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A Study of the Effects of Different Factors on the Heat-Induced Gelation of Cod (*Gadus morhua*, L.) Actomyosin using Response Surface Methodology

Mercedes Careche*, James Currall & Ian M. Mackie

Ministry of Agriculture, Fisheries and Food, Torry Research Station, 135 Abbey Road, Aberdeen AB9 8DG, UK

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ABSTRACT

The effects of pH, concentration of protein, ionic strength, time and temperature of heating were studied on heat-induced cod actomyosin gels according to a 'central composite design'. Response surface analysis modelled the effects of each factor on gel strength (GS) and log gel strength (LGS) as well as the interaction between pairs of factors. Lowering the pH and increasing the concentration of protein had the effect of increasing the LGS. There were interactions between concentration and pH and pH and ionic strength. The effect of temperature at 1 M KCl pH 7 differed from that at 0·15 M KCl pH 6: two maxima were presented at 0·15 M KCl pH 6 whereas only one was produced at 1 M KCl pH 7. At 1 M KCl pH 7, time of heating had no effect on LGS in the range studied whereas at 0·15 M KCl pH 6, its effect was dependent upon the temperature of heating.

INTRODUCTION

Heat-induced gelation of fish muscle proteins, particularly myosin and actomyosin, is thought to be due to thermal denaturation of the protein molecules and interaction of the denatured polypeptide chains to form crosslinkages (Ferry, 1948; Powrie & Tung, 1976) resulting in a three-dimensional protein network in which water can be entrapped. Among the factors that can influence the gelling properties of a protein are its dynamic structural characteristics which are modified by the electrical and osmotic

* Present address: Instituto del Frio, Ciudad Universitaria s/n 28040, Madrid, Spain

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conditions of the system (Ashgar *et al.*, 1985) as well as the heating conditions of the gelling process itself (Lanier *et al.*, 1982). Fish muscle myosin and actomyosin can form gels at very low concentrations and have been used as model systems to study the gelling phenomenon (Siegel & Schmidt, 1979).

When dealing with the effects of different factors on one response, the traditional experimental approach in food research has been the 'one variable at a time' where various factors are held constant while the levels of another are varied (Ashgar *et al.*, 1985; Wicker *et al.*, 1989). The advantage is that for the particular conditions chosen, the effects of these factors can be seen in great detail. The major disadvantage, on the other hand, is that it examines the effect of each variable independently and, as a consequence, may under- or over-estimate the level of each variable for a specific response. Besides, the examination of several variables using this technique may not precisely measure interaction effects.

To describe the effect of several environmental factors, including their interactions on a desired response, Response Surface Methodology (RSM) can be used (Myers, 1976). RSM permits factors of interest to be varied simultaneously in full and fractional factorial designs. Mathematical models, generally first and second order polynomials, are generated to define the levels of the most significant factors required to optimise the response. Thus RSM has already been used in the study of several ingredients on the texture of cod surimi gels (Hastings & Currall, 1989).

In this work, RSM was used to study the effects of the concentration of protein, pH, ionic strength of the medium, time and temperature of heating on the apparent strength of cod actomyosin gels. The purpose of this study was to examine the effect of each factor, show the interaction effects between pairs of factors and, from the results obtained, to select the optimal conditions for further studies in order to improve the understanding of the heat-gelling process in cod. A first screening experiment was conducted from which more adequate designs were selected for further experiments.

MATERIALS AND METHODS

Fish source

Fresh gutted cod (*Gadus morhua*, L.) were purchased from the Fish Market about 24 h after catching and kept on ice until it was handled in the laboratory in an early post-rigor condition. The fillets were then removed and passed through a Moulinex mincer, fitted with 5 mm diameter holes. This operation was carried out in a chillroom held at 4°C.

Actomyosin preparation

Natural actomyosin was isolated according to Kawashima *et al.* (1973) from 200 g of the cod mince. All the handling operations were carried out between 0 and 4°C. Protein concentrations were determined following the method of Kalb and Bernlohr (1977).

Preparation of heat-induced gels

Actomyosin solutions at concentrations required for each experiment were dialyzed overnight against different concentrations of KCl solutions containing phosphate or citrate buffers (50 mM) at the pHs required.

