INFLUENCE OF FREE FATTY ACIDS (FFA) ON THE FUNCTIONAL PROPERTIES OF BLOOD PLASMA

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INTRODUCTION

The main functional property of blood plasma proteins is their gel forming ability. We have previously shown that the addition of FFA to plasma results in reduced gelling ability (Fretheim and Gumpen, 1978; Fig. 1). The underlying mechanism is that the thermal stability of serum albumin is markedly increased when FFA are made available for complex formation with the protein (Gumpen et al., 1979; Fig. 2). On normal industrial heating to about 70-75°C the stabilized protein does not denature and, as a consequence, does not form a gel.

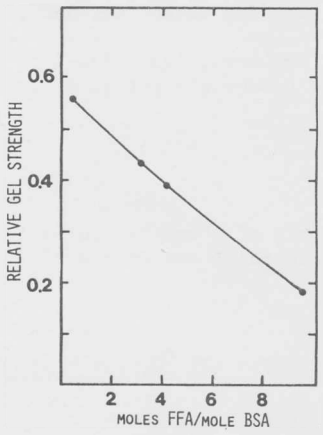

Fig. 1
Effect of FFA on the relative strength of gels obtained on heating blood plasma (Fretheim and Gumpen, 1978).

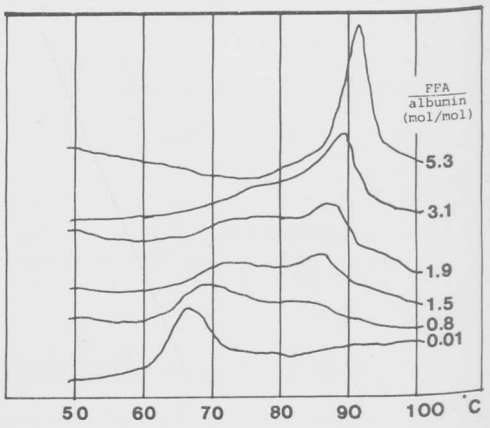

Fig. 2
DSC-thermograms of FFA-albumin complexes. Protein concentration was 5% (w/v) in 0.9% NaCl, pH 7.0 (Gumpen et al., 1979).

In the present communication we try to answer two questions regarding the implications of these phenomena for the texture of food products made from whole blood or containing blood plasma: (1) Are plasma gels rigid enough to be of textural significance in products like blood pudding and cooked meat sausages? (2) Are sufficient amounts of FFA available in meat emulsions for stabilizing the albumin, thereby reducing its contribution to texture formation?
Our results indicate that both questions should be answered with a "Yes".

MATERIALS AND METHODS

Frozen blood plasma was bought from Ellco Protein AB, Kävlinge, Sweden. Fresh blood was obtained at a slaughterhouse by draining from stunned animals into flasks containing enough 35% trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, analyt.reag.) to result in a final concentration of 0.7% anticoagulant. Meat (bovine) used in sausage production was obtained fresh at a slaughter- house. Finest food grade soybean oil was bought at a supermarket. Washed blood cells were prepared from fresh blood by centrifugation at 1200 g for 20 min and washing three times with 0.9% NaCl, each time followed by centrifugation; no visible hemolysis occured. "Meat particles" were prepared by milling freeze dried cooked lean meat in a micro beating mill (Culatti). The bovine serum albumin (BSA) employed was a crystallized and lyophilized preparation essentially free from fatty acids (Sigma Chemical Co.) Lauric acid (99.5%) was obtained from Fluka AG, Switzerland. Other reagents were of analytical grade.

Gels were produced from 10 ml samples in screw capped glass vials of 25 mm i.d. All samples were heated by submerging the tightly capped vessels in a programmed water bath. After 3 min at 60°C, the temperature was increased by 1°/min to 74°C and kept there for 1 min. The samples were then cooled in an ice/water mixture and allowed to age at room temperature overnight before measurement of gel rigidity at room temperature on the following day. The rigidity measurements were performed directly on the samples in the glass vials, using an Instron Universal Testing Machine fitted with a cylindric plunger of 5 mm diameter. The plunger speed was 2.5 mm/min, and the initial slope of the force-distance curves was taken as a measure of gel rigidity.

