GELATION PROPERTIES AND BINDING ABILITY OF BOVINE MYOFIBRILLAR PROTEINS

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ABSTRACT

The present study examined the heat-gelation and the binding properties of myofibrillar proteins extracted from pre-rigor bovine *Pectoralis profundus* muscle.The effect of pH ($5 \leq pH \leq 7$), ionic strength ($0.1 \leq \mu \leq 0.6$), and added sarcoplasmic proteins on the dynamic rheological properties of myofibrillar proteins were evaluated during heating. For all extracts, optimum gelation was observed at 0.6 M NaCl and in the pH range 5.5-6.0. Moreover added sarcoplasmic proteins increased gel strength of myofibrillar proteins, only at low ionic strength. The effects of freeze-dried myofibrillar proteins, alone or mixed with sarcoplasmic proteins, on the cohesiveness of cooked restructured meat products were compared to the effect of a commercial binder (a mixture of ovalbumin and milk proteins). Water- holding and binding capacities appeared to be highly dependent on both the type and the quantity of the added binder. A significant increase in cohesiveness and water-holding capacity was obtained from 2% myofibrillar protein addition.

INTRODUCTION

The growing demand for restructured meat products has resulted in continuous efforts to improve the texture of these products by addition of gel-forming ingredients. This gel-forming property is considered to be responsible not only for texture but also for the water and the fat holding in restructured meat products (MACFARLANE *et al,* 1977; SEIDEMAN *et al*, 1982; SMITH, 1988). Among gel-forming ingredients, most studies conducted on the mechanisms of heat induced gelation have investigated mainly myosin (ISHIOROSHI *et al*, 1979; EGELANDSDAL *et al*, 1986; HERMANSON *et al*, 1986). MACFARLANE *et al* (1977) and SIEGEL *et al* (1979) examined the capacity of the myosin to bind meat pieces. However little is known about the relation between gelling and binding properties of bovine myofibrillar proteins. So the objectives of our study were: (1) to determine the optimal conditions for heat-gelation of myofibrillar proteins in model systems by analysing the effect of pH, ionic strength and added sarcoplasmic proteins; (2) to determine the binding properties and to analyse the relationship between heat-gelation and binding properties.

MATERIALS & METHODS

1 - Preparation of protein extracts

Sarcoplasmic and myofibrillar proteins were extracted from pre-rigor bovine *Pectoralis profundus* muscle. Minced muscle was homogenized in a 0.15 M NaCl, 3mM $MgCl_2$ buffer (pH 6.5) in a Waring Blendor, then let for 60 min. at 4°C. The homogenate was centrifuged at 10000 x g for 15 min. and the supernatant containing the sarcoplasmic fraction was saved. The precipitate was dispersed in 4 volumes of a 0.6 M NaCl, 40mM K-phosphate buffer at pH 6.5. Myofibrillar proteins were extracted during 30 min. at 4°C and recovered in the supernatant after a centrifugation at 10000 x g during 15 min. Myofibrillar proteins were then precipitated by dilution and washed with distilled water while the sarcoplasmic protein fraction was desalted by diafiltration and concentrated by ultrafiltration. Myofibrillar and sarcoplasmic proteins were then freeze-dried.

2 - Rheological measurements

The protein extracts were dispersed in a 40 mM phosphate buffer adjusted to different ionic strengths (0.1, 0.4 and 0.6 M NaCl) and variable pH (5.0, 5.5, 6.0, 7.0), so as to have a protein content of 20 mg/ml.These dispersions were then dialysed 24 h against the same buffer. A constant stress oscillatory rheometer, CARRIMED CSL 100, was used to follow heat-gelation.This rheometer was fitted with a cone and plate cell (4 cm diameter,1°58 angle). One milliliter of each protein solution, placed in the gap of the cell, was heated from 20 to 80°C at a constant rate of 0.7°C/min. Then, the temperature was decreased to 20°C with a constant rate of 5°C/min and the rheological measurements performed at 0.1Hz frequency and a 2% strain. Three parameters were determined: the storage (G') and the loss (G'') moduli and the phase angle (∂).

