FPRF Technical Services Newsletter

DECEMBER 19, 2006

VOLUME 1, NUMBER 3

"Look deep into nature, and then you will understand everything better"

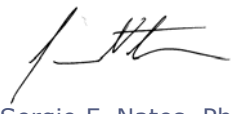
—Albert Einstein

President's Column

The primary rationale for the government to play a role in basic research is that private companies perform too little such research themselves, relative to what is best for society. In addition, government-funded basic research can also stimulate private-sector R&D indirectly. By supporting graduate students and postdoctoral researchers in academic labs where basic research is conducted, federal grants help to train many of the researchers who are hired by companies. This training enhances the productivity and profitability of the companies' R&D investments.

In general, the government tends to focus on basic research, whereas private firms focus much more on applied research and development. However, the distinction between basic and applied research is not well defined, and the division of effort between the two has become less pronounced as the potential commercial value of basic life-sciences research has become more broadly recognized.

On the other hand, companies may not yet have fully mastered the complex new research technologies with which they work; the pool of relatively inexpensive research discoveries may be for the time being useless, awaiting additional advances in basic science. Indeed, strong consumer demand for new products may encourage firms to invest in R&D beyond the point of diminishing returns.



Sergio F. Nates, Ph.D.

Country Focus - Brazil (by Gianni Carniglia)

It is estimated that by the end of 2006, the rendering industry in Brazil will reach a volume close to five million metric tons valued at over one billion $US. During 2006, significant increases in cattle, chicken and hog by-products have been generated as the result of commercial and new sanitary regulations imposed within the different sectors of livestock in Brazil. During the year, prices have fallen for proteins and fats from animals. On the other hand, feed production for animals – the main usage of rendered by products – has grown 3.5% (48.7 million tons). At the same time exports increased, especially for poultry by products. Nevertheless, profits have decreased as the exchange rate rises.

The rendering industry in Brazil consists of 326 registered plants (MAP - Department of Livestock Agriculture and Provision), and among them meat packers and recyclers comprise the largest segment. A group of 35 businesses are grouped at SINCOBESP (Union of Animal By product Recyclers), an organization dedicated to teach, certify

and lobby with the government. Long-term, Brazil is facing many challenges including integration and association membership. Likewise, they will need to comply with norms established by the Department of Agriculture (IN 15/2003), which monitors sanitary, "Best Manufacturing Practices" (BMP) and HACCP conditions in plants. As today, only 40% of the plants overseen by MAP can comply with just 70% of the requisites established by BMP. Brazil is also facing a new challenge as many industry players move into biodiesel production. By 2007, one of the largest meat packers (Bertin), will begin to use tallow for biodiesel production. It is estimated that they will provide 14% of the biodiesel used in Brazil or about 110 million liters per year.

R&D Update

05A-4 Final Report

Diffusion of Protease into Meat & Bone Meal for Solubility Improvement and Inactivation of the BSE Prion

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Technical abstract
Government imposed feed bans have created a need for new applications for meat and bone meal (MBM). Many potential new applications require MBM protein to be both soluble and prion free. Prior studies have shown that certain proteases, including Versazyme, are able to degrade prions in a system where the prions are readily accessible proteolytic attack, which is not necessarily the case when prions are distributed within MBM. These same proteases may also serve to hydrolyze normal MBM protein, resulting in an increase in solubility.

The overall rate of proteolytic MBM digestion depends greatly on whether the protease can penetrate deep within individual particles, or if the protease can only act near the surface of the particle. This research examined the barriers to the diffusion of Versazyme into particles of MBM. Confocal microscopy demonstrated differences in the density distributions between bone fragments and soft tissue particles of MBM. By tracking the diffusion of fluorescently labeled Versazyme through individual particles, it was found that bone particle show full Versazyme penetration within 30 minutes, while penetration of soft tissue particles can take up to 4 hours, depending on the particle's diameter.

A prion surrogate was chosen from the normal protein composition of MBM, and was used to measure the effect of several factors on Versazyme diffusion. Results showed that particles were more susceptible than the soft tissue particles to degradation. Three factors controllable by unit operations in an industrial scale process were also tested. It was found that removing the lipid content, hydrating MBM prior to incubation both significantly improved results compared to control samples. In a test of particle size, the smallest collected diameter range demonstrated the largest degradation of the prion surrogate, suggesting milling would be beneficial.

Introduction

Meat and bone meal (MBM) is a product from the rendering of the unmarketable animal tissue, primarily the bones and offal from slaughtered livestock, the carcasses of deadstock, and meat products that have exceeded their 'sell-by' dates (Garcia, Rosentrater et al. 2006). Prior to the United Kingdom's outbreak of bovine spongiform encephalopathy (BSE) in the 1980s, almost all MBM was utilized as a high protein ingredient in animal feed. Today, most countries do not allow MBM containing any amount of ruminant tissue to be fed to ruminant animals. In the United States, MBM with ruminant tissue is used in feed for non-ruminant farm animals (especially poultry and swine), companion animals, and aquaculture species, which, with the exception of cats, have never been shown to contract BSE

under normal circumstances (Matthews and Cooke 2003; Oidtmann, Hoffmann et al. 2003). In the European Union, MBM is banned from the feed of any animals that may become human food (Taylor and Woodgate 2003). In the EU, MBM is now primarily either incinerated or used for its energy content in operations such as cement plants (Heilemann 2002; Struckmann, Dieckmann et al. 2004), or used as an ingredient in pet food. The Canadian government has recently passed a law that will ban certain cattle tissues (known as "specified risk materials") from all animal feeds, pet foods, and fertilizers (Caparella 2006).

