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Original Paper

Quantity and Functionality of Protein Fractions Isolated from 3 Ecotypes of Indigenous Chicken in Kenya

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Abstract

The aim of this study was to evaluate the effect of the cluster ecotype and the part of chicken on nutritional composition, and functionality of sarcoplasmic and myofibrillar proteins that are most relevant to the technological features of chicken meat. Over 50 chickens from each ecotype cluster purchased, slaughtered and the meat stored under refrigeration at -20^{o}C and later on transferred in cooler box on ice and flown to South Africa, at the Durban University of Technology. Protein fractions were extracted with a cocktail of Sodium Chloride buffer (50mM NaCl, 50mM Tris HCl; 75mM DTT and 1mM EDTA at pH 7) and quantified by Bradford method. One dimensional Sodium Dodecyl Polyacrylamide Gel Electrophoresis (SDS PAGE) was applied to separate protein fractions. Emulsifying capacity, emulsifying stability, solubility, and in vitro digestibility were determined on the total protein isolates. Significant differences in band expressions were recorded for the myofibrillar and the sarcoplasmic proteins. The three ecotypes had high quality proteins with all the limiting and essential amino acids at concentrations higher than FAO/WHO recommended daily allowance for adults and children. Distinct protein bands at larger molecular weight proteins >100 kDa, corresponding to Myosin Heavy Chain, medium fractions 75 kDa and 45 kDa and even lower molecular weight fraction <25 kDa were present in the chicken breast and the thighs. It concludes that Indigenous chicken protein isolates' nutritional and functional properties are affected by part of chicken and ecotype clusters.

Keywords

Indigenous chicken, protein isolate, functionality, sarcoplasmic, myofibrillar

1. Introduction

Researchers have recently used the microsatellite markers for the genetic evaluation of chicken variability (Qu et al., 2006, Ngeno et al., 2015). Indigenous chicken (IC) in Kenya have recently been classified genetically into 3 ecotype clusters based on allele type of LEI0258 marker genotype for Major Histocompatibility Complex (MHC) of the variable region (Ngeno et al., 2015). Limited work if any has been done to evaluate meat quality of IC in Kenya according to (Ngeno et al., 2015) based on this classification. Yet the clusters of indigenous chicken in Kenya show distinct morphological, sensorial and other quality characteristics (Oloo et al., 2017). This may be attributed to the difference in genetic makeup or the differences in the traditional rearing practices by the separate communities which predominate the areas where these chicken ecotypes are found. The source of this variability and how it impacts the meat quality with regard to the protein isolates' functionality and expressions is yet to be studied. Poultry meat is made up of water, proteins, fat, minerals, and carbohydrates (Omana et al., 2010; Bender, 1992). These components determine the functional and sensory quality of the meat. Chicken is a source of high quality protein because they contain all the essential amino acids needed by human body; they are highly nutritious (Friedmann, 1996). Protein composition affects overall meat characteristic of appearance, texture, and mouth feel (Mudalal et al., 2014). Two major types of chicken muscle proteins (myofibrillar and actin) are responsible for muscle texture and water holding capacity (WHC) (Ooizumi & Xiong, 2004). The sarcoplasmic proteins on the other hand, play only a minor role (Petracci et al., 2013). The solubility of sarcoplasmic and myofibrillar proteins on the other hand are reported to be correlated to water retention capacity of chicken meat resulting in low cooking losses (Li-Chan et al., 1987; Warner et al., 1997). Lower protein solubility has an influence on the functionality and or defects of chicken proteins such as the pale soft exudative (PSE) condition (Mudalal et al., 2014).

Meat proteins are generally categorised in terms of their solubility as; salt, water, or alcohol soluble. Chicken protein composition especially of breast; have a crucial impact on processability of meat, its nutritional, and sensory quality (Mudalal et al., 2014). The knowledge of protein composition and structure is important to connecting of functionality and applications in various foods. Protein composition and structure may vary across different ecotypes or genotypes and this is likely to affect their functionality (Arise, 2016). The high molecular weight proteins are known to confer functional properties to the skeletal muscle. Myosin, actin, titin and nebulin are the major of the skeletal sarcomere and are most important in this regard. Myosin with a (molecular weight) MW of 540 kDa and Actin of approximate MW 43 kDa are most noticeable (Clark et al., 2002). The aim of the current study was to evaluate the effect of the cluster ecotype and the part of chicken of indigenous chicken in Kenya on the chemical composition, protein properties and functionality with emphasis on sarcoplasmic and myofibrillar proteins that are most relevant to the technological features of chicken meat.

