, the intent of this work was to gauge the impact oxidation (of rendered protein meals) has on finished pet food shelf-life and to what degree the process of producing pet food from oxidized ingredients has on oxidation measures.

2. Objectives:
   a. Determine the effect of incorporating increasing levels of oxidation in rendered protein meals used to produce extruded pet food on markers of oxidation in finished product.
   b. Determine the effect of increasing rancid ingredients on the shelf-life and acceptability of extruded pet foods.

3. Industry Summary: Rendered protein meal contributes a significant portion of the quality proteins to pet foods. However, fats within the meals can oxidize and create supply chain issues. The level of that oxidation and whether it impacts pet food shelf life have not been directly measured. To evaluate this meat and bone meal (BMBM) and poultry byproduct meal (CBPM) that had no preservatives added were obtained from cooperating renderers. The material was split into three groups: control with no preservative (CO), natural preserved with mixed tocopherols (MT), and synthetic preserved with ethoxyquin (ET). The meals were allowed to oxidize until they reached a stable peroxide (PV) level (63 and 41 days; PV 86.4, 8.9, 2.2 meq/kg for BMBM, and PV 88.4, 4.4, and 2.2 for CPBM for CO, MT, and ET, respectively). These meals were then incorporated into an extruded pet food and level of oxidation was determined immediately and over the course of 18 weeks in high temperature conditions. The PV decreased out of the extruder (e.g. to 10.1 and 14.4 meq/kg for CO, for BMBM and CBPM, respectively). In high temperature (105°F) the CO preserved BMBM had the highest PV at 18 weeks, ET the lowest, and MT intermediate (15.5 vs 6.7, and 3.3 meq/kg, respectively). The CBPM treated with ET remained lowest, MT intermediate, and CO had the highest PV (4.4, 23.2, and 53.2 meq/kg, respectively). The rise in anisidine values and volatile aldehydes like hexanal lagged behind the PV for most treatments and led to different conclusions regarding product oxidation. Further, a preliminary sensory test with an untrained panel was unable to make clear distinctions between treatments until 18 weeks and then only for the CO for BMBM. Longer term ambient shelf-life data are pending to 24 months. These data confirm that ingredient PV is not a strong measure for finished product shelf life because it is diminished during process, fails to parallel the oxidation in the product, and does not provide a meaningful measure of human acceptability of a pet food. Tracking loss of essential nutrients (like linoleic acid or vitamin A) is likely a more effective measure in which to base stability decisions.

Scientific Abstracts

Increasing pressure has been put on ingredient suppliers to assure a low level of oxidation – commonly a low peroxide value. Our objective was to determine the effect of increasingly oxidized protein meals on the shelf life of extruded pet foods. Approximately one metric ton of unpreserved chicken by-product meal (CBPM) and unpreserved beef meat and bone meal (BMBM) were collected and left unpreserved (CO) or preserved with either ethoxyquin (ET), or mixed tocopherols (MT). These were allowed to oxidize at ambient conditions (25°C and 51% RH) while being monitored for peroxide value (PV) and anisidine value (AV) until they plateaued (41 and 63 days, respectively) at a PV of 88.44, 4.43, 2.22 meq/kg and AV of 1.08, 0.55, 0.00 g/g for CBPM-CO, CBPM-MT, CBPM-ET, respectively and at a PV of 86.42, 8.88, 2.23 mEq/kg and AV of 12.23, 7.14, 0.00 g/g for BMBM-CO, BMBM-MT, BMBM-ET, respectively. Each meal was then incorporated into a model extruded cat food diet (~30% protein). Samples of kibble for each treatment were collected and stored at an elevated temperature and humidity (40°C and 70%) for 18 weeks. At time 0, PV and AV were greater for CBPM-CO and BMBM-CO ($P<0.05$; 14.41, 10.07 meq/kg and 15.56, 10.08 g/g, respectively) versus the preserved treatments CBPM-BMBM-MT, CPBM-ET, BMBM-MT, and BMBM-ET (2.78, 2.22, 2.22, 2.22 meq/kg and 3.85, 1.79, 9.62, 3.03 g/g, respectively). At elevated storage temperatures, the PV for CBPM-ET remained low (4.44 meq/kg), CBPM-MT was intermediate (23.21 meq/kg) and CBPM-CO increased to 53.15 meq/kg by 18 weeks ($P<0.05$). The AV for CBPM followed a similar pattern. The PV of BMBM under elevated temperatures behaved differently; wherein, BMBM-ET was low (3.33 meq/kg), but BMBM-MT had the highest PV (15.48 meq/kg) and BMBM-CO was intermediate (6.66 meq/kg) by 18 weeks ($P<0.05$). BMBM-ET had the lowest ($P<0.05$) AV and BMBM-MT and BMBM-CO were greater, but did not differ from each other (average 16.75 g/g) at 18 weeks. The results from this study demonstrate that oxidation occurred regardless of treatment; but, was rapid and extensive in meals without preservative. The ingredient oxidation levels were diluted by food production and their oxidation may not completely account for later food product deterioration.

Key Words: Pet food, shelf-life, oxidation, acceptability

Introduction

Pet foods are a significant user of rendered protein meals. There is increasing pressure by pet food companies to produce foods from naturally preserved ingredients and to guarantee the shelf-life of these foods for 12 months or more. It is assumed that erosion of shelf-life starts with oxidative rancidity of the raw ingredients. Thus, increasing pressure has been put on ingredient suppliers to assure a low measure of oxidation – commonly a low peroxide value. The acceptable ranges for these measures have been somewhat arbitrarily set. Further, a majority of pet foods are extruded and dried, which is a heat intensive process that often causes volatile compounds to be vaporized. The products of oxidation are just such volatile compounds. Thus, the questions that arise are whether the standards currently being used for purchasing specifications of rendered protein meals are relevant to the finished products shelf-life or whether producing foods with previously oxidized protein meals will shorten the shelf-life of the foods and decrease their acceptability.

The hypothesis of this study was that extrusion of protein meals during pet food manufacturing drives off lipid volatiles, thereby resetting the oxidation clock. Further, that the ingredient peroxide value may be only indirectly linked to the product shelf life. To test the hypothesis, the objective of this experiment was to evaluate the level of oxidation in rendered protein meals on shelf life and acceptability in extruded pet foods.

Materials and Methods

Rendered Protein Meal

Approximately one metric ton of unpreserved BMBM and 735 kilograms of CBPM were acquired from cooperating rendering plants. The meals were split into six equal subsamples of about 133 kg into separate 64-L pails. The first two 133 kg sub-samples of BMBM and CBPM were left untreated and labeled BMBM-C01, BMBM-C02, CBPM-C01, and CBPM-C02. Each untreated 133 kg sub-sample was placed inside a fiber drum that was lined with a plastic bag. The second set of 133 kg sub-samples of BMBM were weighed into a horizontal “counterpoise” paddle batch mixer (133 kg, Hayes and Stolz; Fort Worth, TX). A hand-pump pressurized sprayer was used to apply a 1:10 dilution of Naturox™ (Kemin Industries, Des Moines, IA) and canola oil, and mixed for five minutes. Approximately 1,200 ppm of Naturox™ (Kemin Industries, Des Moines, IA) was applied to each sub-sample and meal. Each sub-sample drum was labeled: BMBM-MT1, BMBM-MT2, CBPM-MT1, and CBPM-MT2. After mixing, each 133 kg sub-sample was placed inside a fiber drum that was lined with a plastic bag and a lid was secured on top of each drum. Between the two similar treatments of the samples, for example BMBM-MT1 and BMBM-MT2, an air hose and brushes were used to remove any remaining residue from the equipment. Using the equipment and procedures described above, the third set of 133 kg sub-samples from each meal had a 1:10 dilution of ethoxyquin (Kemin Industries, Des

Moines, IA) applied while the contents were mixing. The rendered protein meal was allowed to mix for five minutes after all of the antioxidant had been applied. The pressurized sprayer was suspended on a digital scale to visually allow a reading of how much antioxidant/canola oil was being applied. To each corresponding subsample and meal, 150 ppm of ethoxyquin (Rendox™ , Kemin Industries, Des Moines, IA) was applied. Each subsample drum was labeled: CBPM-ET1, CBPM-ET2, BMBM-ET1, and BMBM-ET2. After mixing, the samples were stored as described above. The mixer was sanitized prior to experimental procedures and in-between each treatment using a five percent bleach solution and allowed to thoroughly dry. All twelve drums were kept at room temperature (25°C, 51% RH). The BMBM was stored for 63 days and the CBPM for 41 days prior to extrusion. Each drum was allowed to oxidize and was analyzed for PV and AV approximately every five days during storage (table 3.1 and 3.2). Prior to processing, each treatment was analyzed for volatile compounds using gas chromatography.

