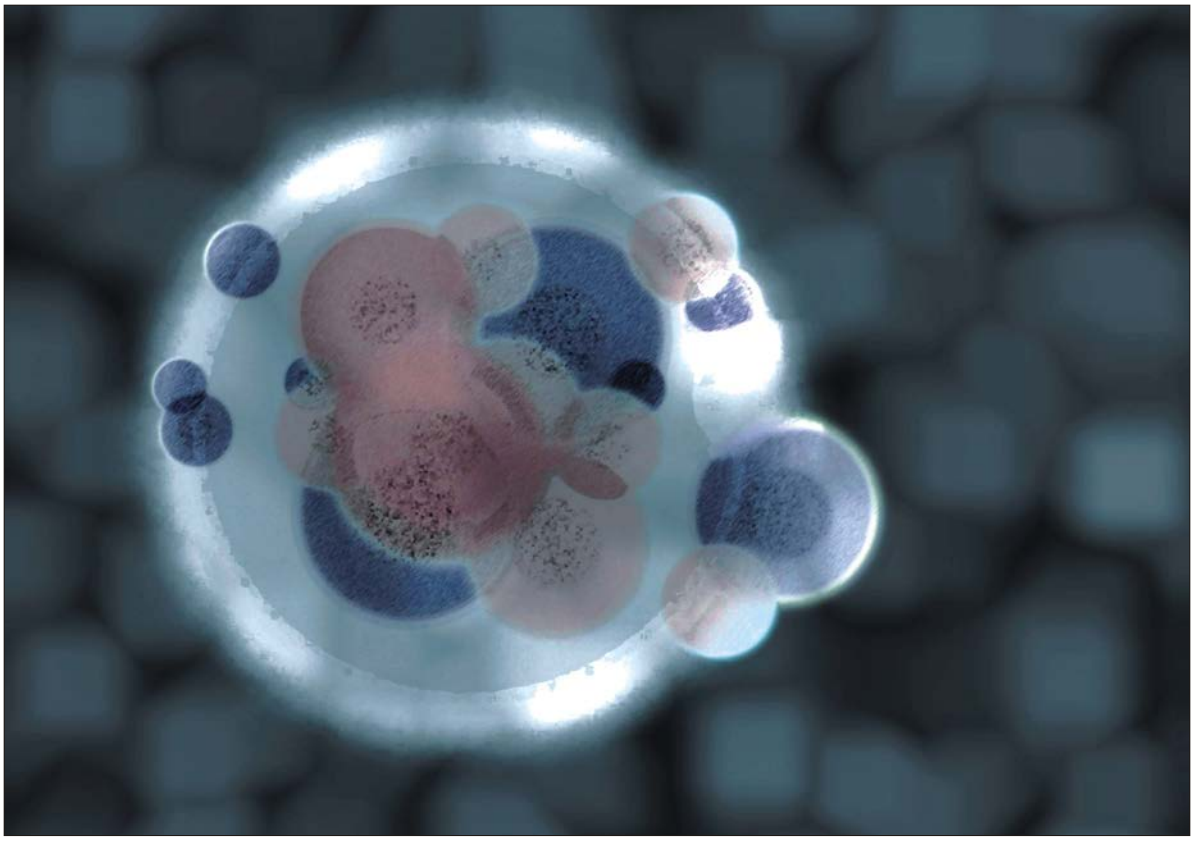

# Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 7 (2): 247-254, 2007
ISSN 1727-3048


# The Improvement of Rheological Properties of Wheat Dough Using Porcine Globin Glycated with Sugar

Aree Innun, Shigeru Hayakawa, Masahiro Ogawa and Yuanxia Sun
Department of Biochemistry and Food Science, Kagawa University, Ikenobe,
Miki, Kagawa 761-0795, Japan

**Abstract:** Globin (Gb) and Gb-sugar conjugates were formulated to dough preparation and their effects on the rheological properties of dough were studied using Farinograph. Gb-sugar conjugate was prepared by conjugating hemoglobin (Hb) with sugar (glucose, allose or psicose) via Maillard reaction and subsequently heme was removed from Hb-sugar conjugate. The number of attached sugar molecules on a molecule of Gb was 1-9 molecules depending upon type of sugar used. The results of Farinograph suggest that water absorption of wheat flour significantly increased by the addition of Gb and glycated Gbs. Stability and weakening of dough were obviously improved by the additions of allose- and psicose-glycated Gbs. It can be concluded that the addition of glycated Gbs, especially allose- and psicose-glycated Gbs, to wheat flour improved rheological properties of dough.