3 - Binding ability measurements

Thin (0.4 mm thick) meat strips were prepared from bovine *pectoralis profundus* muscles (1 day post-mortem) using FRADIN's process (1986). Different treatments were performed and led to five groups of products: (1) Restructured meat strips (RMS), no additives; (2) RMS and dried myofibrillar proteins (1%, 2% or 4%); (3) RMS and a mixture(weight ratio: 1:1) of dried myofibrillar and sarcoplasmic

proteins (1%, 2% or 4%); (4) RMS and dried sarcoplasmic proteins (1%, 2% or 4%); (5) RMS and dried commercial binder, PMF from PROTINA Company (France) (1%, 2% or 4%). Each treatment was performed by mixing equal portions of meat strips with the appropriate additives in a horizontal blendor. Then each fraction of 100 g was mould, vacuum packed and stored at 4°C during 24 hours before cooking for 30 min, under vacuum conditions and in a waterbath set at 60°C or 80°C. Steaks were cut in 5cm x1.5 cm x1 cm (Lxlxh) pieces. The cohesion of the product was measured by a triple beam test set up on an Instron testing machine.

RESULTS

1 - Rheological measurements

1-1 Effects of ionic strength and pH

The values of the storage modulus (G') measured after cooling the myofibrillar proteins gel at 20°C, are reported versus pH for different ionic strengths in figure 1. It can be seen from these curves that the effects of ionic strength and pH are interdependent. The maximal gel strength is obtained at high NaCl contents (>0.4 M) . At these NaCl contents, optimal pH is between 5.5 and 6.0. At low ionic strengths the gels exhibit lower rigidity and an increase of pH from 5.0 to 7.0 only induces a small increase in G'.

1-2 Effect of added sarcoplasmic proteins

Sarcoplasmic proteins, even though they have a small gelling capacity, improve the gel strength of myofibrillar proteins when they are added at a 1:1 weight ratio and at low salt contents (0.1 and 0.3 M NaCl) (figure 2). The storage modulus(G'), so, is increased from 100 to 200 N/m2 at 0.1 M NaCl. The effect is still more pronounced at 0.3 M NaCl. In contrast, at a higher salt concentration (0.6 M NaCl) , the effect is slightly negative.

2 - Cooking loss and binding ability

2-1 Cooking loss

Losses from steaks cooked at 60°C and 80°C with or without binders are shown in figures 3 and 4 respectively. All binders from 1% addition improve the water retention when compared with the control. Although cooking losses are fairly different for the two temperatures (6-17% at 60°C and 16-28% at 80°C) similar effects of binders can be observed. Myofibrillar proteins, when used alone or mixed with sarcoplasmic proteins (weigth ratio:1/1) always result in the highest water retention capacity compared with sarcoplasmic proteins alone and PMF binder .They improved water retention when compared with control by 4, 7 and 12 points of percentage at respectively 1, 2 and 4% levels added and at a 80°C cooking temperature.

2-2 Binding ability

The effect of ingredients and of their levels of addition on the binding strength of beef restructured steaks, cooked at 60°C and 80°C, is given in figures 5 and 6. A positive effect on the binding strength is obtained from 1% protein extract addition. Stresses at yield were significantly higher at 80°C than at 60°C and ranged from 40-80 KPa and 15-65 KPa, respectively, these values being influenced by the level of added binders. Although differences between binders appear mainly for a 4% addition at 80°C, large differences can be noted as soon as a 2% level is reached at 60°C. Myofibrillar proteins alone or mixed with sarcoplasmic proteins have the highest binding effect. They can even double the stress at yield obtained with the two other binders for a 2% addition and at 60°C. This phenomenon could be explained by the fact that in contrast with the other proteins, the bovine myofibrillar proteins prepared in the above conditions present a peak of rigidity at a temperature around 55°C (DE LAMBALLERIE *et al*, 1992). Moreover if at 80°C the cohesion increases up to 4% of addition, at 60°C a plateau value is obtained from a 2% addition of myofibrillar proteins with or without added sarcoplasmic proteins.