Diets

All ingredients were individually weighed prior to mixing in a Wenger double ribbon mixer for three minutes before the micro-ingredients were added and allowed to mix an additional three minutes. After mixing, the diets were then bagged in 22.7 kilogram paper bags in preparation for extrusion. Experimental pet food diets similar to a typical cat food (~30% protein) were produced (table 3.3; BIVAP Extrusion Laboratory; Kansas State University; Manhattan, KS).

Processing

Treatment Sequence

Experimental diet production required sequential days. The sequence of treatment production was: start-up material (a blend of chicken by-product meal, brewers rice, corn, wheat, and beet pulp), CBPM-C01, CBPM-C02, BMBM-C01, BMBM-C02, CBPM-MT1, CBPM-MT2, CBPM-ET1, and CBPM-ET2. Prior to the second day of extrusion, the pre-conditioner was thoroughly cleaned. The treatment sequence for the second day of extrusion was: BMBM-MT1, BMBM-MT2, BMBM-ET1, and BMBM-ET2.

Extruder Parameters

Each diet was mixed as an individual replicate and extruded accordingly. The raw mixed ingredients were extruded on a single screw extruder (Wenger X-20, Wenger Manufacturing; Sabetha, Kansas) using a typical pet food screw profile. The extruder screw profile included 1-Inlet screw, 2-Single flight full-pitch screw, 3-Small shear lock, 4-Singleflight full-pitch screw, 5-Small shear lock, 6-Single flight screw, 7-Medium shear lock, 8-Doubleflight single pitch screw, 9-Large shear lock, 10-Double flight cut cone screw (Figure 3). The extruder barrel jacket

temperature for zone one was 30°C, zone two was 70°C, and zone three was 90°C. The extruder die shape and size was a five millimeter circle with a knife setup of six solid blades.

Target Extruder Conditions

The target extruder conditions for moisture were 27-30% and a dry feed rate of 180 kg/h. The steam was added at a rate of two-thirds the water in the pre-conditioner while the remainder of the moisture was added to the extruder barrel and recalculated to achieve the processing moisture goal. The target bulk density was 350 g/L. The bulk density measurement was duplicated for each treatment and an average bulk density was calculated. The target extruder screw speed was 340 to 450 rpm based on the control diets performance. Once the parameters for the control diet were set, they were held constant for the remainder of the experimental treatment processing.

Dryer Conditions

The extruded kibble exiting the extruder was pneumatically conveyed to a dual pass dryer/single pass cooler (Wenger 4800 Series, Wenger Manufacturing; Sabetha, Kansas). The dryer was set at 99°C and ten minutes per pass and ten minutes through the cooler. The dryer conditions were adjusted to achieve a target final moisture of 7.5%. To confirm the pet food achieved the final moisture level, the kibbles were analyzed (AOAC 930.15). The pet food was not coated with flavors or fats upon exiting the dryer to eliminate confounding factors.

Shelf-Life

Three kg of kibble per treatment were placed in freezer storage bags, each bag was punctured with a pin sized hole to facilitate air exchange. Each bag was labeled with their respective treatment and storage duration. Samples for ambient storage (~22°C and 45% relative humidity) were prepared for 0, 3, 6, 9, 12, and >12 months. These were stored in a covered plastic tote. Samples for “accelerated” conditions were prepared for 0, 3, 6, 12, and 18 weeks. They were held at 40°C and 70% relative humidity in an environmental chamber (Cincinnati Sub-Zero Stability Temperature/Humidity Chamber; Cincinnati, Ohio). Samples for each time point in accelerated storage were arranged vertically throughout the environmental chamber.

Sample Analysis

The rendered protein meals and samples collected for the shelf-life evaluation were evaluated for peroxide (AOCS Official Method Cd 8-53) and anisidine value (AOCS Official Method Cd 18-90). The rendered protein meals prior to extrusion and the kibble samples for the shelf-life evaluation were analyzed for volatile compounds via gas chromatography (GC) head space analysis for hexanal and other 10-carbon or smaller aldehydes.

Fat Extraction from Pet Food

Since there is no official method for extraction of oils from pet food for the determination of peroxide value (PV) or anisidine value (AV), the technique was modified from the procedures of Williams and Hron (1996) in the following manner: 1 kg of pet food was ground through a 1 mm screen in a Wiley mill (Model 4: Thomas Scientific; Swedesboro, New Jersey). Subsamples of each respective CBPM treatment (400 g) and BMBM treatment (900 g) sample were required. The BMBM pet food was split into two equal 450 g subsamples to aid extraction. Each sample was weighed into a 1,000 mL beaker to which an equal amount of hexane was added. The ground pet food and hexane were allowed to mix for five minutes using a magnetic stir plate. Separation of oil and hexane for the pet food was done by vacuum filtration that consisted of a standard laboratory vacuum pump, a vacuum hose, liquid trap, Büchner funnel, Erlenmeyer flask with hose adapter, and Whatman Grade 41 filter paper (GE Healthcare Life Sciences; Pittsburgh, Pennsylvania). Once the hexane and oil were separated, the mixture was transferred to a 1,000 mL round bottom flask attached to a rotating evaporator (Rotavap Büchi R-114: Brinkmann Instruments, Inc.) that was partially submerged in a water bath (Büchi B-490: Brinkmann Instruments, Inc) at 50°C. The rotating evaporator was used to gently separate the hexane from the oil (15 minutes). The isolated oil was transferred to a 50 mL conical tube (BD Biosciences) and centrifuged (Sorvall Legend X1R: Thermo Fisher Scientific) at a 5,000 rpm for fifteen minutes at 35°C. The isolated oil was then analyzed for PV (AOCS Official Methods Cd 8-53) and AV (AOCS Official Methods Cd 18-90).

Volatile Compounds Measurement

Extraction Procedure of Volatile Compounds

Volatiles in dry dog foods were evaluated by headspace-solid phase microextraction (HS-SPME) as described by Koppel (2013). Five sub-samples of BMBM received from each of two locations, five sub-samples of CBPM received from each of three locations, and the one sub-sample of turkey meal were analyzed in triplicate. The samples were ground to a particle size of 1 mm using a Wiley mill (Model 4: Thomas Scientific; Swedesboro, New Jersey), then 0.5 g of each sample was weighed into a 10 mL screw-cap vial with a polytetrafluoroethylene/silicone septum. To this, 0.98 mL distilled water was added to the ground sample in the vial along with an internal standard 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). The final concentration was 0.2 mg/kg. The vials were equilibrated for 10 min at 40 °C in the autosampler (Pal system, model CombiPal, CTC Analytic;, Zwingen, Switzerland) and agitated at 250 rpm. After the equilibrium, 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 volatile capacity in food products (Ceva-Antunes et al., 2006). After sampling, the

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

Chromatographic Analyses

The isolation, identification, and semi-quantification of the volatile compounds were performed on a gas chromatograph (Varian GC CP3800; Varian Inc.; Walnut Creek, California), 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, Pennsylvania; 30 m x 0.25 mm x 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.
The compounds were identified using two 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, Pennsylvania) was used to determine experimental Kovats indices for the volatile compounds detected.

Human Rancidity Panel

At each time point in the shelf-life study, a team of ten untrained volunteers were enlisted to analyze each sample for their aroma. Each panelist was given a score sheet to rank samples based on their perception of the products relative level of rancidity. Scores were one to five, with a higher score for more rancid notes. The average rancidity score for each sample was recorded at each time point. All sample jars were washed thoroughly and dried in an air oven at 100°C between each sensory evaluation period. The samples were randomly assigned to a jar number and labeled one to twelve to correspond with the twelve dietary treatments. Approximately 50 kibbles of each sample were placed in the four ounce jars and covered with a lid. All samples were stored in a freezer at -18°C until the sensory analysis by the trained panelists is arranged.

Statistical Analysis

Oxidative rancidity and stability

Results were summarized according to protein type, treatment, and time of sample analysis from rendered protein meal arrival to processing. Variation among means was determined by treatment and time of sample analysis. The data were analyzed as a completely randomized design and the means were separated by significant F values with $\alpha = 0.05$ of the GLM procedure using statistical software (SAS Institute, Inc.; Cary, North Carolina).

