Protein Concentrate and Adhesives from Meat and Bone Meal

Sang K. Park[a], D.H. Bae[b], and N.S. Hettiarachchy[a,*]

[a]Department of Food Science, University of Arkansas, Fayetteville, Arkansas 72704, and [b]Department of Applied Biology and Chemistry, Konkuk University, Seoul 133-701, Korea

ABSTRACT: Defatted meat and bone meal (MBM) was prepared by the removal of extractable fat with isopropyl alcohol. Proteins in the defatted MBM were extracted with 0–4% NaCl concentrations with 0.05% $CaCl_2$ or 0.05% $MgCl_2$ at pH 5.5–6.6 for 1 h at 50°C and precipitated at pH 4.0–4.5. By using the salt extraction procedure, MBM protein concentrate (MBMPC) (32 g) was obtained from defatted MBM (100 g). Recovery of protein was dependent on the extraction temperature employed; recovery values ranged from 33.2 to 51.4%. At 4% NaCl concentration, $MgCl_2$ increased protein solubility by 30%, compared to the control. The adhesiveness of MBMPC at various pH values ranging from 5.0 to 9.0 was investigated. At pH 6.0–8.0, adhesiveness of MBMPC showed the highest value (78.2 kg). The adhesiveness increased linearly as the MBMPC concentration increased up to 20% with respect to temperature for MBMPC adhesiveness, the greatest adhesiveness was in the range of 70 to 90°C. Improved adhesiveness and water resistance were observed with 0.05% glutaraldehyde treatment.

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KEY WORDS: Adhesives, bond strength, meat and bone meal, tensile strength, water resistance.

About half of every animal processed for human consumption is inedible. In 1991, the total weight of inedible raw materials from animal production was approximately 36 million pounds (16.4×10^6 metric tons). In 1989 approximately 25% of recycled fats and oils originating from meat production was exported, and, according to Reference 1, the U.S. Department of Agriculture's Foreign Agricultural Service subsidized this effort ($1,000,000 annually). Most defatted meat and bone meal (MBM) (approximately 11 billion pounds, or 5.0×10^6 metric tons) was used domestically in animal feeds, resulting in about 80% of all rendered products being recycled into U.S. markets (1). Nevertheless, the MBM market has not been realized, because of animal disease perceptions or the fear that animal diseases may be transferred through leading rendered products. Although there is no evidence that a disease has been transferred from rendered products, thousands of tons of MBM are not recycled for that reason (2). Until now, the principal disease concerns have been with pseudorabies, bovine spongiform encephalophy (BSE), and salmonella (3). With the U.S. Food and Drug Administration's new regulation and goals requiring zero salmonella in feed and ingredients, this issue may become costly and deleterious for the animal and supporting industries (4).

One way to create new channels for marketing MBM products is to produce adhesives from them. Concern over continuing emission of formaldehyde, a probable carcinogen, and volatile organic components from petroleum-derived synthetic adhesives has created the necessity for the plywood industry to investigate new types of adhesives. Once the engineered wood industry (5) adopts acceptable alternative adhesives, these pressures will exclude the use of formaldehyde-containing products.

According to Fats and Proteins Association's report, generalized MBM (beef + chicken + pork) contains approximately 56% crude protein (dry matter basis). MBM protein consists of 0.58% cysteine, 2.87% lysine, and 0.32% tryptophan, polar amino acids that can interact with wood (6,7). However, reactive groups in these amino acids are unavailable due to internal hydrogen bonding. These reactive groups in amino acids need to be exposed by breaking the internal bonds under controlled conditions to provide a balance between hydrophobic and polar groups. This balance together with chemical modification is needed to produce adhesives with desirable bond strength and water-resistant properties. The objectives of this study were to isolate protein concentrate from MBM and use this protein to produce adhesives for plywood industry.

MATERIALS AND METHODS

MBM sample. MBM sample was provided by Fats and Protein Foundation, Inc. (Bloomington, IL). Proximate composition of MBM as determined by using American Oil Chemists' Society methods (8) is shown in Table 1. One hundred grams of MBM was defatted twice using isopropyl alcohol (meal/solvent = 1:3, wt/vol) at 24°C for 12 h. The material remaining was vacuum-packed, and stored at −10°C.