2. Method

2.1 Chemical Composition

Proximate analysis (moisture, protein, lipid and ash) of breast and thighs of indigenous chicken were estimated in 3 replications for each sample using AOAC (1990) official methods. Moisture content was evaluated by weighing 5 grams of sample and drying in a conventional oven at 103^{o}C for 5 hours until constant weight was reached. Crude protein content was estimated by Kjeldhal method while edible fat (intra-muscular fat) was estimated by petroleum ether extraction using Soxhlet method. Ash content was evaluated by incineration in a muffle furnace at 525^{o}C for 5 hours.

2.2 Amino Acid Composition Determination

Amino acid content of the indigenous chicken fillets were determined on lyophilized, ground and homogenous samples by Pico-Tag method as described by (Bidlingmeyer et al., 1984). A total of 16 amino acids were analysed.

2.3 SDS PAGE -Analysis

Frozen chicken breast and thigh muscles were selected to separate the extracted proteins according to their molecular weights by SDS-PAGE according to the procedure of (Mudalal et al., 2014) with slight modifications. Ten (10) grams of samples was mixed with 10 mls cocktail of sample extraction buffer (50 mM $NaCl_{2,}$ 50 mM Tris-HCL (pH=7.0); DDT (75 mM DTT) and 1 mM EDTA (pH=7). Resulting sarcoplasmic and myofibrillar proteins were mixed with equal volume of standard loading Buffer as previously described by (Mudalal et al., 2014). Samples were then heated at 95^{o}C for 5 minutes in a water bath cooled on ice and then applied to a gel at a volume of 15 uL of both sarcoplasmic and myofibrillar proteins and loaded into the already prepared gels. The small gels were set to run at a constant voltage of 180 V. Reservoir buffer was made up of 50 mM Tris, 0.384 M Glycine, and 0.1% (wt/vol) SDS. A mixture of standard proteins (11-190 kDa) was used as the molecular weight marker and the gels were stained in Coomassie brilliant R-250.

2.4 Emulsifying Activity and Stability

Emulsifying activity was determined according to the procedure described by (Lawal et al., 2007). Equal volumes (5 mls) of protein (isolates from chicken) were mixed with equal volumes of pure sunflower oil and homogenized. The emulsifying activity was calculated as the height of the emulsified layer over the overall content of tubes and expressed as a percentage. Samples were then heated at 80^{o}C for 30 minutes and the remaining emulsified layer used to calculate emulsifying stability.

2.5 Protein Solubility

To quantify protein solubility, difference in extractability using different ionic strength solutions was exploited. This was modified from the procedure used by (Warner et al., 1997). After solubilization of proteins, the supernatant was carefully poured out temporarily to separate the sediment. Resultant protein concentration was quantified by Bradford with (Bovine Serum Albumin) BSA as the standard (Bradford, 1976). For total solubility, a high ionic strength of 1.1 M KI and 0.1 M potassium phosphate buffer of (pH 7.2) was used. The difference in total protein solubility from that of sarcoplasmic protein

solubility was used to determine the myofibrillar protein content (Zhang et al., 2009).

2.6 In Vitro GI Digestion (Sangsawad et al., 2017)

In vitro digestion of boiled chicken meat was performed according to (Garrett et al., 1999) and adopted by (Sangsawad et al., 2017), with slight modifications. The boiling of chicken meat was done in six times volume of water in a temperature-controlled furnace at temperature of 95±1℃ for 15 minutes; equivalent to well done. Cooked samples (2 g dry solid) were homogenized (IKA Works Asia, Bhd, Malaysia) in 100 mls of deionized water (DI) for 1 min. The pH was adjusted to 2.0 ± 0.02 with 1M HCl and pepsin (2.86% of substrate, dry basis) was added, and digestion performed at 37^{o}C for 1 h in a shaker set at 95 rpm speed. Subsequently, the pH was adjusted to 7.5 ± 0.02 by adding 5 M NaOH. Pancreatin enzyme (4.00% of substrate, dry weight basis) was added and the reaction was carried out at 37^{o}C for 2 h in the shaker set at 95 rpms. The enzymatic digestion was terminated by submerging the sample in a 95^{o}C water bath for 10 min and then cooling on ice to room temperature. The digested mixtures were then centrifuged at 10,000 g for 10 min. The peptide content of the supernatant was determined using Bradford assay, with BSA as the standard.

2.7 Statistical Analysis

The resulting data was subjected to statistical analysis. A One-Way Analysis of Variance (ANOVA) was employed to determine statistical difference using SAS PROC GLM procedure version 9.3 (SAS Institute Inc, Cary, NC). All data was presented as means ± standard deviation. Duncan's Multiple Range test ($p<0.05$) was used to identify significant differences among means.

