

Alkali-aided protein extraction from chicken dark meat: Textural properties and color characteristics of recovered proteins

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ABSTRACT Textural properties, water-holding capacity, and color characteristics of alkali-extracted chicken dark meat have been studied. Alkali extraction was carried out at 4 different pH values (10.5, 11.0, 11.5, and 12.0). At higher pH of extraction, cooking loss and water loss were found to be significantly decreased ($P < 0.05$). The lightness (L^* value) of the recovered samples treated at higher pH was found to be significantly lower ($P < 0.05$). Whiteness of uncooked samples also decreased significantly at higher extraction pH values. Protein samples extracted at higher pH values were found to be harder, and the maximum (4,956 g of force) value was shown by samples prepared at pH 11.5. Chewiness values were significantly increased ($P < 0.05$) for protein samples extracted at pH values of 11.5 and 12.0. Dynamic viscoelastic behavior of samples was assessed in

the temperature range of 7 to 100°C. The dynamic viscoelastic behavior of raw chicken dark meat as revealed by storage modulus indicated considerable gel-forming ability. The maximum storage modulus (G') value of 439 kPa was measured at 66.7°C. Storage modulus was found to decrease for the recovered protein samples and be lowest at higher pH values. However, the recovered protein samples did show substantial gel-forming ability when stored with cryoprotectants. Tan delta values denoted 2 clear transitions for raw dark meat; however, only 1 major transition at 50.1°C was evident for pH-treated samples, probably reflecting the loss of collagen in processing. In conclusion, this process of protein recovery may offer the possibility to use the underused poultry resources for preparation of functional foods.

Key words: chicken dark meat, alkali extraction, texture, color

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INTRODUCTION

A major challenge facing the North American poultry industry is to find new processes to transform underused poultry resources (mainly dark meat) into valuable foods. One of the alternatives is to recover the proteins from these underused poultry resources without compromising protein functionality. Alkaline extraction of proteins by the pH shift method is widely used for recovery of proteins from fish dark meat (Kristinsson and Hultin, 2003). The major advantages of this process include economical feasibility, high recovery yield, and improved functionalities of the recovered proteins compared with surimi (Undeland et al., 2002; Kristinsson et al., 2005). Besides these advantages, this process has shown to be suitable for the recovery of proteins with improved gel properties in fatty fishes containing more than 5% fat content (Undeland et al., 2002; Kristinsson

and Hultin, 2003; Kristinsson and Ingadottir, 2006). Hence, this method is expected to be suitable for poultry dark meat as well, wherein the fat content is similar to that of fatty fishes.

The color of dark meat is one of the major problems affecting broiler dark meat utilization, which in turn affects consumer satisfaction (Fletcher, 1997; Betti and Fletcher, 2005). It is reported that the major problems with surimi and other materials rich in dark muscle are caused by their poor gel-forming properties, as well as with color and lipid oxidation (Okada, 1980; Hultin and Kelleher, 2000). The gel-forming ability of myofibrillar proteins is of much interest because it is responsible for enhanced texture. The proteins responsible for gelation properties are myosin and actomyosin (Ashgar et al., 1985). Dynamic measurements involving small deformations under constant or sinusoidal oscillating stress may give more reliable information on the viscoelastic nature of the gel when compared with a large strain test (Oakenfull et al., 1997).

The most important property that determines the organoleptic quality of any meat product is texture (Ordóñez et al., 2001). An adequate texture shows in-

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creased consumer acceptance (Stanley, 1976). Human perception of meat quality mainly depends on the complex interaction of sensory and physical processes during chewing. If such a condition is simulated by an instrument, it can give information on the textural quality of food products. Texture can be evaluated by measuring the response of food when subjected to shearing, chewing, compressing, or stretching. Hence, texture profile analysis (TPA) is widely used for assessing textural properties of food (Tabilo et al., 1999).

Extraction and recovery of proteins by treating at high pH, followed by acid precipitation, have been attempted on poultry meat to concentrate the proteins (Jelen et al., 1982; McCurdy et al., 1986). However, data showing the textural and rheological properties of alkali-extracted proteins from chicken meat are scarce. Hence, the aim of the present investigation was to establish the color characteristic, as well as textural properties, of recovered proteins after alkali extraction of chicken dark meat.

MATERIALS AND METHODS

Frozen chicken thigh meat was obtained from Lilydale Inc. (Edmonton, Alberta, Canada) and used for this study. The chicken thigh meat was thawed at 4°C overnight. It was then minced using a meat grinder (Waring Pro, Woodbridge, Ontario, Canada), vacuum-packed in 400-g plastic bags, and stored at -30°C until analysis.