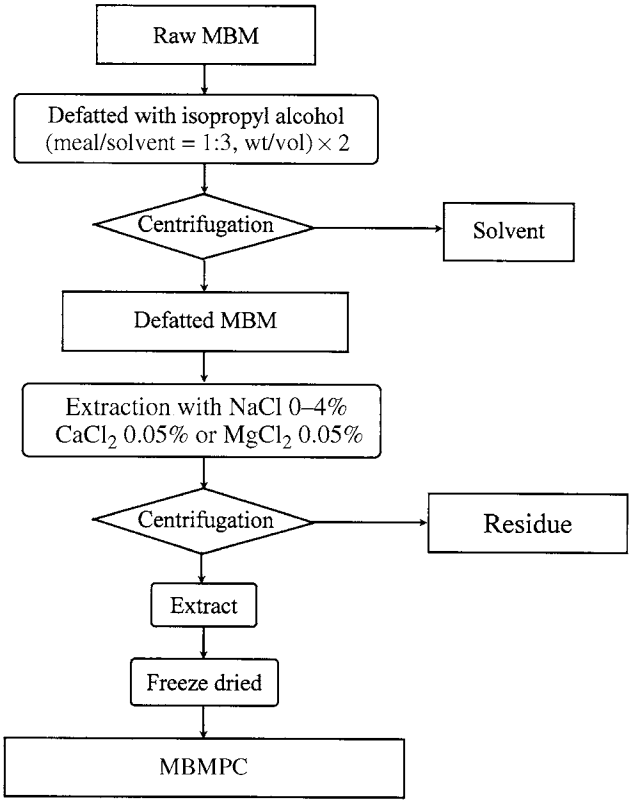
Preparation of salt-extractable MBM protein. Sodium chloride concentrations of 1.0, 2.0, 3.0, and 4.0% (wt/vol) were prepared in phosphate buffer, pH 6.2, with no added divalent chloride salt and with 0.05% (wt/vol) of either $CaCl_2$ or $MgCl_2$. The

*To whom correspondence should be addressed at Department of Food Science, University of Arkansas, 2650 North Young Ave., Fayetteville, AR 72704. E-mail: nhettiar@mail.uark.edu

TABLE 1
Proximate Analysis (%) of Defatted Meat and Bonemeal (MBM)[a]

Component	As received	Dry basis
Moisture	5.20	—
Protein × 6.25	48.12	50.76
Fat	19.37	20.37
Ash	26.44	27.76

[a]Source of meal: Fats and Protein Association (Bloomington, IL). Analytical methods: Reference 8.



SCHEME 1

buffer consisted of 0.067 M monopotassium phosphate and 0.067 M disodium phosphate.

The flow diagram of MBM protein concentrate (MBMPC) prepared from raw MBM by using salt extraction is shown in Scheme 1. Defatted MBM (10 g) samples were extracted with 50 mL of appropriate salt solution for 10 min at room temperature. The slurry was centrifuged at 7,000 × *g* for 30 min. The supernatant was filtered through glass wool and subsequently through Whatman No. 41 filter paper before pH and soluble protein at 24°C were measured. MBMPC was analyzed for total nitrogen by micro Kjeldahl method (9). Percent extractability of total nitrogen was calculated as follows:

$$\text{percent extractability (\%)} = (VC_e/WC_s) \times 100 \quad [1]$$

where V is the volume of recovered extract, C_e is the constituent concentration in the extract, W is the sample weight used, and C_s is the constituent concentration in the sample.

Adhesive formulation. MBMPC (10, 15, 20 g/100 mL, wt/vol) samples were dispersed thoroughly in distilled water. Optimal conditions (concentration, pH, and temperature) for producing adhesives with desired adhesiveness, viscosity, and water resistance were established. MBMPC solutions were treated with either acid or alkali (pH 4 to 11) and were heated at 60, 70, 80, or 90°C for 30 min. Glutaraldehyde and glyoxal (0.01–0.2%, wt/vol) as cross-linking agents for bond strength and water resistance were evaluated.

