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Effect of Bicarbonate and Phosphate Buffer Treatments on the Structure and Thermal Stability of Spent Layer Meat

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Abstract

Spent layer breast meat and leg meat samples washed with 0.05 M sodium bicarbonate solution and 0.04 M phosphate buffer(pH 8.3) showed decreases in heat denaturation temperature indicating the destabilization of myofibrillar proteins. The destabilization was attributed to the solubilization of 95 Kdalton and 55 kdalton proteins from the myofibrils observed in gel-electrophoretograms. Transmission electron microscopy further indicated the breakage of Z-lines.

Key words : spent layer meat, bicarbonate, phosphate

Introduction

Washing of poultry meat and mechanically deboned poultry meat has been attempted by several researchers in order to reduce color intensity and fat content of these products. Ball and Montejano[1] washed broiler thigh meat with sodium bicarbonate solution and sodium acetate solution as an extraction media. The authors reported 73 to 88% reduction of total pigment content in treated meat. Hernandez *et al.*[2] examined the effect of washing on the color characteristic of mechanically deboned turkey meat. Samples were washed with phosphate buffers(0.04 M, pH 6.4, 7.2 and 8.0) and the resulting meat pastes were centrifuged to remove the solutions. Extensive extraction of pigments was observed when the buffer with a pH of 8.0 was used. Treated samples showed a 64% reduction in redness and a 51% reduction in lightness. Results of previous research works indicate heme pigments can be effectively removed from poultry meat by washing. However, the effect of washing treatments on the physicochemical conditions of myofibrillar proteins which are closely realted to the quality of further processed poulty meats have not been provided. In this study, spent layer breast meat and leg meat samples were washed with 0.05 M sodium bicarbonate solution and 0.04 M phosphate buffer(pH 8.3) and the effect of washing treatments on the thermal stability and structure of muscle proteins were investigated.

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Materials and Method

Materials

Spent layers(15 to 17 months old white leghorns) were slaughtered by severing the carotid artery. After bleeding and defeathering, intestines were removed and carcasses were put into ice slush(0℃) and aged overnight(12~15 hr). Aged carcasses were individualy vacuum paccked into Cryovac bags(W.R. Grace and Co., Duncan, SC, USA) and blast frozen(−35℃). For each study, frozen carcasses were thawed at 4℃ overnight(12~15 hr). Breast, thigh and drumstick part were removed from the carcass and deboned without skin by hand. Meat samples were then ground using a meat grinder(Model 4812, Hobart Manufacturing Co., Troy, OH, USA) with a 0.48 cm plate. Representative portions of ground meat samples were treated with cold tap water and buffer solutions as described in following sections and subjected to the analyses.

Measurement of thermal properties

Thermal properties of samples were checked using a Perkin Elmer DSC-4 with a Perkin Elmer Thermal Analysis Micro-processor controller.

Ground breast meat, thigh meat and drumstick meat were treated with 5 times(v/w) of cold(4~6℃) tap water, 0.05 M sodium bicarbonate solution and 0.04 M phosphate buffer(pH 8.3) of 7 min, respectively. Treated samples were then rinsed with 5 times (v/w) of cold tap water and the excess solutions were

removed using a triple layer of cheese cloth. For each run, approximately 10 to 12 mg of samples were accurately weighed and sealed into Perkin Elmer aluminum hermatic pans(kit number 2190-0062) using a crimper. Ten to 12 ml of distilled water were sealed into a pan and use as a reference. The heat flow pattern was scanned from 10℃ to 90℃ at the temperature increment rate of 10℃/min.

Plots of thermograms were then analyzed using the partial area program of the Perkin Elmer Thermal analysis Data Station(TADS). Total enthalpy of transition was calibrated per g sample and per g protein. The protein content of samples were determined by the macrokjeldahl procedure.[3] A factor of 6. 25 was used to estimate protein content based on the amount of nitrogen in a sample.

