2003 Final Report

Fats and Proteins Research Foundation

Non-Feed Applications for Meat and Bone Meal

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Also enclosed for your review is Dr. Maffia's Industry Summary – Non-Feed Applications for Meat & Bone Meal Technical Report – is some 120 pages – Preparing on CD.

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Project Title: Non-Feed Applications for Meat and Bone Meal

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Industry Summary

Industry Summary – Introduction:

In order to develop technologies for the improvement of the market potential for the nearly 6 billion kg per year of inedible material from animal production, several processing and application technologies were studied regarding meat and bone meal (MBM). Specifically, the MBM in rendered products was subjected to a similar examination given to collagen from corium by the Widener University Collagen Research Group (CRG). In the past and on a continuing basis, this group has focused on the development of applications for corium that include:

direct infrastructure applications
environmental remediation
artificial tissue
controlled release of immobilized therapeutic medicines.

The Collagen Research Group (CRG) at Widener University consists of undergradaute and graduate students, professors from chemical, civil, mechanical and electrical engineering, and colleagues from industry, government and other universities. The group has been in operation at Widener since the early 1990's and has involved about 100 students and professionals. Recently, the CRG has formed research alliances with the Nanotechnology Institute (Southeastern Pennsylvania – a consortium of academic institutions and business organizations) and the United States Department of Agriculture. These alliances have helped in the identification and testing of growth areas for the application of collagen fibrils.

Although the CRG performs some fundamental research, most of the effort has been in the development of new applications for the protein recovered from bovine corium. These applications include environmental remediation, separation and purification, infrastructure, and high value end areas such as controlled morphology matrices of variable geometries for cell growth and controlled release of active agents.

When the CRG first began the studies on bovine corium, it became clear that the initial processing would be the key to the development of applications. Consequently, a strong effort was placed on the development of pretreatment technology to increase the surface area of the material without compromising the chemical and physical properties. Due to the more heterogeneous nature of MBM (than bovine corium), the collagen processing steps were not completely transferable. More front end processing work is required to more fully evaluate the potential of MBM in the higher valued, protein-based applications.

Industry Summary - Objectives:

The key areas of investigation in this initial study were the direct application of MBM in concrete manufacturing and asphalt production. Additionally the raw MBM was pretreated in a manner similar to that currently used for raw corium. The assessment of the efficacy of MBM, alone and as a composite system in a variety of potential applications and therefore high value product outlets was also begun.

The main objectives of the research for the FPRF over the past year were as follows:

1. to study various pretreatment methods that have proven successful in the processing of bovine corium

2. to develop a series of protocols for the production of high surface area, active protein from the feed MBM

   and subsequently

3. to perform screening studies on the application of MBM in a variety of technologies with a broad scope.

Consequently, the main focus studies for MBM were:

1. development of protocols for the milling, washing, extraction and drying of the material

2. direct application of MBM in a variety of infrastructure applications, especially as an additive to concrete manufacture and in the production of asphalt.

Figure 1: Student demonstrating the dispersion process

Industry Summary – Results and Future Directions:

The initial results on the mechanical pretreatment of MBM produced mixed results. A chemical pretreatment or initial segregation of particles may prove beneficial to subsequent mechanical disruption. Further research effort in this area is suggested.

Composite structures with collagen also provided mixed results – the collagen structures did not appear to be negatively affected by the presence of the MBM, but there was also no quantifiable improvement as yet. Additional work in this area is required, especially with material that has been pretreated as above.

Scientific Abstract

Using Meat and Bone Meal (MBM) supplied by the USDA (ERRC, Wyndmoor, PA), the Collagen Research Group (CRG) at Widener University has performed a wide range and significant number of tests regarding processing and applications. The testing included MBM pretreatment experiments, similar to that performed with collagen and applications ranging from admixtures for concrete and asphalt to composite protein structures.

Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), and Digital Microscopy, in additional to the usual gravimetric tests, were used to characterize the incoming material. DSC, in particular was used to identify any phase transitions or other heat effects associated with a temperature scan. The only transitions noticed were those expected with water, presumably from the water contained in the original sample.

Electron microscopy and digital microscopy revealed the heterogenous nature of the particles in MBM. This suggests the ability to fractionate the sample into different size and density cuts by air elutriation. The different cuts, fractionated by terminal velocity, may correspond to different protein contents. These individual cuts may also have differences in physical properties that may make them amenable to certain applications.

