Final Project Report

Title: Evaluation of techniques used to extend shelf-life and methods for analysis of rendered protein meals in pet foods.

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Background: Rendered protein meals represent a significant contribution of the quality proteins used to produce modern pet foods. These protein meals carry with them a modest quantity of fat (15 to 20%), which can oxidize readily if not properly preserved. The fastest growth areas in pet food are naturally preserved diets. Keeping these foods shelf-stable is a recurring challenge because natural preservation is more expensive, less effective, and prone to cross-contamination with synthetic preservatives in the supply stream. Synthetic preservatives have proven very effective; but the pet food manufacturing companies are pressured to use the less effective, yet more costly natural-mixed tocopherols. There exist questions regarding the true efficacy of some forms of the natural antioxidant preservatives, whether topical application of the mixtures is effective, and if the methods used to detect the application or the resulting stability are accurate.

Objectives:

1) To determine the efficacy of rapid methods (e.g. SafTest) relative to conventional methods for measures of oxidation in rendered protein meal.

Procedures:

Experiment 1: Characterization of baseline oxidation level, antioxidant efficacy, and particle size distribution of rendered protein meals used in the production of pet foods.

Materials & Methods: In this experiment, five samples of beef meat and bone meal (BMBM) were received from each of two locations (B1 & B2) and five samples of chicken byproduct meal (CBPM) were received from each of three different locations (C1, C2, and C3), and one turkey meal (TM) sample was received. At time of receipt, samples were placed in freezers (0ºC) until each respective protein meal was analyzed. To these samples, standard nutritional analysis was performed (Table 1 and 2). The initial steps in the process were establishment of the assays in our laboratories for peroxide and anisidine value. Samples were analyzed for p-anisidine value (AV), thiobarbituric acid reactive substances (TBARS), peroxide value (PV) via titration by two laboratories and PV via rapid method (SafTest; peroxysafe) (Table 3, 4, and 5). The PV and AV's were determined by the AOCS Official Method Cd 8-53 and the AOCS Official Method Cd 18-90. The samples were also analyzed for TBARS (non-AOCS method) and PV at an external laboratory. Particle size analysis was evaluated by standard Ro-Tap (Testing Sieve Shaker Model B: Combustion Engineering, Inc., Mentor, OH) (Table 6 and 7; Figure 8 and 9) and visual observations of the samples were captured by scanning electron microscope (Figures 10 and 11). The original samples were stored in a freezer at 0°F for about five months and were reanalyzed for PV and AV prior to additional analysis of secondary oxidation products by the AldeSafe and AlkalSafe method (Table 10 and 11). Due to lack of rendered protein meal from sources C1 and C2, these samples were not included in the additional analysis of secondary

oxidation products. The data were analyzed as a completely randomized design and the means were separated by significant F values with α < 0.05 using SAS statistical software (SAS Institute, Inc., Cary, NC). Due to lack of replicate samples, turkey meal was excluded from statistical analysis and simply included for informational purposes.

There is no official method for extraction of oils from rendered protein meals for the determination of PV or AV. The procedure that we used to perform the PV and AV analysis of samples follows in succession. Approximately 200 grams of the respective rendered protein meal was weighed into a 1,000 mL beaker using a digital scale. Once the weight of the meal was achieved, 200 mL of hexane was added. The meal and hexane were allowed to mix for five minutes using a magnetic stir rod and a stir plate. Vacuum filtration was then used to separate the meal from the oil and hexane, which consisted of a standard laboratory vacuum pump, vacuum hose, liquid trap, Büchner funnel, Erlenmeyer flask with hose adapter, and Whatman filter paper. Once the hexane and oil were isolated, the mixture was transferred to a 1,000 mL round bottom flask and attached to a rotating evaporator (Rotavap Büchi R-114: Brinkmann Instruments, Inc.) while partially submerged in a water bath (Büchi B-490: Brinkmann Instruments, Inc) at 50°C. The rotavap was used to gently evaporate the hexane from the oil. Approximately fifteen minutes later, the isolated oil was transferred to a 50 mL conical tube (BD Biosciences) and centrifuged (Sorvall Legend X1R: Thermo Fisher Scientific) at a relative centrifugal force of 4,000xg for fifteen minutes at 25°C. The isolated oil was then analyzed for PV and AV value.

Analysis for volatile organic aldehydes was performed by 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) colum (Restek, U.S., Bellefonte, PA, USA; 30 m x 0.25 mm x 0.25 um film thickness). The intial 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. Most 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 C7-C40 saturated alkane mix (Supelco Analytical, Bellefonte, PA, USA) was used to determine experimental Kovats indices for the volatile compounds detected. For purpose of this analysis all aldehydes with carbons 6-10 were identified and quantified for comparison among treatments and against other methods of detecting secondary oxidation products.

Particle size analysis of each corresponding samples was performed according to the ASAE S319.3 without using a flow agent. Each sieve was adequately cleaned, weighed, and recorded before the material was added. Each corresponding sample of rendered protein meal for CBPM and BMBM was weighed to 100 g in a weigh boat on a digital scale. The sample was then transferred onto the top sieve and the lid to the sieve stack was replaced. The sieve stack was then securely placed into the Rotap and allowed to operate for fifteen minutes. Each sieve had two agitators, a small rubber ball and a brush to aid particle separation. After the allotted time, each sieve was weighed and the difference was determined from the original weight of the sieve.

Data were evaluated as a plot of particle size relative to the weight accumulated (Plot 8 and 9). A determination was made regarding the average particle size, particles per gram, and surface area.