Sodium dodecylsulfate-polyacrylamide gel electrophoresis

Ground breast meat, thigh meat and drumstick meat were treated with cold tap water, bicarbonate solution and phosphate buffer as described in the previous section. After treatment, solutions were filtered through whatman #1 filter papers. Filtrates were then decanted into polycarbonate tubes and centrifuged at 27,000×G for 10 min at 4℃. After centrifugation, the supernatants were dialyzed against deionized water for 24 hrs at 4℃ and freeze-dried using a pilot scale freeze drier(Virtis 15 SRC-X, th Virtis Co., Inc., Gardiner, NY, USA). Dried samples were then put into vials wrapped with aluminum foil and kept in a dessicator until analysis. Exactly 10 mg of freeze dried samples were dissolved in 1 ml of sample buffer(0.0625 M tris, 2.5% SDS, 12.5% glycerol, 5% mercaptoethanol, pH 6.8) and SDS-PAGE was performed following the method of Laemmli.[4]

Transmission electron microscopy

Ground breast and ground drumstick samples were washed with the solutions as described in the previous sections. After draining of the solutions, meat particles were immersed in a fixing solution(3% glutaraldehye in 0.15 M sodium cacodylate buffer, pH 7.1) and kept in an ice bath for 2 hr. The samples were stained with 0.5% uranyl acetate in 0.1 M sodium accetate buffer(pH 7.1) overnight and dehydrated using graded cold acetone solutions. Dehydrated samples were then embedded in expoxy resin. After embedding, sections were cut, mounted on a copper grid and sequentially stainned with 30% uranyl acetate and 0.2% lead acetate solutions. Stained samples were mounted on a Hitachi transmission electron microscope(Model 11 E) and representative parts were photographed at 7.500× magnification. For the quantitation of structural changes, fields were selected in a random fashion(zig-zag motion) and specific morphological parameters(swelling of myofibrils, loss of M-line, breakage of Z-line and mitochondrial swelling) were observed as described by Voyle *et al.*[5] The degree of morphological changes was scored based on 0~2 scale as shown below:

0: No change
1: Partial change, but not clear
2: Definite change

The mean values of scores wer used as quantitative indexes of the change.

Statistical analysis

Analysis of variance(AOV) tests were performed using the general linear model program developed by SAS(Statistical Analysis system Institute Inc., Cary, NC, USA). The mean differences were examined using the Student-Newman-Keuls test, a part of SAS program.

Results and Discussion

Thermal properties

The thermal denaturation patterns of the treated samples and control samples were compared in order to examine the effect of washing treatments on the maximum thermal denaturation temperature(T_{max}) and denaturation enthalpy(ΔH) of the major muscle protein components.

Thermograms of ground breast meat showed five endothermicc transition peaks(Fig. 1). In case of water-washed ground breast meat, first peak and the last peak became more prominent. In cases of bicarbonate and phosphate washed ground breast meat, lowering of T_{max}(approximately 5 to 7℃ decrease) of second major peak was observed.

Kijowski and Mast[6] observed similar denaturation pattern in case of ground broiler breast meat and concluded that the peaks correspond to the denaturation of myosin(T_m 50℃), sarcoplasma(T_{max} 63~68℃) and action(T_{max} 80℃) by comparing the thermogram of ground breast meat with that of purified myosin, actin and sarcoplasmic drip, respectively. Therefore, it was postulated that the disappearance of intermediate peaks observed in case of water-washed ground

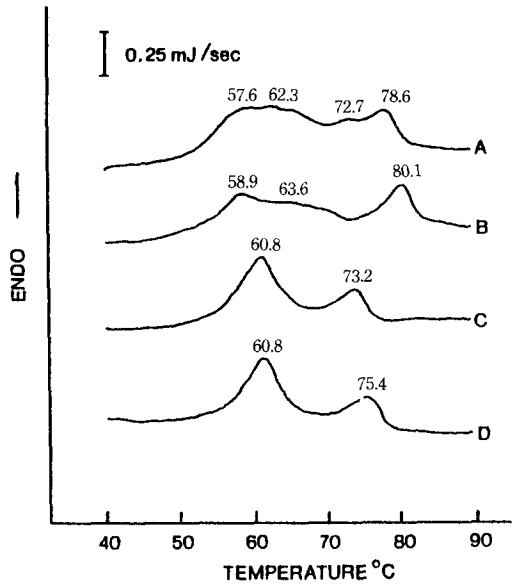

Fig. 1. Effect of washing treatments on the thermal denaturation of breast meat
A: Ground breast(control), B: Water washed, C: Bicarbonate washed, D: Phosphate washed