While established outlets for MBM are threatened, the supply of MBM is tied to meat production and thus relatively unresponsive to changes in demand. The development of alternative outlets for MBM is impeded by a couple of important barriers. Most proposed applications for MBM, other than as a fuel, would take advantage of the functional properties of MBM protein. These functional properties are inaccessible unless the highly degraded MBM protein is somehow made soluble, usually by hydrolysis (Park, Bae et al. 2000; Fasl, Wolf et al. 2004; Jung, Roussel-Philippe et al. 2004; Kontur, Fasl et al. 2005). An application that successfully harnessed the protein's functional properties could be rejected due to concerns of BSE prion contamination.

BSE prions are relatively resistant to hydrolysis, compared to other proteins (Novakofski, Brewer et al. 2005). Prion contaminated tissue can be rendered uninfective by extended alkaline hydrolysis (Whiteman, Sofer et al. 2003; Kalambura, Kricka et al. 2005; Yokoyama, Shimada et al. 2006), but the resulting material is extremely degraded and salty and retains little value. Several research groups have identified enzymes capable of digesting prion proteins (Cho 1983; Langeveld, Wang et al. 2003; Hui, Oka et al. 2004; Tsiroulnikov, Rezai et al. 2004), while other groups have developed methods to increase the prion's susceptibility to protease digestion (Caughey, Raymond et al. 1997; Käsermann and Kempf 2003). However, all past demonstrations have presented the prions to the proteases in a 'best case' scenario; typically raw, homogenized neural tissue diluted with buffer is treaded with the enzyme. These scenarios ignore the mass transport barriers the MBM could impose, limiting access of enzyme to prions distributed within MBM particles. Hypothetically, prions could be protected from enzymatic attack by the matrix of rendered soft tissue or bone in which they would exist. The enzyme may not be able to diffuse into fat-laden particles or calcified bone tissue. Further, the overall rate of proteolytic MBM digestion depends greatly on whether the protease can penetrate deep within individual particles, or if the protease can only act near the surface of the particle. Enzymatic digestion from the surface only might be too slow for practical use.

The present research uses the commercial protease Versazyme, and treats its ability to inactivate BSE prions as a given, based on previous literature. The factors that affect the ability of this enzyme to penetrate MBM particles are studied. The results provide information critical to the design of a process to simultaneously inactivate MBM prions and add functionality to normal MBM protein.

Materials and methods

Meat & bone meal was obtained by the Fats and Proteins Research Foundation (Alexandria, VA) from a member rendering firm and provided to the researchers without revealing the identity of the manufacturer, as described previously (Garcia, Rosentrater et al. 2006). The anonymous manufacturer provided detailed information on their raw material and processing method; this information indicated that the MBM was made entirely from cattle tissue, using a continuous, dry rending process.

Versazyme was purchased from Bioresource Interational (Morrisville, NC). Before use, Versazyme was dissolved in digestion buffer (see table 1) and centrifuged to remove insoluble impurities. All directly compared experiments used Versazyme from a single manufacturing lot.

The fluorescent label used was Alexa Fluor 633 (Molecular Probes, Eugene, OR). All other chemicals used were of reagent or molecular biology grade.

Fluorescent labeling

Before labeling, solutions of Versazyme were fractionated by size exclusion chromatography to remove protein impurities. Using a Biologic DuoFlow Chromatography System (BIO-RAD, Hercules, CA), a Superose 12 10/300 GL column (Tricorn, Uppsala, Sweden) equilibrated with 0.05 M sodium phosphate buffer, 0.15 M NaCl, pH 8.0 at 4°C was calibrated using molecular weight standards. When processing solutions of Versazyme, eluent fractions predicted to contain Versazyme were collected and lyophilized.

The purified enzyme was treated with the fluorescent label according the manufacturer's directions. Briefly, a solution of Alexa Fluor 633 in DMSO was added dropwise to a solution of Versazyme in sodium bicarbonate buffer, pH 8.3. After one hour incubation, the protein-label conjugates were separated from unreacted label using a HiTrap Desalting column (Amersham Biosciences, Uppsala, Sweden).

Confocal microscopy

The imaging system used consisted of an IRBE inverted light microscope, connected to a TCS-SP1 Confocal Scanner Head controlled by LCS-SP2 Leica Confocal Software (all components from Leica Microsystems, Exton, PA). To view the autofluorescence of MBM particles, a 488 nm excitation laser was used at 15% power ,and emission wavelengths from 500 nm to 565 nm were viewed. The dichroic filter used was the RSP500. The gain and offset were adjusted to 847.4 V and 51.0%, respectively. To view fluorescently-labeled Versazyme, the 633 nm excitation laser was set to 50% power, while emission wavelengths from 650 nm to 710 nm were viewed. The gain and offset were adjusted to 468.6 V and 50.2% respectively. The dichroic filter TD 488/568/633 nm was used.