The effect of "particles" on gel rigidity was studied by adding washed blood cells or meat particles to plasma or BSA solutions prior to heat-treatment. With the meat particles rapid sedimentation offered experimental problems. These were satisfactorily overcome by subjec-

ting the sample mixtures in the vials to ultrasonic treatment in a water bath for 5 minutes prior to gelling, in addition to applying gentle stirring with small magnets during the heat treatment, i.e. until increasing viscosity prevented the magnetic bars from rotating.

The thermal stability of albumin was checked by differential scanning calorimetry (DSC) using 15 µl aluminium sample pans in a Perkin Elmer DSC-2. The heating rate was 10°C/min.

The extractability of FFA from the fatty raw material to become bound to albumin in the water phase of a meat emulsion was investigated in a model system prepared by means of a laboratory homogenizer (Ultra Turrax TP 18/10): Meat (58.2%), blood plasma (20%), water (6.4%), NaCl (1.7%) and soybean oil (13.7%) to which different amounts (0-3%) of FFA (lauric acid) were added. The obtained emulsion was partly broken by heating at 50°C for 40 min, then centrifuged at 30 000 g for 45 min at 20°C. The supernatant was microfiltered and recentrifuged at 100 000 g for 90 min at 10°C before being subjected to DSC for determination of albumin stability.

Emulsion type sausages were produced in a 10 l Müller bowl chopper, following a standardized procedure. Composition: 10% meat proteins from lean meat, blood plasma corresponding to 2% plasma proteins, 1.7% NaCl, water to a moisture content of 64%, and enough soybean oil, containing either 0 or 3% lauric acid, to give a fat content of 21%. The emulsions were stuffed in 37/40 mm cattle casings and heat treated in a water bath of 75°C to an internal temperature of 74-75°C (30-35 min).
The firmness of the cooked sausages was measured by penetrometry at room temperature on the following day in accordance with Andersson and Hansson (1979).

RESULTS AND DISCUSSION

Blood plasma proteins are potential contributors to the texture of food products made from whole blood (e.g. blood pudding) or containing blood plasma (e.g. meat sausages). But gels of pure plasma are very soft compared to the firmness of blood- or meat sausages, and one might well think that the contribution of plasma proteins to the overall texture of a sausage is negligible. Fig. 3 shows, however, that an increase in the comparatively low gel rigidity of the continuous phase results in significantly increased rigidity of the corresponding heated suspension of blood cells. If higher ratios of blood cells are employed, and/or if flour is added as in blood puddings, the rigidity is further increased in a similar proportional manner (results not shown). Thus, it appears that the gelling ability of plasma is essential for the texture of whole blood food products.

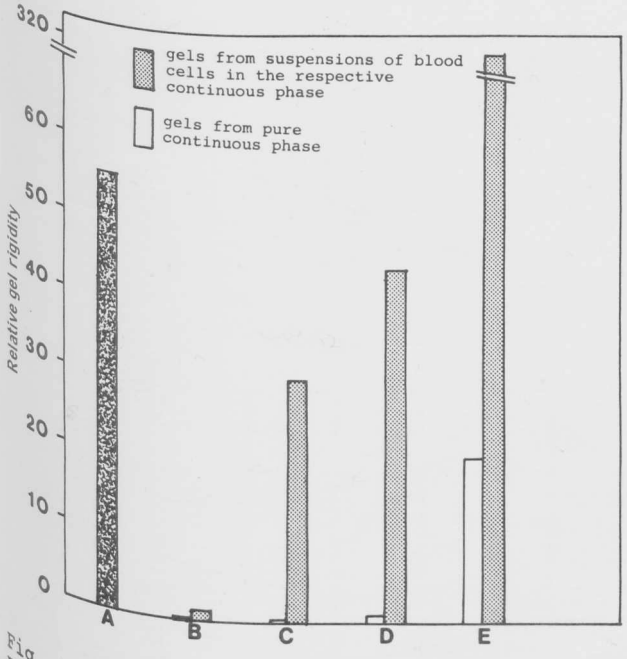

Fig. 3
Effect of the continuous phase on the gel rigidity of heat-treated blood cell suspensions at pH 6.0.
A: whole blood reference. Continuous phases of B-E; B: 0.9% NaCl; C: 7% BSA-solution with 7 mol lauric acid/mol BSA; D: blood plasma; E: 7% BSA-solution with no fatty acid added.
The ratio of washed blood cells to continuous phase was constant (1:1).