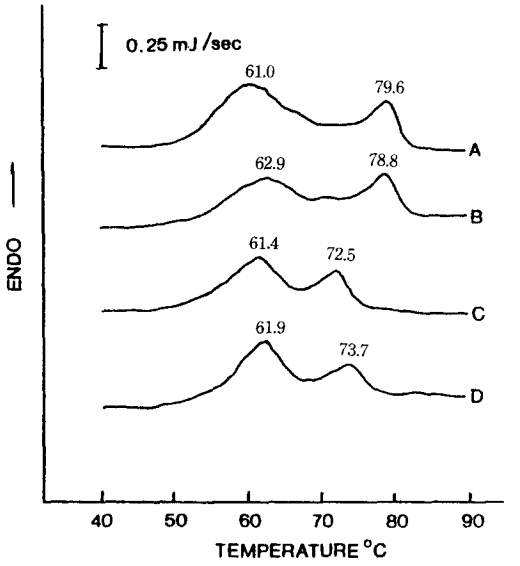

Fig. 2. Effect of washing treatments on the thermal denaturation of thigh meat
A: Ground thigh(control), B: Water washed, C: Bicarbonate washed, D: Phosphate washed

breast meat samples may have been caused by the removal of water soluble sarcoplasmic proteins under the assumption that there is little difference in composition of broiler breast meat and spent layer breast meat.

The lowering of T_{max} of second major peak observed in case of bicarbonate and phosphate-washed ground breast meat required more speculation since it indicated the destabilization of actin which is a major structural protein in a muscle. The lowering of T_{max} of second major peak was also observed in cases of bicarbonate-washed and phosphate-washed ground thigh and ground drumstick samples.(Figs. 2 and 3). This destabilization effect on the thermal transition was believed to be a consequence of the bicarbonate and phosphate washing since the washing was the only additional treatment imposed to the samples.

The lowering of T_{max} in the presence of added NaCl has been observed among different species of meat. Starbursvik and Martens[7] reported approximately a 4℃ decrease in the actin peak of post rigor bovine Semimembranosus due to the addition of 0.15 M NaCl. Kijowski and Mast[8] observed an 8℃ decrease in the actin peak of myofbrils prepared from chicken breast muscle after the addition of 1% NaCl and reported a continuing decrease of the T_{max} of the

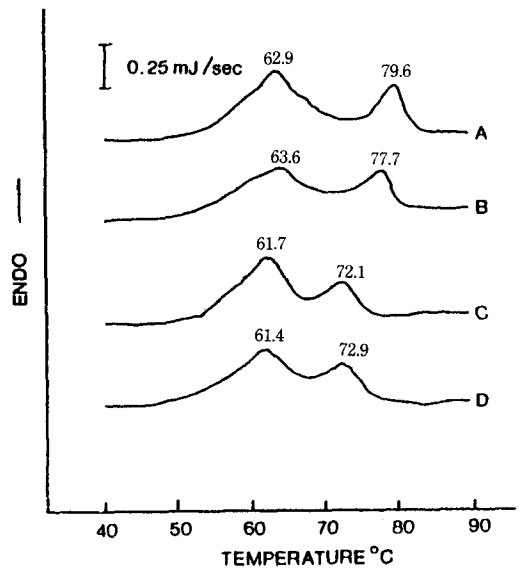

Fig. 3. Effect of washing treatments on the thermal denaturation of drumstick meat
A: Ground drumstick, B: Water washed, C: Bicarbonate washed, D: Phosphate washed

actin peak upon the increase in the NaCl concentration.

