EFFECT OF ADDED FATTY ACIDS ON HEAT-INDUCED GELATION OF MYOSIN

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SUMMARY: The effect of added fatty acids on the interaction of proteins with fats was examined. Each fatty acid increased the viscosity of myosin except lecithin. Differential Scanning Calorimetric Thermograms clearly showed the denaturation of myosin with the addition of fatty acids around 50°C. A rigid gel was formed by the added fatty acids and the gelling properties of myosin were appeared at 65°C. Lecithin did not change the rigidity of the gel and it is assumed that the heat induced gelation is based on the molecular weight, carbon number, and the saturation or unsaturation of the fatty acid.

INTRODUCTION: Meat proteins stabilize the fat and water contents in comminuted meat products such as sausages and this is predominant in myosin as far as the gelation is concerned. The heat gelling properties exhibit a corelation with the crosslinkings of myosin and fat globules in the sausage batter are covered by a protein film in high quality sausages. The appropriate thickness of the film is an important factor(Theno and Schmidt,1978) and this influence on the water holding capacity and the binding properties of sausages. The interaction of proteins with fats depend on their physiological characteristics, however, the mechanism of this phenomenon is not yet known. On the other hand, one of the workers had reported that there is an affinity between myosin and the acyl base of a long chain fatty acid (Borejido,1983) and another worker suggested that the fatty acids enhanced heat gelling properties of myosin(Egelandsdal et al.,1985). Moreover, it can be assumed that an interaction of fat or fatty acids with myosin or other muscle proteins may takes place and more extensive research on these aspects are much essential because fat tissues are important components of muscle and they contain around 0.5% free fatty acids. These fatty acids are getting increased during the conversion of muscle into meat and the main objective of this study was to observe the role of added fatty acids on heat induced gelation of myosin.

MATERIALS AND METHODS: Fatty acids and lecithin were purchased from SIGMA CHEMICAL CO.,LTD. and all other chemicals used were of the highest purity.

Preparation of myosin; The muscles of rabbit were minced within 10 min postmortem, and blended with 0.3 M KCl in 0.15 M phosphate buffer (pH 6.5). Myosin was prepared according to the method described previously (Yasui et al.,1979).

Viscosity; Viscosity measurements were made with Ostwald-type viscometers at 20°C. Flow-time for solvent ranged from 40 to 60 sec. The measurements were carried out with 0.6 M KCl and 20 mM phosphate buffer (pH 7.0) at 2 mg/ml of protein.

Differential Scanning Calorimetric Study (DSC); The effect of heat on the denaturation of myosin was studied using a Rigaku Denki DSC-8240. DSC curved were recorded for heating of 5°C/min in the temperature range from about 25 to 100°C.

Rigidity; Four mg/ml of myosin in 0.6 M KCl and 20 mM phosphate buffer (pH6.0) was heated at 65 °C for 20 min in a glass cuvette. Rigidity of the gel was measured with a band type viscometer by the method of Yasui et al.(1979).

Scanning Electron Microscopy (SEM); SEM observations were made on the heat-induced gels of myosin using a JEOL, JSM-T200 scanning electron microscope.

RESULTS AND DISCUSSION:

The viscosity of myosin was observed with a constant protein content and added various amounts of fatty acids and lecithin (Fig.1). The maximum viscosity was observed when the