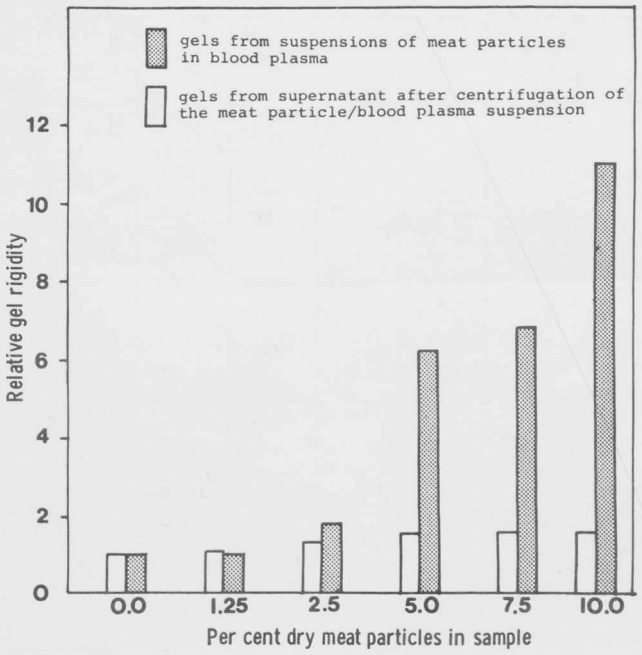

Fig. 4
Effect of meat particles suspended in commercial blood plasma (pH 8.8) on the gels obtained by heating the plasma.

Meat particles probably have a similar influence on the texture of cooked meat sausage as blood cells on the texture of blood sausages. That is, a meat emulsion can be looked upon as a suspension of particles (meat and fat tissue) in a continuous phase of water containing gel-forming proteins. As with blood products, the rigidity of the pure continuous phase may be rather low compared to the firmness of the whole product, e.g. a sausage. Fig. 4 shows how the presence of freeze dried cooked meat particles enhances the rigidity of the plasma gels, although the particles per se have no gelling ability.

In a meat emulsion with blood plasma added, both salt soluble proteins from meat and plasma proteins will contribute to the gelling of the continuous phase. However, since the gelling ability of serum albumin is influenced by the presence of FFA, the relative contribution from plasma proteins depends on the availability of FFA. Textbooks in physiology indicate that a range corresponding to about 0.5-2.0 mol FFA/mol BSA can be regarded as normal in fresh blood. We found in blood from cows subjected to experimental feeding levels ranging from 0.5 to 3.3 mol/mol (unpublished results). According to Fig. 2 one could expect different gelling abilities for the plasmas even within this range of variation. However, commercial blood or blood plasma is always pooled from several animals, thus levelling out extreme values to an average which probably varies within a much more narrow range. On the other hand, a FFA-content of 0.1% in the fat of an emulsion containing 20% fat and 2% plasma proteins, corresponds roughly to 5 mol FFA/mol BSA. For fat tissue fresh from the abbatoir 0.1% of FFA is a normal value. In stored fats the percentage may be much higher. This means that there are always, theoretically, sufficient amounts of FFA in the emulsion for stabilizing the albumin molecules. If the fatty acids are actually available for binding to the albumin, the implication is that the good gelling ability of plasma proteins is of no use unless the products are heated well above the usual 75°C.

Since the affinity of BSA for FFA is rather extreme, extraction of FFA from the lipid phase of the emulsion during or after homogenization must be regarded as a real possibility. To evaluate the extent of extraction, investigations were carried out on model emulsions produced by the laboratory homogenizer. When increasing the FFA-content of the oil employed, there was an increase in the amount of stabilized albumin, as depicted in Fig. 5. The Figure also indicates, however, that FFA-extraction from the oil for complexation with albumin is limited by other factors than the amount of fatty acids in the oil, since a 3% content of lauric acid corresponds to a molecular excess of FFA to BSA of about 200.