Pretreatment experiments of milling neat MBM or a blended sample with collagen were largely unsuccessful. The resulting material did not show any improved properties and there was no synergy by processing collagen and MBM together. This work needs to be repeated over a wider range of concentrations and processing conditions. Also, some pre-segregation, via elutriation or a similar technique may provide a material with physical properties that would provide performance closer to that of collagen.

Additionally, although the MBM did not swell or produce dispersions similar to milled corium (99% Type I collagen), there may be some construction applications for dried matrices that do not require material with such extreme rheological properties. Upon testing the asphalt samples containing various amounts of bone meal, the best results were achieved with when there is one percent bone meal relative to the overall weight.

Concrete results were less conclusive. The samples seemed to yield in stages. The initial yield point was much less than samples without MBM. However, the ultimate yield point was nearly equivalent. Additional testing is required over a more complete range of compositions.

In conclusion, it appears that the application of MBM in infrastructure applications may have some promise and should be studied further. The higher end applications may become more attractive upon the utilization of a fractionated sample. A review of the recent literature indicates that there are research groups active in this area.

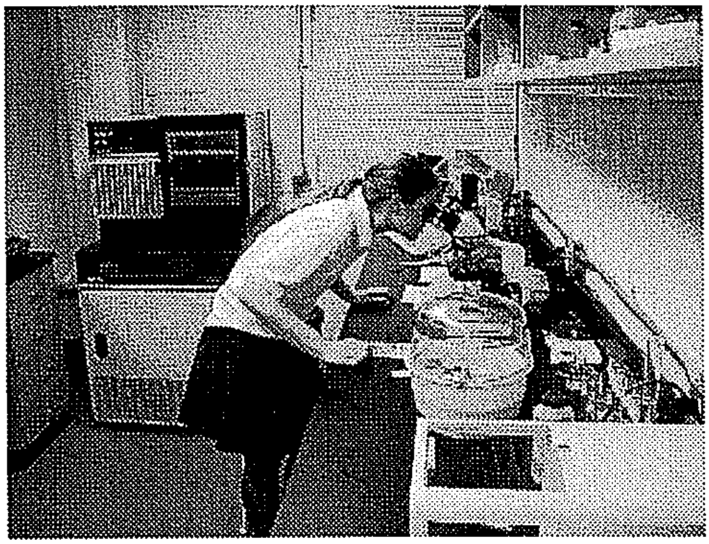
Key Words: Meat Meal, Bone Meal, Asphalt, Concrete, Collagen

Introduction

Background of the Collagen Technology Practiced By The Collagen Research Group (CRG)

Collagen is a biodegradable polymeric fibrous protein found in all animals. In a series of steps, the collagen molecule assembles into a fiber that has the appearance of a rope. The material is insoluble in water, but can retain many times its own mass in water near its charged surface. This and the ability to unravel the fiber thus maximizing the surface area is the key physical property that leads to numerous environmental and biotechnology applications.

Figure 2: CRG student working in the Protein Processing Laboratory



With regard to environmental applications, when added to sludge or any material with suspended solids, a collagen dispersion causes agglomeration, the formation of large flocs, and settling, both at a very rapid rate. The material has proven to be effective in the rapid agglomeration of fine solids in all types of sludge: industrial, water treatment, waste water, inert suspensions, and kaolin.

It has also been discovered that collagen dispersions may also be used in other environmental applications such as, as an aid to filtration, separation of pollutants (including metals and soluble organic molecules) from aqueous streams, selective fractionation of molecules, and oil droplet stabilization. Moreover, because treated collagen can hold hundreds of times its mass in water, there iare potential applications for unraveled collagen in water purification (with minimal energy consumption) and in water siphoning. All of these applications are based on the affinity of the activated surface of collagen, carrying positive charges, for the negative end of the polar water molecule. Processing and applications research (Figures 2,3,4) are accomplished in several laboratories at Widener University by the CRG.

Further processing of the dispersions yields products suitable for biotechnological applications. When the collagen dispersion is frozen and then freeze dried, the resulting material retains the overall dimensions of the original frozen material. However, over 99% of the volume is empty and the structure of the protein is a spongy organic aerogel with controllable pore size, good mechanical properties and a density of one thousandth of water. This solid material can be crosslinked to anchor or memorize its shape, pore size and morphology.

