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Changes in Sarcoplasmic and Myofibrillar Proteins of Spent Hen and Broiler Meat during the Processing of Surimi-like Material (Ayami)

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ABSTRAK

Ayam tua (Rhode Island Red), berumur 18 bulan dan ayam muda (White Leghorn) berumur 2 bulan di proses menjadi bahan seakan surimi, yang dinamakan Ayami. Kaedah pemprosesan ialah pengisaran dua kali melalui plat pengisar 5mm, diikuti oleh tiga tahap pembasuhan dengan air ais. Daging basuhan yang terhasil dicampur dengan krioptotektan dan disimpan dalam bag polietilen pada suhu -15°c. Perubahan kepada protein-protein sarkoplasmik dan miofibrillas diperhati semasa tahap pemprosesan melalui kaedah SDS-PAGE. Pola elekforesis menunjuk pengurangan ketumpatan protein-protein miofibrillas selepas basuhan pertama, dengan sedikit perubahan selepas basuhan kedua dan ketiga. Ketumpatan protein-protein sarkoplasmik berat molikul kurang dari 100.000 Dalton meningkat.Pembasuhan memberi kesan yang nyata terhadap protein-protein sarkoplasmik dan miofibrillar daging ayam tua dan muda. Hasil seperti ini boleh memberi penjelasan terhadap ciri-ciri pengenalan bahan seakan surimi seperti Ayami.

ABSTRACT

Spent hens (Rhode Island Red), aged 18 months and broilers (White Leghorn) aged 2 months were processed into a surimi-like material called Ayami. The processing method includes grinding the material twice through a 5mm grinder plate, followed by three steps of washing with ice cold water, after which the washed meat is mixed with cryoprotectants and stored in polythylene bags at - 15°C. Changes in sarcoplasmic and myofibrillar proteins during the processing stages were monitored using the SDS-PAGE method. The electrophoretic pattern showed a decrease in intensity in the components of myofibrillar proteins after the first wash, with little changes after the second and third wash. For sarcoplasmic proteins, the intensity of components with molecular weights lower than 100,000 Dalton decreased, whereas the components with molecular weights higher than 100,000 Dalton increased. Washing has significant effects on the sarcoplasmatic and myofibrillar proteins of both spent hen and broiler meat. Such results could explain the gelation properties of surimi-like materials like Ayami.

INTRODUCTION

In traditional processing of surimi, deboned fish meat is washed to remove water soluble components that originally contributed to undersirable odour, taste and fishy smell. The washed meat was then mixed with spices, salt and sugar to yield fish gel known as kamaboko (Lee 1984, Piggot, 1986). Washing removed fat, blood, pigments, and water soluble proteins from meat (Grant 1985) which results in an increase of myofibrillar protein especially actomyosin that is responsible in gel formation (Grant 1985; Suzuki 1981). Washing also results in loss of sarcoplasmic and myofibrillar protein in the ratio of 1.7:1 (Lee 1984). Frozen storage of fish meat results in protein denaturation leading to poor gel formation in products like kamaboko and fish ball. The Japanese in 1959 were able to reduce the loss of such functional properties by adding of cryoprotectants such as sucrose (5 - 10%), sorbitol (0 - 5%) and polyphosphate (0 - 3%) (Piggot 1986). Meat proteins are classified into myofibrillar, sarcoplasmic and stroma proteins (Anglemier and Montgomery 1976). Myofibrillar proteins are made up of 12-14 major proteins such as myosin, actin, tropomyosin troponin and actinin (Morrissey *et al.* 1987). These proteins are functional proteins, responsible for the conformational structure and

eating quality of meat and meat products. (Kinsella 1982). Sarcoplasmic proteins form about 25-30% of the total protein content and are mostly water soluble glycolytic enzymes and pigments (Hultin 1985). They are less viscous with a lower water holding capacity (WHC) and molecular weights ranging between 20,000 - 100,000 Dalton (Morrissey *et al.* 1987). Stroma proteins are insoluble in water and salt solution; the main component is collogen (40 - 60%) with smaller amounts of elastin (10 - 20%), reticulin, and glycoprotein (Fawcett and Bloom 1968).

