Monitoring the effects of salt and temperature on myofibrillar proteins in beef

Ruth A. McArdle[1], Joseph P. Kerry[2], Anne Maria Mullen[1], Paul Allen[1], Ruth M. Hamill[1]

[1] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland.
[2]Food Packaging Group, School of Food and Nutritional Sciences, University College Cork, Ireland

Abstract—**The aim of this study was to determine the influence of salt (NaCl) and processing temperature on myofibrillar protein thermal stability using differential scanning calorimetry (DSC). Myofibrillar proteins were isolated from beef *M. semimembranosus.* A two factor central composite design was adopted. Extracted myofibrils were incubated at temperatures ranging from 5 to 90 °C and NaCl concentration ranging from 0% to 1.5%. Temperature and NaCl had a significant effect ($p<0.05$) on onset temperature, peak temperature and denaturation enthalpy, for actin, and peak temperature and denaturation enthalpy for myosin. Examination of the response surfaces for both myosin and actin indicated synergistic effects of processing temperature and NaCl level.**

Keywords— **myofibrillar proteins, NaCl, temperature, differential scanning calorimetry**

I. INTRODUCTION

Most of the existing processes applied in the development of meat products have been developed empirically rather than by systematic scientific design. This approach to testing the effect of a limited number of ingredients, inclusion levels and processing conditions on product quality does little to develop an understanding of the processes going on at the molecular level that affect the final product.

Both NaCl and temperature play an important functional role in processed meat products. They activate proteins to increase hydration and water holding capacity, increases the binding properties of proteins to improve the texture, increase the viscosity of meat batters, facilitate the incorporation of fat to form stable batters and therefore are responsible for the desired textural properties of processed meat [1]. Thus understanding the interaction of meat proteins with NaCl and temperature is crucial to the processing ingredient formulation and assessment of product quality [2]. As myofibrillar proteins are the primary site for trapping water in muscle tissue it is important to understand the effect of NaCl and temperature on these proteins.

In recent years differential scanning calorimetry (DSC) has become one of the most useful methods for monitoring protein denaturation in muscle systems as it pinpoints the interactions among meat components [2]. Previous work has shown that the thermal properties of meat proteins were affected by processing prameters e.g. NaCl concentration and pH [3,4]

The overall objective of this work was to study the thermal behaviour of myofibrillar protein in relation to NaCl and temperature levels. Image analysis was also carried out to visualise the effects of NaCl and temperature on myofibril structure. In an effort to find the critical values of the processing variables Response Surface Methology (RSM) was used.

II. MATERIALS AND METHODS

A. Meat sampling

Beef *semimembranosus* muscles were obtained from a local distribution plant. The muscles were trimmed and cut into 20g pieces, individually vacuum packed and stored at -80°C prior to analysis.

B. Myofibril preparation

Meat samples (20g) were suspended in 20 ml of rigor buffer (0.1 M KCl, 2mM MgCl2, 1mM EGTA and 10 mM K_2HPO_4, pH 7) and homogenised in a waring blender at a low speed for 30 s. The homogenates were centrifuged at 2000 g for 15 min.

After decanting, pellets were resuspended under the above conditions. Homogenisation followed by centrifugation was repeated three more times. Prior to final centrifugation the myofibril suspension was passed through a strainer. After determination of the protein concentration by the biuret method, the final myofibril pellets were made up to a standard concentration of 5g/L.with rigor buffer containing 0%, 1.5% and 3% NaCl and vortexed for 1 min. Samples treated at 47.5 and 90°C were heated for 2 min.

C. Experimental design

The effect of NaCl and temperature level on the thermal parameters of myofibrillar proteins were investigated using response surface methodology. The experimental design employed was a central composite design with two independent variables using design expert software (Design Expert 7.1.6, Stat-Ease Inc., MN, USA) (Table 1).