3. Results

3.1 Chemical Composition of Indigenous Chicken Ecotypes in Kenya

Among the ecotypes, no significant difference was reported in moisture content between the thighs and the breasts ($P>0.05$). In all the ecotypes the thighs recorded higher moisture content than breasts. The mean moisture content reported for the IC ecotypes ranged from 69-72% (Table 1.) This compares favourably with the values reported by Chepkemoi et al. (2017) on the nutritional quality of five different poultry in Kenya. The mean values recorded (breast 71.6% and thigh 71.3%) were lower than those reported by (Chumngoen & Tan, 2015) who got an average moisture of 74.73% among the Taiwan native chicken.

Table 1. Chemical Composition of Indigenous Chicken in Kenya from the Different Ecotypes: Values Are Expressed in (g/100g) of Sample

Ecotype	Chicken Part	Moisture Content	Fat Content	Protein Content	Ash Content
Kakamega	Breast	72.53±1.00[a]	1.75±0.86[a]	21.21±1.50[a]	4.43±1.03[a]
	Thigh	73.32±1.32[a]	3.59±0.95[a]	19.02±2.04[a]	3.88±0.96[a]
Naivasha	Breast	70.40±0.91[a]	1.64±0.31[a]	24.08±1.11[a]	4.61±0.38[a]
	Thigh	71.69±1.83[a]	2.95±0.89[a]	22.99±1.35[a]	4.23±0.49[a]
Taita	Breast	72.46±0.79[a]	2.78±1.00[a]	24.31±0.91[a]	3.00±0.83[a]
	Thigh	69.09±4.97[a]	2.77±0.80[a]	21.97±1.99[a]	2.96±0.40[a]

Values given as mean ± standard deviation (n=30).

[a]-Means and standard deviations within the same column with no common superscript are significantly different at (P>0.05).

3.2 The SDS-PAGE of Proteins from the Kenyan Indigenous Chicken Ecotypes

The SDS PAGE for Kakamega ecotype reveals distinct bands between the myofibrillar and sarcoplasmic proteins. Up to twelve (12) bands were noticeable on the sarcoplasmic proteins and a maximum of 10 were reported on the myofiobrillar protein for the breast muscle (Figure 1). This confirms the report by (Mudalal et al., 2014) that SDS-PAGE analysis for meat proteins from normal and white striated fillets showed different patterns for sarcoplasmic and myofibrillar proteins. The myosin heavy chain (MHC) proteins are dominant and prevalent on the myofibrillar proteins on the thighs as well as on the breast muscles. These proteins are shown to be smeared at the wells evidence of their inability to disintegrate into lower molecular weight proteins and thus their separation is hindered (Figure 1). The Actin (42-45 kDa) molecular weight proteins is a distinct poultry muscle protein as is visibly recorded both on the breast and thigh muscles of the Kakamega ecotype (Figure 1). Sarcoplasmic protein fraction separated into at least 12 distinct bands. This was similar for protein fractions either from the breast or thigh. This was also reported by (Mudalal et al., 2014) though they stated that only 11 of these bands having molecular weight of between 25 and 90 kDa were quantified. A band is observed at the 75 kDa mark. Not as prominent as the Actin band observed at 42-45 kDa (Figure 1).

There is a prominent band at 135 kDa which separates into two with one band spotted at the 100 kDa mark, representing (C-protein). This separation must have been aided by the application of the DTT and then denaturation of the muscles by boiling for 5 minutes at 95^{o}C (Mudalal et al., 2014). They also express very prominent band at the 45 kDa (Actin). The sarcoplasmic protein from breast muscle had seven fully expressed protein bands (lane 2) compared to only 5 for thigh muscle (lane 4) for Kakamega ecotype. The concentration of these specific bands is indicative of the nature of the poultry muscle from those affected by white stripping (Mudalal et al., 2014).