To make the actomysin gels, 10 g aliquots of the protein solutions or suspensions were put into glass flasks (2·5 cm diameter) and then heat-treated in a waterbath at different temperatures for definite times according to the design of each experiment.

Gel strength measurements

The heat-induced gels were kept on ice for 1 h. The apparent gel strength of a specific gel was measured in a Stevens–LFRA texture analyser (C. Stevens and Sons Ltd) adapted routinely by the manufacturer to work with very soft gels and determined by the readings given by the apparatus set at a speed of 0·5 mms^{-1} and 6 mm of penetration into the gel by a plunger of 1·3 cm diameter.

Statistical design

The experiments were designed according to a central composite rotatable design (Cochran & Cox, 1957). This design enabled two objectives to be met. First, all single factor main effects (pH, ionic strength, concentration of protein, time and temperature of heating) and all two factor interactions could be estimated. Secondly, a second order polynomial response surface in the factors could be fitted which allowed estimation of response variables at any combination of the factors. Different groups of experiments were set up and in all of them five levels of each factor were chosen according to the principles of the central composite design. The levels of the factors used in each gel are given in the Table 1 for each experiment. In the first group one experiment with a total of 33 different combinations of five factors was prepared, in the second three experiments each with 20 different combinations of three factors and in the third group two experiments each of 13 combinations of two factors following the designs suggested by

TABLE 1
Experiments and Variables

Experiments	*Levels*				
	Protein conc. (mg/ml)	*pH*	*IS (molar)*	*Time (min)*	*Temp (°C)*
1st experiment variables:	5·00	4·00	0·10	20·00	50·00
T, *t*, *c*, pH, IS	8·75	5·00	0·32	30·00	60·00
	12·50	6·00	0·55	40·00	70·00
	16·25	7·00	0·77	50·00	80·00
	20·00	8·00	1·00	60·00	90·00
2nd experiment variables:	5·00	4·00	0·1	30·00	60·00
c, pH, IS (at low IS)	9·05	4·81	0·2		
	15·00	6·00	0·35		
	20·95	7·19	0·5		
	25·00	8·00	0·6		
3rd experiment variables:	5·00	4·00	0·60	30·00	60·00
c, pH, IS (at high IS)	9·05	4·81	0·72		
	15·00	6·00	0·90		
	20·95	7·19	1·08		
	25·00	8·00	1·20		
4th experiment variables:	5·00	4·00	0·05	30·00	60·00
c, pH, IS (at very low IS)	9·05	4·81	0·09		
	15·00	6·00	0·15		
	20·95	7·19	0·21		
	25·00	8·00	0·25		
5th experiment variables:				20·00	40·00
T, *t* (at very low IS)				22·93	45·86
	20·00	6·00	0·15	30·00	60·00
				37·07	74·14
				40·00	80·00
6th experiment variables:				20·00	40·00
T, *t* (at high IS)				22·93	45·86
	20·00	7·20	1·00	30·00	60·00
				37·07	74·14
				40·00	80·00

T = temperature of heating (°C).
t = time of heating (min).
c = concentration of protein (mg/ml).
IS = ionic strength (concentration of KCl, molar).

Cochran and Cox (1957). In the first two groups of experiments assessment of error was derived from replication of only one treatment combination whereas in the third group each treatment combination was prepared in triplicate.

Statistical analysis

For gel strength and log gel strength, the response variables, a second order polynomial equation of the following form was fitted:

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^2 + \sum_{i<j} b_{ij} x_i x_j$$

Where y is the estimated response, b_0, b_i, b_{ii}, b_{ij} are the equation parameter estimates (constant: b_0, parameter estimates for linear terms: b_i, for quadratic terms: b_{ii}, for interaction terms: b_{ij}), x_i, x_j are the levels of the factors and k the number of factors.

For the response variable the variance was partitioned into linear, quadratic, interaction, lack of fit and error components in order to assess the adequacy of the second order polynomial function and the relative significance of these components.

The significance of the equation parameters for each response variable was assessed using a F-test.

