Studies on Muscle Proteins

II. Changes of Beef-, Pork- and Chicken-Proteins during the Meat Products Manufacturing Processes

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Introduction

In the previous report[1], the author gave an account of the methods, the one to solubilize the muscle proteins before and after heat coagulation, and the other to analyze actually the components of resultant proteins. Applying these methods, the author commenced a series of investigations on the chemical changes of the components of beef-, pork- and chickn-proteins occurring at the respective steps of the meat products manufacturing processes : slaughtering, chilling, curing, smoking, boiling and storing.

Experimental procedures

Materials

In this experiment, the following muscles were employed as the samples.

Beef : M. longissimus dorsi from an individual of female Japanese black cattle, six years old.

Pork : M. longissimus dorsi from an individual of hog (♂, Landrace, ♀ X Birkshire, ♂), nine months old.

These muscles were removed from the carcasses immediately after slaughtering.

Chicken : M. pectoralis superfacialis and profundus from five broiler type chickens (♂, White Cornish, ♂X Plymouth Rock, ♀), sixty five days old.

These birds, killed by being cut the jugular veins and carotid arteries, were bled for 5 min, at 60°*C*, plucked by hand, eviscerated, and then, the muscles were removed quickly from the carcasses.

About one kilogram of each muscle was minced through a chopper plate with 3 *mm* holes, after fats and connective tissues had been removed away. At this stage, as the sample meat just after slaughtering, a part of the minced muscle was separated from the bulk. The rest was removed to a beaker, stuffed compactly without leaving any gap, sealed with a thin sheet of vinyl film and gummy bands, and chilled in a cold room at 4°*C* for 24 hours. After chilling, the minced muscle was mixed well and a part of it was separated as the sample meat after chilling and the rest was cured by the dry curing method through being mixed well with the curing ingredients composed of 2.5 % NaCl, 0.1 % KNO_3 and 0.01 % $NaNO_2$. Then, a part of it was separated as the sample meat just after curing, the rest was put back to the beaker and sealed again, and stored for

curing in a cold room at 4°*C* for 48 hours, and then a part of the cured meat was separated as the sample meat after curing.

Next, in order to achieve the purpose of this experiment consisting in surveying the changes of the proteins in the sample meat at the respective steps of the practical meat product manufacturing, three model sausages were prepared through stuffing the cured meats into the fibrous casing for ham and rolling them tightly with cotton yarn so that the sausages might be formed to have the size of 3.5 *cm* in diameter and 15 *cm* in length. Those model sausages were, then, fumigated with the smoke generated naturally from the saw dust of oaken timber, at 25°*C* for 5 hours. One of them was separated as the sample meat after smoking, the rest were boiled at 75°*C* and cooled in tap water (*ca* 20°*C*) for 1 hour, successively. One of the boiled sausages was separated as the sample meat after boiling and another was wiped with dry gauzes over the surface, packed into a vinyl bag and stored in a cold room at 4°*C* for a week. This was employed as the sample meat after storing. Immediately after their separation, according to the procedure prescribed in the previous report[1], all the sample meats were solubilized to get the sample meat-urea solution.

The sample meat was obtained out of the model sausages, in the following procedure; namely at first, the fibrous casing was peeled off, and the content was cut across with a thickness of 2 *cm* at the midst of it, hacked with a knife and quickly ground in the ceramic mortar, and then, the amount required was weighed.

DEAE cellulose column chromatography

Those sample meat-urea solutions were analyzed by DEAE cellulose column chromatography according to the procedure described in detail in the previous report[1] and the chromatograms obtained are shown in Figure 1. In addition to them, a chromatography by the same buffer system as mentioned above was carried out, under no application of any sample solution to get a blank elution chromatogram, the result of which was illustrated under each chromatogram in Figure 1.

And the differences between the test sample solutions curves and the blank elution curve were plotted as the genuine value of chromatograms, compensated by the blank value of the buffer used, in the figures at the right side of each chromatogram of the sample solutions. On the pork and chicken sample meats, only the genuine ones were shown.

Starch gel electrophoresis

Electrophoresis in 7*M* urea-containing starch gel was also carried out with those sample meat-urea solutions according to the procedure described in the previous report[1].

Preparation of myosin A, B and actin fractions

To identify the components separated by the above analyses, myosin A, B and actin fractions were separated from each fresh muscle.

Myosin A fraction from beef muscle was extracted and purified once by the modified procedure[1] of Portzehl[2] et al., while those from pork and chicken muscles were extracted and purified once by the procedure of Bárány[3] et al.

Myosin B fractions of all kinds of meat were extracted and purified once by the procedure of Portzehl et al[2].

Actin fractions were prepared only from pork and chicken meats according to the procedure of Mommaerts[4], with no addition of ATP, including the preparation of muscle's acetone dry powder of Straub[5], and an aliquot of the actin obtained was transformed to the F-form by the procedure of Krans et al.[6] and analyzed by SGE.

These proteins were solubilized by being stirred with 7.7*M* urea containing buffer, and were analyzed same as in the sample meats.

Measurement of pH

Five grams of each sample meat, separated or ground were homogenized with 10 *ml* of the distilled and deionized water at 10,000 rpm for 5 min, ice cold, to make the meat slurrry, its *pH* being determined immediately by the glass electrode.