Covalent bonds, between adjacent collagen molecules, are formed during crosslinking; thus the resulting material will no longer disperse or retain water. When placed in water, the crosslinked collagen sinks because the specific gravity is slightly higher than that of water. During the process of crosslinking, the material that is produced is also sterile. This material has enormous potential in biotechnology especially in the area of cell culture.

Some of the cell culture applications include substrates for:

a) achieving high cell density in bioreactors leading to increased productivity and reduced reactor sizes
b) hosting unusual and hard-to-culture cells that are used for a variety of applications including biosensors
c) organ and tissue technology that have medical implications (examples are organ regrowth, skin replacement, coating of prostheses and implants, etc.)
d) coating of cell culture devices such as roller bottles or glass beads
e) collagen membranes for cell culture and biomolecule delivery
f) controlled release of pharmaceuticals. In non-biotechnology applications the freeze dried, crosslinked collagen matrix can serve as an organic aerogel.

Other possible uses for this material include encapsulation of a wide variety of organisms, enzymes and synthetic material.

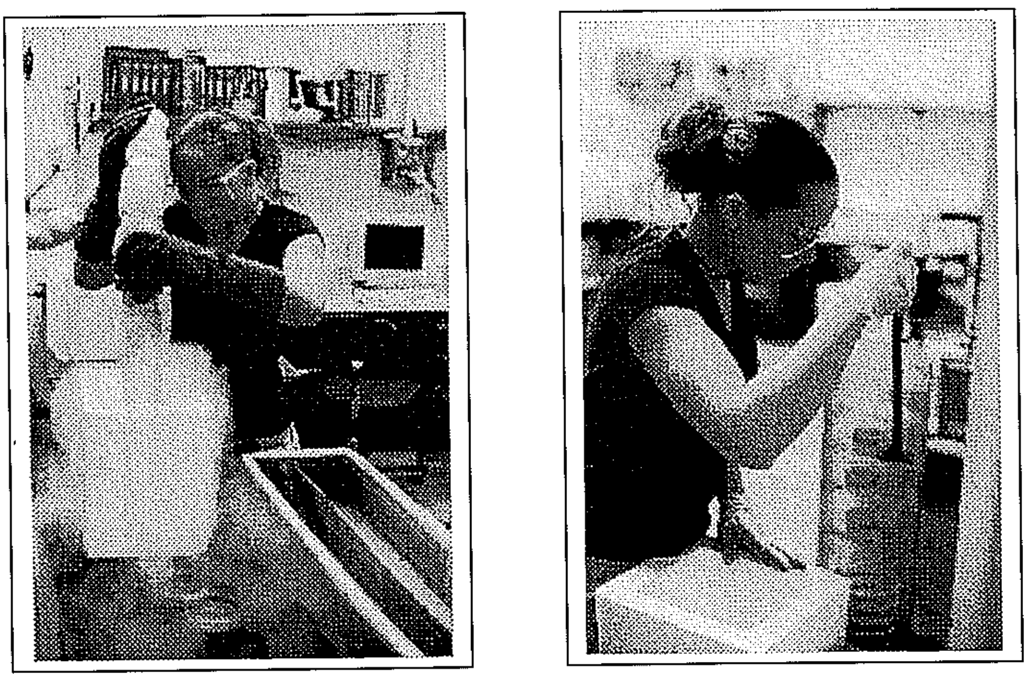


Figure 3: Freezing and lyophilization of collagen dispersions in the Collagen Research Laboratory

