## Faktoren, welche die Denaturierung und das Gelieren von Rinder-Plasmaproteinen beeinflussen

KRISTEN FRETHEIM
Norwegisches Institut für Nahrungsmittelforschung, P.b. 50, N-1432 Ås-NLH, Norwegen

Der Zusatz von Plasma-Proteinen in Brühwurstbräte ist hauptsächlich eine Anwendungsweise billiger Beiprodukte, anstelle teuren Fleisches. Die Plasma-Proteine können gleichzeitig die Qualität des Produktes verbessern, wegen der guten Emulgations- und Gelierungs-Eigenschaften. Das Rezept und die Herstellungsweise des Fleischproduktes beeinflusst die Wirkung der plasma-Proteine. Die vorliegende Arbeit bespricht Grundstudien darüber, wie folgende Variable der Brühwursttherstellung die Denaturierung und das Gelieren dieser Proteine beeinflussen: pH, Konzentration und Type des Salzes und der freien Fettsäuren, sowie Temperaturprofil.

## Factors influencing the denaturation and gelling of bovine plasma proteins

KRISTEN FRETHEIM
Norwegian Food Research Institute, N-1432 ÅS-NLH, Norway

The addition of plasma proteins to minced meat products is primarily a way of utilizing an inexpensive by-product as a substitute for costly meat. At the same time, plasma proteins may contribute favorably towards the quality of the final product due to good emulsification and gelation properties. However, the recipe and processing of the meat product will influence the effect of the plasma proteins. This work concerns itself with basic studies of how the following variables of the production influence the denaturation and gelling of these proteins: pH, concentration and type of salts and free fatty acids, as well as temperature profile.

# H 9:2

Les facteurs qui influencent la dénaturation et la gélification des protéines plasmatiques de boeuf.

KRISTEN FRETHEIM

Institut Norvégien de Recherche Alimentaire, N-1432 Aas-NLH, Norvège

L´addition de protéines plasmatiques à des produits de viande hachée est avant tout une façon d´utiliser un produit bon marché en remplacement d´une viande coûteuse. Les protéines plasmatiques peuvent en outre améliorer la qualité du produit final en vertu de leur propriétés d´émulsion et de gélification. Toutefois le type d´ingrédients utilisés et la façon de préparer le produit à base de viande vont influencer l´effet des protéines plasmatiques. Ce travail porte sur la manière dont les variables de la production nommées ci-dessous influencent la dénaturation et la gélification de ces protéines: le pH, la concentration et le type de sels et d´acides gras libres ainsi que le profil de température.

Факторы влияющие на денатурацию и желатинирование протеина плазмы крупного рогатого скота.

КРИСТЕН ФРЕТХЕЙМ

Норвежский научно-исследовательский институт пищевых продуктов,И-1432 ААС-ИЛН Норвегия.

Добавление протеина плазмы в изделия из промолотого мяса является основным способом использования недорогого побочного продукта заменяющего дорогостоящее мясо. К тому же протеин плазмы может значительно улучшить качество готового продукта,благодаря своей хорошей способности к эмульгированию и желатированию. Однако рецепт и процесс изготовления продукта питания из мяса будет влиять на эффективность протеина плазмы.
Настоящее исследование занимается проблемами того,как нижеперечисленные составные элементы производства влияют на денатурацию и желатирование протеинов: pH,концентрация и тип солей и свободных жирных кислот,а также и кривая температур.

# Factors influencing the denaturation and gelling of bovine plasma proteins

KRISTEN FRETHEIM* and SVEIN AAGE GUMPEN

Norwegian Food Research Institute, P.B.50, N-1432 Aas-NLH, Norway

## Introduction

From a nutritional and resource utilization point of view slaughter blood ought to be used as such as a food ingredient. However, the traditional whole blood dishes consumed in some countries have a limited market which is generally deemed difficult to expand. Centrifugal separation of the blood has therefore been introduced to produce red cell concentrate and plasma. The cell concentrate offers much the same problems of utilization as the whole blood, resulting, in Norway, primarily in its use as fur animal feed. The plasma fraction, containing sodium citrate as an anticoagulant, can be used as an ingredient in minced meat products. This is, however, a new raw material for the meat processing industry, and its use must be optimized.

