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FARMING AND PROCESSING FACTORS FOR IMPROVING QUALITY PROPERTIES OF POULTRY AND RABBIT MEAT

Coordinatore:
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Relatore:
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LIST OF PUBLICATIONS

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1. M. Petracci**, S. Mudalal**, F. Soglia, and C. Cavani (2015) Meat quality in fast-growing broiler chickens. *World's Poultry Science Journal* (in press).
2. **S. Mudalal**, M. Lorenzi, F. Soglia, C. Cavani, and M. Petracci (2015) Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. *Animal*, doi: 10.1017/S175173111400295X.
3. **S. Mudalal**, E. Babini, C. Cavani, and M. Petracci (2014) Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. *Poultry Science* 93: 2108-2116.
4. M. Lorenzi, **S. Mudalal**, C. Cavani, and M. Petracci (2014) Incidence of white striping under commercial conditions in medium and heavy broiler chickens. *Journal of Applied Poultry Research* 23: 754-758.
5. M. Petracci, **S. Mudalal**, E. Babini, and C. Cavani (2014) Effect of white striping on chemical composition and nutritional value of chicken breast meat. *Italian Journal of Animal* 13: 179-183.
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4. M. Petracci, **S. Mudalal**, M. Lorenzi, F. Soglia and C. Cavani (2014) Implications of wooden breast defect on technological properties of chicken breast fillets. *Proceedings of XIV European Poultry Conference,* 23-26 June, Stavanger, Norway, pp. 525
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LIST OF ABBREVIATIONS-NOTATIONS

Abbreviation /Notation	Details
SR	Sarcoplasmic reticulum
WHC	Water-holding capacity
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
CK	Creatine Kinase
PSE	Pale, soft exudative
ES	Electrical stimulation
MHC	Myosin heavy chains
MLC	Myosin light chains
EDTA	Ethylenediamine tetraacetic acid
SDS	Sodium dodecylsulfate
LMM	light meromyosin
HMM	Heavy meromyosin
DMD	Dunchenne muscular dystrophy
BMD	Becker muscular dystrophy
DGC	Dystrophin glycoprotein complex
V	Vinculin
T	Talin
SSPN	Sarcoglycans and sarcospan
Db	Dystrobrevins
Syn	Syntrophins
NOS	Nitric oxide synthase
DPM	Deep Pectoralis Myopathy
NMD	Nutritional muscular dystrophy
NORM	Normal
WB	Wooden breast
WS	Severe white striping
EC	Excitation-contraction
DHPRs	Dihydropyridine binding receptors

CR	Calreticulin
CSQ	Calsequestrin
MH	Malignant hyperthermia
SR-RSCRC	Sarcoplasmic reticulum ryanodine-sensitive calcium release channel
LDH	Lactate dehydrogenase
AST	Aspartate aminotransferase
CPK	Creatine (phospo) kinase
PLAs	Phopholipase
SBY	Standard breast yield
HBY	High breast yield
SEM	Standard error of mean
PUFA	Polyunsaturated fatty acids
CLA	Conjugated linoleic acid
WBC	Water Binding Capacity
PAGE	Polyacrylamide gel electrophoresis
CIE	Commission Internationale de l'Eclairage
AOAC	Association of Official Analytical Chemists
BSA	Bovine serum albumin
EGTA	Ethylene Glycol Tetraacetic Acid
RB	Rigor buffer
GLM	General linear model
H1	Height is measured far from the end of the caudal part of chicken breast by 1 cm toward a dorsal direction
H2	Height measured at the half distance of the chicken breast length
H3	Height measured at the thickest point in the cranial part of the chicken breast
L	Length is measured from the longest dimension of the fillet

W	Width is measured from the longest distance from side to side in the middle of fillet
MDM	Mechanically deboned meat
CVD	Cardiovascular disease
OSA	Octenyl succinic anhydride
GMO	Genetically modified organisms
PPI	Poultry protein isolates
MSPM	Mechanically separated poultry meat
STPP	Sodium tripolyphosphate
ADD	Attention Deficit Disorder
a_w	Water activity
TPA	Texture Profile Analysis
FW	Freezable water
DSC	Differential scanning calorimeter
PA	Phosphate-marinated fillets cooked in the oven and core product temperature 160-76°C
PC	Phosphate-marinated fillets cooked in the oven and core product temperature 160-80°C
PD	Phosphate-marinated fillets cooked in the oven and core product temperature 200-76°C
PE	Phosphate-marinated fillets cooked in the oven and core product temperature 200-80°C
BA	Bicarbonate-marinated fillets cooked in the oven and core product temperature 160-76°C
BC	Bicarbonate-marinated fillets cooked in the oven and core product temperature 160-80°C
BD	Bicarbonate-marinated fillets cooked in the oven and core product temperature 200-76°C
BE	Bicarbonate-marinated fillets cooked in the oven and core product temperature 200-80°C
LAB	Lactic acid bacteria

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plasma membrane. The high levels of free radicals in dystrophic muscle are thought to contribute to muscle degeneration via the oxidation of muscle membranes and recruitment macrophages. Calcium-sensitive pathways also contribute to muscle degeneration in muscular dystrophy. Calcium (*pink spheres*) may enter dystrophic muscle through membrane lesions or through calcium channels. Calcium dysregulation may also lead to abnormal mitochondrial function as well as the activation of the calcium-dependent protease calpain to degrade muscle membrane proteins (Wallace and McNally, 2009).

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SUMMARY

Currently, there are two main growing trends in the global meat market. The first trend is the dramatic increase of demand on poultry meat. In response to this demand, there was a great success in increased growth rate in the last few decades in order to optimize the production of poultry meat. Accordingly, the increase of growth rate induced the appearance of several muscle abnormalities which more recently were white striping and wooden abnormalities. The second trend is related to the growing market of convenient, healthy, and functional processed meat products. In this regards, the Ph.D research project was divided into two parts where the first part was dedicated to evaluate the different implications of recent muscle abnormalities on meat quality traits and their incidence under commercial conditions. While the second part was dedicated to evaluate the possibility to formulate healthy processed meat products such as phosphate free-marinated chicken meat and low sodium-marinated rabbit meat products. The findings of the first part showed that the total incidence of white striped breast fillets under commercial conditions in different genotypes of chicken broilers was very high and reached up to 43%. Considering the effect of genotype, the results showed that high-breast yield hybrids exhibited a higher overall incidence of WS compared with standard-breast yield hybrids. In particular, heavy weight flocks (3.8-4.2 kg) had significantly higher percentages of WS (56.4 *vs.* 28.5%, $P \leq 0.001$) than medium flocks (2.2-3.0 kg). The results showed that the increase of incidence was highly relevant to the increase of growth rate, which can be considered the most determining factor. In addition, the high rate and the severity of WS was higher with increasing the slaughter age and weight. In the context of the quality traits, it was found that breast meat affected by white striping and wooden abnormalities had a different chemical composition than normal breast meat. Both types of defected meat exhibited lower protein content and higher fat and collagen contents than normal. Moreover, both types of abnormal meat had different nutritional properties as a result of compositional change. To illustrate, white-striped fillets exhibited higher energy content (450.7 *vs.* 421.1 KJ/100g; $P<0.01$) with respect to normal meat. From technological viewpoint, white striped and wooden breast meat exhibited lower processing ability (water holding/binding capacity and texture) characterized by lower marinade uptake and higher cooking loss in comparison to normal meat. In addition, white striped meat exhibited softer texture (low Allo-Kramer shear force) while wooden meat showed harder texture (high

hardness value by compression test and high values in texture profile analysis) in respect to normal meat. White striped meat had lower protein solubility as well as lower content of some functional myofibrillar protein fragments such as actin and, fast, and slow-twitch light chain myosin (LC3 and LC1) that has been observed by SDS-PAGE analysis. On the other hand, the histological analysis showed the presence muscle fiber degeneration followed by lipidosis and fibrosis in both muscle abnormalities. In addition, there was an increase in the level of calcium (Ca^{2+}) and sodium (Na^{+}) as well as presence calcium-ATPase (114 kDa) (an indicator of muscle damage) in abnormal meat. In the conclusion, overall findings related to the first part of the Ph.D project (Part A) showed that the total incidence of recent muscle abnormalities (white striping and wooden breast) was relatively high under commercial conditions. In addition, the impact of muscle abnormalities was not confined on the impairing the visual appearance (marbling appearance due to white striations and hard, pale, ridge-like bulge areas due to wooden) but there was also a dramatic adverse change in the other quality traits such as colour, water holding/binding capacity, texture, and the histological status of the meat tissues which may lead to economical consequences on the poultry industry. The overall findings of the second part (B) of the Ph.D showed that sodium bicarbonate can be used as phosphate replacer while keeping to certain extent similar quality traits. Moreover, phosphate-marinated fillets exhibited some differences in the quality traits (texture profile, water binding capacity, colour, freezable water, etc.) under different heat treatments (dry cooking procedure at different core and oven temperature) in comparison to bicarbonate-marinated fillets. On the other hand, a study was designed to evaluate the possibility to formulate low sodium-marinated rabbit meat product. It was found that it is possible to replace sodium chloride up to 30% by potassium chloride without impairing microbiological traits (total aerobic mesophilic and lactic acid bacteria maximum cell loads), sensorial acceptability (perceived saltiness and overall liking) and technological traits (pH, colour, texture, cooking loss, and yield). In conclusion, the finding of this study showed that it is feasible imparting an added value for processed rabbit meat products by reduction of sodium content which could increase market interest.

-UNIT I-

CHAPTER 1

Literature review: Meat structure and Muscle abnormalities

1.1 Abstract

During the past few decades, there was a tremendous increase in the poultry meat demand. Furthermore, current forecast and projection studies pointed out that the expansion of the poultry market will continue in future. This growing demand in the last 30 years had led to progressive improvements in genetic selection to produce fast-growing broilers which induced the appearance of several spontaneous or idiopathic muscle abnormalities and an increased susceptibility to stress induced myopathy. These muscle abnormalities have different implications on the composition and the muscle structure and as a consequence on the quality traits of fresh and processed meat products. This chapter will deal with meat composition and muscle structure and their roles in the quality traits of raw and processed meat products. In addition, this chapter will discuss the impact of the most common muscle abnormalities such as deep pectoral myopathy (DPM) and pale, soft and exudative like meat (PSE) on meat quality traits (water holding capacity, soft texture and pale color). In addition, it will also discuss the most emerging recent muscle abnormalities that occur in chicken breast such as white striping and wooden breast. Both those two muscle abnormalities were the subject of different studies that have been carried out during the Ph.D thesis. Therefore, this chapter aimed to review the consequences of genetic selection on muscle quality traits. It describes the relevance and the effect of main breast abnormalities on nutritional, technological, sensorial, and microbial quality of raw and processed meat.

Keywords: Broiler, genetic selection, fast growing, breast meat, abnormalities, nutritional value, technological traits, microbiological stability.

1.2 Introduction

In early societies, chickens were valuable farm animals, mainly used for the production of eggs and feathers, but also as a barnyard scavenger; for entertainment (cock fighting, animals show), and perhaps least important, as a source of meat. As a result, little effort was made to

increase meat production (Fletcher, 2004). Until the 19th century, there were no concentrated efforts made to increase the commercial production of both eggs and meat. As industrial cities began to grow, the production of many foodstuffs became more concentrated, with the sole purpose to supply high-population centers with food. In the late 1940s and early 1950s, poultry producers began to integrate vertically poultry production stages as well as take advantage of the scientific progress in nutrition, genetics and disease control (Fletcher, 2004). Only from that time, onwards genetic selection programs have resulted in the divergence of laying hens from meat-type broilers (Table 1.1) (Schreurs, 2000).

Table 1.1 Relationships among evolution of marketing forms and selection criteria of chicken broilers and appearance of breast meat quality abnormalities.

Item	**Year**				
	1940	1960	1980	1995	2010
The main form of commercialization	- live bird	- whole carcass	- cut-up	- cut-up - processed products	- cut-up - processed products
Main selection Criteria	- live performances	- live performances - carcass yield	- live performances - carcass yield - cut-up yield	- live performances - carcass yield - cut-up yield - meat yield	- live performances - carcass yield cut-up yield - meat yield
Meat quality Abnormalities			- deep pectoral disease	- deep pectoral disease - PSE-like	- deep pectoral disease - PSE-like - white striping - wooden breast

In the late 1950s, 8 weeks were needed to produce a 2.1 kg bird, while today, a bird of the same market weight can be produced in approximately 5 weeks (Havenstein *et al*., 2003; Berri *et al.*, 2007; Aviagen, 2007, 2012).

The average breast meat yield (included *Pectoralis major* and *Pectoralis minor* without bone and skin) for Athens-Canadian Randombred strain of broilers in the 1957 was 13.5% for birds slaughtered at 2.2 kg of live weight, while in 2001, the average breast yield of the Ross 308 broilers slaughtered at the same weight was 15.8%. However, in the last ten years; breast meat

yield of the same hybrid has been tremendously increased by more than 5 percentage points, and so that nowadays the breast meat exceeds one fifth of the weight of the bird (Aviagen, 2012) (Table 1.2). The majority of these changes (85 to 90%) have been brought by the quantitative selection practiced by commercial breeding organizations (Havenstein *et al*., 1994a, b).

Table 1.2 Progress in breast weight and yield in commercial broiler hybrids from 1957 to 2012

Year	Hybrid	Body weight (g)	Age (d)	Breast weight (g)	Breast yield (%)
1957[1]	Athens-Canadian Randombred	2,078	57	280	13.5
2001[1]	Ross 308	2,207	43	349	15.8
2007[2]	Ross 308	2,200	36	410	18.6
2012[2]	Ross 308	2,200	35	464	21.1

[1] Havenstein *et al*. (1994b); [2] Aviagen (2007, 2012)

The reasons of the increasing demand of chicken breast meat are attributed to the healthy nutritional profile, a peculiar sensory property that makes the breast meat very flexible for any type of home-preparation style as well as for manufacturing processed products. Additionally, mild flavor and high tenderness of breast meat allow imparting a wide range of desired flavor profiles and textures of processed meat products which meet market needs and targeting different groups of consumers. Finally, the breast meat is very suitable for quick and easy home-cooking and this is very important in modern societies where people tend to spend less and less time in home meal preparation (Fletcher *et al.*, 2002; Petracci *et al*., 2013a).

1.3 Muscle structure and meat composition

1.3.1 Muscle structure

Entire skeletal muscle is considered as an organ of the muscular system. Muscle consists of skeletal muscle tissue, adipose tissue, connective tissue, nerve tissue, blood or vascular tissue, nerve tissue, tendon, cartilage, and bone. Muscle fiber has a long and cylindrical shape and each fiber is considered as a muscle cell. Normally, muscle is made up of hundreds, or even thousands, of muscle fibers, organized together as bundles and covered in a sheath of connective tissue. The whole muscle is surrounded by a connective tissue sheath called the epimysium

(Keeton and Eddy, 2004). Part of the epimysium extents inward to separate the muscle into different compartments where each one contains a bundle of muscle fibers. Each bundle of muscle fiber is called a fasciculus and is surrounded by a layer of connective tissue called the perimysium. Within the fasciculus, each individual muscle cell, called a muscle fiber, is surrounded by connective tissue called the endomysium (Davies, 2004; Anonymous, 2014) (Figure 1.1).

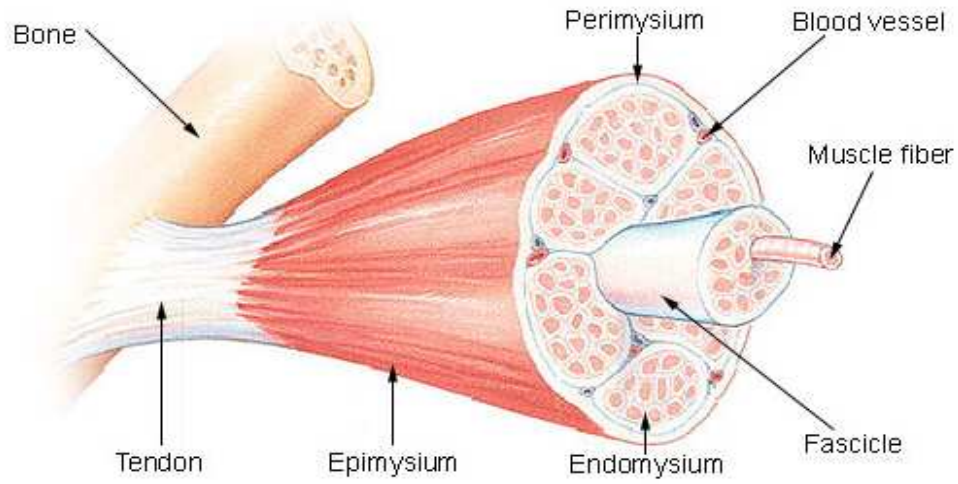


Figure 1.1 Structure of skeletal muscle (Source: Anonymous, 2014)

The main functions of connective tissue to support and protect for the delicate cells (soft and fragile) and enable the cells to resist the generated compression due to muscle contraction. In addition, presence of connective tissues provides protected pathways for blood and nerve supplies. Skeletal muscles are supplied with a sufficient quantity of blood vessels and nerves which depends mainly on the primary function of skeletal muscle. In general, in each myofibril there are two types of myofilaments which are thick myofilaments (myosin) and thin myofilaments (actin) (Davies, 2004; Anonymous, 2014).

Muscle cells are attached by a plasma membrane (the sarcolemma) which forms a physical barrier against the external environment and also mediates signals between the exterior and the muscle cell. The cytoplasm of a muscle cell (sarcoplasm) that normally have subcellular elements along with the Golgi apparatus, abundant myofibrils, a modified endoplasmic reticulum known as the sarcoplasmic reticulum (SR), myoglobin and mitochondria. Transverse (T) -tubules

go through the sarcolemma, which permit the impulses to penetrate the cell and activate the SR. The SR is a type of network surrounds the myofibrils and it stores and provides the Ca^{2+} that is required for muscle contraction (Figure 1.2) (Taylor, 2004, Davies, 2004, Anonymous, 2014).

Myofibrils are contractile units that consist of an ordered arrangement of longitudinal myofilaments. Myofilaments can be either thick filaments (comprised of myosin) or thin filaments (comprised primarily of actin). The characteristic 'striations' of skeletal and cardiac muscle are readily observable by light microscopy as alternating light and dark bands on longitudinal sections. The light band, (known as the I-band) is made up of thin filaments, whereas the dark band (known as the A-band) is made up of thick filaments. The Z-line (also known as the Z-disk or Z-band) defines the lateral boundary of each sarcomeric unit. Contraction of the sarcomere occurs when the Z-lines move closer together, making the myofibrils contract, and therefore the whole muscle cell and then the entire muscle contracts (Figure 1.2) (Davies, 2004, Davies and Nowak 2006; Choi and Kim, 2009; Anonymous, 2014).

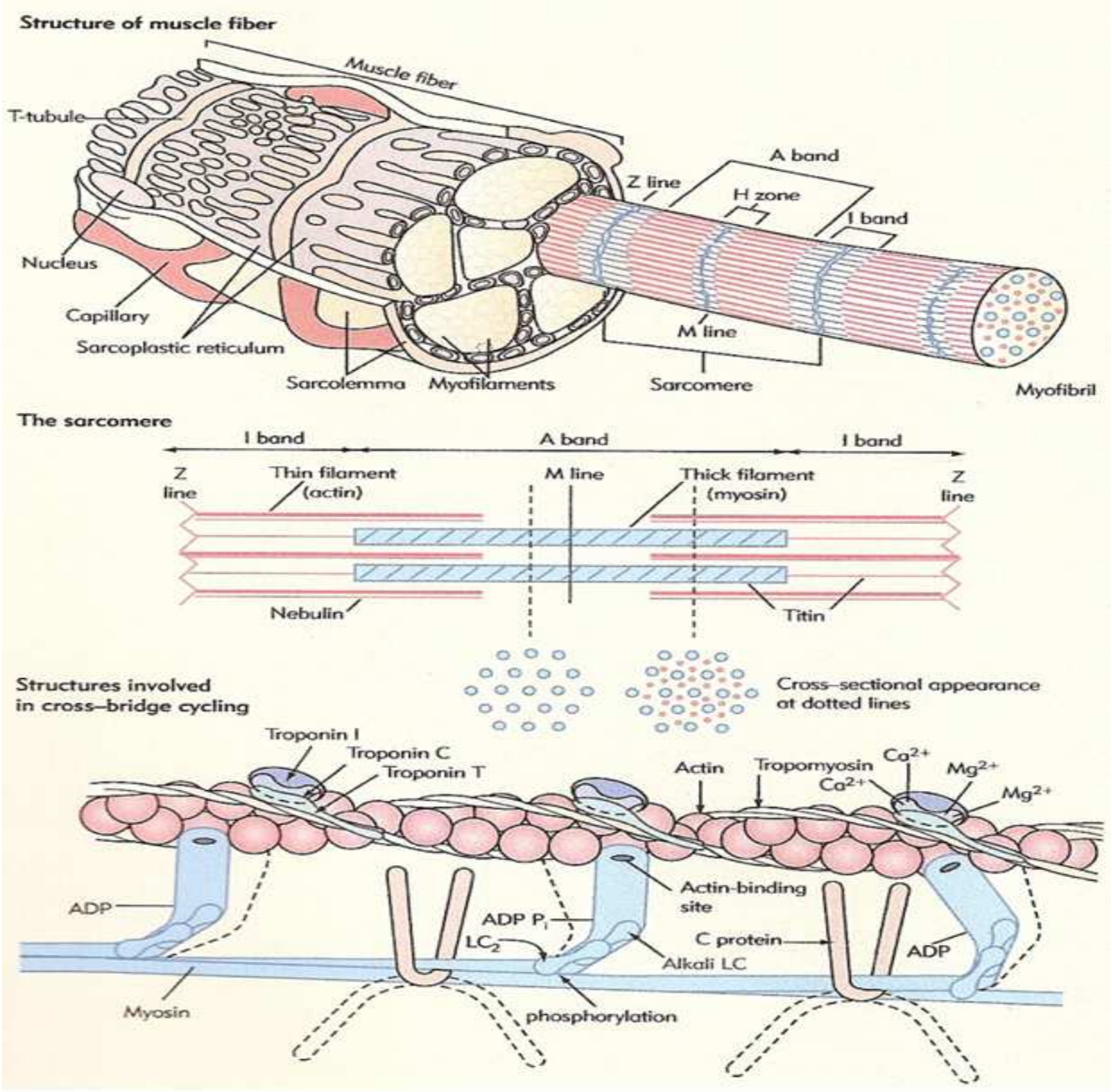


Figure 1.2 Schematic diagram of muscle fiber structure (Source: Hopkins, 2005)

1.3.2 Meat composition

Meat composition and muscle structure are usually affected by genetic selection due to histological and biochemical changes that are induced by growth increase. Meat composition and muscle structure play a crucial role in the quality traits of raw and processed meat products. In general, meat composes from water, protein, lipid, carbohydrate, minerals, vitamins, and nucleic acids (Lawrie, 1991; Keeton and Eddy, 2004; Kauffman, 2012).

Water is the major part of the composition of muscle tissue (about 75% water). The ability of meat to bind and hold either native or added water plays a vital role in the quality traits as well as the economic value of meat products. Water is generally distributed in different form in muscle structure. It can be structurally organized in layers around polar molecules (bound water) and between layers of cellular materials (steric water). Most of water in the muscle is entrapped by different forces and it is difficult to move (Figure 1.3). Bound water (about 5%) usually binds with proteins; it has an ice-like structure (Bertram *et al.*, 2002; Brewer, 2004; Pearce *et al.*, 2011).

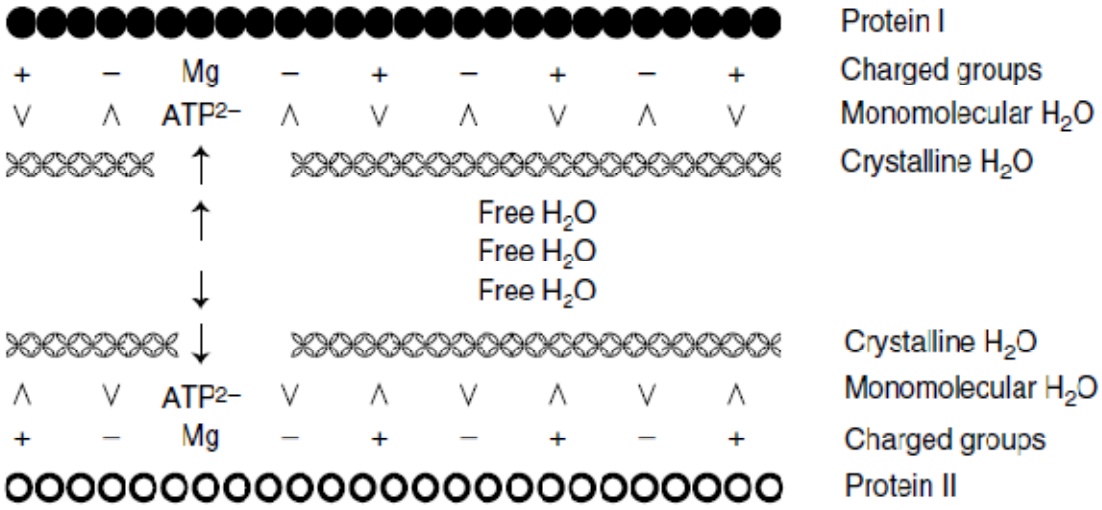


Figure 1.3 Arrangement of intrafilamental water (Source: Brewer, 2004).

Bound water is unfreezable and is unaffected by thermal processing, in addition it is not involved in biochemical reactions. On the other hand, steric or immobilized water can move from compartment to another compartment under various types of stress. Immobilized water is located within the network of cellular protein membranes (intracellular water) of contractile fibers or in the sarcoplasm. Additionally, water is present in the extracellular spaces (Brewer, 2004; Pearce *et al.*, 2011).

Water-holding capacity (WHC) is one of the most important quality measures for the meat industry. WHC can be defined as the ability of meat to retain onto its native and added water under applied force such as heat and pressure (Bertram *et al.*, 2008; Cheng and Sun, 2008; Albarracìn *et al.*, 2011). Water-holding capacity (WHC) is mainly represented by water located in the intermolecular spaces of contractile proteins (myofilament of actin and myosin) of muscle

tissue and it is immobilized under the effect of capillary force. The spaces between myofilaments can vary from 320 and 570 Å according to pH, ionic strength, osmotic pressure and sarcomere length. In general, these spaces are maintained by electrostatic force. Variables that can change the spatial alignment of the myofilaments can modify the quantity of immobilized water. In this context, WHC is affected by both intrinsic and extrinsic factors that can change the net charge such as pH, the presence of divalent cations (Mg^{2+}, Ca^{2+}), denaturation of proteins (rapid decrease in pH at high temperature), and the presence and condition of plasticizing agents (adenosine triphosphate (ATP), enzymes (ATPase), and cofactors necessary for plasticizer function in the prevention of myofibrillar protein cross linking) (Brewer, 2004; Huff-Lonergan and Lonergan, 2005; Cheng and Sun, 2008; Lee *et al.*, 2010; Pearce *et al.*, 2011).

The negative charge of contractile proteins is usually interacted with divalent cations such as Mg^{2+} and Ca^{2+} which decreases the repulsion forces and pulling contractile proteins together (Figure 1.3). As a consequence, there is less interpretation spaces that reduce the number of water molecules. This effect can be mitigated by monovalent captions such as Na^{+} and K^{+} (Brewer, 2004; Cheng and Sun, 2008; Lee *et al.*, 2010; Pearce *et al.*, 2011).

When Mg-ATP^{2-} binds to myosin, electrostatic repulsion is increased between contractile proteins which allows binding of myosin to actin and inhibiting ATP release. Ca^{2+} releases from sarcoplasmic reticulum (SR) due to nerve impulse stimulation and then it binds to troponin-C. Ca^{2+} counteracts the effect of Mg-ATP^{2-} imparting more spaces for water. In addition, presence of Ca^{2+} can induce conformational change in the proteins which increases the activity of Mg-ATP^{2-}-ase that catalysis of Mg-ATP^{2-} to Mg-ADP^{-} (ADP: adenosine diphosphate), generating energy for muscle contraction. In pre-rigor meat, interaction between actin and myosin is inhibited by Mg-ATP^{2-}. In this case, the changes in pH can easily generate electrostatic repulsion between proteins (Figures 1.4 and 1.5), and additionally high ionic strength can attract water by osmotic force. In general, the increase of inter-protein space is restricted due to the attachment of actin filament to the Z-line and myosin filament to the M-line. When the oxygen supply is finished during the *post-mortem* period, cells start to generate energy by the anaerobic process which producing lactic acid and decreasing the pH while ATP is produced by creatine kinase (CK). Production of ATP is usually stopped when CK is depleted. In the case, the cross-linking between actin and myosin is maintained, which leading in shortening of sarcomere (Brewer, 2004; Cheng and Sun, 2008; Pearce *et al.*, 2011).

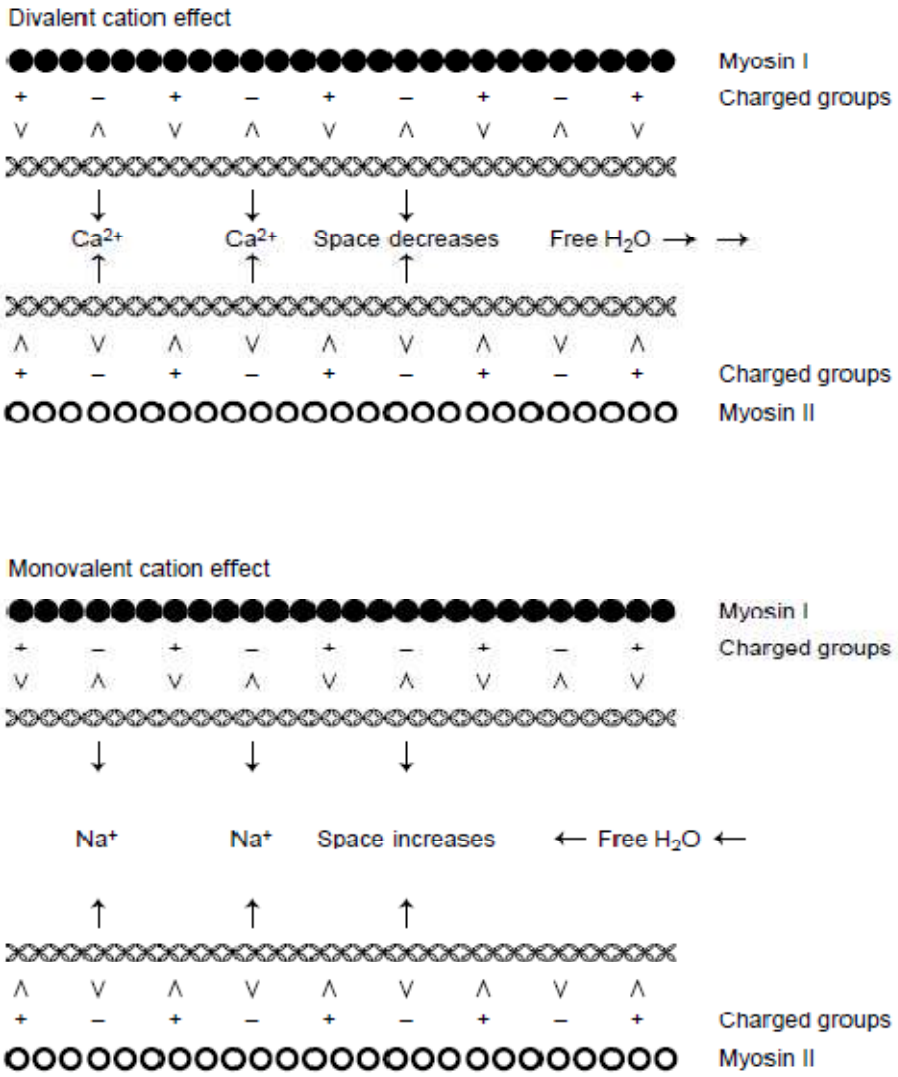


Figure 1.4 Effects of divalent and monovalent cations on free water (Source: Brewer, 2004).

Temperature plays major roles on the rate and extent of these reactions because (1) several reactions are controlled by enzymes and (2) changes in pH at high temperature can denature proteins, both structural proteins and enzymes, leading to loss of functionality. In general, at pre-rigor temperatures >20°C, there is no shortening of the muscle due to the high activity of the Ca^{2+} pump in the SR that keep low Ca^{2+} concentrations around the myofilament and blocking the effect of the troponin-tropomyosin system on actin-myosin interaction (Brewer, 2004).

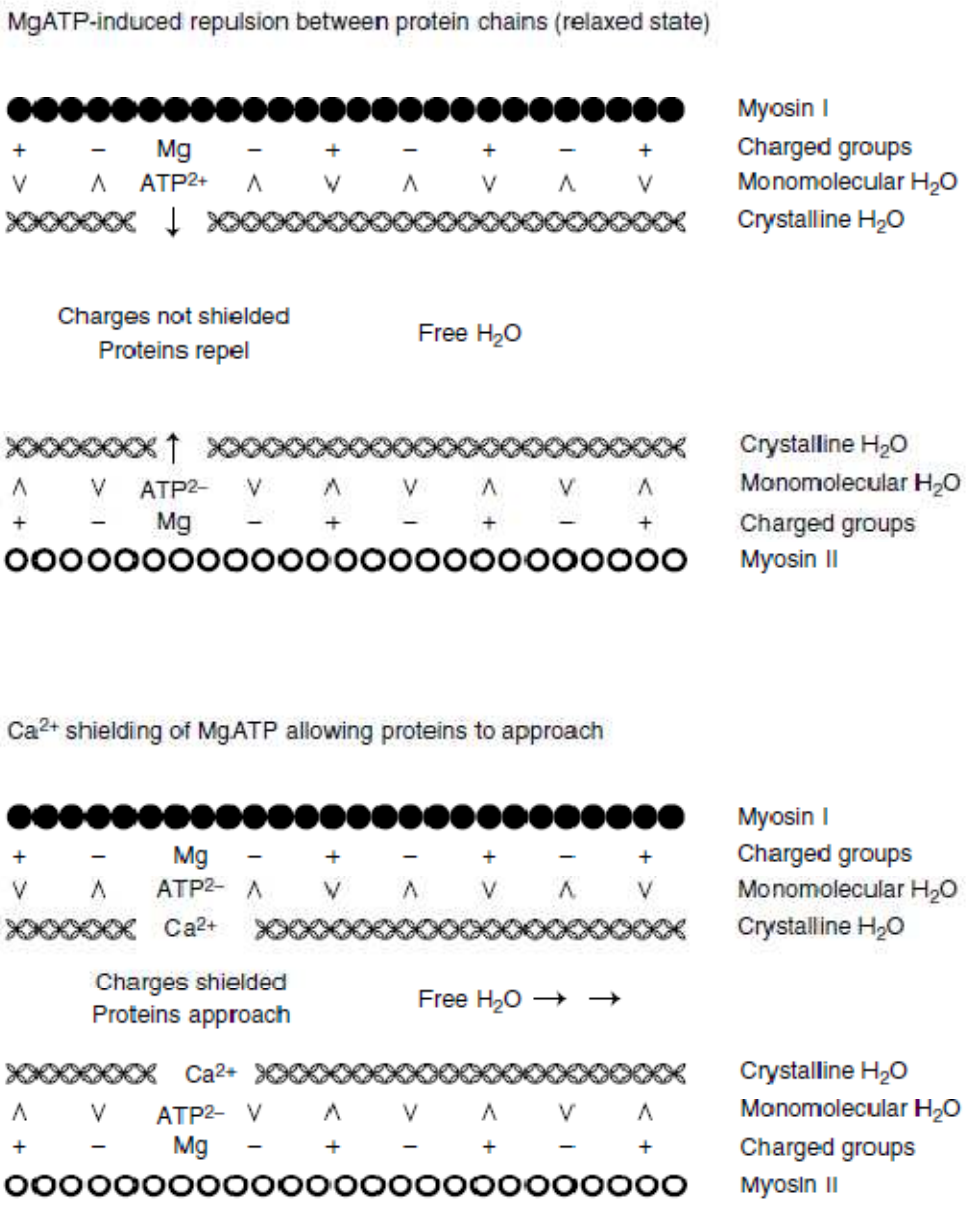


Figure 1.5 Cation (Ca^{+2}) shielding of Mg-ATP^{-2} and its effect on water held between proteins (Source: Brewer, 2004).

Increase of the pH before rigor-mortis can increase the concentration of hydroxyl ions which increase the repulsion force (Figure 1.6). On the other hand, WHC is normally very low at pH 5.4-5.5 (which is very close to isoelectric point) but minimum value occurs when pH arrives around 5 which is the isoelectric point of actomyosin (Figure 1.7). Salt-soluble proteins are completely soluble above pH 5.9 but 95% are insoluble at pH below 4.9, with peak solubility occurring between pH 5.7 and 6.0. The inclusion of water in muscle tissue at pH between 5.1 and 4.4, swells the muscle fibers through the across and along of fiber axis. Increase of muscle fiber

diameter is more important for total muscle swelling than increases in sarcomere length. Fiber diameter is more affected by pH than sacromere and it is negatively correlated with pH. At pH close to 4, white fibers proceed to swell while red fibers shrink. This variation in response can be explained by differences in buffering capacity, where red-fiber muscles exhibit lower buffering capacity than white-fiber muscles under acidic conditions (Brewer, 2004; Cheng and Sun, 2008; *Pearce et al.*, 2011).

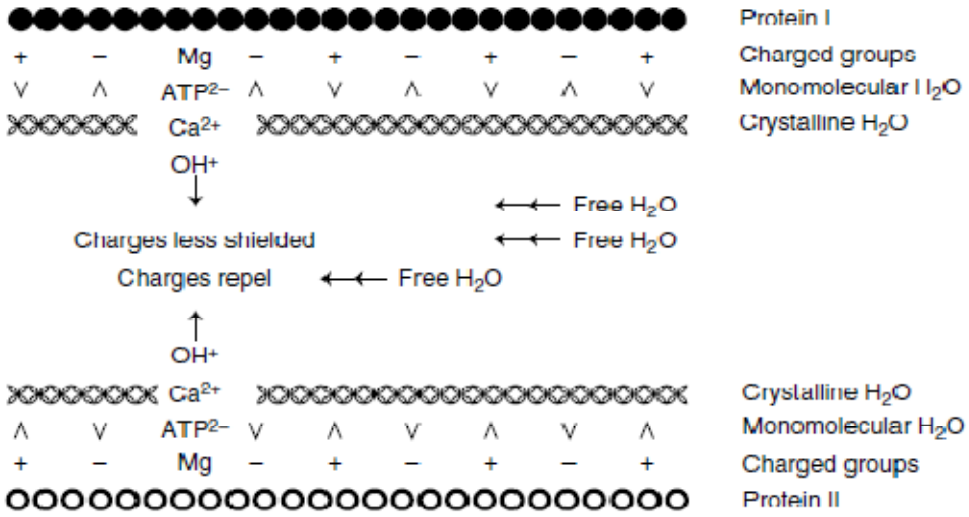


Figure 1.6 Effects of excess hydroxyl ion (pH increase) on the water located in pre-rigor meat (Source: Brewer, 2004).

The highest precipitation of sarcoplasmic proteins takes place between pH 4.8 and 5.2 regardless of temperature; but, at 37°C or more, high pH cannot protect these proteins. The pH-induced precipitation of sarcoplasmic proteins onto myofibrillar proteins which decreases WHC as well as other quality traits of meat. Hence, controlling pH can increase WHC. In this context, one of the strategies is an injection of adrenaline (epinephrine) prior to slaughter can lower intracellular glycogen content at slaughter so that anaerobic glycolysis is limited during post mortem leading to less lactic acid and hence high pH and WHC. Moreover, addition of alkaling agents such as phosphates and sodium bicarbonate also increases post-mortem pH and WHC to a certain extent (Brewer, 2004; Bertram *et al.*, 2008; Cheng and Sun, 2008; Albarracìn *et al.*, 2011).

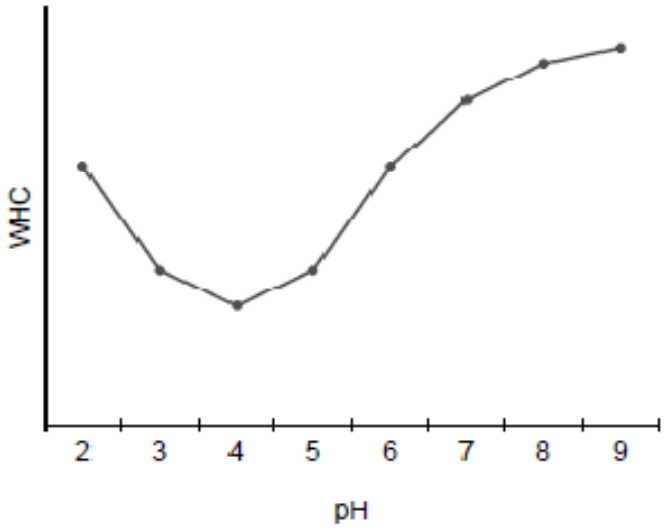


Figure 1.7 Effects of pH on water-holding capacity (Source: Brewer, 2004)

As a result, the shortening of the sarcomere and low pH have an adverse impact on the water-holding capacity of meat. Immediately after slaughter, the meat has the optimum WHC due to composite effects of high levels of ATP and high pH, while after 12-24 h of the *post-mortem* period, WHC starts to sharply decrease as a result of enzymatic hydrolysis of ATP and accumulation of lactic acid which reduces the pH. These conditions induce protein denaturation which decreases its solubility. The solubility of proteins reduces sharply when pH falls from 6 to 5. ATP breakdown takes place at pH between 6.9 and 5.8 (Fletcher, 2004; Brewer, 2004; Sathe, 2012). Based on what is previously mentioned, slight reduction in WHC is normally observed in the early stage of the *post-mortem* period, which is attributed to the direct effect of pH on muscle proteins. From other hand, during rigour there is a sharp decrease in WHC which is explained by the disappearance of ATP (two thirds of responsibility) and the rest (one-thirds) due to pH (Brewer, 2004).

