J. Korean Soc. Food Nutr.
19(3), 219~223(1990)

Acetylation of Fish Protein from Alaska Pollack

Jeong-Hwa Hong*, Jin-Ho Choi and Dae-Seok Byun

Dept. of Nutrition and Food Science, National University of Pusan, 608-023, Korea.

Abstract

Myofibrillar protein from Alaska pollack was modified with acetic anhydride at pH 7.5 and 25℃ and changes in functional properties as affected by the degree of modification were determined. Acetylation of myofibrillar protein resulted in protein with unique functional properties dependent upon the degree of acetylation. By selecting appropriate degree of modification, it was possible to control protein solubility, heat coagulability, calcium precipitability, foaming and emulsion capacity.

Introduction

High sensitivity of fish proteins to temperature, pH, salts and contact with polar solvent resulted in a substantial loss in functional properties when prepared as dry protein concentrates or isolates for use in food ingredients.[1] This problem was largely overcome by the approach described by Groninger[2] for the preparation of a functional protein. He showed that the acylation of fish myofibrillar protein with succinic anhydride produced a modified protein that was potentially useful as a functional ingredient in some food systems.

Acylation reactions appear to have the most potential for chemically modifying food proteins. Basically, the acylation of protein involves the reaction of a nucleophilic group with reactants that are electrophilic to form amide products.[3] In most cases, the anhydrides of acetic and succinic acids are the acylating agents of choice because of their ease of use, low cost, relative safety, and capacity to produce acylated dertvatives that are important from a functional standpoint. Moveover, succinic acids are present in the tricarboxylic acid cycle, and with Coenzyme A, acetic acids can be transformed to acetyl-Co A by the action of acetate thiokinase. Thus, derivatives of them are least likely to be toxic.[4]

This investigation has been focused on the acetylated fish protein derivatives in order to better characterize and evaluate it as a potential food ingredient. The performance of acetylated fish myofibrillar protein has been compared with that of unmodified protein. The relationship between functional properties and extent of acetylation was more specifically defined.

Materials and Methods

Materials

Alaska pollack fillets were used because of commercial availability. Those fillets not used immediately were rapidly frozen and stored at -15℃.

Preparation of myofibrillar protein

Myofibrillar protein was prepared by the method of Groninger.[2] Briefly, the comminuted fish muscle was washed in 0.1M NaCl to remove most of the sarcoplasmic proteins. The insoluble muscle proteins, myofibrils, were solubilized with 0.6M

* Present address : Dept. of Food and Nutrition, Injae University, Kimhae, Kyungnam.

NaCl.

Acetylation

Acetic anhydride was added to myofibrillar protein solution by varying ratios. During the reaction, the pH was maintained to 7.5 with 2M NaOH. After pH of the solution was stabilized, the same volume of absolute ethanol was added. The resulting precipitate was centrifuged at 10,000 × g for 20 min. Pellets were washed with 50% ethanol followed by recentrifugation. Pellets were dried under nitrogen gas and dispersed in small volume of distilled water.

Analytical determinations

Protein was determined by the method of Bradford.[5] The extent of acetylation of protein was estimated by the method of Adler Nissen.[6] Degree of acetylation was expressed as percent reducton of available lysine.

Determination of functional properties

For protein solubility, heat coagulation and calcium precipitability, pH of protein suspensions (2 % w/v) was adjusted by adding the same volume of 0.1M sodium phosphate (pH 7.0) or sodium actetate buffer (pH 4.8). Solubility was calculated as % protein content in superantant to that in suspension. Heat coagulation was determined by modification of the Kramer and Kwee method.[7] The supernatant, obtained from solubility determination, was heated at 98℃ in a water bath for 30 min, cooled to room temperature, and centrifuged at 10,000 × g for 20 min. Protein content of supernatant was determined by the Bradford method.[5] Heat coagulation was represented as % of protein coagulated by heat to total protein of the suspension before heating. To determine calcium precipitability, calcium chloride was added to the supernatant obtained from the solubility test.[8] Protein content of the supernatants before and after centrifugation at 10,000 × g was determined by the Bradford method.[5] Precipitability was expressed as % of protein precipitated by calcium chloride to protein of the supernatant before addition of calcium chloride.