**Key words:** Blood application, dough, globin, Maillard reaction, rare sugar

## INTRODUCTION

Animal blood is a by-product from slaughterhouse in many countries of the world (Goldstrand, 1988). A number of economic considerations have been paid to generate benefit for slaughterhouse by-products by using blood as food additives instead of animal feed ingredients. Blood which is rich in valuable proteins has been studied widely (Anderson, 1988; Knipe, 1988; Wismer-Pederson, 1979). However, most of the studies have been mainly focused on plasma and its protein components rather than red blood cell fraction (Knipe, 1988). Hemoglobin (Hb) is an abundant protein in red blood cell and thus the conversion of this protein to a food grade protein is a great deal of attention. However, a reddish brown color and undesirable odor of native protein are serious barriers for its application to human food. For food application purposes, therefore, further heme-removing treatment is required to obtain a colorless globin (Gb). A number of studies in respect to nutrition values (Marquez *et al*., 2005), chemical and functional properties (Autio *et al*., 1984; Gomez-Juarez *et al*., 1999; Hayakawa *et al*., 1982; Nakamura *et al*., 1984; Ramos-Clamont *et al*., 2003) of Gb has been conducted. According to these studies, Gb exhibited good functional properties of foaming, emulsifying, heat-induced gelation and water-binding capacity and also contained high level of essential amino acids, especially lysine. In addition, an effective fortification with Gb can improve the nutritional quality to wheat based products since wheat proteins are deficient in lysine and threonine (deMan, 1979).

Maillard Reaction (MR), a non-enzymatic reaction which is initiated by the condensation of a carbonyl group of reducing sugar with free amino groups of protein. MR has been reported to influence the color, flavor, texture of food products and improve functional properties of protein (Aoki *et al*., 1999; Fogliano *et al*., 1999; Gerrard and Brown, 2002; Miller and Gerrard, 2005; Sun *et al*., 2004). Moreover, the products of MR (MRPs) have been elucidated to possess antioxidant activity (Benjakul *et al*., 2005; Manzocco *et al*., 2001; Sun *et al*., 2004; 2006). The antioxidant activity of MRPs produced from protein glycated with rare sugar (D-allose and D-psicose) has been demonstrated to have higher activity than that of produced from protein glycated with alimentary sugars (Sun *et al*., 2004; 2006).

Wheat dough, a combination of two main ingredients of wheat flour and water, is an intermediate homogeneous compound for bread-making. The quality of dough, thus, is strongly related to the final quality of finished bread product. Many food additives have been used in bakery industries to improve the qualities of dough such as hydrocolloids (sodium alginate, κ-carrageenan, xanthan gum and hydroxypropyl methylcellulose), skim milk power, lactic acid and carbohydrates (Rosell *et al*., 2001; Singh *et al*., 2002; Miyazaki *et al*., 2004), which some of

**Corresponding Author:** Aree Innun, Department of Biochemistry and Food Science, Kagawa University, Ikenobe, Miki, Kagawa 761-0795, Japan Tel: 0092-41-5000123 Fax +81-87-891-3021

them are artificial chemical. In general, consumers do not prefer food product which contains chemical additives; therefore, using a natural substance to improve dough quality would be acceptable for bread consumer. In this study, the effects of Gb and Gb glycated with sugar (glucose, allose or psicose) on the rheological properties of dough were investigated using Farinograph.

## MATERIALS AND METHODS

**Chemicals:** Bovine and porcine Hbs used as standard protein and low viscosity carboxymethyl cellulose (CMC) sodium salt were purchased from Sigma Chemical Co. (MO, USA). Rare sugars, D-allose (All) and D-psicose (Psi), were obtained from Kagawa Rare Sugar Cluster (Kagawa, Japan). D-glucose (Glc) and other chemicals were purchased from Wako Pure Chem. Ind., Ltd. (Osaka, Japan). Wheat flour for dough preparation was a commercial Haruyokoi product (Yokoyama Milling Co., Hokkaido, Japan). All chemicals used in this study were analytical grade.

**Preparation of Gb and Gb-sugar conjugates:** Gb and Gb-sugar conjugates were prepared from Hb and Hb-sugar conjugates, respectively. Hb was prepared from porcine blood which was collected from local slaughterhouse. The preparation was carried out at 4°C according to the procedure of Sato *et al.* (1981) with a slight modification. Firstly, a whole blood was mixed immediately with an anticoagulant of 15% sodium citrate solution at final concentration of 0.3% after being derived from carcass. The separation of the whole blood into red blood cell and plasma was accomplished by centrifugation at 5000×g for 15 min. Then, the separated red blood cell was twice washed with equivalent volume of saline and centrifuged at 5000×g for 15 min. The Hb was liberated from red blood cell by adding cold distilled water to the red blood cell fraction until reaching a final volume of equivalence to an initial volume. Hb solution was recovered after sonication and centrifugation. Finally, Hb solution was dialyzed at 4°C against distilled water and lyophilized.