DISCUSSION & CONCLUSION

Heat-gelation of myofibrillar proteins is highly dependent on pH and ionic strength. The maximal rigidity is obtained for high salt contents (>0.4 M NaCl) and pH in the 5.5-6.0 range. These values correspond to the conditions prevailing in meat , which would favor heat-gelation in restructured meat products. For most, if not all proteins, gel strength generally is related to their degree of initial dissociation. As regards myofibrillar proteins and more specially myosin, it has long been established that they were mainly soluble at highest salt concentrations (0.4 - 0.6M). It is therefore not surprising to observe an increase in the gel strength of myofibrils as ionic strength raised. Another interesting feature is the antagonist effect of ionic strength and pH (figure 1). At pH 5.5, protein aggregation was overcome by increasing salt concentration to at least 0.6M. At pH 6.0, 0.4M NaCl are sufficient to reverse the lower but still

negative effect of pH on protein dissociation and solubility, an assumption supported by the low G' values obtained at 0.1M NaCl for all pH investigated. The low rigidity of gels at low ionic strengths is apparently contradictory to the results reported by ISHIOROSHI *et al* (1979), HERMANSSON *et al* (1986). This can be explained by the fact that proteins were not previously solubilized at a high ionic strength (0.6 M NaCl) before adjusting it at low value by dialysis (BOYER *et al*, 1992). This can also be related to the fact that protein extracts were freeze-dried before their utilisation.

It has been shown that sarcoplasmic proteins have a poor heat-induced gelling ability (ACTON *et al*, 1983). However their addition to myofibrillar proteins improves the gel strength at low NaCl contents. Under these salt contents, sarcoplasmic proteins may bind water, increasing local myofibrillar concentration which induces an increase in gel strength.This water binding ability is limited at high ionic strengths which may cause their unfolding (MORIOKA *et al*, 1990).

The high binding ability of myofibrillar proteins with or without sarcoplasmic proteins is in agreement with the literature which has established that the myofibrillar proteins are the most important contributors to binding quality in meat systems (MACFARLANE *et al*, 1977; FORD *et al*, 1978). The higher binding strength expressed by myofibrillar proteins compared with commercial binder, may be explained not only by their gelling properties but also by the fact that they are meat proteins, which may favor their interactions with the proteins at the meat surface and may lead to a greater cohesive structure.The poorer binding strength of sarcoplasmic proteins alone may be due to their very poor heat-gelling ability (MACFARLANE *et al*, 1977; FORD *et al*, 1978). However, this study confirms the results of these authors who found a significant contribution of sarcoplasmic proteins to the cohesion of restructured meat products when they are used at low ionic strength in conjunction with extracted myosin.

The mechanisms of binding in meat products is very complex and is not fully understood. One of the important factor that determines the efficiency of binding is the heat-gelation ability of the binder. However, the relationship between gelling and binding ability requires more research to be understood.

Fig.1: Effect of ionic strength and pH on the storage modulus G', after cooling the myofibrillar gel at 20°C. Protein content, 15 mg/ml; 40 mM potassium phosphate buffer; —▫—, 0.1 M NaCl; ···◆···, 0.4 M NaCl; ···✳···, 0.6 M NaCl.

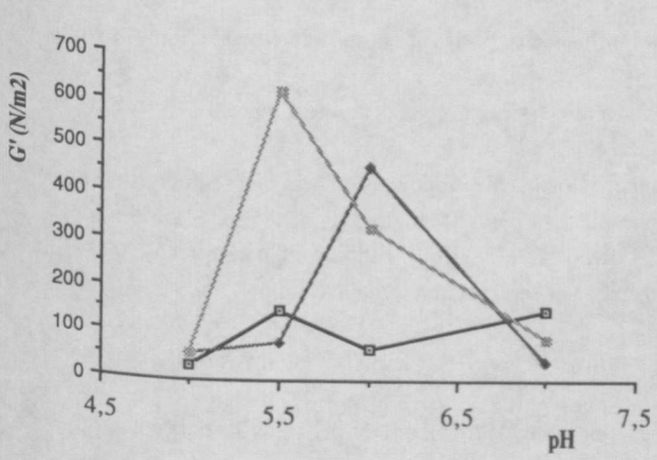


Fig.2: Effect of added sarcoplasmic proteins (weight ratio 1/1) on the storage modulus G', after cooling the myofibrillar gel at 20°C. Protein content, 15 mg/ml; 40 mM potassium phosphate buffer; ■, without sarcoplasmic proteins; ⊠, with sarcoplasmic proteins.