A dilute solution with or without labeled enzyme was mixed with MBM and this suspension was quickly transferred to a viewing dish. Using the continuous scan function the field of view, magnified to 20 times its true size, was moved until a suitable particle was located, and then the magnification was increased by 4 or 8-fold depending on the size of the particle. The z-plane focus was then adjusted to show the plane passing approximately through the center of the particle. In experiments with enzyme, images were recorded at 0, 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 240, and 300 minutes. The process was conducted with three bone and three soft tissue particles, which were selected at random.

Enzymatic digestion

Experiments involving the proteolytic digestion of MBM used a solution of Versazyme (0.2 mg/ml in protein solubilization experiments; 0.1 mg/mL in target protein experiments) in digestion buffer (see table 1) at 50°C. MBM was added to this solution at 1% (w/v). Flasks of this suspension were incubated in a SWB 5050 (Labnet, Edison, NJ) shaking water bath set to 175 rpm and 50°C. Control samples omitted Versazyme.

Table 1. Reaction and analysis solutions

	Digestion buffer	Mineral Extraction Solution	Protein Extraction Solution	SDS-PAGE loading buffer
pH	8.0	7.4	8.0	8.0
Tris (M)	0.01	-	0.01	0.01
EDTA (M)	-	0.5	-	0.001
Urea (M)	-	-	7.0	-
Thiourea (M)	-	-	2.0	-
Sodium dodecyl sulfate (% w/v)	-	-	2.5	2.5
N-lauroylsarcosine (% w/v)	-	-	1	-
2-Mercaptoethanol (% w/v)	-	-	-	5.0

Dithiothreitol (M)	-	-	0.05	-
Protease inhibitor cocktail (% v/v)	-	-	0.02	-
Sodium azide (% w/v)	0.05	-	-	-

Protein concentration assay
The amount of protein released into solution during an enzymatic digestion was determined by a standard bicinconinic acid protein concentration assay (Smith, Krohn et al. 1985), using bovine serum albumin to construct the standard curve. Prior assaying, test samples were incubated at 100°C for 5 minutes to inactivate the enzyme.

Analysis of target protein hydrolysis
After a four hour digestion, reactions were incubated at 90°C for 5 minutes to denature the enzyme. To remove soluble protein, reaction suspensions were centrifuged and the supernatants were discarded. The pellets were resuspended in water, recentrifuged and the supernatant was again discarded; the remaining solids were dried in a vacuum oven at 40°C overnight.

Each sample was then partially defatted by mixing with chloroform for 5 minutes and then pouring through a Büchner funnel fitted with Whatman #50 filter paper. To extract bone mineral that might inhibit target protein solubility, samples were then shaken with 10 ml of *mineral extraction solution* (see table 1) at 4°C for 3 days. After this treatment the samples were centrifuged and the supernatant was discarded.

After defatting and demineralizing, the target protein was extracted from the solid material by mixing for four hours with 10mL of a very aggressive protein solubilizing solution, described in table 1 (*protein extraction solution*). This suspension was centrifuged at 4°C and the supernatant was collected.

Ultrafiltration was used to exchange the protein extraction solution for SDS-PAGE loading buffer (table 1). Equal volumes of supernatant from each treatment were loaded into Amicon Diaflow Ultrafiltration Cells (Amicon, Lexington, MA) with 5,000 Da molecular weight cut off Molecular/Por Cellulose Ester ultrafiltration membranes (Spectrum, Rancho Dominguez, CA). The samples were ultrafiltered by applying 45 psi nitrogen gas pressure. When the volume in the cell approached 0 ml, 5 ml of water was added. Again the volume was reduced down to near 0 ml, and then 1 ml of the SDS-PAGE loading buffer was added, and stirred without applied pressure for 5 minutes to recover as much protein as possible from the membrane. The samples were removed from the cell and diluted to 1.5 ml with additional SDS-PAGE loading buffer.

These concentrated samples were analyzed by SDS-PAGE using the Phastsystem (Pharmacia, Uppsala, Sweden). Phastgels with an 8-25% polyacrylamide gradient were used according to the protocol for SDS-PAGE found in the Phastsystem user's manual. The gels were stained overnight with SYPRO Ruby Protein Gel stain (Sigma, St. Louis, MO), and then destained, all according to the manufacturer's directions.

To quantitate the target protein band on the gels, they were scanned on a FLA-5000 Fluorescent Image Analyzer (Fujifilm, Tokyo, Japan) controlled by Image Reader FLA-5000 v2.1 (Fujifilm, Tokyo, Japan) software. The resulting image was analyzed using Multi Gauge v2.02 (Fujifilm, Tokyo, Japan) quantitation software. The concentration of target protein was determined by linear interpolation between the concentration of a standard band of known concentration and a clear background region on the gel.