We report in this paper the preliminary results of our work on how the denaturation and gelling of plasma proteins may be influenced.

## Materials and methods

Blood was obtained directly from the animals by draining it into flasks containing enough 40% trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, analytical reagent) to result in a final concentration of 0.7% anticoagulant. The samples were cooled on ice and, within a couple of hours, centrifuged to give deep yellow plasma. The obtained plasma was stored on ice and used in experiments within six days.

The pH of the plasma was adjusted from its initial value of about 7.8 by adding 0.15 M HCl while stirring efficiently but cautiously. Similarly, the sodium chloride and calcium chloride ($CaCl_2 \cdot 2H_2O$) concentrations were adjusted by the addition of appropriate amounts of 30% and 60% aqueous solutions, respectively.

The concentration of free fatty acids (FFA) in plasma was increased by treating it with FFA-coated Celite according to Spector and Hoak (1969); palmitic or stearic acid was employed. The colorimetric method of Anderson and McCarty (1972) was used to determine the various concentrations of FFA; standard curves were obtained either by analyzing appropriate solutions of the respective acids or by analyzing a serum reference sample used in clinical laboratories, "Seronorm Lipid" (Nyco, Norway).

Influences on the denaturation temperatures of the plasma proteins were studied by differential scanning calorimetry, using 15µl aluminum sample pans in a Perkin-Elmer DSC-2 equipped with an Intracooler II and a glove-box. The standard heating rate was $10^{\circ}$/min., and duplicate thermograms were always recorded. Temperature calibration ($\pm 1^{\circ}$ standard deviation) was performed using benzil and diphenyl ether.

The procedure followed for gelation studies has been designed by Hegg and Martens (1978). Plasma samples (1.00 ml) were placed in Sorvall centrifuge tubes (part no. 120). A programmable water bath (Heto, type 02 PG 623, Denmark) was then employed to heat the samples in accordance with the temperature program selected, generally $1^{\circ}$/min. from a center temperature of 60 to $73^{\circ}$, simulating sausage cooking (Figure 1). When the desired temperature had been reached the samples were immediately cooled in ice water. The heat treated samples were centrifuged at $4^{\circ}$ at 15000 rpm in a JA-20 rotor for 60 min., and the weight of the pellet, determined by weighing the supernatant, was taken as a relative measure of gel strength. Gelation experiments were generally duplicated.

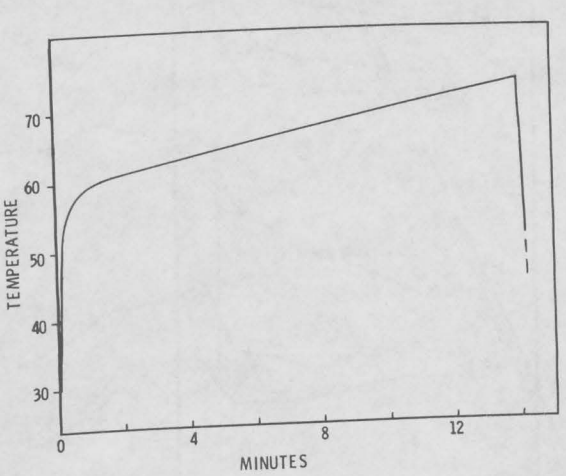


Figure 1: Temperature program of the heat treatment employed for determination of relative gel strength.

* By whom the paper will be presented

Results and discussion

To minimize the distance from laboratory experiments to practical applications, attempts were made to carry out the work on commercially available, frozen plasma. However, the liquid plasma obtained on thawing contained precipitated aggregates, and pH lowering caused further precipitation already at about pH 6.5 - as opposed to below pH 5.8 with the laboratory produced plasma. Commercial plasma, fresh or frozen, also exhibits a varying degree of red color which implies a possibility for uncontrolled side effects from lysed blood cells. We have therefore prepared plasma in the laboratory and maintained pH $\geq$ 5.8 to avoid protein precipitation.