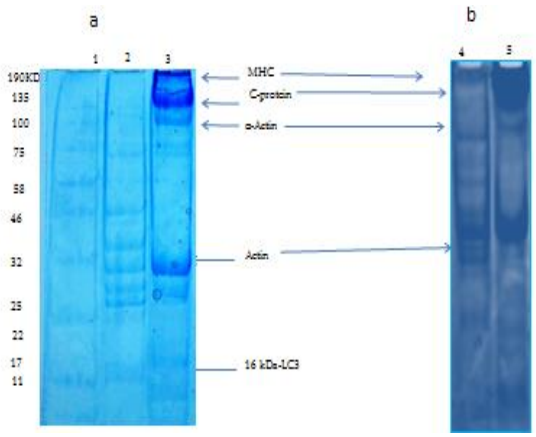

Figure 1. SDS-PAGE Profile of Sarcoplasmic and Myofibrillar Proteins for Kakamega Ecotype. Diagram (a) Breast and (b) Thigh. Lane 1: Marker; Lane 2: Sarcoplasmic; Lane 3: Myofibrillar; Lane 4: Sarcoplasmic; Lane 5: Myofibrillar

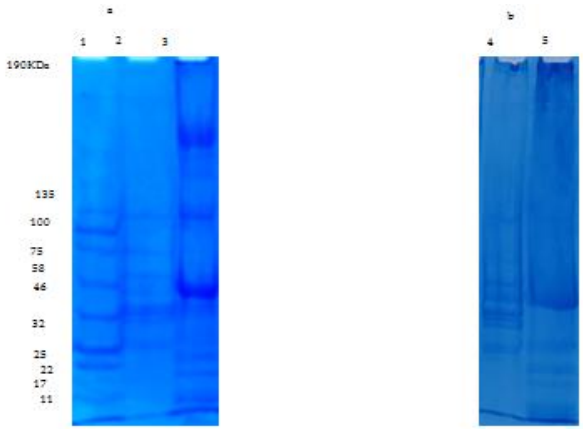

Figure 2. SDS-PAGE profile of Taita Ecotype. Diagram (a)-Breast and (b) Thigh. Lane 1: Marker; Lane 2: sarcoplasmic (deleted breast); Lane 3: Myofibrillar; Lane 4: Sarcoplasmic and Lane 5: myofibrillar

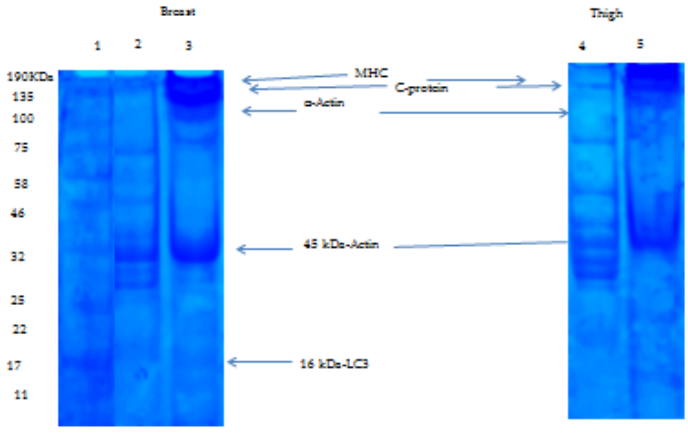

Figure 3. SDS-PAGE Profile of Naivasha Ecotype. (a) Breast and (b) Thigh. Lane 1: Marker; Lane 2: Sarcoplasmic; Lane 3: Myofibrillar; Lane 4: Sarcoplasmic; Lane 5: Myofibrillar

3.3 The Characteristic Bands of Myofibrillar and Sarcoplasmic Muscles

The SDS PAGE pattern for all the ecotypes and the parts of chicken demonstrate the presence of sarcoplasmic and myofibrillar proteins patterns (Figures 2-4). This is similarly reported by several studies (Khiari et al., 2014; Fritz et al., 1989; Zanetti et al., 2014). The SDS-PAGE for the myofibrillar proteins isolated from the three ecotypes show three distinct bands at a molecular weight above the 135 kDa as (MHC, C-proteins and the alpha-Actin).

Figure 5 shows the SDS PAGE profile of sarcoplasmic proteins from Kenyan indigenous chicken displaying some of the most prominent bands identified.

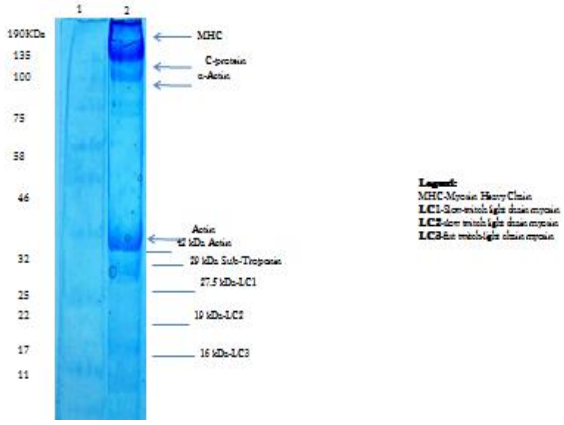

Figure 4. SDS-PAGE Profile of Myofibrillar Protein from Kenyan Indigenous Chicken Showing Some of the Most Prominent Bands Identified. Lane 1: Marker and Lane 2: myofibrillar