Foaming capacity was determined by the method of Wang and Kinsella.[9] The height of the foam was recorded as an index of foaming capacity of protein.

Turbidimetry was evaluated as a method for measuring emulsifying properties of proteins.[10] Emulsions were made by homogenizing 5 ml of 1% protein solution and 5 ml of soybean oil. The emulsions were serially diluted to give absorbance of 0.01−0.6 at 500 nm.

Results and Discussion

Extent of Chemical Modification

The degree of chemical modification, expressed as percent available lysine reduced, increased with the concentration of acetic anhydride (Fig. 1). Up to 0.2g of acetic anhydride/g protein, the reduction was linear. The maximum extent of modification was 93.8%. With this plot, degree of acetylation

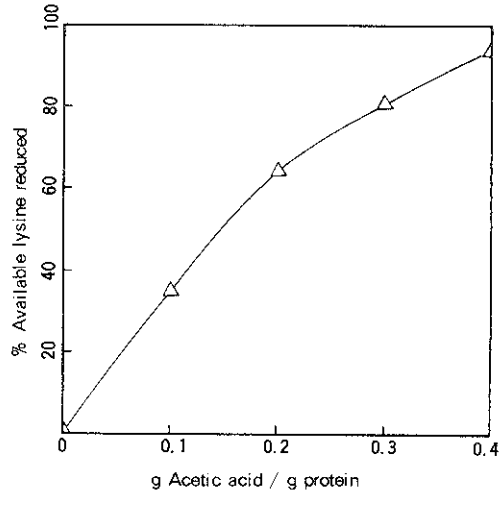

Fig. 1. Extents of acetylation with varying amount of acetic anhydride added.

could be controlled by selecting appropriate ratio between the amount of acetic anhydride and protein.

Protein Solubility

As shown in Fig. 2, solubility of protein at pH 4.5 drastically decreased upon acetylation. All the acetylated protein showed protein solubility of ca. 1.5%. This low solubiluty of protein might be advantageous for recovering proteins just by lowering pH of the solution.

At pH 7.0, protein solubility gradually decreased as the degree of acetylation increased. only 60% of protein was solubilized at over 80.6% acetylation. At higher levels of acetylation, the protein would have acquired a number of hydrophobic groups and the presence of large numbers of hydrophobic groups may be expected to reduce the solubility of the protein in water.

Heat Coagulation

As shown in Fig. 3, acetylated proteins showed very low heat sensitivity at pH 7.0. Little proteins were precipitated upon heating at pH 7.0. Even at pH 4.5, proteins of 35% acetylation showed no precipitation on heating. However, heat sensitivity

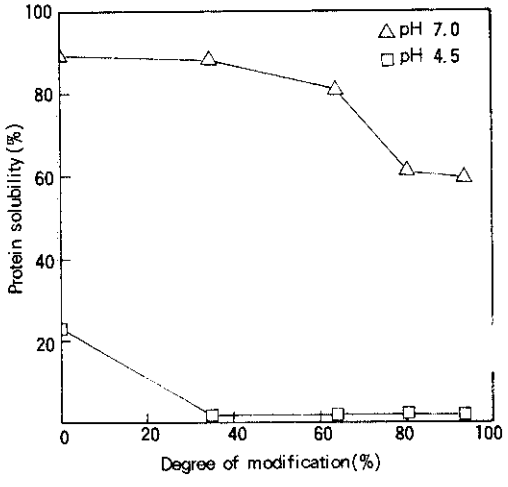

Fig. 2. Changes in protein solubility as affected by acetylation.

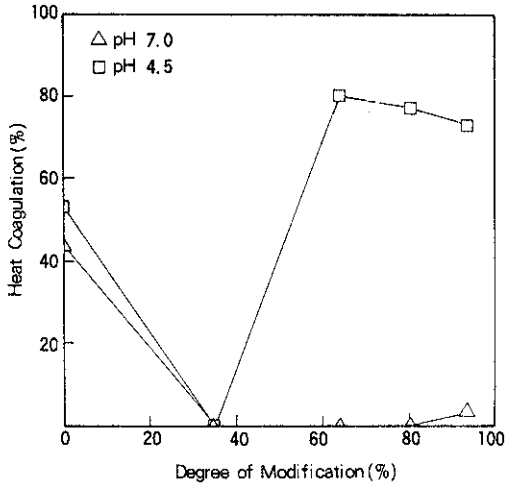

Fig. 3. Changes in heat coagulation as affected by acetylation.

at pH 4.5 were significantly increased in the cases of proteins of 64.1% acetylation or over.