One sample of CBPM and one sample of BMBM were analyzed using a scanning electron microscope (SEM) (S-3500N: Hitachi Science Systems, Ltd.). A small quantity of material was mounted on an aluminum stub using a Pella Brand double-sided adhesive carbon tab. The material was placed at one edge of the tab and then the stub was tilted and tapped to allow the sample to spread across the surface of the adhesive carbon tab. The excess sample was removed with a short burst of compressed air from a "duster" can. The samples were then sputter coated with an alloy of 60% gold and 40% palladium to a thickness of ~4 nanometers using a Desk II Sputter/Etch Unit (Denton Vacuum, LLC). This process is required to make non-metal material conductive, which allows the SEM to use electrons to form an image of the sample. An image of BMBM and CBPM was provided and magnified to 100 and 200 μm respectively.

Results & Discussion: The full compliment of samples initially requested did not materialize, so we adjusted the project plan to move forward with the expectation that the critical objectives outlined by the proposal could still be addressed. For the samples received, the main effect means for the proximate analysis are found in Table 1. The BMBM samples were slightly lower in protein and fat than the CBPM ($P<0.001$). The ash levels in the BMBM were higher than expected (average 35.41%) and nearly 3-fold greater ($P<0.001$) than the CBPM (average 12.12%). This is not problematic in itself, but level of ash may influence the oxidative stability of a product due to a higher content of transition metals present. This question should be addressed at some point in the future. Separating the means into plants within protein meal

category (Table 2), there were some compositional differences among plants for categories. Within the BMBM plant B1 the concentration of protein was lower, but fat and ash levels were higher than plant B2. Further, within he CBPM samples plant C1 and C3 had the highest protein and lowest ash content of the three. This level of ash (< 11%) would typically qualify the ingredient as a "low ash". Fat content differed among the CBPM plants and was greater in all cases than that of the BMBM samples. How this higher level of fat influences stability is not yet known.

The main effect means comparing oxidation measures for the samples received can be found in tables 3. The PV's for BMBM and CBPM were 10.42 and 58.08 meq/kg, respectively from laboratory 1; 4.65 and 3.01 meq/kg, respectively from external laboratory; and 11.52 and 42.96 meq/kg, respectively by the rapid "SafTest" method. The Anisidine values were not different among the main effect means, but the TBARS were slightly greater for the CBPM than BMBM (0.6 vs 0.4, respectively; P<0.001). The main effect means of aldehyde levels (Table 3a) did no differ between protein meals.

When evaluating the plant means among the various protein meals (Table 4) the PV – Lab 1 from plant B1 did not differ from B2 ($P > 0.05$). The PV – Lab 1 for the CBPM from plant C1 was greater ($P < 0.05$) than either C2 or C3, but C2 and C3 did not differ from each other. The TM had a PV – Lab 1 of 6.7 mEq/kg, which was numerically the greatest PV recorded for turkey meal among the three PV methods. The PV – Lab 2 for plant B1 was greater than that of plant B2 ($P < 0.05$) and the PV – Lab 2 for C1 was less ($P < 0.05$) than either C2 or C3, and C2 and did not differ from C3. The PV – Lab 2 for TM was 3.4 mEq/kg, which was different

numerically from the other two PV methods. The PV - SafTest for location B1 did not differ

from B2. For CBPM, the PV - SafTest for location C1 was greater ($P < 0.05$) than either C2 or

C3, but C2 and C3 did not differ from each other. The PV - SafTest for TM was 0.7 mEq/kg,

which was numerically the lowest recorded PV for TM among the three PV methods. The

anisidine value (AV) for material received from B1 did not differ from B2. Whereas, the AV of

the CBPM C2 was less ($P < 0.05$) than either C1 or C3, but C1 and C3 did not differ from each

other. The AV for TM was 0.6. The TBARS of material from location B1 differed from that of

B2 ($P < 0.05$). For CBPM, the TBARS of material from C1, C2, and C3 differed from each other

($P < 0.05$). The TBARS of TM was 0.1 mg MDA eq/g oil. The level of aldehydes within the

protein meals for the various plants differed for each of the various carbon lengths. Hexanal is

most commonly measured as it can be a breakdown product from the oxidation of linoleic acid.

An essential fatty acid for dogs and cats, and a prominent fatty acid in animal protein meals. In

this instance the level of hexanal detected was lower for BMBM from plant B2 than from plant

B1. Futher, the hexanal level for CBPM from plant C3 was lowest ($P<0.05$) and greatest

($P<0.05$) for the plant C1 with C2 intermediate between them. This pattern is consistent with the

results observed for other oxidation measures and may serve as a prime marker for the

development of future methods.

In table 5, the PV – Lab 1 was highly correlated with the PV - SafTest method ($R = 0.98$), but

not with PV – Lab 2 ($R = 0.46$). The PV - SafTest was not correlated with the PV – Lab 2 ($R = 0.37$). The AV method did not have a strong correlation with the PV – Lab 1 ($R = 0.61$), PV -

SafTest ($R = 0.63$), or PV - Lab 2 ($R = 0.22$). The PV measures primary oxidation products;

whereas AV measures secondary oxidation products and would be later to develop during the

oxidation process. The TBARS were not well correlated with titration PV (R = 0.09), SafTest PV (R = 0.13), or lab 2 PV (R = 0.48). TBARS and AV were not correlated (R = 0.01), but they both measure secondary oxidation products; whereas TBARS measures malondialdehydes and AV measures aldehydes present in a sample. This would suggest that there is merit to additional evaluation regarding methods for determining secondary oxidation products in rendered protein meals to verify if a more effective method could be developed. To apply these correlation coefficients further, most of the aldehyde measures were related to the PV-Lab 1 and AV. The most prominent seems to be the Hexanal's correlation to PV-Lab 1 (R = 0.84) and Heptanal's correlation to AV (R + 0.77).