In the production of surimi-like materials, the yield and physico-chemical changes occur as a result of processing spent hen and buffalo meat into surimi-like materials (Johiria and Babji 1990, Gna and Babji 1991, and Babji *et al* 1992). Since gelation of products like 'Ayami' and 'Beefrimi' are largely dependent on the processing method and changes in the protein components, further study is necessary to explain the changes due to various processing variables. This study looked at the changes in the sarcoplasmic and myofibrillar protein components of spent hen and broiler meat as a result of grinding and three steps of washing in the production of surimi-like material called Ayami. In this study chilled tap water was used for washing, even though other washing solutions have been suggested by Yang and Froning (1992).

MATERIALS AND METHODS

Source of Meat

Fresh dressed carcasses of spent hen (Rhode Island Red) aged 18 months and broiler chicken (White Leghorn) aged 2 months were obtained from a local supplier and frozen at -15°C for two days. The carcasses were thawed overnight at -5°C, deboned manually, and stored at -15°C for 1 week until ready for processing.

Washing Procedures

Partially frozen meat (-5°C) were ground using a Hobart grinder (3.8 mm plate) twice. Iced water (5°C) was added in the ratio of 3:1 (water:meat). Mixing was done manually for 30 sec and the mixture allowed to settle down for 5 m. The fat and top water layer were poured off; and the remaining mixture was filtered through a test sieve of size 425 µm. A cheese cloth was placed beneath the sieve to trap escaped meat particles. Filtrate from the sieve was combined with those trapped in the cheese cloth, then pressed manually with a screw press. This procedure was repeated twice to obtain a whitish washed surimi-like material from the broiler and spent hen meat. Mixing of 2% w/w sucrose, 2% w/w sorbitol and 0.3% w/w sodium pyrophosphate on wet basis with the washed meat was carried out in a Hobart bowl-chopper for 5 m. The surimi-like product called 'Ayami' was packed in polyethylene bags and stored at -15°C until ready for further analyses. Results on yield and physico-chemical changes during processing and storage have been reported earlier by the same group of researchers Gna and Babji (1991) and Babji *et al.* (1991).

Analytical Procedures

Extraction of Sarcoplasmic and Myofibrillar Proteins

Myofibrillar protein was extracted following the method of Hay *et al.* (1973). A 20 g meat sample was homogenised with 80 ml of 0.25 M sucrose, 1mM disodium ethylenedinitrilo tetraacetate (EDTA), 0.05 M Tris [tris (hydroxymethyl) aminomethane] pH 7.6 extracting solution, for 15 sec at 45 sec intervals. This was repeated 3 times. The extract was stirred for 1 h at 4°C, then centrifuged at 2500 x g at 4°C for 10 m using a Coolspin Model MSE centrifuge. The supernatant was used as the source for sarcoplasmic protein. The residue was dissolved in 80 ml of 0.05 M Tris pH 7.6, 1mM EDTA extracting solution, stirred for 10 m at 4°C. The homogenate was passed through a 3 layered cheese cloth to remove connective tissue protein. The crude myofibrillar protein was purified by washing with the following solutions separately: 0.15 M KCl, 0.03 M Tris pH 7.6; 1mM EDTA, pH 7.6; deionized water; 0.15MKCl, 0.03M Tris pH 7.6. For each wash, the myofibrillar protein was centrifuged at 2500 x g at 4°C for 10 m. It was stirred for 10 m at 4°C before each washing. The final myofibrillar protein was dissolved in 0.15 MKCl, 0.03 M Tris, pH 7.6. The myofibrillar and sarcoplasmic proteins were kept at -18°C.

Preparation of Sample for Electrophoresis

Samples of washed broiler and spent hen meat at various steps of the processing operation were diluted with buffer solution (2.3 ml 10% SDS solution, 5.2 ml distilled water, 1.0 ml glycerol, 0.5 ml 2-mercaptoethanol, 1.0 ml 0.625 M Tris-HCl pH 6.8 solution and a drop of Bromophenol

Blue) to 1000 ug ml, boiled for 5 m and then cooled before placing in the gel for electrophoresis.