Adhesiveness. MBMPC adhesives were analyzed for their bond strength according to the modified American Standard Test Method (ASTM) 906-82 (10). Adhesive performance was determined by measuring the force (kg) required to break glued joints using an Instron Universal Testing Machine model 4500 (Canton, MA) by a tensile test method. Wood pieces were made of Western Hard Maple cut into a rectangular shape with dimensions of 0.25 × 2 × 6 cm. One hundred milligrams of MBMPC adhesives was placed on each side of a wood piece and spread on a marked area (2 × 2 cm^2) to give protein concentrations of 10–15 mg/cm^2. Three wood pieces were then glued together in the shape of an "H." A special holder was designed to firmly and properly position the assembled H- shaped wood pieces for tensile testing.

Water resistance. The samples were evaluated to determine their ability to withstand an extremely moist condition, which has been shown to be a major problem with protein-based adhesives. To determine the effect of water on bonded wood joints, the moisture cycle for performance testing of the American National Standards (ANSI) A208.1(11) was used. This testing method was intended as a measure of adhesive durability. The weight of two glued wood pieces was determined accurately within ± 0.2%. The glued wood pieces were placed in a rack to facilitate maximum contact with water and with heated dry air. The glued wood pieces were submerged in hot water (50–60°C) for 0–30 h. Following hot-water soaking, glued wood pieces were dried at 82 ± 5°C. The glued wood pieces were ready for performance testing when a constant dry weight (± 0.2%) had been achieved and the pieces had been cooled for 1.5 h in ambient air. These results were compared to glued wood pieces made with phenol formaldehyde resin. The commercial phenol formaldehyde resin was GP 3144 particle board resin from Georgia-Pacific Resin, Inc. (Decatur, GA).

TABLE 2
Protein Solubility, Percent Extractability, and pH as Affected by NaCl Concentration[a]

NaCl (%)	Percent pH[a]	Percent extractability[b] (%)	Protein solubility[c] (mg/mL)
0.0	6.24[e]	45.54	3.62[d]
1.0	6.05[d]	49.57	3.94[c,d]
2.0	5.96[c]	52.59	4.18[c]
3.0	5.71[b]	54.48	4.33[b]
4.0	5.53[a]	57.12	4.54[a]

[a]Means with columns having different roman superscript letters are different ($P < 0.05$).
[b]Calculated per Equation 1.
[c]Measured by AOCS micro-Kjeldahl method (8).

Viscosity. Viscosities of MBMPC dispersions were determined using a Brookfield Rheometer DV-III (Stoughton, MA) with spindle number CP 40. Before measuring viscosity, the rheometer was calibrated without spindle. When calibration was complete, the spindle was installed on the rheometer. After it had been prepared, the adhesive (0.5 mL) immediately was placed into a sample cup. A reading was taken by attaching the sample cup to the cone. All measurements were made at 25°C at 7 rpm.

Statistics. Analysis of variance (11) was used to test for the effect of treatment methods on adhesiveness. When *F*-values were significant, mean differences within each sample were compared by using a least significant difference test. Differences were considered statistically significant at a probability level of 0.05.

RESULTS AND DISCUSSION

Extractability of MBM protein. The salt extraction procedure of MBM was optimized to produce adequate quantities of MBMPC, and this concentrate was used to conduct experiments to test MBM in wood adhesive applications. Extractability of MBM protein was improved when NaCl concentration increased to 4%.

Table 2 shows that protein solubility increased by 25% in MBM as NaCl concentration increased from 0 to 4% ($P < 0.05$). Preliminary experiments showed that increasing the ionic strength by adding NaCl resulted in partial solubilization of insoluble fractions in MBM. The increases of extractability were probably associated with solubility of salt-soluble myofibrillar proteins. Also, the increased protein solubility could have resulted from insoluble actomyosin protein dissociation with a subsequent increase in myosin and actin solubility at high NaCl concentration (13).