High drip loss, poor WHC, and colour of pale, soft exudative (PSE) pork have been attributed to rapid *post-mortem* glycolysis at high temperature which leads to myosin denaturation. Moreover, these conditions may lead to sarcoplasmic proteins denaturation and subsequent precipitation onto the myofibrils (Schreurs, 2000; Petracci and Cavani, 2012a). Exposing the carcass to electrical stimulation (ES) can raise the *post-mortem* glycolysis rate and reduce WHC. When ES-induced pH decline (<6.0), while the carcass is still at >30°C, decreases Ca^{2+} enhanced

myofibrillar ATPase activity, which implies myosin denaturation. ATPase activity is inversely correlated with WHC (Schreurs, 2000; Brewer, 2004).

Poor of circulatory competency makes metabolic heat to accumulate, where the temperature of carcass exceeds 42°C. When pH decline is dramatic (usually during the first 45-60 min *post mortem*), the combined effect can produce denaturation of myofibrillar proteins so that WHC is ultimately quite low, even if the ultimate pH (24 h) is within normal ranges (Schreurs, 2000; Brewer, 2004; Huff-Lonergan and Lonergan, 2005).

WHC can be improved by the addition of salt to ground tissues, even grinding activates ATP hydrolysis and glycolysis. The addition of up to 2% NaCl increases the swelling of muscle tissue when water is added. But, the inclusion of 3-5% NaCl leads to decreased swelling followed by a high increase in salt concentration between 5% and 10% NaCl. Salt at lower concentration (2%) replace calcium on meat proteins by sodium, while at moderate concentration (3-5%), magnesium and potassium are exchanged with sodium. At higher concentration (5-10%), salt increases the ionic effect on meat proteins. The additions of sodium phosphate salts have been associated to increase WHC as characterized by lower drip and cooking loss. This improvement is attributed to their effect on pH, commercial phosphates can impart high pH (9-10) values to the meat which is higher than pI and increasing the net negative charge on myofibril proteins causing them to repel each other and allowing water to enter (Brewer, 2004; Doyle and Glass, 2010).

Proteins are major organic compounds of the muscle tissue, and also are responsible about the structural and biological properties of muscle in living animals. Muscle proteins can be classified according to their solubility characteristics into three categories: sarcoplasmic proteins, the metabolic proteins that are soluble in water or dilute salt solutions; myofibrillar proteins, the contractile proteins that are soluble in concentrated salt solutions; and stromal proteins, the connective-tissue proteins that are insoluble in both (Lawrie, 1991; Lee *et al.*, 2010; Sathe, 2012; Kauffman, 2012).

The sarcoplasmic fraction (30-35% of the total muscle proteins) contains about 200 different proteins, the major part of them are glycolytic enzymes. According to the centrifugal sedimentation forces sarcoplasmic proteins could be divided into four classes: nuclear, mitochondria, microsomal, and cytoplasmic fractions (Xiong, 1997). Sarcoplasmic proteins have

a weak positive effect on the technological properties (water holding capacity) with showed weak and fragile gel (Miyaguchi *et al*., 2000; Sathe, 2012)

Myofibrillar proteins constitute about 55-60% of the total muscle protein and they are salt soluble proteins. On the contrary of sarcoplasmic proteins, myofibrillar proteins have a major role in the technological properties of the meat (texture and water holding capacity). Myofibrillar proteins are responsible about the gel formation during meat processing which imparts the functional characteristics of processed meat products (Asghar *et al.*, 1985; Li-Chan *et al*., 1987; Sathe, 2012). Myosin (thick myofilaments) and actin (thin myofilaments) are the major part of myofibrillar proteins. Myosin is a large fibrous molecule (~ 500 kDa), built from two large subunits called myosin heavy chains (MHC) that form the head and four small subunits called myosin light chains (MLC) where they are attached to myosin head (Bechtel, 1986; Lee *et al.*, 2010).

Myosin can be extracted with NaCl or KCl solutions (>0.15 M). The addition of $MgCl_2$ and ATP or pyrophosphate can prevent the extraction of actin. Oxidation of thiol groups in myosin molecules could lead to aggregation and so ethylenediaminetetraacetic acid (EDTA) and mercaptoethanol is usually added to prevent its aggregation during extraction (Kijowski, 2001). Sodium dodecylsulfate (SDS) has the ability to dissociates myosin molecule into subunits of high (200 kDa of fast-twitch and slow-twitch heavy chain myosins) and low molecular weights (fast-twitch light chain myosin LC1, LC2, and LC 3 having 25, 18, and 16 kDa, respectively and slow-twitch light chain myosin LC1 and LC2 having 27.5 and 19 kDa, respectively) which can be separated by electrophoretic techniques. Under the effect of some enzymes, myosin can be hydrolyzed into some functional fragments such as: light meromyosin (LMM) of 150 kDa and heavy meromyosin (HMM) of 350 kDa. Heavy meromyosin could be also hydrolyzed into S-1 and S-2 fragments with a molecular weight of 115 kDa and 60 kDa, respectively, when under a long period of enzyme digestion (Figure 1.8) (Bechtel, 1986; Choi and Kim, 2009). It was found that S-1 protein has excellent functional properties in processed meat products due to its high water binding capacity (Borejdo and Assulin, 1980; Borejdo, 1983). Some myofibrillar proteins, such as myosin heavy chain (MHC, ~ 200 kDa) and actin (~ 42 kDa) are largely responsible for the textural properties of processed meat products.

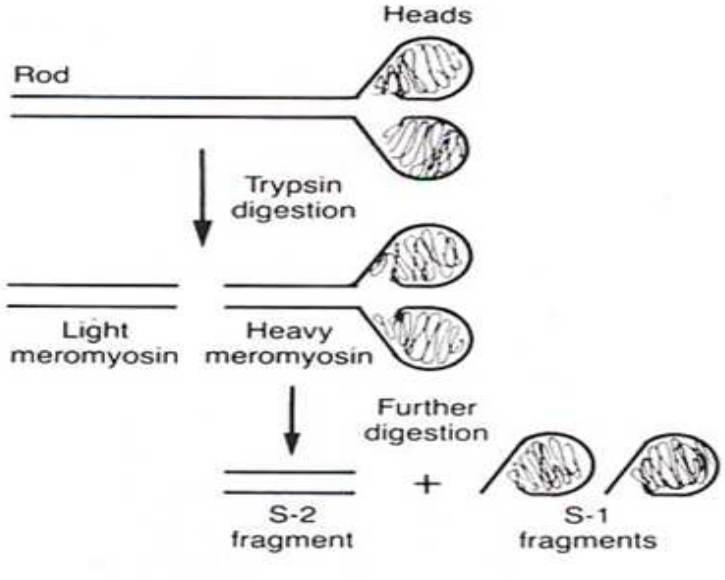


Figure 1.8 Schematic of the generation of LMM, HMM, S-1, and S-2 fragments (Source: Bechtel, 1986).

Actin is a globular protein (G-actin) with a molecular weight of 42 kDa; it contributes about 22% of the myofibrillar protein (Kijowski, 2001). G-actin molecules polymerize into a double-stranded fibrous form (F-actin) which is considered the backbone of the thin filament and also provides binding sites for tropomyosin and troponin complex which regulates the activity of myosin ATPase (Huxley, 1963). In the presence of calcium ions, F-actin contacts with the myosin heads of the thick filaments and there is a rapid breakdown of ATP, ultimately resulting in muscle contraction (Bechtel, 1986; Choi and Kim, 2009).

Some models showed that actin alone had no binding properties (Fukazawa *et al.*, 1961; Samejima *et al.*, 1969) but there is "synergistic effect" between actin and myosin due to the formation of the actomyosin complex. It was found that the strongest gels were formed when the myosin-actin weight ratio was 2.7 (Yasui *et al.*, 1980; Sathe, 2012).

Connective tissue proteins (Stromal proteins) are mainly composed collagen, elastin, and lipoproteins of the cell membrane, but collagen quantitatively predominates (Kijowski, 2001). The toughness of the meat is generally associated with collagen, which is made of three helically twisted polypeptide chains stabilized by intramolecular and intermolecular bonds (e.g., hydrogen bonds). As animals age, more covalent bonds are formed inside and between collagen molecules, which contribute to the toughness of the meat. Collagen is not able to form a gel at temperatures less than 80°C (Asghar *et al.*, 1985; Kijowski, 2001; An *et al.*, 2010).

Several studies showed that the solubility of myofibrillar and sarcoplasmic proteins are highly correlated with water retention (drip loss and moisture uptake). Protein solubility has a major role in the physical properties of the meat. In this regard, lower protein solubility imparts poor functionality like in the case of PSE meat (Camou and Sebranek, 1991; Sathe, 2012)

Tenderness and juiciness of fresh and processed meat products are considered the most important quality criteria that are used by the consumer during the selection. Tenderness of meat depends on different pre- and post-slaughtering factors which form the cyto-architectural design of the finished products while the juiciness depending mainly on the ability of meat to hold and bind water during processing. Juiciness and tenderness can be improved by marination and tumbling processes (Robbins *et al.*, 2002; Alvarado, 2007).

1.4 Genetic improvement and muscle traits

Genetic selection may increase the growth rate of muscle by inducing hypertrophy in the existing fibers due to fusion of satellite cells and to a lesser extent by hyperplasia because the total number of fibers is generally fixed before hatching (Schreurs, 2000; Picard *et al*., 2002; Scheuermann *et al*., 2004; Berri *et al.*, 2007). In particular, fibers number may increase with increasing growth rate and feed efficiency in the same strain, while the cross-sectional area of muscle fiber may increase with age (Dransfield and Sosnicki, 1999). On the other hand, selected modern chicken hybrids showed higher density of fast-twitch fibers which characterized by higher diameter and lower rate of protein degradation in respect to unselected birds (Dransfield and Sosnicki, 1999; Schreurs, 2000; Branciari *et al*., 2009). The lower rate of protein degradation in broilers compared with that in layer strains plays a major role in increasing body weight, muscle weight and relative maturity (Schreurs, 2000).

Selection towards increased growth rate and breast-yield has obviously put more stress on the growing bird which may induce the appearance of several *ante*- and *post-mortem* histological and biochemical modifications in the muscle tissue (Petracci and Cavani, 2012a). Increase of fiber size determines a lower capillarization leading to large diffusion distances for oxygen, metabolites and waste products which can compromise fiber metabolism (McRae *et al*., 2006). In addition, it results to homeostatic dysregulation which leads to cellular dysfunction. It was hypothesized that both growth- and stress-related myopathies may result from disruption of intracellular cation homeostasis (calcium and sodium).

1.5 Muscle abnormalities

Muscle has plastic tissue structure that makes it able to adapt highly to different functional demands. The structure and functionality of muscle are usually changed as the growth and age change. In some cases, it is difficult to differentiate the muscle abnormalities due to the aging process from one which is due to loss of functionality as a result of myopathy. Muscle abnormality or myopathy normally occurs when the muscle loses its ability to meet the demands placed upon it. This failure in response may be due to the presence of too big or continuous or acute stress, as well as due to trauma or genetic defects (Mahon, 1999).

As mentioned before, muscle has high capability to adopt or recover from stress conditions by repairing system or regeneration process to replace the dead tissue. There are different forms of muscle abnormalities: hypotrophy or atrophy, hypertrophy, degeneration, regeneration, and cytochemical alteration (Anthony, 1998; Mahon, 1999).

Therefore, muscle disorders or diseases can be classified into two main groups according to source of insult or stress. The first group is when the muscle has defects in the fibers (myopathic) as a result of congenital, dystrophies, inflammatory, metabolic, ion channel, and secondary disorders. The second group is when the muscle has defects in the nervous system (neurogenic) which could be central, peripheral, and junctional. Again, muscle disorders can be classified according to the origin of insult as acquired or genetic.

In hypotrophy, muscle fibers have smaller size or diameter than normal fibers as a result of growth retardation due to lack of nutrition, endocrine, neural or mechanical stimulation or inherent genetic defect. Atrophy is case of abnormal fibers that are resulted due to changing in the size from native to small and rounded or thin and angular. The causes that are responsible about atrophy are several which could be deficient in nutrients, hormones, nervous stimulation or mechanical tension. Both hypotrophy and atrophy induced by hormonal and neural reasons and occur, particularly in specific populations of fibers (Anthony, 1998; Mahon, 1999).

Hypertrophic muscle abnormality is characterized by fibers size larger than normal as a result of increased workload or failure in the division and multiplications of normal mature muscle fibers. Hypertrophic fibers may contain some architectural defects like splits, whorls or hyaline changes. In general, detection of fibers in hypertrophic case is relatively hard, especially when all fibers are similarly larger than normal. This problem can be overcome by morphometric measurements. The appearance of hypertrophic fibers may be induced by the presence of

atrophic fibers. Accordingly, atrophic fibers leave more space in the muscle and change the workload which activates the growing of hypertrophic fibers to compensate these changes, this case called compensatory hypertrophy. The synthesis of hypertrophic fibers is a sophisticated process which includes a combination of myofilament and skimmer assembly, myofibril splitting and satellite cell activity (Anthony, 1998; Mahon, 1999).

Muscle fibers are very excitable and differentiable and in addition they have very sensitive membranes. Hence, when they subject to stress, it easily leads to irreversible cell death (necrosis) or degeneration. As a response of inflammatory system, macrophages start to attack the cells and eliminate the cell debris. Because the muscle fibers are very long, degenerative process may target whole cell or some selected areas which make histological evaluation of single section samples is not sufficient. There are several features that characterize degenerated or damaged fibers such as: loss of membrane integrity, loss of myofibrillar material, hypercontraction, and hyalinization. Moreover, activity of some metabolic enzymes like succinate dehydrogenase decreases companied by different structural changes. The level of some enzymes usually increases in bloodstream like creatine kinase (CK). In severe cases, urine could contain high level of myoglobin. Finally, macrophages invade the degenerated cells by hydrolysis enzymes, which completely lead to loss of muscle fibers. Muscle triggers the fibrosis process to replace lost fibers by connective tissues. In response of degeneration process, a regeneration process of partial or whole fibers may occur if the insult or damage is not too big or continuous. The regeneration process consists from several stages: fast activation, proliferation and migration of satellite cells, which proceed to normal muscle fiber development (from myoblasts to myofibres). The regeneration process can be recognized and characterized by nucleation of muscle fibers, high basophilic staining, fibers splitting or fusion, and manifestation of many basophilic fibers. The ability of muscle to regenerate depends on several factors like genetic, neural, hormonal, age, and vascular supply (Mahon, 1999; Mitchell, 1999).

The ratio between contractile myofibrillar and metabolic components of muscle fibers is normally changed as well as the number of aerobic structures like mitochondria and their enzymes. These changes may be induced by enzyme defects, ischaemia, exercise, electrical stimulation, drugs, denervation, re-innervation or altered tension. In some cases, storage of metabolite like lipid or glycogen is increased as a result of increased vacuoles. Alteration in

metabolic and contractile components can induce transformation of fiber type as a consequence of neural disorders (Mahon, 1999; Mitchell, 1999).

Chronic atrophy and degeneration is normally followed by fibrosis (fast proliferation of the connective tissues) and lipidosis (proliferation of fatty tissues). Additionally, muscle fibers transform to rounded and separated from adjacent fibers by thick endomysium which may activate ischaemia. In several inflammatory disorders, the level of white blood cells or macrophages increase between and within the fibers as immune response, this increase accelerates muscle fibers destruction. In addition, inflammatory disorders increase blood vessel density (increase capillary to fiber ratio) as a result of increased metabolic demands. Moreover, thickness of blood vessel wall also increases as a consequence of inflammation, immune, or a specific vascular disorder. On another side, neuromuscular disorders may occur because muscle fibers are connected with nervous system where fibers are driven by motor neurons (motor units) (Mahon, 1999; Mitchell, 1999).

Experimentally, the etiological causes of muscle abnormalities and myopathological mechanisms can be evaluated by exposing cell culture, animals or human muscles to experimental insults or altering the physiological demands. In this case, muscle is normally exposed to different sources of insults or stress such as exercise, changing the gravity, disuse, tenotomy, overuse, stretch, myectomy of synergist or anatogonist, denervation, electrical stimulation, transplantation, temperature modifications, perturbation of endocrine or vascular system, changing nutrition, irradiation, application of drugs, toxins, metabolic inhibitors, growth factors, infectious agents or genetic manipulation (Mahon, 1999; Mitchell, 1999).

1.5.1 Myopathic muscle diseases

In these types of muscle disorders, muscle fibers are directly affected by primary lesions which are usually distributed equally and symmetrically. They occur commonly in the weight - bearing muscle. There are several symptoms of that normally company muscle disorder such weakness, wasting, fatigue, and pain in severe cases. The main common features that appear during histological analysis are high contrast in fiber size, degenerated and regenerated fibers, and modification of internal fiber characteristics (Mahon, 1999; Mitchell, 1999).

Congenital muscle abnormality is non-progressive, non-destructive myopathy, and it is characterized by the appearance of undeveloped muscle fibers that stop to grow at myotube stage or other stages, cessation of growth of a fiber type population, the disproportion in fiber type,

aberration in the distribution of various types of filament (e.g. desmin, tropomyosin), and central cores due to disorder in the distribution of mitochondria. In some cases, gene deletion that affects the ryanodine receptor may be responsible about these disorders (Mahon, 1999; Mitchell, 1999).

Dystrophies are a group of progressive, destructive myoptahies which usually occur due to deleted genes coding for membrane or membrane associated proteins (Emery, 1993; Partridge, 1993). Dunchenne (DMD) and Becker muscular dystrophy (BMD) are the most well known dystrophies which are caused due to lack of dystrophy (a type of protein connecting contractile filament and the plasma membrane) (Matsumura and Campbell, 1994). Dunchenne is characterized by progressive wasting and weakness in the muscle during the human childhood and also death in early adulthood may happen. The gene defect for DMD was identified in an X chromosome gene that encodes the 427-kDa intracellular protein dystrophin. Dystrophin is part of a multimeric protein complex, the dystrophin glycoprotein complex (DGC) (Figure 1.9).

The dysfunctionality of the membrane of myofibers is jointed with abnormal intracellular calcium and dysregulated calcium-responsive pathways (Figure 1.10). The patients with DMD and mdx mice showed higher levels of calcium (Robert *et al.,* 2001; De Backer *et al.*, 2002). Some researchers speculated that the presence of microlesions in the sarcolemma could be the reason of entering extracellular calcium to dystrophic fibers (Bodensteiner and Engel, 1978). Several authors found that calcium-permeable channels induced by stretch (Vandebrouck *et al.*, 2002; Yeung *et al.*, 2005). The increase of calcium level has different consequences on the metabolic system of the myofibers. Firstly, it can induce the activity of calcium-dependent proteases (like calpain) which they can lysis different proteins located on the membrane of myofibres leading to myonecrosis (Iwata *et al.*, 2003). Secondly, increase of cellular calcium level can also lead to abnormal mitochondrial function (Millay *et al.*, 2008)

In general, there is a strong relation between calcium dysregulation and the occurrence of pathophysiology of muscular dystrophy. Therefore, recovering calcium homeostasis or controlling calcium-dependent proteases can be a good strategy to reduce muscle degeneration. Increase the level of intracellular calcium can be managed by different strategies:

- It was found that feeding mdx mice with calcium channel blocker streptomycin mitigated the muscle damage (Yeung *et al.*, 2005)
- Injecting mdx mice with protease inhibitors (Bonuccelli *et al.*, 2003)

- With transgenic overexpression of the calpain inhibitor calpastatin (Spencer and Mellgren, 2002)

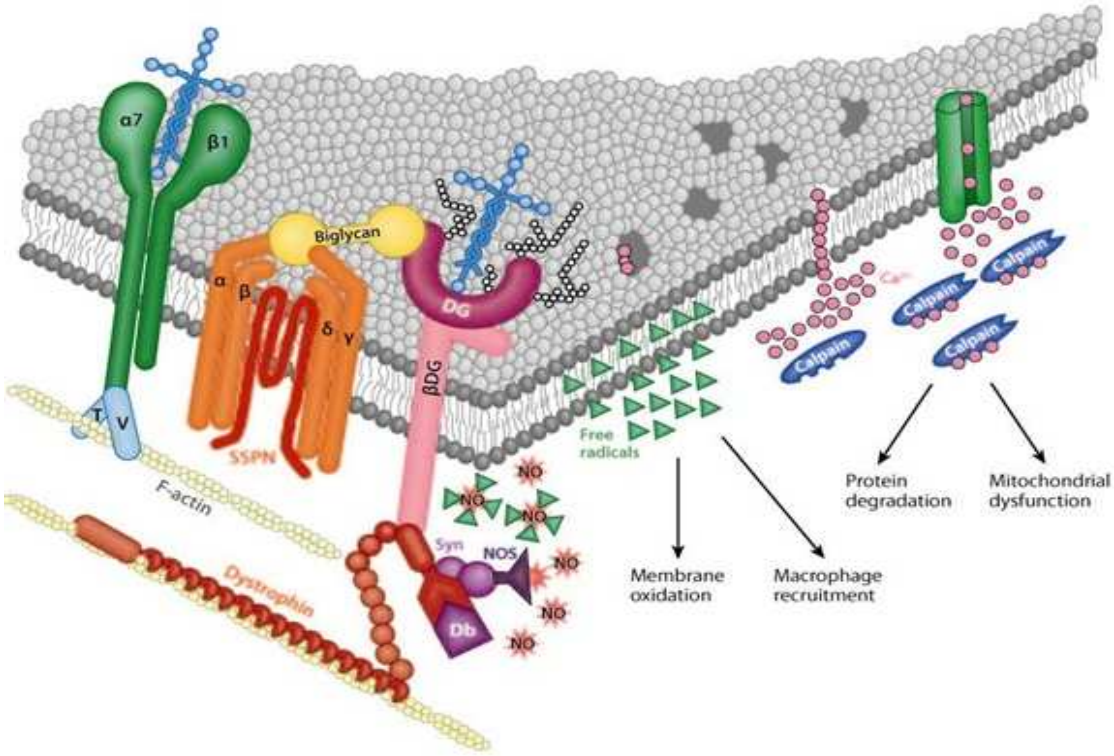


Figure 1.9 Mechanisms for membrane degeneration in dystrophic muscle. The α7β1 integrin linkage system and dystrophin glycoprotein complex (DGC) are distinct, multimeric protein complexes that link the extracellular matrix component to the actin cytoskeleton in muscle cells. (*Left*) A model of these complexes in normal muscle. The α7β1 integrin dimer binds laminin (*blue cruciforms*) extracellularly and associates intracellularly with actin-binding proteins, including vinculin (V) and talin (T). The DGC consists of the sarcoglycan subcomplex of α, β, γ, *δ* sarcoglycans and sarcospan (SSPN); this subcomplex associates with the matrix through biglycan. The α-dystroglycan subunit is heavily glycosylated, and these carbohydrate moieties are necessary for laminin binding. The β-dystroglycan subunit associates directly with dystrophin, and dystrophin binds directly to filamentous actin. The carboxy-terminal domain associates with dystrobrevins (Db) and syntrophins (Syn) that, in turn, bind nitric oxide synthase (NOS). Absence of components of either complex can cause muscular dystrophies characterized by sarcolemmal disruptions and muscle degeneration (*right*). In the absence of proper membrane-matrix attachment, tears may simply develop as a result of the mechanical stress. An increase in free radicals (*green triangles*) is thought to be caused by the displacement of NOS from the plasma membrane. The high levels of free radicals in dystrophic muscle are thought to contribute to muscle degeneration via the oxidation of muscle membranes and recruitment macrophages. Calcium-sensitive pathways also contribute to muscle degeneration in muscular dystrophy. Calcium (*pink spheres*) may enter dystrophic muscle through membrane lesions or through calcium channels. Calcium dysregulation may also lead to abnormal mitochondrial function as well as the activation of the calcium-dependent protease calpain to degrade muscle membrane proteins (Wallace and McNally, 2009).

From the other side, it was found that myofibres lack dystrophin are susceptible to oxidative damage by free radicals (Rando *et al.*, 1998). Neuronal nitric oxide synthase (nNOS) directly

binds the syntrophins in the DGC, when myofibrils have a deficiency in dystrophin; nNOS is moved from the plasma membrane into the cytoplasm (Brenman *et al.*, 1996). NOS generate nitric oxide (NO) which has the ability to react with free radicals and regulates vascular tone. It was found that in *mdx* muscle, a decrease in membrane-associated nNOS causes coincident reduction in NO (Wehling *et al.*, 2001) which may cause free radical-mediated damage. Several hypotheses showed that NO has a dual function in boosting oxidative reactions by reacting with superoxides and from the other side, acting as an antioxidant (Figure 1.10) (Miles *et al.*, 1996).

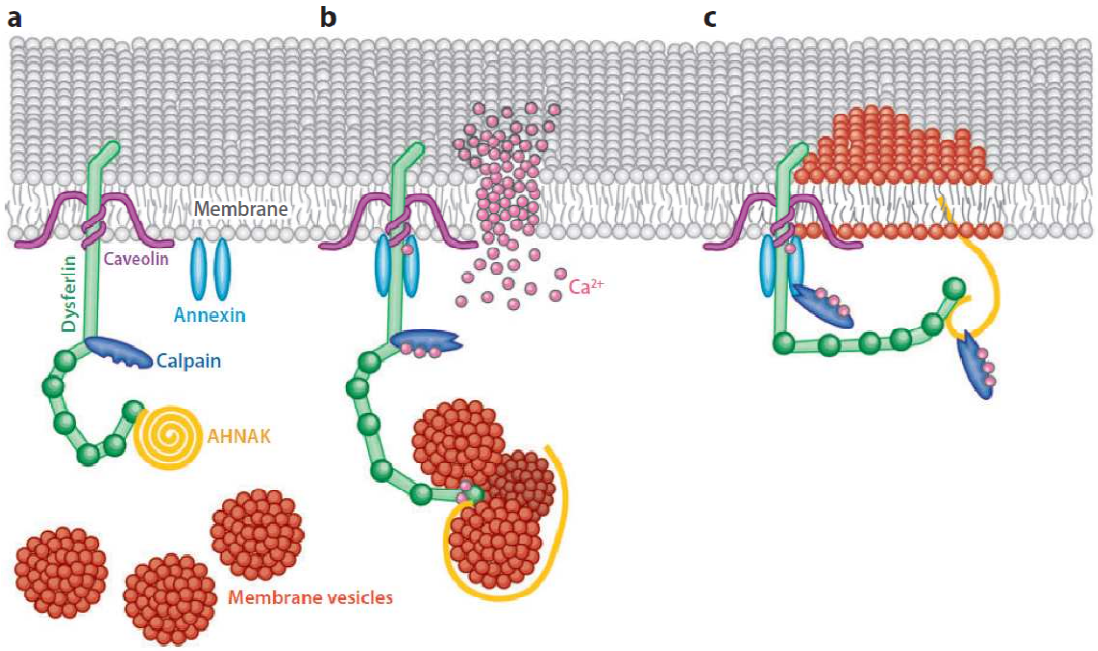


Figure 1.10 A model for dysferlin-mediated membrane repair. (*a*) Dysferlin (green) is localized at the sarcolemma as part of a membrane repair complex. Caveolin-3 (*purple*), a muscle-specific caveolin, interacts with dysferlin. Dysferlin also interacts with AHNAK (*yellow*) and Chopin (*dark blue*), a calcium-activated protease. (*b*) Tears in the sarcolemma result in an influx of calcium (pink *spheres*), which activates and alters the binding properties of proteins in the membrane repair complex. Annexins (*light blue*) bind dysferlin and phospholipids with higher affinity in the presence of calcium, the C2A domain of dysferlin binds phospholipids in a calcium-dependent manner, and calpains are activated. These interactions are thought to encourage the recruitment of internal vesicle structures (*red*). (*c*) Within seconds of activation, membrane lesions are resealed, calcium concentrations are normalized, and the repair complex is deactivated. The deactivation of the complex may be mediated in part by calpain-dependent cleavage of annexins and AHNAK. Mutations in the genes encoding proteins of this sarcolemmal repair complex cause LGMD2B and Miyoshi myopathy (dysferlin), LGMD1C (caveolin), and LGMD2A (calpain-3) (Wallace and McNally, 2009).

Several markers of oxidative stress have been widely examined in *mdx* mice and DMD patients. It was found that the level of byproducts of free radical-mediated lipid damage was higher in DMD muscle (Kar *et al.*, 1979). Myofibres in dystrophic muscle exhibited high levels of the antioxidant molecules like vitamin E and coenzyme Q (Touboul *et al.*, 2005) and antioxidant enzymes such as superoxide dismutase-1, catalase, and glutathione peroxidase (Ragusa *et al.*, 1997; Disatnik *et al.*, 1998). This increase can be explained as an attempt to protect against oxidative damage. Several studies showed that an increase of antioxidants in the feed of mdx mice by including green tea extract or low-iron diet reduced the level of muscle degeneration (Bornman *et al.*, 1998; Buetler *et al.*, 2002).

Histological analysis showed that these dystrophies are characterized by the appearance of hyaline fibers, gross degeneration and regeneration of muscle fibers and fibrosis. All these changes are normally companied by an increase in the level of creatine kinase (CK) in the blood stream. It was found that several animals like mouse, hamster, mink, chicken and quokka exhibited similar muscle disorders like dystrophies. But because of mdx mouse, CMD cat, and GRMD dog have a similar gene deletion, they are used as experimental true models (Mitchell, 1999).

Additionally, inflammatory myopathies are the most common of muscle myopathies which are normally treated with steroids. In mild inflammatory response, they are characterized by little increase of white blood cells and restricted degenerated and regenerated fibers while in severe inflammatory response there is muscle destruction companied by different degrees of regeneration. The main causes of these types of myopathies are bacterial, viral, protozal, or parasitic. It leads frequently to a focal reaction which makes it difficult to diagnosis in several cases. In other hand, the causes of non-infective, inflammatory myopathies are not well known and in some cases could be due to vascular pathology (Mitchell, 1999).

In some cases, metabolic myopathies usually occur due to defect or losing enzymes or related carrier molecules involved metabolism. Several disorders in glycolytic metabolism, lipid storage, or mitochondrial cytoplasm are examples of these metabolic myopathies which characterized by the production of abnormal mitochondria and lipid storage. The main symptoms of these myopathies are low ability to exercise, rapid fatigue, and production of lactic acid, muscle damage and as consequence muscle degeneration, weakness, and wasting (Mitchell, 1999).

Many disorders in different channels that are responsible in regulating ions in the cells may occur which lead to muscle weakness, severe muscle necrosis, increase the level of CK and potassium, high temperature, and eventually death. There are different channels that are usually affected by disorders such as chloride channels (myotonia), sodium channels, calcium (dihydropyridine receptors), and potassium or calcium channels (malignant hyperthermia) (Gronert, 1986; Maclennan and Philips, 1992). Pigs have mutation in the ryanodine receptor gene (calcium release channels) are exposed to malignant hypothermia, which is related to the porcine stress syndrome (Fujii *et al.*, 1991)

Secondary myopathies are frequently destructive and occur due to external insult like toxins, drugs, trauma, or infectious agent which causes membrane damage. As consequences, different degrees of muscle degeneration, inflammation, and regeneration may occur according to the severity and duration of insult. In addition, some cases metabolic disorders in endocrine system may occur. Pituitary hypersecretion causes muscle weakness and lobulation of fibres, increase of type 1 muscle fibers can be induced by hypothyroidism. Moreover, some non-specific muscle abnormalities may occur due to hyperthyroidism and hyperparathyroidism (Mitchell, 1999).

1.5.2 Neurogenic muscle diseases

These types of muscle abnormalities occur due to defects in the nervous system which leads to disorders in the structure and function of the muscle. Defects may occur in lower motor neuron, peripheral nerve, or the neuromuscular junction which leads to weakness and wasting of the muscle companied by slight signs of degeneration and regeneration.

Central neurogenic disorder is a type of spinal muscular atrophy, which leads to disorders in the arrival of trophic and excitatory supplies to the motor unit of muscle fibers (denervation). Denervation induces the generation of atrophic muscle fibers distributed between fibers of healthy motor unit. Atrophic fibers may degenerate or become re-innervated from frontal braches of adjacent intact fibers which lead to transformation of their fiber type characteristics. Intensive denervation, may lead to grouped atrophy, which eventually leads to muscular wasting companied by fibrosis and fatty replacement. In general, central neuronal myopathies affect upper motor neurones (cerebellar system) and hence leading to disturbance in muscle functionality showing little muscle pathology.

Occurrence of insult such as trauma, pressure, and peripheral neuropathy in peripheral nerve may cause denervation which eventually leads to muscular atrophy. According to the severity

and duration of insult, subsequent re-innervation may occur, leading to fiber type grouping. Junctional neuromuscular disorders may affect the previous two neurogenic diseases.

1.6 Muscle abnormalities in poultry

1.6.1 Deep *Pectoralis* Myopathy (DPM)

This myopathy usually occurs in supracoracoid and it is not related to dietary deficiency or toxicity. It is generally thought DPM is due to ischemia and increase the growth of muscle in the inelastic sheath (Figure 1.11). It was found that over development of supracoracoid is not connected with its blood vessel. Deep pectoral disease, also known as Oregon disease or green muscle disease was first described in 1968 as “degenerative myopathy” in turkeys and it was subsequently studied at the Oregon State University.

DPM was firstly recognized in chicken breeders, but it also appeared in fast-growing birds about 30 years ago (Siller, 1985). Despite the efforts that have been exerted by poultry breeders and producers to reduce its occurrence, DPM is still today a significant quality issue in poultry processing plants. *Pectoralis minor* muscles affected by DPM are trimmed which causing downgrading of the breast meat and subsequent economic loss for the poultry industry, while the quality of the rest part of the breast is not affected and it is accepted for human consumption (Kijowski *et al*., 2014).

However, this abnormality is still today a relevant quality issue in poultry processing plants. It is interesting to note that this anomaly does not hit slow- and medium-growing chickens. In 2006, it was estimated that the incidence rate was around 1% in broilers weighing more than 3 kg (Bianchi *et al.*, 2006). Poultry industry reports confirmed that its incidence is not much different today. No public health implications are associated with deep pectoral myopathy, but it is aesthetically undesirable. The fillet should be removed, whereas the rest of the carcass is still fit for human consumption. However, required trimming operations cause the downgrading of the products and produce an economic loss for the industry, especially because it affects the more valuable part of the carcass. In addition, the presence of DPM also causes significant commercial complaints when whole carcasses are sold to small cut-up or processor units or butcher shops.

The incidence of DPM is certainly related to the tremendous development of the pectoral muscles achieved in modern commercial hybrids (about 20% of the body weight), but it has been also favored by the relatively low activity during growing period. *Pectoralis minor* muscles are

confined in inelastic compartments which are insufficiently enlarged if these muscles are poorly exercised by the birds. This lack of movement in fast-growing chickens is not mainly due to intensive farming environment, but rather to a genetic predisposition as evidenced by their behavior under organic farming conditions (Castellini *et al.*, 2002).

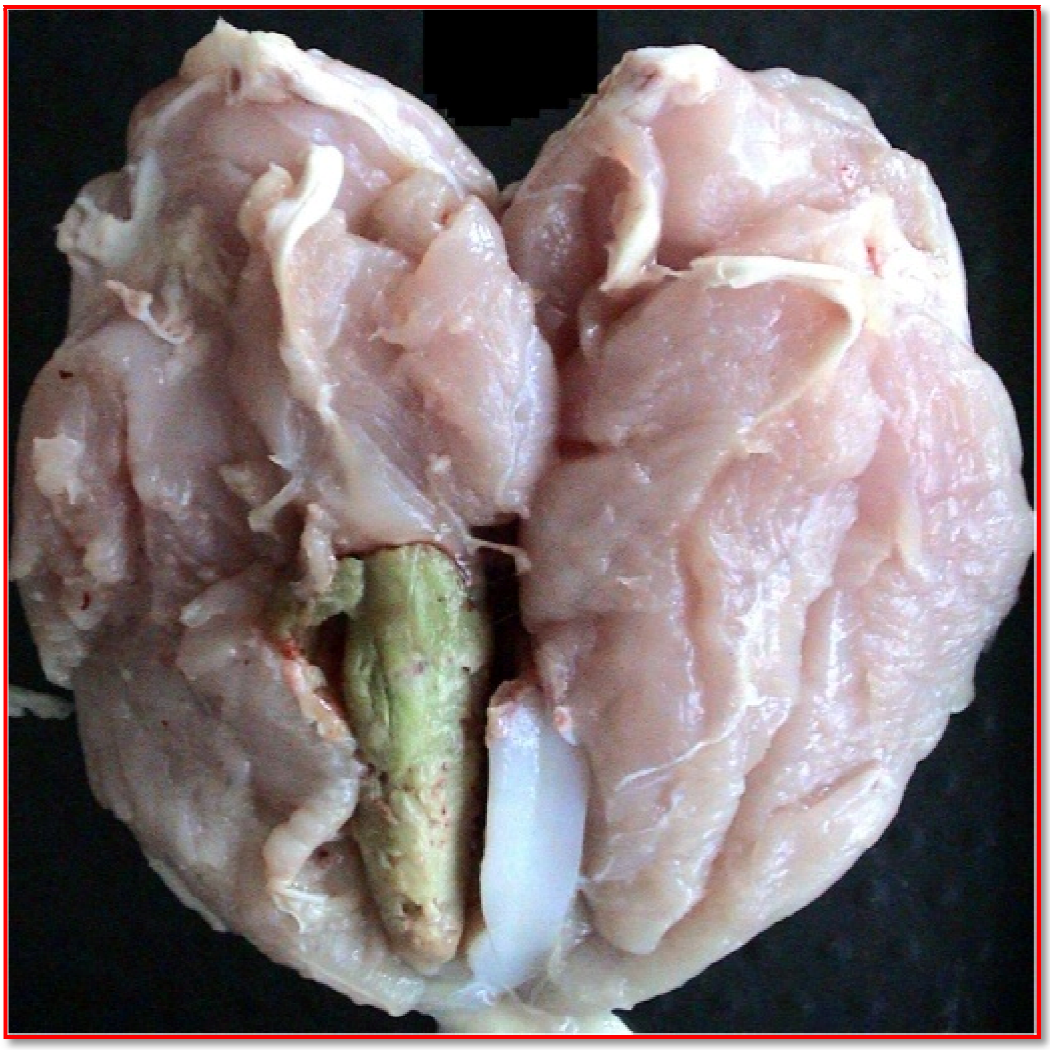


Figure 1.11 Deep *Pectoralis* Myopathy (Source: Petracci and Cavani, 2012a)

Recently, Lien *et al.* (2012) found that susceptibility to induction of DPM appeared to develop at approximately 26 d of age in male broilers and 36 d of age in female broilers. Increased bird activity (flock nervousness, flightiness, struggle, and wing flapping) induced by factors such as feed or water outages, lighting programs and intensity, human activity, and excessive noises in and around the chicken houses should be looked at as a trigger for the development of DPM in broilers. As selection for breast meat yield is continuing and broilers are

growing to heavier body weight, the occurrence of DPM will likely continue to increase (Lien *et al.*, 2012). The same authors suggested that creatine kinase levels could be used as tools in genetic selection programs to screen for green muscle disease susceptibility.

It was found that selection for increased muscle size resulted in modification of vascular structure in relation to the muscle or skeleton which change the flow of blood circulation in the deep pectoral muscle. The exercise-stimulated deep pectoral muscle of heavy-type strains exhibited an increase in pressure within the fascial compartment by one-fifth more than light-type strains (Martindale *et al.*, 1979). These findings showed that the myopathy was the result of ischemia brought about by an increase in internal pressure in the muscle that occludes the cranial and pectoral arteries resulting in a loss of blood supply and leading to a necrotic lesion. In all types of poultry, the muscle increases in weight by about 20% during activity, but because of its anatomical compartment, the increase of the size due to muscular activity is so marked in the heavy-type breeds that the muscle becomes strangulated and ischemic (Siller, 1985). The same phenomenon does not occur in light-type breeds because there is enough space available to accommodate the swelled muscle. It is unknown why the compartment in birds selected for increased musculature has not also enlarged enough to allow for the normal function of the muscle.

1.6.2 PSE- like meat

The term PSE was originally described for pork meat which characterized by light colour, flaccid texture and poor water-holding capacity. The suggestion that a pale, soft, and exudative (PSE-like) conditions exist in poultry was mentioned some decades ago (Barbut, 2009). In 1996, a comprehensive Symposium entitled “Atypical poultry meat in relation to PSE pork: causes, biochemistry, processing and resolutions” was organized in Louisiana and this gave evidence to the importance this problem since twenty years ago. As for DPM, despite the advancements of knowledge, there is still a significant proportion of breast meat having PSE-like characteristics. This is because no genetic marker for commercial selection has been shown.

Several studies conducted in different European and North American countries between 1998 and 2007 estimated the incidence of PSE-like breast meat which was economically significant (Petracci *et al.*, 2009). In Canadian poultry industry, it was found that the rate of PSE in young turkey breast muscles ranges from 18 to 34%, while in mature turkey hens from 5 to 41% and in chicken broilers was from 0 to 28% (Barbut, 1996, 1997a, 1997b). In the USA, the incidence

range of PSE defect in turkey breast muscles was 30-41% (Owens *et al.*, 1998, 2000) and 37-47% in chicken broilers (Woelfel *et al.*, 2002). In England, The incidence of PSE was about 20% of examined chicken broilers (Wilkins *et al.*, 2000). Fletcher (1999) found that about 7% of packages which contain four broiler breast fillets per package had one or more fillets that were noticeably different in colour from the other fillets in the same package.

PSE-like meat is also a major source of breast meat downgrading in particular for the retail market of boneless, skinless breast fillets (Figure 1.12). Although there are no established border lines for optimum colour, extremities of either dark or light meat have been identified as negative qualities. At least, processors need to colour sorting of fillets prior to packaging (Fletcher, 2002).