Hb-sugar conjugates were prepared by incubating Hb with different sugars (Glc, All or Psi) via Maillard reaction. A 5% (w $v^{-1}$) Hb solution was preliminarily prepared with 10 mM phosphate buffer (pH 7.2) before each sugar was added to the protein solution with the sugar concentration of 0.7% (w $v^{-1}$). The mixed solution was lyophilized and the mixture powder was incubated for 24 h in dry state at 40°C and 60% relative humidity using an incubator (LHL-113, Espec Corp., Osaka, Japan).

Gb was prepared from Hb by heme precipitation using the method of Hayakawa *et al.* (1986). Briefly, Hb was dissolved in distilled water to the protein concentration of 4% (w $v^{-1}$). The protein solution was heated at 70°C for 30 min and cooled to room temperature. Two percent CMC solution was slowly added to the cooled protein solution and thoroughly mixed. The soluble Gb was recovered by centrifugation at 5000×g for 15 min. After appropriate pH adjustment, the Gb solution was dialyzed against distilled water and then lyophilized. Gb-sugar conjugate was prepared from Hb-sugar conjugate in the same manner as Gb sample. The protein samples were kept at -20°C until use. Schematic diagram in the preparation of Gb and glycated Gbs is shown in Fig. 1.

**Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):** SDS-PAGE was carried out according to the method of Laemmli (1970) using 15% polyacrylamide gel and a staining mark of commssie billiant blue R-250.

**MALDI TOF-mass spectrometry:** Protein samples were analyzed using a matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometer (Autoflex, Brüker Daltonics, Germany) with a nitrogen laser. Sinapinic acid saturated in TA solution (33% acetonitrile mixed with 0.1% trifluoroacetic acid) was used as a matrix. The sample was dialyzed against deionized water to eliminate interferential ions. The measurement was conducted according to the operator's manual of flexControl™ (Brüker Daltonics).

**Determination of rheological properties of dough:** Rheological properties of dough were determined using a Farinograph DOCORDER E330 (Brabender Instuments Inc., Germany). The results are expressed by Farinogram with the following parameters: Water absorption (%), dough stability (min), dough weakening (Brabender Units, BU) and dough development time (DDT, min). A description of the instrument and the obtained parameters had been explained by Rasper (1979). The determination was conducted at 30°C and a constant speed of 63 rpm with 300 g of wheat flour. Pure water was used to prepare the control dough instead of using 2% (w $v^{-1}$) protein samples solution which was measured using the method of Lowry *et al.* (1951).

**Determination of distributions of Gb and glycated Gbs in dough:** Distribution of Gb and glycated Gb in the mixture of dough was observed on SDS-PAGE. Dough samples obtained from Farinograph measurement were used to extract proteins before loading to SDS-PAGE. Firstly, dough samples were washed with saline solution for several times until the starch was adequately removed.

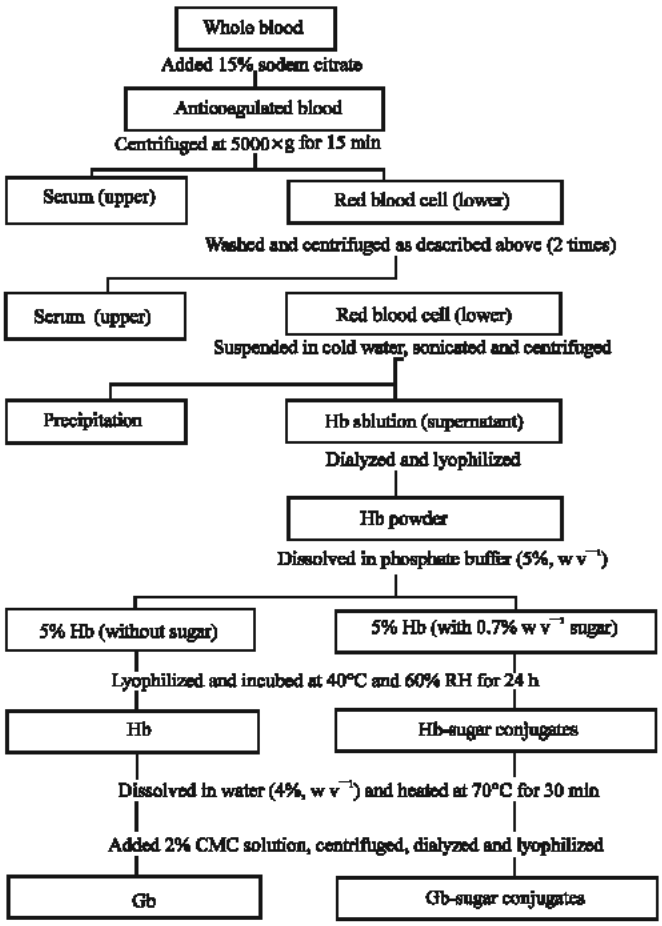


Fig. 1: Schematic diagram of Gb and glycated Gbs preparations

Twenty milligrams of washed sample (gluten) was randomly picked up and then dissolved in a mixture of 1% SDS (1 mL) and 2-mercaptoethanol (10 μL) at 30°C for 24 h with shaking. The resulting protein mixture solution was collected after centrifugation at 7000×g for 10 min. A clear supernatant of 16 μL was mixed with 4 μL of SDS-PAGE running buffer containing 0.02% coomassie brillant blue and was loaded on 15% polyacrylamide gel.

