한국농화학회지 제 39권 제 2호(1996)
Agricultural Chemistry and Biotechnology
Vol. 39, No. 2, pp. 123~126(1996)

Characterization of Plasma Proteins from Bloods of Slaughtered Cow and Pig and Utilization of the Proteins as Adhesives[1)]

Eunhee Park[1], Hwahyoung Lee[2] and Kyung Bin Song[1]*

[1]Departments of Food Sci. & Tech. and [2]Forest Products,
Chungnam National University, Taejon, Korea

Abstract : Simple and rapid method of purification of plasma proteins from bloods of slaughtered animals was developed and the proteins were applied to plywood products as a blood glue to utilize waste materials. Plasma protein was obtained by adding 2% trichloroacetic acid (TCA) or 0.6 N HCl as optimal concentration to the supernatant, after centrifugation of bloods. Molecular properties of beef and pig plasma proteins were examined on SDS-PAGE. Application of blood glue to plywood was quite satisfactory compared to the synthetic amino resin by tensile-shear test for the strength of adhesive bonding(Received December 8, 1995; accepted March 6, 1996).

Introduction

Considerable amount of blood coming from slaughtered animals as waste materials have caused many problems. Especially, bloods are mostly discharged through sewage after diluting with tap water without suitable wastewater treatment. Thus, improper disposal of blood seriously causes water pollution. Therefore, appropriate utilization of bloods should be considered to overcome this problem.

Shortage of food supply requires the new sources of food protein. There have been many attempts to search new protein resources such as plant sources and single cell proteins.[1)] Since blood contains considerable amount of protein, it has been suggested as a potential resource of dietary proteins, although it lacks Ile and Met.[1,2)] Blood has been utilized in many countries as an ingredient in many sausages and puddings.[1,3)] Typical uses of beef plasma are ingredients in meat emulsions of cooked sausages, dietary iron supplements, and ground beef patties.[4-7)] However, one of the disadvantage of blood plasma as an food ingredient might be objectionable dark color imparted to food products. Besides the utilization of bloods in food industry, blood glue had been used as a protein adhesive in wood products industry until early 80's, though it has been mostly replaced by petroleum-based adhesives which were more economical[8-12)]. However, they still remain important in certain areas due to their unique bonding characteristics. Therefore, it is desirable to overcome high cost of blood glue to compete with amino resin such as urea formaldehyde (UF) resin and urea melamine formaldehyde(UMF) resin.

In this study, to utilize bloods isolated from slaughtered animals and prevent water pollution, simple and rapid method of purification of plasma proteins was developed and characteristics of the blood glue applicable to plywood manufacture were investigated.

Materials and Methods

Materials

Cow and pig bloods were freshly collected right after the slaughter and immediately used for the next processing. Veneer used for plywood was *Pinus radiata* from New Zealand. All the Chemicals used were of analytical grade.

Methods

(1) Isolation of blood plasma protein

After collecting the blood from the slaughterhouse, ethylenediaminetetraacetic acid (EDTA, 2 g/L) was immediately added to prevent coagulation and centrifuged at 11590×g for 30 min to remove blood cells. To the supernatant, different concentrations of trichloroacetic acid(TCA) solution and HCl solution were added to precipitate the plasma protein. The weight of precipitate was measured after freeze drying or hot-air drying and

Key words : plasma protein, TCA precipitation, blood glue
*Corresponding author
[1)]Part of this study was applied for a patent.

redissolved in the water and its pH was measured.

(2) Ammonium sulfate fractionation

To the blood plasma, different amounts of ammonium sulfate (20%, 30%, 40%, 50%, 60%, 70%) were serially added to reach the final concentration. After centrifugation at each step, its precipitate was examined on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

(3) SDS-PAGE

SDS-PAGE was performed according to the method of Laemmli.[13]

(4) Amino acid analysis

Amino acid composition of blood plasma protein was analyzed after acid hydrolysis at 110℃ for 24 hr (Picotag amino acid analysis system, Waters Assoc. Co.).

(5) Preparation of blood glue

Using the TCA precipitate of blood plasma, blood glue was prepared according to the modified method of Lambuth.[11-14]

(6) Manufacture of plywood

Plywood was manufactured under the optimum hot pressing condition,[12] 120℃, 3 min, 10 kg/cm^2, using the veneer whose dimension is 250 mm×250 mm.