Protein concentration of the meat sample solutions was controlled by the same procedure as described previously[1], and all the operations were carried out at the room temperature unless otherwise stated.

Results and discussion

DEAE cellulose column chromatography

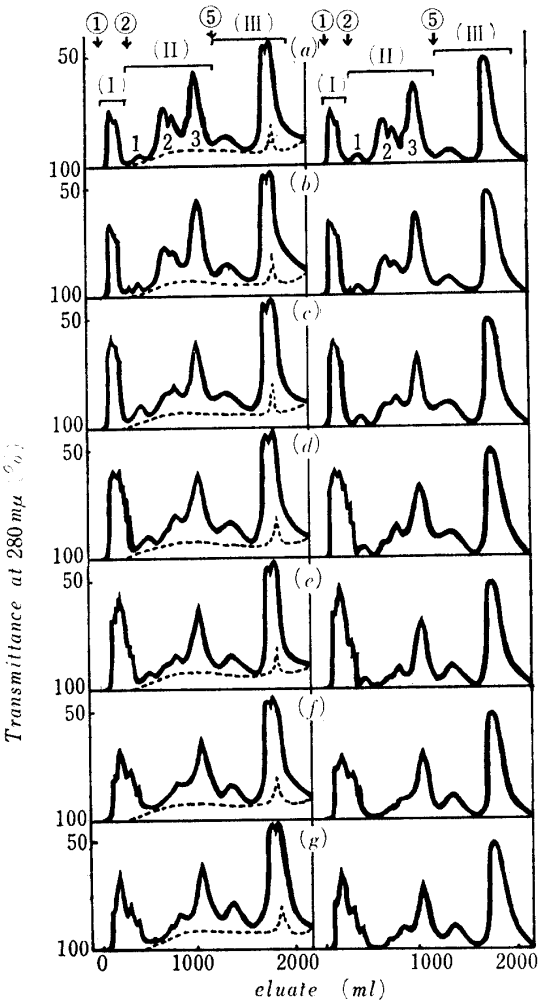

Fig. 1. DEAE cellulose column chromatograms with NaCl- and NaOH- gradient elution of the sample meat solutions of the beef at eacth step of the manufacturing processes in 7*M* urea containing 0.05*M* Tris-HCl buffer, *pH* 8.6.

Ten ml of the solution was chromatographed in each analysis. Left shows the elution curves of the sample meat solutions and Right shows the genuine elution curves drawn by plotting the differences between them, respectively.
(a) Raw ; just after slaughtering, (b) After chilling ; 4°*C*, 24 hours, (c) Just after curing, (d) After curing ; 4°*C*, 48 hours, (e) After smoking ; 25°*C*, 5 hours, with oaken smoke, (f) After boiling and cooling ; boiled, 75°*C*, 1 hour and cooled, about 20°*C*, 1 hour in tap water (g) After storing ; 4°*C*, one week.
—— : The elution curves of the sample meat solutions, --- : the blank elution curve without any sample solution.
(I), (II) and (III) show the fraction number divided by the property of the eluting buffer.
Numbers in circle indicate the changing point of each buffer described in the previous paper[1].

The respective remarks in the following figures (Fig. 2, 3) have the same meanings as those of the remarks in this figure.

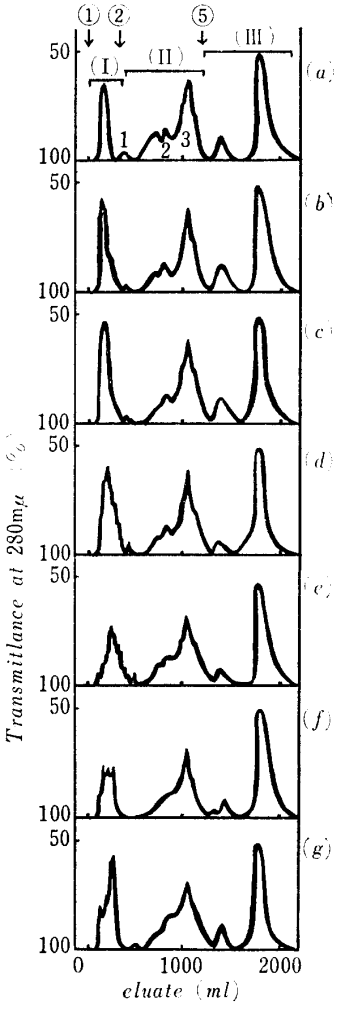

Fig. 2. The genuine DEAE cellulose column chromatograms with NaCl- and NaOH-gradient elution of the sample meat solutions of the pork, obtained by the same procedures as those shown in Fig. 1.