**Statistical analysis:** The results were analyzed statistically using t-test. A 2-tailed p value <0.05 was considered as statistically significant. The results were expressed by mean values.

## RESULTS AND DISCUSSION

**Preparation of Gb and glycated Gbs:** Figure 2A presents SDS-PAGE patterns of Hb with different species. There were double bands at molecular weight (MW) of about 15-16 kDa appeared in porcine Hb. Hb is a tetrameric molecule which consists of 2 α-monomeric subunits and

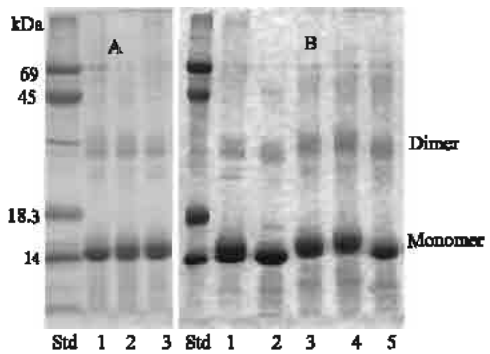


Fig. 2: SDS-PAGE patterns of protein samples (A); standard protein (Std), commercial bovine Hb (1), commercial porcine Hb (2) and collected porcine Hb (3). (B); collected porcine Hb (1), Gb (2), Gb glycated with Glc (3), Gb glycated with All (4) and Gb glycated with Psi (5)

2 β-monomeric subunits. Bovine Hb, in spite of the presence of two subunit forms (α and β) apparently

showed a single band at MW of 15 k. This is probably due to the inefficiency of the electrophoretic separation; the mixture of similar mass proteins could not be separated clearly. The theoretical MWs of bovine and porcine Hbs calculated from their amino acid sequence were 15053 and 15039 Da for α-subunit and for β-subunit were 15954 Da and 16034 Da, respectively (http://ca.expasy.org/). This theorem demonstrates that the difference of mass between α- and β-subunits of bovine Hb is 901 Da which is smaller than that of porcine Hb, 995 Da. Considering on the contamination of collected Hb from other proteins, the results indicate that the collected porcine Hb showed a comparable purity to the commercial Hb. Figure 2B shows SDS-PAGE patterns of all samples prepared in this study. Gb sample showed a single band with MW of 15 kDa. The modification of Gb with different sugars led to the increase in MWs, especially when it was modified with Glc or All. It could be concluded that MWs of Gb glycated with aldo-hexose (Glc and All) were higher than that glycated with keto-hexose (Psi); higher MW suggests higher degree of glycation.

The results of MALDI-TOF mass spectrometry are shown in Fig. 3. The native Hb sample (a) had two major peaks with the mass of 15037 and 16036 m $z^{-1}$. The first peak corresponds to α-subunit (15039 Da, theoretical MW) and the second peak corresponds to β-subunit (16034 Da, theoretical MW). In Gb sample (b), however, only a single peak was observed at m $z^{-1}$ 15037 which matches with the α-subunit of Hb. The loss of β-subunit in Gb sample implies that the β-subunit has been co-precipitated with CMC during the heme-removing process

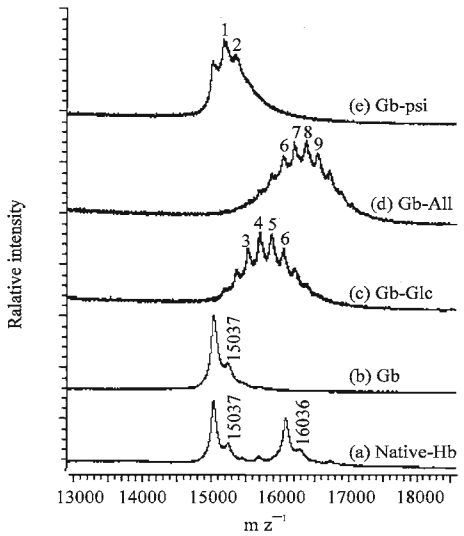


Fig. 3: MALDI-TOF mass spectra of native Hb, Gb and Gb glycated with different sugars