Extraction Method

The extraction was carried out as per the methods described earlier (Liang and Hultin, 2003; Betti and Fletcher, 2005) with modifications. For the extraction of protein isolate, 400 g of frozen chicken thigh meat was thawed at 4°C overnight. The meat was further mixed with an ice-distilled water mixture (1:2.5; wt:vol) using a food processor (Wolfgang Puck Appliances, Hollywood, FL) for 15 min. Further, the pH of the resulting meat slurry was adjusted using 2 M NaOH with constant mixing. Individual lots were adjusted to pH 10.5, 11.0, 11.5, and 12.0 for alkaline hydrolysis-protein solubilization and allowed to stand still at 4°C for 30 min. The slurry was centrifuged using an Avanti J-E refrigerated centrifuge (Beckman Coulter Inc., Palo Alto, CA) at 25,900 × g for 20 min at 4°C. Three layers were formed after centrifugation: an upper fat layer, a middle aqueous layer of soluble proteins, and a bottom sediment layer. The middle aqueous layer of soluble proteins was then carefully removed and its pH was adjusted to 5.2 using 2 M HCl. Because pH 5.2 is the isoelectric pH, the proteins are precipitated. The proteins thus precipitated were separated by centrifugation at 25,900 × g for 20 min at 4°C. These separated proteins were further mixed with iced water and the pH

of this solution was readjusted to pH 6.2, which is the ultimate pH of this type of meat, to regain its original functionality. This readjustment of pH was followed by centrifugation (25,900 × g, 20 min, 4°C) for the final separation of the proteins. Hence, this method of protein recovery is also called pH-shift method. After determining the moisture content of the protein extract, the final moisture content was adjusted to 80% then mixed with cryoprotectants (5% sorbitol, 4% sucrose, 0.3% sodium tripolyphosphate, 0.4% sodium bicarbonate, and 0.03% sodium nitrite) using a Kitchen-Aid meat processor (model KFP 7500B, Kitchen-Aid, St. Joseph, MI). The processor was prechilled and operated in a cold room (6 to 10°C) to prevent the temperature of the protein isolate from exceeding 15°C throughout the mixing process. The recovered proteins were then stored at -30°C for 1 mo, after which samples were drawn for analysis.

Cooking Loss

Samples weighing 25 to 30 g (W1) were packed in plastic tubes. The tubes were then heated at 95°C until the internal temperature of the samples reached 75°C. The temperature was checked using thermocouples inserted in the center of the sample. The samples were considered cooked when the internal temperature reached 75°C. After cooking, the meat was weighed again (W2) to determine the loss in weight during cooking:

$$\text{Cooking loss (\%)} = (W1 - W2/W1) \times 100.$$

Expressible Moisture

The expressible water content was determined using a texture profile analyzer (TA XT Express, Stable Micro Systems Ltd., Surrey, UK). A known quantity of fresh sample (approximately 300 mg) was placed on a preweighed filter paper (Whatman No.1, Whatman International Ltd., Maidstone, UK) and sandwiched between 2 glass plates. Using the texture profile analyzer under adhesive test mode, the sample was tested with a target force of 1,000 g. The hold time for this test was 2 min, which was sufficient to express the water content of the sample. After the test, the filter paper along with the absorbed water was immediately weighed. Expressible water was measured as the quantity of water released per gram of meat and was expressed as a percentage:

$$\text{Expressible water (\%)} = (\text{wet paper} - \text{dry paper} / \text{meat weight}) \times 100.$$

Color Characteristics

A Minolta CR-400 (Konica Minolta Sensing Americas Inc., Ramsey, NJ) colorimeter using illuminant D65

as the light source was used to assess the color (CIE L* a* b*) of alkali-recovered protein samples; L* refers to lightness, a* refers to redness, and b* refers to yellowness. Color of recovered proteins was measured on the frozen samples stored at -30°C for a month. Six readings per treatment sample were taken and the average reading was recorded. Whiteness was calculated as per the following formula:

$$\text{Whiteness} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}.$$

Textural Profile Analysis

The TPA was carried out using a texture profile analyzer (TA-XT Express, Stable Micro Systems Ltd.). Samples (weighing 6 g) cylindrical in shape (1.0-cm height and 4.0-cm width) were prepared from the recovered protein samples. The analysis was carried out in TPA mode, using the TA-XT Express software included with the equipment. A double compression cycle test was performed up to 50% compression of the original portion height with an aluminum cylinder probe of 5-cm diameter. A time of 1 s was allowed to elapse between the 2 compression cycles. The trigger force used for the test was 5 g, with a test speed of 5 mm/s. When the test was finished, the software calculated the values for hardness (maximum force required to compress the sample), springiness (ability of the sample to recover its original form after deforming force was removed), chewiness (the work needed to chew a solid sample to a steady state of swallowing), cohesiveness (extent to which the sample could be deformed before rupture), and resilience. The analysis was carried out for 5 replicates.