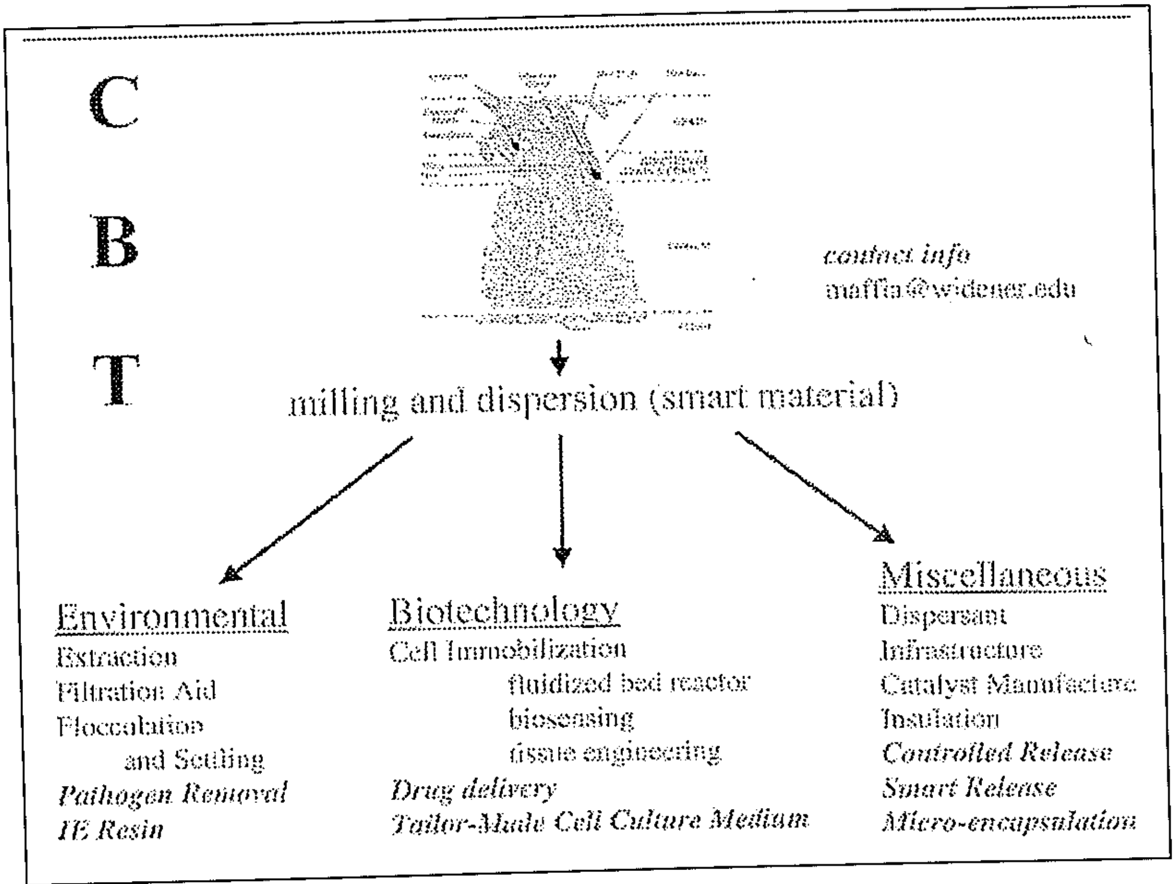


Figure 4: Processing and Applications Map for Collagen

Summary of the Collagen Processing and Applications Technology

Raw fibrillar type I bovine corium is the starting material for Collagen Based Technologies (Figures 4 and 5). Corium is the dermis layer of the hide and is rich in collagen-based connective tissue. In the initial steps in the processing, a dilute solution of the corium itself is milled in a ball mill containing zirconia media for one to two weeks. Once milling is completed, the resulting material is strained, washed, and then subjected to low temperature centrifugation, The supernatant is decanted and discarded. This process is repeated several times until no fats or other floating materials appear in the upper phase and the supernatant is clear. During this procedure the collagen fibrils are unraveled and the molecular structure remains intact (Figure 6).

The lower phase containing collagen is then blended in a solution containing an organic acid to form a dispersion and allowed to thicken. The resulting dispersion has improved physical properties and results in enhanced performance when used in various environmental applications. The above dispersion may be further processed to form physically improved collagen macroporous structures or substrates capable of utilization in various biotechnological applications. During the blending stage material for encapsulation or controlled release is added.

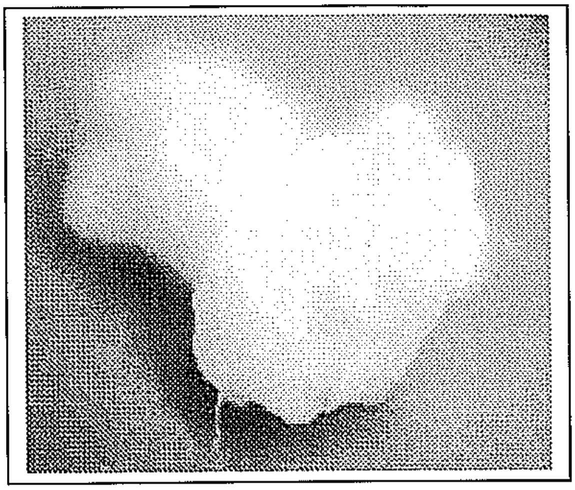


Figure 5 Photograph of the unmilled corium, which is delivered as a 15 % solids (ground) slurry from a meat processing plant.