due to strong interaction between β-subunit and CMC molecules. In the case of Gb-Glc (c), four major peaks were observed with the mass of m $z^{-1}$ 15523-16009, indicating that 3-6 molecules (162 Da per hexose residue) of Glc were attached to a Gb subunit. Similar result was observed in Gb-All sample (d) with four major peaks which showed the mass of m $z^{-1}$ 16009-16495, representing the attachment of 6-9 molecules of All. Gb modified with Psi (e) had three peaks, the first of which showed the mass of m $z^{-1}$ 15037, the original peak of Gb α-subunit. The remaining two peaks with the mass of m $z^{-1}$ 15199 and 15361 are compatible with the attachment of 1-2 molecules of Psi. Thus, the number of aldo-hexose attached to a Gb molecule was found to be significantly higher than that of keto-hexose.

The results obtained from mass spectrometry which associated with the results obtained from electrophoresis suggest that the extent of glycations of Gb-Glc and Gb-All was higher than that of Gb-Psi. This result is in agreement with other literatures (Yoboah *et al*., 1999; Sun *et al*., 2004, 2005, 2006) that the reactivity of All is higher than that of Psi. The high glycation rate in aldose sugar is due to high electrophilicity of aldehyde group (Yeboah *et al*., 1999, Sun *et al*., 2004, 2005, 2006).

**Rheological properties of dough:** The effects of Gb and glycated Gbs at final concentration of about 1.3% (based on wheat flour weight) on rheological properties of dough were determined using Farinograph and their results were expressed by Farinograms. The summarized data of rheological properties of dough obtained from Farinogram are presented in Table 1. In comparison between control dough (no additional protein) and dough containing Gb, the addition of Gb to wheat flour led to an significant increase in water absorption. Moreover, the addition of Gb to wheat flour gave stronger dough as shown in a lower weakening value. However, stability and DDT were not significantly affected by the addition of Gb. In comparison between dough containing Gb and dough containing glycated Gbs, the addition of glycated Gbs, especially Gb-All and Gb-Psi, obviously improved rheological properties of dough. Water absorption of

Table 1: Rheological properties of dough containing Gb or glycated Gbs analyzed by Farinograph in comparison with the control dough (without additional protein)

| Sample | Water absorption (%) | Stability (min) | Dough weakening (BU) | DDT (min) |
|---|---|---|---|---|
| Control | 63.60$^{a}$ | 7.0$^{a}$ | 76.6$^{d}$ | 1.46$^{a}$ |
| Gb | 67.67$^{b}$ | 8.7$^{a}$ | 55.7$^{c}$ | 2.00$^{a}$ |
| Gb-Glc | 68.00$^{b}$ | 7.2$^{a}$ | 45.8$^{c}$ | 2.00$^{a}$ |
| Gb-All | 68.00$^{b}$ | >12 | 10.0$^{a}$ | 1.93$^{a}$ |
| Gb-Psi | 67.33$^{b}$ | >12 | 26.25$^{b}$ | 1.90$^{a}$ |

The same letter (s) in the same column are not significantly different ($p>0.05$, $n = 3$)

dough was significantly increased by the addition of glycated Gbs as well as Gb. Stability of dough was extended significantly by the additions of Gb-All and Gb-Psi. Weakening value of dough was lowest in dough containing Gb-All, suggesting that dough containing Gb-All was highest resistant dough to mixing. From the overall results, it can be concluded that the addition of Gb to wheat flour improved rheological properties of dough in some parameters while the addition of glycated Gbs, especially Gb-All and Gb-Psi, obviously improved rheological properties of dough. As a result, functional properties of Gb in wheat dough can be improved by glycating with sugar.

The significant increase in water absorption of dough containing Gb or glycated Gbs is probably due to the introduction of high water-binding property of Gb (Autio *et al*., 1984) and/or the formation of fine network of dough which can hold and retain water molecule inside. The stability value is an indication of dough strength, with higher value suggesting higher dough strength while the weakening value is related to elasticity of dough. Thus, a longer stability and a smaller weakening are desirable characteristics of dough. The improvements of dough stability and weakening by the addition of glycated Gbs may be attributed to the sugar moiety of Gb-sugar complexes. Singh *et al*. (2002) reported that the addition of free sugar to wheat flour had improving effects on the quality of dough. The individual sugar characteristics would also affect the qualities of dough. It was found that α-lactalbumin glycated with different sugars exhibited different chemical properties (Sun *et al*., 2006).

