CHICKEN BLOOD PLASMA PROTEINS: PHYSICOCHEMICAL, NUTRITIONAL AND FUNCTIONAL PROPERTIES

M. T. E. DEL RIO DE REYS, S. M. CONSTANTINIDES, V. C. SGARBIERI and A. A. EL-DASH

ABSTRACT

Chicken blood plasma protein was prepared by collecting blood during the slaughter of animals using 0.5% sodium citrate solution as an anticoagulant. The blood cells were separated by centrifugation, and the plasma recovered by freeze drying either before or after dialysis. Disc polyacrylamide gel electrophoresis gave a pattern with nine protein bands, which were reduced to seven bands when the sample and gels were treated with urea. Sodium dodecyl sulfate (SDS) gel electrophoresis furnished nine protein bands with molecular weights ranging from 24,000–115,000. Gel electrofocusing revealed three protein bands with isoelectric points of 5.7, 5.3 and 4.8, respectively. Digestibility of the proteins was above 90%, and the protein efficiency ratio (PER) was 2.8 in comparison with 2.5 for casein. Addition of plasma to wheat flour for bread making at 2.5 and 5% levels raised the PER of bread from 0.87 to 1.67 and 2.02, respectively.

INTRODUCTION

ALTHOUGH ANIMAL BLOOD, a by-product of the slaughter houses, contains proteins of high biological value, it is generally wasted in most countries throughout the world thus creating also a serious pollution problem. Utilization of this material at present is limited in scope and restricted to animal feeding purposes. The Brazilian poultry industry, for example, has a production capacity of 300 million chickens per year, with a potential yield of 18 million liters of blood or 12.6 million liters of plasma. This plasma contains about 4.5% protein, which amounts to a total of at least 567 tons of protein of high biological value which is wasted annually. When other slaughtering industries are considered, this represents a substantial quantity of protein. In the past 15 years, several authors have studied the utilization of bovine and porcine blood for human consumption (Tybor et al., 1975; Young et al., 1973; Delaney, 1973; Delaney et al., 1975). No study of chicken blood properties and its utilization in food was reported, however. In the present paper some of the physicochemical and nutritional properties of chicken plasma proteins and their utilization in bread making were investigated.

EXPERIMENTAL

Sample preparation

Blood was collected in the bleeding line of a slaughter house. An aqueous sodium citrate solution (5%) was added as an anticoagulating agent at the level of 100 ml/liter of blood. The blood was centrifuged at 4000 × G at 10°C for 15 min. The supernatant (plasma) was either submitted to dialysis against deionized water (48 hr, 5°) with 6–8 changes of water and freeze dried or freeze dried without dialysis.

Authors Del Rio de Reys, Constantinides, Sgarbieri and El-Dash are with Departamento de Planejamento Alimentar e Nutrição – UNICAMP, Caixa Postal, 1170, 13.100 Campinas, SP, Brazil.

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Chemical analyses

Moisture, total nitrogen (Kjeldahl), lipid, and ash contents were determined according to the AOAC (1975) methods of analysis. Nonprotein nitrogen was determined according to Cristol and Monnier (1936). Amino acid determinations were performed on acid hydrolysates (6N HCl, 105°C, 22 hr) using a Beckman 120°C amino acid analyzer, following the procedure of Spackman et al. (1958). Tryptophan was determined by the colorimetric method of Spies (1967).

Protein solubility

Protein solubility as a function of pH was determined by suspending freeze-dried plasma in distilled water (1:40 w/v), and the pH was adjusted to 2.3, 4.0, 4.5, 5.0, 7.0, 8.0, 9.0, 10.0 and 11.0 with 2N solutions of HCl or NaOH. The suspensions were then agitated for 2 hr at 25°C and centrifuged (16,000 × G) for 15 min. The soluble nitrogen was determined in the supernatants and the protein content calculated using the factor 6.25.

Protein solubility as a function of NaCl was determined by suspending freeze-dried plasma (1:40 w/v) in solutions of 0.1, 0.25, 0.5, 0.75 and 1.0M NaCl. The pH was adjusted to 4.5 for the undialyzed and to 5.0 for the dialyzed plasma. The suspension was agitated for 2 hr at 25°C and centrifuged; protein content was determined in the supernatants as described above.