The collection of thermograms in Figure 2 reflects the effect of pH on the denaturation of the plasma proteins. Due to interference from the citrate present, especially at higher pH values the high temperature ($\geq 80^{o}$) parts of the thermograms should be disregarded. When compared to a corresponding set of curves for 0.9% NaCl solutions of bovine serum albumin (BSA) (Gumpen et al., 1978) the similarity is striking. The dominant peak (pH values $\geq$ 6.2) is readily recognized as being due to BSA, which also gives rise to a less well defined peak in the region $80-85^{o}$. The thermogram of denaturing globulins, constituting about 60% of the total protein content of plasma (Rauen, 1964), is superimposed on that of BSA, and these proteins are primarily detected as an increased heat absorption above $72^{o}$. As judged by the position of the peak maximum, BSA in plasma is $4-5^{o}$ more stable at pH 6-6.5 than at pH 7.8.

For our purpose, denaturation of the proteins may be considered a prerequisite for their forming a gel. Figure 3 shows, however, that the pH values which promote denaturation of BSA do not serve to increase the gel strength of the heated plasma. (We allow ourselves to disregard the minor change in ionic strength caused by pH adjustment). The results of Figure 3 probably reflect two further mechanisms involved in gel formation: (1) The isoelectric point (pI) of BSA is about 5.3 (Wallevik, 1973), which implies that, above this value, increasing the pH will also increase the net, negative charge of the protein. The electrostatic repulsion between the molecules is thereby strengthended, disfavoring the association of them into a gel. This effect has been unambiguously demonstrated with ovalbumin by Egelandsdal (1978). (2) When approaching the pI of the protein, the lack of electrostatic repulsion will allow the molecules to aggregate too quickly and strongly for the three-dimensional network of a gel to be established (Hegg and Martens, 1978). The curve of Figure 3, passing a maximum at pH 6.2 to indicate lower relative gel strength at pH 5.8, may possibly be related to this phenomenon. But the plasma globulins complicate the picture; their contribution towards the gel strength of the heated plasma has not yet been established and cannot be disregarded.

Since Wirth (1975) indicates pH values of 5.8-6.0 to be normal for bologna type sausages, a pH of 5.8 was selected for the initial investigation of salt effects on gelling and denatur-

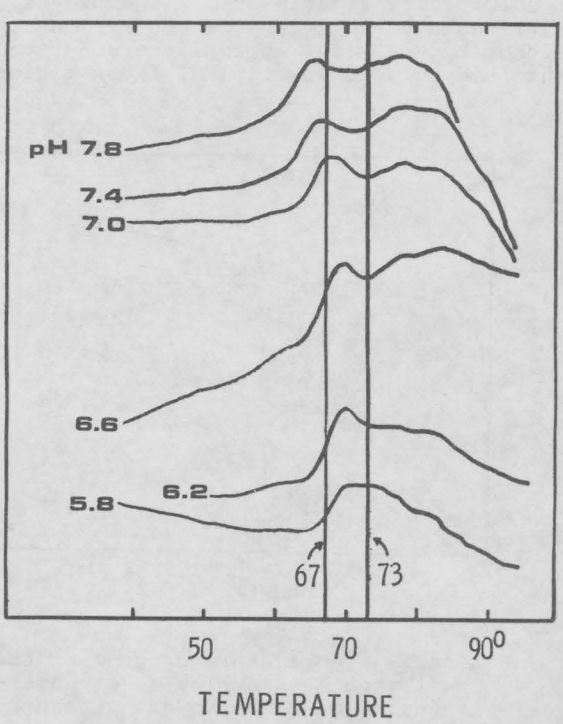


Figure 2: Effect of pH on the thermogram of bovine plasma.