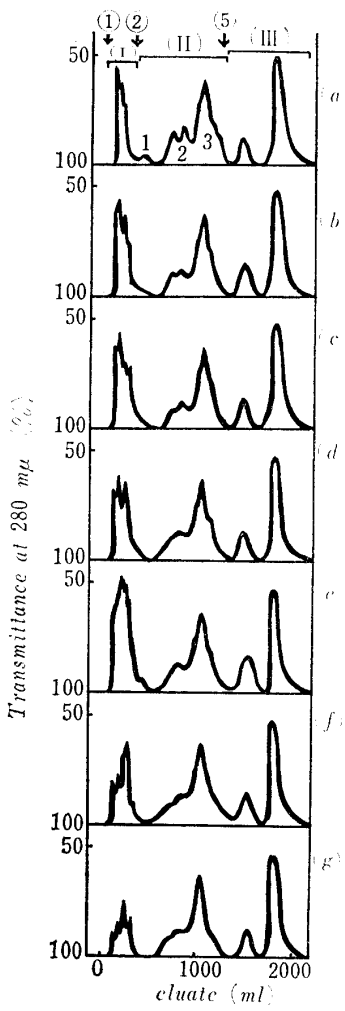

Fig. 3. The genuine DEAE cellulose column chromatograms with NaCl- and NaOH-gradient elution of the sample meat solutions of the chicken, obtained by the same procedures as those shown in Fig. 1.

In Figure 1, 2 and 3, there are shown the DEAE cellulose column chromatograms of the sample meat-urea solutions at each step of meat product manufacturing procsses of three kinds of meat. As described in the previous report, each chromatogram is considered to be capable of being divided into three fractions, that is, the fraction (I) which was eluted with the buffer ①, the fraction (II), with the buffer ②-④ of NaCl gradient and fraction (III), with NaOH gradient. Generally, the peaks in the fraction (I) and (II) were exceedingly liable to undergo changes during the manufacturing processes.

Throughout the entire manufacturing period, only one peak with a few shoulders and changes in quantity was found, in the fraction (I) among the three kinds of meat, and the area occupied by the peak was prone to increase up to the smoking step, followed by an abrupt decreasing occasioned by boiling. After storing for a week, a few shoulders appeared in the peak. The increase of this peak seems to be caused by the increase of the resultants owing to the denaturation of sarcoplasmic proteins and myosin A, which makes their net charges be changed with the lapse of time post mortem, and the decrease of the peak after boiling process is considered to be caused by, in part, the releasing, into the water through the permeable fibrous casing, of the low molecular substances accumulated in the meat until the stage of smoking.

In the fraction (II), three peaks were found. These peaks consisted of the following vintages ; namely, peak 1, the smallest and the fastest one, peak 2, the one accompanied by a shoulder, its eluate being yellowish coloured due to the existence of heme protein, myoglobin, and peak 3, the highest and sharpest of all, located in the last of the NaCl

gradient. Of these peaks, peak 2 changed remarkably in accordance with the abvance of the manufacturing process, that is, it became smaller and its shoulder also became smaller after the 48 hours' curing, and disappeared completely after smoking. After boiling, it became still smaller, as if it had been the shoulder of peak 3. Peak 3 which was the highest and sharpest as was noticed also in the previous report[1], changed only a little throughout the entire manufacturing processes even after boiling, its height became a little decreased but its sharpness remained as before.

In the fraction (III), two peaks, namely, the small one and the large one, which were generally considered to be due to the column denatured proteins and scarcely changed throughout the entire manufacturing processes, were found.

This may be worth noticing as something characteristic, the fact that the small peak was relatively large in the pork's and chicken's chromatogram, whereas it was small in the beef's.

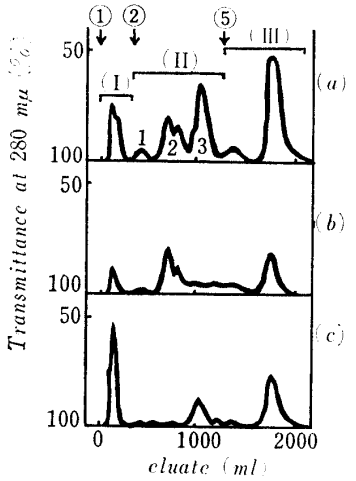

Fig. 4. The genuine DEAE cellulose column chromatograms with NaCl- and NaOH-gradient elution of the solutions of myosin A and B fractions separated from the beef just after slaughtering in contrast to the raw meat in 7*M* urea-containing 0.05*M* Tris-HCl buffer, *pH* 8.6.

(a) Raw meat, (b) Myosin A fraction, (c) Myosin B fraction. Protein concentration was about 2 % in the raw meat solution and 0.5% in the others.
The procedures in detail were same as Fig. 1.

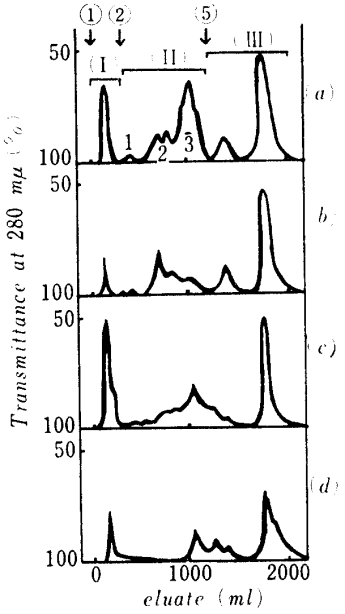

Fig. 5. The genuine DEAE cellulose column chromatograms with NaCl- and NaOH- gradient elution of the solutions of myosin A, B and actin fractions separated from the pork, obtained by the same procedures as those shown in Fig. 4.

(d) Actin fraction
Other remarks are the same as those in Fig. 4.