The lowering of T_{max} due to bicarbonate or phosphate washing observed in this study was close to the change caused by 1% NaCl in the case of chicken

Table 1. Effect of washing treatment on total enthalpy of transition[a]

	Breast		Thigh		Drumstick	
	Joule/ sample	Joule/ g protein	Joule/ g sample	Joule/ g protein	Joule/ g sample	Joule/ g protein
Control (Not washed)	2.93[a]	12.77[a]	2.64[a]	13.48[a]	2.72[a]	13.19[a]
Water-washed	2.38[b]	13.23[a]	2.39[ab]	14.40[a]	2.26[a]	12.64[a]
	2.09[b]	14.53[a]	2.18[b]	14.86[a]	2.22[a]	13.48[a]
Phosphate-washed	2.05[b]	14.78[a]	2.18[b]	14.78[a]	2.05[a]	12.69[a]

[a]Values represent means of three replications. Within each column, means having the same letter(a-b) are not significantly different($P>.0$

myofibrils[8]. The removal of sarcoplasmic proteins did not seem to be related to this phenomenon since the change(lowering of T_{max}) was not observed in case of the water-washed samples. Therefore, it was postulted that structural changes of myofibrillar components similar to the change in the presence of added NaCl may have been caused by the washing treatments.

Bicarbonate-washed thigh and drumstick meat and phosphate-washed thigh and drumstick meat showed significantly($p<.05$) lower total enthalpy of thermal transition on a g sample basis compared to the controls(Table 1). However, the enthalpy values on protein content basis were not significantly($p>.05$) different from those of control samples. It was reported that the thermal transition energy of myofibrillar proteins and sarcoplasmic proteins was approximately the same in case of beef and rabbit muscles.[9] If this is the case in chicken meat, the non-significant differences in thermal transition energy of the sample on a g protein basis can be explained priori.

The low thermal transition energy on g sample basis of the treated samples appeared to be the simple reflection of their lower protein content.

Extraction of myofibrillar proteins

Electrophoretograms of solubilized proteins in the washing solutions(tap water, bicarbonate solution and phosphate solution) indicated the extraction of a 90~95 kdalton protein, a 55 kdalton protein and a 33 kdalton protein due to the bicarbonate and phosphate washings(Fig. 4). The 90~95 kdalton protein may be α-actinin(M.W. 95,000) and the other proteins may be desmin(or H-protein, M.W. 55,000) and tropomyosin(M.W. 33,000), since the estimated molecular weights are within the normal range of variance (±10%).

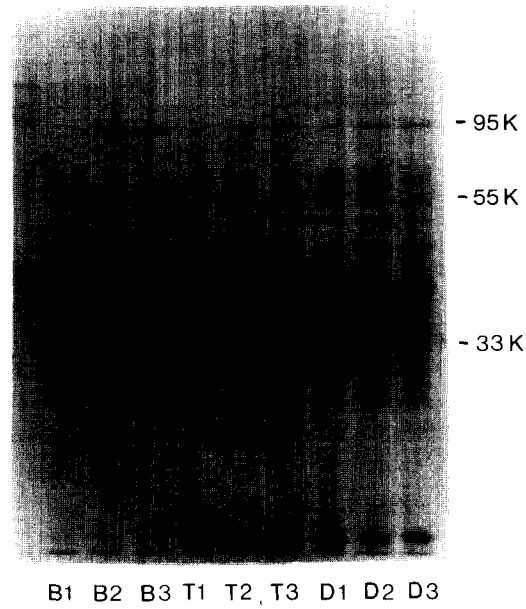

Fig. 4. SDS-PAGE electrophoretogram illustrating the solubilization of some myofibrillar proteins due to bicarbonate-washing or phosphate-washing

B1=Water extract of breast meat(breast control); B2=Bicarbonate extract of breast meat; B3=Phosphate extract of breast meat; T1=Water extract of thigh meat(thigh control); T2=Bicarbonate extract of thigh meat; T3=Phosphate extract of thigh meat; D1=Water extract of drumstick meat(drumstick control); D2=Bicarbonate extract of drumstick meat; D3=Phosphate extract of drumstick meat

Among the three components, α-actinin and desmin are the major components which contribute to the maintenance of the highly organized myofibrillar structure. Therefore, it was postulated that the extraction of these proteins may result in the destabilization of myofibrillar structure.