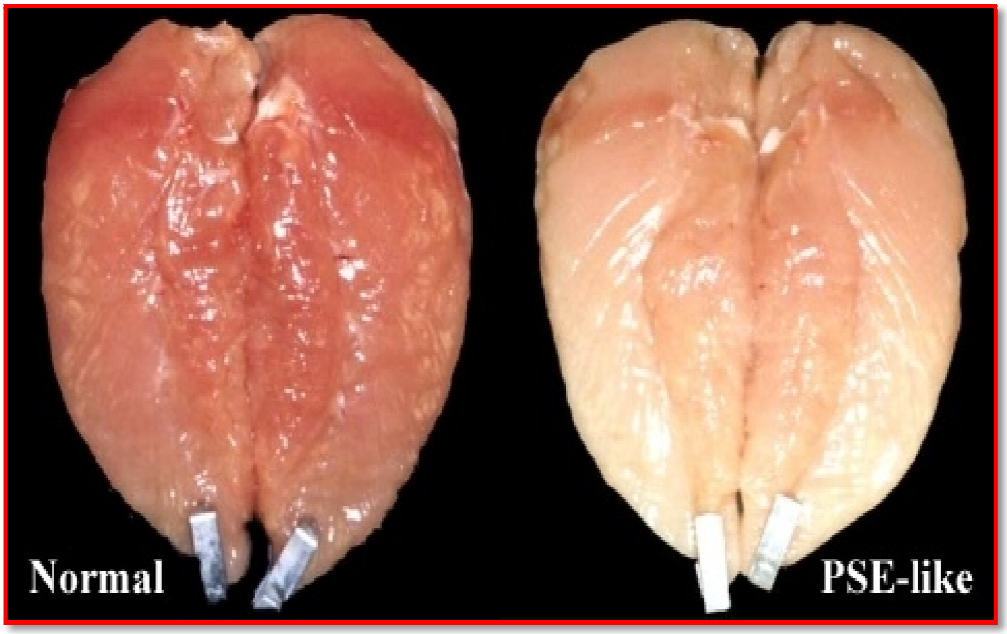


Figure 1.12 Colour differences between normal and PSE-like breast meat (Petracci and Cavani, 2012a)

However, the PSE pork meat is associated with a rapid glycolytic process during a *post-mortem* time (pH lower than 6.0 just after 45 min after the death) which leads to extensive acidification, and thus the achievement of extremely low ultimate pH values (<5.7/5.8), and these changes seem to be the main origin of PSE-like conditions in chickens (Duclos *et al.*, 2007).

Sandercock and Mitchill (2003) suggested that genetic selection for increased growth rate and breast yield has altered inter-compartmental cation regulation in muscle cells of modern chicken hybrids, which reflected adaptive responses to high metabolic demands. The impositions of stress upon broiler birds (i.e. heat pre-slaughter stress) further exacerbate these problems and

underlie additional quality decrements such as PSE-meat. It has been generally recognized that stress during pre-slaughter phases (i.e. catching, transportation and lairage) is associated with detrimental effects on meat quality. Exposure to high temperatures during these phases may exacerbate negative effects. Faster growing or heavier birds have been shown to be more susceptible to heat stress because of the reduced thermo-regulatory capacity compared with their genetic predecessors. It has been demonstrated that acute heat stress increased superoxide free radical production in chicken skeletal muscle. This mechanism may be responsible for the transport stress- and heat stress-induced muscle damage and for the changes in muscle and meat quality observed in broilers.

According to previous studies, there are two categories of conditions that are involved in the occurrence of PSE meat. The first category related to genetics, in which the selection for muscle growth has induced histological and biochemical changes (intracellular calcium homeostasis and sarcolemmal integrity) in the tissues of the muscle which are relevant to PSE defect. These modifications may result from excessive myofiber hypertrophy and inadequate development of support tissues and vascular supply. The second category is related to environmental factors (catching, transportation and lairage) (Sosnicki *et al.*, 1998). Recently, it has been observed that genetic selection for growth has resulted in the opposite effect of PSE meat conditions. Le Bihan-Duval *et al.* (2001) found that selected birds for muscle yield had lower glycogen storage and higher ultimate pH which could have a positive effect in processing ability.

Finally, integrated approaches by employing "omics" science are still needed to study the relationship between genome and functional properties of meat in order to optimize the transformation process of muscle to meat.

1.6.3 White striping and wooden breast abnormalities

A new quality defect has been recently observed related to the appearance of muscle abnormality in chicken breast described as the manifestation of white striations parallel to muscle fibres mainly on the ventral surface of broiler breast fillets (Kuttappan *et al.*, 2009) (Figure 1.13). Histological observations for white striped meat indicated an increase in degenerative and atrophic fibers associated with loss of cross striations, variability in fiber size, floccular/vacuolar degeneration and lysis of fibers, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells), mononuclear cell infiltration, lipidosis, and interstitial inflammation and fibrosis (Kuttappan *et al.*, 2013a).

Even though that white striping has some similarities to nutritional muscular dystrophy (NMD) which relevant to vitamin E deficiency, but it was found that including vitamin E in different level in feed had no effect on the incidence of white striping (Kuttappan *et al.*, 2012a). Breast fillet affected by white striping exhibited higher weight and dimensions (heights in top cranial, middle, and end of the fillet) than normal fillets (Kuttappan *et al.*, 2009, Petracci *et al.*, 2014a). In addition, white striping abnormality was highly associated with heavier birds. In other hand, white striped meat showed a different chemical composition. Moreover, hematologic and serologic profiles of white striped meat showed no systemic infectious or inflammatory condition while elevated serum enzyme levels (creatine kinease) were relevant to the muscle damage associated with the degenerative myopathy (kuttappan *et al*., 2013b; Ferreira *et al.*, 2014).

The aetiological causes of white striping are still poorly known. Until now, there are several factors that could be involved somehow with the occurrence of white striping which can be summarized as follows: genotype (high>standard breast-yield; Petracci *et al.*, 2013b), sex (males>females; Kuttappan *et al.*, 2013c), growth rate (high>low; Kuttappan *et al.*, 2012a; Kuttappanet *et al.*, 2013c), diet (high>low energy diet; Kuttappan *et al.*, 2012a), and slaughtering weight (heavy>light; Kuttappan *et al.*, 2013c).

In other hand, new myopathy "wooden abnormality" accompanied with white striping has recently been observed. Wooden defect appeared in chicken breast meat characterized by macroscopic and histologic lesions. Wooden muscle can be discriminated from normal muscle by visually hard, outbulging, pale, and often accompanied with white striping (Figure 1.13).

The histological changes in wooden meat showed different levels of polyphasic myodegeneration with regeneration companied by accumulative with interstitial connective tissue (Fibrosis). The etiological causes for this myopathy are still undefined (Sihvo *et al.*, 2014).

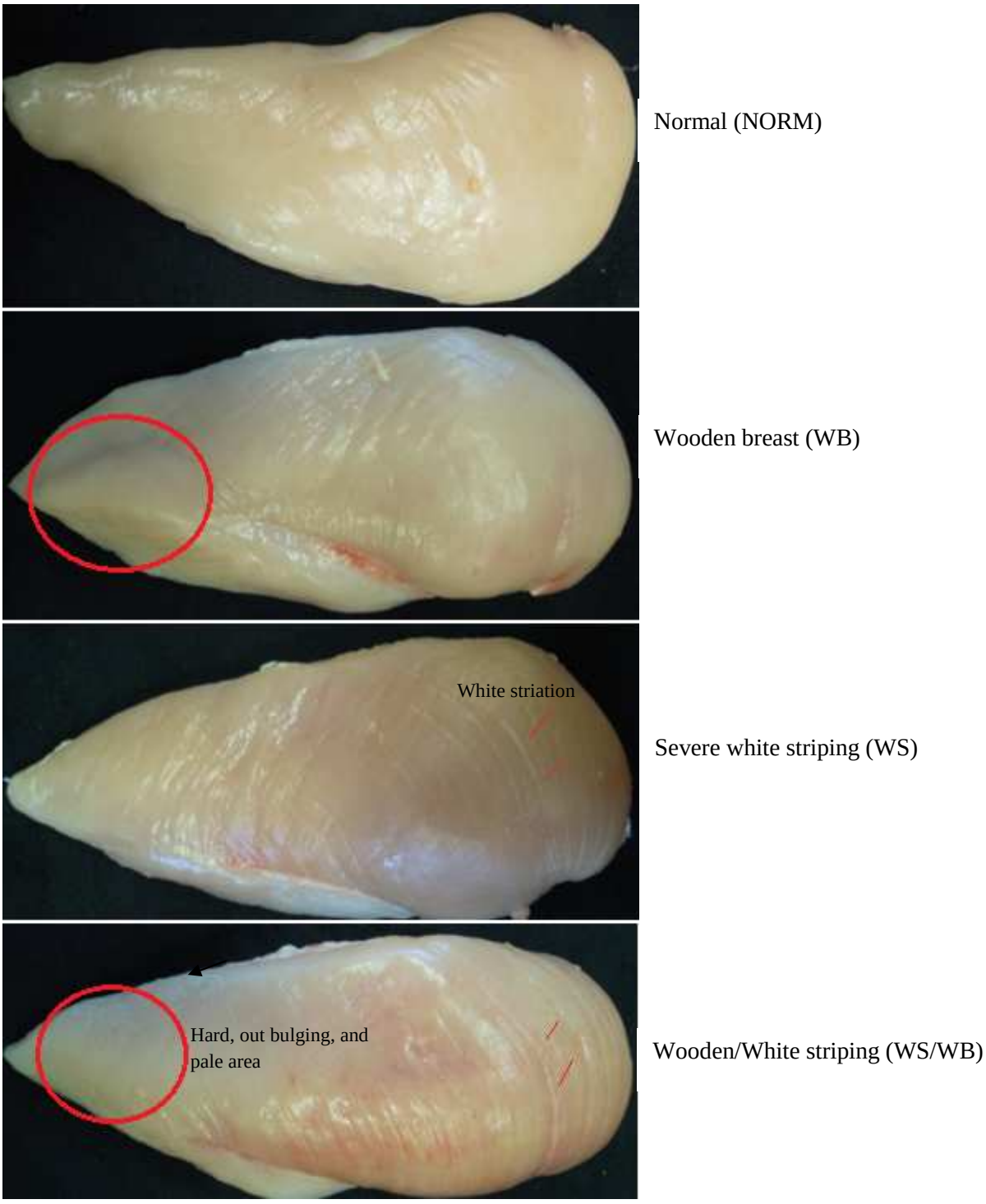


Figure 1.13 Different levels of white striping and wooden abnormalities in the chicken breast meat.

1.7 Muscle abnormalities: pathophysiological mechanisms

A better understanding of normal physiological function of the muscle can help to find explanations or the pathophysiological mechanisms for several muscle abnormalities which are caused by genetic factors or environmental stress.

1.7.1 Skeletal muscle form and function

The main function of skeletal muscle is to generate force by contraction which is used for movement or to support the skeletal structures. Actin and myosin filaments are the main contractile proteins that are organized in pattern to give a striated appearance. Troponin I, C and T, tropomyosin, and alpha-actinin are considered as regulatory contractile proteins (Harvey and Marshall, 1986; Szczesna *et al.*, 1996; Watanabe *et al.*, 1997). The contraction of muscle is usually started by reception of excitatory electrical signal at the neuromuscular junction which induces the interaction of contractile proteins by depolarization of the muscle membrane in the presence of certain calcium level which called "excitation-contraction" coupling (EC).

Sarcoplasmic reticulum (SR) releases the stored calcium under regulated physiological conditions. EC coupling consists of three phases take place at triads which are specified sites at junctional constant between the T-tubule and sarcoplasmic reticulum. The first phase is voltage sensing which measures intra-membrane charge movements by charging sensor molecules located in the membrane of T-Tubule (high in dihydropyridine binding receptors, DHPRs). It is thought that DHPRs are responsible about the transmission the second phase. In the third phase calcium releases from SR in the myoplasm through different types of calcium channels that mediating the release of calcium from internal stores which leads to increase the level of myoplasmic calcium (Schneider, 1994).

Calcium has a different role in the body not just for muscle contraction but also it is responsible about regulating different metabolic and cellular activities (proteins-proteins interaction, phosphorylation, gene expression, cell growth, etc.) (Ashley, 1995; Berridge, 1997). In addition, calcium is present as immobilized form which works as a reservoir and it is used according the requirements of organisms. Calcium is remarkably toxic because its concentration cannot be easily regulated by metabolic synthesis and degradation as other biological messengers. It is thought that the toxicity of calcium is a consequence of its regulatory role.

Hence, Increase the intracellular calcium level due to pathological effect can be explained by over-stimulation of multiple calcium-sensitive pathways.

Intracellular levels of phosphate are always high because phosphorylated compounds are continuously used to generate and regenerate energy. And so when the calcium level is very high, phosphate-calcium precipitate may occur which lead to inhibition of oxidative phophorylation and as a consequence mitochondrial dysfunction and physical damage. Because the dual characteristics of calcium between functionality and toxicity, intracellular free calcium is precisely regulated by different mechanisms. In normal conditions, cells keep cytosolic free calcium level 10^4-fold less than extracellular calcium due to very low permeability of the cell membrane as well as controlling the release, uptake and extrusion of calcium by mobile proteins and storage of calcium in the membrane-bound compartment (Ashley, 1995)

Cytosolic Ca^{+2} levels can be regulated by two groups of binding proteins. The first group of binding proteins works as high capacity storage for calcium, which are present in intracellular compartments and sarcoplasmic and endoplasmic reticulum such as calreticulin (CR) and calsequestrin (CSQ). While the second group of proteins are cytosolic proteins such as troponin C and parvalbumin which exhibit their action in the nucleous and the cytoplasm (Zimmer *et al.*, 1995). Unused calcium ions cannot stay as free in the cytoplasm and should be stored by accumulation in a suitable complex form without generating calcium-phosphate precipitate (Pozzan *et al.*, 1994). Because calcium has several roles in controlling different metabolic and biochemical reactions in the cell, it is important to keep intracellular free calcium homeostasis.

Hence, release, buffering and re-uptake of calcium are controlled by precise regulation in order to maintain calcium homeostasis (Konishi *et al.*, 1991). Therefore, any failure in the system of calcium homeostasis leads to toxic effect and adverse consequences which must be taken into considerations in the pathology of the muscle and myopathies. In some cases due to excessive muscle contraction, calcium level is raised and if the energy depletion proceeds, then extrusion is restricted and increase of calcium may lead to exercise-induced myopathy.

Malignant hyperthermia (MH) is a pharmacogenetic hypermetabolic disorder due to an inherited defect in sarcoplasmic reticulum RyR or calcium release channels which leads to increase the concentration of cytosolic calcium. It is normally induced by exposure the animals to halothane anaesthesia separately or in concomitment with muscle relaxants. MH is usually characterized by several physiological disorders such as uncontrolled muscle contraction,

increase of metabolism, hyperthermia, and aberration in sarcolemmal membrane followed by release in intracellular muscle enzymes into the blood stream. Moreover, due to the increase of calcium level in the cells, muscle contraction increases sharply (hypermetabolism) in order to eliminate calcium from the cytoplasm by pumping (ATPase) into the SR, mitochondria, and by extrusion through the sarcolemma which generate high amount of energy (hyperthermia).

Sarcoplasmic reticulum ryanodine-sensitive calcium release channel (SR-RSCRC) plays a major role in regulating calcium release from storage as well as myoplasim calcium level during the process of excitation-contraction coupling. It is expressed by three distinct genes producing different isoforms (RyR1, RyR2, and RyR3) distributed in specific locations in the tissues. Hence, SR-RSCRC disorders may lead to several muscle pathologies, lesion, muscle damage, and dysfunction. In addition, genetic selection as well as other factors like nutrition and environmental stressors may cause disruption in sarcoplamic calcium homeostasis which leads to muscle pathology (Mitchell, 1999).

1.7.2 Indicators of muscle cell damage

In the last years, different clinical indicators have been developed to understand the type, extent and origin of muscle abnormalities. A muscle biopsy is important in histological analysis tool to understand the morphological changes. In addition, blood analysis can be considered more desirable and less destructive. Changing in the integrity of sarcolemma due to muscle lesion or myopathy may lead to leakage of intracellular enzymes and metabolites into the bloodstream. Hence, measuring the activity and levels of different enzymes (lactate dehydrogenase (LDH), aspartate aminotransferase (AST), aldose, and creatine kinase (CK) in blood can be used as indicators for muscle pathology. Creatine (phospo) kinase (CK/CPK) is the most common enzyme that is often used as an indicator for myopathy due to very high activity in respect to other enzymes.

1.7.3 Mechanism of myopathy in poultry

It was found that commercial lines of chickens and turkey had a degree of myopathy rised with age while muscle damage in selected fast growing lines was higher than in their genetic predecessors (or traditional lines). Additionally, selected broilers exhibited higher muscle damage due to acute heat stress than control lines. Several studies that have been done by Mitchell and Sandercock (1994, 1995, 1997) to explain the mechanism of stress induced

myopathy and monensin myotoxicity by evaluating radioisotopic calcium (^{45}Ca) uptake and CK efflux. They found that the increased level of intracellular calcium either by increased calcium crossing inside the cells (by specific calcium ionophores) or release calcium from sarcoplasmic stores leads to changes in membrane integrity and efflux of CK. It was found that both increase of calcium and sodium may increase the enzyme level and causing membrane damage due to activation of phopholipase (PLAs). Monensin increased the entry of sodium by sodium-proton exchange into the cells, which also allow the entrance of calcium by sodium-calcium exchange mechanism in the sarcolemma as well as calcium release into cytoplasm by SR calcium channel or ryanodine receptor. The disturbance in ion balance underlying the myopathies in poultry is consistent with the mechanisms proposed by mammals. Monensin A is a type of antibiotic produced by streptomyces cinnamonensis. It forms complexes with different monovalent cations such as Li^+, Na^+, K^+, Rb^+, Ag^+, and Ti^+. Recently, it was found that monensin may have ability to transport sodium ion through the membrane in both electrogenic and electroneutral conditions (Huczyński *et al.*, 2007). Increase the level of sodium and calcium in the muscle of Duchenne muscular dystrophy patients reduced the concentration of potassium and magnesium (Jackson *et al.*, 1985).

Based on the former previous studies, it was found the levels of Na, K, Mg and Ca based on inorganic matter content were higher in broiler lines than layers and traditional lines. Broiler lines had a higher content of creatine kinase four times than layer and traditional lines. In other hand, broiler lines exhibited lower initial and ultimate pH than layer and traditional lines (Sandercock *et al.*, 2009). Increase of sodium concentration in selected broiler muscle in comparison to unselected lines indicates to presence of changes in muscle cation homeostasis, and as consequences a sign of the initiation of muscle degeneration (Sandercock and Mitchell, 2004).

Skeletal muscle has the ability to regenerate when exposes to stress like exercise, injury, and disease. Regeneration process is triggered by activating small population of quiescent stem cells (satellite cells) to proliferate, differentiate, and fuse into multinucleated myotubes (Montarras *et al.*, 2005; Collins *et al.*, 2005; Kuang *et al.*, 2007). The presence of abnormalities during the regeneration process in proliferation or differentiation in satellite cells can lead to muscle dysfunction and can induce the appearance of muscle disease (Mitchell, 1999).

1.8 Conclusion

Ever-increasing genetic pressure to improve growth rate and breast yield of broiler chickens has led to very high occurrence of several abnormalities in the breast muscles during the last 20 years (Petracci *et al*., 2009; Kijowski *et al*., 2014; Lorenzi *et al*., 2014). However, until a few years ago, economical loss due meat downgrading has been largely counterbalanced by gains due to increasingly high growth rates of the birds and greater breast meat yields. Nowadays, occurrence of emerging breast meat abnormalities, such as white striping and wooden breast, is determining high percentages of downgrading no longer sustainable for the poultry industry. Beside to the increase in downgrading percentages, there is also a decrease in the nutritional, sensory and technology quality which may impair current consumer attitude towards poultry meat in the near future. As a consequence, either the industry should consider a step back with the increase in growth rates and breast development in order to reduce related problems; or should it go forward? However, the answer to this question may be different depending on the geographical market. In fact, the most vulnerable markets seem to be those where production systems are more efficient and have higher growth rates, birds are slaughtered at higher slaughter weights/ages, and the majority of poultry products are sold under the forms of whole carcasses and pieces. In these developed poultry markets, increasing the occurrence of myopathies may also negatively affect consumer attitude toward perceived animal welfare. Greater specialization of broiler chicken hybrids following the peculiarities of the producing areas could be the answer. Meanwhile, poultry industry may mitigate the negative effects of abnormalities on meat quality by modulating growth rate of birds through farming strategies and by incorporating downgraded meat into processed products.

CHAPTER 2

Incidence of white striping under commercial conditions in medium and heavy broiler chickens

2.1 Abstract

The Poultry meat producers are currently facing several types of emerging muscle abnormalities as a result of genetic selection, which lead to many economical and quality implications. The appearance of thin (<1 mm, considered as moderate) to thick (>1 mm, considered as severe) white striping (WS) striations parallel to muscle fibers on the surface of broiler breast fillets is one of the most troubling issues in the poultry industry. White striped breast meat was characterized by low visual acceptance, nutritional value and processing ability. The aim of this study was to evaluate the incidence of white striping in two different years (2012 and 2013) under commercial production in Italy taking into consideration the effect of market class (medium and heavy birds), genotype (standard and high breast yield hybrids), age and weight at slaughter, growth rate, and sex. In the first survey, which has been carried out in 2012, it was found that the total incidence of white striped breast fillets was 12.0% (8.9 and 3.1% in moderate and severe degree, respectively). Considering the effect of genotype, high-breast yield hybrids exhibited a higher overall incidence of WS compared with standard breast yield birds (15.2 *vs.* 10.0%; $P \leq 0.001$). On the other hand, the second survey in 2013 showed a dramatic increase in the incidence in both levels (moderate and severe) of WS. In particular, the total incidence of WS for both medium and heavy broilers was high (43.0%), with 6.2% of samples were considered as severe. Heavy flocks had significantly higher percentages of both moderate (46.9 *vs.* 25.8%; P≤0.001) and severe (9.5 *vs.* 2.7%; P≤0.001) WS than medium flocks. Considering the effect of genotype, high breast yield hybrids exhibited a higher incidence of both moderate (40.2 *vs.* 33.2%; P≤0.001) and severe WS (7.2 *vs*. 5.0%; P≤0.001) compared with standard breast yield birds. In addition, within the medium class, the occurrence of WS reached higher levels in flocks of males. Within the heavy class, male flocks reached higher slaughtering weights (3.8-4.2 kg) exhibited a higher incidence of WS than flocks slaughtered at lower weights (3.0-3.8 kg) at a similar age. The main broiler genotypes used for commercial production were affected by a high rate of WS; hybrids selected for higher breast yields were more prone to the

WS abnormality. In addition, severe cases of WS are even more prevalent at higher slaughter age and weight, although reduced growth rate was associated with a lower incidence and severity of WS. In conclusion, both surveys revealed that the incidence of WS was relatively very high under commercial conditions which may lead economical consequences for the poultry industry. The differences in the findings between both surveys indicated the importance of the role of farming factors on the incidence of WS where different farming conditions led to a different occurrence rate of WS. Finally, the growth rate can be considered one of the most determining factors which were relevant to the incidence level.

Keywords: Broiler, breast fillet, white striping, slaughter weight, genotype, incidence.

2.2 Introduction

Although genetic selection for growth rate has led to significant economic benefits to poultry broiler producers, but there has been a growing increase in the occurrence of breast meat abnormalities such as deep pectoral muscle disease, pale-soft-and-exudative condition, white striping (WS), and wooden breast. Breast meat affected by muscle abnormalities has low quality and poor aesthetic appeal. As a consequence, this defected meat, particularly in severe cases, are usually downgraded and transformed into further processed meat products or by-products. Inclusion breast meat as a high cost raw material in the formulation of further processed meat products may lead to economic losses for the poultry industry. Previous studies showed that the occurrence level of the most of these muscle abnormalities was associated with increased growth rates and breast yield of birds due to genetic selection in the last 50 years (Kuttappan *et al.*, 2012b; Lien *et al.*, 2012; Petracci *et al.*, 2012a, 2013b, c; Sihvo *et al.*, 2014).

WS is a recent abnormality characterized by the appearance of thin (<1 mm as moderate) or thick (>1 mm as severe) white striations on most of the ventral surface of breast fillets. Severe white striped fillets are used in processed meat products because of their unacceptable visual appearance. In fact, it was found that over 50% of consumers had no desire to purchase fillets showing moderate or severe WS (Kuttappan *et al.*, 2012b). The impact of WS is not confined to unacceptable visual appearance, as the WS abnormality also reduced the nutritional value as well as the water holding and binding capacities (Petracci *et al.*, 2013b; 2014a; Mudalal *et al.*, 2014a). The different implications of WS on meat quality traits have been explained in chapter 3.

Moreover, about 50% of heavy broilers (live weight around 3.5 kg) farmed under experimental conditions exhibit moderate to severe WS (Kuttappan *et al.*, 2013c).

Many studies revealed that several parameters such as: sex, age, body weight, growth rate, and genotype were highly associated with an increased occurrence rate of WS. Regarding genotype, Kuttappan *et al.* (2013c) evaluated three high yield and one moderate yield classic breeds and reported that all breeds exhibited WS abnormalities in a certain proportion. Considering the sex of birds, Kuttappan *et al.* (2013c) found that male birds showed a higher percentage of severe WS compared to females, which was attributed due to differences in the growth rate. The same authors also observed that WS was highly associated with heavy birds and high growth rates (kuttappan *et al.*, 2012b).

Currently, in both the US and Europe, there is a growing trend to produce heavy birds for further processing due to economical outcomes (Brewer *et al.*, 2012). This trend raised some challenges because heavier birds are more prone to have breast meat abnormalities with several quality implications. However, there are no available studies have evaluated the incidence of WS in medium and heavy birds produced under commercial conditions. The aim of this study was to evaluate the incidence of white striping under commercial production in Italy during the years of 2012-2013, taking into consideration the effect of market class (medium and heavy birds), genotype (standard and high breast yield hybrids), growth rate, weight and age at slaughter, and sex.

2.3 Materials and methods

2.3.1 Experimental design of survey conducted on 2012

The study was conducted in one of major Italian commercial processing plants of Amadori group, during the period from July to October 2012. In this study, 28,000 broiler breast fillets (*pectoralis major* muscles) were randomly selected from 56 flocks during a 3-mo period. Chickens of both sexes belonging to commercial meat-type strains (Ross 308, Ross 708, Cobb 500, Cobb 700, and Hubbard) were reared under intensive conditions of commercial production and slaughtered from 45 to 54 d of age (average live weight: 2.75 kg).

The evaluation of the occurrence rate of white striping was performed on the processing line at 3 h of *post-mortem* after the breast-deboning area. Five hundred breast fillets were selected for each flock of birds and used to establish the presence or absence of the white striping. Fillets

showing no white striping were classified as normal, while for those breasts with white striping appearance, it was determined whether the defect level was moderate or severe based on the intensity and thickness of white striation according to the criteria proposed by Kuttappan *et al.* (2012c). The fillets with small thin lines (<1 mm) were considered as moderate, while those having had thick white striations (>1 mm) covering most of the surface area were graded as severe.

2.3.2 Experimental design of survey conducted on 2013

This survey has been carried out in collaboration with Martini Alimentare (plant of Gatteo) Company (Italy) from October to December 2013. During this survey, the following *ante-mortem* variables were collected from each flock to understand their impact on the incidence of WS:

- Genetic strain (Ross708 or Ross308);
- Gender (males or females);
- Skin colour (white or yellow skin);
- Feed (vegetal or commercial feed);
- Average weight;
- Slaughtering age.

The production system of broiler chickens in Italy is mainly characterized by production systems that separate females and males to obtain three different market classes: light, medium, and heavy-sized broilers (Bianchi *et al.*, 2007). The first market class is light female birds which are normally reared up to a live weight of 1.5-1.7 kg to yield 1.0 to 1.2 kg carcasses and used for rotisserie-type products. The second market class is medium birds are usually females (but may also be males) where reared to a live weight of 2.2-3.0 kg to produce 1.5-2.1 kg carcasses, and mainly used for cut-up products. Finally, heavy birds are male broilers, reared up to a live weight of 3.0-4.2 kg to yield 2.2-2.9 kg carcasses and used for the production of cut-up and processed products. Based on the previously-mentioned market classes, a survey was designed to use only breasts from medium and heavy chicken broilers that are commercially used for production cut-up products, while light birds were excluded because they are sold as whole carcasses.

Through this survey, 70 flocks of medium (n=37) and heavy-sized (n=33) broilers farmed and slaughtered under commercial conditions were randomly selected for evaluation the incidence of

WS. The presence of WS was assessed directly in the deboning area of the plant at 3 h *post-mortem* on 500 breasts/flock (total of 35,000 breasts) where they were randomly selected. Flocks of medium-sized birds were slaughtered at a live weight of 2.2-3.0 kg (41-50 days old). This market class was divided according to the sex of birds into males (Medium-M, n=15) and females (Medium-F, n=22). Flocks of heavy-sized birds were produced using only male broilers slaughtered at a live weight of 3.0-4.2 kg (50 to 58 days old). This category was divided according to slaughter weight into light-heavy (L-Heavy-M, n=17) and super-heavy (S-Heavy-M, n=16) subgroups corresponding to birds weighing 3.0-3.8 and 3.8-4.2 kg, respectively. Flocks of both market classes belonged to chicken hybrids having different breast yields and therefore were classified into high (n=47) and standard breast yield (n=23) according to criteria used in previous studies (Petracci *et al.*; 2013b, c). Whole breasts (with both pectoralis major and minor muscles) were selected and used to establish the different levels of WS using the classification criteria proposed by Kuttappan *et al.* (2012c) and previously adopted in a similar study (Petracci *et al.*, 2013b) and as described in "2.3.1 Experimental design of survey 2012". Age and weight at slaughter were also recorded for each flock. Based on these data, growth rates (g/day) were calculated as the ratio between weight and age at slaughter.

2.4 Statistical analysis

Data from the first survey 2012, were analyzed by descriptive statistics (mean, SEM, minimum and maximum values). To evaluate the effect of genotype, flocks were divided into 2 groups: group 1 included 21 flocks belonging to commercial strains with standard breast yield (SBY) and group 2 consisted of 36 flocks from commercial strains with high breast yield (HBY). Subsequently, data on occurrence of WS were analyzed using the ANOVA option of the GLM procedure of SAS software (SAS Institute Inc., 1988), testing the effect of genotype.

While data on the occurrence rate of WS that have been obtained from the second survey 2013 were analyzed as a 4 x 2 factorial with market classes (Medium-F, Medium-M, L-Heavy-M, and S-Heavy-M) and strain (high *vs.* standard breast yield hybrids) as the main effects. In preliminary analyses, no significant effects of interactions were detected, and therefore the interaction term was not considered in the final model. Because of the disproportionate numbers in each subclass, data were analyzed by least squares analyses using ANOVA. Least square means were compared by orthogonal contrasts. The rate of moderate and severe WS expressed as percentages were normalized by the function ArcSin (√ Variable/100) prior to statistical analysis.

The analyses were performed using general linear models present in the SAS program (SAS Institute Inc., 1988).

2.5 Results and discussion

The result of incidence of WS in broiler chickens which was evaluated under commercial conditions during the first survey is shown in Table 2.1. The total incidence of moderate and severe breast fillets in both types of high and standard breast yield hybrid was 12.0% (8.9 and 3.1 with moderate and severe degree, respectively). The range in the incidence of total WS was fairly large and varied from 2.4 to 26.2%. Considerable variations were also observed for moderate (range: 2.4 to 18.6%) and severe (range: 0 to 8.8%) levels (Table 2.1)

Table 2.1 The incidence of white striping (WS) in breast fillets under commercial conditions.

	Degree of white striping[1] (%)		
Item	Moderate	Severe	Total
Mean	8.9	3.1	12.0
SEM	0.53	0.30	0.80
min-max	2.4-18.6	0.0-8.8	2.4-26.2

[1]No. of considered flocks: 56, corresponding to 28,000 examined broiler breast fillets.

Considering the effect of genotype, high-breast yield hybrids (HBY) exhibited a higher overall incidence of WS compared to standard-breast yield (SBY) hybrids (15.2 *vs.* 10.0%; $P \leq 0.001$) (Figure 2.1). This difference in the result was due to the higher incidence of breast fillets affected by both severe (3.9 *vs.* 2.6%; $P \leq 0.05$) and moderate (11.3 *vs.* 7.4%; $P \leq 0.01$) levels of WS in HBY birds in comparison to SBY birds (Figure 2.1).

This study was the first one that has been carried out to evaluate the magnitude of white striping defect under commercial conditions. The breasts were randomly selected from flocks of birds of high yield carcasses slaughtered at quite homogenous different ages (from 45 to 54) which are commonly used for the production of cut-up and further processed products. The overall incidence of WS in broiler breast meat accounted for 12.0% of which 3.1% with severe striping. Kuttappan *et al.* (2012a, b) assessed the incidence of WS in experimental trials by a

growing commercial strain of broiler chickens that were fed with different diets. It was found that the incidence of WS can vary from 52 to 95%. However, it is difficult to compare these results that were obtained under commercial conditions, with one that obtained under experimental conditions.

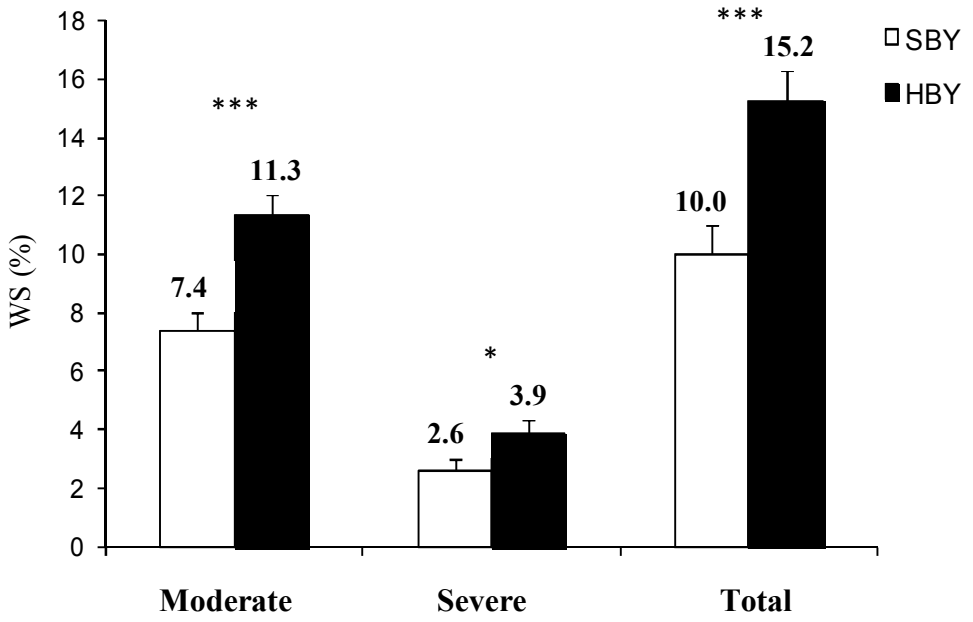


Figure 2.1 The incidence of white striping (WS) in breast fillets (mean ± SEM) in standard-breast yield (SBY, n=21) and high breast-yield (HBY, n=35) hybrids. (*** = P<0.001; * = P<0.05).

The magnitude of WS found in the present study demonstrated the relevance achieved by this quality issue. By the way, fillets having severe white striping are downgraded in commercial plants and cannot be used for fresh market retail which causing a relevant economic damage. This because as recently demonstrated by Kuttapanen *et al.* (2012c) the consumer is able to detect presence of white striping and perceived it as a negative attribute which is frequently associated with marbling appearance and they tend to reject packaged fillets with noticeable WS defects.

The evaluation of the impact of genotype also allowed to demonstrate that among main strains used for commercial broiler production, those marketed especially with the purpose to bear higher breast yield presented much higher incidence of WS. This result was in agreement with Kuttappan *et al.* (2009; 2012 a, b) who found that the higher degrees of white striping were mainly associated with thicker or heavier fillets. Overall, it seems that selection for rapid growth and in particular for breast meat yield is still continuing to induce dramatic quality issues as

extensively reviewed through the last 15 years (Dransfield and Sosnicki, 1999; Mitchell and Kettlewell, 2008; Petracci and Cavani, 2012a).

In general, these results are considered the first estimation about the incidence of white striping under commercial conditions. The findings showed that there were big differences in the incidence rate of WS between experimental and commercial productions. The causes of differences are still unknown. For this reason, a second survey has been carried out in 2013 trying to evaluate the incidence of WS under different commercial conditions with respect to the first survey. Different farming factors such as age, weight at slaughter, sex, genotype, feed, and color of skin have been taken into consideration to understand the magnitude of each variable on the incidence.

The total incidence of WS in breast fillets is reported in Table 2.2. The total incidence of WS in both medium and heavy birds was 43.0% (36.8 moderate and 6.2% severe). The range of the incidence of WS between flocks was highly variable, ranging from 3.0 to 78.1%. In addition, there was considerable variation between flocks in the incidence of moderate (range 2.6-68.1%) and severe (range 0.2-23.8%) WS. Interestingly, severe cases of WS in some flocks were extremely high and reached up to 23%. Fillets showing severe WS are usually downgraded by processors and used for manufacturing processed products (e.g. sausages, nuggets), while fillets with moderate WS are not generally downgraded, and are marketed for fresh retailing. Therefore, the economic loss to poultry industry is basically due to the presence of severe WS as has been mentioned in the first survey.

Table 2.2 The total incidence of white striping in broiler breast fillets under commercial conditions.

Item	Incidence of white striping (%)[1,2]		
	Moderate	Severe	Total
Mean	36.8	6.2	43.0
SEM	2.08	0.66	2.58
Min-max	2.6−68.1	0.2−23.8	3.0−78.1

[1]The presence of white striping was classified as normal, moderate, and severe according to Kuttappan et al. (2012c).

[2]No. of flocks considered: 70, corresponding to 35,000 broiler breast fillets.

The incidence of WS was different between the two market classes (medium and heavy) (Table 2.3). Heavy flocks exhibited significantly higher percentages of both moderate (46.9 *vs.* 25.8%; P≤0.001) and severe (9.5 *vs.* 2.7%; P≤0.001) WS compared to medium flocks. The incidence of severe WS in medium birds was in agreement with previous findings (2.7 *vs.* 3.1%), while the presence of moderate WS was much higher (25.8 *vs.* 8.9%) compared to a previous report in 2013 (Petracci *et al.*, 2013b, data from the first survey). While this apparent discrepancy may be due to different parameters (e.g. strain, age, body weight, growth rate, season, etc.), and moreover, it cannot be excluded that the relevance of this quality issue has increased throughout the last year. On the other hand, the increased incidence of WS in heavy broilers compared to medium broilers was in agreement with previous studies. In particular, Bauermeister *et al.* (2009) and Kuttappan *et al.* (2013c) reported that the overall incidence of WS reached up to 50% by increasing live weight (approximately 3.5 kg). It may be speculated that the differences observed between heavy and medium market classes may be due to differences in weights/ages at slaughter and in the gender of birds.

In addition, orthogonal contrasts were used to discriminate the differences in the incidence of WS between different market class sub-categories to ascertain the influence played by gender and slaughter weight (Table 2.3). By comparing males and females within medium-sized classes, no significant difference in the incidence of severe WS (2.6 and 2.8%, P=0.755) was found, while male broilers had greater levels of moderate WS (31.1 *vs.* 21.7%; P≤0.05) than females. Flocks of female and male birds had similar average live weight (around 2.7 kg) and age (48 days) at slaughter, so that any interference played by these factors can be excluded. This finding was partially in agreement with Kuttappan *et al.* (2013c) who reported that male chickens had a higher incidence of WS compared to female birds. The authors attributed this dissimilarity to the differences in live weights, while in the present study this effect was excluded as previously mentioned.

The reciprocal comparison of the three sub-categories in male gender alone (medium-M, L-heavy-M and S-heavy-M) allowed to evaluate separately the influence of slaughter weight. There was a dramatic increase in the rate of WS moving from medium (33.9%) to light-heavy (52.7%) to super-heavy (60.3%) male broilers. L-heavy-M and S-heavy-M birds also had higher incidences of moderate WS compared with medium-M broilers, while no differences were found between L-heavy-M and S-heavy-M birds.

Table 2.3 Least squares means of moderate and severe white-striping prevalence as affected by market class and genotype on the incidence of white striping (WS) in broilers under commercial conditions (flocks: n=70).

	Flocks (n)	Age (days)	Live weight (kg)	Growth rate (g/day)	Incidence of WS (%)	
					Moderate	Severe
Market class						
Medium-F[1]	22	47.7	2.676	56.1	21.7	2.6
Medium-M[2]	15	48.0	2.684	56.4	31.1	2.8
L-Heavy-M[3]	17	53.3	3.548	66.5	45.2	7.5
S-Heavy-M[4]	16	54.4	3.950	72.6	48.8	11.5
SEM					0.020	0.014
Probability					<0.001	<0.001
Planned contrasts						
Medium vs. Heavy					<0.001	<0.001
Medium-F vs. Medium-M					0.019	0.755
Medium-M vs. L-Heavy-M					0.012	0.001
Medium-M vs. S-Heavy-M					0.001	<0.001
L-Heavy-M vs. S-Heavy-M					0.468	0.032
Genotype						
Standard breast yield	23	51.0	3.192	62.6	33.2	5.0
High breast yield	47	50.5	3.173	62.8	40.2	7.2
SEM					0.011	0.007
Probability					0.035	0.032

[1]Medium size birds of female gender (2.2-3.0 kg)

[2]Medium size birds of male gender (2.2-3.0 kg)

[3]Light heavy size birds of male gender (3.0-3.8 kg)

[4]Super heavy size birds of male gender (3.8-4.2 kg)

Overall, these results confirmed that the severity and incidence of WS increased with increasing slaughter weight were in agreement with previous findings (Bauermeister *et al.*, 2009; Kuttappan *et al.*, 2013c).