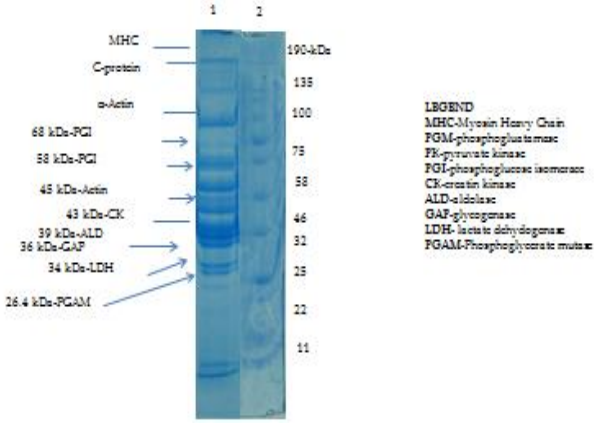

Figure 5. SDS-PAGE Profile of Sarcoplasmic Proteins from Kenyan Indigenous Chicken Displaying Some of the Most Prominent Bands Identified. Lane 1: Marker; Lane 2: Sarcoplasmic Protein Fraction

3.4 Amino Acid Composition of the Kenyan Indigenous Chicken Ecotypes

Except for Histidine and Lysine which were higher in the breasts than in the thigh muscles for all the ecotypes, there were no significant differences reported on the amino acid profile of the breasts and thighs ($P<0.05$) (Table 2). Remarkably, all the amino acids tested were higher than those recommended

by WHO/FAO for adults as well as for children (Organization & University, 2007) Health Organization. The values of Tryptophan and Cysteine were not quantified in this work.

Table 2. Effect of Body Part (Breast and Thigh) and Ecotype on the Amino Acid Profiles (g/100 g Protein) of IC in Kenya

Amino acid	Chicken parts		Ecotype			FAO/WHO2007 recommendations for adults	FAO/WHO2007 recommendations for children
	Breast	Thigh	Kakamega	Naivasha	Taita		
His	2.96±0.03[a]	2.15±0.13[b]	2.51±0.40	2.46±0.52	2.69±0.30	1.6	1.9
Lys	9.02±0.26[a]	7.76±0.24[b]	7.95±0.56	8.43±0.75	8.79±0.58	1.6	5.8
Met	7.96±0.19	6.77±0.89	6.79±0.84	6.90±1.09	8.42±0.13	1.7	2.7
Val	4.33±0.02	3.91±0.22	4.04±0.33	3.99±0.31	4.34±0.01	1.5	3.5
Ile	3.91±0.04	3.62±0.17	3.67±0.29	3.69±0.16	3.94±0.02	1.3	2.8
Leu	7.25±0.10	6.70±0.41	6.81±0.60	6.73±0.35	7.41±0.11	1.9	6.6
Phe	3.42±0.26	3.50±0.27	3.50±0.42	3.25±0.17	3.63±0.38	1.6	6.3
Thr	4.20±0.09	4.04±0.23	4.03±0.35	4.01±0.05	4.33±0.16	0.9	3.4
Total	**43.05**	**38.45**	**39.3**	**39.46**	**43.55**		
Ser	3.42±0.1	3.46±0.19	3.32±0.12	3.39±0.05	3.62±0.22		
Arg	5.73±0.06	5.69±0.34	5.64±0.19	5.51±0.23	5.99±0.37		
Gly	3.89±0.17	4.55±0.37	4.60±0.70	4.20±0.02	3.87±0.27		
Asp	9.09±0.20	8.62±0.39	8.76±0.72	8.68±0.23	9.13±0.24		
Glu	14.23±0.27	14.36±0.71	14.05±0.72	13.96±0.08	14.89±0.83		
Tyr	2.82±0.07	2.79±0.24	2.74±0.22	2.67±0.08	3.02±0.26		
Als	5.19±0.05	4.93±0.09	5.04±0.15	5.04±0.25	5.11±0.83		
Pro	2.98±0.07	3.44±0.18	3.42±0.36	3.10±0.06	3.12±0.26		
Total	**47.35**	**47.74**	**47.57**	**46.55**	**33.86**		

Values given as mean ± standard deviation (n=18).

[ab] Means and standard deviations within the same row with no common superscript are significantly different at ($P<0.05$).