Low heat sensitivity of acetylated proteins might be the result of the increased negative charges upon acetylation. Repulsion between protein molecules kept them from coalescing. At pH 7.0, functional carboxyl groups usually possess strong negative charges with reduced positive charges due to acetylation of ε-amino groups of lysine. Problem arose to explain the high heat sensitivity at pH 4.5 if increased negative charges was the only reason for heat sensitivity. These highly acetylated proteins (64.1% or over) possess much lower net negative charges at pH 4.5 than those at pH 7.0. Functional carboxyl groups tend to have higher pK values than C-terminal. At pH 4.5, functional carboxyl groups will be ionized one-half or slightly more. In contrast, carboxyl groups will be fully ionized at pH 7.0. Thus increased hydrophobic groups upon acetylation might overcome the small increase in negative charges at pH 4.5 resulting in high heat sensitivity.

Calcium Precipitability

Calcium sensitivity of acetylated proteins was decreased remarkably compared to that of control

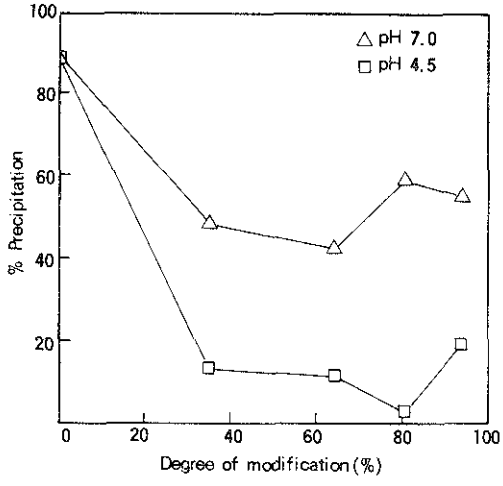

Fig. 4. Changes in calcium precipitability as affected by acetylation.

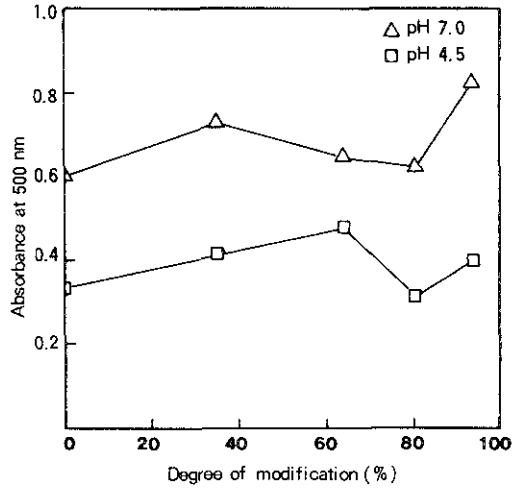

Fig. 5. Changes in emulsion capacity as affected by acetylation.

(Fig. 4). Acetylation of 64. 1% and 80.6% showed the lowest sensitivity to calcium at pH 4.5 and pH 7.0, respectively. The mechanism of precipitation upon addition of calcium ion has not been clearly understood at the present time ; however, combined effects of increased negative charges and hydrophobic groups seemed the reason for this low Ca^{++} —precipitation in this case. Highly acetylated proteins showed slightly higher sensitivity to Ca^{++} ion than low acetylated ones. Increased hydrophobicity appeared to raise the sensitivity to calcium ion.

Emulsifying Capacity

Emulsifying capacity was also improved by acetylation of proteins (Fig . 5). At pH 7.0, acetylation of 93.8% followed by 35% showed significant increase in emulsion capacity. In the case of pH 4.5, however, 64.1% acetylated protein showed the highest emulsion capacity. Slightly low emulsion capacity was observed with 80.6% acetylated protein compared to unmodified proteins. Neither increased hydrophobicity nor negative charges can explain this tendency. Ratios between negative charges and hydrophobicity might play an important role in emulsion capacity because the degree of ionization of functional carboxyl groups should be much higher at pH 7.0 than that at pH 4.5.