(7) Determination of mechanical properties of plywood

[1]Mechanical properties of plywood were examined according to the method of KS Standard F3101-1987 after putting in a constant temperature-humidity controlled room for 1 week. Dry and wet test (60℃, 3hr.) were performed by measuring wood failure and tensile shear strength.

Results and Discussion

Isolation of blood plasma protein

Simple and rapid method of purification of plasma proteins from pig and beef bloods was developed using TCA and HCl. First of all, after centrifugation of whole blood, about 50% of total volume was obtained as plasma. To the blood plasma, various amounts of TCA and HCl were added to precipitate plasma protein and the amount of precipitate is shown in Table 1. As TCA concentration increased from 1% and 6%, the amount of precipitate increased. However, above 10% of TCA treatment, the amount of precipitate decreased. With increase of TCA concentration, pH and solubility of the precipitate decreased. Considering the amount of precipitate and solubility, the optimal condition for isolating plasma protein was 2% of TCA concentration. In case of HCl instead of TCA, according to the same procedure, the optimal condition was found to be 0.6N of HCl.

Characterization of plasma protein

Table 1. Effect of TCA and HCl concentration on the amount of pig plasma precipitate[1]

TCA or HCl concentration	Amount of precipitate (g)	Final pH[2]	Solubiity[3]
TCA 10%	14.6	1.5	0.019
6%	16.5	1.7	0.027
2%	15.6	2.7	0.648
1%	8.4	3.3	1.330
HCl 2.26 N	16.1	1.1	0.302
1.2 N	16.7	1.2	0.507
0.6 N	13.7	1.4	1.412
0.2 N	1.3	2.8	1.524

[1]The amount of plasma used was 40mL. [2]Measurement after redissolving in the water. [3]Absorbance at 280 nm after dilution.

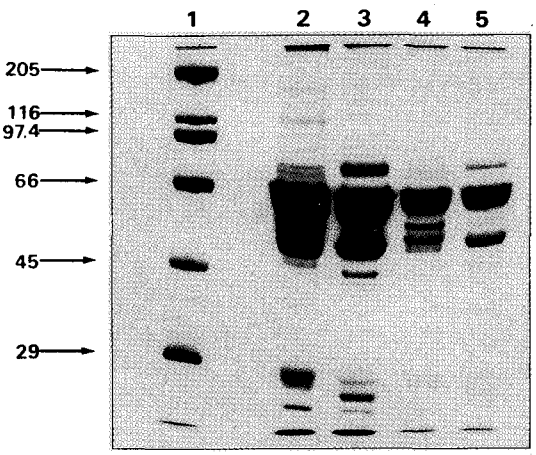

Fig 1. SDS-PAGE profile of blood plasma proteins. Lane 1; molecular weight marker, 2, 4; beef plasma 3, 5; pig plasma.

To study the characteristics of plasma protein obtained by TCA precipitate and compare beef plasma protein with pig plasma protein, SDS-PAGE was performed (Fig. 1). Major components of beef and pig plasma proteins were albumin (66 KDa) on SDS-PAGE(Fig. 1). It has been known that blood plasma protein mainly consists of albumin and globulin fractions[7] and its ratio is dependent on species and other conditions. Besides the major fractions, for pig plasma protein, there are single thick band (72 KDa) and another low molecular weight band (44 KDa), which are absent or negligible in beef blood. In case of beef blood, compared with pig blood, there are larger molecular weight fractions present and a definite band of 20 KDa. However, beef and pig plasma proteins are generally very similar, though there is a slight difference in pattern.

The major fraction of TCA precipitate of plasma was albumin. Considering that the most important component in blood glue is albumin, TCA precipitation proves to be the effective method for isolation of plasma protein.