The pH of salt-soluble protein extract ranged from 5.5 to 6.6 for MBM. Since pH values of MBM protein extracts containing divalent cations were above the isoelectric pH of MBM, the protein side chains would have predominantly negative charges, to which these cations could bind, causing the anionic (chloride ion) effect to dominate (14). Because the extract pH dropped when NaCl concentration increased or when $CaCl_2$ and $MgCl_2$ was added (Table 3), it appeared the isoelectric point was shifted to a lower pH due to anionic effect. This resulted in a salting-in of proteins by $CaCl_2$ and $MgCl_2$ with solubilization by calcium more predominant. Table 3 shows that $CaCl_2$ and $MgCl_2$ increased ($P < 0.05$) protein solubility at 4% NaCl by 24 and 30 in MBM, respectively. Calcium ions may bind to negative charges on the soluble protein fraction, changing the ionic interactions among proteins. The increase in protein solubility by $MgCl_2$ in MBM was attributed to the greater electronegativity of magnesium ions compared to calcium. This property would enable magnesium ions to bind more strongly with polar groups on proteins than calcium ions, resulting in greater protein interaction (15).

TABLE 3
Protein Solubility, Percent Extractability, and pH as Affected by Addition of 0.05% $CaCl_2$ or $MgCl_2$[a]

Divalent salt (0.05%)	pH	Percent extractability (%)	Protein solubility (mg/mL)
Control[b]	5.53[c]	57.13	4.54[b]
$CaCl_2$	5.32[b]	70.71	5.62[a,b]
$MgCl_2$	5.36[a]	74.23	5.90[a]

[a]Means within columns that have different roman superscripts are different ($P < 0.05$).
[b]4% NaCl concentration. For abbreviation see Table 1.

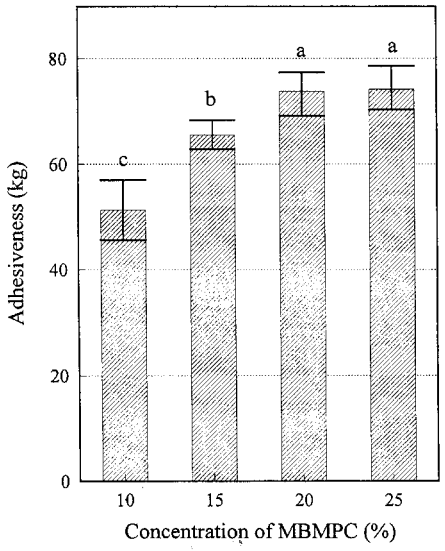


FIG. 1. Effect of defatted meat bone meal protein concentrate (MBMPC) concentration (wt/vol) on adhesiveness. Means within different letters are significantly different ($P < 0.05$).

Initial protein concentration of MBM sample was 48.1%. After defatting with isopropyl alcohol, protein concentration of defatted MBM was 57.3%. By using the salt extraction procedure, 32 g of MBMPC was obtained from 100 g MBM. The corresponding protein yield was 51.76% for MBM concentrate. Final protein concentration of MBM isolate was 77.8%.

Optimal conditions of MBM adhesive. The performance of bonded joints can be influenced by MBMPC concentration, pH, and heating temperature. To accurately measure the adhesiveness of MBMPC, optimization of formulation methods was required.

Figure 1 shows that adhesiveness increased almost linearly as the MBMPC concentration increased from 10 to 20% (wt/vol), after which the increase leveled off at 25%. Adhesiveness of MBMPC as a function of heating temperature at various pH values is shown in Figure 2. To prepare adhesives, MBMPC (20%, wt/vol) were suspended in deionized water (final volume of 100 mL) and the suspensions were heated for 30 min at various temperatures (60, 70, 80, and 90°C) and pH values (5.0–9.0), the latter obtained by treating the suspensions with NaOH or HCl. At pH 6.0–8.0, MBM protein molecules are almost completely and irreversibly uncoiled, mak-