Table 1. Experimental design summary

Sample No.	Run order	NaCl %	Temp °C
8	1	1.5	90.0
9	2	1.5	47.5
10	3	1.5	47.5
5	4	0.0	47.5
11	5	1.5	47.5
2	6	3.0	5.0
3	7	0.0	90.0
7	8	1.5	5.0
6	9	3.0	47.5
1	10	0.0	5.0
12	11	1.5	47.5
4	12	3.0	90.0

D. Differential Scanning Calorimetry

Thermal transition properties were measured using a TA Instruments DSC (Model No. DSC 2010, TA Instruments Inc, Newcastle, USA). The instrument was calibrated using indium (melting point 156.6°C, enthalpy 28.46 J/g) and prior to analysis a base calibration was carried out with two empty hermetically sealed pans (TA Instruments). Sample (7mg) were weighed into coated aluminium pans (TA instruments) and hermetically sealed. A hermetically sealed empty pan was used as a reference sample. Samples were equilibrated at 5°C and then heated from 5°C to 90°C at a heating rate of 10°C per min. Six samples were measure from each treatment.

E. Microscopy

A small amount (a myofibril suspension was dropped onto a slide and a cover slip was placed on top. Myofibrils were examined with a Differential Interference Contrast (DIC) system (Olympus BX-UCB light microscope) using a 60x oil immersion objective lens.

F. Statistical analysis:

The models were subjected to variance analysis (ANOVA) to determine the significance ($p<0.05$), determination coefficient (R^2) and lack of fit. Only the models which satisfy all of these requirements have been analysed.

II. RESULTS AND DISCUSSION

A. Differential Scanning Calorimetry

Thermal analysis of myofibril samples showed two characteristic endothermic peaks, which were related to myosin and actin denaturation respectively [4].

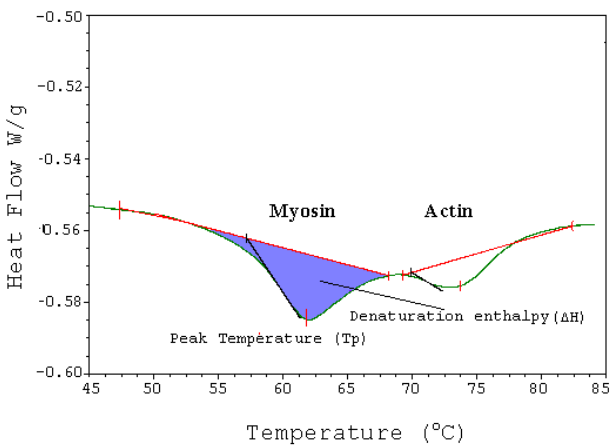

Figure 1. DSC thermogram of non-treated myofibrillar protein

All the models shown were statistically significant, with satisfactory coefficients of determination (R^2) and non significant lack of fit, which indicated that most of the variability in the data could be explained by the models.

The effect of NaCl and temperature on myosin

The denaturation enthalpy is the amount of energy (J/g) needed for total denaturation of a protein. The cubic model indicated no interaction was detected for NaCl and temperature level for myosin denaturation enthalpy. Temperature had an effect on denaturation enthalpy. A progressive reduction of the enthalpy values of myosin was seen with severe denaturation being evident at temperature of 47.5°C or greater (Fig 2a).

The peak temperature (Tp) is the temperature at the time of maxiumum peak value. Two-Factor interaction (2FI) effects on peak temperature for myosin indicates a complex synergistic effect of temperature and NaCl. An increase in NaCl and temperature levels resulted in the decrease ($p<0.05$) in peak temperature of myosin (Fig. 2b). It has been suggested that a reduction in peak temperature indicated protein unfolding which is a pre-requisite for protein gelation [5].