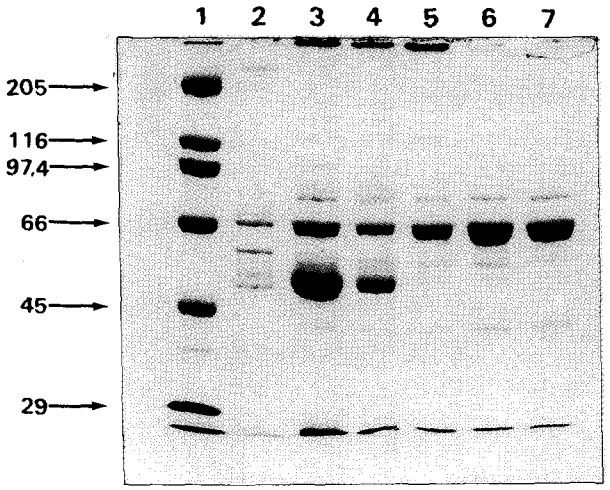

Fig 2. SDS-PAGE profile of ammonium sulfate fractionation of pig plasma. Lane 1; molecular weight marker, 2; 20% precipitate 3; 30% precipitate 4; 40% precipitate 5; 50% precipitate 6; 60% precipitate 7; 70% precipitate.

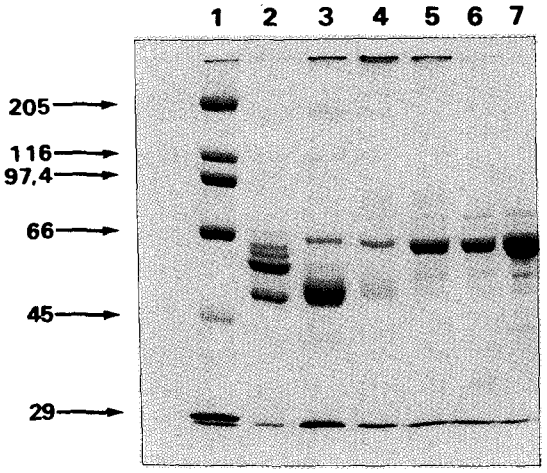

Fig 3. SDS-PAGE profile of ammonium sulfate fractionation of beef plasma. Lane 1; molecular weight marker, 2; 20% precipitate 3; 30% precipitate 4; 40% precipitate 5; 50% precipitate 6; 60% precipitate 7; 70% precipitate.

Also, considering that conventional blood glue was usually manufactured by spray drying or hot-air drying of blood and high energy cost was one of the disadvantage in competing with other types of adhesives, this simple method could eliminate drying step in manufacturing of blood glue.

Ammonium sulfate fractionation

To pursue a purification method of plasma protein, ammonium sulfate was serially added to the blood plasma at various level of concentration to precipitate plasma protein and its molecular weight distribution was examined on SDS-PAGE (Fig. 2, 3)

For pig and beef bloods, both have similar pattern in ammonium sulfate fractionation. Up to 20% of ammonium sulfate, albumin, which is a major component, hardly precipitated but at over 50% of ammonium sulfate, most of it precipitated. This indicates that ammonium sulfate precipitation requires at least 50% ammonium sulfate for effective precipitation.

Table 2. Amino acid composition of pig plasma protein

	Whole pig plasma (%)	TCA precipitate[1)]
Asp	3.4	8.5
Glp	6.3	12.5
Ser	7.4	7.3
Gly	9.3	6.2
His	4.2	2.8
Arg	3.8	4.0
Thr	8.8	7.1
Ala	6.3	7.9
Pro	13.4	6.6
Tyr	4.9	3.3
Val	7.4	7.0
Met	0.9	0.8
Cys	2.0	1.3
Ile	3.3	3.5
Leu	8.4	9.6
Phe	7.4	4.3
Lys	2.6	7.1

[1)]TCA precipitate of pig plasma protein

Amino acid analysis of pig blood plasma

Compared to beef blood, relatively under-utilized pig blood was chosen as a primary source for blood glue. Typical amino acid content of pig plasma was shown in Table 2. Pig plasma protein has unusually high amount of Pro and Gly. On the contrary, TCA precipitation of pig plasma has considerable amount of Asp, Glu and Lys. This difference appears to contribute to the predominant precipitation of albumin fraction among plasma proteins. This phenomenon is also supported by SDS-PAGE profile of TCA precipitate of pig blood plasma.