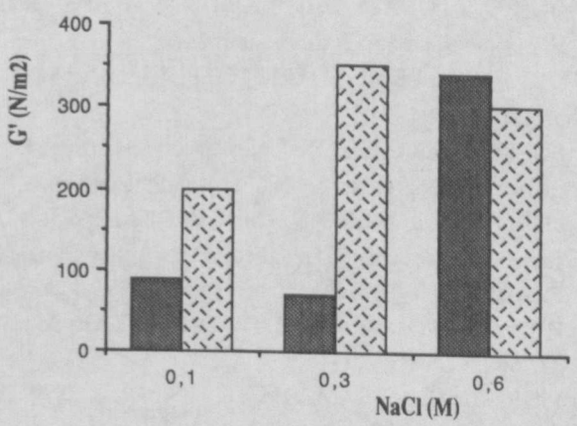


Fig. 3: Effects of type and level of added proteins on the cook loss: 4 binders: —⊡— Myofibrillar proteins; ····◆···· Myofibrillar and sarcoplasmic proteins (weight ratio 1:1); ····▨···· Sarcoplasmic proteins alone; —◇— PMF. Cooking temperature and time: 60°C, 30 min.

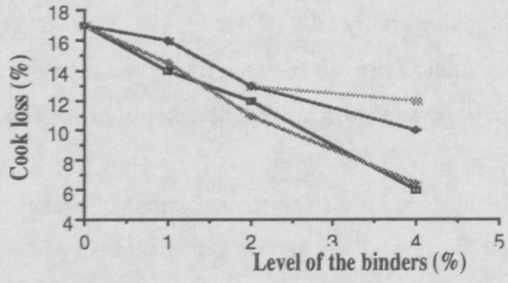


Fig. 4: Effect of the type and the level of added proteins on the cook loss: 4 binders:—⊡— Myofibrillar proteins ····◆····Myofibrillar and sarcoplasmic proteins (weight ratio 1:1); ····▨····Sarcoplasmicproteins alone; —◇— PMF. Cooking temperature and time: 80°C, 30 min.

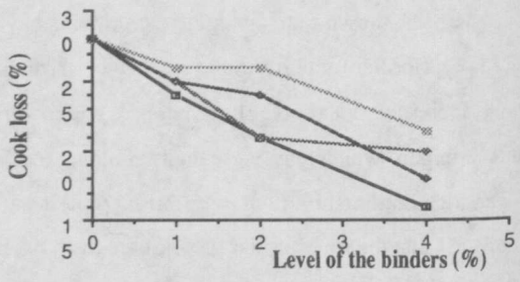


Fig. 5 : Effects of the type and level of added proteins on the binding ability: 4 binders: —⊡— Myofibrillar proteins; ····◆···· Myofibrillar and sarcoplasmic proteins (weight ratio 1:1); ····▨···· Sarcoplasmic proteins alone;—◇— PMF. Cooking temperature and time: 60°C,30 min.

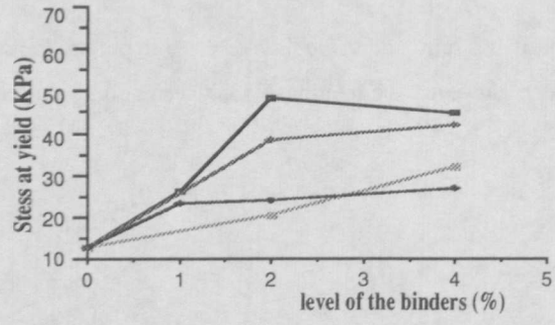
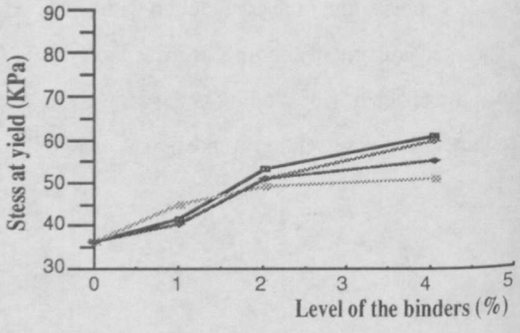


Fig. 6 : Effect of the type and the level of added proteins on the binding ability: 4 binders: —⊡— Myofibrillar proteins; ····◆···· Myofibrillar and sarcoplasmic proteins (weight ratio 1:1);····▨···· Sarcoplasmic proteins alone;—◇— PMF. Cooking temperature and time: 80°C, 30 min.



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