

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2012255643 B2**

(54) Title
Polymers and plastics derived from animal proteins

(51) International Patent Classification(s)
C08H 1/00 (2006.01) **C08L 89/00** (2006.01)
C08J 3/24 (2006.01)

(21) Application No: **2012255643** (22) Date of Filing: **2012.05.14**

(87) WIPO No: **WO12/155244**

(30) Priority Data

(31)	Number	(32)	Date	(33)	Country
	61/485,748		2011.05.13		US

(43) Publication Date: **2012.11.22**

(44) Accepted Journal Date: **2015.12.03**

(71) Applicant(s)
The Governors of the University of Alberta

(72) Inventor(s)
Bressler, David Curtis;Choi, Phillip

(74) Agent / Attorney
Madderns Patent & Trade Mark Attorneys, GPO Box 2752, ADELAIDE, SA, 5001

(56) Related Art
**MARTUCCI, J. F. ET AL., "Creep of glutaraldehyde-crosslinked gelatin films",
Materials Science and Engineering A, 2006, vol. 435-436, pages 681-686
US 2006/0068013 A1
US 3028308 A
APOSTOLOV, A. A. ET AL., "Mechanical Properties of Native and Crosslinked
Gelatins in a Bending Deformation", Journal of Applied Polymer Science, 2000,
vol. 76, pages 2041-2048
GB 683958 A
CA 1059671 A1
US 4649064 A
US 4861698 A**



(51) International Patent Classification:

C08H 1/00 (2006.01) C08L 89/00 (2006.01)

C08J 3/24 (2006.01)

(21) International Application Number:

PCT/CA2012/000458

(22) International Filing Date:

14 May 2012 (14.05.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/485,748 13 May 2011 (13.05.2011) US

(71) Applicant (for all designated States except US): GOVERNORS OF THE UNIVERSITY OF ALBERTA

[CA/CA]; 4000 - 10230 - Jasper Avenue, Edmonton, Alberta T5J 4P6 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BRESSLER, David

Curtis [CA/CA]; c/o 4000, 10230 - Jasper Avenue, Edmonton, Alberta T5J 4P6 (CA). CHOI, Phillip [CA/CA]; c/o 4000, 10230 - Jasper Avenue, Edmonton, Alberta T5J 4P6 (CA).

(74) Agent: BENNETT JONES LLP; 3200 Telus House,

South Tower, 10020 - 100 Street, Edmonton, Alberta T5J 0N3 (CA).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: POLYMERS AND PLASTICS DERIVED FROM ANIMAL PROTEINS

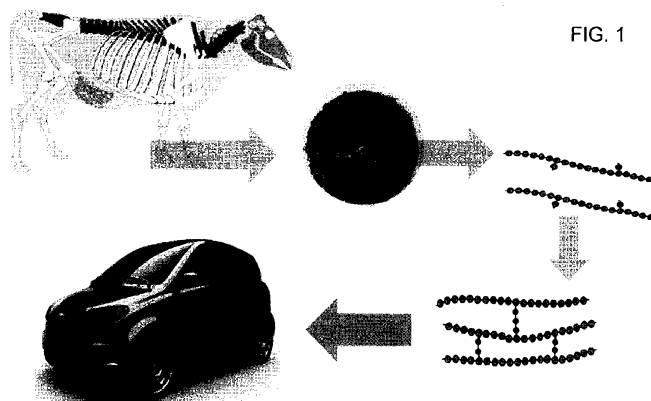


FIG. 1

(57) Abstract: The invention is directed to a method for preparing a polymer derived from an animal protein, such as in a feedstock derived from animal by-products. The method involves hydrolyzing proteins present in a feedstock to obtain hydrolyzed proteins, wherein hydrolysis is conducted under conditions sufficient to digest the proteins and destroy pathogens; extracting a protein fraction from the hydrolyzed proteins; and treating the protein fraction with a crosslinking reagent to form the polymer. The crosslinking reagents may include epoxies. The polymer may be further processed to form a thermoset plastic. The polymer may be used as an adhesive, or in the preparation of a natural fiber composite material.

**POLYMERS AND PLASTICS
DERIVED FROM ANIMAL PROTEINS**

5

Field of the Invention

[0001] The invention relates to polymers and plastics derived from animal proteins.

10

Background of the Invention

[0002] Transmissible spongiform encephalopathies (TSEs) are progressive, fatal diseases affecting the nervous system, causing spongy degeneration in the brain and spinal cord. TSEs include bovine spongiform encephalopathy (BSE or mad cow disease) in cattle, scrapie in sheep, chronic wasting disease in deer and elk, and variant Creutzfeldt-Jakob disease (vCJD) in humans. Although the exact cause of TSEs is unknown, the infectious agent is suspected to be a prion, a misfolded protein which has the ability to self-replicate and accumulate in neural tissue, eventually causing tissue damage and cell death. There is no treatment or vaccine currently available for the disease. Prions are notoriously resistant to routine methods of decontamination.

20

[0003] BSE was first diagnosed in 1986 in the United Kingdom where the majority of the world's cases have occurred. The first North American cases were discovered in 2003. Consumption by cattle of BSE-contaminated ruminant proteins in animal feed has been cited as the most likely means of transmission. Such outbreaks have had major implications for the North American beef industries since foreign markets have closed their borders to beef and cattle exports. Surveillance programs to monitor and assess BSE in cattle herds have been implemented to provide early detection and contain any possible spread in order to keep BSE out of the food supplies of both animals and humans. In humans, vCJD is thought to be linked to the consumption of meat products derived from BSE-infected cattle.

30

[0004] Prior to the emergence of BSE, the primary use for animal protein by-products was as feed ingredients for cattle, poultry, pets and aquaculture in the form of meat and bone meal, meat

meal and blood meal. Subsequently, specific cattle tissues, known as specified risk materials (SRM), have been banned from use in animal feed, pet food and fertilizers, and have been accumulating into landfills at a rate of about 5000 tons per week.

[0005] Rendering is a process whereby waste is "cooked" into ingredients for a wide range of industrial and consumer goods. Regulatory actions to strengthen safeguards against BSE portend significant changes in renderer's business practices, and the value of their products. If inedible animal byproducts have fewer market outlets, the overall economic value of the animal to the producer can decline, and questions arise about how to safely dispose of the SRM. The necessary restructuring of the rendering processing lines to handle SRM and non-SRM in separate lines and costs associated with SRM storing, transporting, and disposal fees have adversely affected profitability to operators and negatively impacted the beef industry.

[0006] Therefore, there is a need in the art for methods of safely handling SRMs and converting animal byproducts into useful products.

Summary of the Invention

[0006a] The present invention relates to polymers and plastics derived from animal proteins, and, in particular, animal proteins derived from byproducts.

[0006b] In a first aspect, the invention provides a method of preparing a polymer derived from specified risk material comprising animal proteins, comprising the steps of:

a) hydrolyzing the specified risk material by thermal hydrolysis or alkaline hydrolysis to produce a hydrolyzed protein; and

b) treating the hydrolyzed protein with a crosslinking reagent to obtain the polymer.

[0006c] In an embodiment, the method comprises the further step of treating the hydrolyzed protein mixture to precipitate ash or other impurities, leaving a protein fraction, which is then mixed with the crosslinking reagent.

[0006d] In an embodiment, the specified risk material comprises brain, eyes, trigeminal ganglia, spinal cord, dorsal root ganglia, tonsils, or the distal ileum of the small intestine, or combinations thereof. In an embodiment, the specified risk material is contaminated by, or at risk of contamination by an infectious agent.

[0006e] In an embodiment, the specified risk material animal proteins are hydrolyzed under conditions to produce an average molecular weight of less than about 100 kDa. In an

embodiment, the specified risk material animal proteins are hydrolyzed by thermal hydrolysis at a temperature of at least about 180° C and at a pressure of at least about 1,200 kPa.

[0006f] In an embodiment, the specified risk material animal proteins are hydrolyzed by alkaline hydrolysis. In an embodiment, alkaline hydrolysis is conducted under elevated temperature and pressure. In an embodiment, alkaline hydrolysis is conducted at a temperature of about 150° C and at a pressure of at least about 400 kPa.

[0006g] In an embodiment, the specified risk material animal proteins are hydrolyzed to an average molecular weight of less than about 70 kDa. In an embodiment, the hydrolyzed proteins have an average molecular weight of less than 40 kDa.

[0006h] In an embodiment, the crosslinking reagent comprises glutaraldehyde, glyoxal, resorcinol, benzaldehyde, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, N-hydroxysuccinimide, N,N-dicyclohexylcarbodiimide, or an epoxy. In an embodiment, the crosslinking reagent is an epoxy. In an embodiment, the epoxy comprises diglycidyl ether of bisphenol A, aliphatic polyglycol epoxy, or resorcinol diglycidyl ether.