Testing different diffusion limiting factors
To prepare partially defatted MBM, 3g MBM was mixed with 80mL chloroform and then poured through a Büchner funnel with Whatman #50 filter paper. To prepare fractions of MBM that were primarily soft tissue or bone particles, a heavy-fluid method adapted from Nash and Mathews (1971)was used. Approximately 4 g MBM and 80 ml of chloroform were added to a 100 ml graduated cylinder and stirred with

a metal spatula to break apart any multi-particle clumps. This suspension was allowed to settle for 5 minutes, during which a large majority of the soft tissue particles float to the surface of the liquid and the bone particles sink to the bottom. The top layer of particles and the liquid were then poured through a Büchner funnel with Whatman #50 filter paper, leaving behind the bone particles. Once dry, the meat fraction was collected off the filter paper, and the bone chips out of the cylinder.

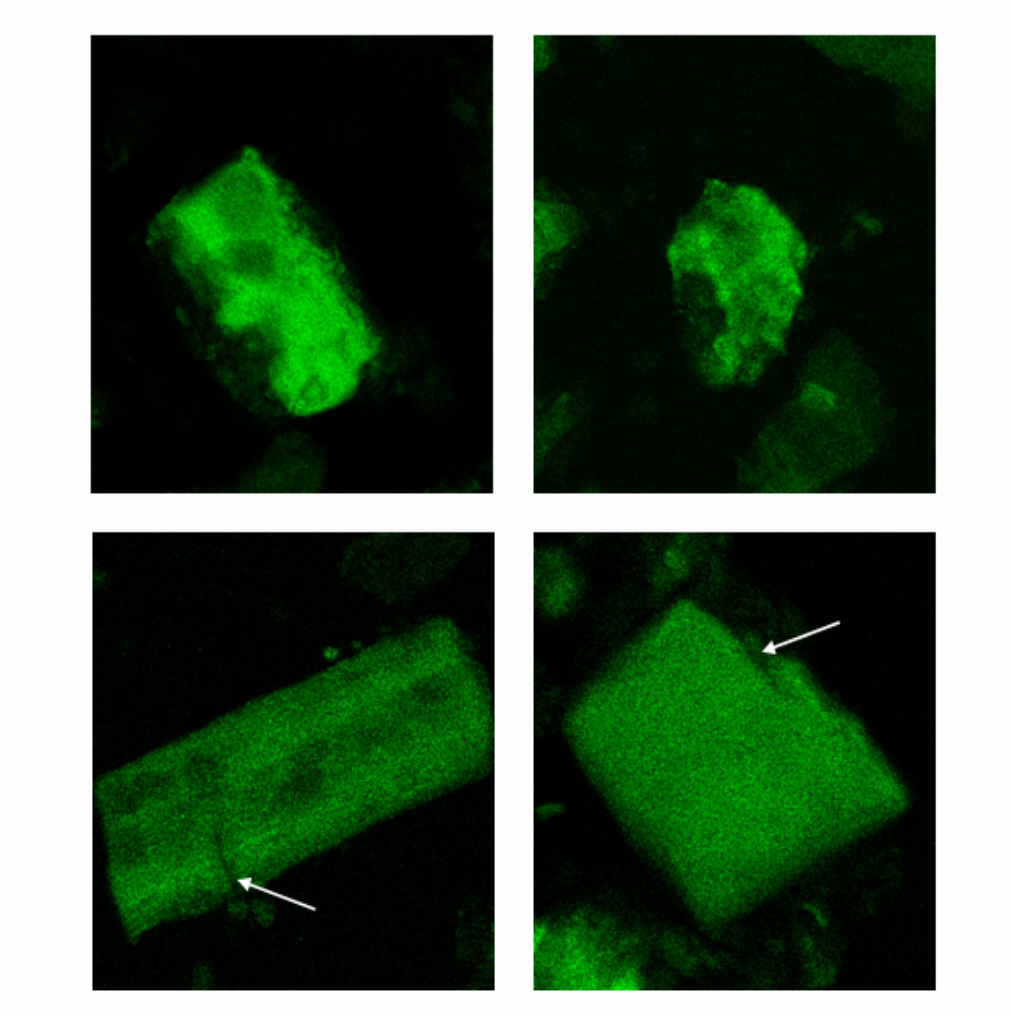
To prepare highly hydrated MBM, 100 mg MBM was suspended in 9 ml digestion buffer was incubated in a shaking water bath set at 25°C, rotating at 175 rpm for 20 hours.

To prepare MBM fractions of different size ranges, 10 g MBM was loaded on the top of a stack of half-height sieves (No. 7, 18, 25, 45, 60, 120, (US Alternative sieve designation system)), and shaken for 20 minutes on a Ro-Tap Testing Sieve Shaker (W.S. Tyler Company, Cleveland, OH). From this fractions labeled 'small' (passing through a sieve with 250 µm openings, but retained on a sieve with 125 µm openings), 'medium' (710 µm, 355 µm), and 'large' (2800 µm, 1000 µm) were obtained.

Results

Soft tissue and bone particles were easily differentiated in microscopic images (figure 1). Qualitatively, soft tissue particles were more irregular is shape and more heterogeneous in density, compared to bone particles. Many bone particles had fissures, extending from the surface of the particle inward.

Figure 1. Representative MBM particles autofluorescing. Particles in top row are soft tissue, particles in bottom row are bone. Arrows indicate 'fissures'.