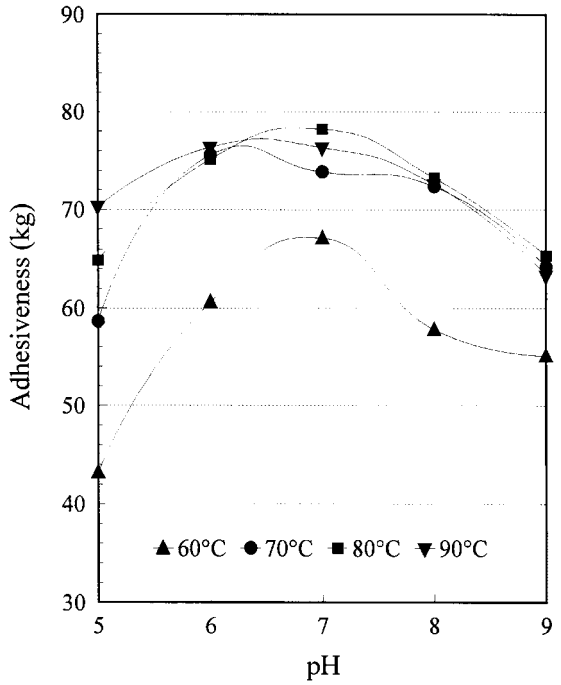


FIG. 2. Effects of heating temperature and pH on adhesiveness of MBMPC adhesive ($P < 0.05$). Each data point represents the average of three determinations. For abbreviation see Figure 1.

ing available the entire adhesive potential of their complex and reactive structure. The increment in adhesiveness of MBMPC adhesive is likely due to the increased van der Waals interaction and to hydrogen bonding to the wood surface by the exposed polar groups of MBM protein (16). An increment in polar hydrophobic groups would lead to an increased interaction (van der Waals force, hydrogen bonding) with polar groups on the wood surface. At 80°C, the adhesiveness of MBMPC adhesive reached the highest values of 78.21 kg. MBMPC with highest adhesion properties were at 70 to 90°C. Heating may have produced higher creep resistance as a result of increased cross-linking and stiffness in the interfacial area (17).

It is generally thought that adhesion of globular proteins is improved by alkali treatment (16). At pH ≥ 11, or above, protein molecules are completely and irreversibly uncoiled. Heating is one of the most important and widely used methods for denaturing a protein, because functional properties such as gelation, emulsification, and foaming must be a reflection of the protein structure of the denatured state. When the temperature of a protein is increased, structural changes occur.

Viscosity. Viscosity is a property of the functional behavior and the physicochemical nature of the proteins, because diffusion through the adherend material affects the adhesion property of MBMPC. Lowered viscosity of adhesive could improve the degree of chemical wetting of the wood surface and reduce the possibility of developing voids in the bondline (17). Owing to its superior flow characteristics, a low-viscosity form of adhesive could also absorb, dissolve, disperse, or desorb contaminants on the wood surface. For this reason a lower-molecular-weight primer was often used under the adhesive to obtain superior wetting to the wood surface and to enhance joint durability. Thus, adhesive with lower viscosity is preferred in actual practice.

Figure 3 shows that viscosity of the MBMPC adhesive solution decreased from 4,800 mPa to less than 2,300 mPa during the first 2 h of storage. The high initial viscosity can be attributed to the formation of protein gel owing to the presence of high concentrations of protein and calcium.

Effects of cross-linking agents. Various cross-linking agents were incorporated into the adhesive formulation to test their effects. Figure 4 shows that glutaraldehyde increased the adhesiveness of MBMPC up to 84.24 kg at 0.1% (wt/vol). When combined with heat treatment, the adhesiveness proportionally increased with the increase in the concentration of the cross-linking agents up to 0.05% (wt/vol) (12). The addition of cross-linking agents in the formulation steps accomplished two purposes: (i) the proteins formed gels with the cross-linking agents by polymerizing into rigid three-dimensional structures, and (ii) the cross-linking agents improved bond strength, elasticity, and flow behavior of protein-based adhesives (18).

Water resistance. Water resistance is an important adhesion property that determines the quality of an adhesive and the type of wood products in which it can be used. Figure 5 shows the effects of glutaraldehyde on water resistance of the bonds. The adhesive formulated with MBMPC showed susceptibility to water after 24 h (21.29 kg). Addition of a cross-linking agent such as glutaraldehyde increased water resistance up to three times over the control MBMPC adhesive (60.49 kg), indicating that the cross-linking agent formed a rigid adhesive structure.