RESULTS AND DISCUSSION

The analysis of the results shows the effect of each variable on the apparent gel strength (GS). The goodness of fit of the models for GS and logarithm of gel strength (LGS) were examined by the F-test and the results are given in Table 2 as well as their lack of fit. Since the models fit the LGS data better, the log transformation was used in the subsequent analyses. The effect of the variables on the LGS were divided into first order (linear), second order (quadratic) and interactive (the interaction between each pair of variables on the parameter studied). For all the experiments the first order and second order effects are described in Table 3 and the interactions in Table 4. A response surface was fitted for the effect of each pair of variables on LGS and examples of these are shown in Figs 3 and 5. Each diagram shows the effect of two variables while the others are constant at the mid-point of their range.

TABLE 2
F Test for the Variables Studied in the Different Experiments

	Gel strength		*Log gel strength*	
	Model	*Lack of fit*	*Model*	*Lack of fit*
1st experiment variables:				
T, *t*, *c*, pH, IS	NS	***	NS	***
2nd experiment variables:				
c, pH, IS (at low IS)	NS	***	*	***
3rd experiment variables:				
c, pH, IS (at high IS)	*	***	***	*
4th experiment variables:				
c, pH, IS (at very low IS)	*	***	***	*
5th experiment variables:				
T, *t* (at very low IS)	NS	NS	NS	*
6th experiment variables:				
T, *t* (at high IS)	***	NS	***	NS

T = temperature of heating.
t = time of heating.
c = concentration of protein.
IS = ionic strength (concentration of KCl).
NS = not significant.
*, **, *** = significant at 5%, 1% and 0·1% levels, respectively.

Screening experiment

In order to determine which of the effects, pH, concentration of protein, ionic strength, time and temperature of heating, contribute to the gel formation of actomyosin in cod, a screening experiment was set up using the RSM. These five factors are known to affect the gelling properties of actomyosin (Acton *et al.*, 1983; Ashgar *et al.*, 1985). The range of variation chosen was as broad as possible for the working conditions and the levels used for each factor are summarised in Table 1.

The analysis of the results in this experiment showed that neither the model fitted for the parameter GS nor the one for LGS was adequate, the lack of fit being significant at the 0·1% level in both cases (Table 2). However, when analysing the regression coefficients (Table 3) it was found that there was a negative, linear effect of the pH on the LGS at the 0·1% level. Figure 1(a) shows the gel strength values as a function of pH independently of the rest of the variables. It was observed that, in general, the lower the pH values the stronger were the gels. There was also a significant (5% level), positive, 2nd order relationship between ionic strength and LGS as shown in Table 3.

TABLE 3
Regression Coefficients for Logarithm of Gel Strength

Experiment	*Temperature*		*Time*		*Ionic strength*		*pH*		*Concentration*	
	Lin	*Curve*	*Lin*	*Curve*	*Lin*	*Curve*	*Lin*	*Curve*	*Lin*	*Curve*
1st experiment variables:										
T, *t*, *c*, pH, IS	NS	NS	NS	NS	NS	+	− − −	NS	NS	NS
2nd experiment variables:										
c, pH, IS (at low IS)	·	·	·	·	NS	NS	− − −	+ +	+ +	NS
3rd experiment variables:										
c, pH, IS (at high IS)	·	·	·	·	NS	NS	− − −	+ + +	+ +	+ +
4th experiment variables:										
c, pH, IS (at very low IS)	·	·	·	·	NS	NS	− − −	NS	+ + +	NS
5th experiment variables:										
T, *t* (at very low IS)	−	NS	NS	NS	·	·	·	·	·	·
6th experiment variables:										
T, *t* (at high IS)	+ + +	− −	NS	NS	·	·	·	·	·	·

IS = ionic strength.
lin = linear relationship between variable and log gel strength.
curve = second order relationship between variable and log gel strength.
NS = no effect.
+, + +, + + + = postive effect (significant at 5%, 1% and 0·1% levels, respectively).
−, − −, − − − = negative effect (significant at 5%. 1% and 0·1% levels, respectively).
· = not measured in that case.