Dynamic Viscoelastic Behavior of Recovered Proteins

The dynamic viscoelastic behavior (DVB) of raw chicken meat and treated samples in the temperature range of 7 to 100°C was measured using a Physica MCR Rheometer (Anton Paar GmbH, Ashland, VA) under oscillatory mode, using a 2.5-cm parallel plate measuring geometry. Approximately 4 g of macerated meat was ground thoroughly to obtain a fine paste. The paste was used for DVB measurement. The gap between measuring geometry and the Peltier plate was adjusted to 1,000 μm . The measurements were made by applying a small amplitude oscillation (0.5%) with a frequency of 1.0 Hz. A heating rate of $2^{\circ}\text{C}/\text{min}$ was achieved through the Peltier plate of the rheometer. The applied stress was compared with the resultant strain. The results of the measurements were expressed as the storage modulus (G') and loss modulus (G''). An average of 3 replicates was used for plotting the results.

Statistical Analysis

Each experiment and each assay were done at least in triplicate. Reported results represent an average of each experimental assay. All data were subjected to ANOVA using the GLM procedure of SAS (SAS, 2006). Differences between least squares means were determined using Tukey's honestly significant differences test and were reported as significant at the $P < 0.05$ level.

RESULTS AND DISCUSSION

Water-holding capacity is one of the important properties that determines the quality of meat. Expressible moisture and cooking loss together give an indication of tenderness of a meat product (El Rammouz et al., 2004), which is further related to the water-holding capacity of the proteins. Cooking loss was assessed in the present investigation to measure the effect of extraction pH on the water-holding capacity of the isolated proteins. Cooking loss decreased for protein samples treated at higher pH values (Figure 1). The decrease was greatest for proteins extracted at pH 11.5 and 12.0. The decrease in cooking loss at pH values above 11.0 indicates higher water-holding capacity of the extracted meat probably due to higher protein content (Moayedi et al., 2010) and to greater entrapment of water resulting from gel formation due to the increased protein hydrophobicity. Extraction of proteins at alkaline pH above 11.0 causes unfolding of proteins leading to an increase in protein hydrophobicity (Omana et al., 2010). The exposed hydrophobic amino acids may lead to hydrophobic interactions resulting in formation of a gel network with improved ability to entrap water (Niwa, 1992). The decrease in cooking loss for extracted proteins also shows that proteins are not strongly oxidized even at this high pH of extraction; oxidation could impair water-binding in meat (Morzel et al., 2006). Cooking loss was found to be the maximum for the raw dark meat. This result was expected because minced dark meat was not mixed with cryoprotectants and contained more fat. Cryoprotectants were added to all of the other samples and it has been reported that cryoprotectants improve water-holding capacity (Henry et al., 1995). These additives protect the washed-extracted myofibrillar proteins against damage due to freezing (MacDonald and Lanier, 1991). This is achieved by increasing the surface tension of water that may stabilize the proteins by favoring solute exclusion from the protein surface so as to enhance the strength of intermolecule hydrophobic interaction (MacDonald and Lanier, 1991). Ensoy et al. (2004) reported higher water-holding capacity for surimi samples prepared from spent layers with added cryoprotectant mixture.

Expressible moisture, which is an important parameter indicating meat texture, tenderness, and juiciness, denotes the ability of meat to hold water (Mallikarjunan and Hung, 1997). Percentage of water loss (expressible

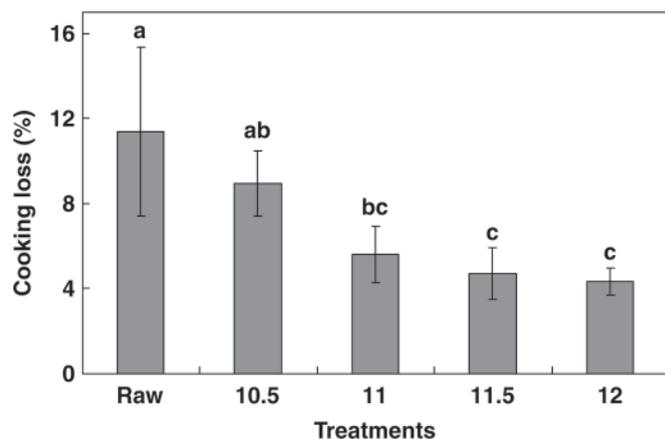


Figure 1. Cooking loss of different treatments during alkali extraction of chicken dark meat. Dissimilar letters (a, b, c) in the figure denote significant difference ($P < 0.05$).