Figure 4 shows typical Farinograms of dough samples. The consistency of control dough decreased from 500 BU after 5 min of mixing time while dough

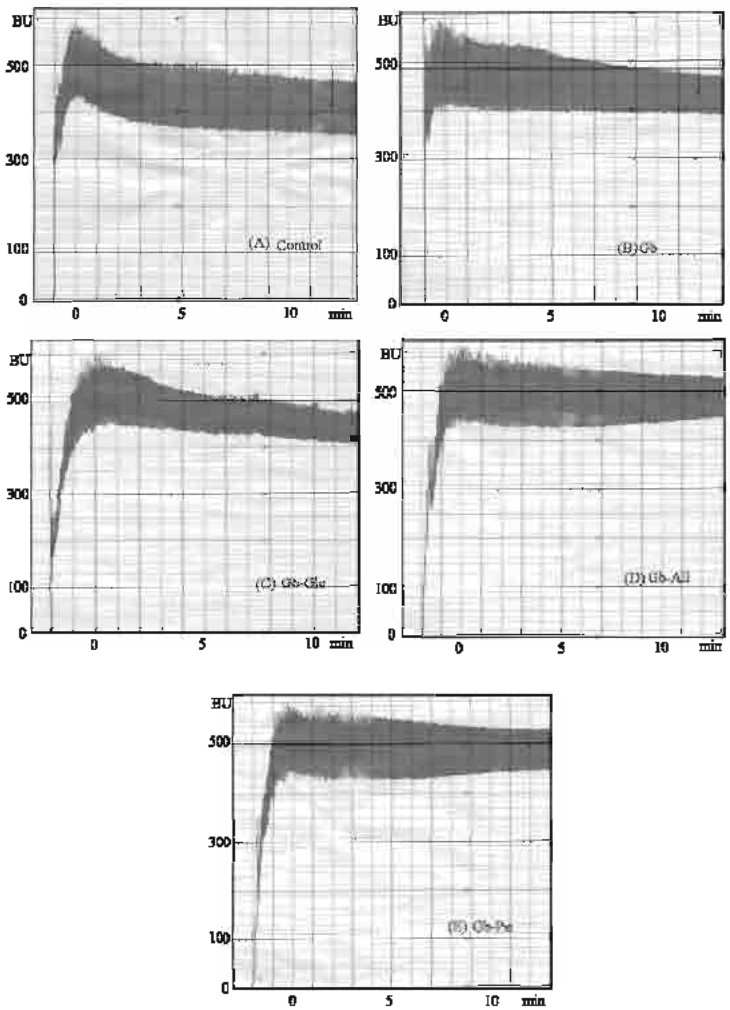


Fig. 4: Typical Farinogram of dough samples; control dough (A), dough containing Gb (B), dough containing Gb-Glc (C), dough containing Gb-All (D) and dough containing Gb-Psi (E)