Gel Preparation

Electrophoresis glass plates (14 x 14 cm) were soaked in chromic acid overnight, then washed with soap and water. The plates were dried and then prepared as a mold for gel preparation. Separating gel (12% gel) was prepared from:

Distilled water, 33.5 ml

1.5M Tris HCl, pH 8.8, 25.0 ml

10% SDS, 1.0 ml

Acrylamide/Bis (30.0:0.8), 40.0 ml

10% Ammonium persulfate, 0.5 ml.

All the materials were stirred and deaerated using a vacuum pump for 15 m; 50 ml 6.6M N,N,N'; N'-tetrametyl-ethylenediamine (TEMED) was added to polymerise the gel. The mixture was poured into the column space between the gel plate.

Casting gel (4%) was prepared from:

Distilled water, 6.1 ml

0.62 M Tris HCl, pH 6.8, 2.5 ml

10% SDS, 100 ml

Acrylamide/Bis, 1.3 ml

10% Ammonium persulfate, 0.05 ml.

All the materials were stirred, and deaerated as above. 10µl 6.6M TEMED was added for polymerization, after which it was added to the top of the separating gel. A comb was placed on top of the casting gel to form 15 columns for electrophoresis.

SDS-PAGE

The polymerized gel in the glass plate was clipped to a casting stand at the inner cooling core and placed in an electrophoresis tank with 200 ml of buffered solution. A 50 µl sample was pipetted into each column with a micro pipette. Electrophoresis was run with a 100 volt power supply at 4°C. The time taken for the dye to reach the end of the plate was 5-6 h. The gels were taken out, dyed overnight with continuous shaking in a SLT Lab instrument shaker (model MPS-4). The gels were destained until clear protein bands were seen on the gel plate. The various solutions necessary for the electrophoresis were as follows:

Acrylamide-bisacrylamide monomer solution is made up of 30.0 g acrylamide and 0.8 gN, N-bis-methylene-acrylamide (bisacrylamide) dissolved in 100 ml distilled water, filtered and stored cold at 4°C in a dark bottle.

Separating gel buffered solution (1.5M Tris HCl, pH 8.8) was prepared from 13.6 g Tris base (hydroximethylaminomethane) dissolved in 100 ml distilled water. It was stored at 4°C.

Casting gel buffered solution (0.625m Tris HCl, pH 6.8) was prepared from 7.57 g Tris-base (hydroxymethylaminomethane) dissolved in 100 ml distilled water and pH adjusted to 6.8 with HCl. It was stored at 4°C.

Tank buffered solution was prepared from 10.0 g dodecyl sulfate (SDS), 144.0 of glycine and 30.3 g Tris-base dissolved in 1.0l distilled water and pH adjusted to 8.3 with HCl. The solution was kept at 4°C. 300 ml of buffered solution was diluted 10 times before placing in the tank for electrophoresis.

Buffered samples were prepared by adding 2.3 ml 10% SDS solution, 5.2 ml distilled water, 1.0 ml glycerol, 0.5 ml 2-mercaptoethanol, 1.0 ml 0.625 M Tris HCl pH 6.8 solution, and a drop of Bromophenol Blue.

Staining solution was prepared by mixing 6.25 ml of 1% Coomasie Blue R-250, 250 ml methanol and 50 ml of 10% acetic acid. Distilled water was added to make up to 500 ml.

Destaining solution was prepared from 500 ml of methanol and 100 ml of 10% acetic acid mixed and diluted to 1.01 with distilled water.

Determination of Molecular Weights of Proteins

The proteins molecular weights were determined by measuring the relative mobility and reading the semi log graph for molecular weights versus relative mobility (Weber and Osborne 1969). Using the standard curve in *Fig. 1*, the relative mobility of various sarcoplasmic and myofibrillar protein components were estimated for their molecular weights. Protein standards used for obtaining the standard curve are carbonic anhydrase (29,000 Dalton) Ovalbumin (45,000 Dalton), Bovine albumin (66,000 Dalton), Phosphorylase B (97,400) and Myosin (205,000 Dalton). The Rf (Relative mobility) for protein standard of molecular weight 29,000 Dalton was slightly higher due to the increase in voltage after 4 h of electrophoresis. This resulted in a standard curve rather than the normal straight line standard curve (*Fig. 1*).