Fluorescently labeled Versazyme was not obviously excluded from any portions of either soft tissue or bone particles, given enough time (figure 2). The images illustrate the pattern of progressive enzyme infusion observed in all particles studied. Labeled enzyme tended to reach the centers of bone particles long before reaching the center of meat particles. It was apparent that Versazyme infused much more freely along fissures in bone particles (figure 3). Under the conditions tested, Versazyme reached the center of particles before they were visibly eroded due to proteolysis.

Figure 2. Fluorescently labeled Versazyme infusing into MBM particles. Top row (left to right) is a soft tissue particle 0, 20, 90 and 300 minutes after exposure to the Versazyme solution. Bottom row is a bone particle 0, 10, 30 and 60 minutes after exposure.

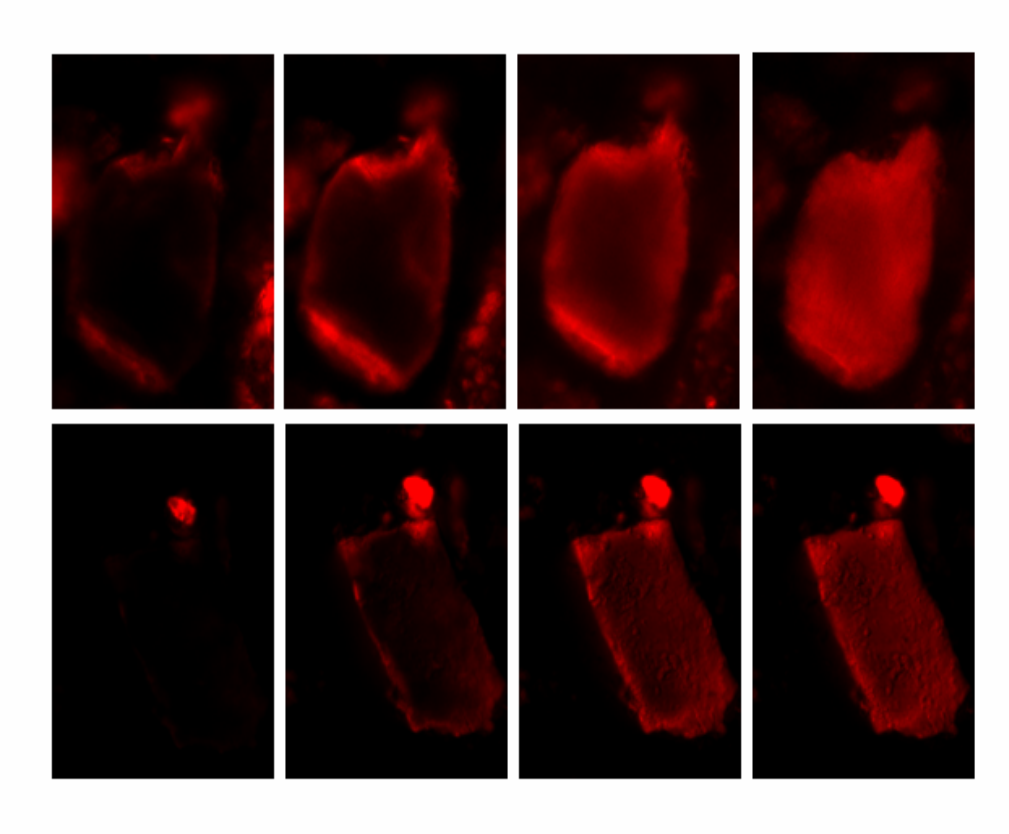
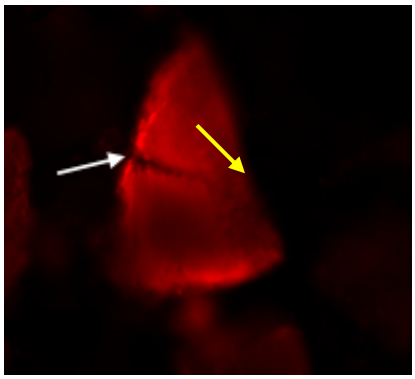
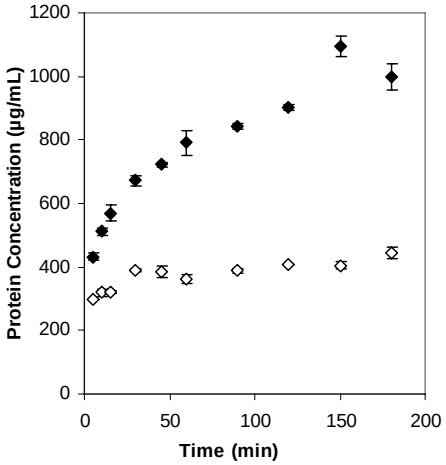


Figure 3. Penetration of fluorescently labeled Versazyme into a bone particle, after 10 minutes incubation. Arrow indicates a fissure in the particle.