The data were analyzed as a completely randomized design and the means were separated by significant F values with $\alpha = 0.05$ of the GLM procedure using statistical software (SAS Institute, Inc.; Cary, North Carolina). For the relationship between accelerated and ambient shelf-life evaluations, regression analysis was performed. Results were summarized according to protein type, treatment, and time of sample analysis of kibble. Variation among means was determined by protein type, treatment, and time of sample analysis of kibble. For the relationship between accelerated and ambient shelf-life evaluations, linear regression was performed by regression analysis. Results were summarized according to protein type, treatment, and time of sample analysis of kibble. Variation among means was determined by protein type, treatment, and time of sample analysis of kibble. The data were analyzed as a completely randomized design and the means were separated by significant F values with $\alpha = 0.05$ of the GLM procedure of SAS statistical software (SAS Institute, Inc.; Cary, North Carolina). For the human rancidity panel the data were analyzed as described previous section.

Results

The feeder screw speed averaged 18.5 RPM with a standard deviation of 0.085 between all treatments. The discharge temperature from the preconditioner to the extruder averaged 95.58°C between all treatments with a standard deviation of 1.240. The shaft speed of the extruder averaged 595 RPM with a standard deviation of 1.6 between all treatments. The percent moisture added to the treatments during processing averaged 28.7%, with a standard deviation of 0.24. The die temperature averaged 115.6°C, with a standard deviation of 4.38 between all treatments. The throughput of the BMBM treatments averaged 220 kg/hr, with a standard deviation of 4.8 kg/hr and the CBPM throughput averaged 204 kg/hr, with a standard deviation of 7.7 kg/hr.

The average bulk density of the kibble for the BMBM treatments out of the extruder was 418.2 g/L (±30.13) and the CBPM treatments averaged 466.5 g/L (±22.87). The kibble out of the extruder for the BMBM treatments averaged a radial expansion of 9.2 mm (±0.39) and the CBPM treatments averaged 8.4 mm (±0.53). The average bulk density of the kibble out of the dryer for the BMBM treatments was 401.8 g/L (±28.25) and the CBPM treatments averaged 459.7 g/L (±36.41). The average radial expansion of the kibble out of the dryer for the BMBM treatments was 9.11mm (±0.236) and the CBPM treatments averaged 8.46 mm (±0.197). The average moisture of the kibble for the BMBM treatments was 3.79% (±0.266) and the CBPM treatments averaged 5.22% (± 1.061) moisture.

The PV and AV of the unpreserved BMBM increased from 3.33 meq/kg and 6.91 g/g at day 2 to 87.99 meq/kg and 14.30 g/g by day 59 (Table 1). The BMBM preserved with mixed tocopherols had a PV and AV of 2.22 meq/kg and 0.00 g/g at day 2 and increased to 8.14 meq/kg and 7.76 g/g by day 59. The BMBM preserved with ethoxyquin had a constant PV and AV from day 2 (2.22 meq/kg and 0.00 g/g) till day 59 (2.22 meq/kg and 0.00 g/g).

The PV and AV of the unpreserved CBPM increased from 4.43 meq/kg and 0.15 g/g at day 2 to 81.20 meq/kg and 3.02 g/g by day 38 (Table 2). The CBPM preserved with mixed tocopherols had a PV and AV of 1.11 meq/kg and 0.00 g/g at day 2 and increased to 4.43 meq/kg and 1.21 g/g by day 38. The CBPM preserved with ethoxyquin had an initial PV of 1.12 meq/kg at day 2 and increased to 2.22 meq/kg from day 7 to 38. The AV held constant at 0.00 g/g from day 2 to 38.

Gas Chromatography/Mass Spectroscopy – Rendered Protein Meals

The hexanal concentration of the BMBM treatment with mixed tocopherols was greater (P <0.0001) than the unpreserved and ethoxyquin treatments, which were also different from each other ($P < 0.0001$; 15,355.82 μg/kg; 5,515.74 μg/kg; and 1,233.18 μg/kg, respectively; Table 4). The hexanal concentration of the unpreserved CBPM was greater than the ethoxyquin CBPM treatment (5,762.33 and 1,550.73 μg/kg; $P < 0.0001$), but did not differ from the CBPM mixed tocopherols (3,128.79 μg/kg; $P > 0.05$). The hexanal concentration of the unpreserved BMBM treatment (5515.74μg/kg) did not differ from the CBPM unpreserved (5,762.33 μg/kg) and mixed tocopherols (3,128.79 μg/kg) treatments ($P > 0.05$). The hexanal concentration of the BMBM ethoxyquin treatment did not differ from the CBPM ethoxyquin treatment (1233.18 and 1550.73 μg/kg; $P > 0.05$).

The heptanal concentration of the unpreserved BMBM treatment was greater than the BMBM mixed tocopherols and ethoxyquin treatments (4,642.83 and 3,081.80 and 1108.29 μg/kg; $P <$ 0.0001). The unpreserved CBPM treatment did not differ from the CBPM mixed tocopherols and ethoxyquin treatments (631.62 and 233.47 and 269.79 μg/kg; $P > 0.05$). The heptanal concentration of the BMBM ethoxyquin treatment did not differ from the unpreserved CBPM treatment (1108.29 and 631.62 μg/kg; $P > 0.05$).

The octanal concentration of the unpreserved BMBM was greater than the BMBM mixed tocopherols and ethoxyquin treatments (7,212.00 and 4,733.21 and 691.75 μg/kg; $P < 0.0001$). The octanal concentration of the unpreserved CBPM did not differ from the CBPM mixed tocopherols and ethoxyquin treatments (462.19 and 251.86 and 154.69 μg/kg; $P > 0.05$). The octanal concentration of the BMBM ethoxyquin treatment did not differ from the CBPM unpreserved, mixed tocopherols, and ethoxyquin treatments (462.19 and 251.86 and 154.69 μg/kg; $P > 0.05$).

The BMBM unpreserved nonanal concentration was greater than the BMBM ethoxyquin treatment (4,515.96 and 767.47 μg/kg; $P < 0.05$), but the unpreserved treatment did not differ from the BMBM mixed tocopherols treatment (4395.62 μg/kg; $P > 0.05$). The nonanal concentration of the CBPM unpreserved, mixed tocopherol, and ethoxyquin treatments did not differ from each other (525.92 and 260.47 and 179.75 μg/kg; $P > 0.05$). The BMBM ethoxyquin

nonanal concentration did not differ from the CBPM unpreserved, mixed tocopherols, and ethoxyquin treatments (767.47 and 525.92 and 260.47 and 179.75; $P > 0.05$).

The decanal concentration for the BMBM and CBPM treatments did not differ from each other ($P > 0.05$).

Shelf Life

The PV of the unpreserved BMBM treatment decreased from 10.07 meq/kg at 0 weeks to 6.66 meq/kg by 18 weeks; whereas, the AV for this treatment increased from 10.08 g/g at 0 weeks to 17.52 g/g by 18 weeks (Table 5). The PV of the mixed tocopherols and ethoxyquin treatments increased from 0 weeks to 18 weeks (2.22 to 15.48 meq/kg and 2.22 to 3.33 meq/kg). The AV of the BMBM mixed tocopherols and ethoxyquin treatments increased from 9.62 to 15.98 g/g and 3.03 to 6.12 g/g from 0 to 18 weeks. The PV of the unpreserved CBPM treatment increased from 14.41 meq/kg at 0 weeks to 53.15 meq/kg by 18 weeks. The AV of the unpreserved CBPM treatment also increased during the 0 to 18 week shelf life (15.56 to 33.41 g/g). The PV and AV of the CBPM mixed tocopherols treatment increased from 2.78 meq/kg and 3.85 g/g at 0 weeks to 23.21 meq/kg and 15.45 g/g by 18 weeks. The PV and AV of the CBPM ethoxyquin treatment also increased from 2.22 meq/kg and 1.79 g/g at 0 weeks to 4.44 meq/kg and 7.53 g/g by 18 weeks.

Gas Chromatography/Mass Spectroscopy – Kibble

The hexanal concentration of the unpreserved BMBM treatment increased from 1,418.86 μg/kg at 0 weeks to its greatest concentration of 1,986.35 μg/kg at 6 weeks and decreased to 1,462.56 μg/kg by 18 weeks (Table 6). The BMBM mixed tocopherols hexanal concentration decreased from 7,861.76 μg/kg at 0 weeks to 2,221.49 μg/kg at 12 weeks, but increased to 3,557.83 μg/kg at 18 weeks. The BMBM ethoxyquin treatment increased from 295.08 μg/kg at 0 weeks to its greatest concentration of 1,779.82 μg/kg at 18 weeks. The CBPM unpreserved treatment increased from 1,062.29 μg/kg at 0 weeks to 4,954.59 μg/kg by 18 weeks. The CBPM mixed tocopherols treatment decreased from 723.93 μg/kg at 0 weeks to 580.50 μg/kg at 3 weeks, but increased to 2,501.54 μg/kg by 18 weeks. The CBPM ethoxyquin treatment increased from 255.50 μg/kg at 0 weeks to 1,263.36 μg/kg by 18 weeks.