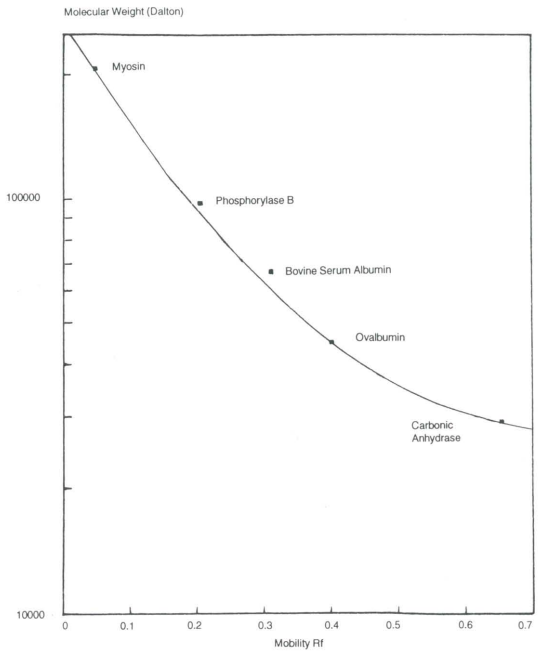

Fig.1: Standard curve for determining the molecular weights of proteins

RESULTS AND DISCUSSION

Sarcoplasmic Proteins

Table 1 and *Fig. 2* show the changes in intensity of sarcoplasmic proteins as a result of processing spent hen and broiler meat into Ayami. Grinding of meat did not show any change in the sarcoplasmic proteins in both treatments. However, washing significantly affected the distribution pattern of sarcoplasmic protein in Ayami. Washing three times resulted in the increase of protein intensity of higher molecular weights (> 100,000 Dalton), while those with 100,000 Dalton and below showed a decrease, especially proteins of molecular weights 28,500, 29,500, 37,000, 40,000, 45,000 and 55,000 Dalton. The washing process seemed to have separated and washed out sarcoplasmic proteins of 100,000 Dalton and below; and this resulted in better gelation properties of Ayami from both spent hen and broiler meat. Graboswaska *et al.* (1976) reported that sarcoplasmic proteins did not possess gelation properties but only coagulated when heated to 80°C. But detailed electrophoretic analyses of sarcoplasmic proteins in this study indicate that more lower molecular weights (< 100,000 Dalton)

TABLE 1
Relative mobility (Rf) and molecular weights of sarcoplasmic proteins of ayami

Band	Rf	Molecular Wt.	Washing effect
1.	0.023	230,000	
2.	0.046	205,000	
3.	0.061	160,000	
4.	0.114	155,000	Increased in
5.	0.136	140,000	intensity (visual observation)
6.	0.159	125,000	
7.	0.189	110,000	
8.	0.231	94,000*	
9.	0.247	90,000	
10.	0.264	82,000	
11.	0.297	72,000	
12.	0.364	55,000#	
13.	0.405	45,000#	
14.	0.455	40,000#	Decreased in
15.	0.504	37,000#	intensity (visual observation)
16.	0.537	35,000*	
17.	0.636	30,000	
18.	0.652	29,500#	
19.	0.678	28,500#	
20.	0.727	27,500	

* Only in Spent Hen
\# Decrease Clearly

were washed out, leaving higher molecular weights sarcoplasmic proteins in Ayami. Gna and Babji (1991) reported improved gelation properties in Ayami products as a result of processing. Yang and Froning (1992) in their study on pH and mixing time on protein solubility of washed MDCM reported significant wash out of several proteins. Fractions, and lower molecular weight soluble proteins (31,000 - 97,000 Dalton) were amongst those contributing to lower yield of washed meat. This has raised the possibility that sarcoplasmic proteins of molecular weights *greater than* 100,000 Dalton could play a role in the gelation process of surimi-like material such as shown with Ayami in this study. Further studies are necessary to investigate the roles of higher molecular weight sarcoplasmic proteins in the gelation process of such surimi-like materials.