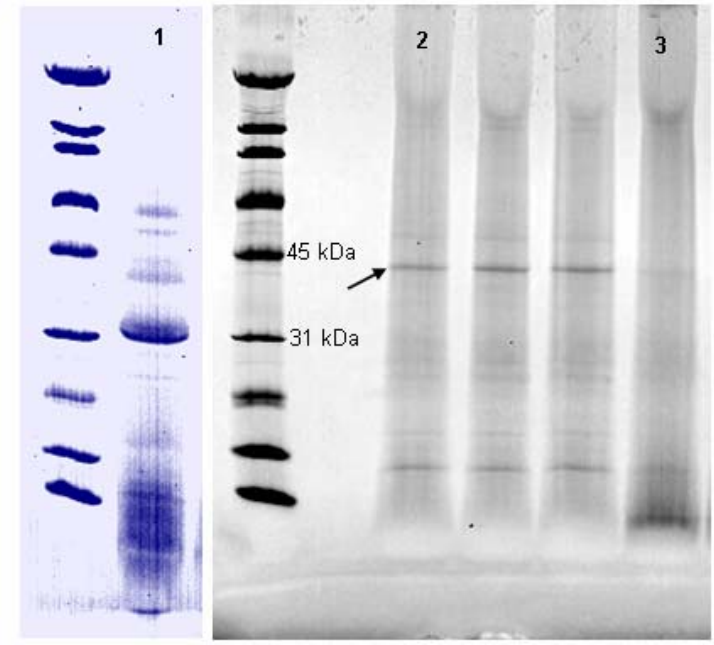
Versazyme catalyzes proteolysis as it diffuses through MBM particles. Normal MBM protein is cut into peptides small enough to dissolve into the surrounding solution (figure 4).

Figure 4. The increase in the solubility of MBM protein and proteolytic digestion progresses. Open symbols represent control experiment with Versazyme omitted, closed symbols represent experiment with Versazyme (n=2, each data point; error bars represent ±1 standard deviation).



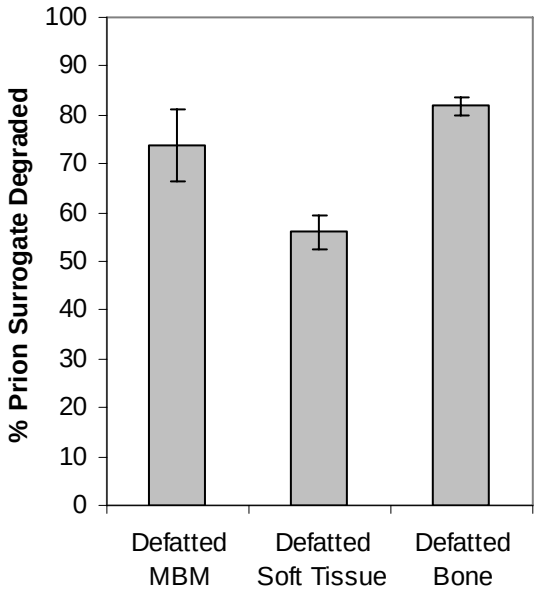
Quantitative measurements on the relative importance of various diffusion-inhibiting factors were achieved using a prion surrogate. Rather than attempting spike MBM with a prion surrogate, a protein that occurs naturally in MBM was selected as the surrogate. A 44 kDa protein (figure 5) was chosen because it met the following criteria: it is insoluble in the conditions used for the enzymatic reaction, it is soluble and can be extracted under special conditions so that it can be quantified by SDS-PAGE and densitometery, it is present in both soft tissue and bone particles at relatively high concentration, it can be hydrolyzed by Versazyme, and it is relatively high molecular weight. No attempt was made to determine the identity of this surrogate protein.

Figure 5. SDS-PAGE Lane 1 is crude Versazyme, lane 2 is extract from MBM incubated without Versazyme, lane 3 is extract from MBM incubated with Versazyme. Arrow indicates band chosen as prion surrogate.



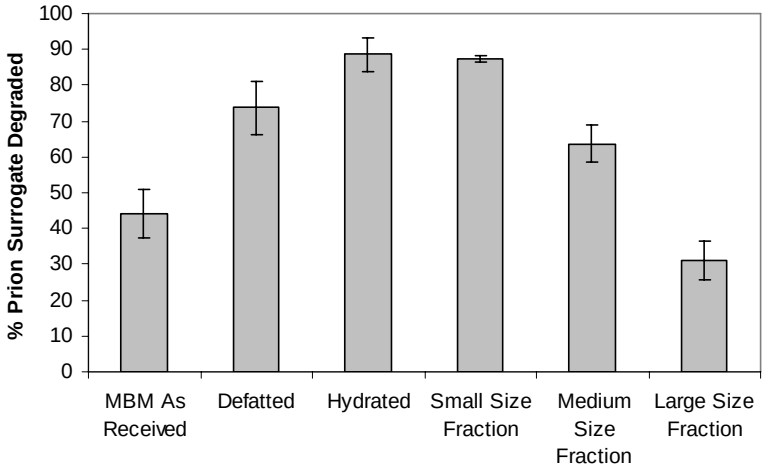
Soft tissue and bone particles differ significantly in the amount of diffusion resistance they present (figure 6). The method used to fractionate MBM into soft tissue and bone particles involves floatation in chloroform, which has the side effect of partially defatting the particles. To control for the effect of this defatting, surrogate degradation in soft tissue and bone particles is compared to surrogate degradation in MBM which was partially defatted in the same manner. Considerably less surrogate was degraded in the soft tissue particles, indicating that they presented greater obstacles to diffusion.