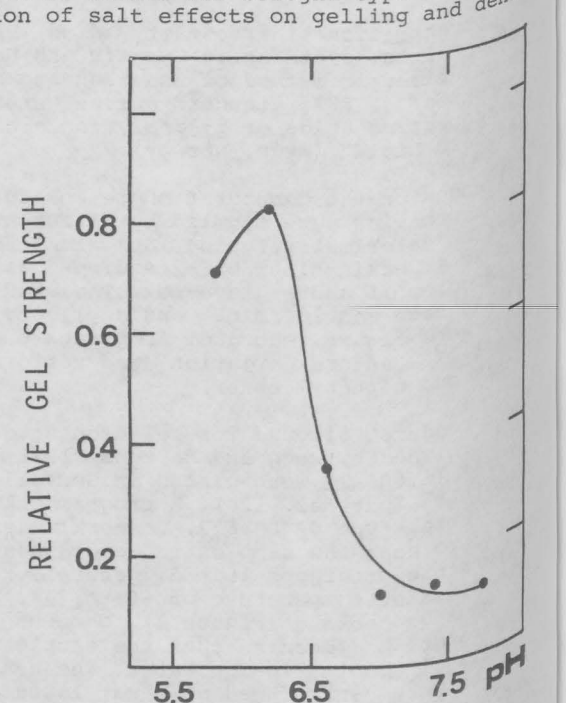


Figure 3: Effect of plasma pH on the relative strength of gels obtained on heating. Dilution due to pH adjustment has been corrected for by assuming gel strength to be proportional to the square of the protein concentration.

ation. The addition of sodium or calcium chloride to give concentrations in the plasma of up to 4.5% had only minor effects on gel strength, however, within ± 10-13% of the value obtained for the gel of plasma itself. As discussed by Hegg and Martens (1978) in their work on ovalbumin, salt ions are assumed to affect gelation primarily by "shielding" the charges of the protein molecules. With a pI of 5.3 BSA can be expected to carry only a minor net charge at pH 5.8, implying that shielding would not make much of a difference. Thus, the lack of significant salt effects is readily understood if BSA is assumed predominant in creating gel strength.

It should be noted that increasing the pH to 6.3-6.5, attainable in batters by adding phosphates, citrate (Wirth, 1975) or caseinates, may imply enough of a net charge increase to make salt concentrations important for gelation. Furthermore, sodium and calcium ions were found to have profoundly different effects on the thermograms of the plasma (Figure 4). We are proceeding to investigate these aspects.

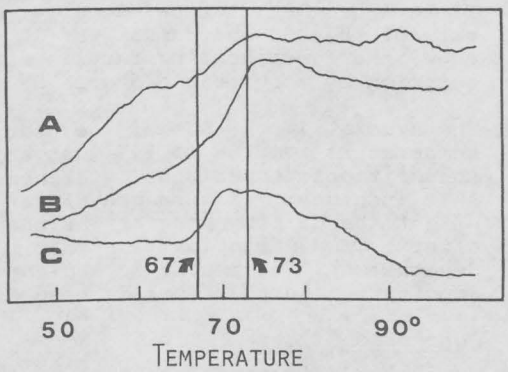


Figure 4: Effect of increased concentrations of sodium and calcium ions on the thermogram of bovine plasma at pH 5.8. A: 0.8% $Ca^{++}$; B: 1.5% $Na^{+}$; C: Pure plasma; pH 5.8; 0.008% $Ca^{++}$; 0.25% $Na^{+}$.

The observed effects of the concentration of free fatty acids (FFA), Figures 5 and 6, can also be understood in terms of BSA being the primary gel-forming protein of bovine plasma. Gumpen et al. (1978) confirmed by differential scanning calorimetry earlier findings (Boyer et al., 1946) that FFA stabilize BSA substantially towards thermal denaturation. Figure 5 reveals the effects in the case of plasma: Fortifying plasma with FFA by the Celite method of Spector and Hoak (1969) shifted the BSA peak maximum from slightly below 70 to about 80° in the case of maximum palmitic acid addition. The gel strength falls off correspondingly, as shown in Figure 6. A positive correlation between the two sets of observations seems evident, but a discussion of the molecular mechanisms involved is beyond the scope of this preliminary work.