3.5 Emulsion Capacity and Stability of Kenyan Indigenous Chicken Ecotypes

Emulsion capacity and emulsion stability of protein isolates from the breasts and thighs of the Kenyan IC ecotypes are presented in Table 3. Only the Kakamega ecotype had a significantly different ($P<0.05$) emulsion capacity. This difference is also expressed in the stability of the emulsion. The Naivasha ecotypes' emulsion capacity is not significantly different between the breast and the thigh protein isolates. Though there is a significant difference in the stability of the emulsions for the Naivasha

ecotype. There were no significant differences on the emulsion capacity and emulsion stability of protein isolates from breast and thighs of the Taita ecotype (Table 3).

Table 3. Emulsion Capacity and Stability of Kenyan Indigenous Chicken Ecotypes

Ecotype	Chicken Part	Emulsion Capacity (ml oil/g protein)	Emulsion Stability (ml oil/g protein)
Kakamega	Breast	22.43±1.38[b]	41.13±8.87[a]
	Thigh	30.16±0.61[a]	41.65±3.81[b]
Naivasha	Breast	31.07±0.51[a]	40.23±4.93[b]
	Thigh	28.75±0.98[a]	44.31±9.02[a]
Taita	Breast	28.04±0.54[a]	28.13±3.13[c]
	Thigh	32.11±2.11[a]	25.54±3.04[c]

Values given as mean ± standard deviation (n=18).

[abc] Means and standard deviations within the same column with no common superscript are significantly different at (P<0.05).

3.6 Protein Digestibility and Protein Solubility

The digestibility of the protein isolates shows a significant difference (P<0.05) between the thigh and the breast muscles for Taita and Naivasha ecotypes (higher digestibility of thigh protein isolates). For Kakamega ecotype, the breasts had a higher digestibility. With regard to solubility, only the Kakamega ecotype showed a significant difference between the breast and the thigh. The solubility of breast protein isolates for this ecotype was 60.9% compared to 62.98% for the thigh protein isolates (Table 4). Naivasha and Taita ecotypes had no significant difference with regard to protein isolates' solubility for the breast and the thigh.

Table 4. Protein Digestibility and Solubility of Isolates of Kenyan Indigenous Chicken Ecotypes

Ecotype	Chicken Part	Protein digestibility (%)	Solubility (%)
Kakamega	Breast	63.50±4.50[b]	60.90±2.08[b]
	Thigh	60.00±6.10[b]	62.98.84±6.89[c]
Naivasha	Breast	66.00±6.10[b]	65.88±0.63[a]
	Thigh	75.50±2.50[a]	66.94±0.06[a]
Taita	Breast	63.90±3.50[b]	67.32±0.02[a]
	Thigh	75.50±0.50[a]	67.22±0.06[a]

Values given as mean ± standard deviation (n=18).

[ab] Means and standard deviations within the same column with no common superscript are significantly different at (P<0.05).

4. Discussion

Protein content was the main focus of this study. Though, no significant differences on crude protein content were noted among the different parts of the ecotypes, the breasts recorded higher values for all the ecotypes than the thigh muscles ($P<0.05$). The values are also higher than what was reported recently by Chepkemoi et al. (2017). These results are supported by the study conducted by Sirri et al. (2010) who noted that the breast chicken muscle had higher protein content. The breast and thigh muscle composition has been found to differ in some studies but are not at all significant for the present study. This, is however in contrast to Guan et al. (2013) who reported that the genotypes of birds influenced their chemical composition. The rate of growth of chicken also affects their protein content (Fanatico et al., 2007; Guan et al., 2013). Indigenous chicken are all slow growing and hence record high quality protein content ranging from 19.0±2.04 to 24.3±0.91%. Khiari et al. (2014) reported protein content of 19.7% among the commercial Turkey (a close rival or alternative of chicken meat) purchased from the market. It is reported that protein content determines nutritional quality especially of protein based diets such as chicken. Similar crude protein content of native chicken meat were reported by Guan et al. (2013). On the contrary, Fanatico et al. (2007) reported slightly higher values from leg muscles among five genotypes of slow growing Chinese chicken. Chumgeon and Tan (2015) recorded the higher protein values both for the broiler and Taiwan native chicken. With regard to fat content, a different trend was observed as higher values of fat content was recorded for the thighs (3.12%) than for the breasts (2.12%). This value was similar to what was reported by Chepkemoi et al. (2017) at an average fat content of 2.23% for indigenous chicken in Kenya. These values were however higher than those reported by Chumgeon and Tan (2015). Fat content determines functionality, sensory quality and nutrition of poultry meat (Aronal, Huda, & Ahmad, 2012). The difference in fat content is normally dependent on the feed (Guan et al., 2013). The results for this study agree with Sirri et al. (2010) and Fanatico et al. (2007). With regard to the Ash content, the same trend was observed. Higher values of Ash content were reported on the breasts than on the thigh muscles for all the ecotypes. Though the ash content is correlated with the mineral content; chicken meat however, is not critically targeted toward contributing to this. These values of ash content are higher than those reported by Chepkemoi et al. (2017). These values were however lower than values reported by Khiari et al. (2014).