Figure 6. Micrograph of milled corium showing the fibrils which have a diameter of ~ 100 nanometers. The scale bar in the lower right hand corner is 1 micron wide.

The collagen applications which have been studied the most are:

1. use of collagen dispersions in the flocculation and rapid settling of suspended solids
2. development of macroporous structures in a variety of different geometries

Other researchers, at university, government and industrial laboratories have used collagen for the manufacture of artificial tissue and synthetic organs. Some of these groups grow collagen on purpose via fermentation of collagen producing micro-organisms.

In the biomaterials area, macroporous collagen matrices, made from collagen dispersions, are excellent cell-culture substrates. The morphology and pore size of the macroporous structures are controllable. Pore size can range from approximately 5 to 100 microns and morphology can be wiry or leafy. The ability to control the pore size and morphology is important because different types of cells prefer different living conditions. Difficult to grow cells, such as rat cortical cells, have been successfully cultured in beads made according to Widener's method (Figure 7). There are many factors during processing, such as acid concentration, thermal cycling, freeze drying conditions, and collagen type, that affect the morphology and pore size.

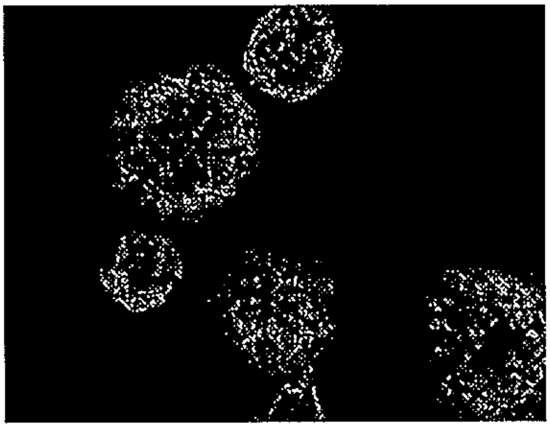


Figure 7 Rat cells growing on porous, high surface area collagen macrostructure

Active Surface Area as the Key Physical Property

Placing milled collagen fibrils in a solution at a pH other than its iso-electric point (IEP) will induce a surface charge (or chemical potential); this is represented in Figure 12. The fibrils respond by dispersing and retaining water between them.

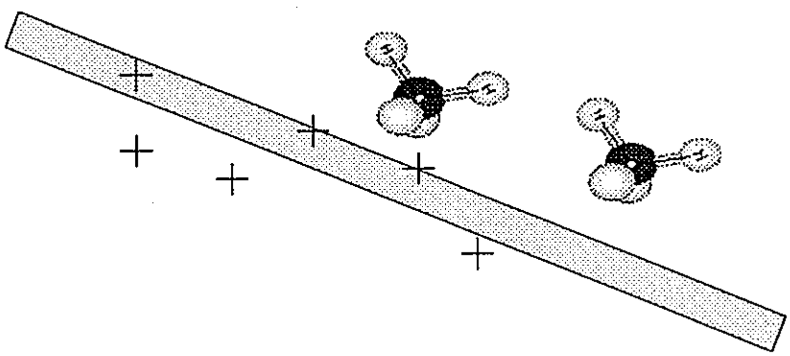


Figure 8 Representation of the response of collagen fibrils to a buffered solution of pH more acidic than the iso-electric point

The higher the specific surface area, that is, square meters per gram of protein, the greater will be the amount of retained water. Fibrils with an effective diameter in nanoscale, that is, 50-100 nanometers, can hold 500 times its mass in water in the vicinity of the charged surface.

Surface area is inversely related to the exposed diameter as shown in the equations in Table 1.

Table 1

Specific Surface Area Equations

$$\text{surface area of a long cylinder} = \pi \, D \, L$$

D = diameter of the fibril

L = length of the fibril

$$\text{volume of a long cylinder} = \pi \frac{D^2}{4} L$$

$$\text{therefore, surface area per volume} = \frac{4}{D}$$

$$\text{and surface area per mass (g)} = \frac{4}{\rho D}$$

where ρ = the density of the fibril

Surface area is difficult to measure. Conventional techniques based on adsorption, such as BET and mercury porosimetry are not effective due to the spongy nature of the proteins.