Protein isolation

Three procedures were tried for the precipitation of protein

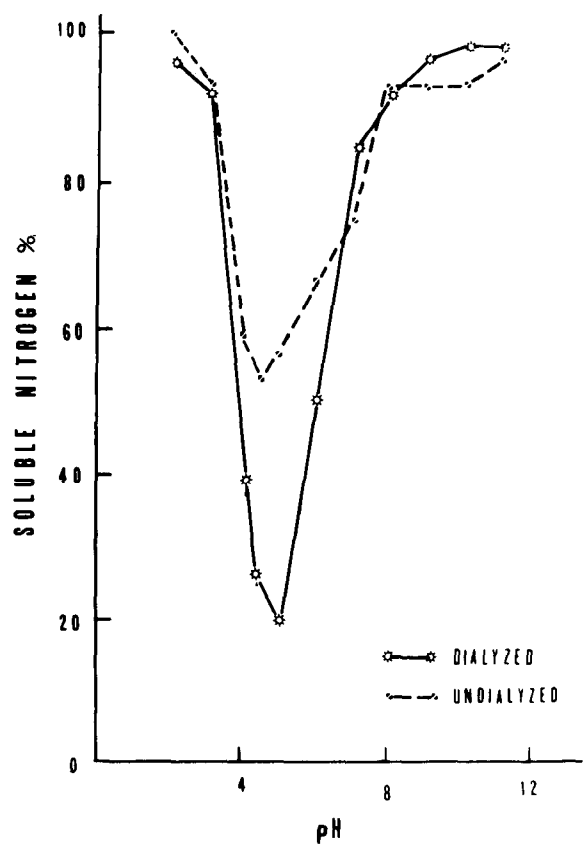

Fig. 1–Solubility curve for dialyzed and undialyzed chicken blood plasma protein as a function of pH.

from the plasma: (1) adjusting pH of the plasma directly to 4.5 with 2N HCl; (2) raising the pH to 9.0 with NaOH solution and then adjusting to pH to 4.5 with 2N HCl; and (3) making the plasma 0.2N with NaOH and then dropping the pH to 4.5 by adding HCl. After adjusting the pH to 4.5, the suspensions were agitated for 3 hr at 25°C and then centrifuged (10,000 × G) for 30 min; the precipitates were freeze dried.

Electrophoretic characterization of the proteins

Electrophoretic patterns of plasma proteins were determined on a simple polyacrylamide gel using vertical tubes of 0.5 × 9.5 cm according to the procedure of Davis (1964), on polyacrylamide gels containing urea according to the Wray and Stubblefield procedure (1970), and by SDS electrophoresis using the method of Weber and Osborn (1969). Electrofocusing on polyacrylamide gels was performed according to the method of Wrigley (1968) using carrier ampholytes in the pH range 3.5–10.

Nutritional evaluation of plasma proteins

The nutritional quality of the plasma proteins was evaluated by three different procedures: (1) amino acid profile as determined by chemical analysis (Spackman et al., 1958); (2) determination of digestibility in vitro (Akeson and Stahmann, 1964); and (3) protein efficiency ratio (PER) as described in AOAC (1975) using groups of six weanling rats (35–40g each) of the Wistar strain.

Functional properties in bread making

Either the freeze-dried dialyzed or undialyzed plasma was added to a commercially roller-milled sample of a medium strength flour (ash 0.53%, protein 10.5%, water absorption 56%) at the level of 2.5, 5 and 7.5%. A baking test was performed according to El-Dash (1978) using the following specifications: mixer speed 63 rpm; mixing temperature 30°C; dough consistency of 500 FU at maximum; mixing time until the dough showed a drop of 10 Farinograph Units (FU) after reaching maximum consistency; and a single step fermentation procedure of 105 min at 30°C. The bread was baked at 210°C for 20 min. Specific volume and internal and external quality of the bread were evaluated.