Another factor of this study was to establish a base-line for particle size characterization and to gain an understanding regarding the "landscape" of the material that suppliers have to overcome in which to effectively stabilize the oxidative components of these meals. In table 6, the main effect means of particle size and surface area did not differ between BMBM and CBPM. But, the particles per gram was greater for BMBM when compared to CBPM ($P \leq 0.05$). When separated into plant origin (Table 7), the particle size of B1 did not differ from B2 ($P > 0.05$); but, within the CBPM particle size from plant C1 was smaller ($P < 0.05$) than either C2 or C3 and C2 did not differ C3 from each other. The particles per gram for location B1 was less than B2 ($P \leq 0.05$) and particles per gram for location C1 differed from C2 and C3 ($P \leq 0.05$). The surface area for B1 was smaller than that of B2 ($P \leq 0.05$), and among the CBPM the surface area for C1 was greater than that of C2 and C3, but C2 and C3 did not differ from each other ($P > 0.05$). While particle size of one class of protein meal to the next may appear similar, there were differences observed between plants. These differences were also observed for the number of particles per

gram and the surface area. This would suggest that the flow characteristics and potential for uniform treatment with an antioxidant preservative would need to be adjusted accordingly to achieve uniform application. This would indicate there is merit to additional evaluation regarding flow properties and material size reduction and how they might influence preservative application and shelf stability. To illustrate this point, Figure 8 and 9 provide examples of each respective meals particle size distribution plotted over geometric mean diameter. The both appear to have a similar bi-phasic pattern to their profile with a substantial portion of the particles of 500 micron mean diameter. The CBPM seems to have a greater portion of these observations than the BMBM. However, as stated above, the mean diameter between the samples was statistically similar ($P > 0.05$). Given they are of the same size, this would suggest that the BMBM samples were heavier (possibly due to ash content) and might have a different flow characteristic during handling and coating with antioxidant preservatives. More work in this area will help to shed more light on the topic.

The corresponding scanning electron micrographs of the same samples are pictured in Figures 10 and 11, respectively. The scanning electron micrographs reveal the non-uniform topography of the particulate in the rendered protein meals. One can discern various components such as bone and upon very close inspection can see "streams" of solidified fat. It also points out that there are a multitude of fissures and crevices where preservatives (and antimicrobials) might be challenged to penetrate. This doesn't provide resolution to the challenges itself, but may inspire more creative ways to approach the problem than has been deployed in the past.

To facilitate an additional analytical methodology to assess secondary oxidation products, the original sample material remaining at the conclusion of the studies were analyzed by the rapid technique "AldeSafe" and "AlkalSafe" by MP Biomedical. To perform the analysis the original samples from the first set of evaluations had been kept frozen (-20°C) for nearly five months prior to this series of tests. Further, the PV – Lab 1 and AV were for these samples was reanalyzed to verify the initial data (Table 3). Due to lack of rendered protein meal from sources C1 and C2, these samples were not included in the additional analysis of secondary oxidation products. Comparing the initial analyzed samples to the frozen, five month old samples, the PV – Lab 1 and AV of source B1 increased, whereas source B2 decreased in PV and increased in AV. The opposite effect occurred for source C3 as the PV – Lab 1 increased and the AV decreased. This further supports the notion that PV is an inconsistent method. As reported in table 12, there was a larger variation in PV results for the sample source B1 (43.4 meq/kg) than for that of source B2 (3.5 meq/kg); source B1 also had a higher average PV – Lab 1 than that of B2, but B1 and B2 did not differ. The average AV and AlkalSafe value for sources B1 (9.5 and 1.8 nmol/mL) and B2 (2.9 and 0.3) were different between sources ($P < 0.05$), but AldeSafe (0.7 versus 0.5 µmol/mL) values did not differ between sources. The overall values for each oxidation measures of B1 (43.4 meq/kg, 9.5, 0.7 µmol/mL and 1.8 nmol/mL) were highest relative to B2 and C3 (3.5 meq/kg, 2.9, 0.5 µmol/mL, 0.3 nmol/mL and 5.3 meq/kg, 4.1, 0.5 µmol/mL, 0.3 nmol/mL, respectively).

The correlation coefficients for the relationships between oxidation measures for the meal samples are presented in Table 13 for the second set of analysis. Initial data suggested that there was a weak correlation among PV and AV (R = 0.610). Given PV measures peroxides (primary

oxidation products) and AV measures aldehydes (secondary oxidation products) that there was a correlation at all could be surprising if measured early in the product lifecycle. However, these products appear to be late in their age curve. Wherein, the PV and AV's have a strong correlation (R = 0.978). The methodology was not altered from previous analysis, suggesting that as the meals oxidized further during storage a stronger correlation between the methods developed. There were also strong correlations between PV and AldeSafe (R = 0.902); AlkalSafe (R = 0.893). The AV method was correlated with the AldeSafe method (R = 0.948) and the AlkalSafe method (R = 0.940). The AldeSafe method and the AlkalSafe method also had a strong correlation (R = 0.965). The AldeSafe method measures malonaldehydes levels in oil/fat and the AlkalSafe method measures alkenal levels. More evaluation amongst these measures is warranted to differentiate which method might have the most merit for practical sample analysis.

The one year shelf life study of the samples is also in progress having passed the 24 week time period. Due to lack of rendered protein meal from sources C1 and C2, these samples were not included in the shelf life study. An interim time course analysis is provided for each plant (Figures 14-16 ambient conditions and Figures 17-19 under accelerated conditions). For BMBM from plant B (Figure 14 and 17) it appears that the samples were quite variable and remained so throughout the duration of the 24 weeks under ambient storage and for 14 weeks at elevated temperatures in accelerated storage. One would expect that the PV and AV levels would increase over time, but this doesn't appear to happen and each declined regardless of storage profile. This is in stark contrast to the low values and consistency for BMBM from plant C over ambient (Figure 15). However, during the latter period of elevated temperature storage (~ 20 weeks or better; Figure 18), there is a slight rise in both PV followed in time by an increase in the AV.