[0006i] In an embodiment, the method comprises the further step of blending the polymer with a reactive natural or synthetic rubber.

[0006j] In a second aspect, the invention provides a polymer comprising hydrolyzed specified risk material animal proteins cross-linked by a cross-linking reagent.

[0006k] In an embodiment, the cross-linking agent comprises glutaraldehyde, glyoxal, resorcinol, benzaldehyde, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, N-hydroxysuccinimide, N,N-dicyclohexylcarbodiimide, or an epoxy.

[0006l] In an embodiment, the hydrolyzed specified risk material animal proteins have an average molecular weight of less than 70 kDa. In an embodiment, the hydrolyzed specified risk material animal proteins have an average molecular weight of less than 35 kDa.

[0006m] In an embodiment, the polymer is blended with a natural or synthetic rubber.

[0006n] In a third aspect, the invention provides a thermoset plastic obtained from the polymer of the second aspect.

[0006o] In another aspect, the invention comprises a method for preparing a polymer derived from a feedstock comprising animal proteins, comprising the steps of:

- a) hydrolyzing the proteins to produce a hydrolyzed protein mixture; and

b) treating the hydrolyzed protein mixture with a crosslinking reagent to obtain the polymer.

[0006p] In one embodiment, the method further comprises the step of treating the hydrolyzed protein mixture to extract a protein fraction.

[0006q] In one embodiment, the animal proteins comprise a fresh meat carcass, a biological tissue, blood meal, meat, bone meal, or a specified risk material, or combinations thereof. In one embodiment, the animal protein comprises a specified risk material, which may comprise tissues such as brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column, dorsal root ganglia, tonsils, the distal ileum of the small intestine, or combinations thereof. In one embodiment, the animal proteins may be at risk of contamination of a pathogen which may comprise a bacteria, virus, fungi, parasite, or prion.

[0006r] In one embodiment, the hydrolysis step comprises thermal hydrolysis. For example, the animal proteins may be subjected to temperatures of at least about 180° C and at a pressure of about 1,200 kPa.

[0006s] In one embodiment, the hydrolysis step comprises alkaline hydrolysis where the proteins are hydrolyzed in the presence of a base. In one embodiment, the base comprises an aqueous solution of an alkali metal hydroxide or an alkaline earth metal hydroxide. In one embodiment, alkaline hydrolysis may take place under elevated temperature and pressure. For example, the alkaline hydrolysis may be conducted at a temperature of about 150° C and at a pressure of about 400 kPa. In one embodiment, a substantial portion of the hydrolyzed proteins produced by alkaline hydrolysis has a molecular weight of less than 35 kDa.

[0007] In one embodiment, the crosslinking reagent comprises glutaraldehyde, glyoxal, resorcinol, benzaldehyde, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, N-hydroxysuccinimide, N,N-dicyclohexylcarbodiimide, or an epoxy. In one embodiment, the crosslinking reagent is an epoxy such as a diglycidyl ether of bisphenol A, aliphatic polyglycol epoxy, or resorcinol diglycidyl ether.

[0008] In one embodiment, the method further comprises the step of blending the polymer with a natural or synthetic rubber.

[0009] In yet another aspect, the invention comprises a polymer obtained by the above method.

[00010] In yet another aspect, the invention comprises a plastic obtained by the above —————

method.

[00011] Additional aspects and advantages of the present invention will be apparent in view of the description, which follows. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention,
5 are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

10 **Brief Description of the Drawings**

[00012] The invention will now be described by way of an exemplary embodiment with reference to the accompanying simplified, diagrammatic, not-to-scale drawings:

15 [00013] Figure 1 is a schematic diagram showing one embodiment of the method of the present invention.

[00014] Figure 2 is a schematic diagram showing one embodiment of the method of the present invention.

20

[00015] Figure 3 is a graph showing total free amines as a function of the crosslinking reagent and molar concentration.

25 [00016] Figure 4 is a Fourier transform infrared spectra of hydrolyzed protein, epoxy resin, and epoxy resin crosslinked polymer.

[00017] Figure 5 is a Fourier transform infrared spectra of hydrolyzed protein prior to crosslinking.

30 [00018] Figure 6 is a Fourier transform infrared spectra of the hydrolyzed protein of Figure 5 after crosslinking with resorcinol diglycidyl ether.

[00019] Figure 7 is a side view of a specimen for testing the adhesive bond strength of a proteinacious adhesive formulation of the present invention.

5 [00020] Figure 8 is a top view of a specimen for testing the adhesive bond strength of a proteinacious adhesive formulation of the present invention.

[00021] Figure 9 is a graph showing the effect of pressing temperature and protein concentration on the adhesive bond strength of one embodiment of a protein-glutaraldehyde
10 adhesive formulation of the present invention.

[00022] Figure 10 is a graph showing the effect of pressing temperature and pressure on the adhesive bond strength of one embodiment of a protein-glutaraldehyde adhesive formulation of the present invention.

15

[00023] Figure 11 is a graph showing the effect of pressing temperature and glutaraldehyde concentration on the adhesive bond strength of one embodiment of a protein-glutaraldehyde adhesive formulation of the present invention.

20 [00024] Figure 12 is a graph showing the effect of protein concentration and pressing pressure on the adhesive bond strength of one embodiment of a protein-glutaraldehyde adhesive formulation of the present invention.

[00025] Figure 13 is a graph showing the effect of glutaraldehyde concentration and
25 pressing pressure on the adhesive bond strength of one embodiment of a protein-glutaraldehyde adhesive formulation of the present invention.

[00026] Figure 14 is a graph showing the effect of protein concentration and glutaraldehyde concentration on the adhesive bond strength of one embodiment of a protein-glutaraldehyde adhesive formulation of the present invention.
30

[00027] Figure 15 is a graph showing the effect of pressing temperature and time on the adhesive bond strength of one embodiment of a protein-DGEBA adhesive formulation of the present invention.

5 [00028] Figure 16 is a graph showing the effect of protein concentration and pressing time on the adhesive bond strength of one embodiment of a protein-DGEBA adhesive formulation of the present invention.

[00029] Figure 17 is a graph showing the effect of protein concentration and pressing
10 temperature on the adhesive bond strength of one embodiment of a protein-DGEBA adhesive formulation of the present invention.

Detailed Description of Preferred Embodiments

15 [00030] The present invention relates to polymers and plastics, and methods for preparing same from animal proteins.

[00031] When describing the present invention, all terms not defined herein have their
common art-recognized meanings. To the extent that the following description is of a specific
20 embodiment or a particular use of the invention, it is intended to be illustrative only, and not
limiting of the claimed invention. The following description is intended to cover all alternatives,
modifications and equivalents that are included in the spirit and scope of the invention, as
defined in the appended claims.

25 [00032] As shown generally in Figure 1, one embodiment of the method utilizes an
agricultural byproduct stream as a source of animal proteins, which are modified to form
crosslinked polymers having desirable physical and chemical properties. In one embodiment, the
crosslinked polymers are further processed to form thermoset bioplastics. Conventionally, a
thermosetting polymer is a prepolymer in a soft solid or viscous state that changes irreversibly
30 into an infusible, insoluble polymer network by curing. Curing can be induced by the action of
heat or suitable radiation, or both. A cured thermosetting polymer is called a thermoset.

[00033] In one embodiment, the invention comprises a method for preparing a polymer derived from a feedstock comprising animal proteins, comprising the steps of:

- a) hydrolyzing the proteins to produce a hydrolyzed protein mixture;
- 5 b) treating the hydrolyzed protein mixture with a crosslinking reagent to obtain the polymer.

[00034] In one embodiment, the invention further comprises the step of treating the hydrolyzed protein mixture to extract a protein fraction, and treating the protein fraction with the
10 crosslinking reagent.

[00035] As shown in Figure 2, the polymers are produced from animal proteins using the methods described herein. The method generally involves at least the following sequential steps: (1) hydrolyzing the proteins in a feedstock to yield hydrolyzed proteins using a process such as
15 either thermal hydrolysis or alkaline hydrolysis; (2) extracting a protein fraction from the hydrolyzed proteins, and (3) treating the protein fraction with a crosslinking reagent to obtain crosslinked thermosetting polymers having physical and chemical characteristics comparable to those of products from non-renewable feedstock. The crosslinked polymers may be further processed to obtain thermoset plastics (4). The thermal, mechanical and physical properties of
20 the resultant plastics may be evaluated to assess their suitability for particular applications (5).