The SDS PAGE of the Taita ecotype reveals that myofibrillar proteins of both the breast and thighs have distinct and similar band profiles or characteristics. There was less smearing at the top of the gel and this may have been as a result of the denaturation for 5 minutes at 95^{o}C (Fritz, Swartz, & Greaser, 1989). The dominant bands are still the MHC (molecular weight above 135 kDa) and actin of 42-45 kDa molecular weights. The observation agrees with the report of Omana et al. (2010) about the location of this abundant actin band. The myofibrilar proteins isolated from the thighs however show least separation of the Heavy Myosin Chain proteins which indicates a difficulty in their separation or reduction to lower molecular weight proteins. Myfibrillar proteins on the other hand had prominent

HMC at the top and some of these were too large as to enter the gel wells for better separation. This may be as a result of the difficulty in reduction of disulfide bonds which results in higher molecular weight species that are transferred out of the gels with greater difficulty than those of lower molecular weight (Fritz Swartz & Greaser, 1989). More bands were expressed in sarcoplasmic proteins than were expressed in the myofibrillar proteins for both the thigh and breast muscle.

With regard to the Naivasha ecotype, the same trend is observed. More bands totaling 12 are visible for the sarcoplasmic proteins on both the breast and thigh muscles. The myofibrillar proteins expression for breast and thighs are very similar (Figure 3). The breast's myofibrillar protein separates better in to 4 distinct bands whereas the thigh myofibrillar proteins have mainly the bands corresponding to MHC and the C-proteins and alpha-actin. This is supported by the work of Omana et al. (2010). At the same time it is evident based on the sizes of the bands that hydrolysis of proteins in this process was greatly reduced and this would suggest intact action of the protein functionality particularly with regard to gel-forming capacity (Kristinsson, 2001). The poor separation or mobility observed on the myofibrillar from the thigh may suggest the occurrence of disulfide bonds just before sample applications particularly in the presence of DTT (Fritz Swartz & Greaser, 1989). Though they reported that this problem could be eliminated by heating or denaturation for longer than 4 minutes which was done in this study; and the addition of 2-mercaptoethanol yet which was not used in ours study.

Protein fractions are determined based on their solubility on a chosen ionic strength of buffer in this case 50mM NaCl was used. The SDS-PAGE patterns revealed the characteristic soluble, regulatory muscles contrasted with the contractile of the abundant myosin and actin. This is in line with the results of Zanetti et al. (2009). From these bands, the next prominent band is Actin which is very indicative of poultry proteins at molecular weight of 42-45 kDa. The slow twitch myosin chains LC1 and LC2 and the fast twitch light chain myosin precede these at molecular weights ranging from 27-16 kDa (Figure 4). The muscles are part of contractile fibre (Mudalal et al., 2014). Zannetti et al. (2013), reported that the fast twitch fibres are major components of breast muscles. This study confirms this finding based on the distinct bands of LC1 and LC2 recognized in this study.

The SDS-PAGE of the sarcoplasmic proteins shows the MHC, C-proteins and the Alpha-Actin as very distinct bands. The presence of alpha actin is a clear indication of the ability of this protein to be extracted by the NaCl. This is supported by the finding of Xiong et al. (2000). The next prominent bands visible on the electrophoretograms are located at the 90 kDa Glucose pyruvate and pyruvate kinase at 68 kDa. The lowest molecular weight protein fraction is the phosphoglycerate mutase at 25 kDa. In total at least 12 distinct bands are observed for sarcoplasmic protein as opposed to 8-10 (maximum) bands observed for the myofibrillar protein fraction for all the ecotypes. This was irrespective of whether the SDS-PAGE was conducted on breast or thigh muscle. The two protein fractions (and myofibrillar) have the highest relevance to nutritional quality and processability (Mudalal et al., 2014). There is a clear distinction in profile of protein sub fractions between the myofibrillar and sarcoplasmic proteins. For the myofibrillar proteins, the actin and alpha actin seemed

to be the most extractable proteins (Y. Xiong, Lou, Wang, Moody, & Harmon, 2000). The results of this work also support the findings of Zhang et al. (2015) who reported the most intense myofibrillar protein bands as containing the MHC at 200 kDa, actin at 43 kDa and tropomysin subunit at 35 kDa. Proteins involved in energy metabolism were identified in the sarcoplasmic proteins (Figure 5). Notably these were expressed by bands corresponding to the Enolase, pyruvate kinase (PK), and the glyceride phosphate dehydrogenase (GAP). This confirms the studies of Zanetti et al. (2013) who reported an increased expression of five proteins involved in the energy metabolism among these were phosphoglycerate kinase, and beta-enolase.