Consequently, only direct measurements from micrographs and performance testing using tools such as swelling ratio have been used to this point to determine surface area. A new technique based on decorin binding (Jaclyn Shea, '05 and USDA) offers promise to quantify the actual active surface area.

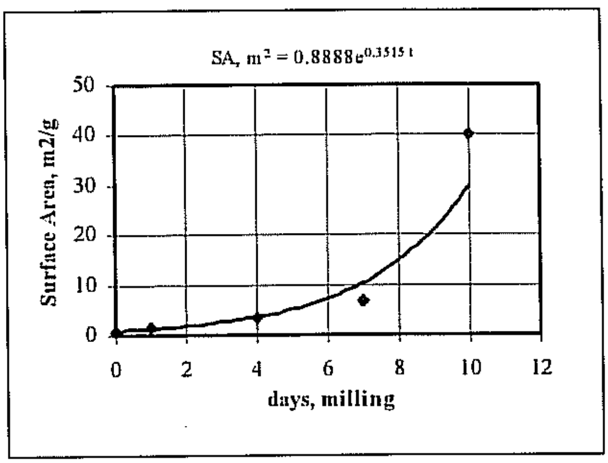


Figure 9 Measured specific surface area using micrographs; surface area per gram (SA) versus milling time (t) is plotted

Meat and Bone Meal Processing

Several processing and application technologies for Meat and Bone Meal have been studied over the past year at Widener University. The goal was to improve the market potential for the nearly 6 billion kg per year of inedible material from animal production.

Meat and bone meal (MBM) in rendered products (and obtained from the USDA) was subjected to a similar examination given to collagen from corium by the Widener University Collagen Research Group (CRG). The key areas of study will be the pretreatment of the raw MBM and the assessment of the efficacy of MBM, alone and as a composite system in a variety of potential applications and therefore high value product outlets.

The key test areas are indicated below:

1. Milling
   a. neat
   b. in heterogeneous solution with collagen

2. Washing and Centrifuging

3. Gravimetric Testing via Drying

4. Physical Measurements
   a. Microscopy (SEM and Light)
   b. Differential Scanning Calorimetry
      i. Dispersibility and Swelling Ratio

5. Infrastructure Experiments
   c. concrete
   d. asphalt
   e. porous matrices
      ii. without collagen
      iii. with collagen

2003 Meat and Bone Meal Project Objectives and Justification

The main objectives of the Meat and Bone Meal Research during the 2003 research program were as follows:

1. to study various pretreatment methods that have proven successful in the processing of bovine corium.
2. to develop a series of protocols for the production of high surface area, active protein from the feed MBM.
3. to perform screening studies on the application of MBM in a variety of technologies with a broad scope.

About 30 % of the inedible portion of animals processed for human consumption is meat and bone meal (MBW). This amounts to about 6 B kg/y. The potential value of this material ranges from negative (for disposal costs) to fuel value to higher value outlets.

Outlet	*Approximate Value per Year*
disposal - no recovered value	negative
fuel value (@ $ 0.005/MJ)	$ 0.5 B
infrastructure	$ 0.5 – 1.5 B
higher value outlets in the environment or in biotechnology	> $ 3-5 B

Almost 80% of the MBW is recycled within the US markets via animal feeds. Due to the fear of problems, such as,

pseudorabies
bovine spongiform encephalophy (BSE)
salmonella

new outlets for MBM are sought.

In preliminary work that has been performed on MBM by the Collagen Research Group at Widener, the starting material (supplied by the USDA) had the following approximate assays:

constituent group	*Meat Meal*	*Blood Meal*
crude protein	58 %	90 %
volatiles	2 %	9 %
crude fat	15 %	min.
ash	21 %	1 %
other	4 %	min.