Foaming Capacity

Foaming capacity was not improved by acetylation at pH 7.0 ; on the contrary, low foaming capacity was observed with 35% acetylated proteins (Fig. 6). In contrast, significant improvement in foam expansion at pH 4.5 was achieved by acetylation. Almost two-fold increase in foaming capacity

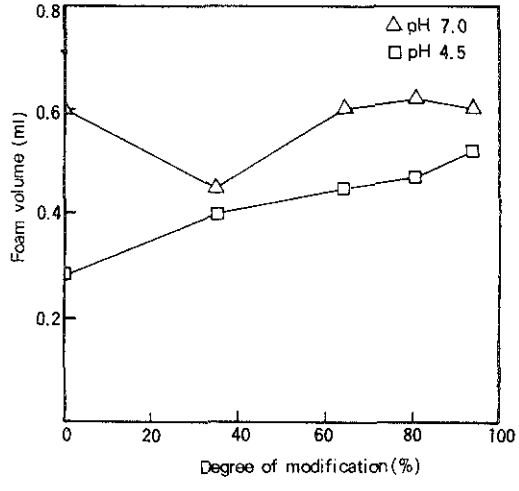

Fig. 6. Changes in foaming properties as affected by acetylation.

at pH 4.5 was obtained with 93.8% acetylated proteins. Apparently higher hydrophobicity combined with low degree of ionization of carboxyl groups lowers interfacial tension between gas and water, facilitating deformation of the liquid and expansion agaist its surface tension.

In conclusion, fish myofibrillar protein can be utilized as food ingredients by acetylation. The degree of acetylation should be carefully selected to satisfy the specific food system because functional properties required might vary from one system to another.

References

1. Tannenbaum, S. R., Ahern, M. and Bates, R. P. : Solubilization of fish protein concentrate; 1. and alkaline process. *Food Technol.* **24**, 96 (1970)
2. Groninger, H. S., Jr. : Preparation and properties of succinylated fixh myofibrillar protein. *J. Agric. Food Chem.* **21**, 978 (1973)
3. Spinelli, J., Koury, B., and Miller, R. : Approaches to the utilization of fish for the preparation of protein isolates ; Enzymic modifications of myofibrillar fish proteins. *J. Food Sci.* **37**, 604 (1972)
4. Franzen, K. L. and Kinsella, J. E. : Functional properties of succinylated and acetylated soy protein. *J. Agric. Food Chem.* **24**, 788 (1976)
5. Bradford, M. M. : A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248 (1976)
6. Adler-Nissen, J. : Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzene sulfonic acid. *J. Agric. Food Chem.* **27**, 6(1979)
7. Kramer, A. and Kwee, W. H. : Functional and nutritional properties of tomato protein concentrates. *J. Food Sci.* **43**, 207 (1977)
8. Choi, Y. R., Lusas, E. W., Rhee, K. C. : Succinylation of cottonseed flour;effect on the functional properties of protein lsolates prepared from modified flour. *J. Food Sci.* **46**, 954 (1981)
9. Wang, J. C. and Kinsella, J. E. : Functional properties of novel proteins;alfalfa leaf protein. *J. Food Sci.* **41**, 286 (1976)
10. Pearce, K. N. and Kinsella, J. E. : Emulsifying properties of proteins;evaluation of a turbidimetric technique. *J. Agric. Food Chem.* **26**, 716 (1978)

(Received November 6, 1989)

명태 근육단백질의 아세틸화에 따른 기능성의 변화

홍정화 · 최진호 · 변대석

부산수산대학교 식품영양학과

요 약

명태(Alaska pollack)의 근원섬유 단백질을 Acetic anhydride로 pH 7.5 25℃에서 화학적으로 수식하였다. 그리고 이에 따른 기능성의 변화를 조사하였다. 근원섬유단백질의 아세틸화는 그 수식정도에 따라 독특한 기능성을 가진 단백질을 만들어내었다. 따라서 적합한 수식정도의 선택에 따라 단백질 용해도, 열 응고성 칼슘 침전성과 거품 및 유화성을 조절할 수 있음을 제시하였다.