Myofibrillar Proteins

Table 2 and *Fig. 3* show the changes in intensity of myofibrillar proteins as a result of processing spent hen and broiler meat into Ayami. The grinding process did not result in changes in the electrophoretic pattern of myofibrillar proteins. However, after the first washing, the intensity of myofibrillar proteins decreased, especially myosin and actin. The second and third washings did not show much reduction of the myofibrillar proteins.

Washing removes fat, blood, pigments, odorous matter, inorganic salts and water soluble enzymes from meat (Grant 1985). Separation of these components results in an increase of functional myofibrillar proteins, responsible in the formation of gel (Grant 1985; Suzuki 1981). Surimi is the result of such washing procedure,

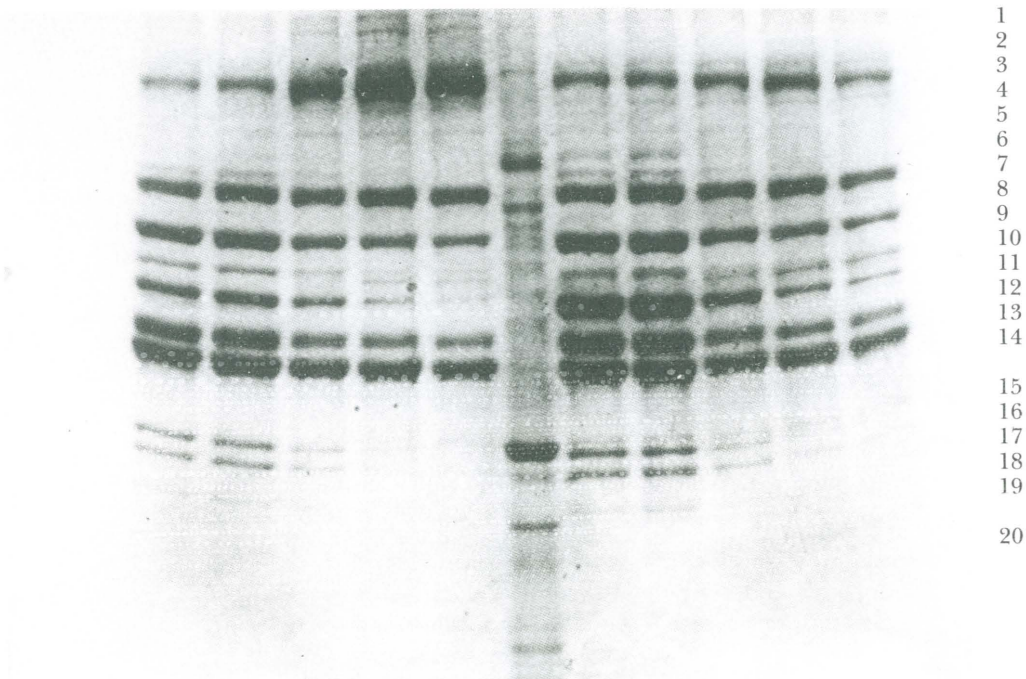

Fig. 2: Changes in sarcoplasmic proteins of broiler and spent hen meat due to various washing and processing procedures

TABLE 2
Relative mobility (Rf) and molecular weights of myofibrillar proteins of ayami

band	Rf	molecular wt.	components
1.	0.045	205,000	myosin
2.	0.116	155,000	
3.	0.134	140,000	actinin and
4.	0.170	120,000	m-protein
5.	0.430	41,000	actin
6.	0.509	37,000	tropomyosin
7.	0.536	34,000	
8.	0.598	31,500	
9.	0.652	29,500	
10.	0.741	27,000	troponin and
11.	0.777	25,500	myosin subunit
12.	0.911	22,000	
13.	0.946	21,000	