The observed effect on gel strength has several implications of practical interest, however: To which level can nutritional status and the stress of hot weather, transportation and pre-slaughter herding raise the level of FFA in the animals' blood? If a possible range of 5-50mg/100ml plasma is assumed, this corresponds to approximately 0.43 - 4.3 moles FFA/mole BSA. According to Figure 6 the higher value renders the gel strength more than 30% lower.

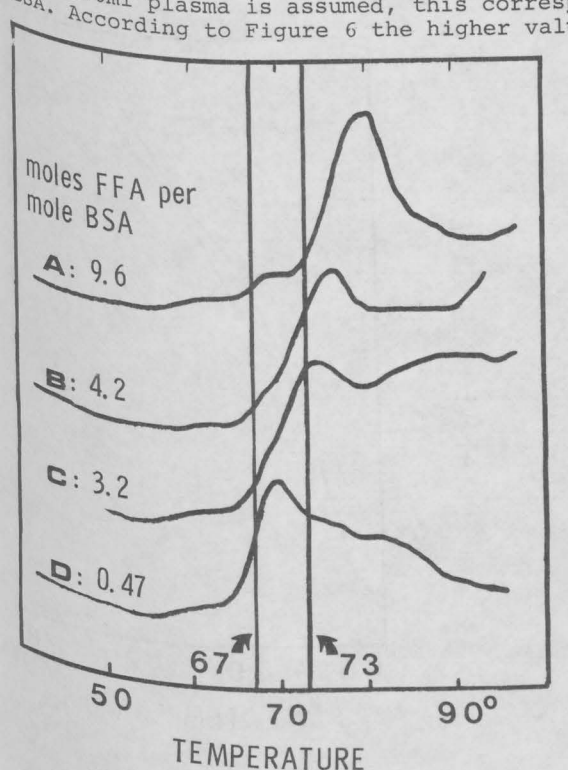


Figure 5: Effect of the concentration of free fatty acids (FFA) on the thermogram of bovine plasma at pH 5.8. FFA added: A and B: Palmitic acid. C: Stearic acid. D: None. (Plasma from a cow in the cow barn).

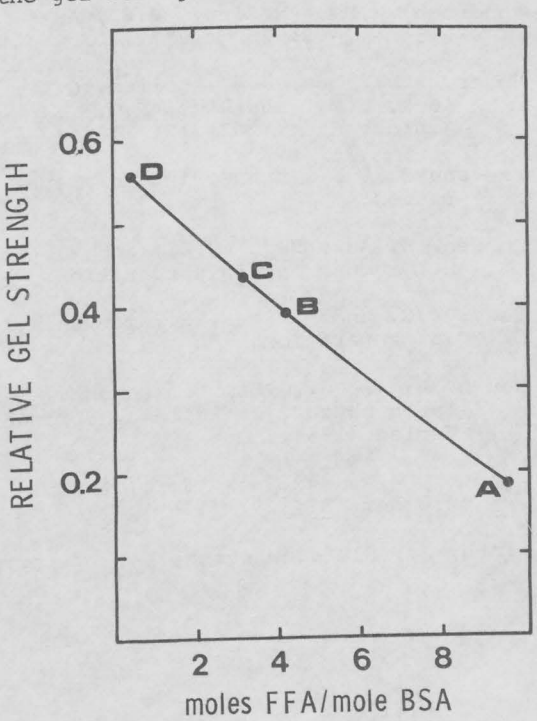


Figure 6: Effect of the concentration of free fatty acids on the relative strength of gels obtained on heating the plasma; cfr. Fig. 5.

Also, is it conceivable that other substances of varying concentration in a batter, e.g. phospholipids, may have a similar effect? Boyer et al. (1946) demonstrated a number of different substances to stabilize BSA, so the effect is not exclusive to FFA. Finally, will the commercial heat treatment of minced meat products alleviate the problem or increase it? The data of Figures 3 and 6 were produced by subjecting the plasma samples to a maximum temperature of 73°. Figure 5 shows temperature to be critical in this connection, since going from 0.47 to 4.3 moles FFA/mole BSA shifts the bulk of denaturation from taking place below 73 to above 73°. Acknowledgedly, to ensure satisfactory detection of denaturation the heating rate of the DSC-instrument was 10°/min. as opposed to 1°/min. in the gelation experiment. However, the lower heating rate is not expected to decrease the temperature of maximum heat absorption by more than a degree or two.