This is more in keeping with what one would expect. For the sole CBPM sample from Plant C there does not appear to be any appreciable increase in the background levels of oxidation products during the 24 week ambient or accelerated storage period (Figure 16 and 19).

Conclusions: There were differences observed between results among the three PV methods, which confirm the inconsistencies reported between quality control laboratories in the industry. Thus, there is a need for additional evaluation of the methods used to determine oxidative stability of rendered protein meals. Some of these method difference deserve further evaluation; especially, measures of the secondary oxidation products. Particle size, particles per gram, and surface area of protein meals varied substantially among rendering plants. This variability may explain some of the inconsistent results observed for naturally preserved protein meals used in pet foods. This latter area merits additional work under a more specifically designed evaluation.

Table 1. Proximate analysis of BMBM and CBPM main effect least square means.

	BMBM	CBPM	SEM	P-Value
Crude Protein, %	48.89	66.75	1.164	<0.0001
Moisture, %	2.81	3.35	0.27	0.17
Crude Fat, %	10.15	14.98	0.526	<0.0001
Crude Fiber, %	2.24	0.84	0.169	<0.0001
Ash, %	35.41	12.12	0.814	<0.0001

Table 2. Proximate analysis (means ± standard deviation) of BMBM, CBPM, and TM samples received for comparative oxidative composition.

	BMBM		CBPM			TM
Supplier/Source	B1	B2	C1	C2	C3	T1
N=	5	5	5	5	5	1
Crude Protein, %	$46.8\% \pm 2.02^{d}$	$51.0\% \pm 1.51^{c}$	$70.7\% \pm 2.42^{a}$	$61.2\% \pm 3.13^{b}$	$68.4\% \pm 1.29^{a}$	61.8%
Moisture, %	$2.8\% \pm 0.31^{bc}$	$2.9\% \pm 0.62^{bc}$	$3.8\% \pm 1.56^{ab}$	$4.0\% \pm 0.41^{a}$	$2.4\% \pm 0.31^{c}$	1.9%
Crude Fat, %	$11.9\% \pm 1.17^{d}$	$8.4\% \pm 0.87^{e}$	$13.2\% \pm 0.86^{c}$	$15.1\% \pm 1.40^{b}$	$16.6\% \pm 0.21^{a}$	20.7%
Crude Fiber, %	$2.9\% \pm 0.57^{a}$	$1.6\% \pm 0.27^{b}$	$0.3\% \pm 0.22^{d}$	$1.1\% \pm 0.17^{c}$	$1.0\% \pm 0.20^{c}$	1.0%
Ash, %	$37.4\% \pm 2.20^{a}$	$33.4\% \pm 2.21^{b}$	$10.5\% \pm 1.43^{d}$	$15.4\% \pm 1.885^{c}$	$10.5\% \pm 0.90^{d}$	14.2%

[a,b,c,d] Means within a row that lack a common superscript differ $P \leq 0.05$.

Table 3. Main effect mean oxidation measures of BMBM and CBPM.

	BMBM	CBPM	SEM	P-Value
PV – Lab 1, mEq/kg	10.42	58.08	27.351	0.23
PV – Lab 2, mEq/kg	4.65	3.01	0.192	<0.0001
PV – SafTest, mEq/kg	11.52	42.96	24.05	0.37
Anisidine Value	2.45	3.57	0.924	0.41
TBARS, mg MDA eq/g oil	0.04	0.06	0.0023	<0.0001

Table 3a. Main effect mean aldehyde levels from GC-MS analysis of BMBM and CBPM.

	BMBM	CBPM	SEM	P-Value
Hexanal (ppm)	2.54	3.41	0.752	0.42
Heptanal (ppm)	0.65	0.63	0.176	0.95
Octanal (ppm)	1.11	0.58	0.366	0.32
Nonanal (ppm)	0.86	0.99	0.352	0.80
Decanal (ppm)	0.017	0.103	0.1536	0.01

Table 4. Comparison of oxidation measures (means ± standard deviation) in various samples of BMBM, CBPM, and TM.

	BMBM		CBPM			TM
Supplier/Source	B1	B2	C1	C2	C3	T1
N=	5	5	5	5	5	1
PV – Lab 1, mEq/kg	16.85 ± 24.671^{b}	4.0 ± 1.857^{b}	169.35 ± 167.973^{a}	2.66 ± 0.996^{b}	2.22 ± 0.004^{b}	6.65
PV – Lab 2, mEq/kg	5.25 ± 0.269^{a}	4.05 ± 0.066^{b}	2.12 ± 0.145^{d}	3.39 ± 0.226^{c}	3.52 ± 0.067^{c}	3.40
PV – SafTest, mEq/kg	21.64 ± 31.086^{b}	1.39 ± 0.736^{b}	127.93 ± 159.896^{a}	0.49 ± 0.212^{b}	0.47 ± 0.089^{b}	0.70
Anisidine Value	3.57 ± 2.877^{ab}	0.57 ± 0.912^{bc}	5.92 ± 3.058^{a}	0.30 ± 1.025^{c}	4.47 ± 1.835^{a}	0.64
TBARS, mg MDA eq/g oil	0.04 ± 0.006^{e}	0.04 ± 0.007^{d}	0.05 ± 0.003^{c}	0.07 ± 0.004^{b}	0.07 ± 0.005^{a}	0.06

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

Table 4a. Comparison of oxidation measures (means ± standard deviation) in various samples of BMBM, CBPM, and TM.