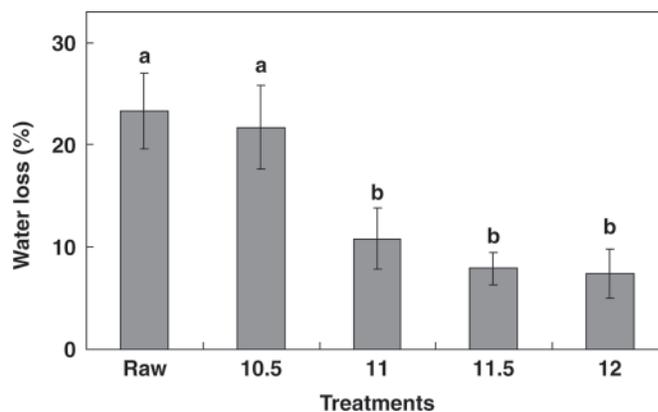


Figure 2. Expressible moisture of different treatments during alkali extraction of chicken dark meat. Dissimilar letters (a, b) in the figure denote significant difference ($P < 0.05$).

moisture) was found to decrease with increase in pH of extraction (Figure 2). Water loss was significantly ($P < 0.05$) lower for pH treatments of 11.0, 11.5, and 12.0 and was less than 10%. Similar results were obtained by Kristinsson and Liang (2006) while analyzing press loss of alkali-extracted fish protein isolates. Lower expressible moisture indicates that the protein network of the gel has a higher water-holding capacity (Rawdkuen et al., 2009). An approximate inverse relationship between expressible moisture and hardness of samples was observed by textural analysis (see below and Table 1). Rawdkuen et al. (2009) also reported lower expressible moisture for samples with higher breaking force. As hypothesized for cooking loss, the exposure of more hydrophobic groups for proteins extracted at higher pH values leads to formation of gel with improved water-holding capacity. In addition, a higher protein content at higher pH of extraction may also have contributed to enhanced water-holding capacity (Moayedi et al., 2010). Water loss of the protein sample treated at pH 10.5 was found to be similar to raw dark meat, indicating less exposure of hydrophobic groups.

The major drawbacks of products prepared from minced meat rich in dark meat are poor gelation properties and the problems associated with color and lipid oxidation (Okada, 1980; Hultin and Kelleher, 2000). The color characteristics of the recovered proteins (before and after gelation) extracted as a function of pH values are given in Table 2. In general, at higher pH

of extraction, the L^* value (lightness) of the recovered proteins was found to be significantly ($P < 0.05$) lower compared with the raw dark meat. This lower L^* value for the extracted proteins may be due to the effect of frozen storage at -30°C , which may render oxidation of the remaining pigments in the extracted and recovered proteins. For example, it is reported that frozen storage caused a decrease in all color parameters in the case of myofibrillar protein isolate from mechanically deboned chicken meat (Uijttenboogaart et al., 1993). Higher L^* values for water-washed mince gels from rockfish and whiting were also observed compared with alkali isolate gels (Choi and Park, 2002; Yongsawatdigul and Park, 2004). Cooking increased the L^* for the respective treatments. Cooking in general produced light color meat in the samples compared with raw dark muscle. This increase in L^* value is due to an increased reflection of light arising from light scattering by denatured proteins (Young and West, 2001). With an increase in extraction pH, there was significant decrease in a^* value (redness) both in cooked and uncooked samples. The higher redness in the raw dark meat is due to a high amount of native heme proteins (Moayedi et al., 2010). After cooking, the a^* values decreased for all treatment samples. The decrease may be due to protein denaturation (Young and West, 2001), in which heme, which normally bonds to globin proteins, could no longer bind, resulting in less redness. The results were consistent with previous studies (Choi and Park, 2002; Undeland et al., 2002). The samples prepared at extraction pH

Table 1. Textural profile analysis of recovered proteins after protein extraction by the pH-shift method¹

Parameter	Raw	pH 10.5	pH 11.0	pH 11.5	pH 12.0
Hardness (g)	2,836 ^b (767)	3,151 ^{ab} (1,318)	3,782 ^{ab} (1,292)	4,956 ^a (1,366)	4,334 ^{ab} (424)
Springiness	0.98 ^a (0.21)	1.17 ^a (0.41)	0.97 ^a (0.05)	1.24 ^a (0.46)	1.36 ^a (0.42)
Chewiness	1,521 ^b (641)	2,840 ^{ab} (1,941)	2,753 ^{ab} (777)	4,492 ^a (1,289)	4,584 ^a (1,573)
Cohesiveness	0.54 ^b (0.06)	0.73 ^a (0.03)	0.77 ^a (0.05)	0.77 ^a (0.03)	0.77 ^a (0.02)
Resilience	0.30 ^b (0.04)	0.43 ^a (0.03)	0.52 ^a (0.07)	0.51 ^a (0.06)	0.52 ^a (0.03)

^{a,b}Dissimilar superscripts in the same row denote significant difference ($P < 0.05$).