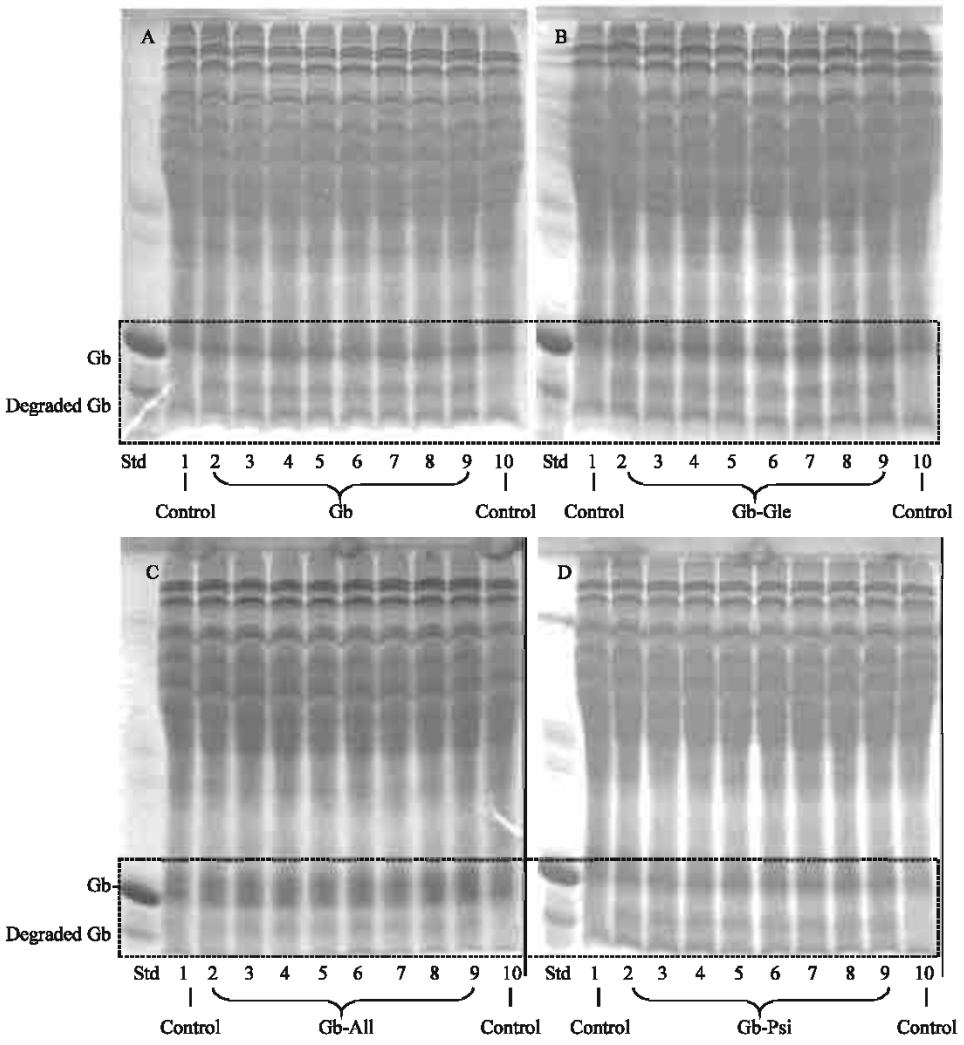


Fig. 5: SDS-PAGE patterns of the proteins mixture, extracted from dough with Gb (A), dough with Gb-Glc (B), dough with Gb-All (C) and dough with Gb-Psi (D), are shown in lanes 2-9 of each figure. The control dough, without additional protein, is shown in lanes 1 and 10 of each figure. Native Gb (15 μg) was used as a protein standard (Std)

containing Gb and Gb-Glc decreased after 8 min of mixing time. In the case of dough containing Gb-All and Gb-Psi, the consistency remained almost constant at 500 BU throughout the mixing time.

**Distribution of Gb and glycated Gb in dough:** The distribution of Gb and glycated Gbs in dough was observed on SDS-PAGE and their results are presented in Fig. 5. According to the MW of native Gb, the target positions are then mentioned in the rectangles. Each page of SDS revealed that the dense bands of lanes 2-9 were not significantly different from the control dough (lanes 1 and 10). Although the bands of Gb or glycated Gbs were contained in the bands of gluten proteins, they could not be recognized clearly due to the appearance of large amount of gluten proteins. Still, there were minor bands with the same MW as degraded Gb marker appeared in every lane of 2-9 and were absent in lanes 1 and 10 of each figure. This result implies that Gb and glycated Gbs homogeneously remained in the mixture of gluten proteins. Dough samples were washed with saline solution several times in order to remove the starch and consequently obtain gluten proteins. During the process of washing, Gb or glycated Gbs would be washed away from the mixture of gluten proteins if they had no interaction with the gluten proteins. Although the exact

interaction between Gb or glycated Gbs and gluten proteins has not been characterized in detail, here, it can be concluded that there were some interactions between Gb or glycated Gbs molecules and gluten proteins molecules.

## CONCLUSION

In the present study, Gb was prepared from porcine Hb using soluble CMC method. Gb-sugar glycation was prepared by incubating Hb with different sugars (Glc, All or Psi) via Maillard reaction. Subsequently, Gb and glycated Gbs were applied to the preparation of dough. The results of Farinograph indicated that the addition of glycated Gbs, in particular Gb-All and Gb- Psi, pronouncedly improved rheological properties of dough. The Gb and glycated Gbs were homogeneously distributed in the mixture of wheat gluten. It could be concluded that Gb glycated with sugar can be use as enhancer in the preparation of dough.

## ACKNOWLEDGMENTS

The authors would like to thank the Food Research Branch of Kagawa Prefectural Industrial technology Center for providing the Farinograph equipment and also their staff for the assistance.

## REFERENCES

Anderson, B.A., 1988. Edible Meat Products: Their Production and Importance to the Meat Industry. In: Edible Meat By-Products, Advances in Meat Research. V.5. Pearson, A.M. and T.R. Dutson (Eds.). Elsevier Applied Sci. London and New York, pp: 147-165.

Aoki, T., Y. Hiidome, K. Kitahara, Y. Sugimoto, H.R. Ibrahim and Y. Kato, 1999. Improvement of heat stability and emulsifying activity of ovalbumin by conjugation with glucuronic acid through the Maillard reaction. Food Res. Intl., 32: 129-133.

Autio, K., M. Kiesvaara, Y. Malkki and S. Kanko, 1984. Chemical and functional properties of blood globin prepared by a new method. J. Food Sci., 49: 859-862.

Benjakul, S., W. Lertitttikul and F. Bauer, 2005. Antioxidant activity of Maillard reaction products from a porcine plasma protein-sugar model system. Food Chem., 93: 189-196.

deMan, J.M., 1979. Principles of Food Chemistry. 2nd Printing, The AVI Publishing Company, Connecticut.

Fogliano, V., S.M. Monti, T. Musella, G. Randazzo and A. Ritieni, 1999. Formation of coloured Maillard reaction products in gluten-glucose model system. Food Chem., 66: 293-299.