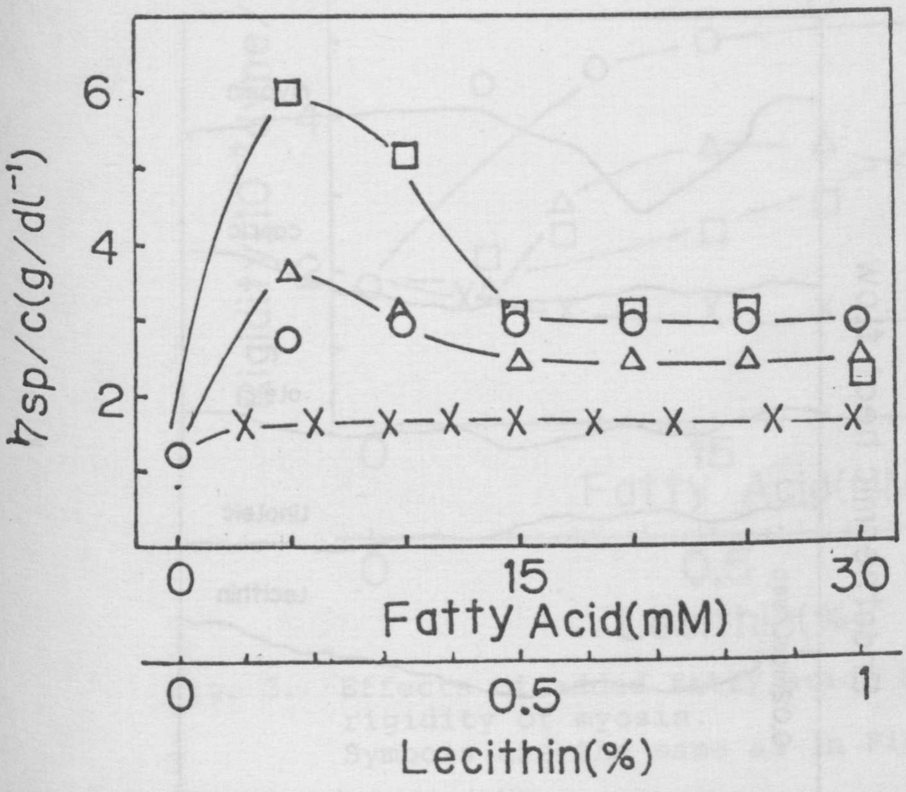

Fig. 1. Effects of added ratty acids and lecithin on the viscosity of myosin.
O : capric acid, □ : oleic acid
△: linoleic acid, ✕ : lecithin

oleic and linoleic acid concentrations were kept at 5 mM. It was gradually decreased above 5 mM and became constant at 15 mM level. In case of capric acid the viscosity increased until 15 mM and thereafter it became constant. On the other hand, lecithin did not reveal any affect on the viscosity and this indicates the aggregation of myosin varies according to different type of fatty acids. This variation may be influenced by the physico-chemical characteristics such as molecular weight, carbon number, and the saturation or unsaturation of the fatty acid.

DSC is a convenient technique which can be used for any type of samples in liquid, solid, or powdered form. Therefore, we can use this method for the denaturation studies to get some useful information. Recently this is widly used in biological studies and Fig. 2 represents the DSC thermograms of myosin with added individual fatty acid. A high endothermic peak with 2 minor peaks on it's shoulders were observed in myosin at about 50°C and the area of this peak expresses the enthalpy for the denaturation of myosin. When capric acid is added, the denaturation of proteins is evident by disappearing the myosin

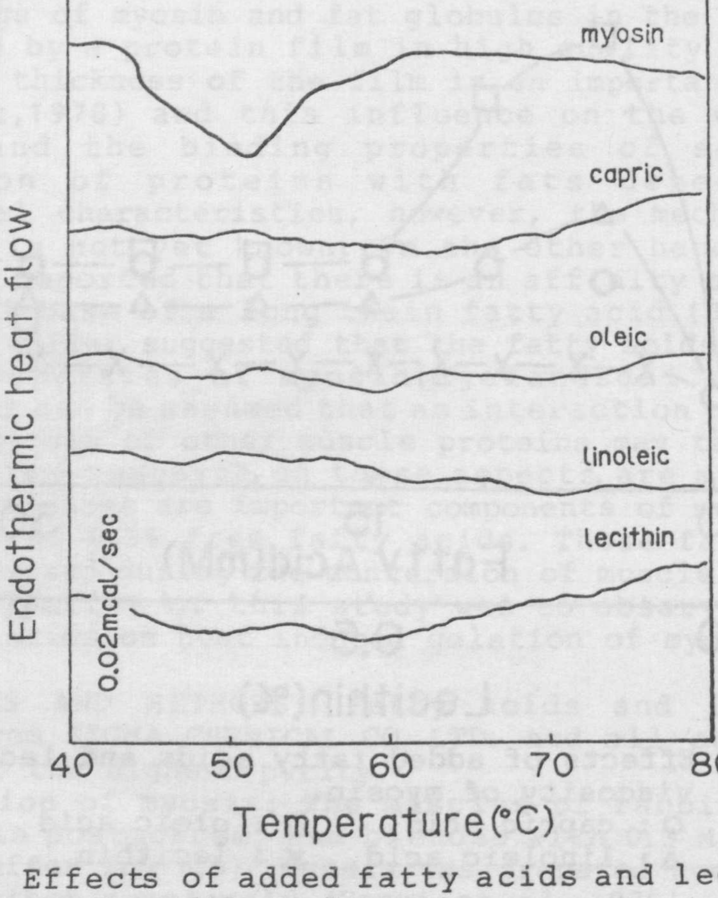

Fig.2. Effects of added fatty acids and lecithin on the DSC profiles of myosin.
Protein concentrations were 10 mg/ml.

peak completely. Just addition of capric acid caused the denaturation of myosin structure before the thermal treatment. The behavior of oleic and linoleic acids was very much similar to that of capric acid and a slight amount of undenatured myosin might have existed in the sample. But in case of lecithin the undenatured myosin was existed when compared to above fatty acids.