[00036] The detailed steps of the process are as follows. A feedstock containing animal proteins is used as the starting material. As used herein, the term "feedstock" means an animal product which comprises proteins. Feedstock may include fresh meat carcasses or other animal
25 tissues, such as blood meal (i.e., the dried blood left over after carcasses are processed at a rendering plant), meat, bone meal, and specified risk material. The feedstock may be contaminated with, or at risk of contamination with infectious agents which may include, but not limited to, bacteria, viruses, fungi, parasites, and prions. As used herein, the term "prion" means a proteinaceous-infectious agent which causes transmissible spongiform encephalopathies in
30 humans and animals.

[00037] As used herein, the term "specified risk material" means tissues removed from animals slaughtered for human consumption and within which infectious agent may be present. Specified risk material includes, but is not limited to, the brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column, dorsal root ganglia, tonsils, the distal ileum of the small intestine, and combinations thereof.

[00038] The feedstock is preferably a waste material which is typically disposed of or used in a rendering plant. In one embodiment, the feedstock may be processed to reduce particle size if necessary, such as by hammermilling, chopping, grinding or blending. A reduced particle size may facilitate the hydrolysis step which follows.

[00039] The first step of the process involves hydrolyzing the proteins in the feedstock to obtain hydrolyzed proteins. As used herein, the term "hydrolyze" refers to the cleavage of amide bonds in a polypeptide to produce carboxylic acid groups and amino groups. Hydrolysis of the proteins generally results in the production of proteins and peptides with varying molecular weight, as well as free amino acids. As used herein, the term "hydrolyzed protein" is the mixture of proteins, peptides, and/or free amino acids produced by the hydrolysis of the proteins present in the feedstock. In one embodiment, hydrolysis comprises thermal hydrolysis or alkaline hydrolysis, or a combined alkaline thermal hydrolysis.

[00040] In one embodiment, thermal hydrolysis is conducted at a temperature of about 180° C, and at a pressure of about 1,200 kPa. In one embodiment, duration of thermal hydrolysis is at least about forty minutes. Thermal hydrolysis may be conducted in a suitable thermal hydrolysis reactor which uses high pressure and saturated steam to denature organic material and destroy pathogens. Suitable reactors and their operation are well known in the art and need not be further described herein (see for example, vessels manufactured by Haarslev Inc., Kansas City, MO, USA; or Dupps Company, Germantown, Ohio, USA).

[00041] In one embodiment, hydrolysis of the proteins is conducted in the presence of a base. In one embodiment, the base comprises an aqueous solution of an alkali metal hydroxide or an alkaline earth metal hydroxide. In one embodiment, the base comprises an aqueous

solution of sodium hydroxide (NaOH) or potassium hydroxide (KOH). In one embodiment, the solution is calculated on a mass per mass basis equal or greater than about 9% of the feedstock. In one embodiment, the solution comprises about 15% sodium hydroxide (w/v) in water or about 19% potassium hydroxide (w/v) in water. In one embodiment, alkaline hydrolysis is conducted
5 at a temperature of about 150° C, and at a pressure of at least about 400 kPa. In one embodiment, duration of alkaline hydrolysis is at least about 180 minutes per cycle.

[00042] Alkaline hydrolysis may be conducted in any enclosed pressure vessel as are known in the art. The vessel allows the immersion of the feedstock in the alkali which is then
10 heated. The feedstock remains within the alkali until sufficiently digested to inactivate or destroy any pathogens which might be present, thereby forming a solution void of such agents. Suitable vessels are described, for example, in US Patent Nos. 7,910,788; 7,829,755; and 7,183,453; or may include, but are not limited to, the WR² alkaline hydrolysis Tissue Digestors™ manufactured by BioSAFE Engineering (Brownsburg, IN, USA).

15 **[00043]** Without being bound to any theory, the alkali-catalyzed breaking of the peptide bonds and the addition of water at the break occurs under the above conditions, promoting rapid dissolution and hydrolysis of the proteins into small peptides and amino acids in the form of their sodium or potassium salts.

20 **[00044]** The conditions and type of hydrolysis may be chosen by one skilled in the art to produce a hydrolyzed protein having a desired degree of hydrolysis. In one embodiment, the hydrolyzed protein will have an average molecular weight of less than about 100 kDa, or 80 kDa, or 70 kDa, and preferably greater than about 1 kDa, 5kDa, or 10 kDa. Obviously, more severe hydrolysis will produce relatively smaller peptides and more individual amino acids. Less
25 severe hydrolysis will produce relatively larger peptides. Substantially all proteins subjected to alkaline hydrolysis with elevated heat and pressure are severely hydrolyzed to an average molecular weight less than about 40 kDa. Proteins subjected to thermal hydrolysis have a comparatively bigger and wider range of molecular weight with the average being below 70 kDa. The molecular size of both the thermal and alkaline hydrolyzed proteins were significantly
30 reduced as the concentration of water and alkaline solution per weight of of the feedstock increases, respectively during hydrolysis.

[00045] After hydrolysis, a protein fraction is extracted or separated from the hydrolyzed proteins using a combination of salts to promote the precipitation of ash or other impurities which may be detrimental to crosslinking, and selectively retain the protein fraction in aqueous solution. In one embodiment, the salt solution comprises 4% (w/v) NaCl and 0.05% (w/v) MgCl₂ (w/v) in a phosphate buffer, comprising 0.067 M KH₂PO₄ and 0.067 M Na₂HPO₄, according to the method optimized by Park *et al* (2000) for meat and bone meal. The aqueous protein fraction comprises major active functional groups such as, for example, primary amine (-NH₂), carboxyls (-COOH), sulfhydryls (-SH), hydroxyl (-OH), and carbonyls (-CHO). The major active functional groups are positioned on the side chains of amino acids or at the amino or carboxy end of each amino acid chain. In one embodiment, the invention comprises the protein fraction obtained by the method described herein.

[00046] The protein fraction is then treated with a crosslinking reagent which reacts with the active functional groups to form crosslinked polymers. Suitable crosslinking reagents include, but are not limited to, glutaraldehyde, glyoxal, resorcinol, benzaldehyde, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, N-hydroxysuccinimide, N,N-dicyclohexylcarbodiimide, and epoxies. In one embodiment, the crosslinking reagent comprises an epoxy. As used herein, the term "epoxy" means an epoxy resin comprising monomers or short chain polymers having functional epoxide groups. The epoxide groups react with the amine groups and other functional groups to form covalent bonds. Based on the comparison of properties, a protein-cured epoxy network shows comparatively superior properties and can be formulated in a viable method of preparation. Hydrolyzed protein used as hardener/co-reactant in an epoxy formulation yields moisture resistance and thermal properties comparable to those of existing industrial synthetic products. As epoxy-based resins find use as diluents, pigments, fillers and flame retardants in the surface coating, construction, and electrical industry, epoxy-protein based thermosetting resins formulated in this manner may be useful. In one embodiment, the invention comprises crosslinked polymers obtained by the method described herein.

[00047] One suitable epoxy is diglycidyl ether of bisphenol A (DGEBA) and known commercially as AralditeTM. DGEBA has the formula (2,2-bis[4-(2'3' epoxy propoxy) phenyl]

propane) and is derived from the reaction of bisphenol-A and epichlorohydrin. Crosslinking hydrolyzed protein fractions with DGEBA yields polymers characterized by high tensile strength and limited elongation at the breaking point. These mechanical properties are very similar to commercial epoxy-based polymers.

5

[00048] In one embodiment, the polymer may be blended with other polymers or substances to alter the properties of the polymer and any resulting thermoset plastic. For example, it is possible to impart flexibility to DGEBA-based polymers by blending reactive natural or synthetic rubber into the DGEBA prior to curing with the proteins. Carboxylic acid synthesized acrylonitrile rubbers, with carboxylic group along the chain and chain ends reacts with epoxy groups of DGEBA and polymerizes altogether through chain extension reactions, to impart flexibility of the backbone molecule. In one embodiment, 10 to 40 % by weight of reactive rubber may be incorporated into epoxy resins prior to overall crosslinking with the hydrolyzed protein to obtain various levels of toughness and flexibility of the thermoset plastics.

15

[00049] Other suitable epoxies include an aliphatic polyglycol epoxy (APO) resin or resorcinol diglycidyl ether (RDE). Crosslinking hydrolyzed protein fractions with APO or RDE yields polymers with higher flexibility, lower viscosity, and reduced brittleness. Lower viscosity improves overall processability and potentially increases the amount of proteins incorporated into the matrix.

20

[00050] The crosslinked polymers may be further processed or cured into plastics for use in conventional plastic applications including, but not limited to, packaging, shipping and insulation material. In one embodiment, the invention comprises plastics obtained by the methods described herein.

25

[00051] The cured thermoset polymers produced as described herein, can be used as an adhesive between surfaces such as wood, metal and glass. In one embodiment, the invention comprises a method of using the polymer obtained by the method described herein as an adhesive.