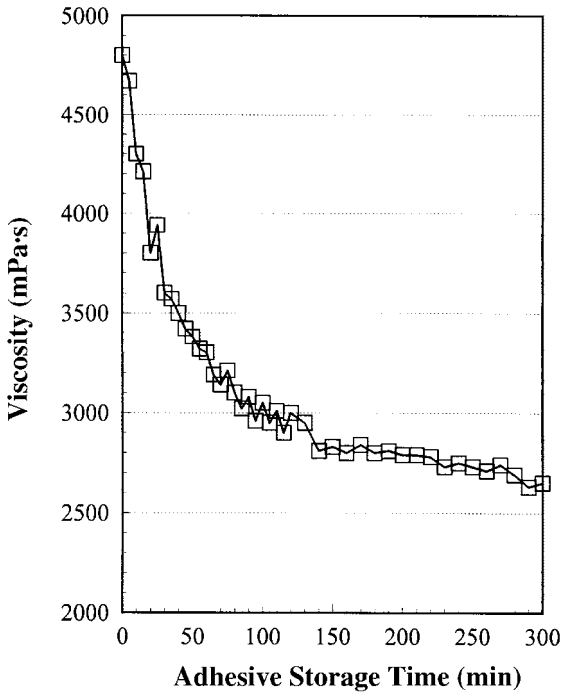


FIG. 3. Changes in viscosity during storage of MBMPC adhesive ($P < 0.05$). Each data point represents the average of three determinations. For abbreviation see Figure 1.

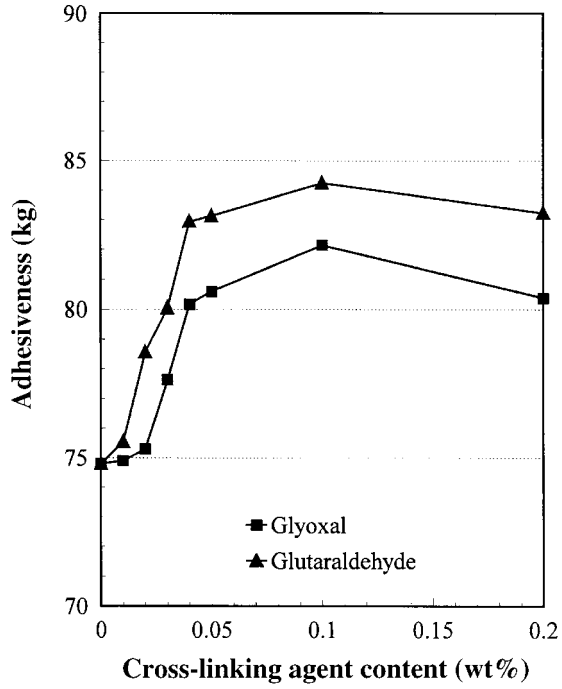


FIG. 4. Effect of concentration of cross-linking agents on adhesiveness of MBMPC adhesives ($P < 0.05$). Each data point represents the average of three determinations. For abbreviation see Figure 1.

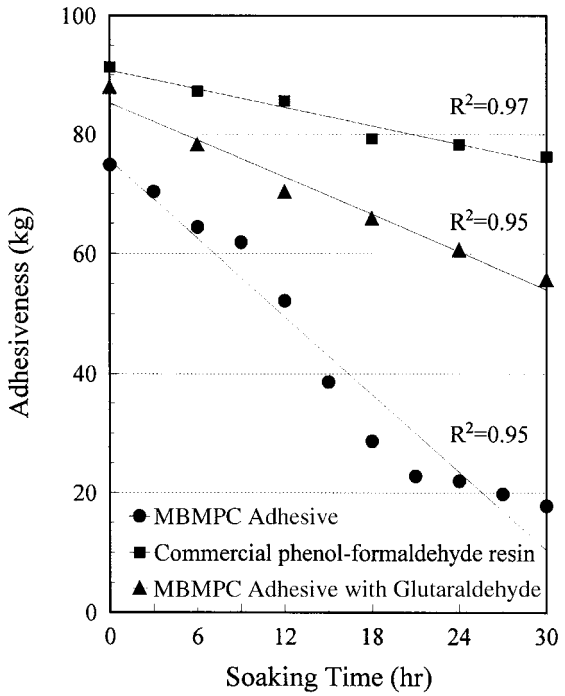


FIG. 5. Effects of cross-linking agents on water resistance of MBMPC adhesive ($P < 0.05$). Each data point represents the average of three determinations. For abbreviation see Figure 1.

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