Amino acid profiles are most important nutritional (protein quality and digestibility) parameters of protein source foods. The values reported for Methionine (at least 6.77 g/100g), and Lysine (at least 7.76 g/100g) which are limiting amino acids in cereals and legumes were very high in the chicken. This affirms the assertion that chicken are a good source of high quality protein and confirms that Kenyan indigenous chicken under free range are equally a good source of the same. High amounts of Lysine, Aspartic acid and Glutamic acid were found in all the three ecotypes. The same trend was observed as well for the breasts and thighs. This result is in line with the finding of Wattanachant et al. (2004); who reported high values for these three amino acids. They also reported very high values of leucine in the breast and thighs of chicken; a result which is supported by our findings. Protein quality is closely related to the efficiency of the protein utilization in human digestive system. The protein digestion efficiency further plays an important role in determining the level of protein required for consumption or type of protein source required to satisfy the nutritional requirements of an individual (Aronal et al., 2012). This information is vital to the choice of quantity of a given food or food source that is required for digestion.

The stability of the emulsions is dependent on the ease of solubility of proteins and this is a function of the protein hydrophobicity. An emulsion refers to of two or more immiscible liquids in which one provides the dispersing phase while the other is dispersed evenly in form of tiny droplets (Klemaszewski et al., 1992). Proteins are amphoteric and have the unique ability to form a layer that binds to both polar and non-polar liquids hence allowing for the two phases to hold as an emulsion. The emulsion capacity is a measure of the volume of oil that can be solubilized by a given amount of protein (Omana et al., 2010). This parameter is useful in qualifying proteins that are best for manufacture of comminuted products such as the frankfurter, bologna and sausages. Some studies show that the emulsion capacity of protein isolates may relate to the pH of protein extraction. In the present study, the pH extraction of the isolates was uniform and hence the differences can be attributed to the ecotypes and parts of the chicken. The hydrophobic-hydrophilic balance of proteins also affects the emulsion capacity. The protein solubility has been shown to correlate with process properties such as WHC, represented by cooking loss, drip loss or moisture uptake (Warner et al., 1997). In another related study, Omana and others demonstrated the foam capacity of proteins has an optimal pH of about 11.5 from whence an increase in pH results in a reduced foam expansion (Omana et al., 2010). All the

extraction of current isolates were done at a pH around neutral where protein solubility is optimal and hence the differences observed in foaming capacity can be ascribed to differences in part of chicken muscle as well as differences in ecotypes.

Protein solubility is also a factor that determines the quality of meat and is in particular an indicator of different meat defects such as PSE and Dark, firm and dry (Warner et al., 1997). Protein Solubility according to Wang et al. (2017) is a key index of functionality as a direct demonstration of the denaturation and aggregation for proteins. Whereas Tang et al. (2006) suggested that changes in conformation of proteins could be due to formation of soluble protein aggregates. Wang et al. (2017) on the other hand, suggested that the increasing exposure of internal hydrophilic sites could be responsible. The higher solubility observed on the breasts for the Kakamega and Taita ecotypes could precisely be due to this very reason. As supported by digestibility and texture results, the breasts muscles were better broken down and therefore isolates exposed more hydrophilic sites responsible for the increased solubility. Marty, Rasale and Das (2015) suggested that cavitation including shear and turbulence induced when they used pulsed ultra sound could disrupt the hydrophobic interactions hence molecular association of protein aggregates thus improving solubility. In the present study, the disruptive force was provided by the homogenizer/blender and the disruption must have been greatest in breast muscle than on the thigh muscle.

5. Conclusions

The expression of proteins from the three Kenyan IC ecotypes showed distinct differences between myofibrillar and sarcoplasmic proteins based on SDS PAGE but very small differences among the ecotypes and their body parts. With regard to functionality, there are differences in emulsification and digestibility capacity among the three ecotypes and also on the breast and thigh muscles. The IC give high concentration of the glutamic, Lysine and Lysine amino acids and at levels higher than the set by FAO/WHO values for adults and children. This underscores the intrinsic nutritive value of indigenous chicken in the diet.

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