However, it is interesting to note that the differences in slaughter weight were not only due to differences in age at slaughter, but also due to differences in growth rate. In particular, within the heavy market class, the greater slaughter weight reached by S-heavy-M birds (3.950 kg) compared with L-heavy-M (3.548 kg) animals was related to a faster growth rate (72.6 *vs.* 66.5 g/day) rather than higher age at slaughter (54.4 *vs.* 53.3 days).

In addition, Kuttappan *et al.* (2012b; 2013c) found that the increase in growth rate increased the incidence and severity of WS. In this context, Kuttappan *et al.* (2012b) found that a low-fat diet can decrease both the growth rate and fillet weight in birds, resulting in a decrease in the percentage of fillets with severe WS compared to birds fed a high-fat diet.

Considering the effect of genotype, high breast yield strains had a higher incidence of both moderate and severe WS compared with standard breast yield hybrids (Table 2.3). A previous study (Petracci *et al.*, 2013b, data from the first survey) also showed that medium-sized broilers from high breast yield strains exhibited a higher incidence of white striped fillets than broilers than standard breast yield hybrids. This result further supports the hypothesis that strains selected for higher breast meat yields are more prone to developing WS abnormalities.

2.6 Conclusions

Both surveys (2012 and 2013) showed that the incidence of WS under commercial conditions was very high which may have economic consequences for the poultry industry. In addition, both surveys indicated that all commercial hybrids had a certain incidence level of WS. The differences in the total incidence of WS between the first and second survey (12 *vs.* 43%) were very great which can be attributed due to an apparent discrepancy in the farming factors (e.g. strain, age, body weight, growth rate, season, etc.). In addition, it may be that the relevance of this quality issue has increased throughout the last year. Both surveys revealed that high breast yield hybrids are more susceptible to white striping abnormality than standard breast yield hybrids. On the other hand, there was a clear effect of sex on the incidence of WS where males had higher rates than female birds. As a matter of fact, the growth rate was one of the most impacting farming factors on the incidence rate. Surprisingly, heavy birds exhibited a higher incidence of WS than medium birds, which was explained due to the apparent differences in

growth rate. According the findings of the second survey, the percentage of fillets affected by severe WS, which are not normally accepted for the cut-up market, was relatively high (6%). In summary, the incidence of white striping abnormality under commercial condition was very high; this may raise some concerns for poultry industry about quality implications. Hence, it is necessary for the poultry industry to adopt some strategies and approaches to mitigate the consequences of this quality issue. In this context, reducing broiler chicken growth rates will decrease the incidence of WS in breast fillets. Moreover, the use of male birds should be limited and production of heavy birds with the strains currently available should be limited to live body weights of fewer than 3.8 kg.

CHAPTER 3

Implications of white striping abnormality on the quality traits of raw and marinated chicken breast meat

3.1 Abstract

The aim of this study was to evaluate the different implications of white striping abnormality on the quality traits of chicken breast meat. Three separated experiments have been carried out to achieve the aim of this study. In the first experiment; 153 fillets were selected in three replications based on white striping (WS) degree (normal, moderate, or severe) and used to assess ultimate pH, color, drip loss, cooking loss, and Allo- Kramer-shear force on raw meat as well to determine marinade uptake, purge loss, cooking loss, total yield, and Allo-Kramer-shear force after marination. The findings of the experiment showed that severe white striped fillets showed higher pH than moderate and normal groups (5.95 *vs.* 5.88 and 5.86; $P \leq 0.05$). Fillets with severe and moderate WS also exhibited lower marinade uptake compared with normal fillets (7.92 and 10.97 vs. 12.67%; $P \leq 0.05$). Moreover, cooking loss increased as the degree of WS increased from normal to moderate to severe groups in both raw (21.27 *vs.* 23.20 *vs.* 26.74%; $P \leq 0.05$) and marinated meat (14.59 *vs.* 14.84 *vs.* 15.93%; $P \leq 0.05$). Finally, non-marinated fillets with severe white striping had lower Allo-Kramer-shear force compared with moderate and normal ones (3.69 *vs.* 4.41 and 4.91 kg/g; $P \leq 0.05$). The second experiment was dedicated to evaluate the effect of white striping on chemical composition and nutritional value of chicken breast meat. During three replications, a total of 108 *Pectoralis major* muscles representing three degrees of white striping (absence= normal; presence of WS classified in 2 levels as moderate or severe) were selected to determine proximate composition (moisture, protein, lipid, ash and collagen) as well as sarcoplasmic and myofibrillar protein profile SDS-PAGE analysis. The results showed that both severe and moderate white-striped fillets had a higher fat content (2.53 and 1.46 *vs.* 0.78%; $P<0.001$), lower protein level (20.9 and 22.2 *vs.* 22.9%; $P<0.001$), and increased of the content low quality protein as evidenced by higher collagen content (1.30 and 1.37 *vs.* 1.43%; $P<0.001$) in respect to normal. Both abnormal fillets exhibited different patterns of myofibrillar and sarcoplasmic fractions when compared to normal

fillets. Moreover, severe white-striped fillets exhibited higher energy content (450.7 *vs.* 421.1 KJ/100g; P<0.01) with respect to normal meat. Finally, the third experiment was designed in order to find some explanations or evidences about reasons standing behind the reduction of processing ability of white striped meat that have been found in the first experiment. During this experiment, a total of 12 *pectoralis major* muscles from both normal and white striped fillets were used to evaluate chemical composition, protein solubility (sarcoplasmic, myofibrillar, and total protein solubility), protein quantity (sarcoplasmic, myofibrillar, and stromal proteins), water holding capacity, and protein profile by SDS-PAGE analysis. White-striped fillets exhibited similar changes in the composition as what's found in the second experiment (less protein and more fat and collagen). There was a decrease in the solubility of sarcoplasmic, myofibrillar, and total proteins as well as an increase in cooking loss (33.7 *vs.* 27.4%; $P < 0.05$) due to presence severe white striping defects. Moreover, gel electrophoresis showed that the concentration of 3 myofibrillar proteins corresponding to actin (42 kDa); LC: slow-twitch light chain myosin (27.5 kDa); and LC3: fast-twitch light chain myosin (16 kDa), and almost all sarcoplasmic proteins were lower than normal. In conclusion, the overall findings of these experiments revealed that white striping had an adverse impact on the nutritional properties (as a consequence of chemical changes) and processing abilities (low water holding/binding capacity and texture). These changes can be attributed due to changes in the quantity and functionality of breast meat proteins as well as changing the chemical composition. These changes in different aspects of the quality traits of breast meat may have an adverse impact on the consumer sensory perception. This side still needs for further investigations.

Key words: White striping, SDS-PAGE, quality traits, composition, myofibrillar.

3.2 Introduction

In the last few decades, the consumption of the poultry meat products has been dramatically increased due to different reasons such as low price, attractive sensory quality traits, higher nutritional value, the absence of cultural or religious effect, and their suitability for processing (Barbut *et al.*, 2008). In response to the growing demand toward the poultry meat, tremendous improvements in strain selection, feed conversion, size of the breast, reduction of fat, and growth optimization had been implemented, which caused a reduction of growing time to half with

double BW when compared to the early 1950s which reflected sharply in the cost of production (Anthony, 1998).

In Europe and North America, the demand is mainly concentrated on breast meat, sold both fresh and processed due to nutritional quality, high tenderness and easiness of culinary preparation (Barbut *et al.*, 2008). In particular, great efforts have been exerted by genetic selection to increase the breast yield. As a consequence, modern hybrid birds showed dramatic development in breast yield, which represents more than 20% of BW (Havenstein *et al.*, 2003). Regarding the nutritional aspects, poultry meat and in particular breast meat fits the modern consumer demand for a low-fat meat with a high unsaturation degree of fatty acids and low sodium and cholesterol levels (Cavani *et al.*, 2009). It is noteworthy that in avian species, lipid accumulation occurs mostly under the skin and so it is easy to separate this fat, while the deposition of intramuscular fat (marbling) is more limited (Mourota and Hermierb, 2001).

Poultry meat may also be considered as a functional food because it provides bioactive substances with favourable effects on human health, *e.g.* long-chain n-3 polyunsaturated fatty acids (PUFA), conjugated linoleic acid (CLA), bioactive peptides, vitamins and antioxidants (Cavani *et al.*, 2009; Gibbs *et al.*, 2010; Ryan *et al.*, 2011). In particular, chicken breast meat is actually considered as a high quality meat because it bears most of features that previously mentioned in regard of nutritional aspects (*i.e.* low energy level, cholesterol content, and high polyunsaturated fatty acid content). Recently, it has been observed that chicken meat has not still kept the same nutritional features as in the past. Some studies showed that today's poultry meat contains higher lipid content compared with that produced some years ago (Wang *et al.*, 2009; Crawford *et al.*, 2010; Kuttappan *et al.*, 2012b).

Several studies have shown that fast growth rate increased the possibility of incidence of abnormalities or distortions mainly in the breast muscles (Dransfield and Sosnicki, 1999; Mahon, 1999). In fact, the most current poultry meat quality concerns up to now; are associated with deep pectoral muscle disease (Bianchi *et al.*, 2006; Lien *et al.*, 2012) and white striping (WS; Kuttappan *et al.*, 2012a,b), PSE-like meat (pale, soft, and exudative-like condition) (Barbut *et al.*, 2008; Petracci *et al.*, 2009; Zhu *et al.*, 2012) as well as poor cohesion (tendency to separation of muscle fiber bundles) related to immaturity of intramuscular connective tissue (Velleman *et al.*, 2003; Voutila, 2009; Petracci and Cavani, 2012a).

White striping defect is a new quality issue that was firstly pointed out by Bauermeister *et al.* (2009) and Kuttappan *et al.* (2009) and has been described as the appearance of white striation parallel to muscle fibre on the surface of pectoralis major muscles. What we know is that several factors can affect its incidence rate, which can be summarized as follows: genotype (high > standard breast yield; Petracci *et al.*, 2013b), sex (males > females; Kuttappan *et al.*, 2013c), growth rate (fast > low; Kuttappan *et al.*, 2012b, 2013c), diet (high > low energy diet; Kuttappan *et al.*, 2012b), and weight at slaughter (heavy > light; Kuttappan *et al.*, 2013c). Some studies that have been done under experimental conditions showed that the incidence rate of WS can be over 50% (Kuttappan *et al.*, 2012a, b). A recent survey has estimated that incidence of white-striped breast fillets was around 12% (Petracci *et al.*, 2013b).

It was found that WS significantly decreased the acceptance of meat for consumers and also affected in a purchase decision because it changed the appearance and exhibited marbling look (Kuttappan *et al.*, 2012c). As a result, the poultry processors are enforced to downgrade breasts showing severe WS and use them to manufacture processed products. The aetiological causes of WS are still not known, but histological evaluations showed that it is usually associated with muscle degeneration and myopathic changes beneath the striation area such as loss of cross striations, variability in fiber size, floccular/vacuolar degeneration and lysis of fibers, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells), mononuclear cell infiltration, lipidosis, and interstitial inflammation and fibrosis (Kuttappan *et al.*, 2013c).

Kuttappan *et al.* (2012a) also hypothesized a similarity in the appearance between WS and nutritional muscular dystrophy; however, they found that dietary vitamin E level was not associated with the modern condition of white striping in broiler breast meat. It was observed that the occurrence of WS increased in large male birds with high breast weight and yield (Kuttappan *et al.*, 2009). It seems that the majority of WS occurred in the finisher age period of the birds especially in the age of 6 to 8 wk (Bauermeister *et al.*, 2009). Some studies have also shown that WS can affect some quality traits of breast meat. Fillets with severe WS have been characterized by higher fat content with more monounsaturated fatty acids and lower protein content compared with normal fillets (Kuttappan *et al.*, 2012b). Otherwise, little impact of WS on technological quality traits of poultry meat was observed (Bauermeister *et al.*, 2009; Kuttappan *et al.*, 2009).

Protein composition of breast meat has a crucial impact on processing, sensory, and nutritional quality traits (Smyth *et al.*, 1999). Particularly, proteins are considered as the most important components of meat from a nutritional and processing viewpoint. Indeed, meat proteins contain all the amino acids essential to the human body, thus making them highly nutritious (Friedman, 1996). Moreover, meat proteins greatly contribute to processing abilities by imparting specific functionalities. The overall properties of meat and meat products, including appearance, texture, and mouth feel are dependent on protein functionality (Xiong, 2004).

It is well known that myofibrillar proteins (i.e., myosin and actin) are mainly responsible for the WHC and textural properties of meat and meat products, whereas sarcoplasmic proteins (i.e., muscle enzymes) play a minor role (Smith, 2010; Sun and Holley, 2011). Solubility of myofibrillar and sarcoplasmic proteins are highly correlated with water retention (Li-Chan *et al.*, 1987; Warner *et al.*, 1997). Protein solubility also has a major role in the physical properties of the meat because lower protein solubility imparts poor functionality, as in the case of pale, soft, and exudative (PSE) -like meat (Van Laack *et al.*, 2000; Bowker and Zhuang, 2013).

In general, there are different processing and compositional aspects are still not sufficiently investigated for breast meat affected by white striping abnormality. Therefore, the main objectives of this study were to evaluate the impact of white striping abnormality on the quality traits (technological and nutritional properties) of chicken breast meat. To achieve the objectives of study, different processing (such as WHC/WBC, texture, color, pH, protein solubility) and nutritional parameters (such as proximate chemical composition and protein composition) have been evaluated through this study.

3.3 Materials and methods

Chemicals and solvents, unless specified, were of analytical grade and purchased from Carlo Erba Reagenti (Rodano, Italy), Merck (Darmstadt, Germany), and Sigma-Aldrich (St. Louis, MO).

During this study, three experiments have been conducted to evaluate the impact of white striping on different quality traits of breast meat as described in (Table 3.1)

3.3.1 Sampling and storage conditions

In all experiments, whole breasts were collected from the deboning area of a commercial processing plant at 1-2 h *post-mortem*. Breast fillets were classified into three degrees of WS

depending on the intensity and thickness of white striations: normal fillet when there are no white striations; moderate when the fillets showed small thin lines (<1 mm); whereas those having thick (>1 mm) white striations covering most of the surface area were graded as severe (according to the criteria suggested by Kuttapan *et al*., 2012c). In each sample collection, breast fillets were bagged by group, packed on ice, and transported to the laboratory.

In the laboratory, the samples were stored under refrigeration conditions (2-4°C) for the next day. The *pectoralis major* muscles were excised from the whole breast muscle. The excess of visual fat, connective tissues, cartilage, and bone fragments were trimmed. After 24 h of *post-mortem*, the samples were reclassified again to three categories (normal, moderate, and severe) as previously mentioned to ensure that each sample is fit to each group because sometimes the thickness of white striation may slightly change during *post-mortem* time.

Table 3.1 The experimental design of the study

Parameters	Experiment 1	Experiment 2	Experiment 3
*Total sample number**	153	108	12
Replication number	3	3	1
Sample (n) /Group	17	12	6
Type of groups	Normal, moderate, severe white striping	Normal, moderate, severe white striping	Normal and severe white striping
Quality traits	- Raw meat: pH, color, drip loss, cook loss, and Allo- Kramer-shear force - Marinated meat: marinade uptake, purge loss, cook loss, total yield, and Allo-Kramer-shear force	Proximate composition (moisture, protein, lipid and collagen), energy distribution as well as sarcoplasmic and myofibrillar protein profile by SDS-PAGE analysis**	Chemical composition, protein solubility (sarcoplasmic, myofibrillar, and total protein solubility), protein quantity (sarcoplasmic, myofibrillar, and stromal proteins), water holding capacity, and protein profile by SDS-PAGE analysis **

* the sample is always whole *pectoralis major* muscle, but according to the analysis, a part of fillets were selected which has been described very well in each experiment.

** For SDS-PAGE analysis, 6 samples from each group were selected.

3.3.2 Quality traits analysis

Each fillet was subjected to different chemical (proximate chemical composition, protein composition, protein solubility, etc.) and physical (color, pH, drip loss, purge loss, cooking loss, marinade uptake, texture, etc.) measurements.

3.3.2.1 Color measurements

The color parameters were measured based on CIE system. CIE organization (Commission Internationale de l'Eclairage) determined standard values that are used worldwide to measure color. The values used by CIE are called L*, a* and b* and the color measurement method is called CIELAB. L* (lightness) represents the difference between light (where L*= 100) and dark (where L*= 0). a* represents the difference between green (-a*) and red (+a*), and b* represents the difference between yellow (+b*) and blue (-b*). Using this system any color corresponds to a place on the graph shown in Figure 3.1. Variables of L*, a*, b* or E* are represented as delta L*, delta a*, delta b* or delta E*, where $\Delta E^* = \Delta (\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})$. It represents the magnitude of color difference, but it does not indicate the direction of the color difference.

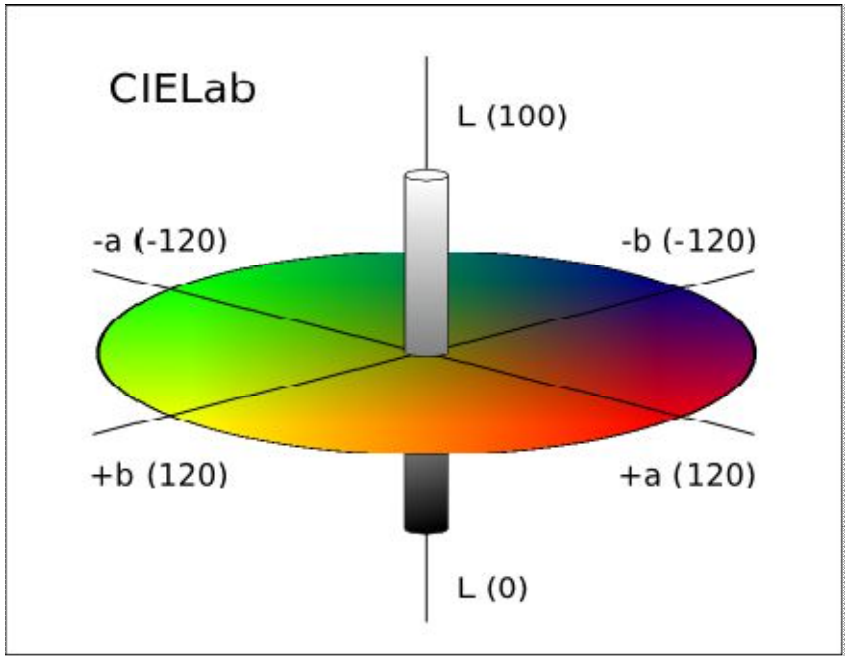


Figure 3.1 A three-dimensional representation of L, a, b CIE Lab (L*, a*, b*) system used to measure the colour parameters.

CIE 1976 (L* a* b*: CIELAB) for color measurements are based on the Opponent-Color theory that assumes that the color is perceived by human eye receptors as a pair of opposites described as the following:

• L* scale: Light *vs.* dark; where a low number (0-50) indicates dark and a high number (51-100) Indicates light.

• a* scale: Red *vs.* green; where a positive number indicates red and a negative number indicates green.

• b* scale: Yellow *vs.* blue; where a positive number indicates yellow and a negative number indicates blue.

Reflectance colorimeter (Minolta Chroma Meter CR-400) with C as illuminant source was used to carry out the measurements. The colorimeter was calibrated with a reference white ceramic tile (Y = 93.9, x = 0.3130 and y = 0.3190) before measurement. Avoiding areas with color defects, color (CIE L* = lightness, a* = redness, and b* = yellowness) was measured in triplicate on the bone-side surface of each fillet. In addition, color was measured on non-marinated and marinated samples after cooking.

3.3.2.2 Breast weight and size measurements

Geometrical measurements were determined in millimeter with a caliper as described by Mehaffey *et al.* (2006) with slight modifications (Figure 3.2). Length (L) was measured from the longest dimension of the fillet. The width (W) was measured from the longest distance from side to side in the middle of fillet. The height was measured in three points. The first height (H3) was measured as vertical distance far from the end of caudal part by 1 cm toward dorsal direction. The second height (H2) was measured at the half distance of the breast length (L). The third height (H1) was measured at the highest point in the cranial part.

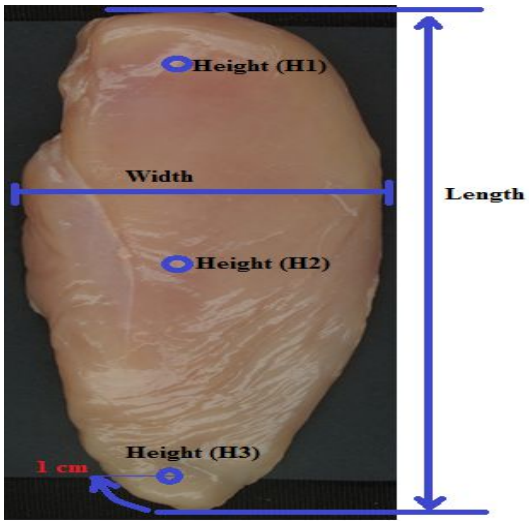


Figure 3.2 Fillet dimension measurements

3.3.2.3 pH measurements

The color, water-holding capacity, flavor, tenderness and shelf life of meat are highly affected by pH. Therefore, pH was measured to understand its effect on those quality traits. It was

determined according to the Jeacocke (1977) procedure of iodoacetate method which was adapted and utilized and used it for measuring the pH for raw, marinated and cooked chicken breast meat (Figure 3.3). From each sample about 2.5 g of meat was collected from the top of the cranial part (Figure 3.3), minced manually, and homogenized with ultra-turrax for 30 s at speed 10000 rpm in 25 mL of iodoacetate (5 mM) and potassium chloride (150 mM) solution. The pH of meat suspension was measured by pH-meter calibrated at pH 4.0 and 7.0.

3.3.2.4 Samples preparation for processing

To evaluate the impact of white striping abnormality on meat quality traits during processing, two parallelepiped cuts as described in the Figure 3.3 were dedicated for this purpose:

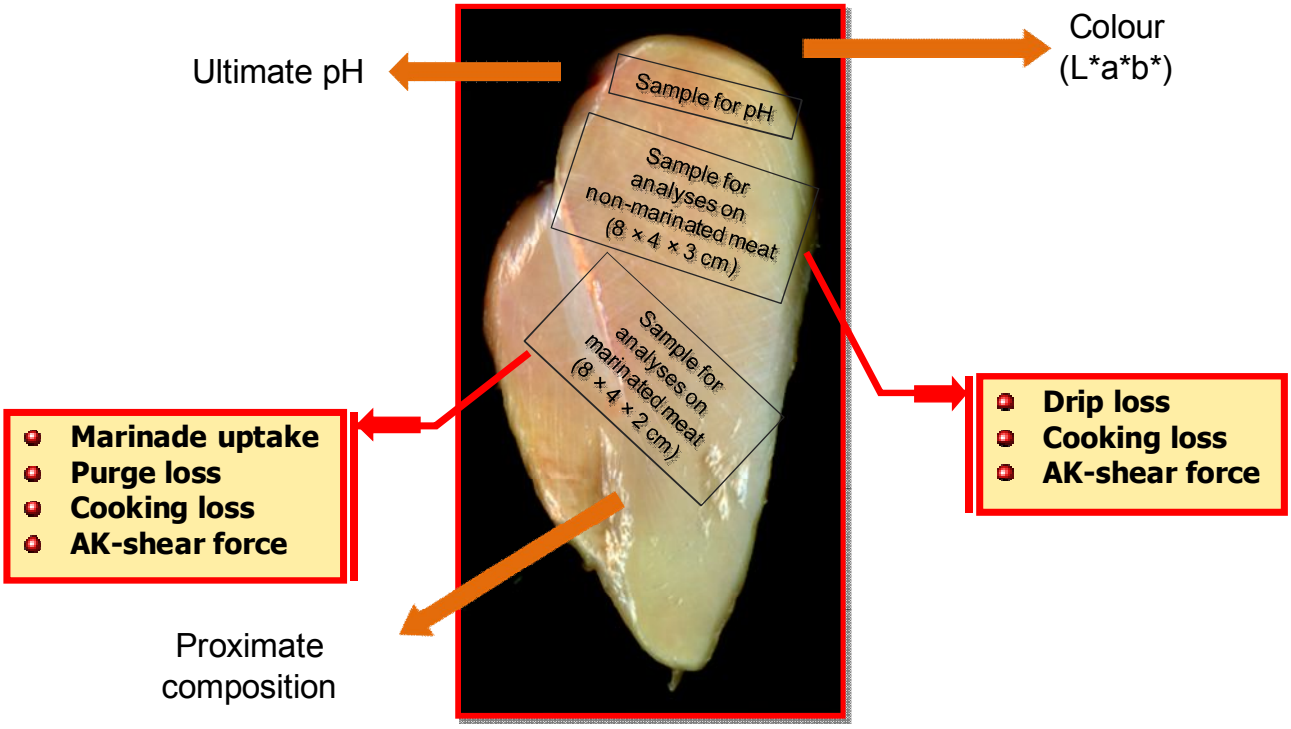


Figure 3.3 The sampling protocol for evaluating the quality traits of marinated and non-marinated meat.

3.3.2.4.1 Non-marinated meat cuts

A parallelepiped cut (8 × 4 × 3 cm) weighing about 80 g was excised from the cranial part of each fillet parallel to muscle fiber directions and used to assess non-marinated quality traits (drip loss, cooking loss, and Warner-Bratzler shear force) (Figure 3.3).

a) Drip loss

Parallelepiped cuts, which were dedicated to non-marinating cooking, were used to determine the drip loss. The cuts were stored in covered plastic boxes over sieved plastic racks for 48 h at 2 to 4°C. After 48 hours, cuts were gently cleaned from the excess of superficial juices with paper towel, weighted again, and drip loss determined as percentage of weight lost by the sample during refrigerated storage period (Petracci and Baeza, 2011) according to the following formula below:

Drip Loss (%) = (initial weight - weight after storage) / (initial weight) × 100

b) Cooking loss

After finishing the measurement of drip loss, meat cuts were packaged under vacuum (-99% ambient pressure for 4s). The biggest sample in the weight was selected to measure the temperature during cooking (in general the samples were homogenous in the weight, about 80 ± 3 g). The meat cuts were cooked in water bath adjusted at 80°C until the core temperature reached 78°C (estimated time 35 min). Subsequently, cooked samples were cooled to room temperature using a cold water bath. The packages were removed, and the meat cuts were dried from superficial liquids with a paper towel and re-weighed again. The cooking loss was calculated based on the difference in weight loss before and after cooking as follows:

$$Cooking\ loss\ (\%) = \left(\frac{Weight\ of\ sample\ after\ storage - weight\ of\ sample\ after\ cooking}{Weight\ of\ sample\ after\ storage} \right) \times 100$$

c) Allo-Kramer shear force for cooked meat cuts

After 3-4 h of refrigerated storage (2-4°C) of cooked meat cut (8 × 4 × 3 cm) to optimize sample preparation during cutting, parallelepiped cut (approximately 4 × 2 × 1 cm) was excised parallel to muscle fibre direction to measure the shear force needed to cut the sample by Allo-Karmer method. The weights of the samples were recorded in gram in order to express the final results. Samples were stored for 3-4 hour in the refrigerator to have similar temperature for all samples before shearing.

Allo-Kramer shear force was evaluated by a TA.HDi Heavy Duty texture Analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) provided with an Allo-Kramer shear cell with 10 blades, using the procedure described by Sams *et al.* (1990). Samples were perpendicularly

sheared to fibre direction with 10 blades probe using a 25 kg load cell at 500 mm/min rate (Figure 3.4). Shear force values were expressed as kilograms force per gram of sample.

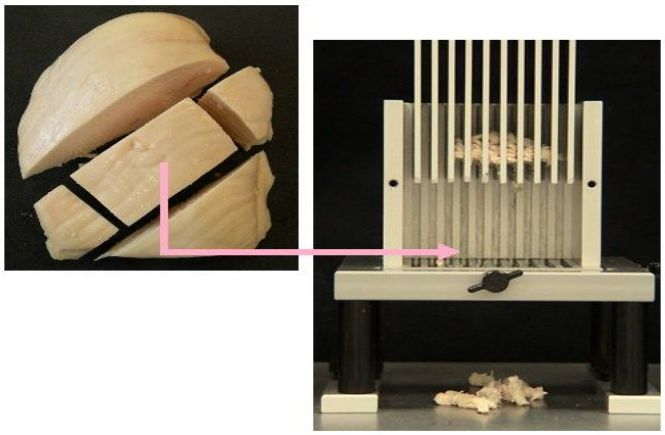


Figure 3.4 Allo-Karmer shear force measurements

3.3.2.4.2 Marinated meat cuts

A parallelepiped cut (8 × 4 × 2 cm) weighing about 60 g was excised from the caudal part of each fillet parallel to muscle fiber directions and used to assess marinated quality traits (marinade uptake, purge loss, cooking loss, yield and Allo-Kramer shear force) (Figure 3.3). Meat Cuts were subjected to tumbling and marination procedure, were individually labeled and marinated by addition of 150 ml marinade solution (7.6% sodium chloride and 2.3% sodium tripolyphosphate) per 1 kg of meat using a small-scale vacuum tumbler (model MGH-20, Vakona Qualitat, Lienen, Germany) that were employed to achieve a standard concentration of salts (1.0 and 0.30%, respectively) in the final marinated meat. Tumbling time was 46 min under vacuum (-0.95 bar) and the total number of revolutions was 800 rounds at a speed of 20 RPM (3 working cycles of 13 min/cycle and two pause cycles of 3 min/cycle).

a) Marinade uptake

Marinade uptake measures the quantity of marinade solution that is able to be absorbed by the meat during the marinating process. After marination, the excess of superficial liquids was removed by paper towel. The marinade uptake was calculated considering the weight difference before and after marination as follow:

$$Marinade\ \ uptake\ (\%) = \left(\frac{marinated\ sample\ weight - raw\ meat\ weight}{raw\ sample\ weight} \right) \times 100$$

b) Purge loss

Purge loss is usually used to evaluate the ability of marinated meat to retain liquids during refrigerated storage conditions. The marinated meat cuts were placed over sieved plastic rack inside covered plastic box and kept for 24 h at 2-3°C to facilitate the diffusion and the balance of the absorbed marinade in the muscle of the sample. After this, samples were weighed again and the percentage of purge loss was calculated as below:

$$Purge\ loss\ (\%) = \left(\frac{marinated\ sample\ weight - sample\ weight\ after\ storage}{marinated\ sample\ weight} \right) \times 100$$

c) Cooking loss and yield

After measuring the purge loss, all meat cuts were packaged in polyethylene packs under vacuum. The samples were cooked in thermo stated (80°C) water bath until the core temperature reached 78°C and the cooking time was about 25 min. Subsequently, cooked samples were cooled to room temperature using a cold water bath and weighed again. Cooking loss and total yield were determined as follows:

$$Cooking\ loss\ (\%) = \left(\frac{Weight\ of\ sample\ after\ storage - weight\ of\ sample\ after\ cooking}{Weight\ of\ sample\ after\ storage} \right) \times 100$$

$$Total\ yield\ (\%) = \left(\frac{Weight\ of\ sample\ after\ cooking}{Weight\ of\ sample\ before\ tumbling} \right) \times 100$$

3.3.2.5 Proximate chemical composition

The remaining part of the fillet after all samples have been excised was minced to obtain homogenous meat mass and used to determine the proximate chemical composition.

3.3.2.5.1 Moisture

The moisture analysis was performed on raw meat according to official methods of AOAC (1990). About 5 g of finely chopped meat was accurately weighted in previously dried and weighted aluminium pan (pans were heated for 1 h at 103°C and cooled in desiccators to obtain stable weight). Samples were dried in conventional oven for 16 hours at 105°C then cooled in desiccators. The dried weights of the samples were weighted to determine the moisture content as a following:

Moisture (%) = (initial weight – dry weight) / (initial weight) × 100

3.3.2.5.2 Total crude fat content

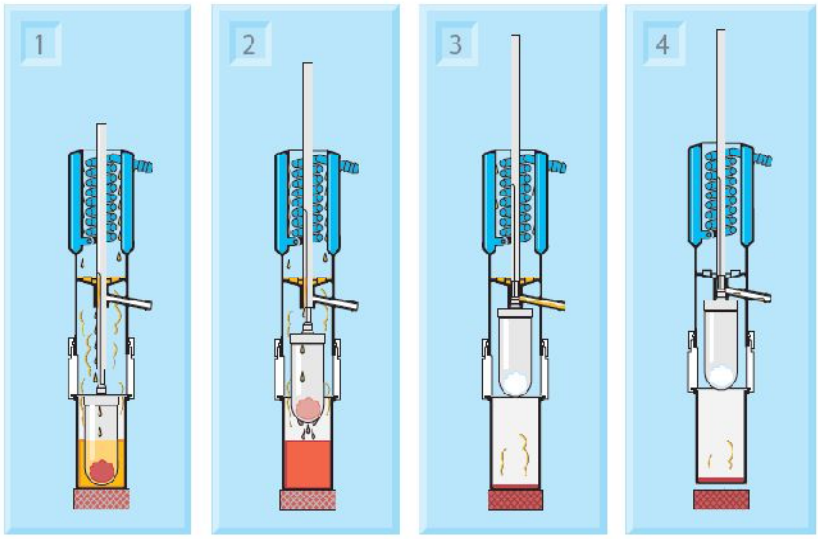
Fat is important to all aspects of meat production and processing. Fresh and frozen meat prepared for manufacturing purposes is specified in terms of fat content (expressed as chemical lean). It can be considered as a part from the commercial importance of the fat content of unprocessed meat, especially manufacturing meat. Fat content is an important technical and regulatory specification for almost all processed meat products. The "Soxhlet" method was used to determine fat content in this study. It is recognized by the Association of Official Analytical Chemists (AOAC) as the standard method for crude fat analysis.

Crude fat content is determined by extracting the fat from the sample using a solvent, then determining the weight of the fat recovered. The sample is contained in a porous thimble that allows the solvent to completely cover the sample. The thimble is used to hold the sample during the extraction and it works as strainer that allows recycling the solvent over the sample continuously. This extends the contact time between the solvent and the sample which ensure enough time to dissolve all of the fat contained in the sample. It is necessary to comminute finely the sample as much as possible in order to optimize solvent extraction process. Before and at the beginning of the solvent extraction step, the sample must be usually dried. Frequently, the moisture analysis is required as well as fat analysis and this can be achieved by accurately weighting the sample after drying and before extraction, as well as before drying. If the moisture analysis is not required, the sample has to be weighed before drying and after solvent extraction. In either case, the sample must be weighed accurately on an analytical balance at each stage of the analysis. The procedure of fat analysis is described as follows:

- All aluminum cups that were used in the analysis have been rinsed by petroleum ether, drained, dried in an oven at 102ºC for 60 min., cooled in desiccators, and accurately weighted.
- About 1.5 of finely minced meat samples were accurately weighted and put in the bottom extraction thimble then covered with fat free cotton.
- The thimble with the sample was inserted in a Soxhlet liquid/solid extractor and 50 ml of petroleum ether was added to previously weighted aluminum cup, the thimble was

lowered inside the extraction solution in the cup where the sample was completely covered with petroleum ether (Figure 3.5).

- The assembled extraction unit was fixed over electric heating block adjusted at 95ºC. The extraction at this stage has been conducted by recirculating the evaporated extraction solvent through the condenser for 45 min (Figure 3.5: Boiling phase 1).
- The thimble was raised up to be separated from the extraction solution in the cup, and in this stage, the extraction was preceded by dripping the evaporated extraction solvent that comes from the condenser over the sample for 45 min (Figure 3.5: Rinsing phase 2).



1. Boiling: rapid solubilization in boiling solvent
2. Rinsing: efficient removal of remaining soluble matter
3. Recovery: automatic collection of distilled solvent for re-use
4. Auto-shut down: the system closes down and the cups are lifted from the hot plate

Figure 3.5 Different phases of fat extraction

- The recirculating of the evaporated extraction solvent was ceased, and the content of the cup was dried for 15 min over a heating block in order to remove the major part of extraction solvent from fat (Figure 3.5: Recovery phase 3). The remaining of extraction solvent was collected from the condenser.
- The aluminum cup containing the extracted fat was dried at 85ºC for one hour in order to remove any residue of the extraction solvent, then cooled inside the desiccators (Figure 3.5: Auto-shut down 4).

Then the content of the cup was weighted and the weight of fat was determined as the difference in the weight of the cup and fat subtracted by weight of empty cups. The percentage fat content was calculated as follows:

$$Crude\ fat\ \% = \frac{Weight\ of\ fat}{Weight\ of\ sample} \times 100$$

3.3.2.5.3 Total crude protein content

Protein content was determined according to the procedure reported by Kjeldahl method (AOAC, 1990). In Kjeldahl method, the basis of protein determination is based on the total nitrogen content (including protein, amine, and ammonia and urea nitrogen fractions) that is usually obtained through three different phases: digestion of the sample, distillation and titration of liberated ammonia. In order to calculate the percentage of protein, nitrogen content is converted to protein content by multiplying with specific coefficient that considers the amino acid composition of the sample.

In the first phase, which is the digestion, about 0.5 g of finely minced meat sample was accurately weighted and put in the Kjeldahl tube. Half tablet of Kjeltabs (containing 3.5 g of K_2SO_4 and 3.5 mg of selenium) and 12 mL of solution containing 96 % sulfuric acid and 4 % ortho-phosphoric were added to the tube containing the sample. The contents of the tube were digested at 400/420°C for 3 h. During the digestion, temperature was gradually increased (50°C/15 min) from 80 to 420°C. The digestion was finished when the contents of tube were completely changed to clear colour. At the end, ammonium sulphate is generated as described in the following equation:

$$Organic\ N + H_2SO_4 \xrightarrow{Heat} (NH_4)_2SO_4 + H_2O + CO_2 + Other\ sample\ matrix\ by-products$$

Both the distillation and titration phases were carried out using a "Büchi 339" distil-titrator unit (Figure 3.6). Digested samples were cooled at room temperature and then moved to the distillation unit. Distillation stage was performed by adding 50 mL of distilled water and 65 mL of sodium hydroxide (solution with 30% of NaOH) in order to neutralize the effect of the acid solution used in the mineralization phase. During neutralization, ammonia starts to liberate in the form of gas, then it condensates in the distillation unit and captured by boric acid in the form of ammonium borate:

$$(NH_4)_2SO_4 + 2\,NaOH \xrightarrow{Heat} \overbrace{2\,NH_3}^{Ammonia\ gas} \uparrow + Na_2SO_4 + 2\,H_2O$$

$$\overbrace{2\,NH_3}^{\text{Ammonia gas}} \uparrow + \overbrace{2\,H_2BO_3}^{Boric\ acid} \longrightarrow \overbrace{NH_4 : H_2BO_3}^{Ammonium\ -borate\ complex} + \overbrace{H_2BO_3}^{Excess\ boric\ acid}$$

Color change

Titration of distilled ammonia was performed using 0.2 N hydrochloric acid (HCL). Protein content was obtained multiplying the concentration of nitrogen by the conversion factor of 6.25 (calculated for meat and meat product) and expressed as a percentage (Equation 3.1+3.2).

$$\overbrace{NH_4 : H_2BO_3}^{Ammonium\ -borate\ complex} + \overbrace{HCL}^{Hydrochloric\ acid} \longrightarrow \overbrace{(NH_4)CL}^{Ammonium\ chloride} + \overbrace{H_3BO_3}^{Boric\ acid}$$

$$Nitrogen\% = \frac{(ml\ of\ acid\ for\ sample - ml\ of\ acid\ for\ blank) \times N\ of\ acid \times 1.4007}{Weight of\ sample in\ gram} \quad \text{(Equation 3.1)}$$

$$\text{Protein}\ \% = Nitrogen\% \times Conversion\ factor \quad \text{(Equation 3.2)}$$

Figure 3.6 Büchi 339 units used to distillate and titrate the sample for the total protein determination (Kjeldal).

3.3.2.5.4 Total ash content

The ash content is a measure of the total amount of minerals present within a food. Ash is the inorganic residue remaining after the water and organic matter is removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components.

There are three main types of analytical procedure used to determine the ash content of foods is based on this principle: dry ashing, wet ashing and low temperature plasma dry ashing. The chosen method for a particular analysis depends on the reason for carrying out the analysis, the

type of food analyzed and the equipment available. Ashing may also be used as the first step in preparing samples for analysis of specific minerals, by atomic spectroscopy or the various traditional methods described below. Ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%, e.g., dried beef.

Dry ashing procedure:

This method was chosen to analyze ash content in this study. Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600^{o}C. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost, *e.g.,* iron, lead and mercury. If an analysis is being carried out to determine the concentration of one of these substances, then it is advisable to use an alternative ashing method that uses lower temperatures.

Ashing sample dish was heated in a muffle furnace at 525^{o}C for one hour and cooled in desiccators. The ashing dish was accurately weighted; About 5 g of finely minced meat was accurately weighted in the ashing dish. The sample was dried in an air oven at 102^{o}C for two hours. The sample was charred in a muffle furnace at 200^{o}C, and then followed by charring step at 525^{o}C for 4 hrs, after the samples have been cooled to 200^{o}C, moved to desiccators for cooling at room temperature. The food sample was weighed before and after ashing to determine the concentration of ash present.

The ash content can be expressed on a wet basis:

$$Ash\ (wet\ basis)\ \% = \frac{M\ ash}{M\ wet} \times 100$$

Where M_{ASH} refers to the mass of the ashed sample and M_{wet} refer to the original mass of the wet samples.