Gerrard, J.A. and P.K. Brown, 2002. Protein cross-linking in food: mechanisms, consequences, applications. Intl. Congress Series, 1245: 211-215.

Goldstrand, R.E., 1988. Edible Meat Products: Their Production and Importance to the Meat Industry. In Edible Meat By-products, Advances in Meat Research. V.5. Pearson A.M. and T.R. Dutson (Eds.). Elsevier Applied Sci. London and New York, pp: 1-13.

Gomez-Juarez, C., R. Castellanos, T. Ponce-Noyola, V. Calderon-Salinas and J.D. Figueroa, 1999. Functional properties of globin protein obtained from bovine blood by decolorisation of the red cell fraction. J. Sci. Food Agric., 79: 793-796.

Hayakawa, S., T. Ogawa and Y. Sato, 1982. Some functional properties under heating of the globin prepared by carboxymethyl cellulose procedure. J. Food Sci., 47: 1415-1418.

Hayakawa, S., Y. Matsuura, R. Nakamura and Y. Sato, 1986. Effect of heat treatment on preparation of colorless globin from bovine hemoglobin using soluble carboxymethyl cellulose. J. Food Sci., 51: 786-790.

Knipe, C.L., 1988. Production and Use of Animal Blood and Blood Proteins for Human Food. In: Edible Meat By-products, Advances in Meat Research. v. 5. Pearson A.M. and T.R. Dutson (Eds.), Elsevier Applied Science, London and New York, pp: 147-165.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature, 227: 680-685.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Manzocco, L., S. Calligaris., D. Mastrocola, M.C. Nicoli and C.R. Lerici, 2001. Review of non-enzymatic browning and antioxidant capacity in processed foods. Trends Food Sci. Technol., 11: 340-346.

Marquez, E., M. Bracho, A. Archile, L. Rangel and B. Benitez, 2005. Proteins, isoleucine, lysine and methionine content of bovine, porcine and poultry blood and their fractions. Food Chem., 93: 503-505.

Miller, A.G. and J.A. Gerrard, 2005. The Maillard reaction and food protein crosslinking. Progress in Food Biopolymer Res., 1: 69-86.

Miyazaki, M., T. Maeda and N. Morita, 2004. Effect of various dextrin substitutions for wheat flour on dough properties and bread qualities. Food Res. Intl., 37: 59-65.

Nakamura, R., S. Hayakawa, K. Yasuda and Y. Sato, 1984. Emulsifying properties of bovine blood globin: A composition with some proteins and their improvement. J. Food Sci., 49: 102-104.

Rasper, V.F., 1979. Texture of Dough, Pasta and Baked Products. In: Rheology and Texture in Food Quality. deMan, J.M., P.W. Voisey, V.F. Rasper and D.W. Stanley (Eds.), the AVI Publishing Company, Westport, Connecticut, pp: 308-354.

Ramos-Clamont, G., S. Fernandez-Michel, L. Carrillo-Vargas, E. Martinez-Calderon and L. Vazquez-Mopeno, 2003. Funtional properties of protein fractions isolated from porcine blood. J. Food Sci., 68: 1197-1200.

Rosell, C.M., J.A. Rojas and C. Benedio de Barber, 2001. Influence of hydrocolloid on dough rheology and bread quality. Food Hydrocolloids, 15: 75-81.

Sato, Y., S. Hayakawa and M. Hayakawa, 1981. Preparation of blood globin through carboxymethyl cellulose chromatography. J. Food Technol., 16: 81-91.

Singh, N., I.K. Bajaj, R.P. Singh and H.S. Gujral, 2002. Effect of different additives on mixograph and bread making properties of Indian wheat flour. J. Food Eng., 56: 89-95.

Sun, Y., S. Hayakawa and K. Izumori, 2004. Antioxidative activity and gelling rheological properties of dried egg white glycated with a rare keto-hexose through the Maillard reaction. J. Food Sci., 69: 427-433.

Sun, Y., S. Hayakawa, O. Masahiro and K. Izumori, 2005. Evaluation of the site specific protein glycation and antioxidant capacity of rare sugar-protein/peptide conjugates. J. Agric. Food Chem., 53: 10205-10212.

Sun, Y., S. Hayakawa, S. Puangmanee and K. Izumori, 2006. Chemical properties and antioxidative activity of glycated α-lactalbumin with a rare sugar, D-allose, by Maillard reaction. Food Chem., 95: 509-517.

Wismer-Pederson, J., 1979. Utilization of animal blood in meat products. Food Technol., 33: 76-80.

Yoboah, F.K., 1999. Reactivities of D-glucose and D-fructose during glycation of bovine serum albumin. J. Agric. Food Chem., 47: 3164-3172.