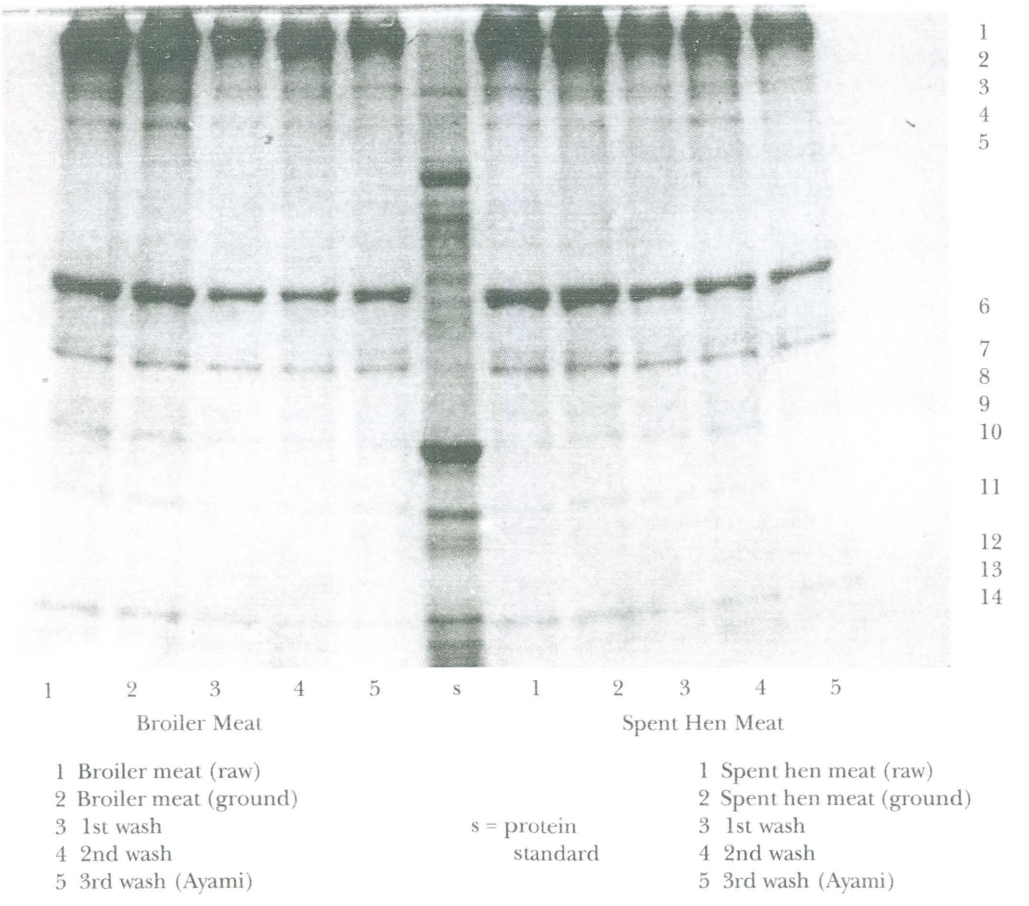

Fig. 3: Changes in myofibrillar proteins of broiler and spent hen meat due to various washing and processing procedures

leaving a material that is white in colour and high in myofibrillar proteins. However, washing has resulted in losses of up to 30% or more of fish meat (Lee 1984). Included in the loss, are water soluble proteins as well as myofibrillar proteins in the ratio of 1.7:1 (Lee 1984).

Gna and Babji (1991) reported the changes in the ratio of salt soluble proteins (myofibrillar) to sarcoplasmic proteins, which increased as a result of washing.

CONCLUSION

Electrophoretic analyses indicate that washing of broiler and spent hen meat results in some major changes in the intensity of sarcoplasmic and myofibrillar proteins. Washing resulted in the reduction of lower molecular weights (<100,000 Daltons) sarcoplasmic proteins and an increase in the higher molecular weights (>100,000 Daltons) sarcoplasmic proteins. The reduction of myofibrillar proteins was only significant after the first washing step, with second and third washing showing little losses. Results from this study suggest that the presence of sarcoplasmic proteins of higher molecular weights could be important in the process of gelation, since surimi-like material (Ayami) obtained after the third washing possesses good gelation properties similar to those commonly associated with surimi.

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