Figure 6. The effect of tissue type on the extent of prion surrogate degradation (n=3, each data point; error bars represent ±1 standard deviation).



Compared to untreated MBM, MBM that has been defatted, rehydrated, or reduced in size presents less diffusion resistance (figure 7). Approximately twice as much surrogate was degraded in particles that were either very small or well hydrated, compared to untreated. Partial defatting had a smaller, but still significant effect.

Figure 7. The relative effect of hydration, defatting and particle size on the extent of prion surrogate degradation (n=3, each data point; error bars represent ±1 standard deviation).



Discussion and conclusions

The treatment of MBM with protease to increase solubility and inactivate prions is technically possible. The practicality of such a system depends largely on whether this can be done rapidly with a relatively small amount of enzyme. This research shows that additional unit operations such as milling, solvent stripping, and hydrating improve the performance of the enzyme in such a treatment and gives some indication of the relative value of each of these operations.

Enzymatic treatments are often dismissed as being prohibitively expensive for price-sensitive applications. This argument is becoming progressively less valid as the enzyme producing industry matures, and enzyme prices drop. The enzyme used this research is marketed primarily as an additive for poultry rations.

Acknowledgements

This research was supported by a grant from the Fats and Proteins Research Foundation of Alexandria, VA. Peter H. Cooke, Darrell O. Bayles, Phoebe X. Qi, Jeng-Jie Wang, Lorelei Bumanlag, and Kelly Grube provided advice and assistance valuable to the success of this project.

ACREC Update – Executive Summary 2006

The faculty and staff of the Clemson University Animal Co-Products Research & Education Center have initiated the second year of sponsored research from the Fats & Proteins Research Foundation. The following is an overview of progress to date alphabetized on researcher name.

Dr. Budd Bodine, has started an exploratory investigation into the use of animal fat triglycerides for synthesis of polymers. They have studied different protocols for maximizing polymerization relevant to the water to organic phase ratios and triglyceride to methacrylic acid ratios. Thus far, the amount of incorporation of methacrylic acid into the triglyceride backbone has not exceeded ~ 10-15 %. When amounts exceed 20% the parameters for cross-linking with bifunctional agents will be determined .

Dr. David Bruce, has reviewed literature on the safety and environmental aspects of using animal fats. Work is ongoing on the calculation of the energy efficiency of operating a commercial diesel engine on animal derived biofuels as compared to traditional diesel fuels. Additionally, a comparison is being made between animal derived and plant derived biofuels.

Dr. Feng Chen, is continuing his work on screening bioactive peptides from animal by-product proteins. The team began a preliminary experiment in June and has obtained the following results: (1) enzymatic hydrolysates from porcine collagen were found to possess strong antioxidant activities using *in vitro* DPPH and metal chelating methods; (2) chromatographic separation of these hydrolysates in an analytical scale tentatively seemed practical and feasible, which helped building a solid foundation for potential scale up. Further investigation found that proteolytic degree of hydrolysis and hydrolyzing time also had significant influence on the antioxidant activity of the collagen hydrolysates.

Dr. John Coates is continuing review the literature for dioxin in animal co-products as well as reviewing economically feasible technologies to remove dioxin and dioxin-like toxicants from animal co-products. Depending on limits promulgated in the United States, there may be the potential for some concern. Reported contaminant levels continue to be below the TEQ of 2 parts per trillion. Some toxicants namely DDTs, p,p'- DDE and Chlordane were reported above the EC limit.

Dr. Paul Dawson, and his collaborators are continuing their work on determining D and Z values of thermally resistant bacteria isolated from raw rendering materials. In order to simulate the heating medium in a rendering operation, ground beef and fat were mixed targeting approximately equal amounts of fat and protein. The determination of the most heat resistant bacteria and their heat inactivation characteristics will allow the rendering industry to establish and document process controls to ensure the final rendered product is free from harmful and other bacteria

that can reduce the quality of the product.

Dr. James G. Goodwin, is continuing his study of the use of a three phase reaction for biodiesel production. Dr. Goodwin and his colleagues have conducted all experiments use a commercially available catalyst, tungstated zirconia (13.4 wt% W) provided by Magnesium Electron Inc. (MEI, Manchester, UK). Goodwin's group have conducted reaction systems studies and transesterification of poultry fat with methanol has been carried out at 120oC in a Parr 4590 batch reactor. The three phase reaction studies involve first the construction of a suitable three phase reactor system, which is now in its final stage. XRD showed the tetragonal structure for the zirconium oxide present in WZ calcined at 700°C. No XRD signals were observed for the formation of WOx crystallites. Building of a three reaction system is almost complete and three phase reaction studies using WZ will start this month.

Dr. Annel Greene and her laboratory team are continuing work on the identification of heat resistant bacteria isolated from rendering products. Microbial results from heat treated and control poultry viscera samples were highly variable even within replicates and contained numerous spreading colonies, and thus, greatly complicated determination of bacterial means. The majority of isolated colonies were Gram positive cocci, but colonies consisting of Gram positive and Gram negative bacilli also were observed. Several of the isolated colonies were identified as *Staphylococcus* species such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus warneri*. Many endospore forming *Bacillus* species such as *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus lentimorbus*, *Bacillus marinus* and *Bacillus pumilus* were observed as well as *Escherichia coli* and *Escherichia coli* O157:H7.