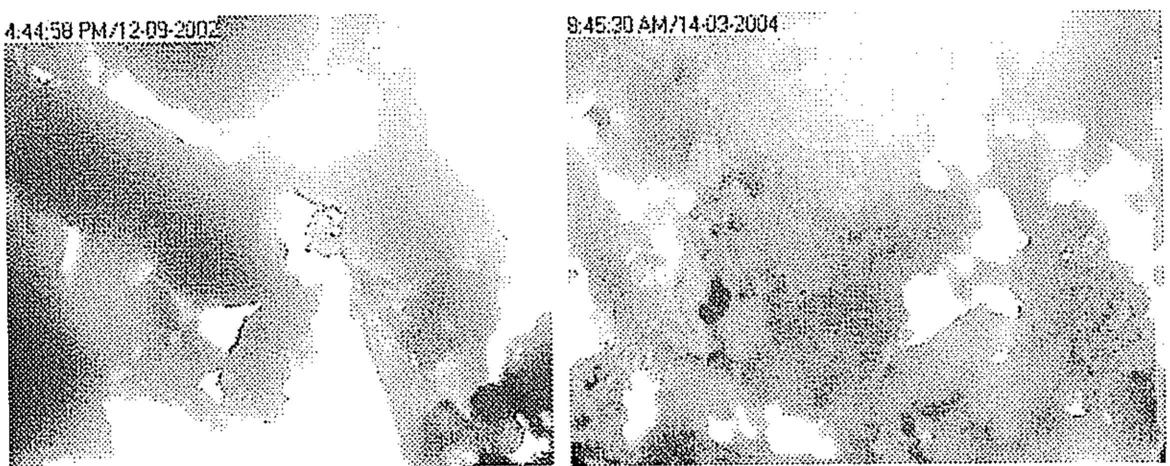


Figure 10 Raw MBM supplied to Widener by the USDA.
The approximate scale is 250 microns across the width of the image.

This material was pretreated to remove volatile components and to increase the surface area of the material. Light microscope images and Scanning Electron Micrographs are presented in the Appendices. The pretreated material has been milled, washed and then centrifuged, with the latter two procedures repeated several times.

PROCEDURES:

In order to fully evaluate the potential of MBM in the applications that were found to be efficacious for bovine collagen, the following pretreatment steps were be studied:

a. dilute wet milling (with and without ultrasonic preparation)
b. washing, extraction, centrifugation and drying (conventional, vacuum drying and freeze drying will be studied)
c. the following analytical testing will be performed for each of the processing techniques:
    1. standard gravimetric testing and characterization
    2. measurement of zeta potential, swelling ratio, rheology of dilute and concentrated solutions, response to pH adjustments, phase diagram using Differential Scanning Calorimetry, Electron Microscopy (in conjunction with the USDA - ARSERRC), Atomic Force Microscopy (in conjunction with the USDA - ARSERRC)

Upon completion of the pretreatment steps the resulting material was tested in the following applications:

d. infrastructure
    1. admixture for concrete
    2. composite structure with concrete
    3. composite/reinforcing material for ice based structures (on-going)
    4. blend with collagen and carrageenan for insulation (on-going)
e. macroporous matrix development
    1. neat or blended with collagen
    2. testing of the aseptic nature and the ability to grow a variety of cells
    3. controlled release of blended and impregnated materials

RESULTS and CONCLUSIONS

The results of the pretreatment testing was reported in the progress report issued on September 30, 2003 and attached in Appendix F.

The results of the concrete testing are presented in Appendix G and the results of the Asphalt Testing are presented in Appendix A.

Pretreatment Studies

Pretreatment experiments of milling neat MBM or a blended sample with collagen were largely unsuccessful. The resulting material did not show any improved properties and there was no synergy by processing collagen and MBM together. This work needs to be repeated over a wider range of concentrations and processing conditions. Also, some pre-segregation, via elutriation or a similar technique may provide a material with physical properties that would provide performance closer to that of collagen.

Dispersion Studies

Additionally, although the MBM did not swell or produce dispersions similar to milled corium (99% Type I collagen), there may be some construction applications for dried matrices that do not require material with such extreme rheological properties.

Asphalt Studies

Upon testing the asphalt samples containing various amounts of bone meal, the best results were achieved with when there is one percent bone meal relative to the overall weight.

Concrete Studies

Concrete results were less conclusive. The samples seemed to yield in stages. The initial yield point was much less than samples without MBM. However, the ultimate yield point was nearly equivalent. Additional testing is required over a more complete range of compositions.

Overall Conclusion

In conclusion, it appears that the application of MBM in infrastructure applications may have some promise and should be studied further. The higher end applications may become more attractive upon the utilization of a fractionated sample. A review of the recent literature indicates that there are research groups active in this area.

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