Supplier/Source	BMBM		CBPM		
	B1	B2	C1	C2	C3
N=	5	5	5	5	5
Hexanal (ppm)	3.76 ± 3.219^{ab}	1.33 ± 0.175^{b}	5.44 ± 2.151^{a}	2.85 ± 0.515^{ab}	0.15 ± 0.000^{b}
Heptanal (ppm)	0.98 ± 0.845^{a}	0.31 ± 0.026^{b}	0.88 ± 0.480^{ab}	0.39 ± 0.134^{ab}	ND
Octanal (ppm)	2.01 ± 2.026^{a}	0.20 ± 0.048^{b}	1.08 ± 0.766^{ab}	0.26 ± 0.183^{b}	0.12 ± 0.044^{b}
Nonanal (ppm)	1.26 ± 1.207^{ab}	0.37 ± 0.062^{b}	2.03 ± 1.554^{a}	0.50 ± 0.188^{b}	0.08 ± 0.041^{b}
Decanal (ppm)	0.017 ± 0.0101^{b}	ND	ND	0.040 ± 0.0000^{b}	0.116 ± 0.0198^{a}

[abc] Means within a row that lack a common superscript differ $P \leq 0.05$.
ND: not detected

Table 5. Coefficient of correlation analysis (Pearson) of methods used to determine oxidative stability of rendered protein meals, respectively.

	PV – Lab 1	PV - SafTest	PV - Lab 2	PV	TBAR
PV – Lab 1	1.00	0.98*	0.46*	0.61*	0.09
PV - SafTest		1.00	0.37*	0.63*	0.13
PV – Lab 2			1.00	0.22*	0.48*
PV				1.00	0.01
TBARS					1.00

* Coefficient of correlation significant at $P < 0.05$.

Table 5a. Coefficient of correlation analysis (Pearson) of methods used to determine oxidative stability of rendered protein meals, respectively.

	PV – Lab 1	AV	Hexanal	Heptanal	Octanal	Nonanal	Decanal
PV – Lab 1	1.00	0.61*	0.84*	0.56*	0.33*	0.86*	0.49*
AV		1.00	0.59*	0.77*	0.38*	0.68*	0.29*
Hexanal			1.00	0.76*	0.55*	0.87*	0.66*
Heptanal				1.00	0.75*	0.89*	0.75*
Octanal					1.00	0.61*	0.64*
Nonanal						1.00	0.66*
Decanal							1.00

* Coefficient of correlation significant at $P < 0.05$.

Table 6. Particle size analysis of BMBM and CBPM main effect least square means.

	BMBM	CBPM	SEM	P-Value
Particle Size, Dgw	492.3	511.9	16.2	0.40
Particles / Gram	37460	20221	2464.6	< 0.0001
Surface Area, cm^2/gram	112.6	102.1	3.8	0.06

[1]Geometric mean diameter

Table 7. Particle size analysis (mean ± Sd) of BMBM and CBPM from 2 and 3 locations, respectively.

Supplier/Source	BMBM		CBPM		
	B1	B2	C1	C2	C3
N=	5	5	5	5	5
Particle Size, Dgw	522.4 ± 41.13^{ab}	462.2 ± 24.00^{bc}	458.2 ± 52.74^{c}	532.8 ± 64.93^{a}	544.8 ± 35.49^{a}
Particles / Gram	28634 ± 3664.75^{b}	46285 ± 8113.00^{a}	31660 ± 17113.89^{b}	14445 ± 5201.95^{c}	14557 ± 5819.25^{c}
Surface Area, cm^2/gram	105.1 ± 5.14^{bc}	120.1 ± 6.17^{a}	115.5 ± 16.16^{ab}	96.3 ± 11.44^{c}	94.5 ± 8.19^{c}

[abc] Means within a row that lack a common superscript differ P ≤ 0.05.
[1] Geometric mean diameter

Figure 8 & 9. Examples of particle size distribution and size for CBPM (left) and BMBM (right), respectively.
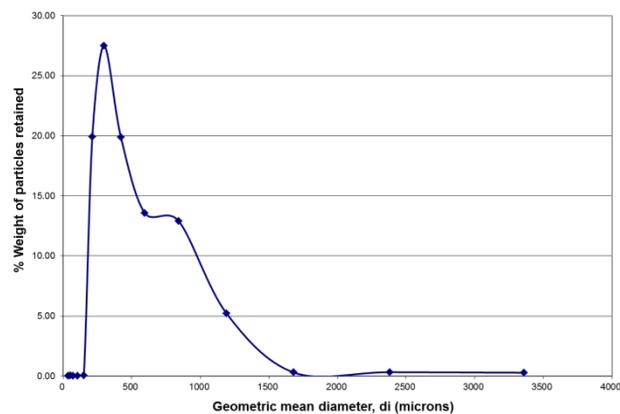
Particle Size, Dgw	486
Surface Area (cm^2) / gram	108.3
Standard Dev., Sgw	1.72
Particles / gram	24,620
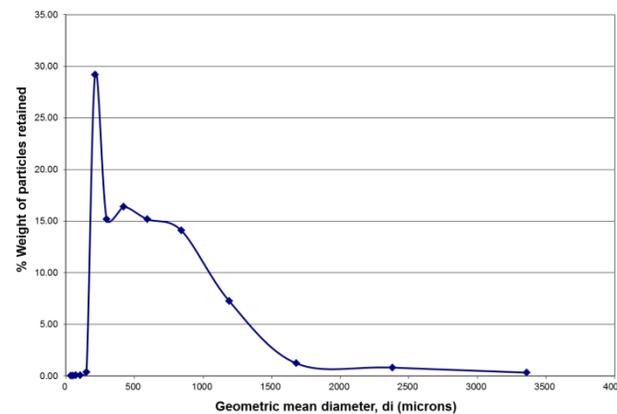
Particle Size, Dgw	502
Surface Area (cm^2) / gram	110.0
Standard Dev., Sgw	1.87
Particles / gram	34,548