TABLE 4
Interactions between Pairs of Variables for Log Gel Strength

Experiment	*tT*	*tc*	*tpH*	*tI*	*Tc*	*TpH*	*TI*	*cpH*	*cI*	*pHI*
1st experiment variables:										
T, *t*, *c*, pH, IS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2nd experiment variables:										
c, pH, IS (at low IS)	·	·	·	·	·	·	·	− −	NS	NS
3rd experiment variables:										
c, pH, IS (at high IS)	·	·	·	·	·	·	·	+	NS	NS
4th experiment variables:										
c, pH, IS (at very low IS)	·	·	·	·	·	·	·	− − −	NS	−
5th experiment variables:										
T, *t* (at very low IS)	NS	·	·	·	·	·	·	·	·	·
6th experiment variables:										
T, *t* (at high IS)	NS	·	·	·	·	·	·	·	·	·

t = time.
T = temperature.
c = concentration of protein
IS = ionic strength (concentration of KCl)
NS = no effect.
+ = positive effect (significant at 5% level).
−, − − − = negative effect (significant at 5% and 0·1% level, respectively).
· = not measured.

It was observed that there seemed to be two maxima: at high and low ionic strengths (Fig. 1(b)). Other authors have also found two maxima for actomyosin gels from other animals (Ashgar *et al.*, 1985). As the model is constrained to be a 2nd order polynomial curve (inappropriate when two maxima are present), it was decided, for further experiments, to split up the range of ionic strengths into low and high values. None of the rest of the variables studied (concentration of protein, time, temperature) had a

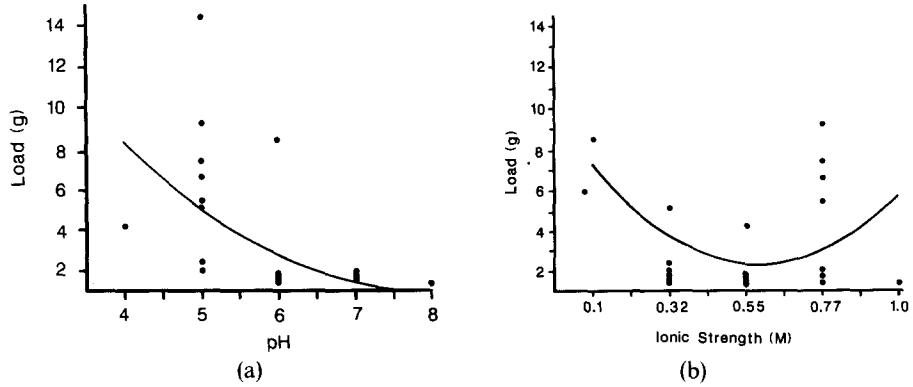


Fig. 1. The effect of (a) pH and (b) ionic strength independently of the rest of the variables on gel strength of heat-induced cod actomyosin gels.

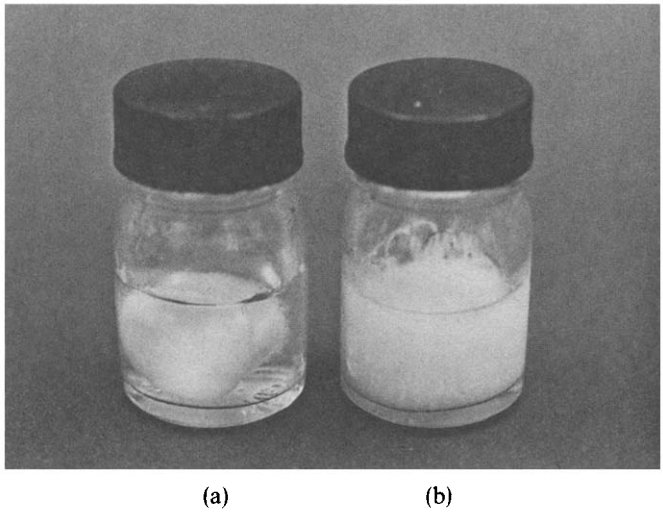
significant effect on the gel strength as predicted by the model although concentration was very close to the 5% significance level. Over the range of gel strengths studied in this experiment there were no significant interactions between pairs of variables (Table 4).

Visually it could be observed that the structure of the gels at high and low pH, was different. In certain cases with very low pH values, a coagulum from which the water had separated instead of a true gel was obtained, as can be seen in Fig. 2.

Following this experiment, further RSM experiments concentrated on the effect of concentration of protein, pH and ionic strength. Time and temperature effects were set constant and studied later on (see below).