30

Examples

[00052] The following are specific examples of embodiments and methods of using the present invention. These examples are offered by way of illustration and are not intended to limit the invention in any manner.

Example 1: Preparation of Polymers and Plastics

[00053] This example demonstrates how the method of the present invention can be used in preparing polymers and plastics from agricultural proteins.

[00054] As the starting material, the feedstock comprised specified risk material obtained from cattle. Thermal hydrolysis was conducted for about forty minutes per cycle at a temperature of about 180° C, and at a pressure of about 1,200 kPa using a thermal hydrolysis reactor (Haarslev Inc., Kansas City, MO, USA; Dupps Company, Germantown, Ohio, USA). Alkaline hydrolysis was conducted for about 180 minutes per cycle at a temperature of about 150° C, and at a pressure of about 400 kPa using a tissue digester (BioSAFE Engineering, Brownsburg, IN, USA). The alkaline solution comprised about 15% sodium hydroxide (w/v) in water. The protein fraction was then extracted from the hydrolyzed proteins using a combination of salts to precipitate ash, and to retain the protein fraction into aqueous solution.

[00055] The physical, chemical and structural characteristics of the protein fraction were found to be correlated to the specific type of hydrolysis.

[00056] The molecular weight analysis revealed that alkaline hydrolyzed proteins are severely cleaved to a molecular weight of less than 35 kDa due to the catalytic property of the hydroxide solution. Thermal hydrolyzed proteins have comparatively wider range of molecular weight, with an average molecular weight of less than 66 kDa.

[00057] The amino acid profile was obtained using reversed-phase HPLC. The protein fraction comprised functional groups including primary amine (-NH₂), carboxyls (-COOH),

sulfhydryls (-SH), hydroxyl (-OH), and carbonyls (-CHO). The functional groups were positioned on the side chain of each amino acid or the end of each main chain.

[00058] Both thermal and alkaline hydrolysis yielded protein fractions which are
5 amenable to crosslinking, providing two distinct routes to prepare final products having different properties. A functional group study showed that most functional groups of protein survived the conditions of both thermal and alkaline hydrolysis. The reduction in functional groups was used to measure the level of crosslinking of proteins with various agents (i.e., glutaraldehyde, glyoxal, resorcinol, and benzaldehyde) at various molar concentrations (i.e., 0 mmol, 0.01 mmol, 0.02
10 mmol, 0.05 mmol, and 0.1 mmol). A linear reduction of amine group was observed as the molar concentration of each of the crosslinking reagents was increased (Figure 3).

[00059] Figure 4 is a Fourier transform infrared spectra of hydrolyzed protein, epoxy resin, and epoxy resin crosslinked polymer. The spectra showed carboxylic acid and epoxy ring
15 reduction and the formation of new ester bonds. Epoxy, being reactive to several of protein functional groups, exhibited superior crosslinking property.

Example 2: Use of RDE as crosslinking reagent

20 [00060] This example demonstrates how the method of the present invention can use resorcinol diglycidyl ether (RDE) as a crosslinking reagent.

[00061] Figure 5 is a Fourier transform infrared spectra of hydrolyzed protein prior to crosslinking. Figure 6 is a Fourier transform infrared spectra of the hydrolyzed protein of Figure
25 5 after crosslinking with RDE. The broad absorption band in the range of 3200-3500 cm^{-1} corresponds to the hydroxyl (O-H) of the hydrolyzed proteins whereas the band at around 1525 cm^{-1} can be attributed to the amine group (N-H) of the protein chain. A comparison of Figures 5 and 6 depicts how both groups have disappeared following crosslinking, demonstrating that epoxy can cap both reactive moieties.

30

Example 3: Method of using proteinaceous polymers as an adhesive

[00062] Proteinaceous adhesive formulations were prepared using hydrolyzed protein containing 91% (dry basis) protein extracted from specified risk material thermally hydrolyzed as described in Example 1 above (CFIA, 2009). The extract was freeze-dried and milled into powder. The hydrolyzed protein was mixed with one of two crosslinking reagents. In the first case, the hydrolyzed protein was dispersed in solutions of glutaraldehyde (Fisher Scientific, 50%). The protein concentrations varied from 0 to 30 percent by weight, and the glutaraldehyde concentration in the solutions varied from 0 to 50 percent. The dispersions were agitated for 3 hours at 250 rpm until a homogeneous solution was obtained. The pH of the adhesive formulations were adjusted to neutral using 0.5 N NaOH and 0.1N HCl solutions. In the second case, the hydrolyzed protein was dispersed in solutions of Diglycidyl Ether of Bisphenol A (DGEBA) epoxy resin (Sigma-Aldrich, epoxide equivalent weight, 172-185 Da). The protein concentrations in the DGEBA varied from 0 to 40 percent by weight, the remaining being epoxy resin.

[00063] In addition, to serve as an industrial benchmark for the proteinaceous adhesive formulations, a urea formaldehyde adhesive was prepared by dispersing about 50 g of urea in 100 mL of aqueous formaldehyde. (The reaction of urea and formaldehyde involves condensation between the nucleophilic nitrogen of urea with the electrophilic carbonyl carbon of formaldehyde.) The mixture was stirred at 60 °C for 15 min and the pH was adjusted to 8.5 by adding drops of 0.5 N analytical grade NaOH (Fisher Scientific), and refluxed for 30 minutes. A white semi-solid urea formaldehyde polymer was produced.

[00064] Specimens were prepared to test the adhesive bond strength of the adhesive formulations. The test specimens were made by obtaining hardwood plywood veneer (oak, Columbia Forest Products) from a Home Depot store in Edmonton, Alberta and cutting the veneer into rectangular pieces having dimensions of 3 x 20 x 50 mm (thickness, width and length) (Figure 7). Each of the formulated adhesive slurries was brushed onto each surface of the test specimens in the amount of about 15 mg/cm² to wet the glued area of the test specimens (Figure 8). The specimens were allowed to equilibrate for 10 minutes and then pressed at

varying temperature and pressure combinations using a thermal press (Model 3890 Auto 'M', Carver, Wabash, IN). In the case of the protein-glutaraldehyde adhesive formulation, the pressing temperatures ranged from 100 °C to 150 °C and the pressures ranged from 1 MPa to 5 MPa. In the case of the protein-DGEBA adhesive formulation, pressing times ranged from 10 to 60 minutes and pressing temperatures ranged from 100 °C to 200 °C. Preliminary study of protein crosslinking with epoxy using differential scanning calorimeter showed high temperature requirement to complete the curing, unless it is catalyzed. The glued test specimens were conditioned at 23 °C and 50% relative humidity for 7 days after hot pressing. The conditioned specimens were tested for dry shear strength in accordance with ASTM Standard Method D2339 using Instron 5967 (Norwood, MA, USA) equipped with a 5 kN load cell at a crosshead speed of 1 mm/min. The shear strength was calculated as the average determination from quintuplicate specimens.

[00065] With respect to the protein-glutaraldehyde adhesive formulations, the adhesive bond strength was comparable and in some cases greater than the adhesive bond strength of the urea formaldehyde adhesive formulation, which was found to be 1.97 MPa. Figures 9 to 14 illustrate the effect of pressing temperature and pressure, glutaraldehyde concentration and protein concentration on the adhesive bond strength of the protein-glutaraldehyde adhesive formulations. (Statistical analysis (not shown here) indicated that temperature and pressure, protein and glutaraldehyde concentration were interacting parameters, so it was impossible to separately observe the influence of one parameter without the other in this study.) Referring to Figure 9, higher protein concentrations generally favoured higher adhesive bond strength. Formulations with protein concentrations exceeding 25 % (w/v) were found to have high viscosity that will in effect increase the contact angle of the adhesive with the wood surface, which leads to unfavourable dispersion properties of the adhesive. Referring to Figure 10, higher pressure generally favoured higher adhesive bond strength. Pressures above 4 MPa started to damage the plywood test specimen.

[00066] With respect to the protein-DGEBA adhesive formulations, the adhesive bond strength was of the same order of magnitude as the urea formaldehyde adhesive and in some cases less. The lack of strength might be attributed to the poor penetration of epoxy protein

adhesive into the wood surface owing to its high viscosity. However, the water resistance of epoxy based adhesives bond is expected to be superior to the rest of the protein-glutaraldehyde or urea formaldehyde adhesives formulations. Figures 11, 12 and 13 illustrate the effect of pressing temperature, time and protein concentration on the adhesive bond strength of the protein-DGEBA adhesive formulations. Longer pressing time and higher pressing temperature during bonding favored greater adhesive bond strength. This could be due to completion of protein curing with epoxy resin. Referring to Figures 12 and 13, increasing total protein concentration up to about 24% per weight of epoxy in the adhesive formulation increased the adhesive bond strength, but increases in total protein concentration above 24% per weight of epoxy reduced the adhesive bond strength. The decrease of the adhesive bond strength for protein concentrations above 24% per weight of epoxy might be attributed to the high viscosity of the mix, leading to poor penetration depth of the adhesive.