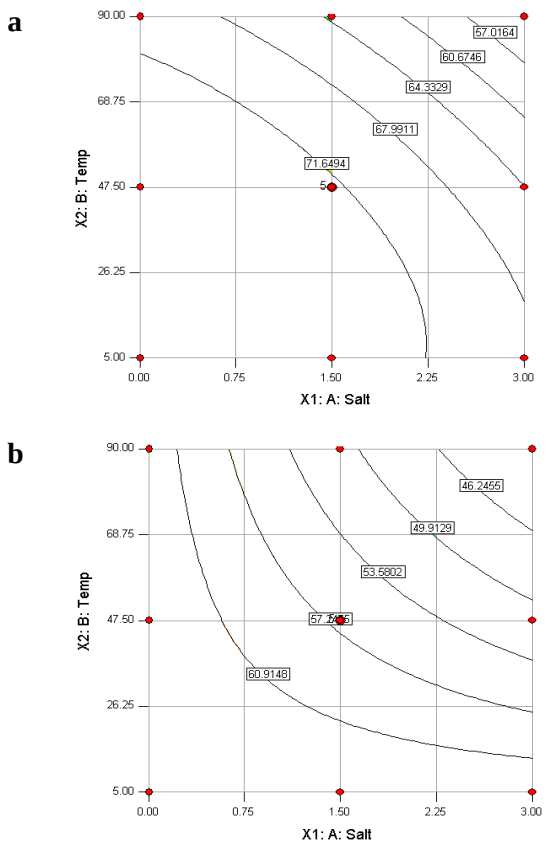

Figure 2. Response surface of (a) denaturation enthalpy (ΔH) and (b) peak temperature (Tp) of myosin in relation to temperature and NaCl.

The effect of NaCl and temperature on actin

Examination of the response surface (cubic model) show that an interactive effect between NaCl and temperature was apparent.

A progressive decrease ($p<0.05$) in denaturation enthalpy values was found with increasing levels of NaCl (0-2%) at low temperatures (26.25°C). However at levels 2.25% or greater complete denaturation has taken place with further increases in NaCl having no effect on enthalpy values. As previously stated an interaction ($p<0.05$) between NaCl and temperature was found, however there was a tendency toward lower enthalpy values as the level of NaCl increased which is apparent in Fig. 3b.

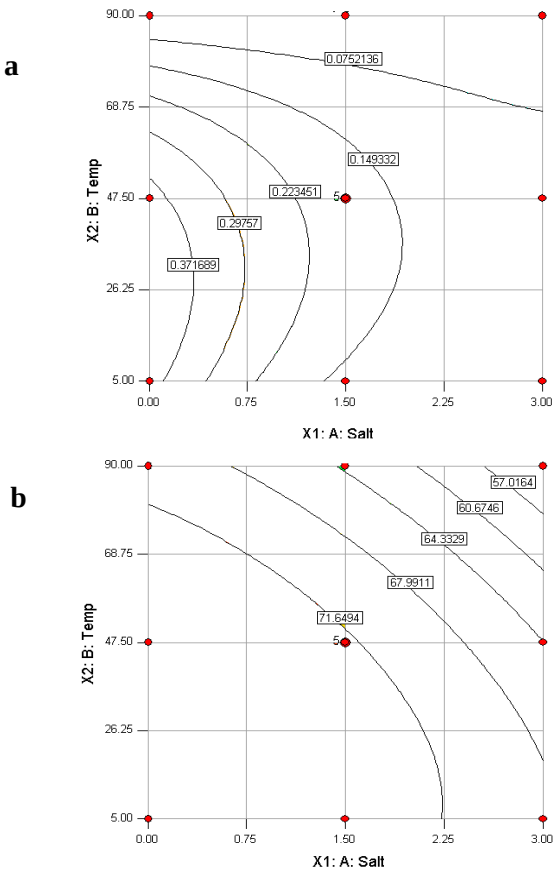

Figure 3. Response surface of (a) denaturation enthalpy (ΔH) and (b) peak temperature (Tp) of actin in relation to temperature and NaCl.

Temperature and NaCl had a significant effect ($p<0.01$) on the peak temperature of actin (quadratic model). An increase in NaCl and temperature levels resulted in the decrease ($p<0.05$) in peak temperature

of actin. At higher NaCl levels (2.25%) at low temperatures (26°C) protein denaturation took place more readily (67.99 °C) when compared to samples with no NaCl processed at the same temperature (71.65°C) (Fig 5). Pighin et al., [6] related the reduction of peak temperature to the protein becoming more susceptible to protein denaturation.

