Functional Aspects of Blood Plasma Protein Fractions

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Introduction

As one aspect of attempts to improve the utilisation of the proteins which are currently wasted at abattoirs, there is renewed interest in blood proteins. Thus they have been spun into meat-like fibres (Young & Lawrie, 1974). But they have also functions as extenders in sausage products (Lysner, 1972); and blood plasma has been sued as a substitute for egg albumen in cake mixtures (Brooks & Ratcliff, 1959 : Johnson, Havel & Hoseney, 1979), because its proteins have similar functional properties. One disadvantage of the latter, however, is the tendency for fish-like odours to develope in spray-dried blood plasma, possibly due to the oxidation of polyunsaturated fatty acids and phospholipids (Brooks & Ratcliff, 1959).

It seemed feasible that individual proteins or groups of proteins, when separated from blood plasma, might have specific functional properties in cake-type systems. The present paper reports some results on groups of proteins which were separated from blood plasma in sufficient quantity to examine this possibility.

Methods

Because fibrinogen is a major component of blood plasma, blood clotting was prevented by the addition of 0.4% sodium citrate at time of collection.

Plasma proteins were separated on columns packed with the ion exchange resin diethylaminoethyl (DEAE) Sephadex A-50. This was found to be superior to vistec DEAE cellulose because it retained high binding capacity even at high ionic strength. It was thus useful for citrated plasma. Elution by stepwise change of ionic strength yielded three fractions of plasma proteins in reasonable amount. Fraction I contained fibrinogen and α_2, β and γ globulins. Fraction II contained α_1 globulins and Fraction III contained serum albumin. 0.002% chlorhexidine was used as antimicrobial agent.

Viscosity was measured by Ferranti-Shirley Cone & Plate viscometer. Gel strength and breaking strength were measured by an Instron Universal Texturometer after heating to various temperatures for varying times.

An interaction index for these parameters was determined for reactions between plasma proteins and egg albumen in cake-type systems. (6% protein in 45% sucrose). The interaction index was defined as :-

$$\frac{\text{actual value - expected value}}{\text{expected value}} \times 100$$

Results and Discussion

Only a selection of the data obtained can be considered here. Solutions of whole porcine and bovine plasma, porcine serum and porcine plasma Fractions I, II & III all exhibited Newtonian behaviour at temperatures of heating between 20°-73°C. At 76°C reversible gels formed. Viscosity increased between 73° & 76°C; but this was particularly marked and rapid between 76° & 79°C.

Comparative data on viscosity (following heating up to 70°C) are shown in Table 1, from which it is evident that values for Fractions I and III are lower than that for whole plasma; whereas values for Fraction II are close to those of the latter.(The viscosity of 6% serum was also low). On the other hand, the viscosity of whole plasma fell to that of serum when heated to 80°C. This implies that fibrinogen is involved since it denatures at 80°C.

The gel strength (GS) and breaking strength (BS) of gels involving whole porcine and bovine blood plasma both increased with time and temperature of heating. The values for GS of whole porcine plasma were higher than those of whole bovine plasma when heating was at 95°C, but lower with heating at 80°C. (since serum albumin is the major constituent of blood plasma, it is of interest that both N- and C-terminal amino acid sequences of both porcine and bovine albumin are reported to be different). For blood plasma from both species there was an increase in BS with time of heating at 80°C and 85°C; but little change with time of heating at 90° or 95°C.

That fibrinogen made some contribution to the gelling as well as to the viscous properties of plasma was suggested by the higher values for plasma in comparison with serum.

There were differences in gelling behaviour of the plasma fractions. Values for GS were highest for Fraction I and lowest for Fraction II (Tables 2 & 3), with heating at 85°C. For BS these differences were even more marked.

On the other hand, with heating at 90° & 95°C, values for gel strength were much higher for Fraction III than those for the other two Fractions; and were similar to those of whole plasma, suggesting that serum albumin is of particular importance for its gelling properties. In this respect the importance of disulphide bonds was shown by the diminution in gel strength caused by such agents as cysteine hydrochloride.