¹Results are presented as means values ($n = 6$). Values in parentheses denote SD.

of 11.5 and 12.0 showed significantly ($P < 0.05$) higher b^* values (yellowness) in uncooked samples. The higher level of yellowness is probably due to higher levels of denatured and oxidized heme proteins (Kristinsson et al., 2005) and increased phospholipids (Moayedi et al., 2010). The whiteness of uncooked samples decreased significantly at higher pH values. The lower whiteness of alkali-treated samples was due to the remaining hemoproteins in the recovered proteins (Choi and Park, 2002). There was no difference in whiteness between samples prepared at extraction pH of 11.0, 11.5, and 12.0. However, there were increases in whiteness values for all of the samples after cooking. The increase in whiteness in cooked gels could be due to heat treatment and changes of native heme protein during protein gelation (Rawdkuen et al., 2009). Whiteness is mainly caused by changes in L^* values; hence, its magnitude is higher than a^* and b^* values. After cooking, L^* value increased significantly, which further increases the whiteness of the cooked gels. This is mainly due to the increased reflection of light due to denatured proteins after cooking (Young and West, 2001).

It has been reported that alkali-aided protein extraction caused less denaturation than an acid-aided process (Kristinsson et al., 2005). This lower denaturation of proteins leads to products with enhanced texture (Chen and Jaczynski, 2007). Texture profile analysis of proteins extracted as a function of various pH values is presented in Table 1. Hardness values were higher for samples prepared at higher extraction pH values and hardness was found to be maximum (4,956 g of force) for samples prepared at pH 11.5. The exposure of more hydrophobic groups due to protein extraction at higher pH (Omana et al., 2010) values leads to more protein-protein and protein-cryoprotectant interactions. These interactions may lead to formation of a gel network with increased hardness. Tadpitchayangkoon and Yong-sawatdigul (2009) hypothesized that such a gel network would differ from a typical gel network of myofibrillar proteins. Elastic gels are derived from numerous cross-links between the myofibrillar proteins. When this gel network is unevenly distributed due to local aggregation of myofibrillar proteins, it will lead to formation of poor gels, which is indicated by lower G' values in rheological analysis. These gels appear harder due to the presence of this local protein aggregation and will break easily with the application of force (Feng and Hultin, 2001). The increase in hardness may also be due to the stronger gel network formed by the concentrated myofibrillar proteins in the protein isolates. Chen et al. (1997) observed an increase in breaking force for myofibrillar proteins concentrated by washing procedures. Previous studies of our group showed that treated samples at pH 10.5 and 11.0 had a higher protein content (Moayedi et al., 2010). Hence, this higher protein content might also contribute to the increased hardness of these samples.

There was no significant difference for springiness values between various samples prepared by different

Table 2. Color characteristics of recovered proteins after the alkali extraction method¹

Treatment	L^*		a^*		b^*		Whiteness	
	Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking
Raw meat	74.49 ^a (1.34)	77.42 ^a (0.62)	7.57 ^a (0.95)	4.22 ^a (0.65)	16.05 ^{bc} (0.55)	14.26 ^b (0.56)	65.22 ^a (1.71)	70.81 ^a (0.75)
pH 10.5	69.15 ^b (1.56)	71.22 ^b (1.55)	0.50 ^c (0.23)	0.10 ^b (0.40)	15.46 ^c (0.90)	15.50 ^a (0.74)	65.24 ^a (1.15)	67.25 ^b (1.48)
pH 11.0	62.74 ^c (3.34)	69.52 ^{bc} (1.32)	1.36 ^{bc} (0.61)	-0.16 ^b (0.67)	14.90 ^c (0.29)	14.96 ^{ab} (0.55)	59.34 ^b (3.19)	66.11 ^{bc} (1.42)
pH 11.5	64.61 ^c (1.40)	67.42 ^c (1.93)	1.86 ^b (0.88)	0.16 ^b (0.34)	17.46 ^{ab} (1.04)	15.48 ^a (0.53)	59.66 ^b (1.89)	63.86 ^c (2.03)
pH 12.0	65.26 ^c (1.15)	68.43 ^c (1.47)	1.09 ^{bc} (0.66)	-1.01 ^c (0.25)	17.88 ^a (1.24)	15.90 ^a (0.41)	60.38 ^b (0.88)	65.09 ^{bc} (1.31)

^{a-c}Dissimilar superscripts in the same column denote significant difference ($P < 0.05$).

¹Values in parentheses denotes SD; n = 6.