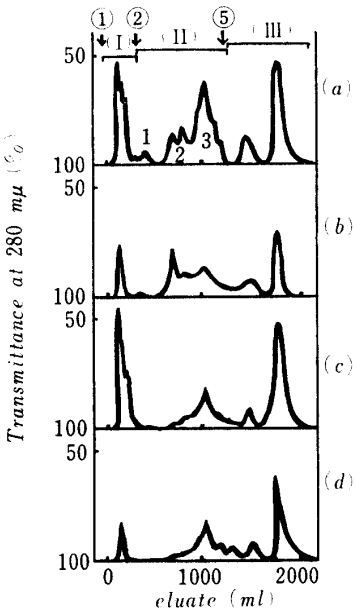

Fig. 6. The genuine DEAE cellulose column chromatograms with NaCl- and NaOH-gradient elution of the solutions of the solutions of myosin A, B and actin fractions separated from the chicken, obtained by the same procedures as those shown in Fig. 4.

All the remarks are the same as those in Fig. 5.

In order to clarify what kind of protein in muscle corresponds to the components of these peaks, myosin A, B and actin fractions separated and purified, were analyzed same way as in the sample meat. Results obtained are shown in the Figure 4, 5 and 6. As

evident from the figures, peak 2 corresponded to myosin A, containing heme protein and peak 3, to myosin B. It was not ascertained whether the main peaks of the purified myosin A and myosin B fractions, which were found in NaCl gradient, represented the bulk, respectively; but at least, the specific fragment of each fraction seemed to be present in the peaks. Therefore, it was suggested that myosin A was comparatively liable to undergo changes during the meat product manufacturing processes, especially by boiling; on the contrary, myosin B had a considerable resistance to the denaturation during these processes as far as this experiment was concerned. In this regard, it was verified by a research[7] that myosin B, in general, was hard to be denatured in the molecular structure. At least, changes of the charges on the surface of myosin B seemed hardly to occur, though no investigation of myosin B after heating at such a high temperature as was seen in this experiment, had been carried out. In the previous study[1] on the thawed raw sample meats, the peak which corresponded to myosin A was not so clearly separated from the one corresponding to myosin B as was seen in this experiment. Hence it is assumed that myosin A may be partially denatured even by the processes of freezing and thawing.

Actin fractions showed the presence of three peaks near the last of NaCl gradient in the fraction (II). Out of them, the fast eluted and largest peak corresponded to the main peak of myosin B and, of all the chromatograms of the sample meat solution, to the highest peak in the fraction (II).

Therefore, it was manifested that the peak in question corresponded to both the main peak of myosin B fraction and that of actin fraction. Actin, as known well, can be transformed alternatively into two forms under the appropriate condition, namely, G-actin which has globular form and F-actin, fibrous form, the latter being constructed by the polymerization of the former.

In the extracted fraction from the acetone dry powder of muscle, three types of actin which are different in the degree of the polymerization of G-actin, seen to be present. The studies on actin fraction of beef muscle will be made in the near future and reported elsewhere.

Thus, it was made clear that, in general, the changes of the muscle proteins in three kinds of the sample meat, which were observed, during the manufacturing processes, in the results from the analyses by DEAE cellulose column chromatography, resembled each other, though different in detail.

Hitherto, the analyses by DEAE cellulose column chromatography in the high concentration of urea-containing buffer solution have been carried out neither on the whole muscle nor on the isolated muscle protein, still less on the heat coagulated muscle protein and the meat product except the previous report[1].

Therefore, the results obtained in this experiment can not be compared directly with those of other works. However, it is known that, in the high concentration of urea-containing buffer solution, miosin A complex is dissociated[8,9,10], and ATPase activity of myosin A is rapidly lost[11], whereas complete deformation of myosin A requires 10-12*M* urea[12,13]. So, even in 7*M* urea, which was selected to solubilize the heat-coagulated muscle proteins, myosin A was considered to maintain its specific property as a monomeric protein, not as an enzyme. As the peak corresponding to myosin A was apt to vanish rapidly with the advance of manufacturing process, the degradative change of myosin A

seemed to be relatively rapid under the environment of this experiment. On actomyosin or myosin B, it is known that its association is disrupted in the high concentration of urea-containing buffer solution[12,14]. In this experiment, the main peak of myosin B fraction of pork and chicken, corresponded to that of actin fraction.

It is considered that the dissociation of myosin B to myosin A and F-actin, may be possible, therefore, the component in the main peak of myosin B fraction may be F-actin. Indeed, the peak in the fraction (I) of the myosin B is very large. This may imply that the isolated myosin B is liable to be dissociated in the 7*M* urea-containing buffer solution, and the resulting myosin A is denatured more easily.

As an additional experiment, an attempt to analyze F-actin fraction of pork was made by using DEAE cellulose column chromatography, to observe the appearance of small three peaks at the position of the main peak of myosin B, but all the peaks were not so large as the latter. Therefore, it is required to ascertain whether the main peak of myosin B fraction is composed of F-actin or myosin B itself. And this is accompanied with the inevitable finding, on the chromatograms, of the hidden area of the nucleic acids and nucleotides, naturally contained in the muscle.

Starch gel electrophoresis : (*SGE*)

The results from starch gel electrophoresis with the sample meat-urea solution obtained at each step of the manufacturing processes except those after storing are shown schematically in Figure 7, 8 and 9.