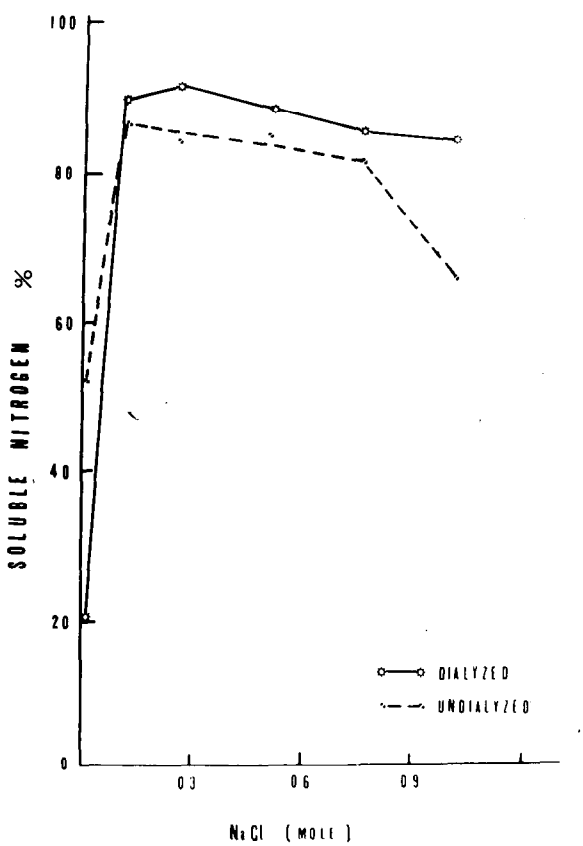

Fig. 2—Influence of sodium chloride concentration on the solubility of the dialyzed and undialyzed chicken blood plasma protein.

RESULTS & DISCUSSION

Chemical analysis of freeze-dried plasma

The composition of the dialyzed and undialyzed freeze-dried plasma is shown in Table 1. The dried plasma is highly rich in protein, varying from a content of 59.4 to 79.6%, depending on the procedure of preparation. The dialyzed plasma is characterized by a higher concentration of protein and a lower content of ash, carbohydrates, and nonprotein nitrogen than the undialyzed plasma.

Influence of pH and NaCl concentration on protein solubility

The plasma protein was found to be 100% soluble in both acid and alkaline solutions. Although the difference in pH at minimum solubility of dialyzed and undialyzed plasma was minimal (4.5 and 5.0, respectively), the differences in solubility were pronounced, as shown in Figure 1. Undialyzed samples showed a low degree of solubility, which may be attributed to the effect of the high concentration of salt present in the undialyzed samples. Sodium chloride greatly increased the solubility of the proteins at the pH of lowest solubility for both the dialyzed and undialyzed plasma. The solubilization effect was more pronounced, however, for the dialyzed plasma, as shown in Figure 2, with the greatest influence in the range 0.1–0.5M of NaCl.

Electrophoretic patterns

Electrophoresis in simple polyacrylamide gels revealed nine protein bands, whereas only seven bands were detected in the gels containing 10M urea. Isoelectric focusing on gels containing a mixture of carrier ampholytes of pH 3.5–10 separated the proteins into three bands of isoelectric pH: 5.7, 5.3 and 4.8 (Fig. 3).

On the other hand, when the plasma was previously treated with urea and mercaptoethanol and run in gels containing SDS, nine polypeptide bands were revealed with molecular weights ranging from 24,000–115,000, as shown in Table 2. A standard semilog plot of molecular weight versus electrophoretic mobility in the SDS gels appears in Figure 4. The fact that an equal number of bands was ob-

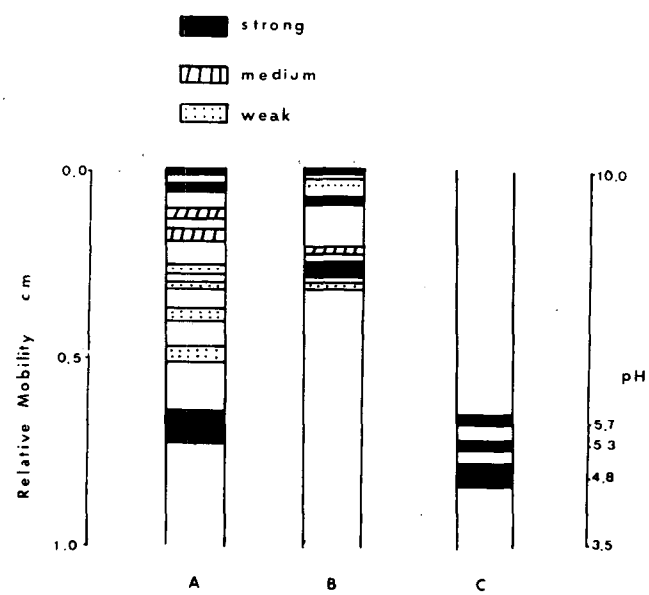

Fig. 3—Electrophoretic pattern of chicken blood plasma protein: A—simple polyacrylamide gel; B—polyacrylamide gel containing 10M urea; and C—electrofocusing (ampholine gradient pH 3.5–10).

served in simple polyacrylamide gels and in the SDS gel electrophoresis appears to indicate a low degree of polymerization of plasma proteins.