B. Image analysis

Myofibrils treated with 0% NaCl at 5°C had distinct morphological structure with A bands clearly visible (Fig 4a). However as the level of NaCl and temperature increased, changes to myofibril structure were apparent. In myofibrils treated with 3% NaCl at 47.5°C a narrowing and elongation of the A bands was observed (Fig. 4c) when compared to myofibrils treated with 0% NaCl at 5°C. This narrowing elongation of the A band appears to be correlated with an increase in myofibril diameter. In samples treated with 3% NaCl at 90°C myofibrils appeared to clump and were morphologically distorted with disorganised A bands with no preferential orientation (Fig. 4d).

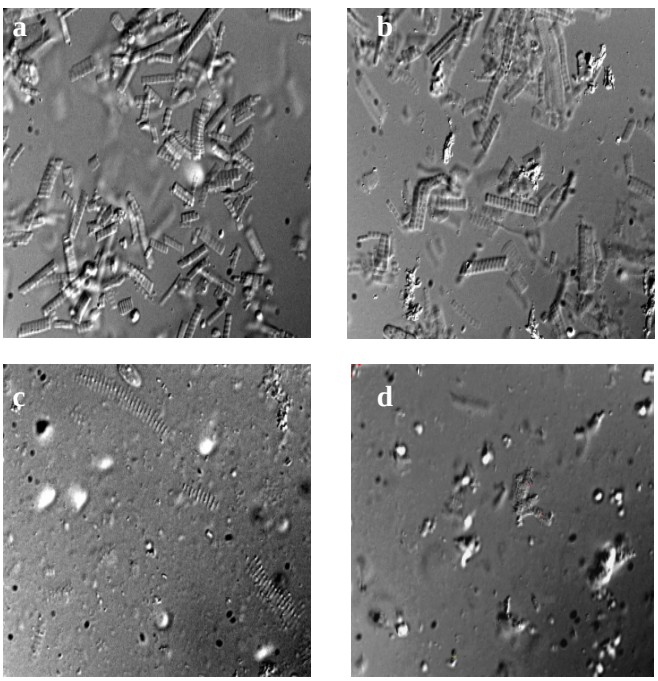

Figure 4. DIC images of myofibrils treated with (a) 0% NaCl at 5°C, (b) 0% NaCl at 47.5°C, (c) 3% NaCl at 5°C and (d) 3% NaCl at 90°C.

IV. CONCLUSION

Examination of the response surfaces indicates a complex synergistic effect of temperature and NaCl for myosin and actin. The use of moderate temperature in combination with low levels of NaCl was found to result in a destabilising effect on myofibrillar proteins. Image analysis has shown that increased levels of temperature and NaCl leads to morphological changes in myofibrils, and analysis is ongoing in order to quantify the changes taking place at each of the temperature / NaCl levels.

Increased understanding of the effects of NaCl and temperature on myofibrillar proteins could lead to improved strategies for optimisation of healthier meat products.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support of the Department of Agriculture, Fisheries and Food, through the Food Institutional Research Measure project number 08/RD/TAFRC/671.

REFERENCES

1. Desmond, E. (2006) Reducing NaCl a challenge for the industry. *Meat Sci* 74 188-196
2. Chin, K. B., Go, M. Y. & Xiong, Y. L. (2009). Konjac flour improved textural and water retention properties of transglutaminase-mediated, heat-induced porcine myofibrillar protein gel: Effect of NaCl level and transglutaminase incubation. *Meat Sci* 81 565-572
3. Shiga, K., Kami, T., Fuji, M. (1988). Relation between gelation behaviour of ground chicken muscle and soybean proteins and their differential scanning calorimetric studies. *Journal of Food Sci* 53 1076-1080
4. Barbut, S. & Findlay, J.C. (1991). Influence of sodium, potassium and magnesium chloride on thermal properties of beef muscle. *Journal of Food Sci* 56 (1) 180-182
5. Quinn, J. R., Raymond, D. P. & Harwalkar, V.R.. (1980). Differential scanning calorimetry of meat proteins as affected by processing treatment. *Journal of Food Sci* 45 1146-1149
6. Pighin, D. G., Sancho, A. M. & Gonzalez, C.B. (2008). Effect of NaCl addition on the thermal behaviour of proteins of bovine meat from Argentina. Meat Sci 79 549-556