The effects of concentration of protein, pH and ionic strength

The effects of concentration of protein, pH and ionic strength were studied in two different RSM experiments: at low and high concentrations of KCl. The range of variation and the levels for each variable are shown in Table 1 (2nd and 3rd experiments). As shown in Table 2, the model for LGS was more significant than the one for GS for both cases. In the range of low ionic strengths, however (0·1–0·6M KCl), the lack of fit was so high that another experiment with a narrower range of IS (0·05–0·25M KCl) was performed and in which the best responses were found. The effects of concentration of protein, pH and ionic strength were therefore studied at very low (0·05–0·25M KCl—4th experiment) and at high (0·6–1·2M KCl—3rd experiment) values of IS.



(a) (b)

Fig. 2. Photograph of heat-induced cod actomyosin gels at pH 4 (a) and pH 8 (b) at 0·55M KCl, 12·5 mg/ml protein, and heated for 30 min at 70°C.

The effect of concentration of protein

Concentration of actomyosin had a linear positive effect on LGS at both low (0·1% level) and high (1% level) IS (Table 3) and a quadratic effect at high (1% level) IS. The linearity of LGS upon concentration has been shown in other species (Acton *et al.*, 1981) although other authors have found a log–log relationship (Yasui *et al.*, 1980; Beas *et al.*, 1988). Figure 3 ((a), (b), (c), (d)) shows the contour diagrams of this variable at low ((a), (c)) and high ((b), (d)) values of IS.

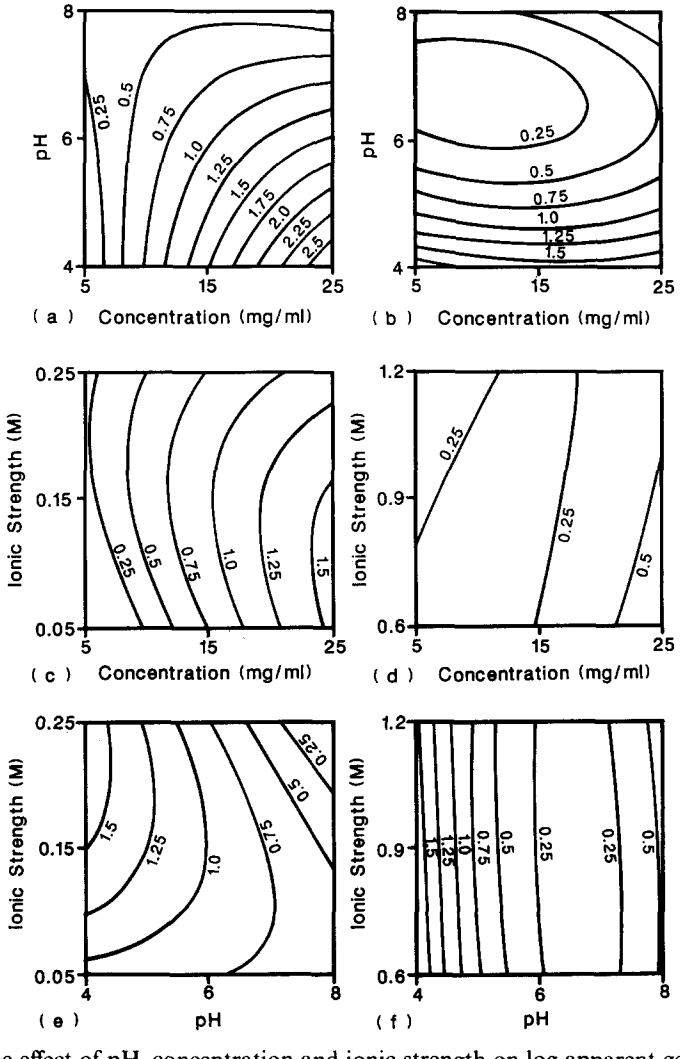


Fig. 3. The effect of pH, concentration and ionic strength on log apparent gel strength of heat-induced cod actomyosin gels at low ((a), (c), (e)) (0·05–0·25M) and high (0·6–1·2M) ((b), (d), (f)) ionic strength ranges.