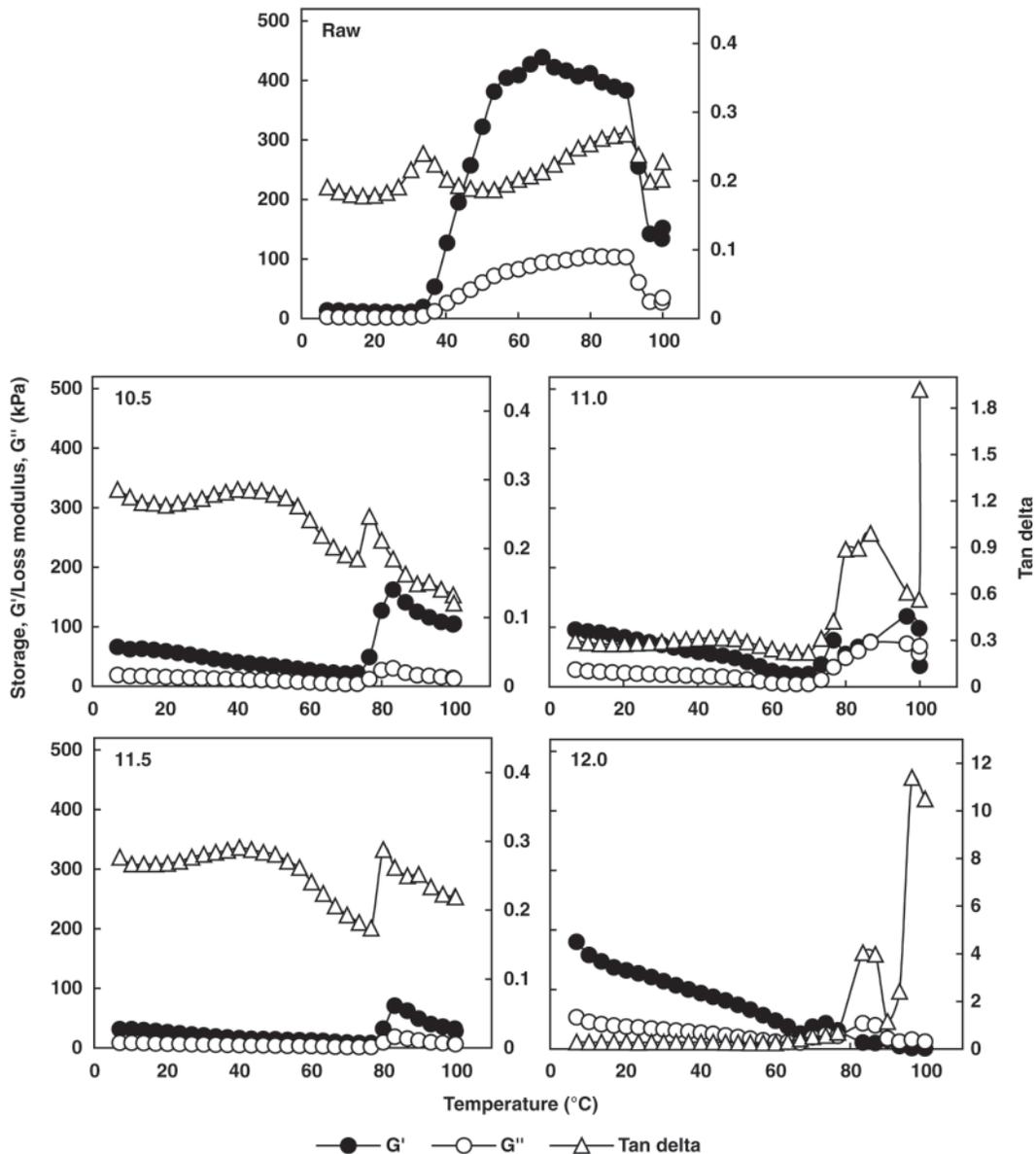


Figure 3. Dynamic viscoelastic behavior of recovered proteins from chicken dark meat after alkali extraction (without cryoprotectants). Dynamic viscoelastic behavior of fresh chicken dark meat is given for comparison. Storage/loss moduli are drawn on the same scale for all of the graphs.

pH. This may be due to the fact that all of the samples were adjusted to equal moisture content. Chewiness values were significantly ($P < 0.05$) increased for protein samples extracted at pH values of 11.5 and 12.0. Chewiness, which indicates the resistance to compression force, is probably the main textural property determining tenderness characteristics (Caine et al., 2003). Higher chewiness values at higher pH may be due to increased water-binding ability of the extracted proteins (Figure 2). The values for cohesiveness and resilience increased significantly ($P < 0.05$) for the pH-treated samples compared with raw dark meat and among the various treatments, there was no significant difference. Caine et al. (2003) speculated that the change in resilience value was mainly due to the i.m. fat content. The alkali-aided process resulted in significant lipid loss. Lipid loss varied between 45 and 53% during alkali

treatments at various pH (10.5 to 12.0) values (Omana et al., 2010). The difference between TPA parameters of the recovered meat from the raw dark meat may be due to the difference in moisture, lipid, and collagen content.