The crucial importance of the heat treatment is revealed in Figure 7. Increasing the final temperature from 68 to 74° just about doubles gel strength (measurements indicated by squares). An additional experiment (star denotation) indicates that temperatures beyond 76° do not result in higher gel strength. In an attempt to separate the time effect from the temperature effect the heating program was changed to have all final temperatures be reached 13 minutes after placing the samples into the bath and starting the linear programming at 59° (disc denotation). The result is a clear indication that the final temperature as such is of prime importance. Further work is needed to assess the kinetics involved.

## Conclusion

General: Indications are that bovine serum albumin (BSA) is the dominating gel-forming protein of bovine plasma. BSA is also predominant in the thermograms of the plasma.
Effects on denaturation (as observed in the thermograms from differential scanning calorimetry): The temperature of maximum heat absorption is 4-5° higher at pH 7.8 than at 6.0-6.5, it is increased about 4° by raising the concentration of NaCl to 4% (pH 5.8), and it is increased by 10-11° when the concentration of free fatty acids (FFA) is raised to 9.6 moles/mole BSA (pH 5.8). The addition of calcium ions seems to have a complex effect on plasma protein denaturation.
Effects on gelation: Maximum gel strength is produced at about pH 6.2, approximately 7-fold higher than at pH 7.8. At pH 5.8 NaCl and $CaCl_2$ had only minor effects. Increasing the concentration of FFA 10-fold from a natural low of approximately 5mg/100ml reduces gel strength by 30% (pH 5.8). The final temperature of heat treatment is crucial; if it is raised from 68 to 74°, gel strength doubles.

## References


Anderson, M.M. & McCarty, R.E., Anal. Biochem. 45, 260 (1972).

Boyer, P.D., Lum, F.G., Ballou, G.A., Luck, J.M., and Rice, R.G., J.Biol. Chem. 162, 181 (1946).

Egelandsdal, B., manuscript in preparation.

Gumpen, S. Aa., Hegg, P.O., and Martens, H., manuscript in preparation.

Hegg, P.O. and Martens, H., manuscript in preparation.

Rauen, H.M., ed., "Biochemisches Taschenbuch", 2. Auflage, Springer Verlag (1964).

Spector, A.A. and Hoak, J.C., Anal. Biochem. 32, 297 (1969).

Wirth, F., Fleischwirtsch. 55, 17 (1975).


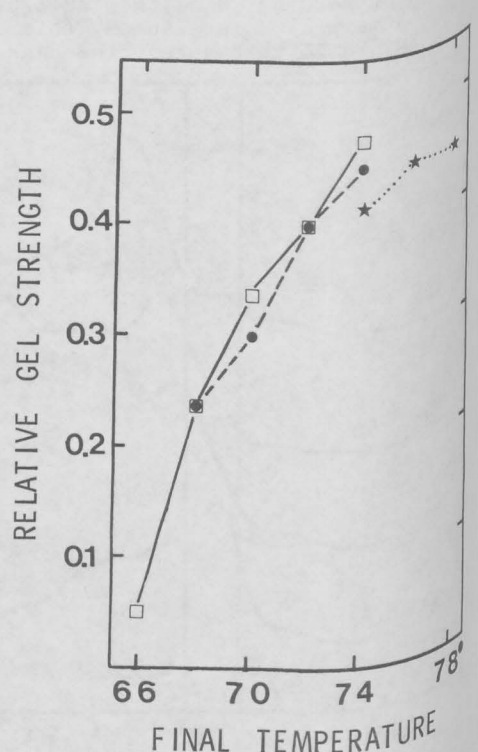


Figure 7: Effect of the final temperature of heat treatment on the relative strength of bovine plasma gels. For details, see text; pH 5.8.