The effect of ionic strength

The IS did not have a significant effect on LGS into the studied ranges (Table 3 and Fig. 3 ((c), (d), (e), (f)). Although there was no significant effect of IS within either the low or the high range of IS, the actual values of apparent gel strength were, in general, higher in the low range of this variable (Fig. 3, contour lines). This explains why there was some effect of IS on the screening experiment and agrees with findings of other authors who found more rigid gels at 0·2M KCl, pH 6 than at 0·6M KCl at the same pH for myosins of rabbit (Ishioroshi *et al.*, 1983) and tilapia (Wicker *et al.*, 1986).

The effect of pH

The LGS of the actomyosin gels was strongly dependent on the pH at both low and high ionic strengths (Table 3, Fig. 3(a), (b), (e), (f)). There was a linear negative relationship (0·1% level) of pH with LGS for both low and high ionic strengths and a quadratic relationship at high ionic strength (0·1% level), confirming the observation made in the screening experiment, that the lower the pH values the stronger were the gels. It has been reported that maximum gel strength occurred for rabbit and beef actomyosin in the pH range 5·5–6·0 (Yasui *et al.*, 1980; Acton *et al.*, 1981), and at pH 6·3 and 6·0 for carp and hake actomyosins, respectively (Itoh *et al.*, 1979; Beas *et al.*, 1988) whereas it has been observed that the strength of gels of mackerel actomyosin (Deng *et al.*, 1976) and tilapia myosin (Wicker *et al.*, 1986) increased as the pH was reduced in agreement with the present observations. The fact that the pH strongly affects the gel forming ability of cod actomyosin can be due to the wider range (log scale) used in this case, compared to others such as IS.

Interactions between pairs of variables

A strong negative interaction (0·1% level) between concentration of protein and pH was found at low ionic strength ranges (Table 4, Fig. 3(a)). This interaction means that the effect of pH is going to be dependent on the protein concentration, and vice-versa, i.e. there is little effect of protein concentration at pH 8 whereas its effect is very strong at pH 4. Also, the effect of the pH is dependent on the concentration of protein: at high concentrations of protein (25 mg/ml) the effect of pH is very strong whereas it is very weak at low concentrations of actomyosin (5 mg/ml). The fact that there is a strong interaction can be related with both variables modifying protein–protein interactions.

No interaction was found between concentration and ionic strength in the range studied at either low or high ionic strengths (Table 4, Fig. 3(c), (d)).

At low ionic strengths there was a negative (5% level) weak interaction between pH and ionic strength (Table 4, Fig. 3e), so that the effect of the pH is

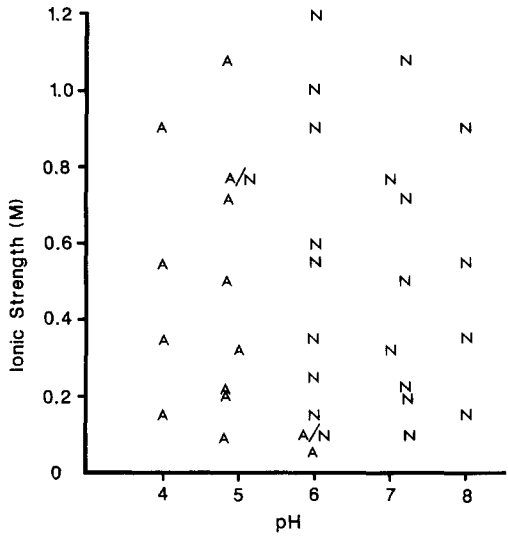


Fig. 4. The effect of pH and ionic strength on the types of heat-induced cod actomyosin gels. A = gels which presented syneresis; N = gels with no syneresis; A/N = starting of syneresis.

higher at the higher ionic strengths. In the range of high ionic strength, on the other hand, there was no interaction between pH and IS (Table 4, Fig. 3(f).

When taking into account the effects of pH and IS, it has to be said that, depending on these two factors, two different sorts of gels could be distinguished visually. At pH values below 5 the gels showed syneresis, whereas above 6 there was none (Fig. 4). The former could have so much syneresis that a coagulum type of structure could be observed at extremely low pH values (Fig. 2). It was observed at pH 5 and 6 that the IS had an influence on the formation of this type of structure: the lower the pH, the higher the IS had to be in order not to have syneresis-type gels. The gels which presented syneresis were much stronger than the other ones. Syneresis below a certain pH has been reported previously (Acton *et al.*, 1981; Ashgar *et al.*, 1985).