The heptanal concentration of the unpreserved BMBM treatment increased and decreased over the 18 weeks shelf life (842.44 μg/kg at 0 weeks; 747.26 μg/kg at 3 weeks; 1,592.52 μg/kg at 6 weeks; 1,004.65 μg/kg at 12 weeks; and 1,008.85 μg/kg at 18 weeks). The concentration of the BMBM mixed tocopherols treatment decreased from 521.51 μg/kg at 0 weeks to 290.67 μg/kg at 3 weeks, but increased to 774.46 μg/kg by 18 weeks. The concentration of the ethoxyquin treatment increased from 97.77 μg/kg at 0 weeks to 520.84 μg/kg by 18 weeks. The CBPM unpreserved treatment increased from 133.24 μg/kg at 0 weeks to 980.11 μg/kg by 18 weeks.

The CBPM mixed tocopherols and ethoxyquin followed the same trend as the unpreserved treatment and increased from 0 weeks to 18 weeks (43.46 to 672.14 μg/kg and 28.75 to 234.28 μg/kg).

The octanal concentration of the BMBM unpreserved treatment fluctuated during the 18 week shelf life (1,857.98 μg/kg at 0 weeks; 1,839.88 μg/kg at 3 weeks; 2,068.78 μg/kg at 6 weeks; 1,816.19 μg/kg at 12 weeks; and 2,378.81 μg/kg at 18 weeks). The BMBM mixed tocopherols treatment decreased from 812.89 μg/kg at 0 weeks to 515.64 μg/kg at 6 weeks, but then increased to 996.08 μg/kg at 18 weeks. The BMBM ethoxyquin treatment increased from 197.17 μg/kg at 0 weeks to 236.74 at 3 weeks, decreased to 146.76 μg/kg at 6 weeks, and increased to 327.26 μg/kg at 18 weeks. The CBPM unpreserved treatment increased from 217.55 μg/kg at 0 weeks to 904.95 μg/kg by 18 weeks. The CBPM mixed tocopherols and ethoxyquin treatments also had an increase in octanal concentration over the 18 week shelf life (75.08 to 339.17 μg/kg and 27.71 to 129.28 μg/kg).

The nonanal concentration of the BMBM unpreserved treatment increased and decreased over the course of the 18 week shelf life (1,696.82 μg/kg at 0 weeks; 1,768.01 μg/kg at 3 weeks; 951.42 μg/kg at 6 weeks; 1,106.41 μg/kg at 12 weeks; and 830.94 μg/kg at 18 weeks). The BMBM mixed tocopherols treatment decreased from 1256.11 μg/kg at 0 weeks to 321.21 at 12 weeks, but increased to 607.18 at 18 weeks. The BMBM ethoxyquin treatment increased from 85.70 μg/kg at 0 weeks to 384.07 μg/kg at 3 weeks, decreased to 146.50 μg/kg at 6 weeks, and increased to 268.72 μg/kg at 18 weeks. The CBPM unpreserved treatment increased from 296.32 μg/kg at 0 weeks to 500.55 μg/kg at 3 weeks, decreased to 284.78 at 6 weeks, and increased to 554.74 at 18 weeks. The CBPM mixed tocopherols treatment decreased from 137.99 μg/kg at 0 weeks to 90.54 μg/kg at 3 weeks, but increased to 181.12 by 18 weeks. The CBPM ethoxyquin treatment increased from 59.63 μg/kg at 0 weeks to 84.45 μg/kg at 3 weeks, decreased to 68.01 μg/kg at 6 weeks, and increased to 94.78 μg/kg at 18 weeks.

The decanal concentration of BMBM unpreserved treatment fluctuated throughout the 18 week shelf life (213.08 μg/kg at 0 weeks; 324.06 μg/kg at 3 weeks; 55.45 μg/kg at 6 weeks; 36.52 μg/kg at 12 weeks; and 38.45 μg/kg at 18 weeks). The BMBM mixed tocopherols treatment decreased and increased throughout the course of the 18 week shelf life (42.70 μg/kg at 0 weeks; 7.59 μg/kg at 3 weeks; 20.34 μg/kg at 6 weeks; 17.34 μg/kg at 12 weeks; and 17.91 μg/kg at 18 weeks). The BMBM ethoxyquin treatment increased from 35.97 μg/kg at 0 weeks to 38.53 μg/kg at 3 weeks, decreased to 10.08 by 6 weeks, and increased to 15.25 μg/kg by 18 weeks. The CBPM unpreserved treatment increased from 7.41 μg/kg at 0 weeks to 10.57 μg/kg at 3 weeks, decreased to 5.54 μg/kg by 12 weeks, but increased to 13.57 μg/kg by 18 weeks. The CBPM mixed tocopherols treatment increased from 3.87 μg/kg at 0 weeks to 7.25 μg/kg at 3 weeks, decreased to 2.83 μg/kg at 6 weeks, but increased to 3.85 μg/kg by 18 weeks. The CBPM

ethoxyquin treatment increased from 1.80 μg/kg at 0 weeks to 2.30 at 6 weeks, decreased to 1.97 μg/kg at 12 weeks, but increased to 2.32 μg/kg by 18 weeks.

Human Rancidity Panel

The BMBM unpreserved treatment had the lowest numeric rancidity score compared to the other BMBM treatments at 0 weeks (2.00), but the treatments did not differ ($P > 0.05$; Table 7). The unpreserved treatment had the highest score at 18 weeks (3.65) shelf life compared to all the BMBM and CBPM treatments ($P < 0.05$). The BMBM mixed tocopherols treatment increased from a score of 2.05 at 0 weeks to 2.45 at 18 weeks, which had, numerically, the lowest score at 18 weeks among the BMBM treatments, but did not differ from the BMBM ethoxyquin treatment ($P > 0.05$). The BMBM ethoxyquin treatment, numerically) had the highest score at 0 weeks (2.10) and had an intermediate score by 18 weeks (2.55), but did not differ from the other BMBM treatment at 0 weeks and the BMBM mixed tocopherols treatment at 18 weeks ($P > 0.05$) . The CBPM unpreserved treatment, numerically) had the highest rancidity score compared to the other CBPM treatments at 0 weeks (2.25), but did not differ from the ethoxyquin treatment ($P > 0.05$). The CBPM unpreserved treatment remained the highest among the CBPM treatments at 18 weeks (2.65), but did not differ from the other CBPM treatments ($P > 0.05$). The CBPM mixed tocopherols treatment had the lowest score at week 0 (1.30; $P < 0.05$), but did not differ from the other treatments at 18 weeks (2.05; $P > 0.05$). The CBPM ethoxyquin treatment had an intermediate score at time 0 (2.10) and 18 weeks (2.20), but did not differ from the CBPM unpreserved treatment at 0 weeks and either CBPM treatments at 18 weeks. Among the BMBM and CBPM at 18 weeks, the unpreserved treatments numerically had the highest rancidity score (3.65 and 2.65), the mixed tocopherols treatments had the lowest rancidity score (2.45 and 2.05), and the ethoxyquin treatments had intermediate scores (2.55 and 2.20).

Discussion

Rendered Protein Meals

The PV of unpreserved BMBM and CBPM provide a vivid representation for the stages of lipid oxidation: initiation, propagation, and termination (Figures 1 and 2). Both rendered protein meals behaved similarly to sunflower oil over a shelf life of 90 days (Crapiste et al., 1999), unrefined Pollock oil over a shelf life of 12 weeks (Sathivel et al., 2008), and instant noodles over a shelf life over several intervals (Gotoh et al., 2007). This was by design. The unpreserved BMBM and CBPM were expected to oxidize rapidly and were timed so that they were extruded into a model cat food diet at the peak of propagation. The unpreserved BMBM was beginning to enter the termination phase prior to processing, so it was none too soon. This may have contributed to the decrease in PV during the 18 week shelf life of this treatment (Table 5). The mixed tocopherol treatments slightly oxidized during storage; whereas, the ethoxyquin treatment remained stable. This supports the conclusion of Hilton (1989) and Frankel (1996) that mixed tocopherols may

not provide as much stability on a mass basis as the synthetic form of antioxidants. The AV of the unpreserved and mixed tocopherols treatments continuously rose in the same overall direction as in the study with Crapiste et al. (1999) and Gotoh, et al. (2007). The ethoxyquin treatments did not give rise to any measurable increase in aldehydes prior to processing as determined by the ansidine values. The preparation of the meals resulted in a high and low level of oxidation as the experiment was designed to measure. The MT treatment lead to some oxidation of the meals, but the initial target was that they oxidize more into a mid-range between the extremes. Regardless, the construct of the study was fully established.