Observation of the patterns obtained from the raw sample meats, made it possible to find the bands and zones, amounting to 17 in beef and pork, 15 in chicken in all. Seen in the order of the movability from the moving front, 8 bands were recognized in beef, 5 bands in pork and 6 bands in chicken. Judging from the fact that they move fast, appearing in the shape of bands, not zones, we may be allowed to assume that they represent the components of sarcoplasmic globular proteins in the meats. On the other hand, some detections were made on the existence of 5 zones moving foreward, in the rear of the bands in beef and chicken, and on that of 8 zones in pork ; and owing to their weakened movability and their disorderly spreading manner they are supposed to be the components of the fibrous proteins. Besides these, three zones moving backward and

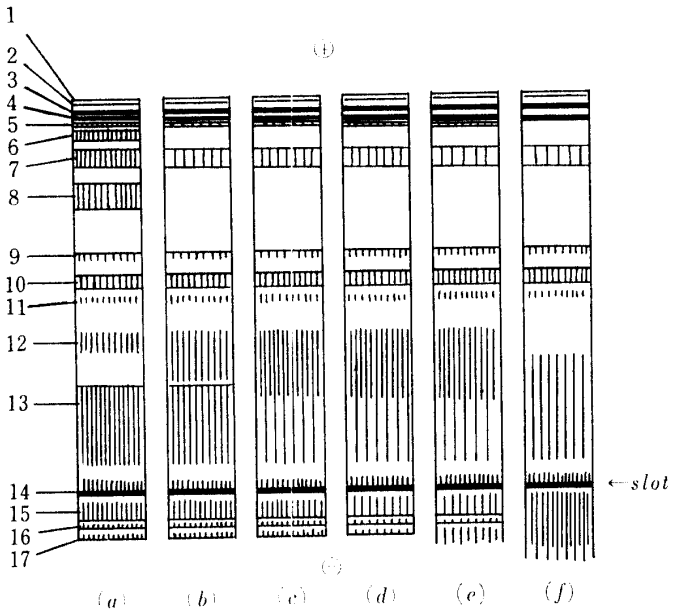

Fig. 7. Starch gel electrophoretic patterns of the sample meat solutions of beef at each step of the manufacturing processes at *pH* 8.6, Tris-HCl buffer, 7*M* urea.
(a) Raw ; just after slaughtering, (b) After chilling ; 4°*C*, 24 hours, (c) Just after curing, (d) After curing ; 4°*C*, 48 hours, (e) After smoking ; 25°*C*, 5 hours, with oaken smoke, (f) After boiling and cooling ; boiled, 75°*C*, 1 hour and cooled, about 20°*C*, 1 hour in tap water.

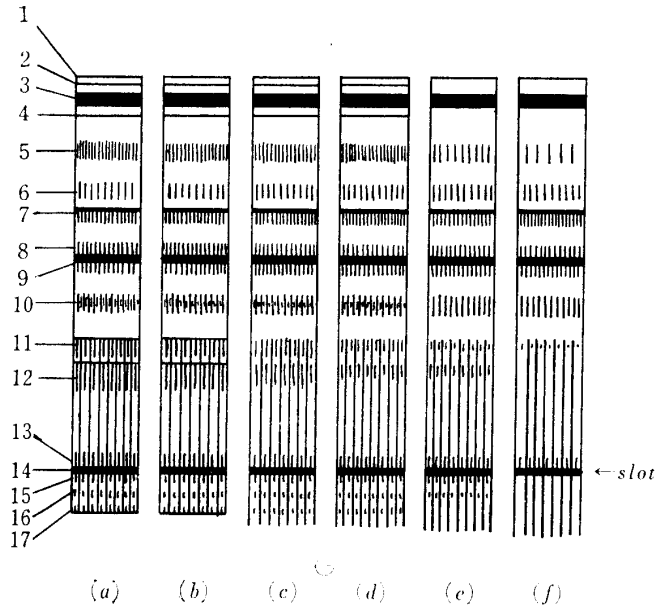

Fig. 8. Starch gel electrophoretic patterns of the sample meat solutions of pork at each step of the manufacturing processes at *pH* 8.6, Tris-HCl buffer, 7*M* urea.
All the remarks are the same as those in Fig. 7.

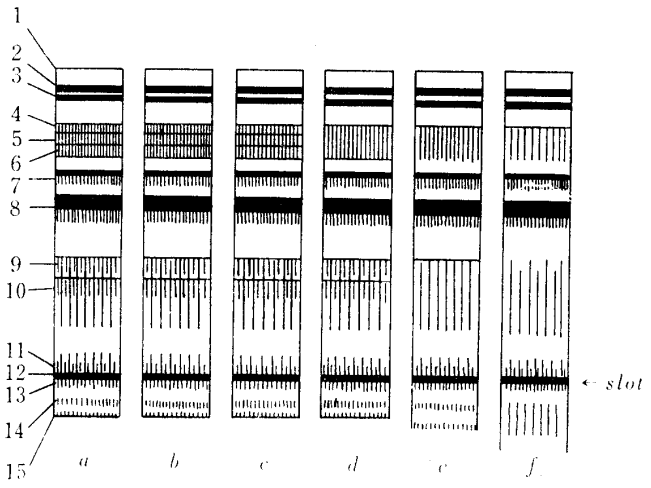

Fig. 9. Starch gel electrophoretic patterns of the sample meat solutions of chicken at each step of the manufacturing processes at *pH* 8.6, Tris-HCl buffer, 7*M* urea.
All the remarks are the same as those in Fig. 7.

a remainder in the slot were commonly found in all kind of meat.