Various protein-protein interactions evidently occured when the plasma proteins were heated with egg albumen in model cake-type mixtures. Those with blood plasma were 3 or 4 fold greater than with blood serum when heating took place at 85° or 90°C; but the interactions were similar at 95°C. For whole porcine plasma, interactions in respect of gel strength and in breaking strength were greatest with heating at 80°C over 30 min. and with low protein concentration (2%). On the other hand, interaction between whole bovine plasma and egg albumen was low at 80°C and increased with heating at 90° & 95°C. Interactions at 85°C over 15 mins. were all greater than those occuring over 30 or 60 minutes for all concentrations of bovine and porcine plasma studied.

As measured by gel strength, the interaction indices for the three Fractions of porcine plasma were highest when heated with egg albumen over 15 minutes; but those for Fraction I were highest when the applied temperature was 95°C, whereas those for Fractions II & III were highest at 85°C. Maximum interactions, as measured by breaking strength, were obtained with heating at 85°C over 15 minutes with all three fractions. Particularly high values for interaction indices were found with Fraction III (Table 3), these being similar to values for whole plasma.

Conclusions

It is evident fibrinogen makes some contribution to the various viscous and gelling properties of whole plasma; its retention during blood collection by the use of anticoagulents is thus indicated. Fraction III (~ serum albumin), however, is the constituent which is mainly responsible for the responses of both serum and plasma to time and temperature of heating. Its gelling properties would appear to be most useful in products heated to relatively high temperature.

Because of its capacity to gel at relatively low concentration (2%) Fraction II could be utilized when only a small quantity of protein was required.

In general, the synergistic interactions between the plasma proteins and egg albumen in cake-type model systems, as shown by the various parameters examined, can clearly be exploited by selecting Fractions with desired functionality. Similarly, it appears that porcine plasma proteins are likely to be more useful for interactions with egg albumen at 80°-90°C; whereas for higher temperatures (> 95°C), the use of bovine plasma would be indicated.

There is much scope for the further utilization of specific proteins, isolated by ion exchange chromatography or other means, from waste abattoir sources.

References

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Johnson, L.A., Havel, E.F. & Hoseney, R.C., (1979), Cereal Chem., 56 (4) 341.
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Table 1. Viscosity of Porcine Blood Plasma and its Fractions using a Ferranti-Shirley Cone and Plate Viscometer

Sample (6% protein in 45% sucrose soln.)	cp at 20°C, shear rate 1600 sec^{-1}
45% sucrose solution	8.8
egg albumen	30.52
whole plasma	38.55
Fraction I	32.9
Fraction II	36.99
Fraction III	32.93

Table 2. Gel Strength & Interaction Indices of Blood Plasma Protein Fractions (P) Egg Albumen (E) and Mixtures of these after Heating for 15 min. @ 85°C.

(All solutions contained 6% protein and 45% sucrose)

Plasma Fraction	Components % Protein	P 6	P + E 4+2	P + E 3+3	P + E 2+4	E 6
I	Gel strength (g)	323	379	377	305	120
	Interaction Index (%)	-	48	70	63	-
II	Gel strength (g)	0	260	237	245	110
	Interaction Index (%)	-	602	331	275	-
III	Gel strength (g)	22	251	253	253	116
	Interaction Index (%)	-	373	266	201	-

Table 3. Breaking Strengths & Interaction Indices of Blood Plasma Protein Fractions (P) Egg Albumen (E) and Mixtures of these, after Heating for 15 min @ 85°C.

(All solutions contained 6% protein and 45% sucrose)

Plasma Fraction	Components % Protein	P 6	P + E 4+2	P + E 3+3	P + E 2+4	E 6
I	Breaking strength (g)	349	1231	1144	627	120
	Interaction Index (%)	-	352	388	219	-
II	Breaking strength (g)	0	290	302	427	110
	Interaction Index (%)	-	683	449	485	-
III	Breaking strength (g)	22	541	495	480	116
	Interaction Index (%)	-	864	617	471	-