If, during and after homogenization of a sausage emulsion, extraction of FFA takes place to an extent that stabilizes the albumin present, will then the texture of the cooked sausages be significantly influenced? An answer to this question was sought in the production experiments, using soybean oil without (reference) and with 3% lauric acid added as the main fat

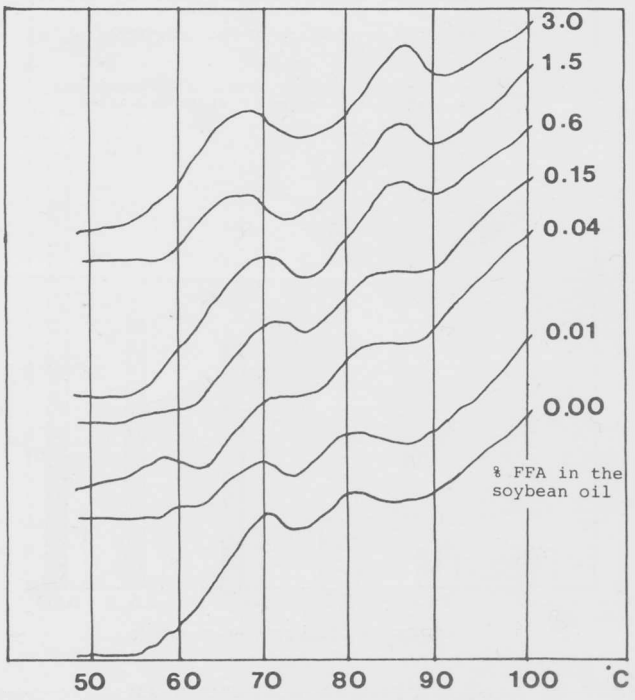

Fig. 5
DSC-thermograms of the aqueous phase of model meat emulsions consisting of lean meat, blood plasma, soybean oil and NaCl. Effect of the FFA concentration of the soybean oil.

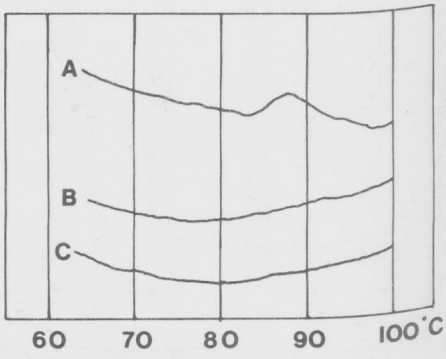

Fig. 6
DSC-thermograms of cooked sausages.
A: sausages made with soybean oil containing 3% FFA and with blood plasma corresponding to 2% plasma proteins in the sausage; B: sausages as in A, except soybean oil with no FFA added; C: sausages as in A, but without blood plasma.

ingredient. The results are shown in Table 1. The difference in firmness between sausages with and without FFA is significant at the 1% level according to Student's t-test. DSC-thermograms of the cooked sausages confirm that the difference is due to a lack of albumin gelation in the samples containing FFA: All proteins are denatured in the reference samples, while there is a peak with T_{max} = 88°C in the thermograms of FFA-containing sausages (Fig. 6). The size of the peak tells us that the main part of the albumin is still native after cooking of these sausages.

Table I

Relative firmness of cooked sausages containing blood plasma
Mean ± S.E. Number of separate productions in brackets.

Per cent lauric acid in fat (soybean oil)	
0	3
100±2(6)	89±2(5)

CONCLUSIONS

In spite of the relatively low strength of plasma gels, the gelling of plasma proteins may contribute significantly to the texture of whole blood food products (e.g. blood pudding) and comminuted meat products containing blood plasma. Since free fatty acids (FFA) are detrimental to the gel forming ability of albumin, the textural effect of the plasma proteins will depend on their access to FFA. The products in question normally contain sufficient amounts of FFA for stabilizing albumin, but the fatty acids are not necessarily available for binding to the protein under practical conditions. By using fat (soybean oil) with a high level of FFA as a raw material in meat sausages, the gelation of albumin was found to fail due to extraction of FFA from the fat phase, and the sausages were less firm than the ones produced with FFA-poor fat. Hence, FFA-rich raw materials and efficient extraction conditions (e.g. long time, intimate contact) in the production of meat emulsions appear disadvantageous if maximum gelation of plasma proteins is desirable.

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