Figure 10 & 11. Scanning electron micrographs of representative samples of BMBM (left) and CBPM (right) magnified to 100 and 200 μm, respectively.
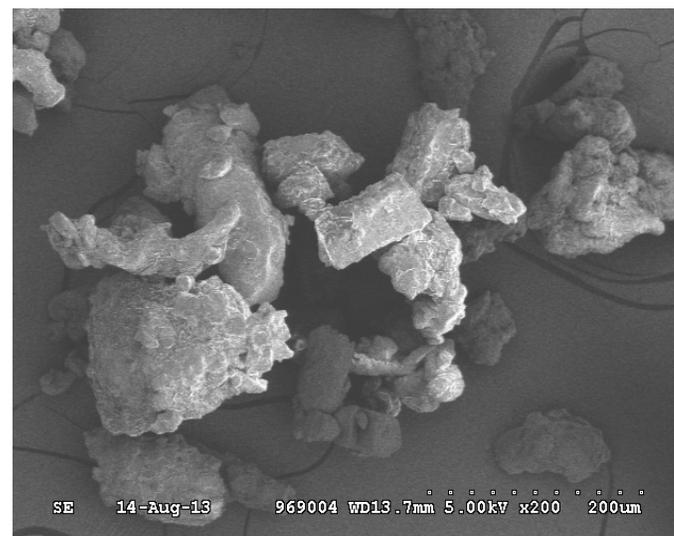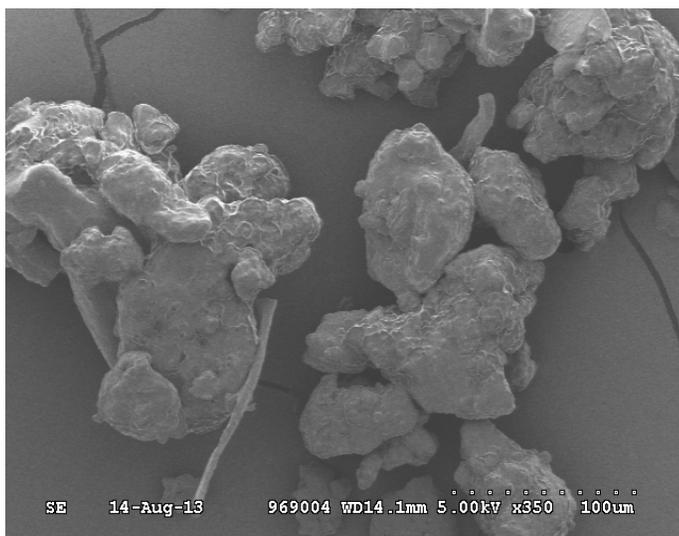

Table 12. Comparison of oxidation measures (means ± standard deviation) of various samples of BMBM and CBPM.

	BMBM		**CBPM**		
Supplier/Source	B1	B2	C3		
N=	5	5	5	SEM	P-Value
PV – Lab 1, mEq/kg)	43.4 ± 53.01^a	3.5 ± 1.20^a	5.3 ± 1.22^a	13.693	0.107
Anisidine Value	9.5 ± 7.67^a	2.9 ± 0.86^b	4.1 ± 0.40^{ab}	2.000	0.085
AldeSafe, μmol/mL	0.7 ± 0.31^a	0.5 ± 0.01^a	0.5 ± 0.01^a	0.080	0.158
AlkalSafe, nmol/mL	1.8 ± 1.41^a	0.3 ± 0.01^b	0.3 ± 0.02^b	0.365	0.021

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

Table 13. Correlation of oxidation measures of various samples of BMBM and CBPM.

	Peroxide Value	Anisidine	AldeSafe	AlkalSafe
Peroxide Value	1.00	0.978	0.902	0.893
Anisidine	-	1.00	0.948	0.940
AldeSafe	-	-	1.00	0.965
AlkalSafe	-	-	-	1.00

* Coefficient of correlation significant at $P < 0.05$.

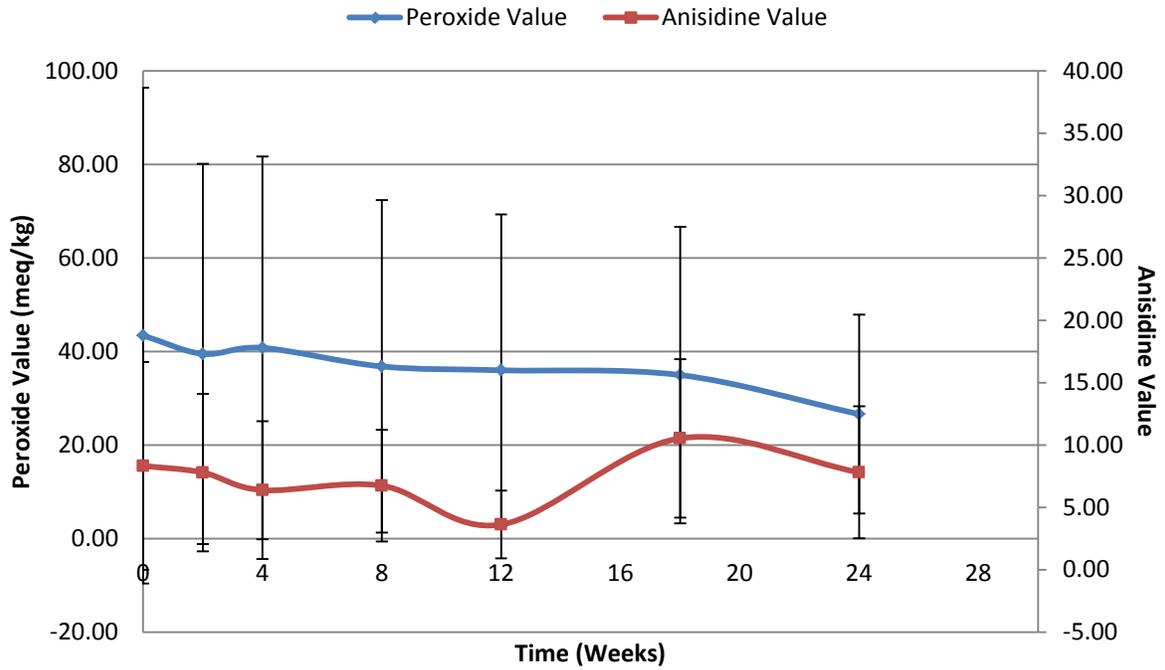Figure 14. Change in PV and AV (mean ± Sd; N=5) of BMBM from Plant B over 24 weeks of ambient storage conditions.