Gas Chromatography/Mass Spectroscopy – Rendered Protein Meals

The aldehydes that were measured using GC-MS ranged in several carbons in length, but we chose to report on number of carbon atoms from 6 to 10, such as hexanal, heptanal, octanal, nonanal, and decanal, because they are important contributors to rancid and unpleasant flavors and odors in oxidized oils (Frankel et al., 1985; DeHaan et al., 2004). It was found in the study with DeHaan et al., (2004) that hexanal, heptanal, octanal, and nonanal compounds were consistently detected in animal fats, but decanal was not always found. This would support the low values of decanal that were found in the BMBM and CBPM samples relative to the other volatile compounds (Table 3). In two studies by Greenberg (1981), the volatile aldehydes of meat and bone meal and poultry by-product meal were identified in order of decreasing abundance as hexanal, heptanal, octanal, nonanal, and decanal. Hexanal was identified as the aldehyde in the most abundance, representing about 43% of the overall relative concentration in meat and bone meal and 40% in poultry by-product meal (Greenberg, 1981). Shahidi et al., (1994) found that during a three week shelf life study of meat, hexanal concentration increased rapidly during the early stages of storage and dramatically decreased after six days; thus, mimicking the similar phases of hydroperoxide formation during lipid oxidation. They suggested caution when hexanal is used as a marker of lipid oxidation because it could correspond with two different points of oxidation. This may explain the higher level of hexanal in the BMBM mixed tocopherols treatment than the BMBM unpreserved treatment and the difference in phases of oxidation (Table 4). The aldehydes listed above have been identified as contributors to the flavor of cooked beef and are more concentrated in beef and chicken meat that are uncured versus cured (Elmore et al., 1999, Ramarathnam et al., 1993). It was also suggested by Ramarathnam et al. (1993) that several factors, both pre-slaughter and post-mortem, have an effect on the compounds formed in meat products, such as the type of feed, storage and sanitation conditions, and processing methods.

Tompkins et al., (1999), found the hexanal and heptanal concentrations in soybean frying oil had a high coefficient of correlation with *p*-anisidine value ($R = 0.81$, $P = 0.0001$ and $R = 0.66$, $P = 0.0009$), but *p*-anisidine value was not significantly correlated with nonanal ($R = 0.33$, $P = 0.1299$). Hexanal and heptanal were breakdown products of linoleic acid and linolenic acid; whereas nonanal is only a breakdown product of oleic acid. Additional analysis of the poor

correlation between nonanal and *p*-anisidine should be further studied. Similar to the *p*-anisidine value and peroxide value relationship, the headspace analysis of a food could be utilized in conjunction with peroxide value to identify both primary and secondary oxidation products (Elizalde et al., 1991).

Processing – Pet Food Production

The lower average bulk density of the BMBM out of the extruder agrees with the higher radial expansion relative to the CBPM treatments due to the higher surface area of the BMBM treatments. The lower bulk densities of the BMBM and CBPM treatments out of the dryer would be consistent with the moisture lost during drying of the kibble. The higher radial expansion and lower density of the BMBM treatments may have had an effect on the lower percent moisture of the kibble after drying. The goal was to keep the radial expansion of all treatments consistent as it has been suggested that kibble with a larger surface area are subject to increased oxidation relative to the more dense kibble (Labuza et al., 1971; Nawar et al., 1985; Rao et al., 1989). The cell structures within the kibble of the BMBM and CBPM treatments would have been interesting to compare as it has been suggested that an increase in radial expansion increases the cells or "air pockets" within kibble; which, may also suggest an increase in subjection to oxygen of the kibble with larger "air pockets" (Camire et al., 1990).

Diets

The experimental diet was similar to that produced for a typical cat food (Table 3), which was representative of a higher inclusion of rendered protein meal (30 to 40% of the formula). The higher inclusion would allow for a better measurement of the oxidation of the rendered protein meal without too much dilution from other ingredients.

Treatment Sequence

The process sequence was organized so as to decrease the possibility of residual transmission of antioxidants between treatments; wherein, the diets with unpreserved BMBM and CBPM were extruded first. During processing of the unpreserved BMBM, large pieces of bone from the BMBM blocked the die; therefore, the remaining BMBM treatments needed to be ground to a 3/64 grind to remove any large pieces of bone. After the diets with the unpreserved treatments were extruded, the diets with CBPM preserved with mixed tocopherols and ethoxyquin were completed in the same day. Prior to extrusion the following day, the preconditioner was cleaned to remove any residual antioxidants to eliminate residual ethoxyquin "carry through" during processing (Hilton, 1989). The remaining diets with BMBM preserved with mixed tocopherols and ethoxyquin were then extruded.

Shelf Life Challenge

The oxidation of the unpreserved treatments was rapid and extensive, but oxidation occurred regardless of treatment (Table 5). The unpreserved treatments were expected to have a shorter shelf life than the other preserved treatments, but the preserved treatments had a shorter shelf life than expected; hence, the high values of AV in the preserved treatments towards the end of the shelf life. In the study with Lin et al., (1998), the effect of fat type (beef tallow and poultry fat) and fat content on lipid oxidation of extruded kibble were measured by the TBARS assay. Their control diet without added fat oxidized quicker than the diet coated with poultry fat or beef tallow. This was assumed to be due to the added preservatives in the fat that contributed to the decrease in oxidation of the poultry fat and beef tallow (Lin et al., 1998). This compares favorably to our data in that the unpreserved BMBM and CBPM treatments oxidized faster than the preserved treatments (Table 5). In the 14 month shelf life study with Lin et al., (1998), the kibble with the added poultry fat oxidized quicker than the kibble with the added beef tallow presumably due to the increase of polyunsaturated fatty acid profile of poultry. This fact also agrees with our results in that the CBPM preserved with mixed tocopherols and ethoxyquin had a higher PV and comparable AV to the BMBM mixed tocopherols and ethoxyquin treatments at the end of the 18 week shelf life (Table 5).

The PV at zero weeks (Table 5) for the BMBM and CBPM unpreserved and mixed tocopherol treatments were less than the PV of the raw rendered protein meal prior to processing. This decrease in PV after processing may have been attributed to the dilution of the other ingredients within the kibble formulation. The PV for all treatments, excluding the BMBM unpreserved treatment, continued to increase over time. The BMBM unpreserved treatment continued into the termination phase as demonstrated prior to processing (Table 1). The secondary oxidation products, or AV, increased for all treatments, but not as dramatically for the unpreserved BMBM as the CBPM (Table 5). The AV of the BMBM unpreserved treatment prior to processing was considerably higher for the unpreserved treatments when compared to the mixed tocopherols and ethoxuquin treatments. Processing may have broken down the aldehydes into smaller compounds, such as organic acids, that the AV assay could not measure after processing. Alternatively, oxidation of the other ingredients within the kibble may have contributed to the increase in AV during the 18 week shelf life.

Gas Chromatography/Mass Spectroscopy Shelf Life - Kibble

The aldehydes that were measured using GC-MS ranged in several carbons in length, but we chose to report on number of carbon atoms 6 to 10, such as hexanal, heptanal, octanal, nonanal, and decanal, because they are considered important contributors to rancid and unpleasant flavors and (or) odors in oxidized oils (Frankel et al., 1985; DeHaan et al., 2004). It was found in the studies with DeHaan et al (2004) and Koppel et al., (2013), that hexanal, heptanal, octanal, and nonanal compounds were consistently detected in animal fats and dry kibble, but decanal was not

always found. This would support the low values of decanal that were found in the BMBM and CBPM treatments relative to the other volatile compounds (Table 6). In the study of Koppel et al. (2013), the total aldehydes contributed more than 50% of the total compounds identified in grain and grain-free dry kibble samples. In two studies by Greenberg (1981), the volatile aldehydes of meat and bone meal and poultry by-product meal were identified in order of decreasing abundance as hexanal, heptanal, octanal, nonanal, and decanal. Hexanal was identified as the aldehyde in the most abundance, representing about 43% of the relative concentration in meat and bone meal and 40% in poultry by-product meal (Greenberg, 1981). Shahidi et al. (1994) found that during a three week shelf life study of meat, hexanal concentration increased rapidly during the early stages of storage and dramatically decreased after six days; thus, mimicking the similar phases of hydroperoxide formation during lipid oxidation. Shahidi et al., (1994) suggests that one should be cautious when using hexanal as a marker of lipid oxidation as it may correspond to two different points in the oxidation process. This increase and decrease was observed in the BMBM unpreserved and mixed tocopherols treatments for all volatile compounds within these treatments. The initial increase of hexanal and other volatiles was observed in the BMBM ethoxyquin and CBPM treatments (Table 6). The aldehydes listed above have been identified as contributors to the flavor of cooked beef and are more concentrated in beef and chicken meat that are uncured versus cured (Elmore et al., 1999, Ramarathnam et al., 1993). This increase in concentration is evident in the unpreserved CBPM treatment relative to the preserved treatments (Table 6). It is also suggested by Ramarathnam et al. (1993) that several factors, both pre-slaughter and post-mortem, have an effect on the compounds formed in meat products, such as the type of feed, storage and sanitation conditions, and processing methods.

