CHANGES IN PROTEIN COMPONENTS DURING PROCESSING OF CHICKEN SURIMI (AYAMI)

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SUMMARY

Spent hens (Rhode Island Red), aged 18 months and broilers (White leghorn) aged 2 months old, were processed into a surimi-like material called Ayami. The processing method includes grinding twice through a 5mm grinder plate, followed by 3 steps washing with ice cold water, after which the washed meat is mixed with cryoprotectants and stored in polyethylene 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. This study showed that washing steps have significant effects on the sarcoplasmic and myofibrillar proteins of both spent hen and broiler meat. Such results could assist in explaining 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). This resulted in increase of myofibrillar protein especially actomyosin that is responsible in gel formation (Grant, 1985; Suzuki, 1981). Washing also resulted in loss of sarcoplasmic and myofibrillar protein in the ratio of 1.7:1 (Lee, 1984). Frozen storage of fish meat resulted in protein denaturation, leading to poor gel formation in products like kamaboko and fish ball. The Japanese in 1959 managed to reduce the loss of such functional property by the addition 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 & Montgomery 1976). Myofibrillar proteins are made up of 12-14 major proteins like myosin, actin, tropomyosin troponin and actinin (Morrissey, et al. 1987). These proteins are functional proteins, responsible in the conformational structure and eating quality of meat and meat products. (Kinsella, 1982). Sarcoplasmic proteins formed about 25-30% of the total proteins and they are mostly water soluble glycolytic enzymes and pigments (Hultin, 1985). They are less viscous with lower water holding capacity (WHC) and molecular weights ranging between 20,000 - 100,000 daltons (Morrissey et al. 1987). Stroma proteins are insoluble in water and salt solution, with collogen (40 - 60%), as the main component and smaller amounts of elastin (10 - 20%), reticulin, and glycoprotein (Fawcett & Bloom 1968). In the production of surimi-like materials, Babji et al. (1992), Gna and Babji (1991), and Johina and Babji (1990) reported on the yield and physico-chemical changes as a result of processing spent hen and buffalo meat into surimi-like materials. 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 washing involved 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 & Froning (1992).

MATERIALS AND METHODS

Source of meat

Fresh dressed carcasses of spent hen (Rhode Island Red) age 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. Ice water (5°C was added in the ratio of 3:1 (water:meat). Mixing was done manually for 30 seconds and the mixture allowed to settle down for 5 minutes. The fat and top water layer was poured off, while the rest 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 broiler and spent hen meat respectively. 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 minutes. The surimi-like product called 'Ayami' was packed in polyethylene bags stored at -15°C until ready for further analyses. Results on yield and physico-chemical changes during processing and storage were 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 seconds with 45 seconds interval. This was repeated 3 times. The extract was stirred for 1 hr at 4°C, then centrifuged at 2500 x g at 4°C for 10 minutes using 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 minutes 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 seperately: 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 minutes. It was also stirred for 10 minutes at 4°C before each washing. The final myofibrillar protein was dissolved in 0.15 MKCl, 0.03 M Tris, pH 7.6. Both 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 minutes 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 minutes. 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 dearated as above. 10 µl 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 column 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 using 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 hours. The gels were taken out, dyed overnight with continuous shaking in 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 follow: 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 liter 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 liter 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 & Osborne 1969). Using the standard curve in Figure 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 hours of electrophoresis. This resulted in a standard curve as shown in Figure 1, rather than the normal straight line standard curve.

RESULTS AND DISCUSSION

Sarcoplasmic Proteins

Table 1 and Figure 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 affect the distribution pattern of sarcoplasmic protein in Ayami. Washing 3 times resulted in the increase of protein intensity of higher molecular weights (> 100,000 Daltons), while those with 100,000 Daltons and below showed a decrease, especially proteins of molecular weights 28,500, 29,500, 37,000, 40,000, 45,000 and 55,000 Daltons. The washing process seemed to have separated and washed out sarcoplasmic proteins of 100,000 Daltons 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 indicated that more lower molecular weights (< 100,000 Daltons) 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 Daltons) were amongst those contributing to lower yield of washed meat. This raised the possibility

that sarcoplasmic proteins of molecular weights greater than 100,000 Daltons 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 Figure 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 was decreased, especially myosin and actin. The second and third washings did not show much reduction of the myofibrillar proteins. Washing removed fat, blood, pigments, odorous matters, inorganic salts and water soluble enzymes from meat (Grant, 1985). Separation of these components resulted 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, leaving a material that is white in color and high in myofibrillar proteins. However, washing have 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) earlier reported the changes in the ratio of salt soluble proteins (myofibrillar) to sarcoplasmic proteins, which increased as a result of washing.

CONCLUSION

Electrophoretic analyses indicated that washing of broiler and spent hen meat resulted 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 suggested 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 3rd washing prosesses good gelation properties similar to those commonly associated with surimi.

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