The results from the analyses with myosin A, B and actin fractions in contrast to the raw sample meat are shown in the Figure 10, 11 and 12, showing some characteristic differences found among the patterns of those.

In beef, the zone corresponding to myosin A could move the half way to the moving front and that corresponding to myosin B could move only one fifth of the distance. The separated myosin fractions, however, seem to behave in a slightly different way in the raw sample meat, namely, the zones in the separated fraction are apt to move faster than in the raw sample meat. Besides, two clear zones (9, 10) which moved a little faster than myosin A zone, were found in the patterns of myosin B fraction and the raw sample meat.

Relying on the consideration on the isoelectric point and molecular weight of G-actin, these are likely to show actins, though no direct proof was obtained in this study, yet. The ascertainment of this will be soon carried out afterward. It is of no small interest that, as evident in Figure 7, these zones were found in every pattern of each step during the manufacturing processes, especially in the pattern after boiling. In comparison of Figure 7 with Figure 10, it is evident that the movability of the zone of myosin A fraction was converted to be a little faster, its stainability being decreased as the result of the heat denaturation by boiling. And the components in the fast moving bands (1-7) which seemed to correspond to the sarcoplasmic proteins, were likely to be liable to undergo changes. So, these bands became generally spread and blunt after smoking, and out of them, two bands (3, 4), stained intensely near the moving front, as described in the previous report[1], as the representation of heme protein, decreased in

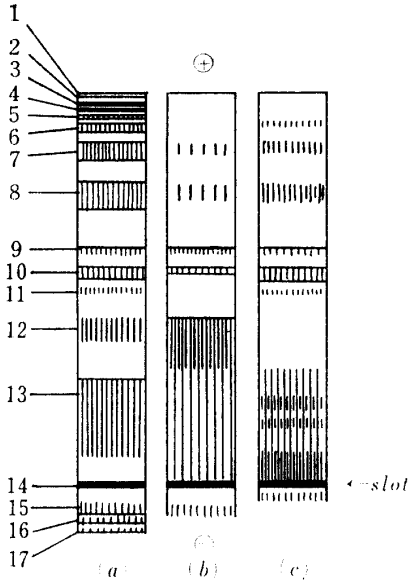

Fig. 10. Starch gel electrophoretic patterns of the urea solutions of myosin A, B fraction and of the raw sample meat of beef, at *pH* 8.6, Tris-HCl buffer, 7*M* urea.
(a) Raw ; just after slaughtering, (b) Myosin A fraction, (c) Myosin B fraction.

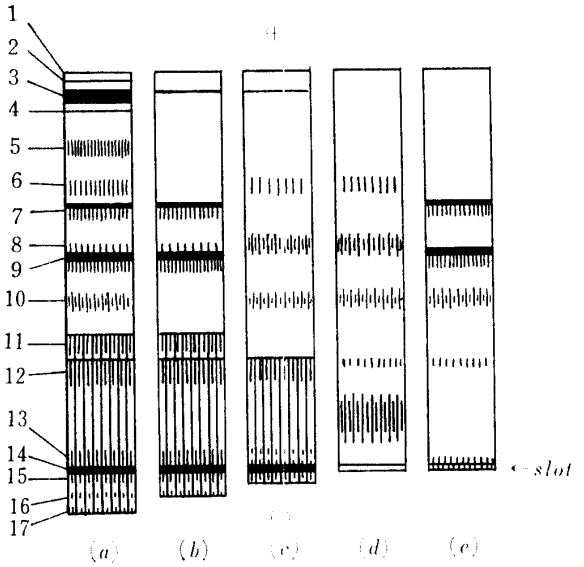

Fig. 11. Starch gel electrophoretic patterns of the urea solutions of myosin A, B, G- and F-actin fraction and of the raw sample meat of pork, at *pH* 8.6, Tris-HCl buffer, 7*M* urea.
(a) Raw ; just after slaughtering, (b) Myosin B fraction, (c) Myosin A fraction, (d) G-actin fraction, (e) F-actin fraction.

stainability rapidly, even after 48 hours' curing. After 24 hours' chilling, two bands (6, 8) had already vanished. The three clear zones moving backward (15, 16, 17) were noticed in the pattern of the raw sample meat, of which the least moving zone was likely to be a part of myosin B fraction, and the next, a part of myosin A fraction, and the zone moving most remarkably was left unexplainable. But they became more obscure with the advance of the manufacturing process, and at last, were reduced to only one widely spread zone after boiling.