Human Rancidity Panel

The goal for using an untrained panel was to explore, in a very preliminary way, circumstances that would mimic an at-home consumer based analysis of the pet food and to determine what the consumer may identify as “rancid.” The term “rancid” is often used to designate the off-flavors and odors caused by lipid oxidation in foods in addition to several other sensory attributes (Jacobsen, 1999). Although, the meaning of the term “rancid” may depend on the food product in question. What is “rancid” for one particular food may differ for another (Jacobsen, 1999). The sensory attributes of various foods can be identified with a trained sensory panel equipped to breakdown the components (Koppel et al., (2013), or a lexicon can be developed as in the study with Di Donfrancesco et al., (2012). This analysis provided a basis for a complimentary sensory analysis that will be completed with trained panelists in a study to follow.

Interestingly, the untrained panelists identified the BMBM ethoxyquin treatment to be more “rancid” compared to the other BMBM treatments in the beginning of the shelf life until 18 weeks. But this treatment had the lowest PV and AV throughout the shelf life. The high sensory scores of the of the BMBM ethoxyquin treatment may be attributed to the strong odor of ethoxyquin itself or some other unidentified olfactory note. In the study with Thompkins et al.

(1999), AV correlated (R = 0.82) well with the development of off-odors as the frying/heating time of oil samples increased. This agrees with our results for the AV of the BMBM unpreserved treatment at 18 weeks; wherein, the untrained panelist identified this treatment as the most "rancid" treatment. But the high sensory scores did not agree with the low AV of the other two BMBM treatments at the beginning of the shelf life. The untrained panelist identified the CBPM unpreserved treatment as the most "rancid" treatment throughout most of the shelf life study. These results correspond to high AV of this treatment as well.

Summary

The model used to create oxidized meals was effective. The foods produced with oxidized meals had some "re-set" to the oxidation levels following extrusion. Oxidation of the kibble occurred regardless of treatment, but was rapid and extensive in meals that started with oxidized protein meal. The ingredient oxidation levels were diluted by food production and their oxidation may not completely account for later food product deterioration. Rendered protein meal stability is essential to the shelf life of extruded pet foods, but may have more to do with vitamin and essential fatty acid losses than measures of oxidation, or sensory evaluation by pet owners.

Table 1 Analysis of oxidation measures on beef meat and bone meal (BMBM) prior to processing (mean ± SD).

		BMBM		
	Time (Days)	Unpreserved	Mixed Tocopherols	Ethoxyquin
Peroxide Value (meq/kg)	2	3.33 ± 1.282	2.22 ± 0.000	2.22 ± 0.000
	7	34.44 ± 32.054	2.22 ± 0.006	2.23 ± 0.006
	12	70.95 ± 10.242	3.33 ± 1.282	2.22 ± 0.000
	17	95.53 ±10.260	2.22 ± 0.000	2.22 ± 0.006
	23	109.51 ± 1.819	2.22 ± 0.000	2.23 ± 0.006
	28	102.29 ± 2.448	4.43 ± 0.000	2.22 ± 0.000
	33	106.75 ± 5.260	5.55 ± 1.287	2.23 ± 0.006
	40	104.52 ± 2.690	5.55 ± 1.299	2.22 ± 0.000
	48	96.53 ± 1.155	5.55 ± 1.299	2.23 ± 0.006
	59	87.99 ± 2.448	8.14 ± 1.144	2.22 ± 0.006
p-Anisidine Value (g/g)	2	6.91 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
	7	7.33 ± 6.443	1.91 ± 0.635	0.00 ± 0.000
	12	17.23 ± 2.806	5.28 ± 0.352	0.00 ± 0.000
	17	9.92 ± 1.542	1.61 ± 1.137	0.00 ± 0.000
	23	12.11 ± 0.00	2.11 ± 0.000	0.00 ± 0.000
	28	6.75 ± 2.465	1.48 ± 0.508	0.00 ± 0.000
	33	15.19 ± 4.284	5.23 ± 1.628	0.00 ± 0.000
	40	18.70 ± 0.375	7.47 ± 1.645	0.00 ± 0.000
	48	17.72 ± 1.801	5.68 ± 2.448	0.00 ± 0.000
	59	14.30 ± 3.299	7.76 ± 0.966	0.00 ± 0.000

Table 2 Analysis of oxidation measures on chicken by-product meal (CBPM) prior to processing (± SD), respectively.

		CBPM		
	Time (Days)	Unpreserved	Mixed Tocopherols	Ethoxyquin
Peroxide Value (meq/kg)	2	4.43 ± 5.110	1.11 ± 1.282	1.12 ± 1.287
	7	15.58 ± 5.138	3.34 ± 1.287	2.22 ± 0.000
	12	35.55 ± 7.700	3.33 ± 1.276	2.23 ± 0.006
	19	45.58 ± 14.070	3.33 ± 1.282	2.22 ± 0.000
	27	67.64 ± 1.305	3.34 ± 1.287	2.22 ± 0.006
	38	81.20 ± 11.367	4.43 ± 0.010	2.22 ± 0.006
p-Anisidine Value (g/g)	2	0.15 ± 0.173	0.00 ± 0.000	0.00 ± 0.000
	7	1.54 ± 0.745	0.37 ± 0.156	0.00 ± 0.000
	12	3.62 ± 2.177	1.80 ± 1.397	0.00 ± 0.000
	19	4.32 ± 3.285	1.96 ± 1.189	0.00 ± 0.000
	27	4.46 ± 2.211	1.50 ± 0.421	0.00 ± 0.000
	38	3.02 ± 3.006	1.21 ± 1.072	0.00 ± 0.000

Figure 1 Oxidation measures on beef meat and bone meal (BMBM) prior to pet food processing (average of ±2 days).