3.3.2.5.5 Total collagen content

The spectrophotometric-based hydroxyproline assay is one of the few assays that allows for the actual quantification of collagen content. The assay was first described in 1950 (Bateman *et al.*, 1996), based on the fact that all collagens contain globular domains and share the common structural of triple helical segments. This triple helical structure is composed of three α-(polypeptide) chains, which range from 10 to 150 kDa per chain. Each chain consists of a

repeating triplet amino acid sequence (Gly-X-Y) n, where X and Y can be any amino acid, but are often proline and hydroxyproline, respectively (Bateman *et al.*, 1996). Collagen is one of the few proteins that containing hydroxyproline amino acid. Thus, based on the absolute quantification of hydroxyproline (which represents a fixed percentage of amino acid composition of collagen in most meat tissue, the amount of collagen content and concentration in tissues can also be derived (Jackson and Cleary, 1967; Gallop and Paz, 1975).

Collagen content was evaluated using the modified spectrophotometric-based hydroxyproline assay (Kolar, 1990). About 4 g of finely minced meat was accurately weighted in 250 ml round bottom flask. 30 ml of sulphuric acid solution (A) was slowly added to the sample; the neck of the round bottom flask was covered with an evaporating glass dish and put it in an air oven at 105^{o}C for 16 hr for collagen digestion. The content of each flask was transferred while they were hot into 500 ml volumetric flask and then the flask was filled with distilled water to the mark. The content of the flask was agitated very well to ensure the homogeneity of the solution.

About 50-70 ml of the sample has been used for filtration. Precisely, 5 ml of the filtrate was transferred into 100 ml volumetric flask and then the filtrate was diluted with distilled water to 100 ml and the content was agitated again. After, 2 ml of the diluted solution was transferred into a test tube, then 1 ml of oxidizing agent (B) was added and the content of the tube was mixed by vortex for 1 min. To develop the reaction, the tubes were left in the dark for 20 min. Finally, 1 ml of colorimetric solution (C) was added to the tube, mixed by vortex for a few seconds and then the tubes were left to complete the colour development in water bath at 60^{o}C for 15 min.

The samples were filled in cuvettes and the absorbance of the solution was measured at 558 nm. Blanks were prepared from all reagents that were used in the analysis except the sample and then the absorbance was measured. The absorbance that has been obtained from blank was subtracted from the sample.

To find the real concentration of collagen in samples, a standard curve (Figure 3.7) was prepared between known concentration of hydroxyproline and the absorbance.

Finally the absorbance was read with UV-visible spectrophotometer (Shimadzu model UV-1601, Shimadzu Italia S.r.l., Milano, Italy) at 558 nm of wavelength (λ). The hydroxyproline content (HYP) was calculated with the following formula:

$$HYP\ (\%) = (h \times 2.5) / (m \times V)$$

Where h= HYP in µg/mL of filtrate, derived from calibration curve, m= sample weight in grams, and V= mL of filtrate for the 100 mL dilution.

The percentage of collagen was calculated by multiplying the percentage of hydroxyproline by 8.

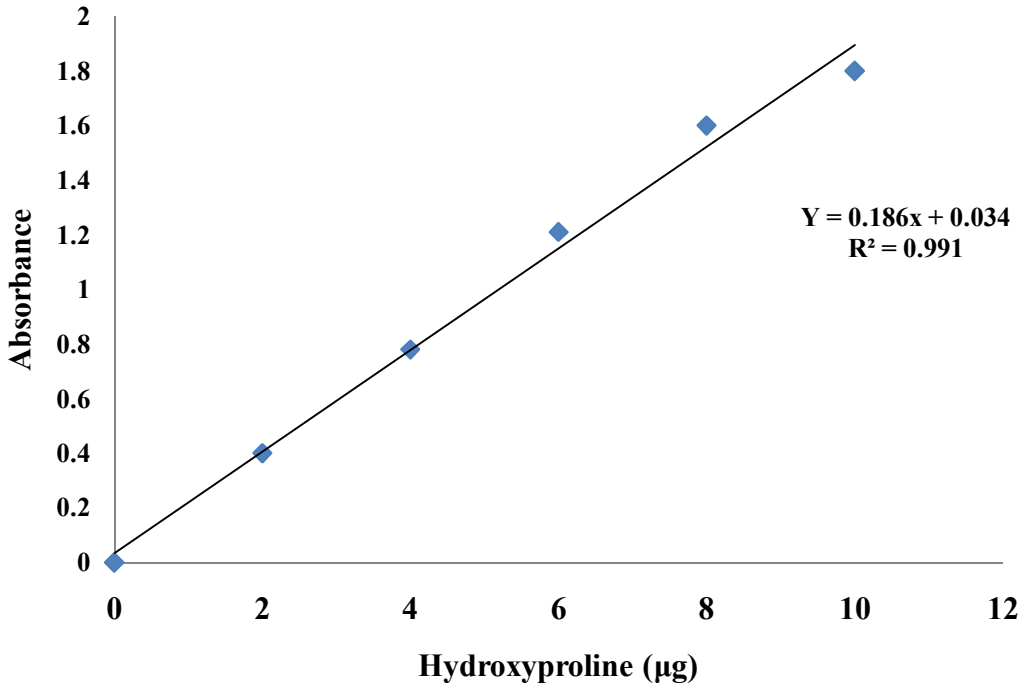


Figure 3.7 standard curves for determination of collagen content by hydroxyproline.

Sulphuric acid solution (A): 750 ml D.W was added in volumetric flask 2 L then 575 ml of concentration sulphuric acid was slowly added to flask content. The solution was left to get cool, after that the volume was completed to the mark.

Buffer solution (pH=6): 30 g of citric acid monohydrate, 15 g sodium hydroxide, 90 g sodium acetate.3H2O were weighted and added into 1 L V.F then 500 ml D.W was added to dissolve them, slowly 290 ml 1-propanol was slowly added, transferred the content to 1 L beaker and adjust the pH to 6 by acid or base while stirring, return back the content to V.F and then complete the volume, keep in refrigerator (2 months at 4°C).

Oxidizing solution (B): 1.4 g of chloroamine T was accurately weighted and put it 100 ml V.F dissolve it by buffer solution (stable for one week in refrigerator)

Colorimetric reagent (C): 10 g of 4-dimethylaminobenzyldhede was accurately weighted, 35 ml of perchloric acid 60% was slowly added while stirring until the content dissolved, then added slowly while stirring 65 ml of 2-propanol (1 day in refrigerator stable).

3.3.2.6 Nutritional index calculation

The Atwater general factor system was used to estimate the total caloric content. This system is based on the total energy of combustion for protein, fat, and carbohydrates after they are corrected by losses in digestion, absorption and urinary excretion of urea. Irrespective of the food, it utilises a single factor for each of the energy-yielding substrates (protein, fat, carbohydrate) where the energy values are 17 kJ/g (4.0 kcal/g) for protein, 37 kJ/g (9.0 kcal/g) for fat and 17 kJ/g (4.0 kcal/g) for carbohydrates.

Moreover, collagen/total protein ratio was calculated as follows (European Commission, 2005):

$$\left(\frac{\text{Collagen}}{\text{Total protein}}\right)\times 100$$

Finally, fat to protein ratio was determined as the following:

$$\left(\frac{\text{Total fat content}}{\text{Total protein content}}\right)\times 100$$

3.3.2.7 Total content of myofibrillar and sarcoplasmic proteins

To determine the total content of myofibrillar and sarcoplasmic proteins; firstly, both fractions were extracted as described in section "3.3.3.10.1 Protein extraction". The extracted proteins were quantified by the Bradford method. The Bradford assay is a protein determination method that involves the binding of Coomassie Brilliant Blue G-250 dye to proteins (Bradford, 1976). The dye exists in three forms: cationic (red), neutral (green), and anionic (blue) (Compton and Jones, 1985). Under acidic conditions, the dye is predominantly in the doubly protonated red cationic form (A_{max}= 470 nm). However, when the dye binds to protein, it is converted to a stable unprotonated blue form (A_{max} = 595 nm) (Fazekas *et al.,* 1963; Reisner *et al.,* 1975; Sedmack and Grossberg, 1977). The blue protein-dye form is usually detected at 595 nm in the assay using a spectrophotometer or microplate.

In any protein assay, the ideal protein to use as a standard is a purified preparation of the protein being assayed. In the absence of such an absolute reference protein, another protein must be selected as a relative standard. The best relative standard to use is one that gives a colour yield

similar to that of the protein being assayed. Selecting such standard protein is generally done empirically. Alternatively, if only relative protein values are desired, any purified protein may be selected as a standard. The two most common protein standards used for protein assays are BSA and gamma-globulin.

In this study, the Quick Start Bradford protein assay was used, where dye color development is significantly greater with BSA than with most other proteins, including gamma-globulin. Therefore, the BSA standard would be an appropriate standard if the sample contains primarily albumin, or if the protein being assayed gives a similar response to the dye. For a color response that is typical of many proteins, the gamma-globulin standard is appropriate.

The standard protocol can be performed in three different formats: 1 and 5 ml cuvette assay, and 250 µl microplate assay. The linear range of these assays for BSA is 125-1,000 µg/ml, whereas with gamma globulin is linear the range 125-1,500 µg/ml.

Procedure: the 1x dye reagent was removed from 4°C storage and left out to warm to ambient temperature. The 1x dye reagent was inverted a few times before use. Duplicate samples of protein solutions were assayed. For convenience, the BSA or gamma-globulin standard sets can be used, but blank samples (0 µg/ml) should be made using water and dye reagent. Each standard and unknown sample solution was pipette into separate clean test tube. The dye reagent was added to each tube and vortex (or invert). The tubes were incubated at room temperature for at least 5 min. Samples should not be incubated longer than 1hr at room temperature. The spectrophotometer was set at 595 nm. The instrument has been set to zero with the blank sample. The absorbance of the standards and unknown samples were measured.

A standard curve was created by plotting the absorbance of each protein concentration at 595 nm (y-axis) versus their concentration in µg/ml (x-axis). Seven different concentration points (2, 1.5, 1, 0.75, 0.5, 0.25, 0.125 mg/ml) of Bovine Serum Albumin Standard have been selected to prepare the standard curve with good linearity. The unknown sample concentration was determined by using the standard curve (Figure 3.8). Some time the samples were diluted, the final concentration of the unknown samples was adjusted by multiplying by the dilution factor used.

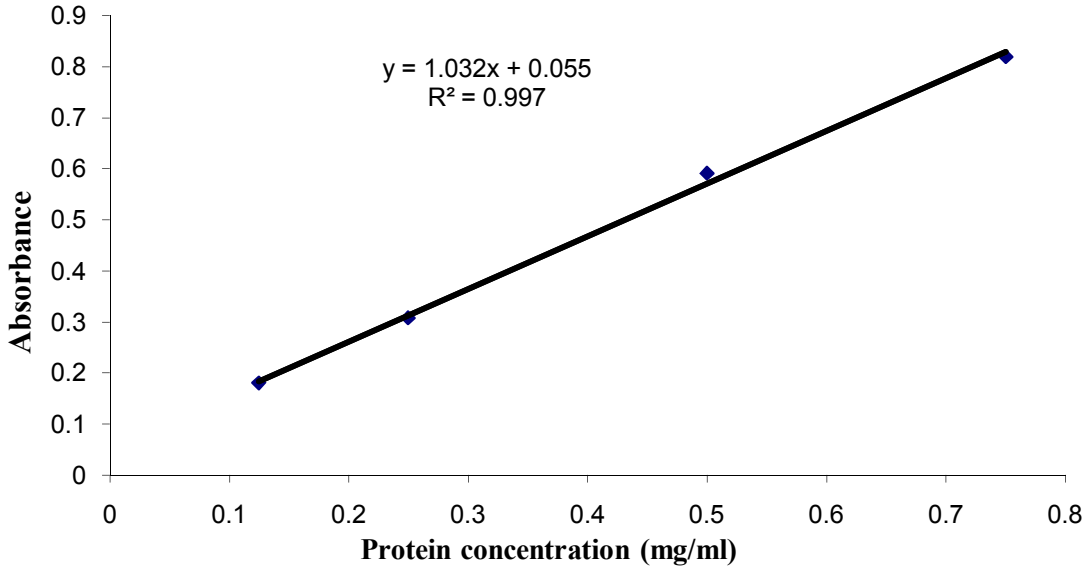


Figure 3.8 Standard curve prepared from known concentrations of BSA versus absorbance.

The absorbance of the blue protein-dye complex was detected at 595 nm using a spectrophotometer UV-1601 from Shimadzu (Germany). The concentration was calculated using a calibration curve obtained with quick start BSA standard set from Bio-Rad (Italy) (Figure 3.8).

3.3.2.8 Protein Solubility

Protein solubility was estimated according to differences in extractability of proteins in different ionic strength solutions (Warner *et al.*, 1997). Sarcoplasmic protein solubility was measured in 3 replications by weighing 1 g of breast meat sample. Ten milliliters of cold 25 m*M* potassium phosphate buffer (pH 7.2) were added to each sample and homogenized by high-speed blender (Ultra-Turrax, T25 basic, New Brunswick, NJ) on the lowest speed (11,000 rpm/min). The homogenized samples were kept under refrigeration conditions (4°C) for 20 h and then centrifuged at 2,600 × *g* for 30 min at 2-3°C.

The supernatant was decanted and protein concentration was measured using the Bradford assay (Bradford, 1976) with bovine serum albumin as a standard as described in section (3.3.2.7). Total protein solubility was similarly determined in a 1.1 M KI, 0.1 M potassium phosphate (pH 7.2) buffer. Myofibrillar protein solubility was calculated by the difference in the solubility of total and sarcoplasmic proteins.

3.3.2.9 Water Holding Capacity (WHC)

Cooking loss was used as a measure for WHC. About 6 g of minced breast meat was weighed into a 50-mL plastic test tube. After the addition of 10 mL 3.5% NaCl solution, the tubes were vigorously shaken for 15 s and then held for 30 min at room temperature. The supernatant was separated after centrifugation for 15 min at 3,000 × g (temperature 2-3°C), and the sediment was weighted (Van Laack *et al.*, 2000). After cooking the tube content at 80°C for 20 min, cooking loss was determined by weight difference.

3.3.2.10 SDS-PAGE analysis

3.3.2.10.1 Protein extraction

Normal and WS fillets were selected to separate the extracted proteins according to their molecular weights by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis. The analysis was repeated twice for each sample. Minced meat sample (2 g) was added to 20 ml of rigor buffer (RB) containing 75 mM KCl, 10 mM KH2PO4, 2 mM MgCl2, and 2 mM EGTA (Ethylene Glycol Tetraacetic Acid) (pH 7.0) and homogenized with a high-speed blender (Ultra-Turrax, T 25 basic) on the lowest speed (11,000 rpm/min). The homogenate was centrifuged at 10,000 × *g*, keeping the temperature at 4°C for 10 min, and the supernatant (S1) decanted and saved. Twenty ml of fresh RB was added to the sediment and the homogenization repeated. A sample (0.5 mL) of this homogenate (P1) was saved and the centrifugation repeated. This process was repeated to obtain S1 up to S4 and P1 up to P4 (Fritz *et al.*, 1989). The S1 was dedicated to sarcoplasmic protein evaluation, whereas P4 was used for myofibrillar protein evaluation. Moreover, a composite sample from S1 to S4 was used for sarcoplasmic fractions.

3.3.2.10.2 Protein separation and quantification by SDS-PAGE analysis

Samples were mixed 1:1 with a standard sample buffer that contained 8 M urea, 2 M thiourea, 3% (wt/vol) SDS, 75 mM dl-dithiothreitol, and 25 mM TrisHCl at pH 6.8 (Fritz *et al.*, 1989), then it was heated at 100°C for 5 min in a water bath, cooled, and applied to the gel. The concentration of extracted protein was measured using the Bradford assay (Bradford, 1976) before loading to gel. Fifteen microliters of myofibrillar protein extract was loaded at 12% Mini-Protean TGX Stain-Free Gel (Bio-Rad), and the same amount of sarcoplasmic extract was loaded

at Mini-Protean TGX any kDa Stain- Free (Bio-Rad). The separated protein bands were identified by comparing their mobilities against those of molecular weight of protein marker (Precision plus Standard protein, all blue prestained, Bio-Rad) made of 10 purified proteins with different molecular weights (10, 15, 20, 25, 37, 50, 75, 100, 150, and 250 kDa). The point to point (semi-log) regression method was used to calculate the molecular weights. The reservoir buffer used in the Mini-protean II cell small electrophoresis unit (Bio-Rad) contained 50 mM Tris, 0.384 M glycine, and 0.1% (wt/vol) SDS (Figure 3.9). Small gels were run at a constant voltage of 80 and 120 V for stacking and running gel, respectively.

Sarcoplasmic and myofibrillar protein gel images were captured by a ChemiDoc MP (Bio-Rad) tabletop scanner with Image Lab Rev 4.0 software (Segrate (MI), Italy) (Figure 3.9). During the acquisition of images, Stain Free Gel application was used with 5-6 s (Auto-Intense Bands) as exposure time. Gels were activated by UV Trans illumination and subsequently managed by Image Lab Rev 4.0 software on a tabletop computer to determine protein concentration. Calibration curve was prepared by standard bovine serum proteins (BSA: 0.1, 0.25, 0.5, 0.75, 1.00, and 1.5 µg/L). Image area was X: 95.0, Y: 71.0 (mm) with a pixel size (µm) X: 68.2, Y: 68.2.

Figure 3.9 SDS-PAGE analysis system from Bio-Rad (source: Electrophoresis guide, Bio-Rad)

One dimensional SDS-PAGE analysis was used to evaluate the molecular weight profile of sarcoplasmic and myofibrillar proteins. The concentration of each band was expressed in 2 ways

as absolute (mg/g of meat) and relative abundance (%). The latter was calculated based on the sum of protein concentration in all bands within the same lane to avoid the small differences due to protein loading among lanes. In each band, the dominant protein was determined based on molecular weight and relative abundance. Electrophoretic protein bands were assigned by comparison with data reported in literature where gel purified sarcoplasmic and myofibrillar proteins were identified by mass spectrometry (Huang *et al.*, 2011; Zapata *et al.*, 2012).

3.3.2.11 Endothermic transitions by Differential Scanning Calorimetry (DSC)

A TA Instruments, differential scanning calorimeter (DSC), model SDT Q600, with computer-assisted data acquisition and curve sensitivity analysis function, was used for all thermal analysis. Changes in the structure of the protein during heating are much clear if use the 2nd order derivative of the heat flow. Small pieces of meat, free from visible traces of fat and connective tissue, were selected for analysis. At least 3 samples with 20-30 mg meat of each sample were accurately weighed in an aluminum pan to 0.001 mg by an electronic balance was used for each individual sample. The samples were scanned at 10°C/min from 20 to 100°C under dry nitrogen purge of 20 mL/min (Hanne *et al.*, 2006).

3.4 Statistical analysis

The differences in quality traits between normal, moderate, and severe white striping fillets were evaluated by ANOVA. The model tested the main effects of WS degrees (normal, moderate, or severe) and replication, as well as the interaction term using the general linear model (GLM) (SAS, 1988) on meat quality traits. Means were separated using Tukey's honestly significant difference multiple range test with $P \leq 0.05$ considered as significant.

3.5 Results and discussion

3.5.1 Experiment 1

The results of quality trait analysis, such as pH, color, drip loss, cook loss, and Allo-Kramer-shear of raw breast meat are presented in Table 3.2. The pH of severe white striped breast fillets was significantly higher than in normal and moderate groups (5.95 *vs.* 5.86 and 5.88; $P \leq 0.05$). There were no differences in the L* of meat among different degrees of WS, but moderate and severe samples showed a significant increase in a* and b*.

In general, severe white striped fillets had significantly higher redness and yellowness values (1.77 *vs.* 1.21 and 3.16 *vs.* 2.37, P<0.001) than normal one, respectively. No differences were observed in drip loss, while cooking loss increased as the degree of WS increased from normal to severe groups (21.27 *vs.* 23.20 *vs.* 26.74%; $P \leq 0.05$). For the texture of non-marinated meat, severe WS resulted in significantly lower shear values compared with moderate and normal groups, which did not differ from each other (3.69 *vs.* 4.41 and 4.91 kg/g; $P \leq 0.05$).

The results for marinade uptake and quality traits of marinated breast meat with different degrees of WS are presented in Table 3.3. All these parameters were significantly affected due to the presence of white striping abnormality. There was a decrease in the marinade uptake as the degree of striping increased from normal to severe (12.67 *vs.* 10.97 *vs.* 7.92%; $P \leq 0.05$). Moreover, severe group had a higher purge loss, cooking loss, and lower total yield compared to moderate and normal groups, which did not differ from each other. Finally, severe white striped fillets had significantly higher Allo-Kramer-shear force values (2.09 and 1.89 *vs.* 1.83 kg/g, P<0.001) than moderate and normal fillets.

Table 3.2 Effect of white striping degree (normal, moderate and severe) on pH, color (L*, a*, b*), drip loss, cooking loss and AK-shear force of broiler raw breast meat (means ± SE).

	Level of white striping[1]			Probability
	Normal	Moderate	Severe	
n.	51	51	51	
pH	5.86 ± 0.01[b]	5.88 ± 0.01[b]	5.95 ± 0.01[a]	0.000
Lightness (L*)	53.81 ± 0.32	53.80 ± 0.38	53.42 ± 0.38	0.644
Redness (a*)	1.21 ± 0.08[b]	1.50 ± 0.10[a]	1.77 ± 0.10[a]	0.000
Yellowness (b*)	2.37 ± 0.26[b]	2.61 ± 0.27[ab]	3.16[a] ± 0.31	0.003
Drip loss (%)	1.04 ± 0.03	1.08 ± 0.04	1.06 ± 0.04	0.827
Cook loss (%)	21.27 ± 0.25[c]	23.20 ± 0.37[b]	26.74 ± 0.48[a]	0.000
AK-shear force (kg/g)	4.91 ± 0.22[a]	4.41 ± 0.12[a]	3.69 ± 0.13[b]	0.000

[1] the occurrence of white striping was classified into normal, moderate and severe according to Kuttappan *et al.* (2012b)

[a-c] Means within a row followed by different superscript letters differ significantly (P < 0.05).

Table 3.3 Effect of white striping degree (normal, moderate and severe) on marinade uptake, purge loss, cooking loss and AK-shear force of broiler marinated breast meat (means ± SE).

	Level of white striping[1]			Probability
	Normal	Moderate	Severe	
n.	51	51	51	
Marinade uptake (%)	12.67 ± 0.43[a]	10.97± 0.38[b]	7.92 ± 0.36[c]	0.000
Purge loss (%)	1.82 ± 0.06[b]	1.97 ± 0.06[ab]	2.11 ± 0.09[a]	0.003
Cooking loss (%)	14.59 ± 0.22[b]	14.84 ± 0.26[b]	15.93 ± 0.28[a]	0.000
Total yield (%)[3]	83.86 ± 0.21[a]	83.47 ± 0.25[a]	82.23± 0.31[b]	0.000
AK-shear force (kg/g)	1.83 ± 0.03[b]	1.89 ± 0.04[b]	2.09 ± 0.06[a]	0.004

[1] the occurrence of white striping was classified into normal, moderate and severe according to Kuttappan *et al.* (2012b)

[a-c] Means within a row followed by different superscript letters differ significantly ($P < 0.05$).

Overall, the findings of quality trait analysis revealed that breast fillets affected by white striping abnormality do not only impair product appearance, but also the main technological traits (water holding and binding capacities and texture) were adversely affected. Fillets affected by WS exhibited reduced ability to hold and bind water. Indeed, WS determined a decrease in the ability to retain liquid during cooking in non-marinated fillets, but also a very poor ability to pick up marinade solution and retain it during refrigerated storage and cooking as indicated by the higher purge loss and cooking loss values. This effect was dramatic in fillets with severe striping, but also the presence of moderate WS was associated with a significant impairment in the functionality of protein. This effect was not pH-dependent because severe striped fillets had a higher pH compared with moderate and normal ones, which is usually associated with greater water-holding capacity (Petracci *et al.*, 2013b). Also, Kuttappan *et al.* (2009) observed high pH values in fillets with severe WS, but no significant differences in water-holding capacity were previously found (Bauermeister *et al.*, 2009; Kuttappan *et al.*, 2009). However, more recently the same authors demonstrated that breast fillets showing severe white striping had reduced protein content and myopathic lesions (Kuttappan *et al.*, 2013a).

Also in the present study, an extensive poor cohesion (tendency to separation of muscle fiber bundles) was visually observed beneath the striation area during sampling preparation similar to that described by Petracci and Cavani (2012a). This observation was also confirmed by Allo-Kramer-shear force assessed on non-marinated fillets, which was lower in severe white striped

fillets by evidencing a softer texture after cooking. It can be hypothesized that WS is associated with muscle degeneration very similar to that occurring in muscular dystrophies as also suggested by Kuttappan *et al.* (2013a). This degeneration may result a strong decrease of muscle contractile proteins (e.g., myosin and actin) which leads to breast meat with a reduced ability to hold and bind water as well as a softer texture. This hypothesis should be further investigated by histological approaches. In addition, water is generally distributed in different form in muscle structure. It can be structurally organized in layers around polar molecules (bound water and between layers of cellular materials (steric water)). Most of water in the muscle is entrapped by different forces and it is difficult to move. Bound water (about 5%) usually bonds with proteins, it has an ice-like structure (Bertram *et al.*, 2002; Brewer, 2004; Pearce *et al.*, 2011). But, the changes in the cyto-artchitectural design due to degeneration may lead to disturbance in the steric water distribution as well as bound water due to loss of contractile proteins.

On the other hand, effect of WS on color attributes (L*, b*, a*) was limited with only a small increase of a* and b*. Overall, these results are in agreement with Kuttappan *et al.* (2009) who found that b* values increased with WS severity, and no effect on L* and a* values. The increase of b* value can be attributed due to increase of fat content that has been observed by Kuttappan *et al.* (2012b) and Petracci *et al.* (2014a). Finally, a difference detected in Allo-Kramer shear force of cooked marinated meat was of little practical importance. It is most likely that marinating determined an overall increase in meat tenderness, which smoothed differences found in non-marinated fillets.

3.5.2 Experiment 2

The results of chemical composition for different degrees of white-striped chicken breast meat have been presented in Table 3.4. The results for normal fillets agreed with those reported in the main food composition database (International Network of Food Data Systems, 2013; USDA, 2013). On the other hand, it was found that there were significant differences ($P<0.001$) in protein and lipid percentages among normal, moderate and severe groups. Protein content decreased as the degree of WS increased from normal to severe groups (22.9 *vs.* 22.2 *vs.* 20.9%; $P<0.001$), respectively, while the opposite trend was observed in lipid (0.78 *vs.* 1.46 *vs.* 2.53%; $P<0.001$) and collagen (1.30 *vs.* 1.37 *vs.* 1.43%; $P<0.001$) contents which exhibited the highest values in severe white-striped fillets. Finally, white striping defects did not show any effect on the moisture content of the breast meat.

These results are consistent with Kuttappan *et al.* (2012b) who found that severe white-striped fillets had higher fat content and lower protein content when compared with normal fillets, but they did not find any differences between moderate and normal fillets. In addition, Mudalal *et al.* (2014b) found a similar trend for the effect of white striping on the chemical composition of breast meat and these results have been mentioned in the section 3.5.2 and Table 3.7.

The changes in chemical composition of white-striped fillets can be due to the occurrence of degeneration process for muscle fibers as some studies have pointed out the presence of different histological changes in the breast of modern chicken hybrids like increased eosinophilia, floccular/vacuolar degeneration and lysis of fibers, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells), mononuclear cell infiltration, lipidosis and interstitial inflammation and fibrosis (Kuttappan *et al.*, 2013a; Petracci *et al.*, 2013c; Sihvo *et al.*, 2014). Hence, these modifications explain the increase of intramuscular lipids (e.g. lipidosis) as well as the higher content of collagen (e.g. fibrosis) in fillets affected by WS, while lower protein level may be an indirect effect of increased accumulation of intramuscular lipid.

Table 3.4 Chemical composition of chicken breast meat affected by different degrees of white striping (WS) (normal, moderate, and severe) (means ± SEM).

Composition	**Degree of WS**			**Probability**
	Normal	**Moderate**	**Severe**	
Samples (n.)	36	36	36	
Moisture (%)	75.10±0.13	75.16±0.15	74.90±0.16	0.635
Total protein (%)	22.90±0.25^{a}	22.20±0.29^{b}	20.90±0.23^{c}	<0.001
Intramuscular fat (%)	0.78±0.09^{c}	1.46±0.11^{b}	2.53±0.30^{a}	<0.001
Collagen (%)	1.30±0.01^{c}	1.37±0.01^{b}	1.43±0.02^{a}	<0.001

$^{a–c}$ Means within a row followed by different superscript letters differ significantly ($P \leq 0.001$).

The results of SDS-PAGE for meat proteins also evidenced great differences in the pattern of sarcoplasmic (Figure 3.10) and myofibrillar (Figure 3.11) fractions. Severe WS resulted in reduction of total amount of sarcoplasmic and myofibrillar proteins.

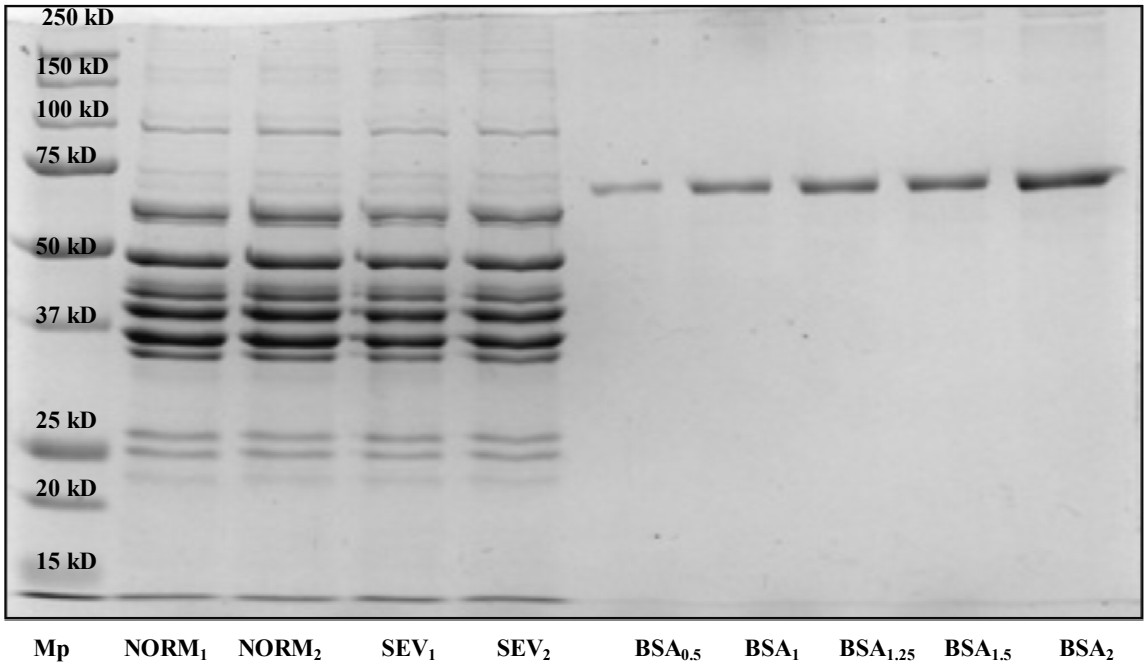


Figure 3.10 SDS-PAGE image for sarcoplasmic proteins of normal (NORM) and severe (SEV) white-striped samples (Mp: Marker proteins; BSA: bovine serum albumin as reference protein with concentration 0.5-2 µg/ml).

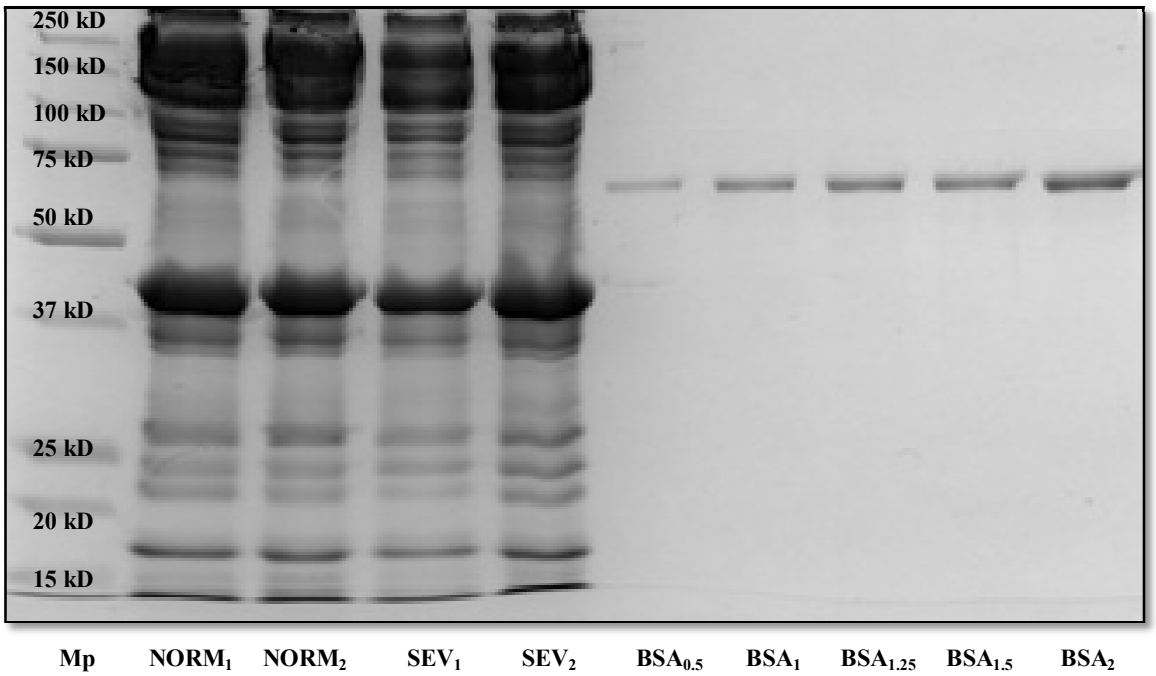


Figure 3.11 SDS-PAGE image for myofibrillar proteins of normal (NORM) and severe (SEV) white-striped fillets (Mp: Marker proteins, BSA: bovine serum albumin as reference protein with concentration 0.5-2 µg/ml).

Certain protein bands of sarcoplasmic proteins showed low concentration, while the other ranges of molecular weights did not exhibit any change (Figure 3.10). Severe white-striped meat showed lower myofibrillar protein concentration at almost all molecular weights (Figure 3.10). Degeneration of myofibrils (Kuttappan *et al.*, 2013a; Petracci *et al.*, 2013c; Sihvo *et al.*, 2014) which coincided with an increase in the levels of some serum enzymes like creatine kinase and alanine transaminase (Kuttappan *et al.*, 2013b) can explain the disparity in the pattern of sarcoplasmic and myofibrillar proteins as well as the differences in the concentration for both types of proteins between normal and severe white-striped fillets.

Based on the data obtained on proximate composition, some nutritional indexes were estimated and the results have been summarized in Table 3.5. The total energy content of the normal chicken breast was in agreement with those reported in the main food composition database (International Network of Food Data Systems, 2013; USDA, 2013). However, severe white-striped fillets had significantly higher total energy content in comparison to normal fillets (450.6 *vs.* 421.1 kJ/100g; $P<0.01$), while moderate white-striped samples did not differ from each other. The effect of WS was not confined in increasing the total energy content, but there was a change in energy contribution from fat and protein. The energy contribution from protein, with respect to total energy, decreased from 93.0% to 78.8%, while the energy contribution from fat increased from 7.0 to 21.2% when normal fillets compared to severe fillets, respectively (Table 3.5). Energy from fat in severe and moderate fillets were significantly higher (95.5 *vs.* 54.9 *vs.* 29.5 kJ/100g; $P<0.001$) than normal fillets. The fat/protein ratio was also significantly increased in moderate and severe white-striped fillets (0.067 and 0.118 *vs.* 0.027; $P<0.05$) in comparison to normal.

Many factors may be involved in favoring this trend, however, it is very likely that intensive genetic selection towards increasing growth rate and breast yield, which have been achieved in modern chicken hybrids, have fostered some modifications in muscular anatomy and metabolism like those of WS (Petracci and Cavani, 2012a) and its consequent strong effect on the nutritional value of breast meat which has been observed in this study.

Certainly, the use of high-energy diets in conjunction with farming systems, allowing a low mobility of the animals and increasing slaughter ages and weights, which have been employed by processors during the last years in order to optimize the production performance of meat to meet the persistent demand on processed products, may be other important factors involved in

this problem (Wang *et al.*, 2009; Crawford *et al.*, 2010; Kuttappan *et al.*, 2012b, Petracci *et al.*, 2013c).

The ratio between collagen and total proteins was significantly increased (Table 3.5) in severe and moderate fillets in comparison to normal (6.73 *vs*. 6.19 *vs*. 5.72; P<0.05). This increase means that the nutritional quality of proteins in white-striped fillets may be reduced due to low digestibility of collagen and the deficiency of some essential amino acids (e.g. tryptophan, sulfur amino acids, and lysine) in connective tissue with respect to myofibrillar and sarcoplasmic proteins (Young and Pellett, 1984; Boback *et al.*, 2007).

Table 3.5 Total energy content, energy distribution, and collagen: protein and fat: protein ratios of chicken breast meat affected by different degrees of white striping (WS) (normal, moderate, and severe) (means ± SEM).

Nutritional index	Degree of WS			Probability
	Normal	Moderate	Severe	
Samples (n.)	36	36	36	
Energy from proteins (kJ/100g)	391.62±0.79^{a}	381.08±1.16^{b}	355.13±0.90^{c}	<0.001
Protein energy (%)	93.00±0.74^{a}	87.40±0.86^{b}	78.82±1.76^{c}	<0.001
Energy from fat (kJ/100g)	29.50±0.88^{c}	54.94±1.03^{b}	95.55±2.72^{a}	<0.001
Fat energy (%)	7.00±0.79^{c}	12.60±0.86^{b}	21.18±1.76^{a}	<0.001
Total energy (kJ/100g)	421.12±1.36^{b}	436.02±1.52^{ab}	450.68±2.93^{a}	0.009
Fat: protein ratio	0.027±0.001^{c}	0.067±0.002^{b}	0.118±0.002^{a}	<0.001
Collagen: protein ratio	5.72±0.09^{c}	6.19±0.19^{b}	6.73±0.12^{a}	<0.001

$^{a–c}$ Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

The impact of WS defect should not be underestimated because a recent survey estimated that current incidence rate under commercial production was around 43.0% (36.8 and 6.2% in moderate and severe degree, respectively) (Lorenzi *et al.*, 2014). At present, if fillets showing severe WS are usually downgraded by processors and used for manufacturing further processed products (e.g. Sausages, nuggets) where chemical composition can be modified during formulation, fillets with moderate WS are not downgraded and marketed for fresh retailing. This means that an increasing share of chicken breast meat currently marketed in the form of cut-up

(whole or sliced) can have rather different nutritional characteristics in respect to those reported on the label and to consumer expectations towards poultry meat (e.g. Low calories and fat).

3.5.3 Experiment 3

The weights and dimensions of normal and WS fillets are presented in Table 3.6. WS fillets exhibited higher weight (290.4 *vs.* 243.1 g, $P<0.05$), length (18.4 *vs.* 19.9 cm; $P<0.05$), and middle (H2, 3.1 *vs.* 2.4 cm; $P<0.01$) and top (H3, 3.5 *vs.* 2.1 cm; $P<0.01$) heights, while breast width and bottom height (H1) did not vary between groups.

Table 3.6 Weight and dimensions (means ± SEM) of normal and WS chicken breast fillets (n=6/group).

Parameter	Normal	WS	Probability
Weight (g)	243.1±10.6	290.4±15.8	*
Length (cm)	18.4±0.1	19.9±0.5	*
Width (W) (cm)	8.9±0.5	9.2±0.6	NS
Bottom height (H1)[1] (cm)	0.7±0.1	0.8±0.1	NS
Middle height (H2)[2] (cm)	2.4±0.1	3.1±0.2	**
Top height (H3)[3] (cm)	3.1±0.2	3.5±0.1	*

[1] H1 was measured far from the end of the caudal part by 1 cm toward a dorsal direction

[2] H2 was measured at the half distance of the breast length

[3] H3 was measured at the thickest point in the cranial part

* = $P<0.05$; ** = $P<0.01$; NS not significant

Table 3.7 Chemical composition (means ± SEM) of normal and WS chicken breast meat (n=6/group).

Parameter	Normal	WS	Probability
Moisture (%)	73.8±0.24	75.4±0.31	**
Protein (%)	22.8±0.63	18.7±0.25	***
Intramuscular fat (%)	0.98±0.23	2.15±0.40	***
Ash (%)	1.34±0.04	1.14±0.02	***
Collagen (%)	1.22±0.03	1.36±0.04	**

** = $P<0.01$;***= $P<0.001$

The results of proximate composition of normal and WS fillets are reported in Table 3.7. All parameters were significantly modified by occurrence of white striping. WS fillets showed significantly lower content of protein (18.7 *vs.* 22.8%; P<0.001) and ash (1.14 *vs.* 1.34; P<0.001), as well as higher percentage of moisture (75.4 *vs.* 73.8%; P<0.001), intramuscular fat (2.15 *vs.* 0.98%; P<0.01) and collagen (1.36 *vs.* 1.22%; P<0.01).

Total sarcoplasmic and myofibrillar protein contents are shown in Figure 3.12. It was found that white striping determined a lower content of both sarcoplasmic (2.6 *vs.* 3.2 g/100g of meat; P<0.001) and myofibrillar (8.7 *vs.* 14.0 g/100g of meat; P<0.05) proteins.