Nutritional evaluation of plasma

The in vitro digestibility of the proteins was 92% for the dialyzed plasma and 83% for the undialyzed plasma, as compared with 96% for casein. The protein efficiency ratio (PER) for the dialyzed and undialyzed plasma and for casein appears in Table 3. It is apparent that, if judged only by this parameter, the plasma protein is superior to casein. The growth-promoting capacity of the diet containing casein, however, was greater than that of the diets containing either dialyzed or undialyzed plasma at the same protein concentration (Fig. 5). The superior growth rate of rats on a casein diet could be explained in terms of a greater food intake and protein consumption.

Protein recovery

Upon isolation of plasma protein by directly lowering its pH to 4.5 with 2N HCl, the protein recovery in the precipitate was fairly low (17.4%). When the pH was first raised to 9 and then lowered to 4.5, the protein recovery increased to 19.6%. A substantial increase to 87.2% was obtained when the plasma was first brought to 0.2N with NaOH before dropping the pH to 4.5 with HCl.

Amino acid composition

The amino acid composition of the dialyzed freeze-dried plasma and that of the isolated proteins (NaOH-treated prior to dropping pH to 4.5) is presented in Table 4. The differences in amino acid composition were quite limited, with the exception of cystine, which was reduced drastically in the isolated protein. This is probably due to the alkaline treatment required before isolation of the protein. The blood plasma proteins were found to be well balanced in amino acid composition, with a high concentration of the amino acid lysine. The only limiting amino acid was found to be isoleucine, which provides only 92% of the F.A.O. reference standard (Bender, 1967).

Bread fortification with plasma

From the above-mentioned results, it was evident that the addition of high-lysine protein of plasma to the low-lysine protein of wheat flour should produce a complementary nutritional effect. It appeared essential, however, to test the effect of plasma proteins on the technological quality of bread. The effect of adding undialyzed and dialyzed

Table 1—Proximate composition of freeze-dried plasma

Components[a] (%)	Plasma	
	Dialyzed[b]	Undialyzed[c]
Crude protein	79.60	59.40
Nonprotein nitrogen	0.75	1.16
Lipid	0.20	0.29
Ash	4.60	20.10
Carbohydrate[d]	14.99	19.05

[a] On dry basis
[b] Moisture content 6.7%
[c] Moisture content 5.5%
[d] Calculated by difference

Table 2—Relative mobilities and molecular weights of blood plasma proteins as determined by SDS gel electrophoresis

Protein bands	Relative mobility	Molecular weights
1	0.023	115,000
2	0.046	105,000
3	0.069	90,000
4	0.12	83,000
5	0.16	73,000
6	0.21	64,000
7	0.23	60,000
8	0.25	56,000
9	0.53	24,000

Table 3—Biological evaluation (PER) of casein and of dialyzed and undialyzed blood plasma protein

Protein source in the diet	Body weight gain (g)	Protein consumption (g)	PER[a] (Found)	PER[b]
Casein	106.7	39.6	2.7 ± 0.44	2.5
Dialyzed plasma	89.0	29.5	3.0 ± 0.39	2.8
Undialyzed plasma	80.4	28.7	2.8 ± 0.39	2.6

[a] Groups of six rats were used in each assay; values in this column are PER ± 1 S.D.
[b] Casein = 2.5.