Figure 15. Change in PV and AV (mean ± Sd; N=5) of BMBM from Plant C over 24 weeks of ambient storage conditions.
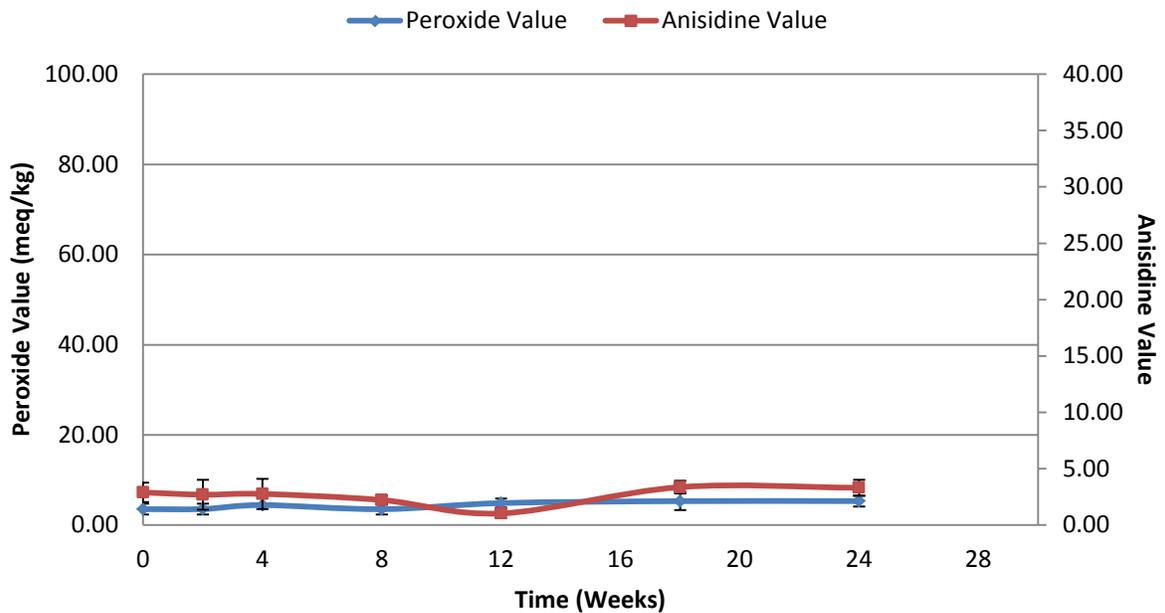
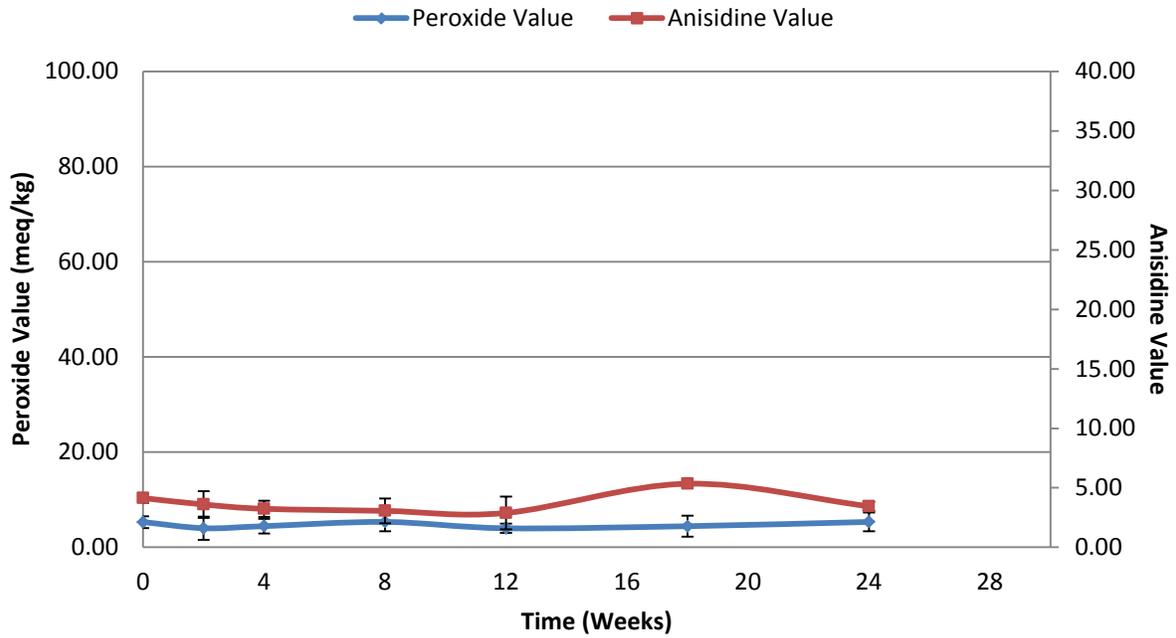Figure 16. Change in PV and AV (mean ± Sd; N=5) of CBPM from Plant C over 24 weeks of ambient storage conditions.