It has assumed in the previous discussion that the

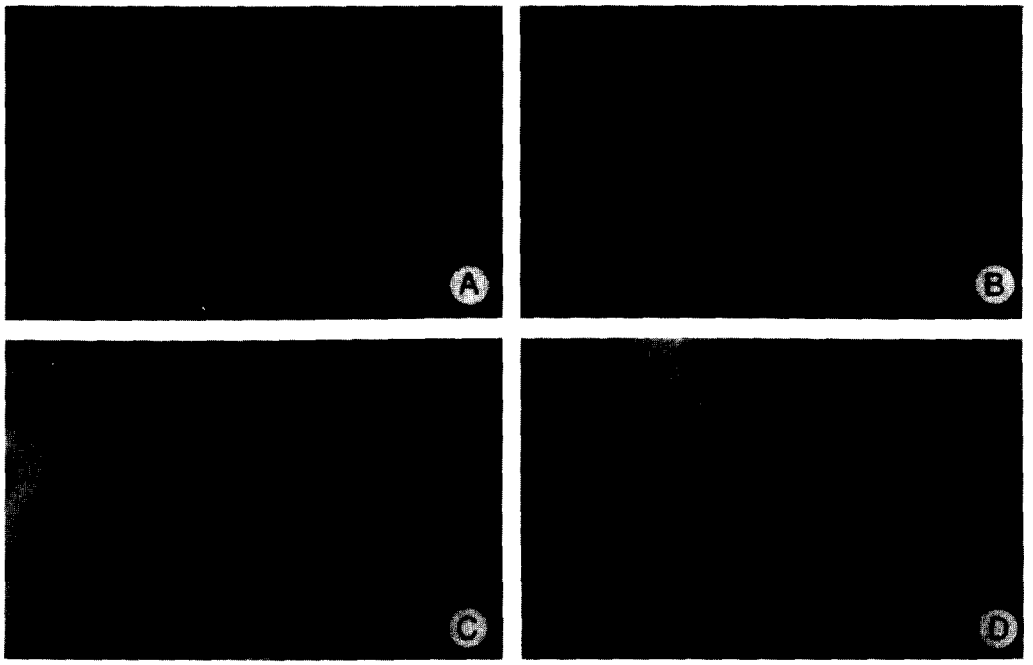

Fig. 5. Effect of washing, bicarbonate-washing and phosphate-washing on the myofibrillar structure of spent layer breast meat

A=Breast meat(not treated, control); B=Water-washed breast meat; C=Bicarbonate-washed breast meat; D=Phosphate-washed breast meat