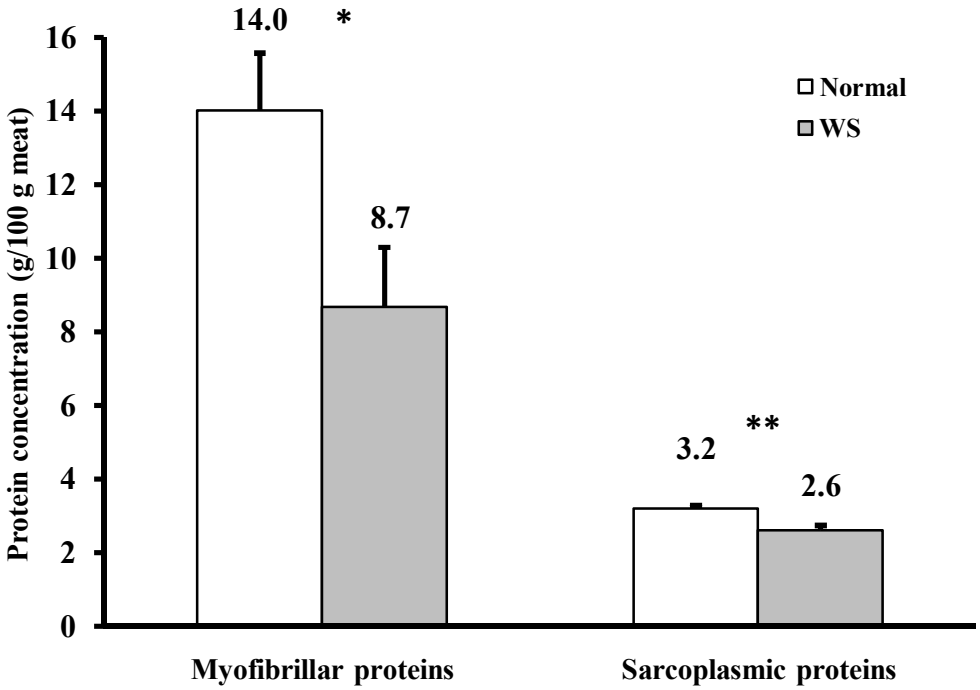


Figure 3.12. Total content (means ± SEM) of sarcoplasmic and myofibrillar proteins (g/100 g meat) of normal and WS chicken breast meat (n=6/group; *=P<0.05; **=P<0.01).

The results of SDS-PAGE analysis for meat proteins from normal and WS fillets showed different patterns of both sarcoplasmic and myofibrillar proteins (Figure 3.13). Eight bands of myofibrillar proteins with molecular weights ranging from 16 to 80 kDa were quantified (Table 3.8). Instead, for sarcoplamic proteins, 12 bands were detected, but only 11, having a molecular weight from 25 to 90 kDa, were quantified (Table 3.8).

An unknown protein of 80 kDa, actin (42 kDa), LC1 slow-twitch light chain myosin (27.5 kDa) and LC3 fast-twitch light chain myosin (16 kDa) were significantly lower in the concentration in WS fillets than in normal ones. No significant difference was detected in protein

concentration of troponin (70 kDa), desmin (53 kDa), partial hydrolysis of troponin (29 kDa) and LC2 slow-twitch light chain myosin (19 kDa) between normal and WS meat.

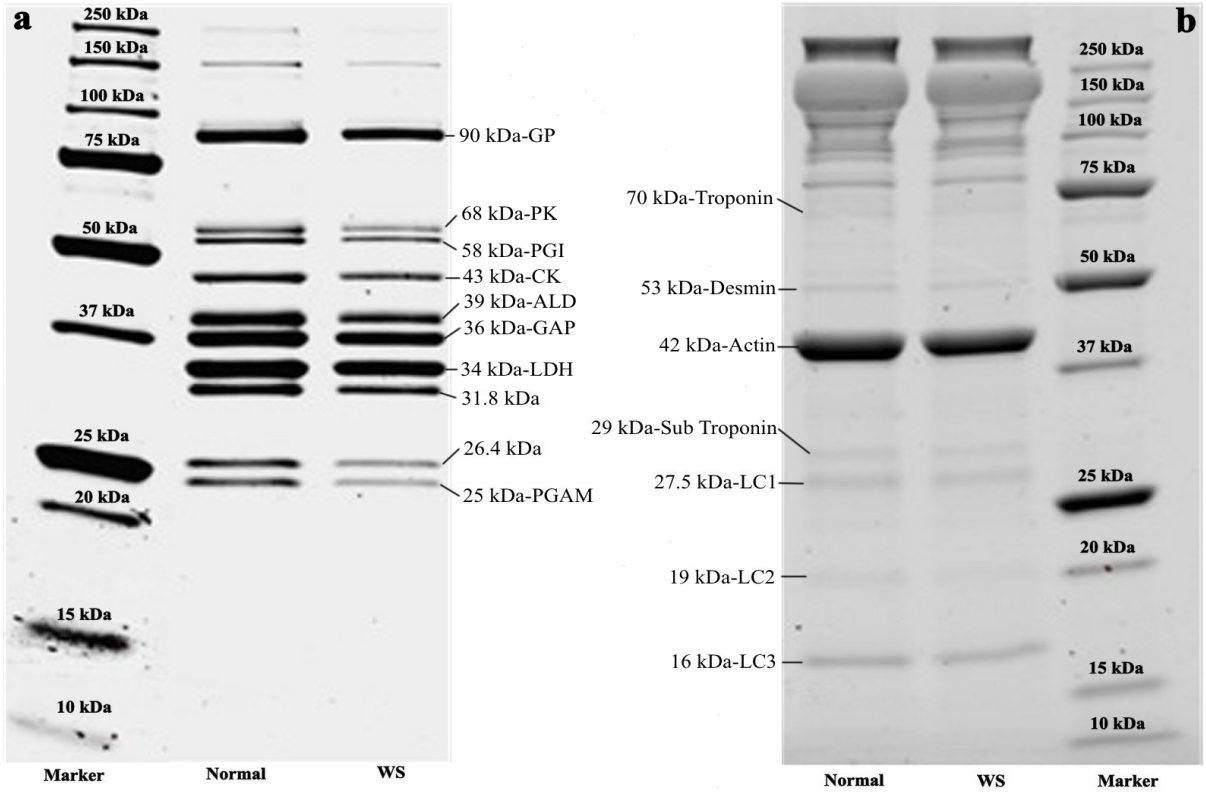


Figure 3.13 Stain free SDS-PAGE of sarcoplasmic (a) and myofibrillar (b) proteins of normal and WS samples. GP, glycogen phosphorylase; PGM, phosphoglucomutase; PK, pyruvate kinase; PGI, phosphoglucose isomerase; EN, enolase; CK, creatine kinase; ALD, aldolase; GAP, glyceraldehyde phosphate dehydrogenase; LDH, lactate dehydrogenase; PGAM, phosphoglyceratemutase, LC2 slow-twitch light chain myosin, LC2 slow-twitch light chain myosin, LC3 fast-twitch light chain myosin.

WS samples also exhibited a lower relative abundance of the 80 kDa band, LC1 slow twitch light chain myosin (27.5 kDa), LC3 fast-twitch light chain myosin, and partial hydrolysis of troponin. G-actin (42 kDa) had an exceptionally higher value of relative abundance in WS meat. The remaining proteins did not show any significant difference in relative abundance. In general, all proteins showed the same trend between the concentration and relative abundance for both normal and WS fillets, except for partial hydrolysis of troponin (29 kDa). In this type of protein, there were no significant differences, but at the same time there was a significant difference in

relative abundance. Actin was the highest in concentration (156.2 and 136.5 mg/g) and relative abundance (81.2 and 86.1%) for both normal and WS fillets.

Table 3.8 Concentration (mg/g)[1] and relative abundance (%)[2] of SDS-PAGE myofibrillar protein bands (means ± SEM) of normal and WS chicken breast meat (n=6/group).

Band No.	Protein name	Molecular weight (kDa)	Concentration (mg/g)		Prob.	Relative abundance (%)		Prob.
			Normal	WS		Normal	WS	
1	Unknown	80	7.6±0.6	3.7±0.7	***	3.9±0.2	2.2±0.3	***
2	Troponin	70	2.3±0.6	1.2±0.4	NS	1.1±0.3	0.7±0.2	NS
3	Desmin	53	2.3±0.7	2.1±0.9	NS	1.1±0.3	1.2±0.5	NS
4	Actin	42	156.3±7.7	136.5±6.3	*	81.2±1.7	86.1±1.5	**
5	Partial hydrolysis of troponin	29	2.0±0.6	1.4±0.4	0.06	0.9±0.3	0.8±0.2	*
6	LC1 slow-twitch light chain myosin	27.5	7.7±1.1	4.0±0.6	***	3.9±0.3	2.4±0.2	***
7	LC2 slow-twitch light chain myosin	19	1.5±0.4	0.6±0.4	NS	0.7±0.2	0.3±0.2	NS
8	LC3 fast-twitch light chain myosin	16	13.6±1.2	9.6±0.7	***	6.9±0.2	6.0±0.3	***

[1] The concentrations of proteins were expressed in mg per g of raw chicken breast meat

[2] The relative abundance was calculated by measuring the concentration of extracted protein on each band divided by the sum of the concentration for all bands in the same gel lane multiply by 100

* = P<0.05; ** = P<0.01; *** = P<0.001

The concentration of sarcoplasmic proteins of band 1 (glycogen phosphorylase), 2 (pyruvate kinase), 3 (phosphoglucose isomerase), 4 (enolase), 6 (aldolase), 7 (glyceraldehydes dehydrogenase), 8 (lactate dehydrogenase), 9 (31.8 kDa), 10 (26.4 kDa) and 11 (phosphoglyceratemutase) were lower in WS meat samples (Table 3.9). On the contrary, there was no significant difference in the concentration of creatine kinase (43 kDa). WS samples

exhibited a lower relative abundance of enolase (47 kDa), aldolase (39 kDa), and phosphoglycerate mutase (25 kDa) than normal ones. Glycogen phosphorylase (22.8 *vs.* 18.3%; P<0.01) and lactate dehydrogenase (25.1 vs. 23.3%; P<0.05) showed exceptionally higher relative abundance in WS fillets when compared to normal ones. The remaining proteins did not exhibit any difference in relative abundance. The concentration and relative abundance of lactate dehydrogenase were the highest in both normal and WS samples when compared with other types of proteins. In general, there was no increase in the concentration of any type of sarcoplasmic proteins.

Table 3.9 Concentration (mg/g)[1] and relative abundance (%)[2] of SDS-PAGE sarcoplasmic protein bands (means ± SEM) of normal and WS chicken breast meat (n=6/group).

Band No.	Protein name	Molecular weight (kDa)	Concentration (mg/g)		Prob.	Relative abundance (%)		Prob.
			Normal	WS		Normal	WS	
1	Glycogenphosphorylase	90	12.1±0.6	9.2±0.6	**	18.3±0.4	22.8±1.1	**
2	Pyruvatekinase	60	2.8±0.3	1.4±0.2	**	4.2±0.2	3.3±0.3	*
3	Phosphoglucose isomerase	58	2.0±0.3	1.0±0.2	**	2.9±0.2	2.4±0.3	NS
4	Enolase	47	4.0±0.3	2.0±0.2	***	6.0±0.2	4.9±0.2	**
5	Creatine kinase	43	1.6±0.2	1.0±0.2	NS	2.4±0.3	2.4±0.3	NS
6	Aldolase	39	2.8±0.2	1.4±0.1	***	4.1±0.1	3.4±0.1	***
7	Glyceraldehyde phosphate dehydrogenase	36	9.4±0.5	5.5±0.6	***	14.3±0.4	13.2±0.6	NS
8	Lactate dehydrogenase	34	15.3±0.7	10.3±0.9	***	23.3±0.5	25.1±0.5	*
9	Unknown	31.8	6.6±0.3	4.0±0.4	***	10.0±0.3	9.6±0.2	NS
10	Unknown	26.4	4.5±0.6	2.6±0.3	**	6.6±0.4	6.3±0.3	NS
11	Phophoglycerate mutase	25	4.9±0.4	2.7±0.30	***	7.4±0.1	6.5±0.2	**

[1] The concentrations of proteins were expressed in mg per g of raw chicken breast meat

[2] The relative abundance was calculated by measuring the concentration of extracted protein on each band divided by the sum of the concentration for all bands in the same gel lane multiply by 100

* = P<0.05; ** = P<0.01; *** = P<0.001

Cooking loss is normally used to measure the loss of liquids as a result of protein denaturation and decomposition of cell membranes during cooking. In the current study, WS fillets exhibited higher values of cooking loss (33.7 *vs.* 27.4%; P<0.001) in comparison to normal fillets (Figure 1.14).

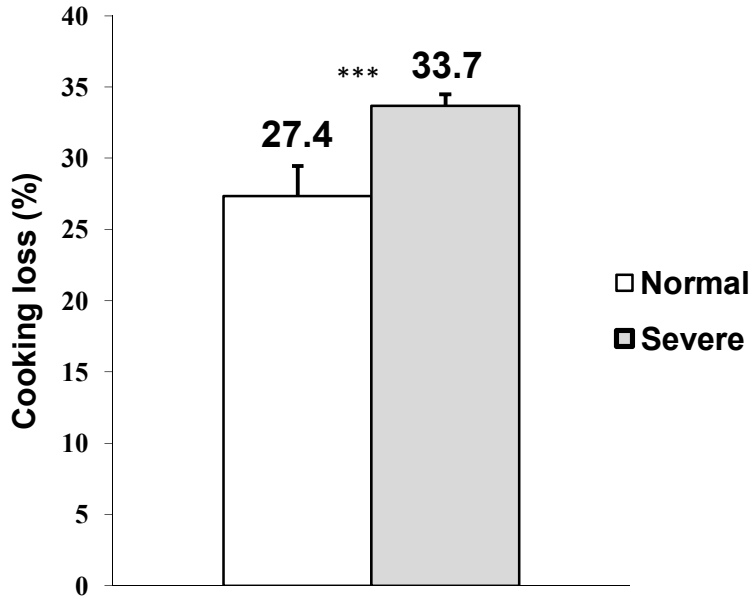


Figure 3.14 Cooking loss (means ± SEM) of normal and WS chicken breast meat (n=6/group; ***=P<0.001).

The ranges of sarcoplasmic, myofibrillar and total protein solubility for both severe and normal fillets were 44.8-52.0, 65.3-85.5, and 110.1-137.4 mg/g meat respectively. The solubility of total, myofibrillar and sarcoplasmic proteins are reported in Table 3.10. Protein solubility is frequently used to evaluate protein denaturation and its effect on water holding capacity.

Severe WS fillets showed lower protein solubility for sarcoplasmic, myofibrillar and total protein fractions when compared with normal fillets. The differences in protein solubility between normal and WS fillets were more tangible in total (137.4 *vs.* 110.1 mg/g; P<0.001) and myofibrillar (85.5 *vs.* 65.3 mg/g; P<0.001) proteins than sarcoplasmic (52.0 *vs.* 44.8 mg/g; P<0.01) proteins. On the other hand, when protein solubility based on total crude protein content was taken into account (Table 3.10), there were no significant differences between normal and WS meat samples.

Table 3.10 Total, myofibrillar and sarcoplasmic protein solubility (means ± SEM) of normal and WS chicken breast meat (n=6/group)

Solubility	Normal	WS	Probability
Total proteins			
mg/g meat	137.4± 5.1	110.1±3.5	***
mg/g protein[1]	604.3± 25.7	587.7± 19.8	NS
Myofibrillar proteins			
mg/g meat	85.5± 5.8	65.3±4.3	***
mg/g protein[1]	375.2± 27.3	348.6± 28.5	NS
Sarcoplasmic proteins			
mg/g meat	52.0±1.1	44.8±1.7	**
mg/g protein[1]	229.0± 9.4	239.1± 11.1	NS

[1]based on total crude proteins content

** = P<0.01; *** = P<0.001

The endothermic transition properties of normal and white striped meat are shown in Table 3.11. Three endothermic transition peaks (48.0-59.6, 59.8-63.5, and 73.3-81.7°C) have been detected by differential scanning calorimetry (Figure 3.15). Peak 1 of white striped meat exhibited significantly higher onset temperature (49.29 vs. 48.03°C, P<0.05) than normal meat while there were no significant differences in the end and peak temperature and the enthalpy. In the second endothermic transition (peak2), normal meat showed significantly higher end temperature (68.13 *vs.* 67.42°C, P<0.001) and enthalpy (0.126 *vs.* 0.039 J/g, P<0.05) than white striped meat while there were no significant differences in the other thermal properties. Finally, during the third endothermic transition (peak 3), there was a significant decrease in onset temperature of white striped meat in comparison to normal meat.

Table 3.11 transition temperature and enthalpy of denaturation for normal and white striped chicken breast proteins.

Endothermic transitions	Thermal properties	Normal	Severe	p-value
Peak 1	T_{onset} (°C)[1]	48.03±0.26	49.29±0.29	<0.05
	T_{end} (°C)[2]	59.63±0.73	60.43±0.14	0.313
	T_{peak} (°C)[3]	54.93±0.51	55.84±0.16	0.117
	H (J/g)[4]	0.174±0.033	0.216±0.020	0.305
Peak 2	T_{onset} (°C)	59.82±0.74	60.94±0.20	0.176
	T_{end} (°C)	68.13±0.04	67.42±0.13	<0.001
	T_{peak} (°C)	63.51±0.23	63.36±0.17	0.601
	H (J/g)	0.126±0.032	0.039±0.004	<0.05
Peak 3	T_{onset} (°C)	73.35±0.10	72.72±0.23	<0.05
	T_{end} (°C)	81.66±0.20	81.88±0.18	0.442
	T_{peak} (°C)	76.65±0.13	76.72±0.15	0.739
	H (J/g)	0.114±0.011	0.142±0.013	0.147

[1] Onset temperature has been measured at the beginning of temperature rising at the baseline

[2] End temperature has been measured when the peak curve meet the baseline

[3] Peak temperature has been measured when temperature of endothermic transition reached the maximum

[4] The enthalpy has been recorded after normalization based on sample weight

Deconvolution for all peaks (Figure 3.16) has been carried out in order to avoid the error in estimation of thermal properties of endothermic transitions due to overlapping between the peaks.

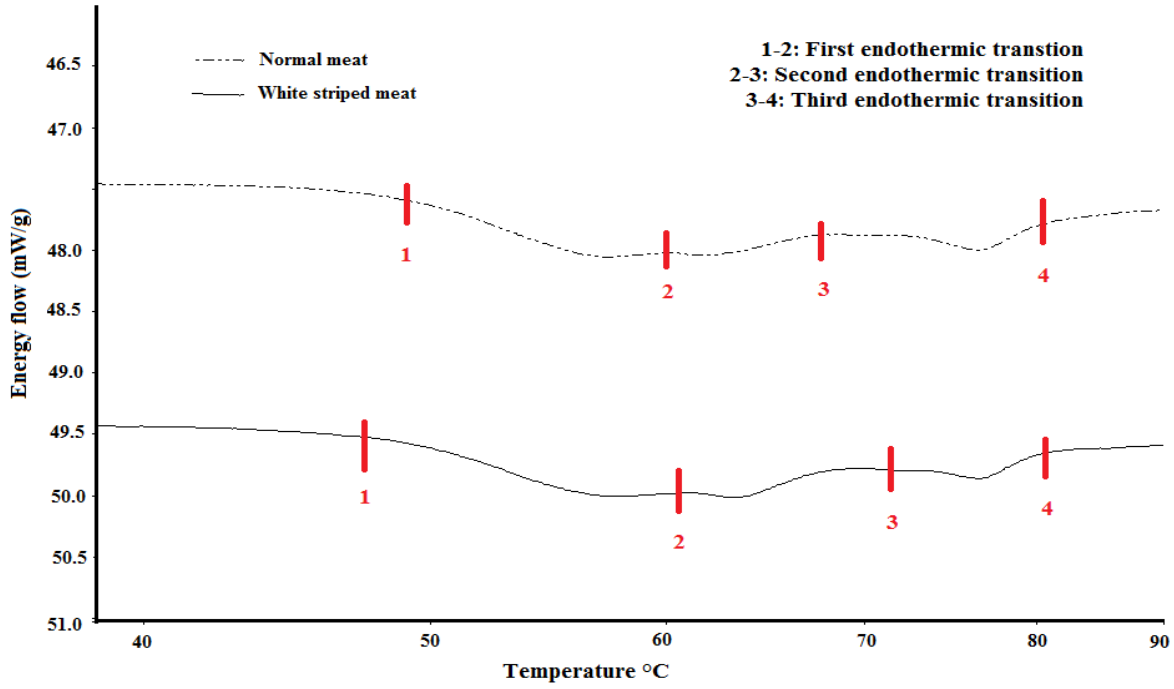


Figure 3.15 Endothermic transitions of both normal and white striped meat proteins.

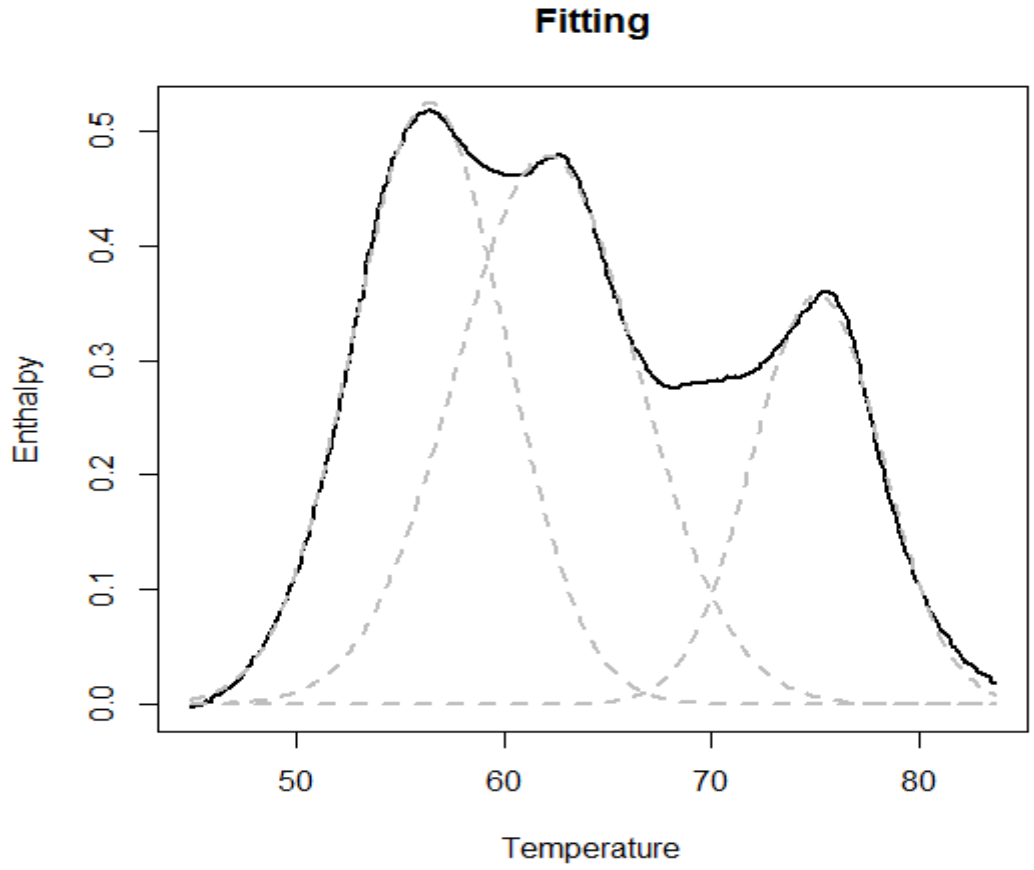


Figure 3.16 Deconvolution of overlapped endothermic transition peaks to the baseline.

The weight and dimension measurements were used to evaluate the growth pattern of breast fillets, because it was found that high-yield breast hybrids were affected by white striping and related histopathological anomalous features more than low-yield breast hybrids (Kuttappan *et al.*, 2013c; Petracci *et al.*, 2013b). From the current data, the WS fillets showed higher weight and dimensions than normal fillets. These results agree with Kuttappan *et al.* (2009) that used a similar approach and found that all of breast dimensions except the length were affected by white striping. In a more recent study, the same authors evidenced that fillet weights and yields increased as the severity of striping increased (Kuttappan *et al.*, 2012b). Even if a small number of samples were considered in the present study, it was confirmed that occurrence of white striping was mainly associated with thicker or heavier fillets as suggested by Kuttappan *et al.* (2013c) and, as a consequence, birds within the same flock showing higher breast sizes are more prone to develop white striping abnormality.

Overall, the results confirmed that white striping defect had a dramatic impact on the chemical composition. Severe white striped breast meat had higher fat and moisture content and lower levels of total crude proteins and ash. Moreover, even if the total protein content was dramatically reduced in fillets showing white striping, but there was an increase in collagen. These results were consistent with Petracci *et al.* (2014) and Kuttappan *et al.* (2012b; 2013a) who found that WS fillets showed higher fat and collagen content and lower protein content as described in section 3.5.2 And Table 3.4.

These dramatic differences in proximate composition can be likely due to muscular degeneration previously observed in white striped breast muscles (Kuttappan *et al.*, 2013a) that can explain the reduction in protein content. In addition, increase of fat accumulation due to lipidosis can explain the higher intramuscular lipid content, while higher content of collagen can be explained by fibrosis (Kuttappan *et al.*, 2013c; Sihvo *et al.*, 2014). Thereupon, lower total protein levels may be an indirect effect of fibre degeneration and atrophy, coupled with increased fat accumulation. This hypothesis is reinforced by the remarkable reduction of both myofibrillar and sarcoplasmic proteins observed in the present study. It is well known that degeneration of muscle in muscular dystrophy is characterized by an extensive loss of sarcoplasmic and contractile protein with replacement of fat and connective tissue (Stacher *et al.*, 1979). Reduction of myofibrillar proteins can be mainly due to increased myofibrillar catabolism (Hillgartner *et*

al., 1981), while a sarcoplasmic protein decline can be a consequence of leakage due to sarcolemma damage and alteration of muscular enzymes (Patnode *et al.*, 1976). On the other hand, similar degenerative processes and histopathological lesions have been also described for some of the major poultry myopathies which have been associated with selection for growth rate of chickens (McRae *et al.*, 2006).

SDS-PAGE revealed that the absolute concentrations of myofilament proteins such as actin, LC1 slow-twitch light chain myosin and LC3 fast-twitch light chain myosin, which are components of contractile fibres, were decreased. Furthermore, the decrease of concentration of specific myofibrillar proteins (actin, LC1 and LC2) may indicate that the degenerative process could be selective in some sites of myofilament. However, it was possible to observe a reduction of both absolute and relative concentrations of LC1 slow-twitch and LC3 fast-twitch light chain myosins. Previously, Stracher *et al.* (1979) reported that myosin from dystrophic chickens contained less LC3 myosin than normal birds and suggested that dystrophic myosin might be embryonic in nature and more susceptible to proteolysis. In general, SDS-PAGE analysis revealed to certain extent that white striped meat had a different quantitative distribution of sarcoplasmic and myofibrillar proteins.

On the other hand, all identified sarcoplasmic proteins (glycogen phosphorylase, pyruvate kinase, phosphoglucose isomerase, enolase, aldolase, glyceraldehyde phosphate dehydrogenase, and lactate dehydrogenase and phosphoglycerate mutase) exhibited lower absolute concentrations with the exception of creatine kinase. Previous microscopic examinations on WS muscle fibers showed that a part of myofibrils had poor functionality of sarcolemma and there was a loss of sarcoplasmic fluids, which contain sarcoplasmic proteins (Stracher *et al.*, 1979; Sihvo *et al.*, 2014). However, when concentrations were expressed as relative abundance, sarcoplasmic bands did not show the same trend. The relative abundance of some bands (glycogen phosphorylase and lactate dehydrogenase) increased while for others decreased in WS samples or did not change. This behavior can be explained assuming that protein turnover has not the same rate during the muscle degeneration and regeneration for all proteins, as suggested by previous histological studies that showed polyphasic degeneration (Kuttappan *et al.*, 2013a; Petracci *et al.*, 2013c; Sihvo *et al.*, 2014).

As a result of quantitative changes in myofibrillar and sarcoplasmic protein contents, both protein solubility and WHC were measured to evaluate if there were also changes in protein

functionality. The results of protein solubility were in agreement with Warner *et al.* (1997) who found very wide changes in sarcoplasmic (50-70 mg/g), myofibrillar (55-130 mg/g) and total protein (100-200 mg/g) solubility for meats having different quality defects such as PSE-like and dark, firm and dry abnormalities. Several studies showed that myofibrillar and sarcoplasmic protein solubility was highly correlated with some processing properties like WHC (drip loss, moisture uptake, cooking loss) (Van Laack *et al.*, 1994; Warner *et al.*, 1997) and texture and gel characteristics (Li-Chan *et al.*, 1987; Sun and Holley, 2010). Even in the current study, WS breast meat showed lower protein solubility (sarcoplasmic, myofibrillar, and total proteins) when based on the weight of fresh meat in comparison to normal meat as usually expressed in the literature, but when the solubility was based on protein content, there were no significant differences. WS fillets also exhibited a lower WHC which was in agreement with previous findings of Petracci *et al.* (2013b) who found lower marinade uptake and increased cooking loss in fillets with severe white striping. Hence, the reduction in protein solubility and cooking loss of WS fillets can be explained by reduction of total protein content and in particular of myofibrillar and sarcoplasmic fractions and to a lesser extent by collagen increase, and not to actual differences in protein solubility. These results can also be supported by the previous studies that showed that the decrease of protein solubility was attributed to protein denaturation as a result of low pH, which formed insoluble aggregates (Fischer and Honikel, 1979; Bowker and Zhuang, 2013). Van Laack *et al.* (2000) found that pale chicken fillets characterized by low pH and WHC had decreased solubility for both sarcoplasmic and total proteins. By contrast, WS fillets were characterized by higher pH values than normal meat (Petracci *et al.*, 2013b). Therefore, the reduction of myofibrillar and sarcoplasmic protein contents could be the main reason for reduction of WHC in WS fillets and this contributes to exclude any similarities between PSE-like and white striping abnormalities.

Endothermic transition is used to monitor the conformational changes and transitions for different types of meat proteins and subunits of proteins during heating. Upon heating, the protein start to unfold faster than aggregation then the denatured proteins reorganize themselves to interact at certain points to give organized three dimensional network structures. In general, the presence of three different endothermic transitions that have been found in this study was in agreement with previous studies. In this regard, DSC studies on meat showed the presence of three denaturation transitions that have been attributed to myosin denaturation (40-60°C),

sarcoplasmic protein and collagen denaturation (60-70°C) and actin denaturation (70-80°C). It was found that the F-actin in chicken breast muscle starts to unfold at 64.2°C and has a single sharp peak at 75.5°C with calorimetric entalphy of 143.4 kcal/mol (Wang and Smith, 1994). In general, there was no agreement between the studies about the transition temperature ranges for meat proteins. In this context, it was found that exudates proteins (as a result of drip loss) in chicken breast muscle exhibited three or four transitions with Tms ranging between 60 and 80°C (Kijowski and Mast, 1988; Wang and Smith, 1994; Murphy *et al.*, 1998) or in other study was between 58 and 72°C (Xiong *et al.*, 1987). On the contrary to some previous study, chicken connective tissue (collagen) showed one thermal transition at 65.3°C (Xiong *et al.*, 1987; Kijowski and Mast, 1988; Murphy *et al.*, 1998).

Three endothermic transitions in 54, 65 and 77°C have been detected by DSC thermograms, probably reflecting the denaturation of myosin (rods and light chain), sarcoplasmic proteins together with collagen and actin, respectively. Heat induced chicken breast myosin gelation proceeds with unfolding LMM, S-1 subfragment and alkali LC, followed by aggregation (LMM and S-1) at temperature lower than 55°C. During this process, DTNB LC unfolds without aggregation at temperature 54°C, and S-2 subfragment unfolds and aggregate at temperature above 55°C. Therefore, Ferry's gelation mechanism (i.e., unfolding, aggregation and matrix formation) need to be extended to unfolding with or without following aggregation and matrix formation, depending on the protein components. In the case of SSP, unfolding of actin occurs at temperatures above 55°C.

From a practical point of view, it is important to control the heating process in such a way (e.g., slow cooking) to allow sufficient time for unfolding and aggregation of protein molecules before the development of a viscoelastic gel matrix. The existence of different denaturation, aggregation, and gelation properties for white versus red poultry muscle proteins would necessitate the design and adoption of different thermal processing conditions for breast versus red meat (Tomasz and Xiong, 2001).

The differences in the thermal properties between normal and white striped meat may be attributed to different amino acid compositions that may change a result of degeneration of muscle fiber and increase of connective tissues due to fibrosis process. Several studies showed that thermal stability during heating is influenced by its amino acid composition. It was found that the thermal denaturation temperatures for the same set of proteins were negatively correlated

to the presence some amino acids such as Ala, Asp, Gly, Gln, Ser, Thr, Val, and Tyr (Ponnuswami *et al.*, 1982). In addition, it was found that proteins that have a high content of hydrophobic amino acid was more thermally stable than one that have high hydrophilic proteins content (Zuber, 1988). The differences in the thermal properties between white striped and normal meat can be attributed to the changes in the proteins profile that have been found by SDS-PAGE analysis which may contribute to change amino acids profile.

3.6 Conclusions

In conclusion, this study revealed from different technological and compositional aspects the influence of WS abnormality on the quality of broiler meat and in particular for those hybrids that were selected for high cut-up yield. In contrast to the few previous studies, it was also found that WS had a dramatic effect on the quality traits of breast, meat particularly in the water holding/binding capacity and texture. Overall, these results indicate that the effects of WS can be serious and critical for the poultry production chain and the necessity of conducting further researches for understanding its biological origins.

In addition, this study concluded that appearance of white striping was associated with a dramatic change in the chemical composition and nutritional value of chicken breast represented by an increase of intramuscular fat, moisture, ash and a consequent reduction of the protein content. In this regard, there was a relevant decrease in protein fractions with higher nutritional value and processing features (e.g. myofibrillar rather than sarcoplasmic), while collagen was increased. In general, these changes likely play the major role in the reduction of the processing properties of meat affected by white striping. Moreover, the disparities in molecular weight profile patterns for myofibrillar and sarcoplasmic proteins which were observed by SDS-PAGE analysis showed different availability of some protein sub-fractions. The effect of WS on the nutritional value of chicken breast was not confined to severe cases which can be separated in the plant production line, but moderate white-striped breasts also exhibited reduced nutritional quality. All these aspects might impair current consumer attitude towards poultry meat in comparison to red meats and hamper the future development of the poultry meat market. Therefore, it is essential, in the near future, to identify the causes of the WS defect and overall emphasize the relevance of meat quality traits among the selection criteria of commercial chicken hybrids.

CHAPTER 4

Implications of combined white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat

4.1 Abstract

One of the consequences of intense genetic selection for growth of poultry is the recent appearance of abnormalities in chicken breast muscles such as white striping (characterised by superficial white striations) and wooden breast (characterised by pale and bulged areas with substantial hardness). The aim of this study was to evaluate the quality traits of chicken fillets affected by white striping and wooden breast abnormalities. In two replications, 192 fillets were divided into 4 classes: normal (n=48; absence of any visual defects), white striping (n=48, presence of white striations), wooden breast (n=48; diffusely presence of hardened areas) and white striping/wooden breast (n=48; fillets affected by both abnormalities). Morphology, raw meat texture and technological properties were assessed in both unprocessed (weight, fillet dimensions, pH, colour, drip loss, cooking loss and cooked meat shear force) and marinated meat (marinade uptake, purge loss, cooking loss and cooked meat shear force). Fillets affected by white striping, wooden breast or both abnormalities exhibited higher breast weights than normal fillets (305.5, 298.7, 318.3 and 244.7 g, respectively; $P<0.001$). Wooden breast, either alone or in combination with white striping, was associated with a significant ($P<0.001$) increase of fillet thickness in the caudal area and raw meat hardness compared with both normal and the white striping abnormality, for which there was no difference. Overall, the occurrence of the individual and combined white striping and wooden breast abnormalities resulted in substantial reduction in the quality of breast meat, although these abnormalities are associated with distinct characteristics. Wooden breast fillets showed lower marinade uptake and higher cooking losses than white striped fillets for both unprocessed and marinated meats. On the other hand, white-striped fillets showed a moderate decline in marinade and cooking yield. Fillets affected by both abnormalities had the highest ($P\leq0.001$) ultimate pH values. In contrast, the effects on colour of raw and cooked meat, drip loss, purge loss and cooked meat shear force were negligible or relatively low and of little practical importance. Thus, the presence of white striping and wooden breast abnormalities impair not only breast meat appearance, but also the quality of both raw and marinated meats mainly by reducing water holding/binding abilities.

Keywords: chicken breast; abnormalities; white striping; wooden breast; meat quality.

4.2 Introduction

In the recent past, there have been tremendous improvements in growth rates and breast yield, which have dramatically increased the commercial production of meat. Notwithstanding, these advances are associated with several important implications in the quality of the obtained meat. In particular, genetic improvements are related to the large increase of birds during growth, resulting in histological and biochemical modifications of muscle tissues that lead to different types of myopathies (Barbut *et al*., 2008; Petracci and Cavani, 2012a). In this context, several studies have shown that breast muscle fibres of fast-growing strains showed a shift towards glycolytic metabolism and are characterized by a larger diameter, lower capillary to fibre ratio, greater inter-capillary distance and lower rate of protein degradation than unselected breeds (Sosnicki *et al.*, 1991; Mahon, 1999; Schreurs, 2000). All these changes have contributed to the appearance of different structural and metabolic abnormalities within muscle that are usually accompanied by myodegeneration and subsequent regeneration (Dransfield and Sosnicki, 1999; Sandercock *et al.*, 2009; Petracci and Cavani, 2012a)

Recently, muscle abnormalities have also been observed in the breast muscles characterized by degenerative myofibrillar proteins that impair the functionality of cell membranes (Kuttappan *et al.*, 2013a; Sihvo *et al.*, 2014). This myodegeneration can be explained by the increased rate of growth of muscle, which exceeds the physiologically sustainable growth rate, leading to muscle damage (Wilson, 1990; Mahon, 1999). In particular, the main abnormalities that have emerged raise concerns over the quality of meat affected by white striping (Kuttappan *et al.*, 2009) and wooden breast (Sihvo *et al.*, 2014) abnormalities. White striped fillets are characterised by the appearance of white striations parallel to muscle fibres on the surface of the *Pectoralis major* muscle (Kuttappan *et al.*, 2009). It was estimated that the incidence of severe white striped breast fillets was about 3.1% under commercial conditions in Italy (Petracci *et al.*, 2013b). Recently, it has also been observed that white striped meat can be accompanied by another type of muscle abnormality called "wooden breast", which is characterised by macroscopically visible hard, bulging and pale area in the caudal part of the fillet (Sihvo *et al.*, 2014). Moreover, wooden breast and white striping exhibit similar histological changes consisting of moderate to severe polyphasic myodegeneration with regeneration as well as varying amounts of interstitial connective tissue accumulation or fibrosis (Sihvo *et al.*, 2014).

The effect of these muscle abnormalities was not confined to histological changes, but the aesthetic characteristics are also strongly impaired. The poultry industry downgrades this defected meat, which is commonly used to manufacture processed products. Aesthetic defects of meats affected by abnormalities may be mitigated or eliminated by including them at certain levels in processed products, but some studies have shown that white striped meat had inferior technological properties, such as reduced water holding and binding capacities and poor texture (Petracci*et al.,* 2013b). In addition, some investigators have started to evaluate the inclusion of wooden breast meat in different proportions in sausage and nuggets (Puolanne and Ruusunen, 2014).

Concomitant with the dynamic and dramatic improvements in growth rate, body size, breast yield and feed conversion by intense genetic selection, muscle abnormalities or myopathies have begun to appear, which adversely affect the poultry meat industry. White striping and wooden breast are the most recent abnormalities and occur with high incidence under commercial growth conditions. Today, the meat industry is forced to downgrade these defective meats due to low aesthetic acceptability. Therefore, there is growing interest in the meat industry to understand what effects these abnormalities have on the different quality traits of raw and processed meat.

Both white striping and wooden breast abnormalities have a similar histological impact on the muscle and can frequently be found in the same breast muscle that are grown under commercial productions as recently reported by Sihvo *et al.* (2014). The quality traits of white striped breast fillets have been previously assessed (Kuttappan *et al.,* 2012c; Petracci *et al.*, 2013b; Petracci *et al.*, 2014), while no information is available on the quality traits of raw and marinated fillets affected by wooden breast. Hence, the aim of this study was to evaluate the impact of separate and combined effects of white striping and wooden breast abnormalities on the quality traits of breast fillets.

4.3 Materials and methods

4.3.1 Sample selection and preparation

Two individual trials were conducted using 192 boneless, skinless, *Pectoralis major* muscles from 52-day-old male Ross 708 broilers (3.7 kg live weight) selected from the deboning area of a commercial processing plant at 3 hours *post-mortem* based on the presence of white striping and wooden breast abnormalities. Breast fillets were grouped into four classes based on the criteria proposed by Kuttappan *et al.* (2012c) and Sihvo *et al.* (2014) to describe white striping and wooden breast abnormalities, respectively, as follows:

- Normal (N): fillets with neither hardened areas nor white striations on the surface;
- White striping (WS): fillets that superficially showed medium to thick white striations;
- Wooden breast (WB): fillets with diffuse hardened areas and pale ridge-like bulges at the caudal end;
- White striping and wooden breast (WS/WB): fillets affected by both WS and WB abnormalities.

The samples transportation and preparation before quality trait analysis have been done as described in 3.3.1 Sampling and storage conditions. At 24 hours of *post-mortem*, colour (CIE L* = lightness, a* = redness and b* = yellowness) was measured according the description in 3.3.2.1 Colour measurements.

Afterwards, each fillet was individually weighed and used for morphometry measurements (width, length and height) according to section (3.2.2.2 Breast weight and size measurements). As shown in Figure 4.1, fillets were subsequently cut to obtain four sub-samples (C1, C2, C3, and C4) that were used for quality analyses. The first sub-sample (C1) was cut from the cranial section and used to assess ultimate pH as described in 3.3.2.3 pH measurements. A raw cylindrical meat cut (C2) 2.5 cm diameter × 1 cm height, weighing about 5 g, was excised from the caudal part of each fillet and used for the compression test. In addition, a parallelepiped cut (C3, 8 × 4 × 3 cm) weighing about 80 g was excised from the cranial part of each fillet and used to assess non-marinated quality traits (drip loss, cook loss, colour after cooking and Warner-Bratzler shear force). Finally, the last parallelepiped meat cut (C4, 8 × 4 × 2 cm) weighing about 60 g was excised from the middle part of each fillet and used, after tumbling, to evaluate the quality traits of marinated breast meat (marinade uptake, purge loss, cooking loss, colour after cooking and Warner-Bratzler shear force).