[00067] Exemplary embodiments of the present invention are described in the above Examples, which are set forth to aid in the understanding of the invention, and should not be construed to limit in any way the scope of the invention as defined in the claims which follow thereafter.

[00068] All experiments were performed in a Biosafety Level II laboratory (University of Alberta, Edmonton, Canada) operating under a Canadian Food Inspection Agency permit for handling specified risk material.

[00068a] Throughout this specification, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[00068b] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia or elsewhere before the priority date of each claim of this application.

References

[00069] The following references are incorporated herein by reference (where permitted) as if reproduced in their entirety. All references are indicative of the level of skill of those skilled in the art to which this invention pertains.

ASTM D2339-98, Standard Test Method for Strength Properties of Adhesives in Two-Ply Wood Construction in Shear by Tension Loading ASTM, West Conshohocken, 15 June 2002.

CFIA. 2009 Bovine Spongiform Encephalopathy Manual of Procedures, (accessed September 09, 2010).

Wilson, J.H.; Kaye, G.I.; Hahn, R.L.; Weber, P.B. and Morris, K.A. System for treating infectious waste matter. United States Patent No. 7,910,788, issued March 22, 2011.

Wilson, J.H.; Kaye, G.I.; Hahn, R.L.; Weber, P.B. and Morris, K.A. System and method for treating infectious waste matter. United States Patent No. 7,829,755, issued November 9, 2010.

Wilson, J.H.; Kaye, G.I.; Hahn, R.L.; Weber, P.B. and Morris, K.A. Method for treating infectious waste matter. United States Patent No. 7,183,453, issued February 27, 2007.

Park, S.K., Bae, D.H., Hettiarachchy, N.S. 2000. Protein Concentrate and adhesives from meat and bone meal. JAOCS. 77(11), 1223-1227.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of preparing a polymer derived from specified risk material comprising animal proteins, comprising the steps of:
 - a) hydrolyzing the specified risk material by thermal hydrolysis or alkaline hydrolysis to produce a hydrolyzed protein; and
 - b) treating the hydrolyzed protein with a crosslinking reagent to obtain the polymer.
2. The method of claim 1, comprising the further step of treating the hydrolyzed protein mixture to precipitate ash or other impurities, leaving a protein fraction, which is then mixed with the crosslinking reagent.
3. The method of claim 1 or 2, wherein the specified risk material comprises brain, eyes, trigeminal ganglia, spinal cord, dorsal root ganglia, tonsils, or the distal ileum of the small intestine, or combinations thereof.
4. The method of claim 1 or 2, wherein the specified risk material is contaminated by, or at risk of contamination by an infectious agent.
5. The method of claim 1 or 2, wherein the specified risk material animal proteins are hydrolyzed under conditions to produce an average molecular weight of less than about 100 kDa.
6. The method of claim 1 or 2, wherein the specified risk material animal proteins are hydrolyzed by thermal hydrolysis at a temperature of at least about 180° C and at a pressure of at least about 1,200 kPa.
7. The method of claim 1 or 5, wherein the specified risk material animal proteins are hydrolyzed by alkaline hydrolysis.
8. The method of claim 7, wherein alkaline hydrolysis is conducted under elevated temperature and pressure.

9. The method of claim 8, wherein alkaline hydrolysis is conducted at a temperature of about 150° C and at a pressure of at least about 400 kPa.
10. The method of claim 1 or 5, wherein the specified risk material animal proteins are hydrolyzed to an average molecular weight of less than about 70 kDa.
11. The method of claim 9, wherein the hydrolyzed proteins have an average molecular weight of less than 40 kDa.
12. The method of any one of claims 1 to 11, wherein the crosslinking reagent comprises glutaraldehyde, glyoxal, resorcinol, benzaldehyde, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, N-hydroxysuccinimide, N,N-dicyclohexylcarbodiimide, or an epoxy.
13. The method of claim 12, wherein the crosslinking reagent is an epoxy.
14. The method of claim 13, wherein the epoxy comprises diglycidyl ether of bisphenol A, aliphatic polyglycol epoxy, or resorcinol diglycidyl ether.
15. The method of any one of claims 1 to 14, comprising the further step of blending the polymer with a reactive natural or synthetic rubber.
16. A polymer comprising hydrolyzed specified risk material animal proteins cross-linked by a cross-linking reagent.
17. The polymer of claim 16, wherein the cross-linking agent comprises glutaraldehyde, glyoxal, resorcinol, benzaldehyde, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, N-hydroxysuccinimide, N,N-dicyclohexylcarbodiimide, or an epoxy.
18. The polymer of claim 16, wherein the hydrolyzed specified risk material animal proteins have an average molecular weight of less than 70 kDa.

19. The polymer of claim 18, wherein the hydrolyzed specified risk material animal proteins have an average molecular weight of less than 35 kDa.
20. The polymer of claim 16 or 17, blended with a natural or synthetic rubber.
21. A thermoset plastic obtained from the polymer of any one of claims 16 to 20.