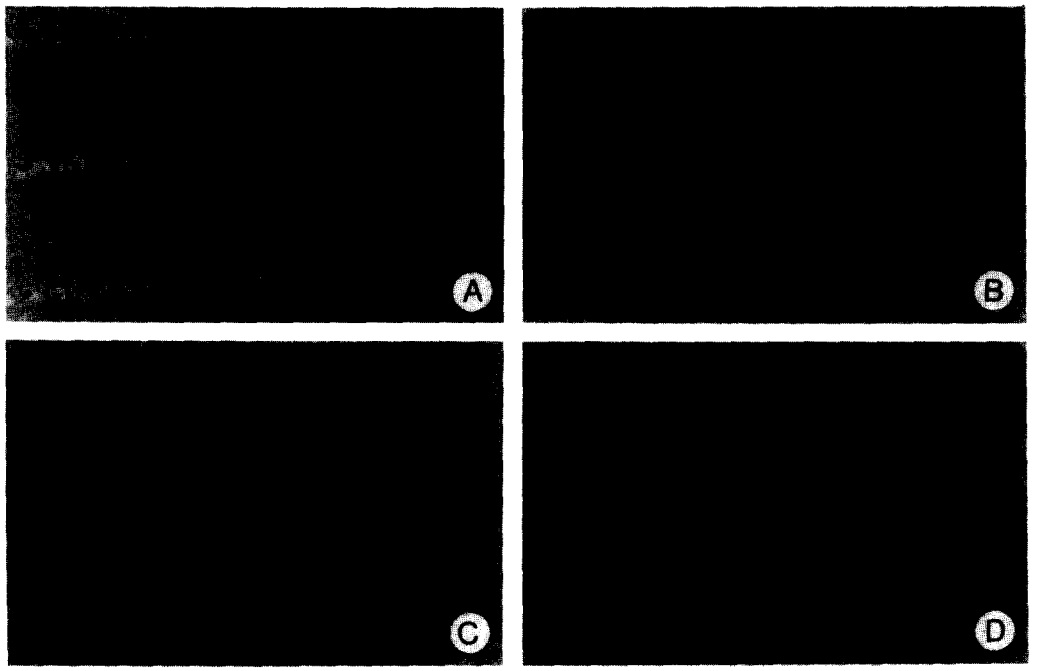

Fig. 6. Effect of water-washing, bicarbonate-washing and phosphate-washing on the myofibrillar structure of spent layer drumstick meat

A=Drumstick meat(not treated, control); B=Water-washed drumstick meat; C=Bicarbonate-washed drumstick meat; D=Phosphate-washed drumstick meat

Table 2. The incidence of structural alterations due to washing treatments[a]

	Control (not washed)	Water washed	Bicarbonate washed	Phosphate washed
		Breast		
Swelling of myofibrils	0.1 y	0.2 y	1.6 x	1.7 x
Loss of M-line	0.0 x	0.1 x	0.9 x	0.9 x
Z-line break-up	0.3 y	0.7 y	1.5 x	1.7 x
Mitochondrial swelling	0.5 y	1.3 xy	1.6 x	1.8 x
		Drumstick		
Swelling of myofibrils	0.3 x	0.2 x	1.7 x	1.3 x
Loss of M-line	0.8 x	0.5 x	0.6 x	1.6 x
Z-line break-up	0.0 z	0.1 z	1.1 x	1.7 y
Mitochondrial swelling	0.3 y	1.7 y	1.7 x	1.9 x

[a]Mean of scores based on; no change(0), partial change(1), definite change(2). Each value is a mean of 2 replications; each replicate consists of 5 observations. Within each row means having the same letter(x-z) are not significantly different($P>.05$)

decrease of the thermal denaturation temperature may be related to the changes in the myofibrillar structure. The change shown in the electrophoretograms supported this assumption.

Structural changes

The result of the previous parts of this study indicated possible changes in the myofibrillar structure due to the treatments. The morphological characteristics of samples observed using TEM also indicated significant change in the myofibrillar structure.

Bicarbonate and phosphate-washed samples showed the breakage of the Z-lines, the swelling of myofibrils and the swelling or rupture of mitochondria, while water wased samples did not show any significant changes in structure except for the swelling of mitochondria(Fig. 5 and 6). The same trends were also indicated by the mean scores(refer to Materials and Methods) of the morphological changes(Table 2).

The breakage of the Z-lines observed among the bicarbonate-washed and phosphate-washed samples appeare to be related to the extraction of 95 kdalton protein and 55 kdalton protein since the moleculr weights of these two components matched those of α-actinin and desmin. However, the extraction of a 33 kdalton component can not be explained by any of the observed changes.

The swelling of myofibrils and the rupture of mitochondria were postulated to be related to the increase in electrostatic repulsion due to the alkalinity of the buffer solutions and the increase in osmotic pressure due to the extraction of sarcoplasmic components, respectively.

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중 탄산 및 인산염 완충액 처리가 노계육의 조직구조 및 열안정성에 미치는 영향

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0.05 M 중탄산 나트륨 및 0.04 M 인산염 완충용액(pH 8.3)으로 처리한 노계육 가슴살 및 다리살 시료의 경우 근섬유 단백의 불안정화를 시사하는 열변성 온도의 저하가 관찰되었다. 이러한 현상을 근섬유 단백중 95 Kdalton 및 55 Kdalton protein의 추출과 관련 가능성이 있는 것으로 판단되었으며 처리 시료의 근섬유구조 변화(Z-line의 파괴)와도 관련 가능성이 있는 것으로 판단되었다.