4.3.2 Meat quality measurements

Meat cuts sampled from the caudal part of fillets (C2, Figure 4.1) were compressed to 40% of the initial height by using a 25 kg loading cell connected to a 50 mm DIA cylinder aluminium probe using TA.HDi heavy duty texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK). The test speed of the probe was 1 mm/sec, while the pre- and post-test speeds were both 3 mm/sec. The compression value was recorded as the maximum force needed to compress 40% of the initial height of the sample and expressed in kg.

Parallelepiped meat cuts excised from the cranial part of each fillet (C3, Figure 4.1) were initially used to determine drip loss following the procedure described in 3.3.2.4.1 Non-marinated meat cuts (a. Drip loss) and cooking loss following the procedure described in

3.3.2.4.1 Non-marinated meat cuts (b. Cooking loss). Colour values (L*, a*, b*) were measured on the upper surface of the cooked cut as described in 3.3.2.1 Colour measurements.

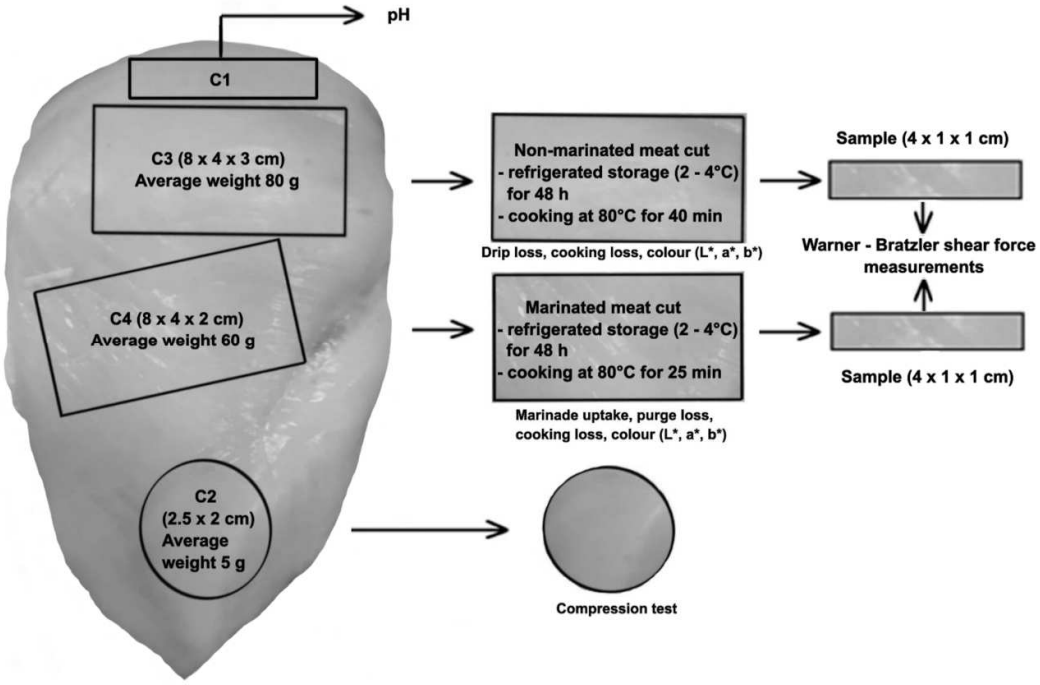


Figure 4.1 Sampling protocol: C1, Meat cut used for pH measurements; C2, Meat cut used for the compression test; C3, Meat cut used for assessing quality traits on non-marinated meat; C4, Meat cut used for assessing quality traits on marinated meat**.**

In addition, Warner-Bratzler shear force was measured according to the same procedure described in 4.4.2.1 Warner-Bratzler. Finally, C4 meat cuts were individually labelled and marinated by tumbling in the same conditions of the procedure described in 3.3.2.4.2 Marinated meat cuts and marinade uptake, purge loss, and cooking loss were determined according to procedure 3.3.2.4.2a and b, respectively. Moreover, colour values (L*, a*, b*) after cooking were measured according to 3.3.2.1 Colour measurements and Warner-Bratzler shear force was measured as described for C3 meat cuts.

4.3.2.1 Warner-Bratzler shear force

Parallelepiped cut (approximately 4 × 1 × 1 cm) was excised parallel to muscle fibre direction to measure the shear force needed to cut the sample by Warner-Bratzler. Parallelepiped samples were sheared in a perpendicular direction respective to the muscle fibres using a triangular blade slot (Lyon and Lyon, 1991) attached to two different tools

(Figure 4.1): 1) Warner–Bratzler shear machine (Bodine Electric Company Chicago 60618, USA); and 2) TA.HDi Heavy Duty texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK). Shear values are represented by the kg peak force necessary to cut the samples.

Figure 4.2 Warner Bratzler (WB) probe used for the shear force determination.

4.4 Statistical analysis

Data were analysed using the ANOVA option of the GLM procedure present in SAS software (SAS Institute, 1988) and testing the main effects for type of meat quality abnormality (N, WS, WB and WS/WB) and replication, as well as the interaction term on meat quality traits. Means were separated using Tukey's honestly test of the GLM procedure (SAS Institute, 1988).

4.5 Results

4.5.1 Weight, dimension and texture of raw fillets

The results for weight, dimension and the compression test of breast fillets are shown in Table 4.1. Fillets affected by WS, WB and WS/WB abnormalities had significantly higher ($P<0.001$) weights and thickness at the top (H1), middle (H2) and bottom (H3) positions compared with normal fillets. None of the abnormalities had any significant effect on either length or width of fillets. However, fillets affected by both defects (WS/WB) exhibited the highest H2 ($P<0.05$). As for H3, fillets affected by only WB or WS/WB exhibited significantly higher ($P<0.05$) values compared with both WS and N fillets, which showed no significant differences. The same trend was observed for raw meat texture. In particular, WS/WB and WB showed significantly higher compression values (3.33 and 4.02 kg, respectively) than WS and N fillets (2.28 and 2.02 kg, respectively; $P<0.001$). There was no significant difference between WS and N fillets.

Table 4.1 Effect of breast abnormalities on weight, dimension and texture of raw chicken fillets.

Parameter	Breast meat category[1]				s.e.m.	Probability
	Normal (N)	White striped (WS)	Wooden breast (WB)	WS/WB		
Weight (g)	244.7[b]	305.5[a]	298.7[a]	318.3[a]	3.4	<0.001
Top height (H1)[2] (mm)	38.1[b]	45.7[a]	43.9[a]	45.7[a]	0.4	<0.001
Middle height (H2)[3] (mm)	24.7[c]	31.2[b]	30.5[b]	33.8[a]	0.4	<0.001
Bottom height (H3)[4] (mm)	8.2[b]	8.7[b]	11.0[a]	11.6[a]	0.2	<0.001
Length (mm)	195.0	196.5	196.8	196.5	0.9	NS
Width (mm)	78.7	81.5	80.3	79.9	0.6	NS
Compression test (kg)	2.02[b]	2.28[b]	4.02[a]	3.33[a]	0.15	<0.001

[1] n=48/group

[a-c] Means within a row followed by different superscript letters differ significantly (P≤0.05)

NS = not significant

[2] H1 was measured at the thickest point in the cranial part

[3] H2 was measured at the half distance of the breast length

[4] H3 was measured far from the end of the caudal part by 1 cm toward a dorsal direction

4.5.2 Quality traits of non-marinated breast meat

The quality traits of non-marinated breast meat samples are shown in Table 4.2. Fillets affected by WS/WB had the highest (P<0.001) ultimate pH values (6.04). In addition, while the pH of WB fillets did not differ compared with N fillets, WS samples had a significantly higher (P<0.05) value than N fillets. Considering the colour of raw meat, the WB group showed higher lightness values (L*) compared with WS and WS/WB fillets (57.0 *vs*. 54.9 and 55.2, respectively; P<0.001), while N samples exhibited an intermediate value. Moreover, WB fillets also had higher (P<0.05) yellowness values, while there were no significant differences among the other groups. The redness was not affected by breast fillet abnormalities.

Compared with the N group, WS/WB fillets had similar drip losses, while WB and WS fillets exhibited significantly higher (P<0.05) and lower (P<0.05) values, respectively. In general, all types of abnormalities showed higher (P<0.001) cooking losses than N fillets. In particular, WB or WS/WB fillets had the highest (P<0.001) cooking losses. However, the presence of abnormalities had no effect on the shear force or colour of cooked meat with the only exception of yellowness, which was higher (P<0.05) in WB and WS/WB than in N fillets.

Table 4.2 The effect of breast abnormalities on quality traits of non-marinated chicken meat.

Parameter	Breast meat category[1]				s.e.m.	Probability.
	Normal (N)	White striped (WS)	Wooden breast (WB)	WS/WB		
Raw meat						
pHu	5.80^{c}	5.90^{b}	5.87^{bc}	6.04^{a}	0.01	<0.001
lightness (L*)	56.0^{ab}	54.9^{b}	57.0^{a}	55.2^{b}	0.2	<0.001
redness (a*)	1.76	1.72	1.67	1.70	0.05	NS
yellowness (b*)	2.72^{b}	2.70^{b}	3.27^{a}	2.64^{b}	0.08	0.017
drip loss (%)	0.93^{b}	0.72^{c}	1.19^{a}	1.03^{b}	0.03	<0.001
Cooked meat						
cooking loss (%)	21.6^{c}	24.7^{b}	28.0^{a}	29.5^{a}	0.4	<0.001
shear force (kg)	2.37	2.35	2.19	2.21	0.04	NS
lightness (L*)	84.0	83.7	84.0	83.5	0.1	0.037
redness (a*)	1.64	1.81	1.79	1.85	0.03	NS
yellowness (b*)	8.01^{b}	8.30^{ab}	8.43^{a}	8.47^{a}	0.05	0.001

[1] n=48/group

[a-c] Means within a row followed by different superscript letters differ significantly (P≤0.05)

NS = not significant

4.5.3 Quality traits of marinated breast meat

The results of marinade uptake and quality traits of the breast meat following tumbling are presented in Table 4.4. All parameters were significantly (P<0.01) affected by the presence of WS, WB, or WS/WB with the exception of cooked meat colour. All groups affected by abnormalities showed lower (P<0.001) values of marinade uptake than N fillets. In particular, WB and WS/WB showed lower values of marinade uptake than the WS group (6.94 and 6.24% vs. 9.33, respectively; P<0.001). Despite the lesser ability to absorb marinade, WB and WS/WB fillets also had greater cooking loss than WS and N meat (17.4 and 18.7 *vs.* 15.3 and 15.0%, respectively; P<0.001).

On the other hand, fillets affected by WS/WB showed significantly lower (P<0.001) values of purge loss than N and WB fillets, while the value of WS was somewhat lower than N and WB, but not statistically significant. Considering the shear force of cooked meat, fillets with both defects showed the highest shear force than the N and WS groups (1.63 *vs.* 1.25 and 1.38 kg, respectively; P<0.001), while WB exhibited an intermediate value (1.45).

Table 4.3 Effect of breast abnormalities on quality traits of marinated chicken meat.

Parameter	Breast meat category[1]				s.e.m.	Probability
	Normal (N)	White striped (WS)	Wooden breast (WB)	WS/WB		
Raw meat						
marinade uptake (%)	13.15[a]	9.33[b]	6.94[c]	6.24[c]	0.26	<0.001
purge loss (%)	1.30[a]	1.20[ab]	1.30[a]	1.10[b]	0.02	0.007
Cooked meat						
cooking loss (%)	15.3[b]	15.0[b]	17.4[a]	18.7[a]	0.2	<0.001
total yield (%)	94.5[a]	92.0[b]	87.3[c]	85.6[c]	0.4	<0.001
shear force (kg)	1.25[b]	1.38[b]	1.45[ab]	1.63[a]	0.03	<0.001
lightness (L*)	84.6	84.8	84.8	84.2	0.1	NS
redness (a*)	2.14	1.88	1.84	1.91	0.04	NS
yellowness (b*)	10.9	10.9	10.9	11.2	0.1	NS

[1] n=48/group

[a-c] Means within a row followed by different superscript letters differ significantly (P≤0.05)

NS = not significant

4.6 Discussion

The rate of growth is one of the most important factors related to the incidence of muscle abnormalities (Mitchell, 1999). Herein, differences in the physical growth pattern between different muscle abnormalities were evaluated by measuring the weight and dimension of fillets. In general, the results of this study revealed that abnormal fillets showed higher weights and greater thickness than normal ones. These findings are consistent with those of Kuttappan *et al.* (2013c) who found that white striping was associated with heavier and thicker fillets. However, our results also showed that wooden breasts generally had higher weight and thickness. This indicates that birds of the same flock with superior breast development are more prone to individual and combined abnormalities. This can be considered as further support for the hypothesis that selection for growth rate and breast yield plays a major role in the occurrence of these emerging aberrations (Petracci and Cavani, 2012a).

However, the increased weight of fillet did not result in any changes in length or width. This is in general agreement with previous studies (Lubritz, 1997; Brewer *et al.*, 2012) which reported that weight had much greater impact on thickness compared with length and width of fillets. The wooden breast abnormality was associated with a remarkable increase in the

bottom fillet height (H3) compared with both normal and white-striped fillets. This is clearly due to the presence of a ridge-like bulge at the caudal end in wooden fillets, as previously described by Sihvo *et al.* (2014). Accordingly, evaluation of bottom fillet height could be proposed as a criterion to discriminate breast fillets affected by the wooden breast abnormality. In this regard, it has been shown that the greater hardness observed empirically in wooden breast fillets can be objectively evaluated using the compression test. In fact, wooden breast fillets, regardless of the presence of white striping, exhibited higher instrumental hardness than normal samples, while the texture of raw meat of white striped fillets was not modified. Additionally, the increase of hardness measured by texture profile analysis was in agreement with the compression values that have been measured for raw meat. It seems that presence of white striping and/or wooden abnormalities has resulted in the reduction of force at maximum compression during the second compression cycle as has been observed by lower springiness values. This may explain due to presence of muscle degeneration and loss contractile fibers that give strength to the muscle (Sihvo *et al.*, 2014)

The higher ultimate pH of white striped fillets compared with the normal group is also in agreement with previous findings (Kuttappan *et al.*, 2009; Petracci *et al.*, 2013b). In this regard, several earlier studies indicated that the positive relationship between the development of breast muscle and ultimate pH as a result of a decrease in glycolytic potential (Berri *et al.*, 2001; Berri *et al.*, 2007). Even if former studies compared unselected and selected lines in terms of growth rate and breast yield, and in our study birds were collected from the same fast-growing hybrid, there was still a large range in growth rates as seen by the large differences in breast weights from birds with the same age. It may be argued that the growth rate for each individual bird is involved in ultimate pH, so that chickens with greater breast development may exhibit higher ultimate pH values. However, the most interesting result is the large difference in pH between the WB and WS/WB groups. In fact, breast muscles showing both abnormalities exhibited pH values even higher than 6, which are common in dark, firm and dry meat (Fletcher, 2002). The cause for this increase in pH due to wooden and white striping abnormalities is not known. In this regard, a previous study clearly showed the presence of massive histological degeneration of muscle fibres in wooden breast and white striped fillets (Sihvo *et al.*, 2014). This anomaly may reduce the glycogen content or modify the onset of acidification during the post-mortem time and lead to an increase in ultimate pH. However, further investigations are needed to confirm these hypotheses.

Concerning colour, wooden fillets showed lighter colour values (L*) compared with WS and WS/WB fillets, while normal fillets exhibited intermediate values. These differences may

be due to both different ultimate pH values and muscle tissue modifications following histological degeneration of abnormal breast muscles. In agreement with our previous study (Petracci *et al.*, 2013b); it appears that even if white striping was associated with higher ultimate pH values, there were no relevant colour changes compared with normal fillets. On the other hand, Sihvo *et al.* (2014) reported that WB fillets were visually pale, an observation that was instrumentally confirmed in the present study where wooden breast fillets without superficial white striping showed a higher lightness and increased yellowness. However, when fillets were affected by both abnormalities, there was no change in meat lightness. This may be explained by the high pH observed in fillets affected by both abnormalities, which have increased meat darkness (Swatland, 2008) that is partially counteracted by the effects of the WB abnormality. However, further studies are needed to elucidate the possible role of these concomitant effects.

As for drip losses, it can be suggested that the higher pH values observed in white striped fillets allowed for an increased ability to retain liquid during storage than normal breast meat. However, in our previous study (Petracci *et al.*, 2013b), we did not find any modifications in either moderate or severe white striped fillets. On the other hand, wooden fillets had higher drip losses than normal ones even if the ultimate pH was similar to both normal and white striped fillets. In this case, it can be speculated that the extensive loss of membrane integrity and the presence of a thin layer of fluid viscous material over the wooden breast (Sihvo *et al.*, 2014) caused an increased loss of liquid during refrigerated storage of the meat regardless of a slightly higher ultimate pH. In this regard, when wooden breast was associated with white striping, drip loss was similar to normal fillets. As previously hypothesised for colour, this may be due to the very high pH observed in fillets with both abnormalities, which is commonly associated with an increased ability to retain liquid (Fletcher, 2002). This may have partially counteracted the negative effects of the wooden breast abnormality.

However, while the negative impact of breast abnormalities on raw meat quality properties seems to be mitigated by the concomitant rise in ultimate pH, the ability to bind marinade solutions and retain liquid during cooking in both non-marinated and marinated meat were also severely impaired. In our earlier study (Petracci *et al.*, 2013b), white striped fillets were shown to exhibit higher cooking losses and lower marinade yields. This was attributed to a dramatic reduction in total crude protein content, and, in particular, of the myofibrillar fraction (Petracci*et al.*, 2014; Mudalal *et al.*, 2014b) which plays a major role in determining of protein functionality during processing (Petracci *et al.*, 2013a). The main novel findings of this study are that the wooden abnormality causes much lower marinade uptake and cooking

losses irrespective to the presence of white striping. These outcomes, together with the observations on raw meat, clearly indicate that wooden breast abnormality resulted in more severe adverse effects on meat quality attributes than white striping. Based on these findings, it may be argued that wooden breast was associated with more damage to muscle tissue than white striping. Previous observations showed that histopathological changes in white striped and wooden breast muscles have similar features (Kuttappan *et al.*, 2009; Sihvo *et al.*, 2014), and thus a common aetiology may be hypothesised. However, Sihvo *et al.* (2014) observed that wooden fillets were affected by an inflammatory process as demonstrated by the presence of T lymphocytes, which were not observed in white striped fillets (Kuttappan *et al.*, 2013a). As a consequence, it might also be proposed that white striping occurs during an early stage of muscle degeneration, while breast fillets become "wooden" only at a later stage of development.

Finally, the shear force of both non-marinated and marinated meat was modestly affected by the presence of both abnormalities. In particular, even if raw wooden fillets showed higher resistance to compression, these differences had little impact on the cooked meat texture. The small differences observed with marinated meat can be associated with lower marinade yield rather than to a direct effect of the wooden breast abnormality. High amounts of liquid losses during cooking are usually associated with increased toughness of meat (Murphy and Marks, 2000). In a recent study, it was also found that inclusion of wooden breasts to some extent in sausages and nuggets increased the shear force and binding strength (Puolanne and Ruusunen, 2014).

4.7 Conclusions

In conclusion, the higher weights of white striped and wooden breast fillets compared with normal breasts can be considered as further support for the hypothesis that selection for growth rate and breast yield plays a major role in the occurrence of these emerging abnormalities. In addition, the presence of either separated or both abnormalities resulted in a large reduction in the quality traits of breast meat. Even if the negative impact of both abnormalities on raw meat quality seems to be mitigated by the concomitant rise in ultimate pH, the ability to bind marinade solutions and retain liquid during cooking in both unprocessed and marinated meat was severely impaired. In addition, the implications of these abnormalities on meat quality were very distinct, as wooden breast fillets exhibited dramatically poorer cooking and processing yields irrespective of the concomitant presence of white striping. It was also established that measurements of height or compression force in

the caudal part of fillets may be used as a tool to discriminate between white striping and wooden breast abnormalities. Further studies are needed to understand the causes of the different behaviour of white striping and wooden breast abnormalities and to identify processing strategies that can minimise their inferior quality.

CHAPTER 5

Histology, composition and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality

5.1 Abstract

Just a few years ago, poultry industry started to face a recent abnormality in breast meat termed as wooden breast which frequently coincided with white striping. The aim of this study was to evaluate the impact of this abnormality on histology, proximate and mineral composition, protein distribution pattern and technological traits of breast meat. Meat affected by wooden or wooden plus white striping exhibited different chemical composition and harder texture than normal meat. In addition, a decrease in water holding/binding capacity as characterized by lower marinade uptake and higher cooking loss has been observed. On the other hand, both muscle abnormalities had higher levels of calcium (Ca^{2+}) and sodium (Na^{+}) and histological lesions than normal meat. In particular, calcium-ATPase (114 kDa), one of the enzymes used as indicator of muscle damage, did not appear in normal fillets but was present in abnormal ones. This study showed that meat affected by wooden or by wooden plus white striping abnormalities had poorer nutritional value, texture, and water holding capacity. Additionally, abnormal fillets showed histological damage which was characterized by fiber degeneration, fibrosis, and lipidosis as well as high level of calcium and sodium.

Key words: Chicken breast meat, wooden breast, white striping, calcium, sodium, SDS-PAGE, chemical composition.

5.2 Introduction

In the last few decades, the production of poultry meat has been dramatically increased. The majority of this increase has been supported by quantitative selection which has tremendously increased the growth rate of muscle by basically inducing hypertrophy in the existing fibres due to fusion of satellite cells (Dransfield and Sosnicki, 1999; Picard *et al.*, 2002; Scheuermann *et al.*, 2004).

Increase of growth rate and breast-yield due to genetic progress has obviously put more stress on the broilers by inducing the appearance of several ante- and *post-mortem* histological and biochemical alterations in the muscle tissue (Dransfield and Sosnicki, 1999; Sandercock *et al.*, 2009; Petracci and Cavani, 2012a). In particular, some of these alterations are attributed to homeostatic dysregulation which leads to cellular dysfunction (MacRae *et al.*, 2006; Sandercock *et al.*, 2006). It was hypothesized that both growth- and stress-related myopathies may result from

disruption of intracellular cations homeostasis (calcium and sodium) (Sandercock *et al.*, 2009). Accordingly, elevations of sodium in muscle cells can facilitate the increase of calcium concentration which activates phospholipase A2 and other proteases (i.e. calpains) leading to membrane dysfunction and creatine kinase losses (Sandercock and Mitchell, 2003; 2004).

At present, the most recent breast myopathies or abnormalities that heavily affect the poultry producers are white-striping (white striations parallel to muscle fibres mainly on the ventral surface) (Kuttapan *et al.*, 2012) and wooden breast (visually hard, out bulging and pale colour) and they may occur separately or together (Sihvo *et al.*, 2014). It was recently estimated that up to 40% of medium (2.2-3.0 kg) and heavy (>3.0 kg) sized birds raised under commercial conditions were affected by different levels of white striping (Lorenzi *et al.*, 2014), while wooden abnormality affects mainly roaster chickens (personal communication). Because of the impaired visual appearance, white striped and wooden breast fillets, especially in severe cases, are downgraded and transformed into processed meat products leading to economic losses for the poultry industry (Mudalal *et al.*, 2014). Previous observations showed that histopathological changes in white striped and wooden breast muscles had similar features such as increase in degenerative and atrophic fibres associated with loss of cross striations, variability in fibre size, floccular/vacuolar degeneration and lysis of fibres, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells), mononuclear cell infiltration, lipidosis, and interstitial inflammation and fibrosis (Kuttappan *et al.*, 2013; Sihvo *et al.*, 2014). These myopathies may have great implications on meat quality. White striping has already been the subject of several studies that have investigated its consequences on consumer acceptability (Kuttappan *et al.*, 2012), histological traits (Kuttappan *et al.*, 2013), chemical composition (Kuttappan *et al.*, 2012; Petracci *et al.*, 2014), protein profile (Mudalal *et al.*, 2014), and processing abilities (Petracci *et al.*, 2013). On the other hand, there are only a couple of studies which evaluated technological traits (Mudalal *et al.*, 2014) and suitability for further processing (Puolanne and Ruusunen, 2014) of breast muscles affected by the wooden breast abnormality. In addition, there are not available data about the mineral composition to evaluate the homeostatic status of breast muscle tissue for both emerging breast abnormalities.

Therefore, the aim of this study was to evaluate the impact of wooden abnormality, with or without white striping, on muscle histology and chemical composition with special emphasis on mineral levels and protein profile which may likely affect textural and water holding capacity (WHC) of the meat.

5.3 Materials and methods

5.3.1 Sample selection and preparation

According to the presence or absence of muscle abnormalities, 96 boneless, skinless, *Pectoralis major* muscles were selected in two replicates from 52-days-old male Ross 708 broilers (3.7 kg live weight) in deboning area of a commercial processing plant after 3 hours *postmortem*. Breast fillets were graded into four classes (Normal, white striping, wooden, and white striping/wooden abnormalities) according to integrating criteria suggested by Kuttappan *et al.* (2012b) and Sihvo *et al.* (2014) which was described in 4.4.1 Sample selection and preparation.

For both replications, approximately 1 cm^3 from one *Pectoralis major* muscle per each group was immediately removed, frozen in liquid nitrogen-cooled in isopentane and used to perform histology and immunohistochemistry analyses as well as morphometrical and morphological evaluations. Subsequently, each group of samples (16/group/replication) were packaged and transported under refrigerated conditions to the laboratory. At 24 hours *post-mortem*, *Pectoralis major* muscles were trimmed from superficial fat, cartilage and connective tissues then used to determine pH, compression test, and drip loss, cooking loss, marination performances and textural profile analysis. The remaining parts of the raw breast fillet were ground at temperature 1-4°C by a food processor for 1 min to obtain homogenous ground meat mass, then ground meat samples were kept in freezer at -20°C for further analysis (proximate composition, mineral composition and SDS-PAGE analysis).

5.3.2 Histological analysis

Histological evaluations were performed on three samples/group for each replication for a total of 18 samples. Approximately 1 cm^3 from each *Pectoralis major* muscle was immediately removed and fixed in 10% buffered formalin for 24 h at room temperature. Specimens were oriented for transverse fiber sectioning, dehydrated in a graded series of ethanol, and embedded in paraffin. From each sample, serial transverse sections (6 μm thick) were obtained, mounted on polylysine-coated slides, and stained with Masson's trichrome. For each section of muscle, the presence of abnormal fibers (giant fibers, fibers with hyaline degeneration, and damaged fibers with round profile) in 10 primary myofiber fascicles (PMF) were assessed and the levels of myodegeneration were graded according the same criteria adopted in our previous study (Mazzoni *et al.*, 2015):

Score F1 – mild: abnormal fibers ranging from 2 to 4 for each PMF (Figure 1A);

Score F2 – moderate: abnormal fibers ranging from 5 to 10 for each PMF (Figure 1B);

Score F3 – severe: abnormal fibers represent the majority of the fibers for each PMF (Figure 1C).

5.3.3 Proximate and mineral composition

Proximate chemical composition (moisture, protein, lipid, ash, and collagen) of breast meat was determined for each sample using official methods of AOAC (1990) which was described in 3.3.2.5 Proximate chemical composition.

Element analysis (Ca, Na, P, K, and Mg) was performed using ICP-OES method after microwave digestion of 3.0 g of meat. Samples were digested by microwave using a Milestone ETHOS ONE oven using 4 mL nitric acid and 1 mL hydrogen peroxide. All reagents were from Merck, Darmstadt (Germany); acids were of Suprapur grade. Elements were quantified by Inductively Coupled Plasma-Optic Emission Spectrometry technique (ICP-OES) using a Perkin Elmer Optima 2100 DV instrument, coupled with a CETAC U5000AT+ ultrasound nebulizer for mercury. Two blanks were run during each set of analysis to check for chemical purity, and the accuracy of the method was verified with reference materials (CRM GBW 09101, human hair control, Shanghai Institute of Nuclear Research Academia Sinica; CRM 201505 and 201605 Trace Element Whole Blood, Seronorm, Billingsad, Norway). All the values of the reference materials were within certified limits. Instrumental detection limits, expressed as wet weight (w.w.). Element concentrations in tissues were expressed as mg kg^{-1} breast muscle wet basis.

5.3.4 Electrophoresis analysis

One dimensional SDS-PAGE analysis was used to evaluate the molecular weight profile of sarcoplasmic and myofibrillar proteins. In two replications, six samples were selected from each group to extract and separate proteins according to their molecular weights (MW) by SDS-PAGE analysis.

In two replications, 6 samples were selected from each group to extract and separate proteins according to their molecular weights by SDS-PAGE analysis. The MicroRotofor lysis kit (Catalog # 163-2141) was used to extract protein fractions. 100 mg of meat tissue was weighted in tubes containing resin. 500 µl of the prepared PSB solution was added to the tube containing resin. The content was ground by pestle, when large tissue pieces have been reduced, an additional 500 µl of prepared PSB solution has been added and grinding proceeded for 5 min. The obtained suspension was centrifuged at 20,000 x g for 30 min at 20°C. The supernatant was transferred into a new tube without disturbing the pellet. The separation was repeated two times with the same buffer. Protein concentration was measured by the Bradford method. Interfering substances that can negatively impact SDS-PAGE includes salts, detergents, denaturants, or organic solvents were removed by ReadyPrep™ 2-D cleanup kit (Bio-Rad, Catalog # 163-2130).

The concentration of extracted protein was measured using the Bradford assay (Bradford, 1976) while samples for SDS-PAGE were prepared according to Fritz et al. (1989). Electrophoresis have carried out according to the procedure described in 3.3.2.10 SDS-PAGE Analysis.

5.3.5 Technological traits (pH, drip and cooking losses and marinade performances)

pH, drip and cooking losses and marinade performances were measured as mentioned in 3.3.2.4 Samples preparation for processing.

5.3.6 Textural traits

Compression test: A raw cylindrical meat cuts having 2.5 cm diameter × 1 cm height and weighing about 5 g was excised from the caudal part of each fillet. It was compressed to 40% of the initial height by using a 25 kg loading cell connected to a 50 mm DIA cylinder aluminium probe using TA.HDi heavy duty texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK). The test speed of the probe was 1 mm/sec, while the pre- and post-test speeds were both 3 mm/sec. The compression value was recorded as the maximum force needed to compress 40% of the initial height of the sample and expressed in kg.

Texture Profile Analysis (TPA)

Cylindrical meat cut (3 cm of diameter and 0.8 cm of height) was excised from the caudal part of breast fillet cooked according to the procedure described in section 3.3.2.4.1 Non-marinated meat cuts (b. Cooking loss) to evaluate TPA values (Figure 5.1). Then samples were axially compressed (compression speed of 1 mm/s) using a cylindrical tool with 5 cm of diameter to 50% of their initial height in a double compression cycle (Figure 5.2). TA.HDi Heavy Duty texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) equipped with a 50 kg load cell was used.

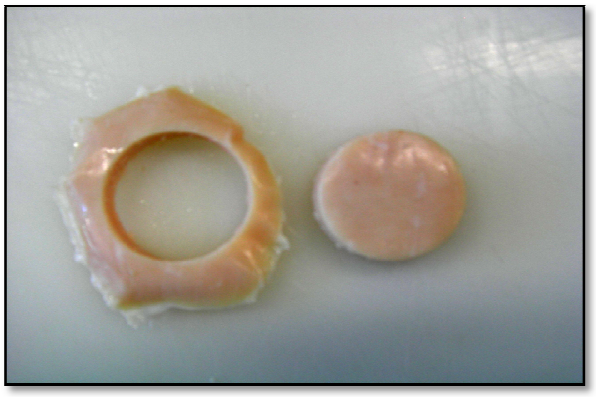


Figure 5.1 The sample used for the evaluation of the Texture Profile Analysis.

TPA was performed by double compression cycle, with the purpose to reproduce the chewing activity. For each sample, the TPA parameters (hardness, cohesiveness, springiness, gumminess and chewiness) were obtained by the elaboration of the double compression curve (force/deformation) represented in Figure 5.2 (De Campos *et al.*, 2008; Lyon *et al.*, 2010).

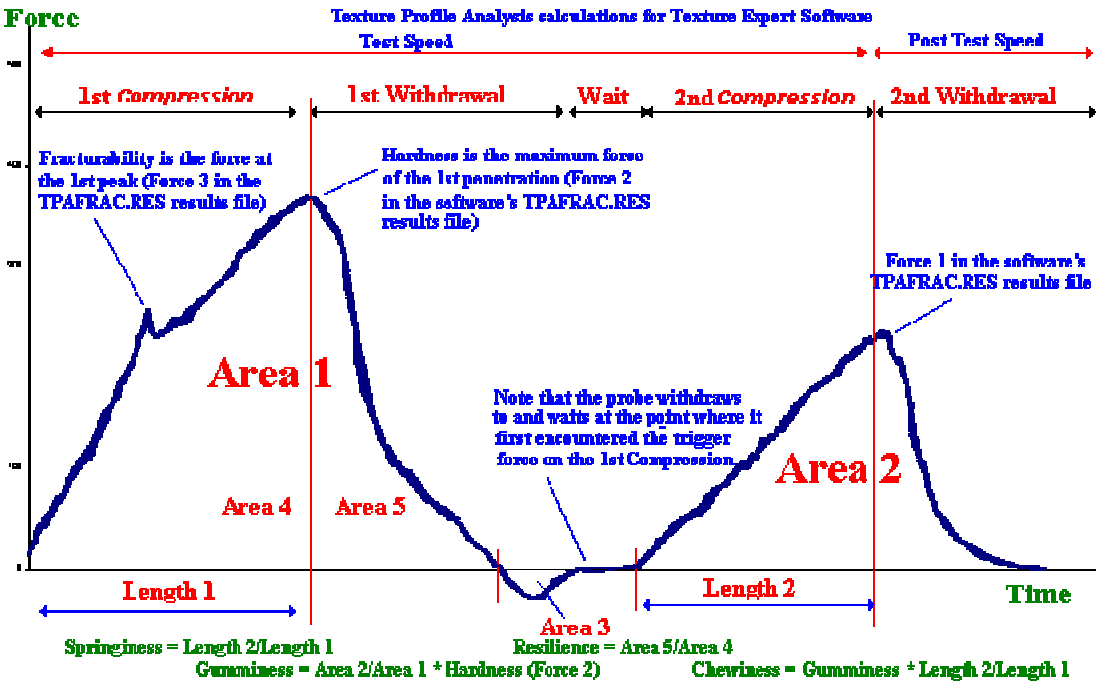


Figure 5.2 TPA general curve

Hardness is defined the peak force of the first compression of the product. The hardness does not necessarily occur at the point of deepest compression, although it typically does for most products. Cohesiveness is how well the product withstands a second deformation relative to how it behaved under the first deformation. It is measured as the area of work during the second compression divided by the area of work during the first compression. (Refer to Area 2/Area 1 in the below graph). Springiness is how well a product physically springs back after it has been deformed during the first compression. The spring back is measured at the down stroke of the second compression, so the wait time between two strokes can be relatively important. In some cases an excessively long wait time will allow a product to spring back more than it might under the conditions being researched. Springiness is measured in several ways, but more typically, by the distance of the detected height of the product on the second compression (Length 2 on the below graph), as divided by the original compression distance (Length 1). The original definition of springiness used the Length 2 only, and the units were in mm or other units of distance. Many TPA users compress their products a % strain, and for those applications a pure distance value (rather than a ratio) is too

heavily influenced by the height of the sample. By expressing springiness as a ratio of its original height, comparisons can be made between a more broad set of samples and products.

Gumminess only applies to semi-solid products and it is equal to Hardness *Cohesiveness (which is Area 2/Area1). Gumminess is mutually exclusive with chewiness for a product would not be both a semi-solid and a solid at the same time. Gumminess is definite as the force necessary to disintegrate a semisolid food until the condition that permits to swallow it. Chewiness only applies for solid products. Chewiness is mutually exclusive with Gumminess for a product would not be both a solid and a semi-solid at the same time. Chewiness indicates the force needed to disintegrate a solid food until the condition that permits to swallow it. It is calculated multiplying hardness × cohesiveness × springiness.

5.4 Statistical analysis

The results were statistically evaluated by using the ANOVA option of the GLM procedure present in SAS software (SAS Institute, 1988). The main effects of meat abnormality type (N, WB and WB+WS) and replication, as well as the interaction term on meat quality traits were evaluated. Means were separated using Tukey's honestly test of the GLM procedure (SAS Institute, 1988).

5.5 Results and discussion

5.5.1 Histological analysis

In pectoral muscle, overall, histological observations showed correlations with the gross lesions used as selection criteria during sampling. Indeed, all N breasts not showing neither hardened nor white striations on the surface had myofibers with a normal profile and endo- and perimysial connective tissues without remarkable alterations: primary myofiber fascicles (PMF) showed few abnormal fibers (Figure 5.3A, F1). On the other hand, muscle fibers with an abnormal polygonal profile (rounded fibers) were found in correspondence to diffuse hardened areas in WB breasts. These fibers showed different diameters and nuclear internalization (Figure 5.3B, F2 score): large caliber fibers were associated with small caliber fibers. Similarly, Sihvo *et al*. (2014) described myodegeneration accompanied by rounded fibers (reduced in number) and central nuclei in broiler wooden breast muscle. In addition, the same authors observed diffuse interstitial thickening with variable amounts of loose connective tissue, granulation tissue, and fibrosis in the areas affected by lesions (Sihvo *et al*., 2014).

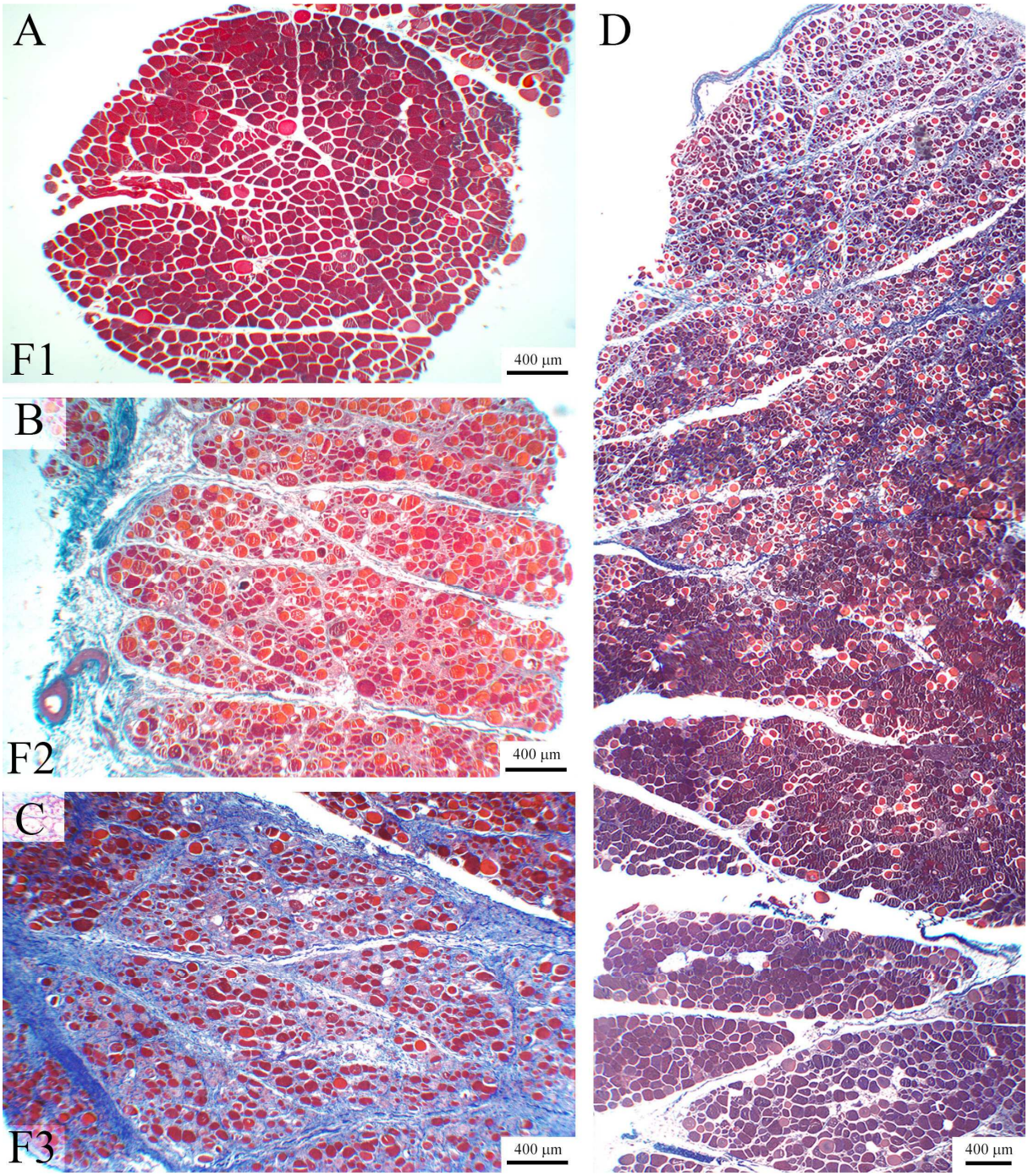


Figure 5.3 Images of mild (A), moderate (B) and severe (C) samples in the histological scale (ranging F1 to F3) used to score the levels of myodegenerations (Masson's Trichrome). A = the polygonal muscle fibers in an F1 score are well packaged and relatively of the same size. B = the fibers show different diameter and the perimysial connective tissue is thickened. C = The number of muscle fibers is reduced; variably sized muscle fibers are rounded and separated or replaced by a loose or more organized connective tissue. In (D) is represented a sample (score F3) with gradual progression of the histopathological lesions. From the surface

(upper part image) up within the muscle (lower part of the image) the histological lesions are gradually disappearing. (Masson's Trichrome).