The fatty acids had an effect on the gelling properties of myosin at 65°C and this is indicated in Fig. 3. In case of capric acid, the maximum rigidity increased until 15 mM and then decreased slightly or may became constant. Linoleic acid too has a similar trend but it is lower than the capric acid. The addition of 30 mM oleic acid increased the rigidity of the gel continuously. On the other hand, the rigidity did not change with the addition of lecithin as explained in viscosity experiment. In comparison with the maximum points, the highest value was observed in capric acid following linoleic and oleic acid which gained the same values. Viscosity and the rigidity patterns were quite different in each case and this explains the different aggregation mechanisms of myosin before and after the heat treatment.

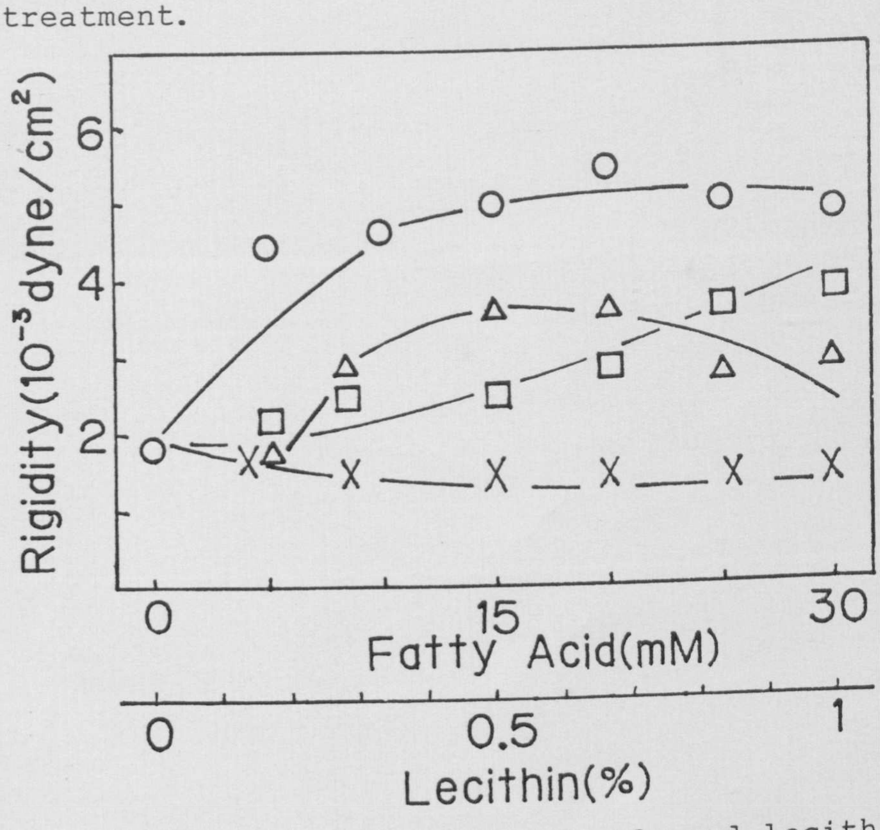

Fig. 3. Effects of added fatty acids and lecithin on the rigidity of myosin.
Symbols are the same as in Fig. 1.