The dynamic rheological test has been used widely to study the heat-induced gelation of myofibrillar proteins (Hamann, 1987; Visessanguan et al., 2000). Because G' measures the energy recovered per cycle of sinusoidal shear deformation, its increase indicates the formation of elastic gel network. Hence, the changes in storage modulus have been used to monitor gelation of proteins (Venugopal et al., 2002). On the other hand, loss modulus measures the energy dissipated or lost as heat per cycle of sinusoidal strain and indicates the viscous element in the sample. The changes in stress-strain phase angle during oscillatory test indicate the sol-gel transi-

tion temperatures, which can be expressed as $\tan \delta$ values.

Dynamic viscoelastic behavior of recovered proteins after extraction (without cryoprotectants) at various pH values is given in Figure 3. The DVB of fresh chicken dark meat indicated considerable gel-forming ability as revealed by G' values. The maximum G' value of 439 kPa was measured at 66.7°C. The increase in G' value showed that proteins underwent ordered aggregation and formation of a 3-dimensional network with entrapment of water in the matrix (Dileep et al., 2005). The G'' value also increased during heating. This indicates formation of a viscoelastic gel network. The maximum rate of increase in G' values was found to be in the temperature range of 40 to 60°C. The forces responsible for gelation have been found to be hydrophobic interac-

tions, disulfide crossbridges, and hydrogen bonds (Hammann, 1992). Storage modulus decreased drastically for extracted proteins prepared at alkaline pH value. The maximum G' value of all of the protein samples was found above 80°C and the value was less than 200 kPa. The proteins completely lost the ability to form an ordered gel network. At pH 12.0, G' value was decreasing even at temperatures above 70°C, wherein proteins usually form strong gel networks. The proteins underwent extensive denaturation; hence, the proteins failed to form crosslinks between each other to form an ordered gel. This decrease in G' value of extracted proteins may also be due to the removal of collagen during the extraction process. During heating, collagen transforms from a quasi-crystalline structure into a random-like structure (Lepetit, 2008). After this transition around