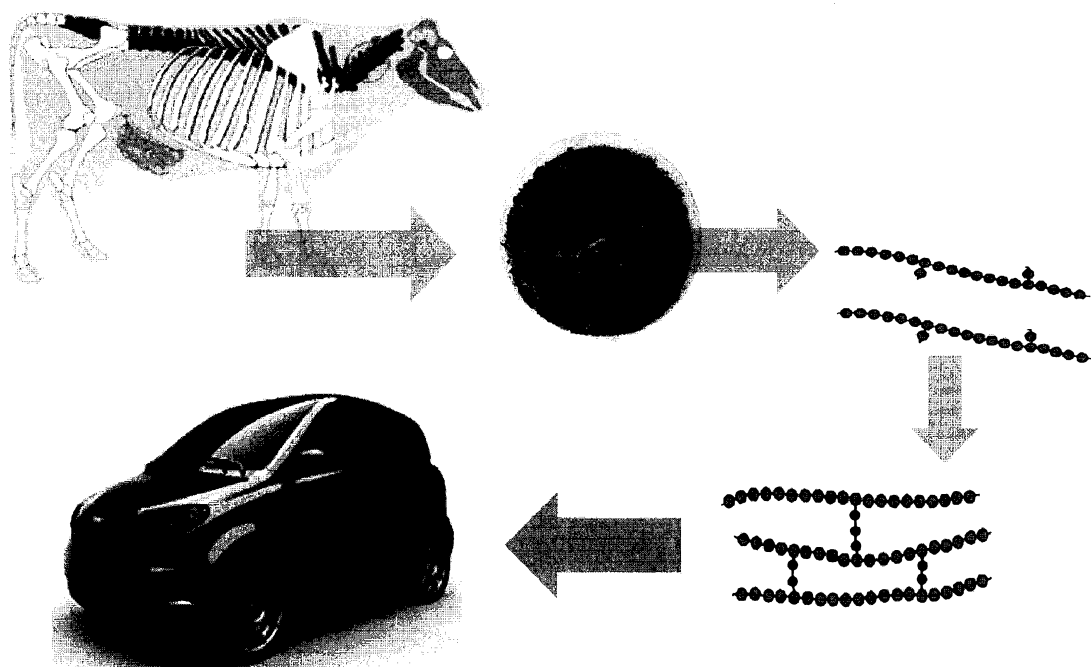


FIG. 1

2/11

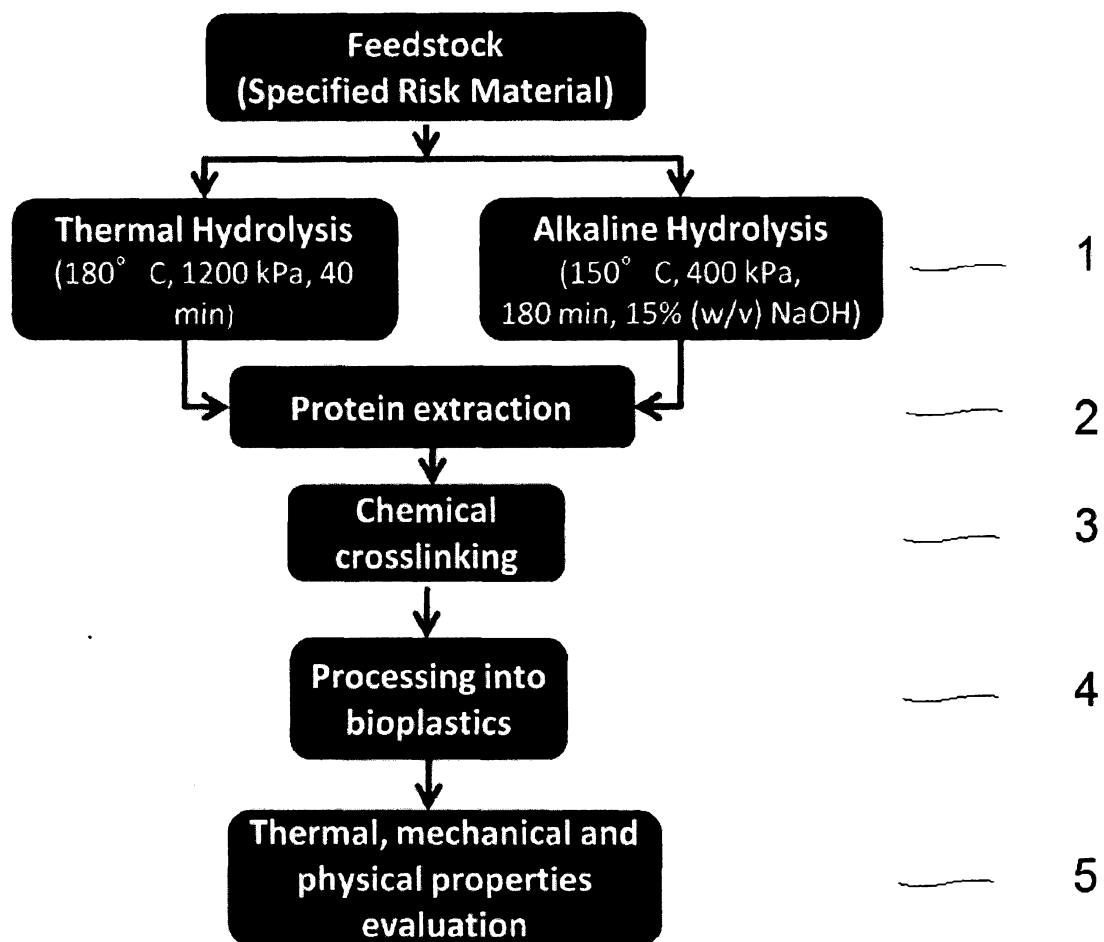


FIG. 2

3/11

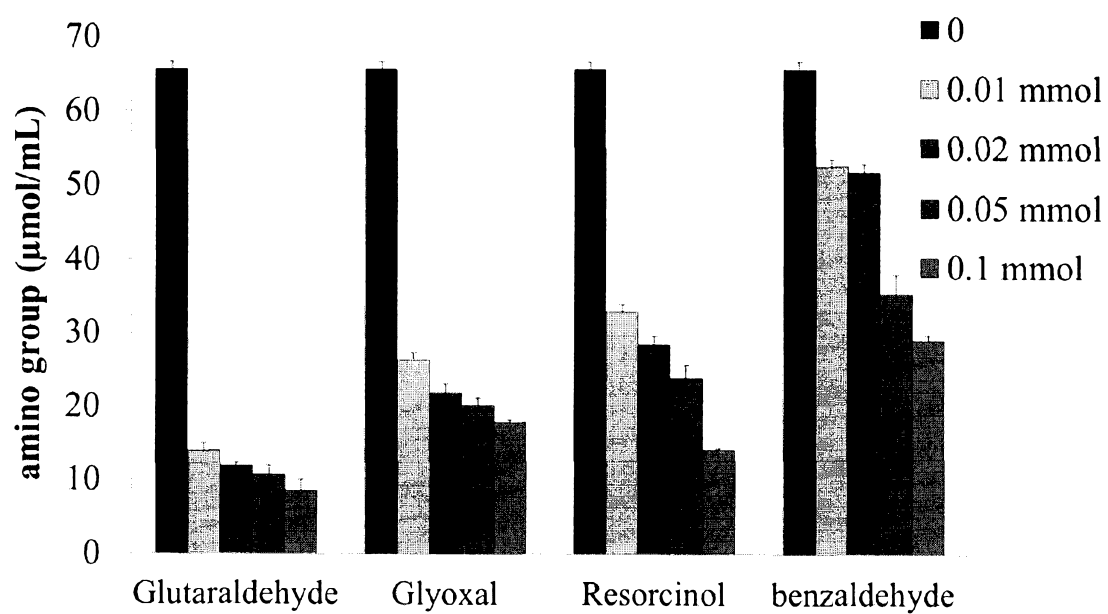


FIG. 3

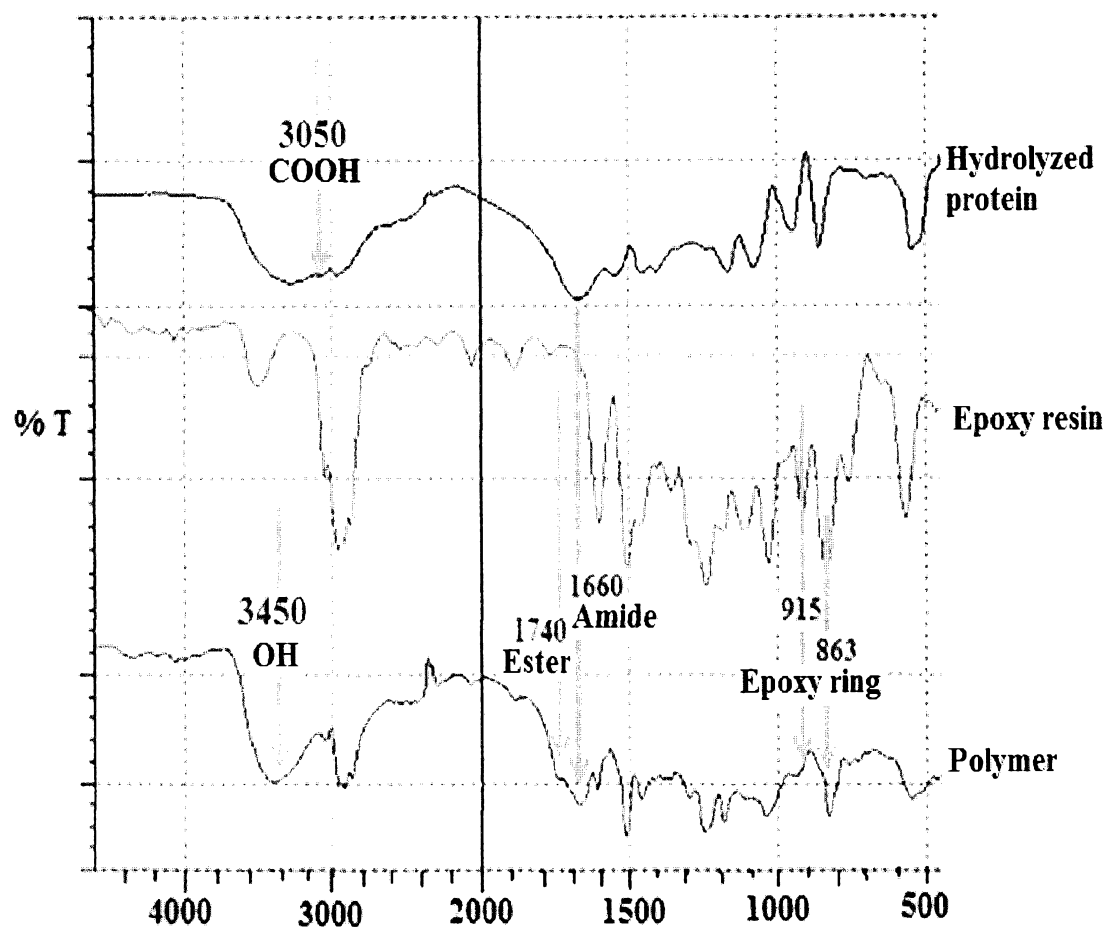


FIG. 4

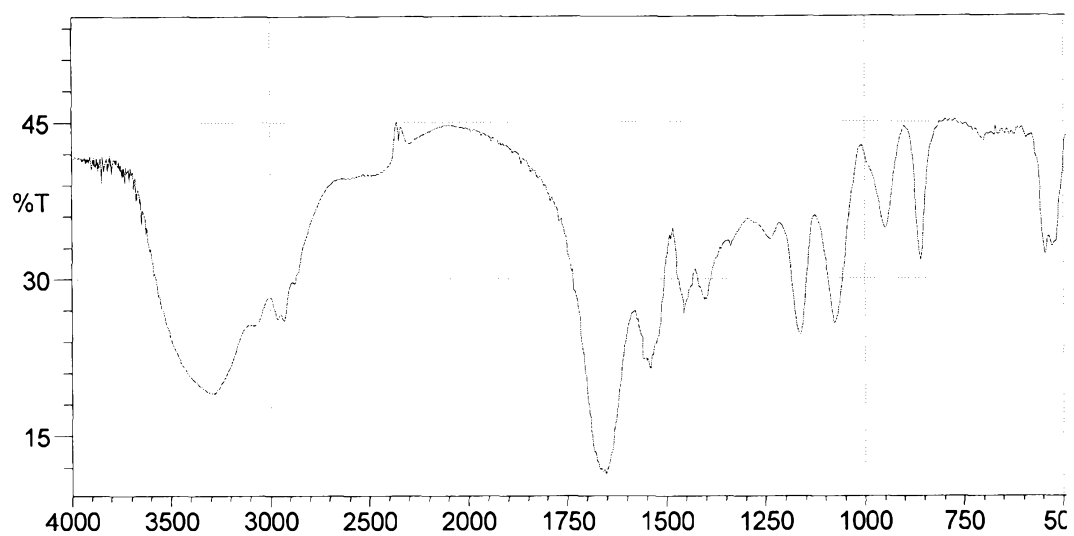


FIG. 5

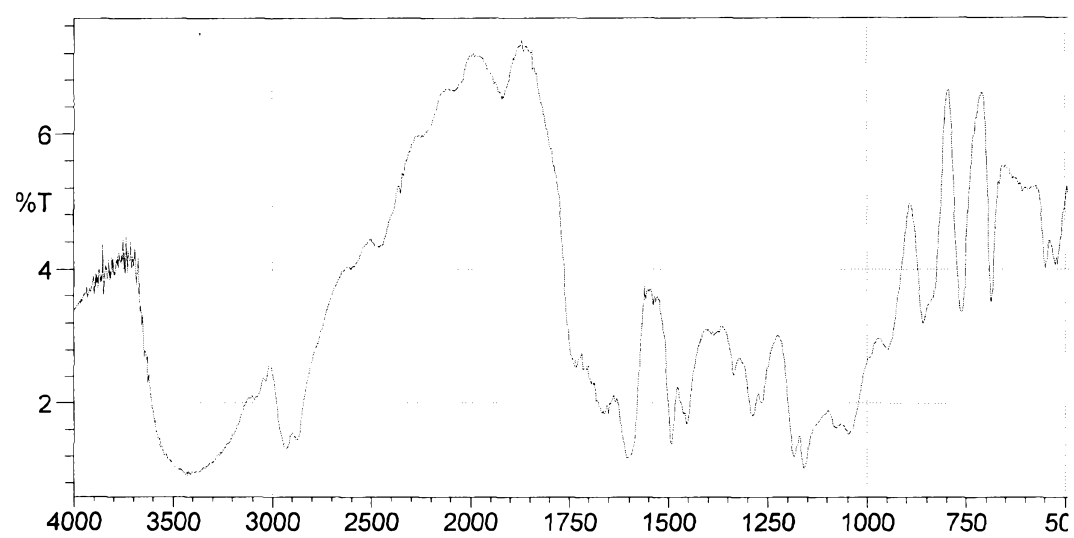