It is known that myosin molecules have different arrangements depending upon pH and IS: at low IS and neutral pHs these molecules are packed as filaments but as the ionic strength is increased above 0·3 they dissociate and disperse as monomers (Huxley, 1963). According to Yamamoto *et al.* (1988), the gel strength depends upon the state of the myosin so that filamentous myosin forms more rigid gels than monomeric myosins. They also reported that the longer the filaments, the stronger were the gels. Wicker *et al.* (1986), on the other hand, found that tilapia myosin gels at 0·1M KCl and pH 6, although more rigid, were less translucent than myosin gels formed at higher pH and higher concentrations of KCl. They suggested that the former gels

could result from a coagulum type of denaturation due to an excess of protein–protein interaction, which is in agreement with our results. In our results we have found not only that IS and pH are related but also that concentration of protein has a strong interaction effect with pH. So any explanation about the first step in the mechanism of gelling of cod actomyosin related to the ionic conditions of the system should take into account these interactive effects among these three variables.

Although it is difficult to differentiate between 'true gels' and 'coagulum-type gels', our results indicate that for cod actomyosin, in the range of the rest of the variables that were studied, combinations of pH-IS of pH 5 and IS $\geq$0·6M KCl to pH 6 and IS 0·15–0·25M KCl should give true gels with fairly high rigidity. Besides, according to the results obtained, the effect of concentration of protein will be much more apparent at lower values of pH (pH 5). For the following studies on the effect of time and temperature of heating, two different combinations of IS and pH were chosen: 0·15M pH 6 where the gels are quite strong but there is no coagulum and 1·0M KCl pH 7 where the gels are weak but will not present syneresis.

The effect of time and temperature of heating

Although time and temperature of heating did not show significant effects on the LGS in the screening experiment, they were included in the main study because of their known importance on the gel formation of surimi actomyosin and myosin from a range of species (Deng *et al.*, 1976; Lanier *et al.*, 1982; Ashgar *et al.*, 1985; Beas *et al.*, 1988; Sano *et al.*, 1988). Their effects were determined in another set of experiments with the levels of the factors described in Table 1 (5th and 6th experiments). Table 2 shows that the model was not significant for the experiment done at 0·15M KCl, pH 6 whereas it was very highly significant (0·1% level) at 1·0M KCl pH 7 and provided a good fit to the LGS data. In the gels at high ionic strength, neutral pH, temperature affects LGS both linearly (0·1% level) and quadratically (1% level). Figure 5 shows the contour diagram for the effect of temperature at

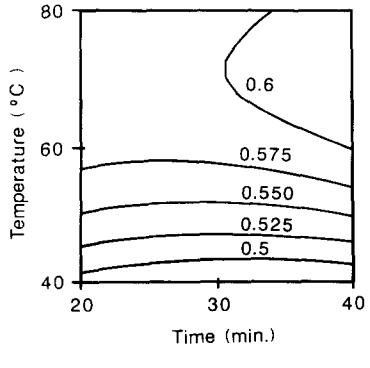


Fig. 5. The effect of time and temperature of heating on log gel strength of heat-induced cod actomyosin gels at 20 mg/ml, 1·0M KCl pH 7.

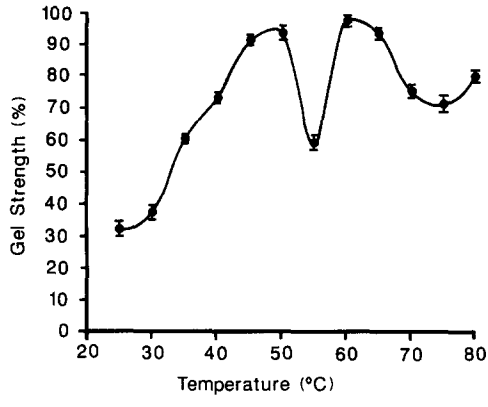


Fig. 6. The effect of temperature of heating on gel strength of heat-induced cod actomyosin gels at 20 mg/ml, 0·15M KCl pH 6 and 30 min of heating.

high ionic strength. It can be seen that the higher the temperature the stronger are the gels, with the more significant effects being within the 40–60°C range and remaining almost constant above 60°C. This behaviour is similar to that found in mackerel actomyosin (Deng *et al.*, 1976) as well as in tilapia myosin (Wicker *et al.*, 1986) for similar values of pH and ionic strength.