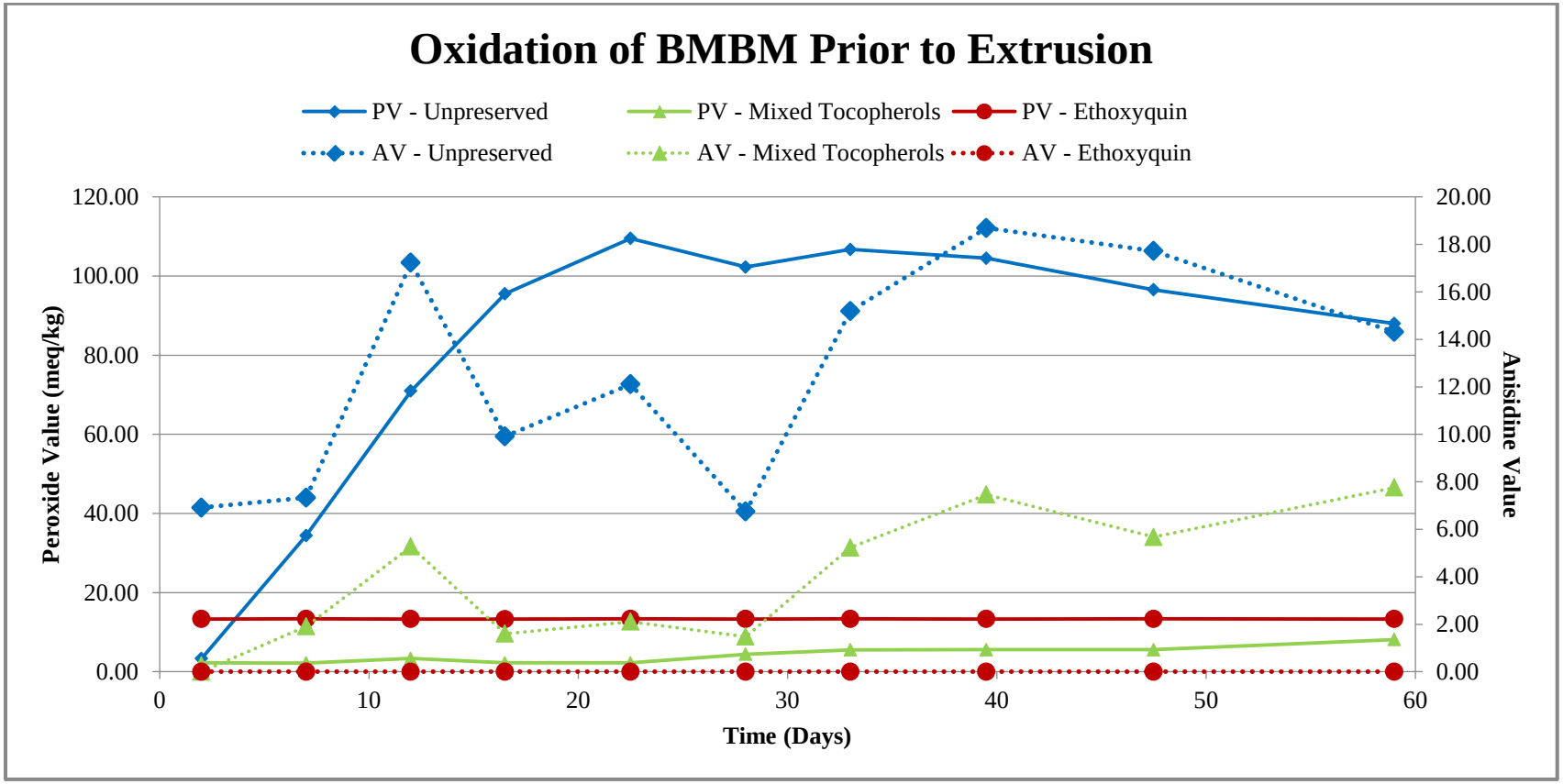

Figure 2 Oxidation measures on chicken by-product meal (CBPM) prior to pet food processing (average of ±2 days).

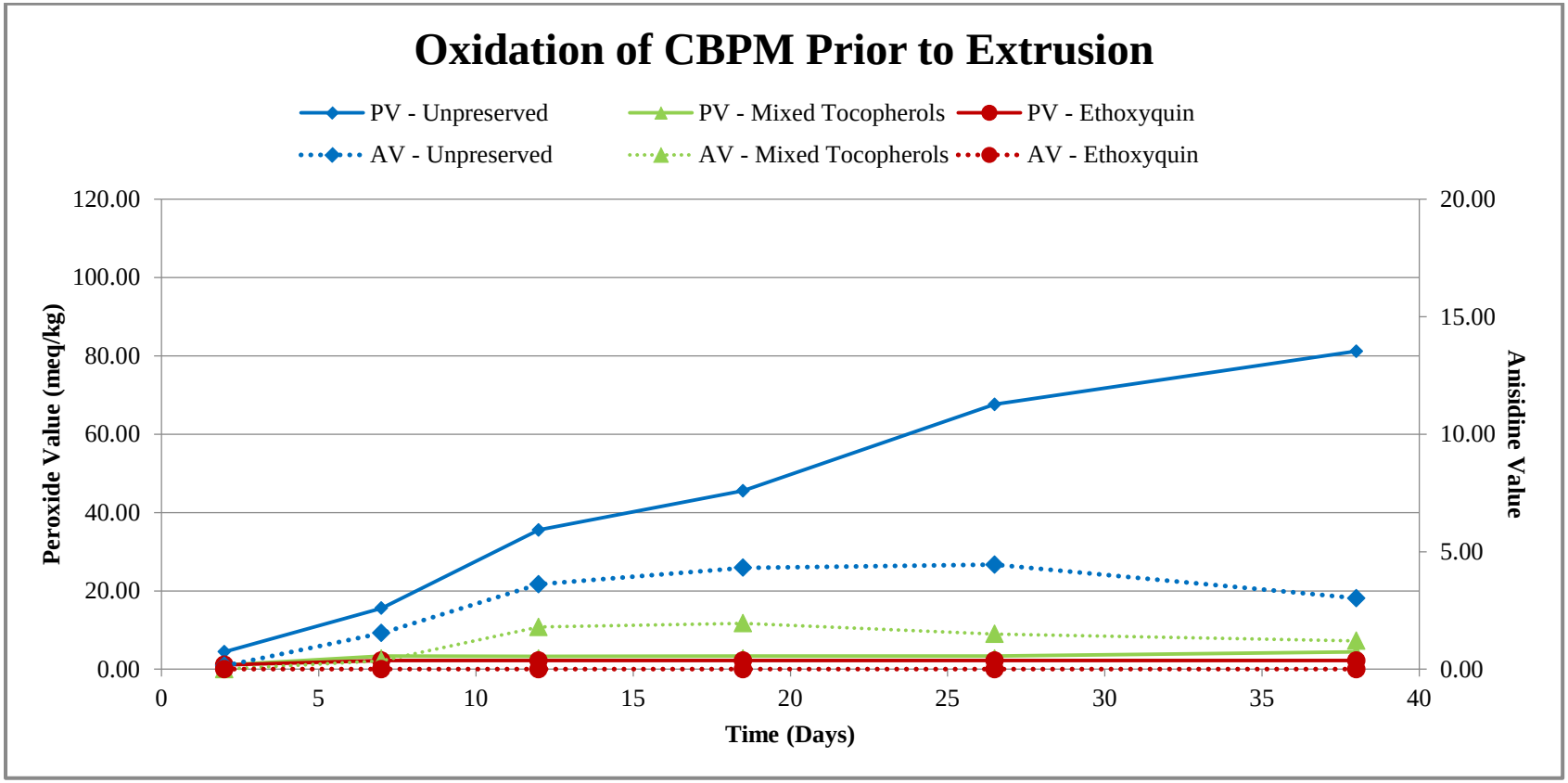

Table 3 Pet food diet produced with oxidized CBPM and BMBM inclusion.

Ingredient	Diet, %	Diet %
Chicken By-Product Meal	37.80	-
Meat and Bone Meal	-	51.37
Rice, Brewers	18.92	14.38
Corn	18.92	14.38
Wheat	18.92	14.38
Beet Pulp	4.00	4.00
Potassium Chloride	0.40	0.40
Monosodium Phosphate	-	0.25
Salt	0.25	0.25
Choline Chloride, 60% Dry	0.20	0.20
Vitamin Premix (Kansas)	0.15	0.15
Trace Mineral Premix (Kansas)	0.10	0.10
DL Methionine	0.10	-
Taurine	-	0.05
Ingredient Total	**100.00**	**100.00**

Figure 3. Extruder Screw Profile

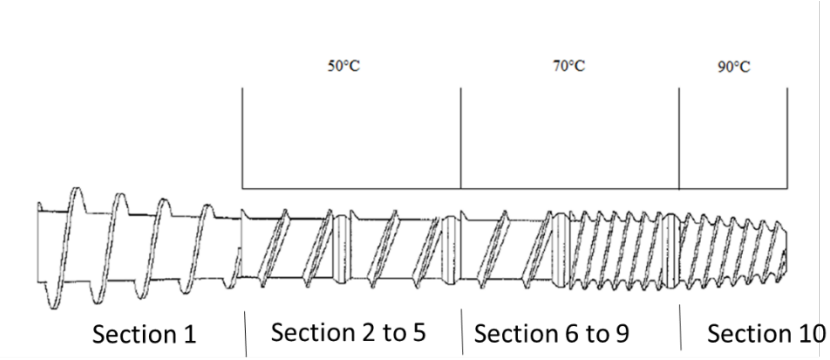

Table 4 Analysis of volatile compounds of rendered protein meals prior to extrusion via gas chromatography.

Compound	BMBM			CBPM			SEM	*P*-Value
	Unpreserved	Mixed Tocopherols	Ethoxyquin	Unpreserved	Mixed Tocopherols	Ethoxyquin		
Hexanal (µg/kg)	5515.74^{b}	15355.82^{a}	1233.18^{c}	5762.33^{b}	3128.79^{bc}	1550.73^{c}	818.615	0.0004
Heptanal (µg/kg)	4642.83^{a}	3081.80^{b}	1108.29^{c}	631.62^{cd}	233.47^{d}	269.79^{d}	233.253	0.0014
Octanal (µg/kg)	7212.00^{a}	4733.21^{b}	691.75^{c}	462.19^{c}	251.86^{c}	154.69^{c}	435.510	0.0011
Nonanal (µg/kg)	4515.96^{a}	4395.62^{a}	767.47^{b}	525.92^{b}	260.47^{b}	179.75^{b}	292.107	0.0014
Decanal (µg/kg)	182.48	657.80	233.32	16.32	9.83	8.54	194.503	0.4480

^{abcd} Means within a row that lack a common superscript differ $P \leq 0.05$.