FIG. 6

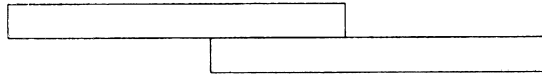


FIG. 7

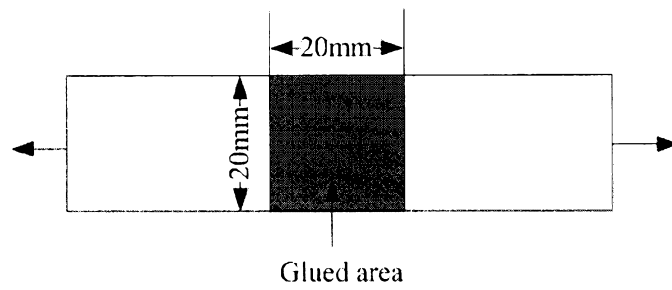


FIG. 8

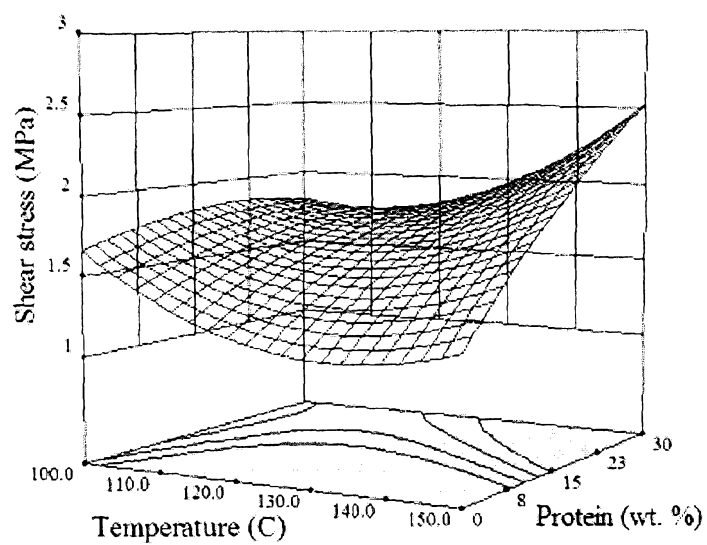


FIG. 9

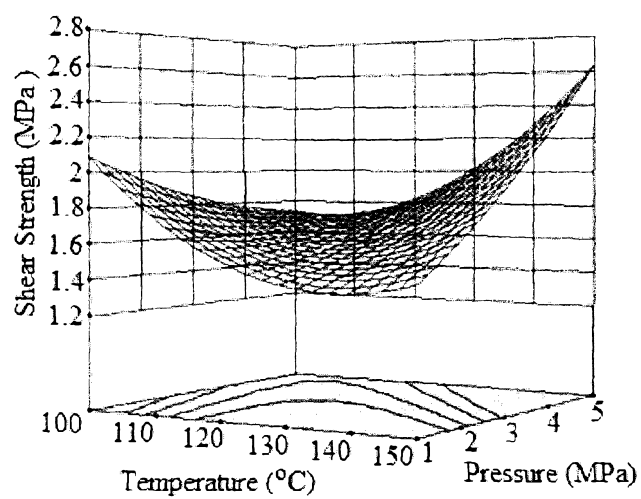


FIG. 10

8/11

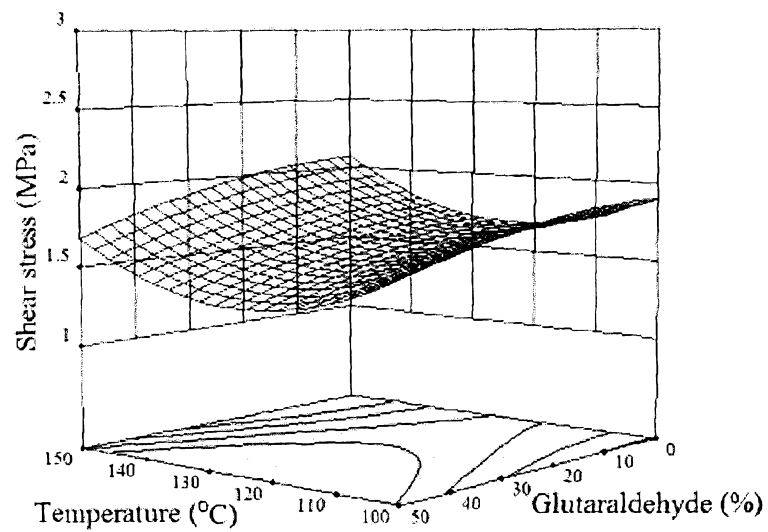


FIG. 11