Utilization as blood glue in plywood adhesive

As one of the application, TCA precipitate of pig plasma was studied as a source of blood glue for plywood and compared with other adhesives. The result of mechanical test of plywood manufactured under various conditions using different adhesive clearly indicates that blood glue is an excellent substitute for amino resin such as urea formaldehyde(UF) resin and urea melamine formaldehyde(UMF) resin (Table 3). All the plywood were satisfactory regarding mechanical property, density, and moisture content. Especially, regarding tensile shear strength, there was no significant difference between

Table 3. Characteristics of plywood prepared with various adhesives

properties / treatment	density	moisture content (%)[5]	Dry test%		Wet test[7]	
			strength[6] (kg_f/cm^2)	wood failure (%)	strength (kg_f/cm^2)	Wood failure (%)
UF[1]	0.54	10.94	19.73	38	7.68	20
UMF[2]	0.55	11.11	17.95	25	12.12	25
BG I[3]	0.56	11.30	19.72	14	9.15	10
BG II[4]	0.56	11.25	17.68	34	9.93	20

[1]Urea formaldehyde resin [2]Urea melamine formaldehyde resin [3]Pig plasma protein as a blood glue [4]TCA precipitate as a blood glue [5]Korean standard requires value below 13% [6]Tensile strength, Korean standard requires greater value than 7.5 kgf/cm^2 [7]Pretreated at 60℃ for 3 hr.

blood glue and other adhesive. Also, in case of wet test (60℃, 3 hr), blood glue was even better than UF resin. Therefore, blood glue is quite promising in plywood industry and furthermore, it could prevent emission of formaldehyde, which is a major problem in formaldehyde-based resin.

References

1. Cadironi, H. A. and H. W. Ockerman (1982) Incorporation of blood proteins into sausage. *J. Food Sci.* **47**, 405-408.
2. Swingler, G. R., Neale, R. J., and Lawrie, R. A. (1978) Nutritive value of proteins isolates and fibers from meat industry by-products. *Meat Sci.* **2**, 31.
3. Wismer-Pedersen, J. (1979) Utilization of animal blood in meat products. *Food Technol.* **8**, 76-80.
4. Cadironi, H. A. and H. W. Ockerman (1982) Blood and plasma protein extracts in sausages. *J. Food Sci.* **47**, 1622-1625.
5. Guzman, J. C., McMillin, K.W., Bidner, T. D., Dugas-Sims, S. and J. S. Godber (1995) Texture, color and sensory characteristics of ground beef patties containing bovine blood proteins. *J. Food Sci.* **60**, 657-660.
6. Graham, A. (1978) The collection and processing of edible blood. *CSIRO Food Res. Q.* **38**, 16-22.
7. Raeker, M. O. and Johnson, L. A. (1995) Thermal and functional properties of bovine blood plasma and egg white proteins. *J. Food Sci.* **60**, 685-690.
8. Brandis, R. L. (1990) In 'Handbook of Adhesives', I. Skeist, Animal Glue. p. 123, Van Nostrand Reinhold Co., New York.
9. Bye, C. N. (1990) In 'Handbook of Adhesives', I. Skeist, Casein and Mixed Protein Adhesives. p. 135, Van Nostrand Reinhold Co., New York.
10. Detlefsen, W. D. (1989) In 'Adhesives from renewable resource', Hemingway, R. W. Blood and Casein Adhesives for Bonding Wood, p. 445, ACS.
11. Lambuth, A. L. (1977) In 'Hand book of Adhesives', Skeist, I, Blood Glues, p. 181, Van Nostrand Reinhold Co., New York.
12. Sellers, T. (1985) Plywood and Adhesive Technology p. 353, Marcel Dekker.
13. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680.
14. Lambuth, A. L. (1989) In 'Wood adhesives', Pizzi, A., Protein adhesives for wood, p. 13.

도축혈액 혈장 단백질의 특성 및 접착제로의 응용

박은희[1], 이화형[2], 송경빈[1,*] (충남대학교 식품공학과[1], 임산공학과[2])

초록 : 도축동물의 대부분 폐기되는 혈액으로부터 기능성 소재를 발굴하고 수질오염을 방지하기위해서 소, 돼지 도축혈액으로부터 간단하고 신속한 방법으로 혈장 단백질을 분리하는 방법을 개발했고 그것을 혈분접착제로서 응용하였다. 혈액을 원심분리한후 혈장단백질은 2% TCA 또는 0.6N HCl을 최종농도가 되게 첨가하여 침전시킴으로써 얻어졌다. 소, 돼지 혈장단백질을 비교 분석하기 위하여 SDS-PAGE를 하였고 얻어진 혈장단백질을 이용하여 혈분접착제로서 합판제조에 사용하여 다른 합성접착제와 접착성 시험을 한 결과 다른 합성 접착제와 비교해서 만족한 결과를 얻었다.

*연락저자