Dr. Xiuping Jiang, continued her study of the antibiotic resistant bacteria. Her lab group completed analyzing approximately 150 fresh rendered meals including feather meal (n=30), meat meal (n=14), meat and bone meal (MBM) (n=37), meat and bone meal from poultry (MBM-P) (n=6), poultry meal (n=51), and blood meal (n=12). The mean total bacterial counts for each meal type ranged from 5 x 103 to 7.5 x 105 CFU/g, whereas the mean enterococci counts were in the range of 4 x 101 to 7 x 103 CFU/g. *Enterococcus* spp. was detected in 122 of 150 samples (81.3%), and accounted for up to 54% of the total bacterial counts in some samples. Additional species identification and genetic determinants for vancomycin resistance are currently being analyzed using real-time PCR.

Dr. Igor Luzinov initiated a project on making plastics and polymer blends from proteins produced by animal co-product industry. The initial results indicate the brittle nature of this plastic; although it has modulus comparable with polystyrene plastic. The next immediate step is to incorporate different impact modifiers such as recycled rubber powder, acrylate and polystyrene-butadiene particles to improve the toughness properties of these plastics. In the future, polymer blends, where the proteins will be mixed with industrially produced biodegradable polymer (polycaprolactone), will be also prepared and tested. Various protein meals such as feather meal (CP 87.2%, fat 8.5%), pet food poultry meal (CP 70.2, fat 11%) and blood meal, have been provided by Fats and Proteins Research Foundation (FPRF).

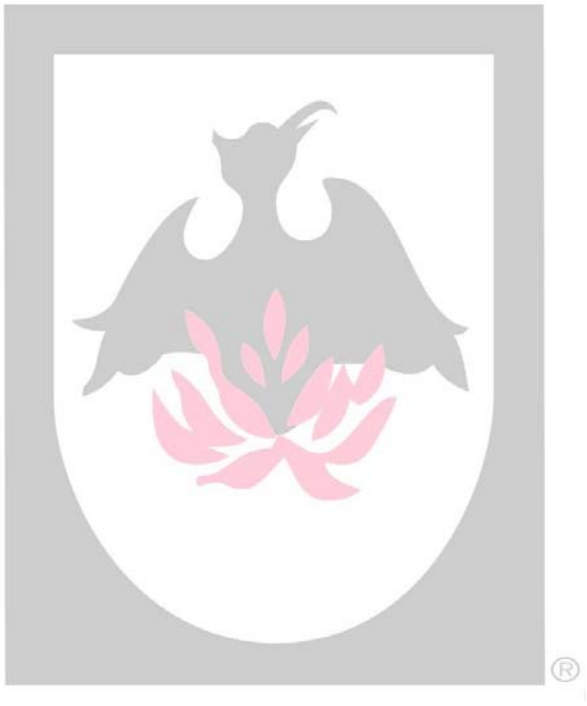
Dr. A. A. Ogale, is continuing his study on extrusion & molding of animal coproduct-based proteins for geostructural applications. In this study, two techniques were used to mix the feathermeal (FM) protein with a plasticizer, glycerol. The proposed research is directed at the use protein by-products for nonfood applications. Specifically, the extrusion and molding of animal by-product proteins is being investigated to develop geostructural sheets for applications such as reinforcements of temporary roads on soft/weak soils and for oil spill containment.

Thomas R. Scott, is continuing his study on the determination of super mammary lymph node proteins and growth factor activity. In this study, the researchers are assessing the efficacy of the supernatant proteins and isolated protein factors recovered from super mammary lymph nodes of dairy and beef cows in order to determine the usefulness of the proteins for biomedical and veterinary purposes. Steps to improve the clarity of the extract samples have been incorporated into the preparation of lymph nodes.

Noteworthy Article

Garcia, R.A., R.A. Flores and C.E. Mazenko (2006) Factors contributing to the poor bulk behavior of meat and bone meal and methods for improving these behaviors. Bioresource Technology. *Article In Press.*

Meat and bone meal (MBM), a product of the rendering industry, is a potential feedstock for numerous bio-based applications. Design of processing equipment for MBM is difficult due to MBM's bulk behaviors; it flows less easily than many other granular materials, and it tends to foul the surfaces of processing equipment. This study examines the major factors contributing to MBM's poor bulk behavior, including moisture content, fat content, particle size distribution and temperature, and the relative importance of these factors. Potential methods for improving MBM's bulk properties, including use of an anti-caking agent, dehydration, fat extraction, milling and refrigeration are also studied. The effects of these factors were determined by a standard laboratory measurement, the Hausner ratio, as well as by the rate of surface-fouling and dust generation using a pilot-scale aspirator. In contrast to past studies with other granular materials, moisture content was shown to have an insignificant effect on MBM's bulk behavior. The results, however, show that MBM fat content is a major determinant of the bulk behavior of the MBM. Reduction of fat content resulted in major changes in MBM's bulk behavior, by all measures used. Less dramatic changes were achieved through refrigeration to solidify the fat and/or treatment with an anti-caking agent.



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