Table 5 Analysis of oxidation measures (mean ± Sd) under accelerated shelf life conditions (40°C, 70% RH) of kibble produced from oxidized rendered protein meals.

		BMBM			CBPM		
	Time (Wks)	Unpreserved	Mixed Tocopherols	Ethoxyquin	Unpreserved	Mixed Tocopherols	Ethoxyquin
Peroxide Value (meq/kg)	0	10.07 ± 1.345	2.22 ± 0.006	2.22 ± 0.006	14.41 ± 1.259	2.78 ± 0.641	2.22 ± 0.000
	3	8.33 ± 0.652	6.10 ± 0.641	2.77 ±0.629	14.98 ± 1.917	3.33 ± 1.282	2.18 ± 2.140
	6	9.85 ± 2.275	10.79 ± 1.166	2.46 ± 0.566	36.25 ± 5.618	4.91 ± 1.132	1.96 ± 1.960
	12	8.86 ± 0.000	13.29 ± 0.023	2.22 ± 0.006	50.96 ± 20.490	14.40 ± 1.328	2.22 ± 1.700
	18	6.66 ± 0.006	15.48 ± 0.006	3.33 ± 1.287	53.15 ± 12.754	23.21 ± 3.834	4.44 ± 1.800
p-Anisidine Value (g/g)	0	10.08 ± 2.794	9.62 ± 0.930	3.03 ± 0.370	15.56 ± 0.393	3.85 ± 1.166	1.79 ± 1.200
	3	10.38 ± 1.016	7.79 ± 0.941	0.88 ± 0.803	6.47 ± 0.410	2.46 ± 0.629	1.29 ± 0.930
	6	13.86 ± 0.306	11.12 ± 1.068	3.95 ± 0.647	11.28 ± 1.039	4.34 ± 0.266	2.66 ± 2.570
	12	18.77 ± 1.651	15.52 ± 0.675	6.57 ± 0.485	22.04 ±5.433	10.72 ± 0.416	7.02 ± 6.660
	18	17.52 ± 0.156	15.98 ± 2.026	6.12 ± 0.704	33.41 ± 5.987	15.45 ± 1.744	7.53 ± 7.400

[abcdefghijklmno] Means within a row and column that lack a common superscript differ $P \leq 0.05$.

Table 6 Concentration of volatile compounds of accelerated (40°C, 70% RH) kibble via gas chromatography.

Compound	Time (Wks)	BMBM Unpreserved	BMBM Mixed Tocopherols	BMBM Ethoxyquin	CBPM Unpreserved	CBPM Mixed Tocopherols	CBPM Ethoxyquin	SEM	*P*-Value
Hexanal (µg/kg)	0	1,418.86^{ijklm}	7,861.76^{a}	295.08^{op}	1,062.29^{jklmno}	723.93^{mnop}	255.50^{p}	274.812	<0.0001
	3	1,545.81^{hijkl}	3,516.53^{c}	388.69^{op}	1,813.94^{fghij}	580.50^{nop}	331.92^{op}		
	6	1,986.35^{efghi}	2,624.21^{de}	361.84^{op}	2,575.39^{def}	941.55^{klmnop}	743.61^{mnop}		
	12	1,574.72^{hijkl}	2,221.49^{efgh}	588.73^{nop}	3,113.67^{cd}	1,637.76^{hijk}	811.59^{lmnop}		
	18	1,462.56^{hijklm}	3,557.83^{c}	1779.82^{ghij}	4,954.59^{b}	2,501.54^{defg}	1,263.36^{ijklmn}		
Heptanal (µg/kg)	0	842.44^{bc}	521.51^{cdef}	97.77^{ghi}	133.24^{ghi}	43.46^{hi}	28.75^{i}	123.664	<0.0001
	3	747.26^{bcd}	290.67^{fghi}	114.27^{ghi}	139.40^{ghi}	65.99^{hi}	30.07^{hi}		
	6	1,592.52^{a}	386.55^{efgh}	118.94^{ghi}	354.25^{efghi}	97.45^{ghi}	92.72^{ghi}		
	12	1,004.65^{b}	672.35^{bcde}	210.57^{fghi}	426.41^{defg}	332.00^{efghi}	170.63^{fghi}		
	18	1,008.85^{b}	774.46^{bcd}	520.84^{cdef}	980.11^{b}	672.14^{bcde}	234.28^{fghi}		
Octanal (µg/kg)	0	1,857.98^{b}	812.89^{cde}	197.17^{hijk}	217.55^{ghijk}	75.08^{jk}	27.71^{k}	136.938	<0.0001
	3	1,839.88^{b}	546.15^{defgh}	236.74^{ghijk}	336.02^{fghijk}	90.20^{jk}	46.25^{k}		
	6	2,068.78^{ab}	515.64^{defghi}	146.76^{ijk}	462.84^{efghij}	111.57^{jk}	64.61^{k}		
	12	1,816.19^{b}	724.24^{cdef}	181.13^{hijk}	607.95^{cdefg}	253.97^{ghijk}	109.88^{jk}		
	18	2,378.81^{a}	996.08^{c}	327.26^{ghijk}	904.95^{cd}	339.17^{fghijk}	129.28^{ijk}		
Nonanal (µg/kg)	0	1,696.82^{a}	1256.11^{b}	85.70^{lm}	296.32^{hijkl}	137.99^{klm}	59.63^{m}	80.500	<0.0001
	3	1,768.01^{a}	767.97^{def}	384.07^{ghij}	500.55^{gh}	90.54^{klm}	84.45^{lm}		
	6	951.42^{cd}	408.06^{ghi}	146.50^{klm}	284.78^{hijklm}	104.06^{klm}	68.01^{lm}		
	12	1,106.41^{bc}	321.21^{hijk}	159.45^{jklm}	408.64^{ghi}	143.49	83.25^{lm}		
	18	830.94^{de}	607.18^{efg}	268.72^{hijklm}	554.74^{fg}	181.12^{ijklm}	94.78^{klm}		
Decanal (µg/kg)	0	213.08^{b}	42.70^{cd}	35.97^{cdef}	7.41^{g}	3.87^{g}	1.80^{g}	9.293	<0.0001
	3	324.06^{a}	7.59^{g}	38.53^{cde}	10.57^{fg}	7.25^{g}	2.04^{g}		
	6	55.45^{c}	20.34^{defg}	10.08^{fg}	5.73^{g}	2.83^{g}	2.30^{g}		
	12	36.52^{cdef}	17.34^{defg}	14.79^{efg}	5.54^{g}	3.61^{g}	1.97^{g}		
	18	38.45^{cde}	17.91^{defg}	15.25^{efg}	13.57^{efg}	3.85^{g}	2.32^{g}		

^{abcdefghijklmnop} Means within a row and column that lack a common superscript differ $P \leq 0.05$.

Table 7 Sensory (rancidity) characteristics on kibble samples (40°C, 70% RH) kibble samples.*

		BMBM			CBPM				
	Time (Weeks)	Unpreserved	Mixed Tocopherols	Ethoxyquin	Unpreserved	Mixed Tocopherols	Ethoxyquin	SEM	*P*-Value
Human Rancidity Score	0	2.00^{efghij}	2.05^{efghij}	$2.10^{defghij}$	$2.25^{cdefghij}$	1.30^{k}	$2.10^{defghij}$	0.236	0.0067
	3	$2.10^{defghij}$	1.85^{hijk}	2.60^{bcdef}	2.05^{efghij}	1.95^{fghijk}	1.85^{hijk}		
	6	1.65^{jk}	2.65^{bcde}	2.90^{bc}	2.05^{efghij}	$2.10^{defghij}$	1.90^{ghijk}		
	12	$2.50^{bcdefgh}$	$2.45^{bcdefghi}$	3.05^{ab}	2.75^{bcd}	1.80^{ijk}	1.90^{ghijk}		
	18	3.65^{a}	$2.45^{bcdefghi}$	2.55^{bcdefg}	2.65^{bcde}	2.05^{efghij}	$2.20^{defghij}$		

^{abcdefghijk} Means within a row and column that lack a common superscript differ $P \leq 0.05$.

* Based on a scale of 1 to 5 (5 being very rancid) utilizing an untrained panel.

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