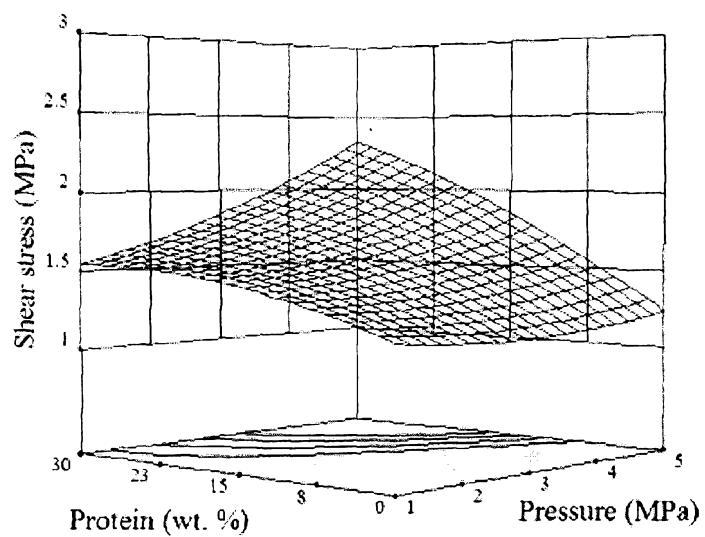


FIG. 12

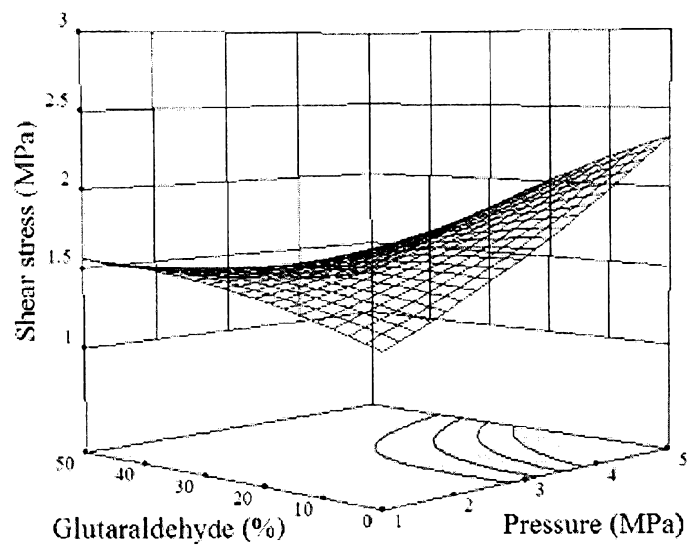


FIG. 13

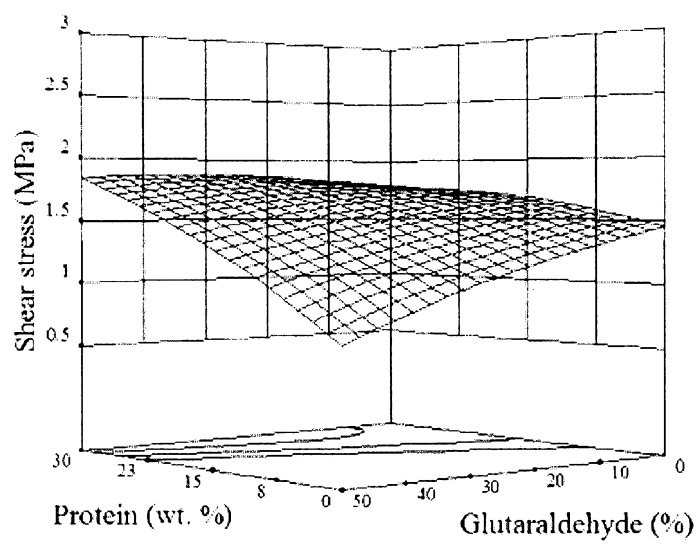


FIG. 14

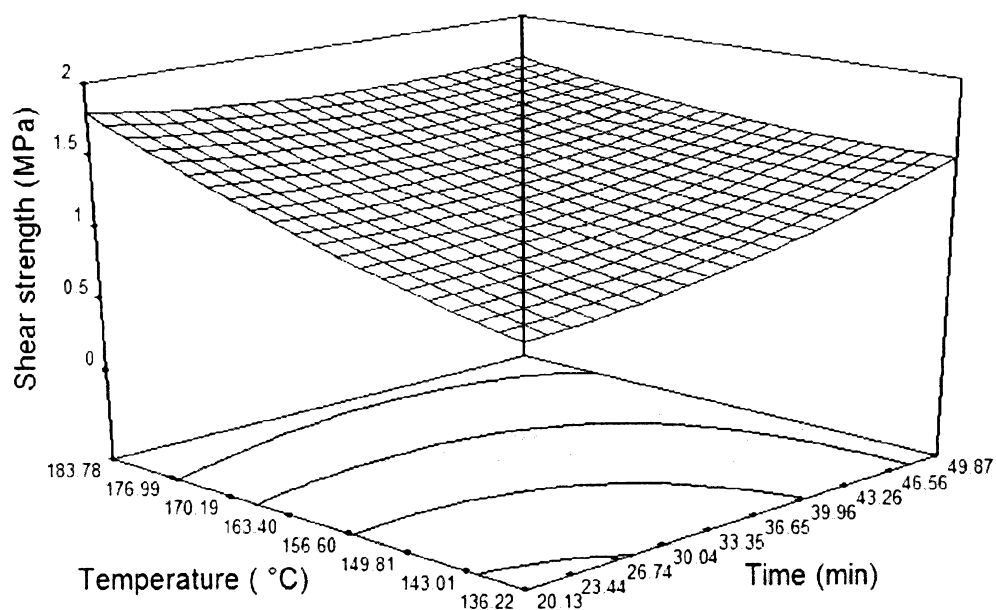


FIG. 15

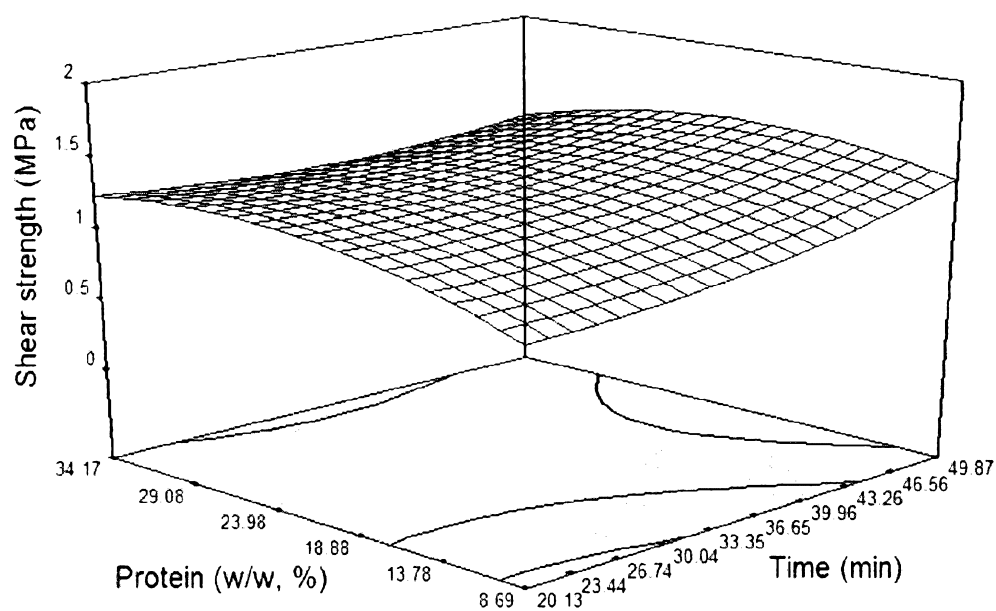


FIG. 16

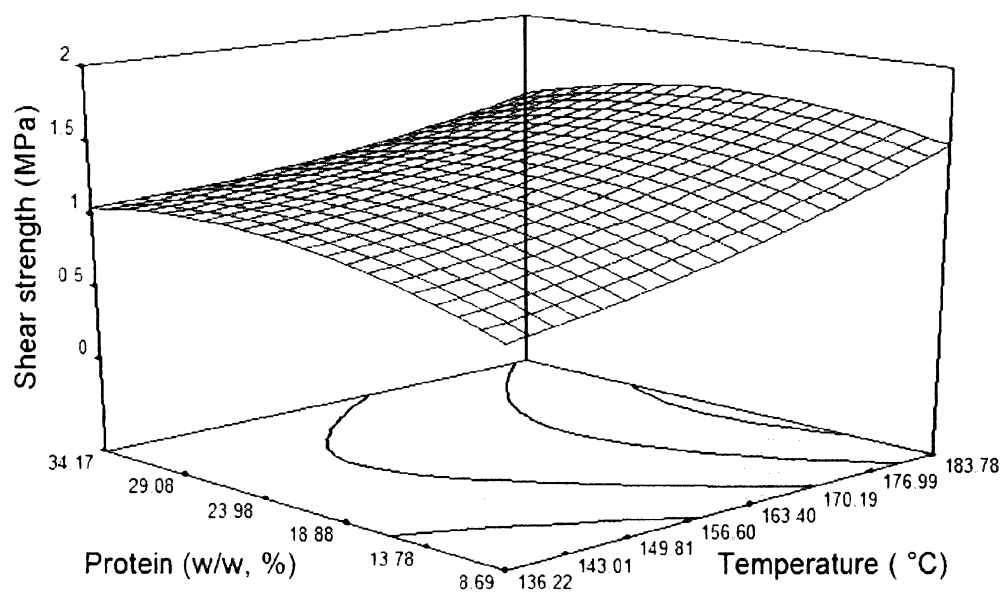


FIG. 17