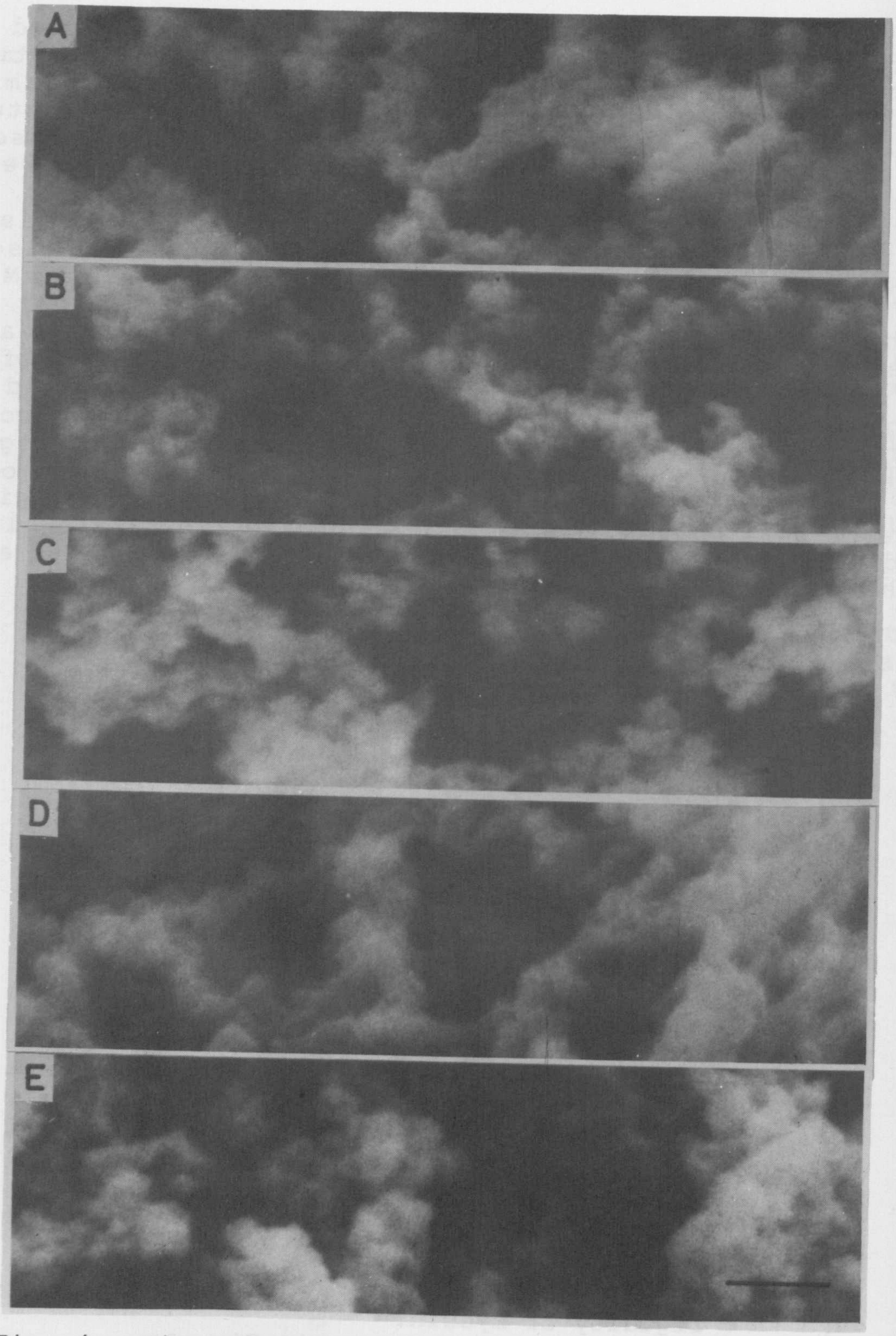

Fig. 4. Microstructures of heat-induced gels of myosin. A: myosin , B: myosin + 15 mM capric acid C: myosin + 15 mM linoleic acid, D: myosin + 15 mM oleic acid, E: myosin + 0.1 % lecithin Bar length is 1 μm.

According to these results, it can be suggested that these fatty acids effect on the heat induced gelation of myosin and this depend on the carbon number, saturation or unsaturation of the fatty acid. However, the used fatty acids in this study was just only 3 and the effect of other fatty acids is now under investigation. When the rigidity results are compared with DSC profiles, capric acid added myosin revealed a higher rigidity than the intact myosin. In case of oleic, linoleic, and lecithin mixed samples contained a certain amount of intact myosin and this might have caused the weak rigidity of the gel. The appropriate gelation on the myosin structure was caused by the added capric acid. The results of microstructure of heat induced gels observed with SEM are indicated in Fig. 4. There was a strong gel with a fine microstructure in capric acid added myosin when compared with myosin by itself. But in weak gels such as linoleic,lecithin, and oleic acid revealed a course structure when compared with that of capric acid. These results significantly coincide with those of rigidity.

CONCLUSIONS: It can be concluded that the free fatty acids can effect on the aggregation properties of myosin molecules and this is basically depend on the physico-chemical characteristics of the fatty acid. These fatty acids can denaturate myosin molecules and enhance the gelling properties. Therefore, it can be assumed that the free fatty acids presence in the sausage batter can cause different micro-structured gels with different rigidities.

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