Figure 17. Change in PV and AV (mean ± Sd; N=5) of BMBM from Plant B over 24 weeks of accelerated storage conditions.
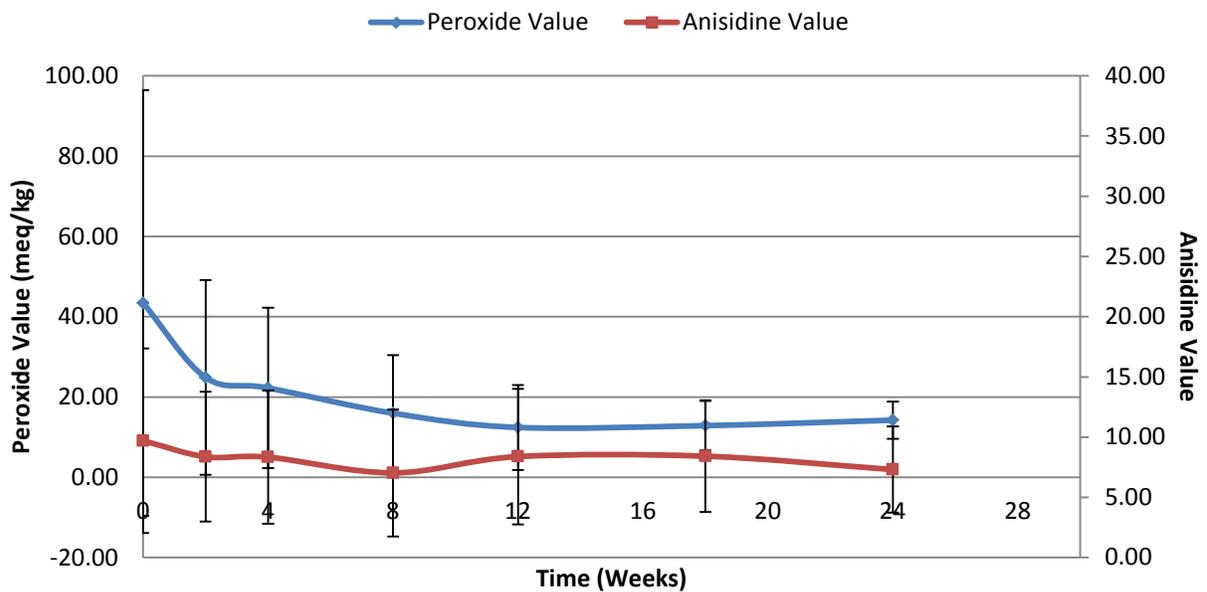
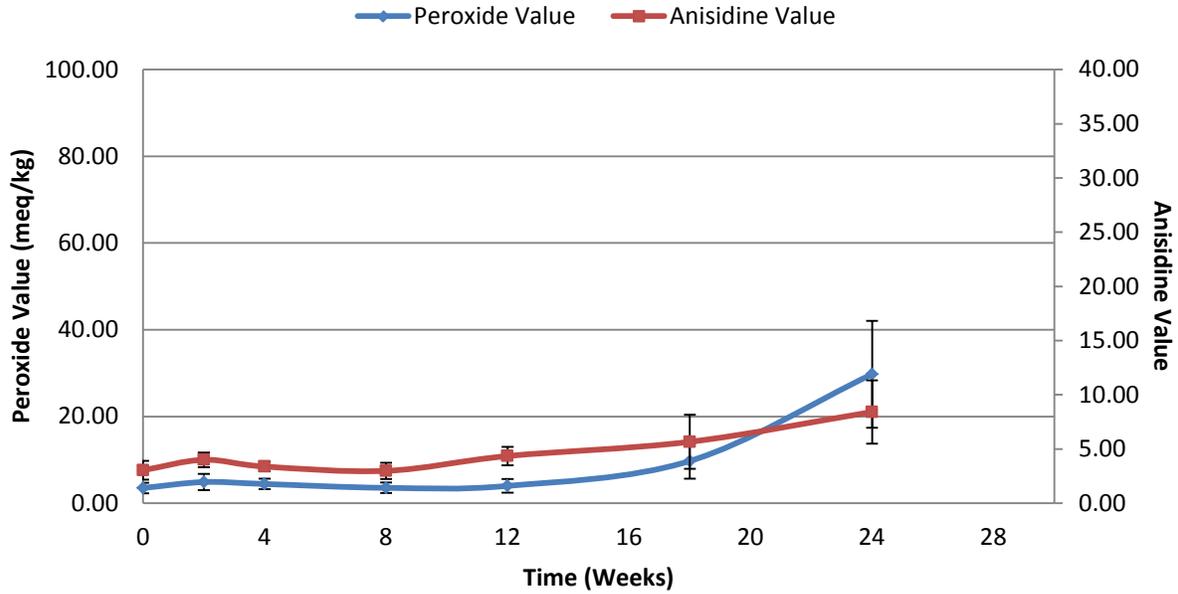Figure 18. Change in PV and AV (mean ± Sd; N=5) of BMBM from Plant C over 24 weeks of accelerated storage conditions.

Figure 19. Change in PV and AV (mean ± Sd; N=5) of CBPM from Plant C over 24 weeks of accelerated storage conditions.