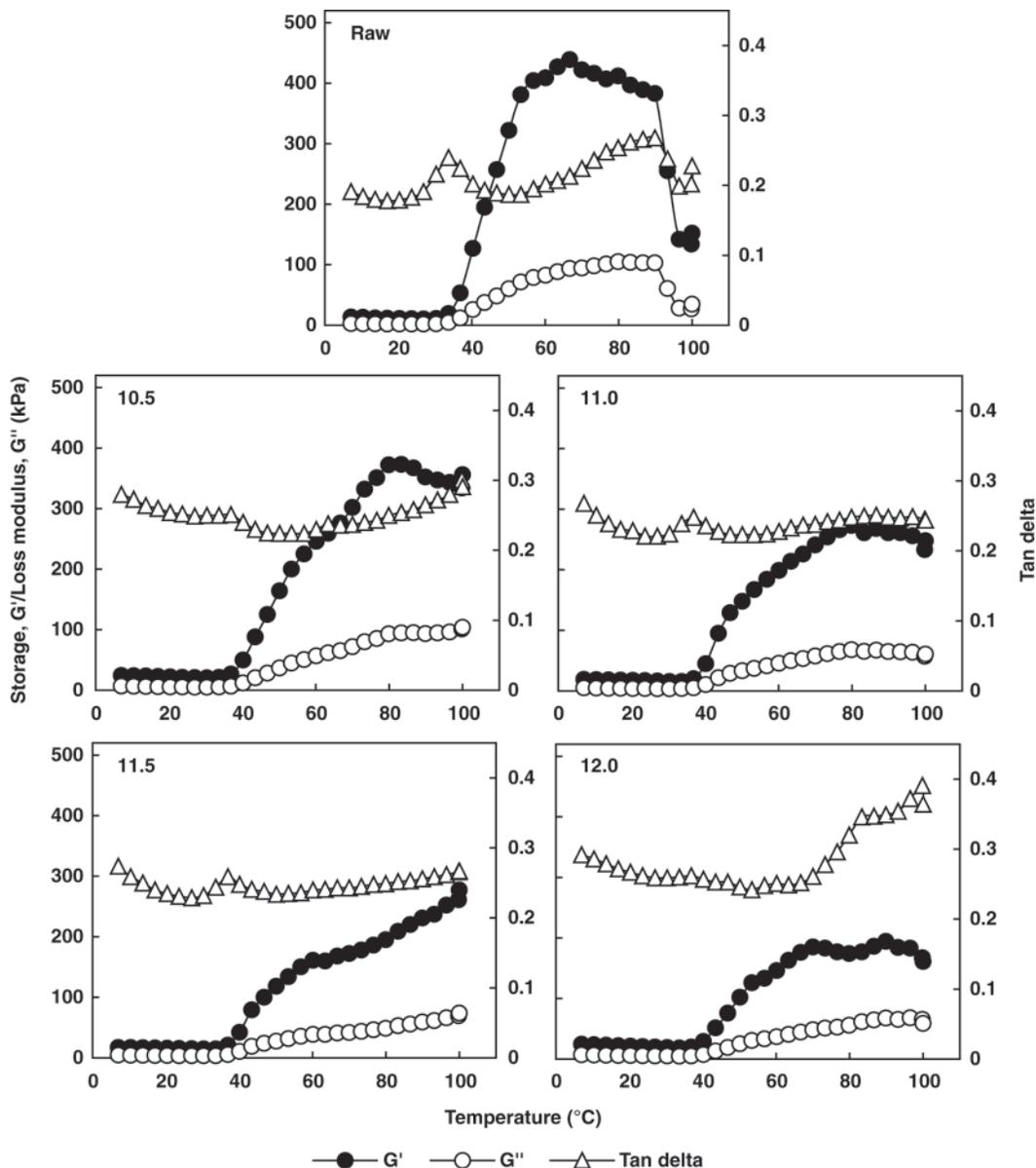


Figure 4. Dynamic viscoelastic behavior of recovered proteins from chicken dark meat after alkali extraction, stored at -30°C for 1 mo with added cryoprotectants. Dynamic viscoelastic behavior of fresh chicken dark meat is given for comparison. Storage/loss moduli are drawn on the same scale for all of the graphs.

60°C, collagen behaves according to the theory of rubber-like elasticity (Kopp and Bonnet, 1987), leading to an increase in meat elasticity. Hence removal of such a component might cause a decrease in elastic modulus of the extracted meat.

The addition of cryoprotectants to the extracted proteins helps to regain the elastic properties of the recovered proteins (Figure 4). The maximum G' values of the recovered protein samples were found to decrease with increase in treatment pH, indicating the lower ability of proteins to form an elastic gel network at higher pH. The proteins extracted at a pH value of 12.0 showed the lowest G' value of 194 kPa. Even though the recovered proteins showed high hardness values (Table 1) compared with raw dark meat, G' values were considerably lower. The reason may be due to the aggregation of these denatured proteins due to heating. Tadpitchayangkoon and Yongsawatdigul (2009) observed that denatured fish protein isolates became aggregates during heating, which caused increasing protein-protein interaction resulting in rigid gel networks. However, the protein-water binding was reduced, leading to high breaking force with low deformation. Alkali-aided proteins from Atlantic menhaden also showed lower deformation compared with those of conventional surimi (Perez-Mateos and Lanier, 2006).

Storage modulus (G') was found to be marginally decreasing until the onset of gelation at 30.3°C. Further increase in temperature resulted in an increase in G' values for all of the samples. The G' value of raw dark chicken meat increased until 66.7°C, above which it decreased. However, in the case of recovered protein samples, the G' values were increasing even above 80°C, showing that the gel network formed is stable even at elevated temperature. Tan delta values denote 2 clear transitions for raw dark meat; one at 50.1°C and the second one at 96.5°C. Wright et al. (1977) demonstrated that the transition at 55°C was due to the denaturation of myosin molecule and the one at 63°C due to collagen and the sarcoplasmic protein denaturation. However, only 1 major transition at 50.1°C was evident for pH-treated samples. This may be due to the fact that the collagen layer is removed during the extraction process. The difference in gelation behavior of the recovered proteins compared with raw meat would be due to the partial denaturation of the proteins during high pH of extraction (Kristinsson and Hultin, 2003; Davenport and Kristinsson, 2004) as well as due to collagen removal.

Significant decrease of cooking loss and water loss was observed for samples prepared at higher pH of extraction. Increase in pH of extraction caused reduction in L^* value of the treated samples stored at -30°C for 30 d, which further reflects the whiteness values of respective samples. Hardness increased for samples prepared at higher pH values compared with raw dark meat. The pH-treated samples were found to have increased chewiness values. Even though the hardness of pH-extracted samples was higher, the samples showed less elasticity,

which was indicated by lower storage modulus values. However, the gels obtained from recovered meat with added cryoprotectants showed a stable gel formation, which was not disrupted even at temperatures above 80°C. Raw dark meat showed a maximum G' value of 439 kPa, indicating a considerable gelling ability of the dark meat, which decreased with pH of extraction. Instead of 2 transitions observed in raw dark meat, pH-treated samples showed only 1 transition, probably due to the removal of the collagen layer during the process. In conclusion, the recovered proteins can be used for preparation of functional foods. This study revealed the possibility of usage of underused chicken dark meat by alkali-aided protein recovery.

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