There was no significant effect of time on LGS (high ionic strength, neutral pH) in the range studied. This result agrees with that of Deng *et al.* (1976) for mackerel and beef actomyosin. No interactions between time and temperature were found in this experiment (Table 4).

As the time and temperature had no significant effect at low ionic strength in the designed RSM experiment, these two factors were studied in 'one factor at a time' approach type experiments. In one experiment with the following factors constant, 20 mg/ml of actomyosin, 0·15M KCl, pH 6 and 30 min of heating, the temperature only was varied. In the other experiment the effect of the time of heating was studied at two temperatures (45° and 65°C), all other conditions being unaltered.

The results for the effect of temperature are given in Fig. 6. Actomyosin started to gel at temperatures as low as 30°C. There were two maxima at around 45° and 60°C, and two minima, at 55° and 75°C. The shape of the curve obtained explains why a second order polynomial equation could not fit adequately.

The behaviour found at low ionic strength is similar to that found for tilapia myosin (Wicker *et al.*, 1986) heated under similar conditions. Similar results to those at low ionic strength, pH 6, were found for carp actomyosin, but at a different ionic environment (Sano *et al.*, 1988) as well as for surimi from different species of fish (Montejano *et al.*, 1984; Kim *et al.*, 1986),

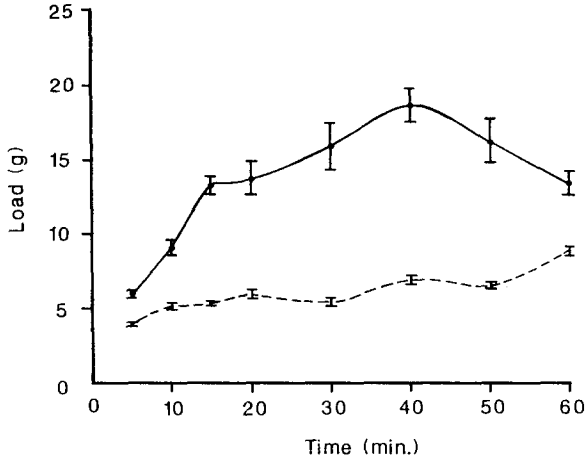


Fig. 7. The effect of time of heating on gel strength of heat-induced cod actomyosin gels at 0·15M KCl, pH 6 at 45°C (– – –) and 65°C (——).

whereas in actomyosin from red muscle the curves did not present two maxima either at high or low ionic strengths (Samejima *et al.*, 1981; Ishioroshi *et al.*, 1983).

The first peak of gel strength can be related to the 'high temperature setting' (Montejano *et al.*, 1984). In this region, actomyosin molecules can undergo conformational changes bringing about active interaction of the molecules, such as the formation of intermolecular cross-linkages which leads to the formation of gel structure (Sano *et al.*, 1988). The drastic loss of gel strength at 55°C is at a temperature range where the 'modori' phenomenon occurs. Modori consists of an alteration of gel structure with a loss of its textural characteristics. Several authors attribute the loss of gel forming ability (Beas *et al.*, 1988; Sano *et al.*, 1988) directly to this phenomenon and this could be an explanation of our results.

Figure 7 represents the effect of time of heating at 45° and 65°C, at pH 6 and 0·15M KCl. The greater effect of time was observed at 65°C, with a maximum around 40 min.

Further studies are needed to improve the understanding of the formation of cod actomyosin gels taking into account the interaction effects of all the factors studied in this paper.

CONCLUSIONS

RSM can be used to study the various factors which affect the gelling properties of fish muscle proteins.

In cod actomyosin, protein concentration, pH and IS are important factors that influence the strength of heat-induced gels. In the range studied there are interactions between concentration and pH and ionic strength and pH. The factors, time and temperature of heating have different effects depending on the pH and the IS of the system, showing that all the factors studied in this paper are inter-related.

Although the strongest gels are given by very low pHs, these gels can result in coagulum-type ones, so a pH about 6, and IS of 0·15M KCl, heating at 65°C for 30 min is recommended, with the concentration of protein as high as possible.

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