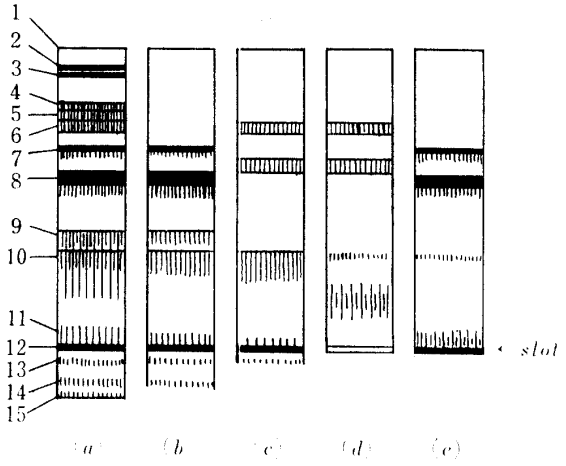

Fig. 12. Starch gel electrophoretic patterns of the urea solutions of myosin A, B, G- and F-actin fraction and of the raw sample meat of chicken, at *pH* 8.6, Tris-HCl buffer, 7*M* urea. All the remarks are the same as those in Fig. 11.

In pork and chicken, the patterns of myosin A fractions, which were prepared by the procedure of Bárány et al[3]., showed a clear and typical zone which moved very slowly (12 in pork, 10 in chicken), three zones in pork (6, 8, 10), two zones in chicken (6, a zone 7' between 7 and 8) which moved faster, a zone moving backward

(15 in pork, 13 in chicken) and a component in the slot (14 in pork, 12 in chicken), respectively.

In addition to the zones corresponding to the typical zones of myosin A, the patterns of myosin B fractions showed not only the zones moving a little faster, just corresponding to the zones (11 in pork, 9 in chicken) found in the pattern of the raw sample meat but two other zones equal to those in question (7, 9 in pork, 7, 8 in chicken) unchangeable throughout the entire manufacturing processes. As the zones moving backward, two zones (15, 16 in pork, 13, 14 in chicken) and a component in the slot were found, too.

In the pattern of the actin fractions extracted with the water from the acetone dry powder, there were found four zones in pork and three zones in chicken, namely, the most slowly moving and diffused zone, located between the zone (12) and (13) in pork, (10) and (11) in chicken, in the pattern of the raw sample meat, a zone corresponding to the zone (10), the two zones moving relatively fast and corresponding to the zones (6, 8) in pork, and two zones (6, 7') in the pattern of myosin A fraction in chicken, were found. Since the components extracted with the water from acetone dry powder were considered to be crude G-actin, these zones were assumed to indicate the polymers which were different in the degree of polymerization. Out of these zones, (6), (8) in pork and (6), (7') in chicken were found in the pattern of myosin A fraction which was extracted and purified with Hasselbach and Schneider solution containing 0.01*M* potassium pyrophosphate, so, they were assumed to be the contaminants of G-actin fraction, which could not be eliminated through once-purification-process. As known well, the construction of myosin B is composed of myosin A and F-actin. So, the transformation of the crude G-actin fraction into F-actin was attempted according to the procedure of Krans et al.[6] and the resultant was analyzed by SGE. It is of interest that in the pattern of F-actin, the disappearance of the most slowly moving zone was instantaneously followed by the appearance of the remainder in the slot, not present in the G-actin fraction. But the fast moving zones (6, 8) in pork and (6, 7') in chicken, were retained and they migrated to the location of the above mentioned zones in question (7, 8) and (7, 9), respectively. In this regard, the respective two zones unchangeable throughout the entire manufacturing processes seemed to represent the components of F-actin. In comparison of Figure 8 with 11 and Figure 9 with 12, it is evident that the sarcoplasmic proteins (1-5) of pork and (1-6) of chicken, myosins (10-13) of pork and (9-11) of chicken, and the components which moved backward were considerably changed during the manufacturing processes, especially after smoking and boiling process, but the specific two zones (7, 9) which seemed to correspond to the components of F-actin were kept throughout the entire manufacturing processes without any change.

There seem to be some resemblances between the results from the investigation on the chicken meat with the urea-containing SGE by Neelin and Rose[15] and those of this study, though no identification of the individual protein component was carried out in the former. The investigation by the urea-containing SGE on myosin A and H-meromyosin was made by Mueller and Perry,[16] and three to four zones were recognized. On actin, many investigations were conducted. Above all, Krans et al.[6] have reported the existence of four zones in the SGE pattern, under the condition similar to this study. Although these results were obtained under the different condition from this experiment, they seem to have some coincidences with the results obtained in this study.

On myosin B or actomyosin, no investigations by SGE are found for the present. In this experiment, out of the zones which appeared in the pattern of myosin B, fraction there were detected some zones different from those of the isolated myosin A and actins (G-, F-) fraction. Therefore, it is suggested that myosin B is not necessarily dissociated completely in 7M urea-containing buffer solution.

Table 1. The *pH*-changes of the three kinds of meat at each step of the meat product manufacturing processes

Steps of the manufacturing processes	Kind of meat		
	Beef	Pork	Chicken
		pH	
Just after slaughtering	6.60	6.23	5.90
After chilling	5.53	5.39	5.70
Just after curing	5.33	5.59	5.70
After curing	5.45	5.51	5.73
After smoking	5.16	5.33	5.41
After boiling and cooling	5.59	5.73	5.85
After storing	5.52	5.60	5.82

The procedure of the *pH*-measurement and the respective processes were described in text.