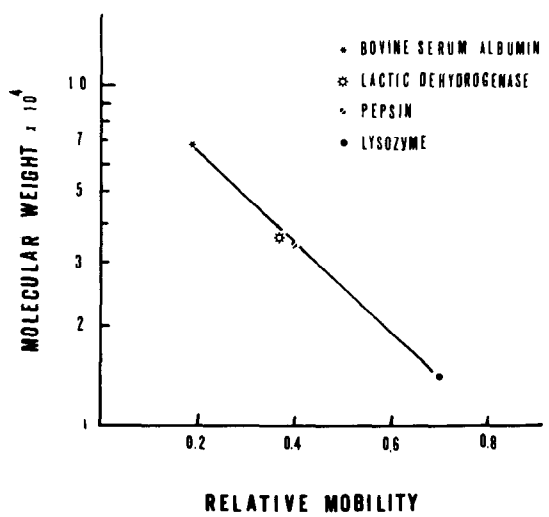

Fig. 4—Semilog standard plot of MW vs relative mobility in the SDS-gel electrophoresis.

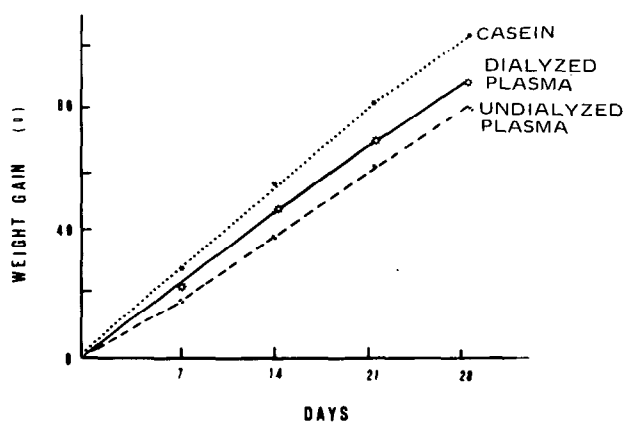

Fig. 5—Growth rate curves for rats on 10% protein diets furnished by the dialyzed and undialyzed chicken blood plasma, compared with a 10% casein diet.

Table 4—Amino acid composition of the proteins in the dialyzed plasma and of the isolated proteins (g/16g N)

Amino acid	Dialyzed plasma	Isolated proteins[a]
Lys	8.2	8.4
His	2.4	2.2
NH_3	2.1	1.6
Arg	6.0	5.6
Asp	14.7	13.1
Thr	7.3	6.0
Ser	9.7	8.0
Glu	25.6	24.9
Pro	6.3	5.6
Gly	5.0	5.2
Ala	6.8	6.8
1/2 Cys	3.9	0.9
Val	7.8	7.1
Met	2.3	2.3
Ile	3.9	3.9
Leu	11.3	9.8
Tyr	4.8	4.3
Phe	5.4	5.8
Trp	1.6	1.4

[a] Isolated after bringing the plasma to 0.2N NaOH before dropping pH to 4.5 with HCl.

Table 5—Effect of undialyzed and dialyzed chicken blood plasma on bread specific volume and total quality score

	Undialyzed		Dialyzed	
Plasma % of flour	Specific volume (cm^3/g)	Total score	Specific volume (cm^3/g)	Total score
0.0	4.89	76.2	4.89	76.2
2.5	4.85	74.1	5.12	73.8
5.0	4.66	62.0	5.35	69.8
7.5	4.06	50.0	5.60	65.1

plasma is presented in Table 5. The use of over 2.5% undialyzed plasma caused a rapid deterioration in the bread specific volume and internal characteristics of the loaf; this was attributed in part to the high ash content of the undialyzed plasma. On the other hand, the addition of dialyzed plasma resulted in a marked improvement in the specific volume, as it increased from an original of 4.89 cm^3/g to a value as high as 5.6 cm^3/g at the 7.5% level. Although an improvement in the bread external characteristics was also noted at the latter level, the internal characteristics evidenced deterioration with 5% plasma. Both dialyzed and undialyzed plasmas affected the crumb color, bread aroma, and taste when used over the 2.5% level. Addition of 5% plasma slightly darkened the crumb, produced a yellowing in the interior of the loaf, in addition to aroma and taste similar to egg bread.

The bread protein, on a dry basis, increased from 10.8% in the control to 14.8% in the bread with 5% of the flour replaced by dialyzed plasma. The PER of the control bread was 0.86 and was raised to 1.64 and 2.02 in the bread fortified with 2.5 and 5.0% dialyzed plasma, respectively. These results indicate a strong nutritional complementary effect between wheat protein and chicken blood plasma protein.

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