Changes of pH

The pH values of the three kinds of meat at each step of the manufacturing processes are summarized in Table I. The *pH* values of the raw sample meats just after slaughtering generally dropped abruptly after chilling. The changes of *pH* in this stage are rather naturally caused as the result of post mortem glycolysis. However, the values were apt to be slightly elevated during the curing process, dropped after smoking, and again elevated after boiling. During the storing period, slight drops were recognized. Out of those changes, it is noteworthy that the pH-dropping after smoking process was especially remarkable. This is, perhaps, caused by the penetration of the components in the oaken smoke including several organic acids into the meat, resulting in the changes observed on the DEAE cellulose column chromatograms and starch gel electrophoretic patterns.

Thus, the main components of the proteins, found as the peaks in the DEAE cellulose column chromatograms, as well as the bands and zones in the patterns of SGE of the sample meat solution at each step of the manufacturing processes of three kinds of the sample meat, and their changes during the manufacturing processes were made clear to some extent, though some components supposed to be actins and others were left to be ascertained in detail.

Stricter resolution of the results obtained in the present study seems to be required, and further investigations are to be done on the following items, namely, the location of the components of the muscle proteins in the chromatograms and patterns, their behaviours in the more precisely separated and purified forms, and their changes in detail during the manufacturing processes, especially heat coagulation process.

Summary

The research on the chemical changes of the muscle proteins occurring throughout the entire manufacturing processes, with beef, pork and chicken utilized commonly as raw

materials in the meat industry, was carried out.

The sample meats were solubilized by homogenizing with 7.7*M* urea-containing 0.055*M* Tris-HCl buffer (*pH* 8.6). Analyses were made just after slaughtering, after chilling, just after curing, after curing, after smoking, after boiling and cooling, and after storing, and at the same time, myosin A, B and actin fractions were separated and purified. The urea-solution of every sample meat was analyzed by means of DEAE cellulose column chromatography and starch gel electrophoresis. The results obtained are summarized as follows :

On the DEAE cellulose column chromatograms, it was recognized that a peak probably corresponding to myosin A decreased rapidly with the advance of the manufacturing processes, on the other hand, the peak possibly attributable to myosin B or its fragment located near the last of the NaCl gradient was left almost unchanged throughout the entire manufacturing processes, notwithstanding the treatments of curing, smoking, boiling and cooling, and storing.

On the patterns of starch gel electrophoresis, the components in the fast moving bands which probably corresponded to the sarcoplasmic proteins including the heme protein were generally subject to changes, and also myosin A was prone to undergo changes, especially after smoking, being decreased in its stainability and becoming spread. However, there were two clear zones left without almost any change at the midst of each run, which seemed to correspond to the parts of F-actin. In addition, a few zones moving backward were found, which became a single spread zone after smoking. These tendencies of chemical changes, independent of the kinds of meat, were found in common, though some minute analytical differences were recognizable.

Aknowledgements

The author expresses his thanks to Prof. N. Andō, Kyushu University, Prof. T. Tsugō, the University of Tokyo, and Prof. M. Kojima, Kagoshima University for their kindly guidance and suggestions, and to Mr. M. Yahisa, Mr. K. Arita and Mr. T. Kamei for their technical assistances.

References

1. KAKŌ, Y. : *Memoirs Fac. Agr. Kagoshima Univ.* : **6**, 161 (1967)
2. PORTZEHL, H., G. SCHRAMM and H. H. WEBER : *Z. Naturforsch.*, **5b**, 61 (1950)
3. BÁRÁNY, M., K. BÁRÁNY, T. RECKARD and A. VOLPE : *Arch. Biochem. Biophys.*, **109**, 185 (1965)
4. MOMMAERTS, W. F. H. M., : *J. Biol. Chem.*, **198**, 445 (1952)
5. STRAUB, F. B. and G. FEUERR : *Biochim. Biophys. Acta*, **4**, 445 (1950)
6. KRANS, H. M. J., H. G. VAN EIJK, and H. G. K. WESTENBRINK : *Biochim. Biophys. Acta*, **65**, 166 (1962)
7. YASUI. T., Y. HASHIMOTO and Y. TONOMURA : *Arch. Biochem. Biophys.*, **87**, 55 (1960)
8. WEBER, H. H., and R. STOVER : *Biochem. Z.*, **259**, 269 (1933)
9. TSAO, T-C. : *Biochim. Biophys. Acta*, **11**, 368 (1953)
10. SZENT-GYÖRGYI, A. G., and M. BORBIRO : *Arch. Biochem. Biophys.*, **60**, 180 (1956)
11. TAKASHINA,H. and M. KASUYA : *Arch. Biochem. Biophys.*, **118**, 475 (1967)
12. STRACHER, A. : *J. Biol. Chem.*, **236**, 2467 (1961)
13. SMALL, P., W. W. KIELLEY and W. F. HARRINGTON : *Biochim. Biophys. Acta*, **49**, 462 (1961)
14. BÁRÁNY, M., K. BÁRÁNY and W. TRAUTWEIN : *Biochim. Biophys. Acta*, **45**, 317 (1960)
15. NEELIN, J. M. and D. ROSE : *J. Food Sci.*, **29**, 544 (1964)
16. MUELLER, H. and S. V. PERRY : *Biochem. J.*, **85**, 431 (1962)