Score F1 – mild: abnormal fibers ranging from 2 to 4 for each PMF (Figure 1A);

Score F2 – moderate: abnormal fibers ranging from 5 to 10 for each PMF (Figure 1B);

Score F3 – severe: abnormal fibers represent the majority of the fibers for each PMF (Figure 1C).

In the present study, proliferation and thickening of the perimysial network (fibrosis) was also observed, which appeared to separate muscle fibers. In addition, an increase in intramuscular fat was observed. In some cases, multifocal degenerative aspects of some fibers together with inflammatory cell infiltration (probably lymphocytes) were evident (Figure 5.3C). Finally, samples from fillets having both white stripes and hardened areas (WB/WS) exhibited profound degenerative myopathic lesions together with replacement of chronically damaged muscle with adipocytes and fibrosis of muscle tissue which was in agreement with the observations of Kuttappan *et al*. (2013a) in WS breast samples. In the present study, microscopic observations showed complete reorganization of skeletal muscle structure (F3 score) characterized by replacement of muscle fibers with boundless proliferation of peri- and endomysial connective tissue stained by Masson's trichrome. As a result of the severe fibrosis, connective tissue was the most abundant tissue in PMF. Fibers appeared to be decreased both in number and diameter and were round of small caliber. Many degenerate and/or necrotic fibers were accompanied by an interstitial inflammatory infiltrate (Figure 1C) in agreement with previous findings (Kuttappan *et al*., 2013a; Ferreira *et al.*, 2014; Sihvo *et al*., 2014).

An interesting macroscopic appearance was observed in majority of F3 samples, where there was a clear and gradual decrease of histopathological lesions from the surface towards the inside of the muscle: histological sections corresponding to these macroscopic lesions showed a gradual modification of the architecture of muscle tissue from external (surface muscle) to internal (about 1 cm deep) (Figure 5.3D).

5.5.2 Proximate and mineral composition

Proximate and mineral compositions are shown in Table 5.1. The presence of WS and WB either separated or together changed significantly the composition of breast meat. In particular, the presence of both abnormalities had a stronger effect on chemical composition than that of white striping or wooden abnormality alone. Fillets affected by both abnormalities and wooden alone showed significantly higher moisture content than WS and normal fillets. The presence of WS and WB either separated or alone has led to reduction of protein content and increase of fat and collagen content in comparison to normal fillets. There were no significant differences in protein and fat contents between WS and WB while they showed intermediate values in ash and collagen contents.

In general, meat affected by muscle abnormalities was characterized by higher fat and collagen contents and lower protein content than normal meat. In particular, the differences in the composition were stronger when wooden abnormality occurred in conjunction with WS. Similar changes in the chemical composition of white striped meat have been observed by Mudalal *et al.* (2014) and Petracci *et al.* (2014) where they found that white striped fillets showed higher fat and collagen content and lower protein percentages. And these results were described in chapter 3. Additionally, Kuttappan *et al.* (2012a) also found similar trends, that severe WS fillets had a higher fat content and lower protein content when compared with N fillets, although they found no effects on moisture and ash content. In particular, even if there were no available studies about the changes in the chemical composition of breast meat affected only by wooden abnormality or both abnormalities. It could be speculated that these differences may be explained due to the presence of similar histological changes in both abnormalities as have been observed in this study (Figure 5.3) where it was found that both abnormalities exhibited moderate to severe polyphasic myodegeneration with regeneration as well as a variable amount of interstitial connective tissue accumulation or fibrosis (Figure 5.3).

Table 5.1. Effect of wooden breast (WB) and wooden breast/white striping (WB+WS) on chemical and mineral composition of raw meat (n=32/group).

Parameter	Category				SEM	Sig.
	Normal	WS	WB	WB+WS		
Moisture (g kg^{-1})	741^{b}	742^{b}	753^{a}	751^{a}	1.7	***
Protein (g kg^{-1})	228^{a}	219^{b}	214^{b}	204^{c}	1.9	***
Fat (g kg^{-1})	8.7^{c}	13.6^{b}	12.5^{b}	19.8^{a}	0.78	***
Ash (g kg^{-1})	13.7^{a}	13.1^{ab}	12.6^{bc}	12.1^{c}	0.16	***
Collagen (g kg^{-1})	10.9^{c}	11.4^{bc}	11.8^{b}	12.6^{a}	2.8	***
Na (mg kg^{-1})	393^{b}	475^{b}	637^{a}	741^{a}	37.9	***
Ca (mg kg^{-1})	84^{b}	103^{ab}	201^{a}	131^{ab}	21.9	*
Mg (mg kg^{-1})	364^{ab}	406^{a}	350^{ab}	320^{b}	9.0	*
K (mg kg^{-1})	3,754^{ab}	4,117^{a}	3,794^{ab}	3,611^{b}	70.0	*
P (mg kg^{-1})	2,202^{a}	2,238^{a}	2,059^{ab}	2,079^{b}	29.1	*

Significance: *** P < 0.001; * P < 0.05.

$^{a-c}$ Means within a row followed by different superscript letters differ significantly (P≤0.05).

Hence, myodegeneration of muscle fibres may lead to decrease in protein content while fibrosis may result in increase of collagen tissue. Replacing of degenerated muscle fibers by adipose tissues through lipidosis process may also increase the fat content. The increase of moisture content may be explained due to occurrence of moderate to severe edema (fluid accumulation) as a result of the

inflammatory process that has been observed by Sihvo *et al.* (2014). On the other hand, the changes in the ash content may be explained as a consequence of the overall change in the chemical composition.

In respect to mineral content, all mineral contents that have been evaluated in this study (Na, Ca, Mg, K, and P) were significantly affected due to presence of muscle abnormalities. In general, the presence of WS and WB separated or together, has caused to increase the level of the mineral contents or in some cases there were no changes. WB and WS/WB had significantly higher content of Na (637 and 741 *vs.* 475 and 393 mg kg^{-1}, $P<0.05$) than WS and Normal fillets. Moreover, WB fillets showed higher content of Ca that normal fillet while WS and WS/WB had intermediate values. WS fillets had significantly ($P<0.05$) higher contents of Mg (406 *vs.* 320), K (4,117 *vs.* 3,611), and P (2,238 *vs.* 2,079 mg kg^{-1}) than WS/WB, respectively. On the other hand, WS exhibited intermediate values in Mg and K contents in comparison to normal fillets while there were no significant differences between WB and normal fillets. In addition, there was no significant difference in P content between WS and normal fillets.

This overall increase of Na and Ca and also in the other minerals especially in the presence of both abnormalities (WS and WB) may be explained due to the presence of several histological lesions such as multifocal degeneration and necrosis, characterized by fragmented hypereosinophilic amorphous fibers that have been observed in this study (Figure 5.3). In addition, several studies that have been done by Sandercock and Mitchell (2003; 2004) to explain the mechanism of stress induced myopathy showed increased level of intracellular calcium either by increased calcium crossing inside the cells (by specific calcium ionophores) or release of calcium from sarcoplasmic stores which leads to changes in membrane integrity. It was found that both increase of calcium and sodium may increase the enzymes level, causing membrane damage due to activation of phopholipase. The perturbation of ion balance underlying the myopathies in poultry is consistent with the mechanisms proposed for mammals such as Duchenne muscular dystrophy where patients had higher levels of calcium and sodium (Wallace and McNally, 2009).

The dysfunctionality of the membrane of myofibers is jointed with abnormal intracellular calcium and dysregulated calcium-responsive pathways. The patients with Duchenne muscular dystrophy and mdx mice showed higher levels of calcium (Robert *et al.*, 2001; De Backer *et al.*, 2002). The increase of calcium level has different consequences on the metabolic system of the myofibers. Firstly, it can induce the activity of calcium-dependent proteases (like calpain) which they can lyses different proteins located on the membrane of myofibres leading to myonecrosis (Iwata *et al*., 2003) and some these alterations have been observed in this study by histological analysis (Figure 5.3). Secondly, increase of cellular calcium level can also lead to abnormal

mitochondrial function (Millay *et al.*, 2008). In general, there is a strong relation between calcium dysregulation and the occurrence of pathophysiology of muscular dystrophy. Additionally, it was found an increase of sodium concentration was considered as signs of the initiation of muscle degeneration (Sandercock and Mitchell, 2004) and also these signs have been detected by histological analysis in this study (Figure 5.3).

5.5.3 Electrophoresis analysis

The results of SDS-PAGE analysis for sarcoplasmic and myofibrillar meat proteins obtained from normal, wooden, and WB+WS fillets were expressed as relative abundance to avoid the small differences due to protein loading among lanes (Table 5.2 and 5.3). Moreover, to exclude the variations in protein quantification between gels, different concentrations of the standard protein BSA were loaded on each gel to build a separated standard curve. Only standard curves with good linearity (R^2> 0.98) were considered and no band lower or higher than the linear range of densitometer was considered. The presence of wooden alone or with WS exhibited an effect on the distribution patterns of both sarcoplasmic and myofibrillar proteins (Figure 5.2). The relative abundance of nine bands of myofibrillar proteins having MW ranging from 16 to 220 kDa has been determined (Table 5.2).

All myofibrillar protein bands except LC1 slow-twitch light chain myosin (27.5 kDa) did not show any significant difference in the relative abundance between all groups. On the contrary, WS/WB fillets exhibited a significantly lower relative abundance (8.4 *vs.* 12.5, $P<0.05$) of LC1 slow-twitch light chain myosin (27.5 kDa) than normal fillets while WS and WB exhibited intermediate values. This trend could be explained by under expression of LC1 or increased degeneration of LC1 in affected fillets than in normal ones.

For sarcoplasmic proteins, the relative abundance of 11 bands having MW from 25 to 114 kDa has been determined in WS, WB, and WB+WS samples while only 10 bands having MW from 25 to 90 kDa have been determined in N group (Table 5.3). There were no significant differences in the relative abundance of TPI1 (Triosephosphateisomerase, 26.4 kDa), carbonic anhydrase, and pyruvate kinase (PK) between all groups. One band corresponding to calcium-ATPase (114 kDa) did not appear in normal meat, but was present in the other groups. This is particularly interesting as calcium-ATPase is a transport protein in the plasma membrane of the cells that functions to eliminate calcium cations. As it is very important for cells to keep the low concentration of calcium cations for proper cell signalling (Carafoli, 1991; Carafoli and Stauffer, 1994), the increase of calcium level in WB and WB+WS groups may be dependent on the presence of calcium-ATPase which was not found in N group.

Table 5.2 Effect of wooden breast (WB) and wooden breast/white striping (WB+WS) abnormalities on relative abundance (%) of myofibrillar proteins in raw meat (n=6/group).

Protein	Molecular weight (kDa)	Category				s.e.m.	Sig.
		Normal	WS	WB	WB+WS		
1. LC3	16	14.1	14.6	14.1	15.8	0.69	n.s.
2. LC2	19	1.2	3.0	1.1	1.7	0.22	n.s.
3. LC1	27.5	12.5[a]	10.1[ab]	9.3[ab]	8.4[b]	0.48	*
4. Sub-troponin	29	4.1	4.5	4.9	5.0	0.16	n.s.
5. Tropomyosin	34	4.7	4.3	5.2	4.6	0.14	n.s.
6. Actin	42	34.1	32.2	34.4	34.6	0.91	n.s.
7. Desmin	53	5.2	6.1	6.6	6.3	0.25	n.s.
8. Troponin	70	4.9	6.7	6.7	6.5	0.33	n.s.
9. MHC	220	15.9	17.8	15.6	15.4	0.91	n.s.

Significance: * P≤0.05; n.s., not significant.

MHC, myosin heavy chain; LC, myosin light chain.

[a-b] Means within a row followed by different superscript letters differ significantly (P≤0.05).

In general, there were significant differences in the relative abundance of sarcoplasmic protein bands of phosphoglyceratemutase (25 kDa), lactate dehydrogenase (34 kDa), glyceraldehydes dehydrogenase (36 kDa), aldolase (39 kDa), creatine kinase (43 kDa), phosphoglucose isomerise (58 kDa), pyruvate kinase (68 kDa), glycogen phosphorylase (90 kDa), and calcium-ATPase (114 kDa) between groups. WB+WS fillets exhibited a significantly higher relative abundance of phosphoglycerate mutase (PGAM: 7.2 *vs.* 6.2%, P<0.05), creatine kinase (CK: 10.9 *vs.* 9.6%, P<0.001), and glycogen phosphorylase (GP: 7.7 *vs.* 5.4%, P<0.05), while a lower relative abundance of lactate dehydrogenase (LDH: 18.6 *vs.* 22.3%, P<0.05), glyceraldehydes dehydrogenase (GAP: 11.2 *vs.* 13.3%, P<0.05), and aldolase (ALD: 7.2 *vs.* 9.6%, P<0.05) than normal fillets. There were no significant differences in the relative abundance of PGAM, TPI1, carbonic anhydrase, LDH, PGI, and PK between normal and WS fillets while ALD, CK, and GP exhibited intermediate values. WS fillets showed intermediate values of relative abundance of PGAM, LDH, and CK with respect to WB fillets, while for other bands there were no significant differences.

Table 5.3 Effect of wooden breast (WB) and wooden breast/white striping (WB+WS) abnormalities on relative abundance (%) of sarcoplamic proteins in raw meat (n=6/group).

Protein	Molecular weight (kDa)	Category				s.e.m.	Sig.
		Normal	WS	WB	WB+WS		
1. PGAM	25	7.2[a]	7.2[a]	6.3[ab]	6.2[b]	0.14	*
2. TPI1	26.4	7.3	7.3	7.3	6.8	0.14	n.s.
3.Carbonicanhydrase	31.8	9.6	9.0	9.9	9.0	0.28	n.s.
4. LDH	34	18.6[b]	18.6 [b]	21.2[ab]	22.3[a]	0.44	*
5. GAP	36	11.2[b]	13.1 [a]	13.3[a]	12.8[a]	0.30	*
6. ALD	39	7.1[b]	8.4 [ab]	8.6[ab]	9.8[a]	0.31	*
7. CK	43	10.9[a]	10.1[ab]	9.5[b]	9.6[b]	0.14	***
8. PGI	58	7.7[a]	7.8 [a]	7.3[a]	5.4[b]	0.33	*
9. PK	68	5.6	4.9	4.5	4.7	0.18	n.s
10. GP	90	13.6[ab]	11.7 [b]	11.7[b]	14.4[a]	0.35	*
11. Calcium ATPase	114	n.d[b].	3.2 [a]	3.3[a]	2.7[a]	0.30	***

Significance: *** P < 0.001; * P < 0.05; n.s., not significant, n.d. not detected.

[a-c] Means within a row followed by different superscript letters differ significantly (P≤0.05).

PGAM, phosphoglyceratemutase; lactate dehydrogenase, LDH; glyceraldehydes dehydrogenase, GAP; aldolase, ALD; creatine kinase, CK; phosphoglucose isomerise, PGI; pyruvate kinase, PK; glycogen phosphorylase, GP.

Previous studies that evaluated the meat tissue affected by myscle abnormalities showed that a part of myofibrils had poor functionality of sarcolemma and that there was a loss of sarcoplasmic fluids, which contain sarcoplasmic proteins (Stracher *et al.*, 1979; Sihvo *et al.*, 2014). Due to the differences in the relative abundance between different glycolytic enzymes, it can be speculated that protein turnover has not had the same rate during muscle degeneration and regeneration of all proteins, as suggested by previous histological studies that showed polyphasic degeneration (Kuttappan *et al.*, 2013; Sihvo *et al.*, 2014). In addition, Mudalal *et al.* (2014) found different sarcoplasmic protein patterns between N and WS meat by using SDS-PAGE analysis.

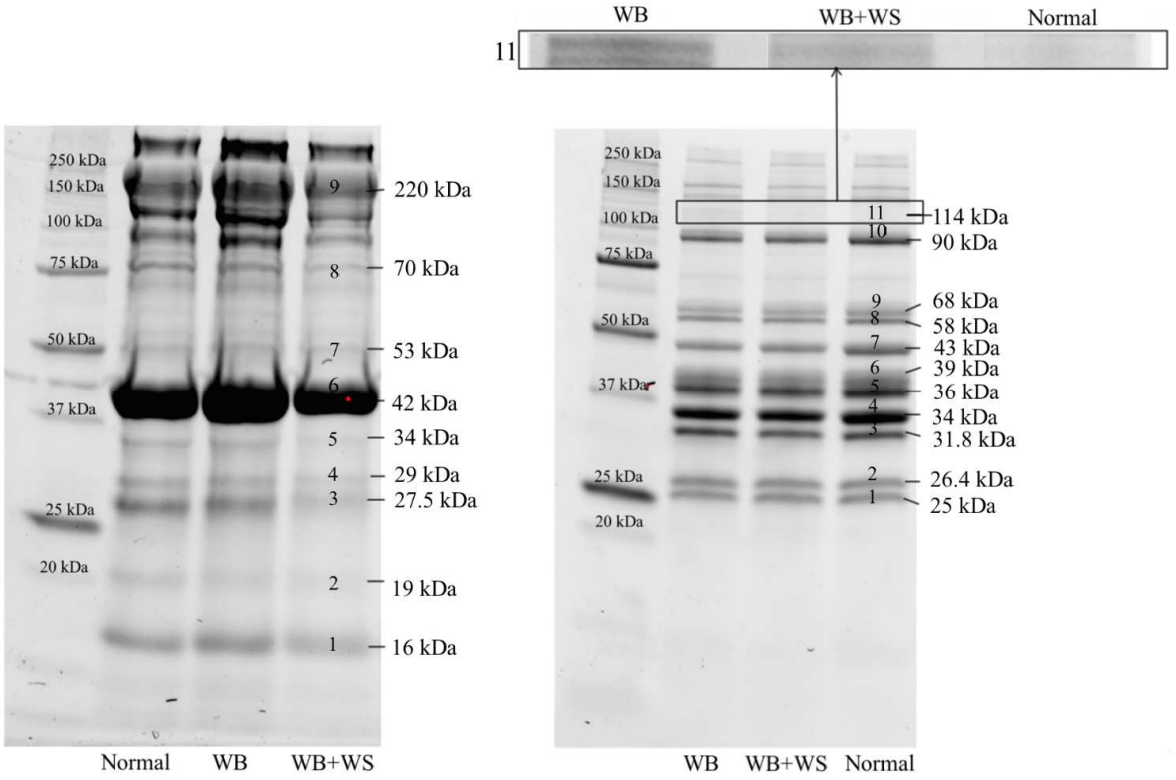


Figure 5.4 SDS-PAGE analyses of myofibrillar and sarcoplasmic proteins for normal and abnormal samples.

5.5.4 Technological traits

The results of the effect of wooden abnormality on pH, drip and cooking losses and marination performances are shown in Table 5.4. Overall, fillets affected by both abnormalities (WB+WS) had significantly higher ($P<0.001$) ultimate pH value than N and WB fillets which did not differ each other. Additionally, there was a general decrease in the ability to retain liquid during refrigerated storage (i.e. drip losses) and cooking as well as to pick-up of marinade solutions (i.e. marinade uptake) in WB and WB+WS fillets. These results were in agreement with our previous findings (Mudalal *et al.*, 2014). Impairment of water holding/binding ability in WB+WS samples cannot be ascribed to differences in ultimate pH where in this study was higher than normal (i.e. higher pH are associated with higher WHC), but it can be attributed to the negative effects of WB abnormality which have been described through histological analysis (Figure 5.3) and characterized mainly by degeneration of muscle fibres companied by fibrosis and lipidosis. Alterations in fibre membrane integrity may have contributed to the loss of liquid during refrigerated storage and cooking. Moreover, degeneration of muscle fibres (Figure 5.3) may affect the ability of meat to bind water because it is well known that the majority of water (>85%) in the cell is held in the myofibrils and most of water is retained (steric) by capillary forces which is generated due to the arrangement of thick and thin filaments within the myofibril (Huff-Lonergan and Lonergan, 2005). This

cytoarchitectural design may be disorganized due to the histological changes that have been previously described. From the other hand, the change in the chemical composition is likely to have played a major role in the reduction of WHC. To illustrate, decrease of protein content (in particular contractile proteins due to fibres degeneration) which has major role (due to high hydrophilicity) in binding water molecules during storage and processing may contribute to this reduction. Additionally, increase fat content (high hydrophobicity) may reduce the ability of meat to bind water.

Table 5.4. Effect of wooden breast (WB) and wooden breast/white striping (WB+WS) on pH and water holding ability of non-marinated and marinated meat (n=32/group).

Parameter	Category			SEM	Sig.
	Normal	WB	WB+WS		
Non-marinated meat					
Ultimate pH	5.82^{b}	5.87^{b}	6.05^{a}	0.019	***
Drip loss (g kg^{-1})	9.9^{b}	12.2^{a}	10.3^{ab}	0.36	*
Cooking loss (g kg^{-1})	215^{c}	278^{b}	297^{a}	4.3	***
Marinated meat					
Uptake (g kg^{-1})	128^{a}	69^{b}	62^{b}	5.4	***
Cooking loss (g kg^{-1})	153^{c}	175^{b}	189^{a}	3.1	***

Significance: *** $P < 0.001$; * $P < 0.05$

[a-c] Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

5.5.5 Textural traits

The results of compression test and Texture Profile Analysis are shown in Table 5.5. Both WB and WB+WS raw fillets exhibited significantly higher compression force than N fillets (4.2 and 3.3 *vs.* 1.9 kg; $P<0.001$). Moreover, the texture profile analysis revealed the presence of significant differences ($P<0.05$) between groups in hardness, gumminess, springiness, and chewiness of cooked meat, where all values except springiness were higher in WB and WB+WS groups than N group. WB+WS and WB groups differed each other significantly only in springiness and chewiness that had higher values in WB fillets.

The instrumental tests conducted in this study confirm that the WB fillets are characterized by a very noticeable hardness in raw meat as shown by the dramatic increase of compression force values which confirmed our earlier findings (Mudalal *et al.*, 2014). Therefore, instrumental compression tests can be helpful to objectively establish the presence of the wooden abnormality in raw breast fillets. In addition, it was found that textural traits of cooked meat are dramatically modified in WB fillets, which were harder, more gummy, less elastic (lower springiness) and thus

requiring more energy to crumble (higher chewiness). In general, the changes in texture profile are considered a consequence of complex chemical changes (Wattanachant *et al.*, 2004) which are more relevant to muscle fibers and connective tissues. The SDS-PAGE analysis in this study showed primarily that there was a different pattern in myofibrillar proteins between normal and abnormal meat which may indicate compositional changes. While the increase in the quantity of collagen (as a result of fibrosis) may also contribute to the textural changes, in particular, the structural changes in collagen may also have a role in these changes but this aspect until now was not investigated. On the other hand, the increase in hardness, gumminess, and chewiness values in WB and WS/WB fillets can be explained due to higher cooking loss which depends on lower WHC (Table 5.4) which is normally led to shrinkage of muscle due to protein denaturation and hence increasing the packing density of the fibers after cooking (Huff-Lonergan and Lonergan, 2005).

Table 5.5 Effect of wooden breast (WB) and wooden breast/white striping (WB+WS) on textural traits of raw and cooked meat (n=32/group).

Parameter	Category			SEM	Sig.
	Normal	WB	WB+WS		
Raw meat					
Compression test (kg)	1.9^{b}	4.2^{a}	3.3^{a}	0.23	***
Cooked meat					
Hardness (kg cm^{-2})	19.1^{b}	22.1^{a}	21.6^{a}	0.53	*
Cohesiveness	2.80	2.87	2.93	0.065	n.s.
Gumminess (kg cm^{-2})	52.5^{b}	63.3^{a}	61.3^{a}	2.0	*
Springness (mm)	1.66^{a}	1.66^{a}	1.58^{b}	0.017	*
Chewiness (kg × mm)	89.3^{a}	107.3^{c}	97.8^{b}	3.7	*

Significance: *** $P < 0.001$; * $P < 0.05$; n.s., not significant

[a-c] Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$).

It is clear that sensory properties of breast meat are deeply impaired by WB abnormality which does not only affect the appearance and texture of raw breast meat, but also it can lead to a decline of sensory traits when meat is either cooked at home or used to manufacture processed products. In this context, a recent study evaluated the impact of inclusion of different levels of WB on the quality traits of coarse meat products (Puolanne and Ruusunen, 2014). Even if comminuting is able to reduce negative effects of inclusion of WB in meat batters, it was found that replacing lean meat with meat affected by the wooden abnormality at certain levels (15 to 30%) in formulating of comminuted sausages and nuggets has changed the texture and other quality traits of the finished products (Puolanne and Ruusunen, 2014).

5.6 Conclusions

In conclusion, meat affected either by WB alone or by WB plus WS had a different chemical composition characterized by higher fat and collagen content and lower protein content. Additionally, the defected breast fillets exhibited lower processing ability which was characterized by lower WHC and harder texture. All these changes may raise some questions about the suitability of these types of meat for processing and consumer acceptance. On the other hand, the results of mineral composition indicated that there was ion dysregulation which may be relevant to the occurrence of pathophysiology of muscular abnormality. The discrepancy in protein patterns of sarcoplasmic and myofibrillar proteins may also indicate the presence of muscle fibre degeneration. All these results were also confirmed by histological analysis that revealed that the aberration in the cation homeostasis may be somehow standing behind the pathophysiology in the muscle abnormality.

UNIT-II-

CHAPTER 6

Literature review: functional ingredients and innovative formulations in processed poultry and rabbit meat products

6.1 Abstract

In our modern life, the consumer preferences are dramatically changing toward more convenient, easy to prepare, and attractive food products, this attitude has pushed the meat industry to employ several additives (sodium chloride, nitrites, phosphates, etc.) during formulation to meet the dynamic consumer demands. Accordingly, different health organization started to warn about the health implications of the usage of additives in processed meat products. Hence, in response to these changes meat industry has launched different innovative strategies to improve the health image of meat and meat products taking into the consideration the technological and economical aspects. These strategies are based on two approaches which are usually used individually or together. The first approach is to reduce the level of unhealthy substances such as sodium chloride, phosphates, nitrites and nitrates, cholesterol, unsaturated fatty acids, and total fat. The second approach is to increase the level of healthy substances such as natural antioxidants, omega-3 fatty acids, probiotics and bioactive peptides. This chapter will deal with the first approach, in particular, focusing on functional ingredients to improve sensory and processing ability of meat as well as health image. In this regard, the functional ingredients that will be discussed are inorganic salts (i.e. sodium chloride, phosphates, and bicarbonate) and organic compounds which are mainly derived from plant and animal origins. In addition, it will deal with functional ingredients that are commonly used in processed meat products, their mechanisms of action, the scope of applications and current market trends. Functional ingredients could be effectively used by adopting integrated approaches which manage the use of these ingredients in a way to allow producing clean labels, accepted meat products and at the same time optimize the cost of formulations. In addition, this chapter will deal with the technical aspects of these ingredients that are relevant to innovative formulation of processed meat products.

Key words: Processed meat, formulation innovation, functional ingredients, and technological traits.

6.2 Introduction

During the last few decades, the consumption of poultry meat has tremendously increased and also the current forecast and projection studies point out that poultry market will be keep growing in future (Anonymous, 2011). The main reasons for the success of poultry meat production as have been explained in "Chapter 1" which briefly are: i) the healthy and nutritional image of poultry products (high-protein content, low-fat content coupled with a balanced n-6 to n-3 polyunsaturated fatty acids ratio, low cholesterol, and presence some of functional components); ii) their suitability for processing which enables the meat producers to launch more attractive, convenient, easy to use products; iii) lower price compared to red meats; and iv) absence of cultural or religious effect (Cavani *et al.*, 2009). Poultry meat is more favorable for processing in comparison with other type of meats because it has neutral flavor, consistency and good texture, and light color. These characteristics allow for producers to impart desired flavor profiles (i.e. spicy *vs.* mild flavor) and textures according to market needs and consumer segments (i.e. adult *vs.* children) (Barbut, 2012). These unique features of poultry meat, made it more qualified for innovation during processing. In this context, rabbit meat has similar features to poultry meat, but it is still not exploited in innovation process due to relatively the high cost of production and also due to cultural aspects that consider the rabbit as a pet animal (Cavani *et al*., 2009; Petracci and Cavani, 2012a).

Despite of the previous limitations, there is a potential to exploit rabbit meat in a more effective way by employing the nutritional properties of rabbit meat to produce healthy products that are low in fat with a high unsaturation fatty acids and low sodium, cholesterol and high in heme-iron levels. In addition, employing functional ingredient during processing of rabbit meat products can alleviate the cultural image about rabbit as a pet animal, while processing of rabbit meat can give a real opportunity to mask its wild flavour which may improve the sensory acceptance of rabbit meat (Dalle Zotte and Szendro, 2011; Petracci and Cavani, 2012a).

According to the final destination of meat muscle and the degree of size reduction applied on the muscle, processed poultry meat products could be grouped in 4 categories: i) whole-muscle products such as marinated whole carcass or cut-ups where the cyto-architectural design and

geometric distribution of intra- and extra-cellular water are maintained intact; ii) formed/restructured products manufactured by chunks or pieces of meat bonded together such as rolls and hams; iii) ground products made of coarse minced meat, such as burgers and sausages where meat fibrous structure is still detectable to some extent; iv) emulsified products such as frankfurters which are made of finely comminute meat slurry in which meat fibre structure is disappeared (Figure 6.1).

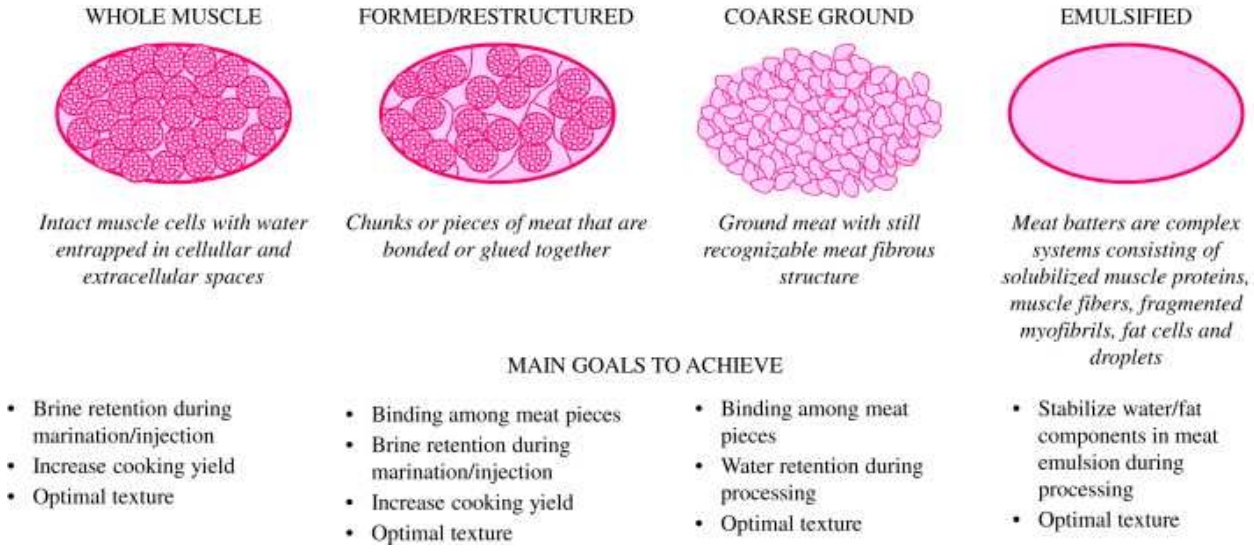


Figure 6.1 Classification of poultry meat products according to structure of raw meat materials and the roles of the functional ingredients in each class of product.

The functional properties (in this chapter, the use of the term “functional ingredients” is just from a technological viewpoint: texture and water holding capacity) of raw meats depend mainly on chemical composition which varied according to the anatomical position of the muscle, genotype and age of animal, and feed composition which have great impact on quality of processed meat products (Givens *et al.*, 2011). White meats like breast fillet and tender have a soft texture, light color, low-fat and high-protein content, therefore they are normally dedicated to produce premium meat products. From other hand, dark meats like thigh and drumstick have more harder texture, darker color, higher fat content, and stronger flavor, but due to the their lower economic value; they are widely used to optimize the costs of product formulation (Bianchi *et al.*, 2009).

In the context of the functional properties of meat and meat products, there are some obstacles and challenges which can be grouped into 3 main categories. The first, natural variability in the

quality of raw meat due to variability in ante-mortem, post-mortem and processing factors (Petracci *et al.*, 2009; 2010). The second, appearance of some recent muscle dystrophies or abnormalities due to the improvements in growth rate and muscle yield such as pale, soft and exudative (PSE) meat, immaturity in intramuscular connective tissues, white striping and wooden which impaired water binding capacity, color, and appearance of meat (Barbut *et al.*, 2008; Petracci and Cavani, 2012a; Petracci *et al.*, 2013b; Sihvo *et al.*, 2014).

Finally, the shift towards further processed products and changing in consumers' attitude towards foodstuff has underscored the necessity for higher standards in poultry meat quality in order to improve sensory characteristics and functional properties (Barbut *et al.*, 2008; Cavani *et al.*, 2009). Integrated approaches can be employed to manage all former obstacles and challenges to alleviate their consequences on functional properties of processed meat products.

Using functional ingredients to optimize the functional properties of processed meat can reduce the effect of natural variability in the quality of raw meat and at the same time can provide more flexibility for processed meat producers to introduce a broad spectrum of products for meeting the consumer demands and at the same time optimizing of the cost formulations.

The addition of functional ingredients helps to modify the overall technological and sensory characteristics of meat products such as water holding capacity (WHC), fat holding capacity and texture properties. These ingredients can be divided based on their mechanisms of action into two main groups: salts which are added in order to enhance functionality of muscle proteins (myofibrillar), and organic compounds of different plant and animal origins which play an indirect effect on water/fat retention and texture modulation by working alongside of myofibrillar proteins (Lamkey, 1998). The first group basically consists of sodium chloride, phosphates, citrates and alkaline salts (i.e. bicarbonates), while the second one includes starches, cereal flours, hydrocolloids (i.e. carrageenans, alginates), collagen derivatives, blood proteins (plasma and globin fractions), milk proteins (caseinates and whey proteins) as well as plant proteins (i.e. soy, pea) and fibers (Table 6.1).

In this chapter, both inorganic salts and organic compounds which are usually used as "functional ingredients" have been reviewed, focusing on the roles of these ingredients and their impact on economic values and quality issues. Moreover, the applications of these ingredients in formulating healthy meat products have been explained. Functional ingredients will be addressed based on their roles in water binding (either "native" or added during processing), fat-binding

capacity, adhesion and cohesiveness of product fragments together, and improving the texture and cooking yield. The possibility of using functional ingredients to replace other traditional ingredients that having nutritional drawbacks will be also discussed.

Table 6.1 Main categories of functional ingredients used in poultry meat products manufacturing.

Ingredients to enhance the functionality of muscle proteins	Ingredients with a direct functional effect on the meat system
Sodium chloride (table salt)	Vegetable compounds
Phosphates	Starches
Citrates	Flours
Carbonates	Proteins
	Fibers
	Hydrocolloids (i.e. carrageenans, alginates)
	Animal compounds
	meat by products (collagen derivatives, blood proteins)
	milk proteins (caseinates and whey proteins)
	egg albumen

The types of the functional ingredients which could be selected in meat formulations depend mainly on the type of manufacturing techniques, raw materials, and intended purpose for finished products (Figure 6.1). From a technological viewpoint, improving water binding capacity to the utmost level by functional ingredients can achieve a reduction in cost of formulation; with the increasing the ability of meat to bind very cheap raw materials such as water and MDM (mechanically deboned meat) during processing.

6.3 Ingredients to enhance the functionality of muscle proteins

Main functional salts used in manufacturing of poultry meat products are sodium chloride, phosphates, citrates and carbonates. Sodium chloride and phosphates are widely used in all poultry meat products, while the use of citrates and especially carbonates are more limited (Table 6.2). In the next sections, the different applications and mechanisms of these salts in processed meat product will be discussed.

Table 6.2 Classification, dosage and main product applications of the main functional salts used in poultry meat processing.

Salts	E-number	Current dosage	Main product applications			
			Whole-muscle	Formed/ Restructured	Coarse ground	Emulsified
Sodium chloride	No	up to 2.0%	+++	+++	+++	+++
Phosphates[1]	Yes	up to 0.3%	+++	+++	+++	+++
Citrates	Yes	up to 0.5%	++	++	++	++
Carbonates[1]	Yes	up to 0.2%	+	+	+	+

[1] allowed in most countries only in cooked products

+++ = high; ++ = medium; + = low

In most countries, sodium chloride is the sole salt that classified as ingredient and also it is usually used at higher dosages. The mechanism of actions and purposes of usage of these functional salts in poultry meat products are summarized in Table 6.3.

5.3.1 Sodium chloride

Sodium is one of important micronutrients that needed to regulate the volume of extracellular fluid and plasma. It also engages in the active transport chain for some molecules on the cell membrane (Adrogue and Madias, 2007). The increase of dietary sodium intake was considered one of the important contributing factors in increasing the incidence of hypertension. Hypertension plays a major role in increasing the occurrence of cardiovascular disease (CVD) and end-stage renal disease (Desmond, 2006). Sodium dietary intake in western societies is relatively high, and this can present a main risk to salt-sensitive population who is more prone to have high blood pressure and a result more serious diseases such as cardiovascular diseases, diabetes, and kidney disease (Doyle and Glass, 2010).

Table 6.3 Mechanisms of action and other effects of the main functional salts used in poultry meat processing.

Salts	Increase ionic strength and promote ion exchange	Improve muscle swelling by chloride (Cl^-) effect	Improve muscle swelling by sequestrating Calcium (Ca^{2+})	Increase meat pH	Other effects
Sodium chloride	+++	+++	O	O	- Provide and enhance flavor - Alter microbial growth
Phosphates	+	O	+++	+	- Protection against lipid oxidation and discoloration - Flavor protection and enhancing
Citrates	+	O	o/?	+	- Protection against lipid oxidation and meat discoloration
Carbonates	+	O	O	+++	- Might decrease shelf-life due to vey high increase of meat pH

+++ = high; ++ = medium; + = low; o = none

In several developed countries, dietary sodium intake exceeded the nutritional recommendations (Ruusunen and Puolanne, 2005). In this regard, it was found that sodium intake in some countries exceeded the recommended daily intake by two or three times or more. The daily sodium intake of Finnish men was 9.9 g/day (The National FINDIET 2002 Study, 2003) while in U.K was 8.2 g/day (Godlee, 1996). The recommended dietary intake of sodium chloride can vary according to health status and the age of the individuals, but in general, according to the most public health organizations and regulatory authorities the recommended daily intake of sodium chloride must not exceed 6 g /day, while for genetically sensitive person, salt intake should be in range between 1 and 3 g/day (Desmond, 2006).

In healthy human, the body has several hormones and the sympathetic nervous system which adjusts the concentration of sodium in plasma within a specific range and the body can eliminate the excess of dietary sodium by excretion the sweat and urine to keep the fluid balance. Due to the aging process or some chronic diseases, the body loses its ability to regulate the electrolyte and so the efficiency of kidney and excretory systems reduce which causing increase of sodium level companied by increase the volume of plasma and eventually trigger hypertension.

There is a persistent increase in pre-hypertension or hypertension cases, some researchers pointed out that it could reach more than 60% of adults (Dickinson and Havas, 2007). Different factors can affect on hypertension such as age, physical activity, body mass index, and dietary sodium and potassium level. Dietary sodium is considered as a major contributing factor in hypertension (Hollenberg, 2006). By excluding the other cardiovascular factors even the blood pressure, it was found that high sodium intake had a direct effect in increasing mortality and risk of coronary heart disease (Tuomilehto, 2001). Cardiovascular disease is considered as major risk for 25% of the adult population in the world (Adrogue and Madias, 2007). Despite that the dietary advices has resulted in reduction of sodium intake in the half of hypertensive patients in the US (Ayala *et al.,* 2010). But recently, a survey pointed out that a high percentage of consumers in US outpaced the recommended daily tolerable upper limits of sodium intake (Doyle and Glass, 2010).

Increase the awareness of consumer in recent years towards the relationship between high sodium intake and hypertension has resulted in the increase of demand of low salt meat products. Accordingly, meat producers have started to develop low-salt products to meet this demand. Overall, the reduction of sodium in meat products is not easy approach, because its effect is not confined on the perceived saltiness, but it could have some consequence on flavor, texture, and microbiological stability. Even those, meat producers have succeeded to launch several types of low-salt meat products into the market with acceptable technological properties and microbiological stability. Low-salt meat products showed weak flavor characteristics in comparison to normal-salt products. This problem can be alleviated or overcome by satisfactory seasoning substitute (Ruusunen and Puolanne, 2005).

In the meat industry, sodium chloride is considered as a multifunctional ingredient that has the ability to improve the texture and WHC by solubilization/extraction of the salt-soluble myofibrillar proteins in raw meat, promoting the taste, enhancing the flavors of meat and

ameliorate microbiological stability. The theory about the role of sodium chloride in improving WHC of meat products has extensively been reviewed (Ruusunen and Puolanne, 2005; Puolanne and Halonen, 2010). In meat matrix, sodium chloride dissociate into sodium (Na^+) and chloride (Cl^-) ions, Cl^- ions are adsorbed more strongly than Na^+ ions to positively charged groups of myosin. Binding of chloride ions to myosin and actin filaments increases the electrostatic repulsive forces between fibers, causing unfolding of the protein structure matrix and expanding the spaces between actin and myosin (Hamm, 1986). Moreover, the adsorption of Cl^- ions with positively charged groups of myosin results in a shift of the isoelectric point towards a more acidic pH value. And so the gap between the ultimate pH of meat and isoelectric point increases giving more flexibility to improve water binding capacity (Feiner, 2006a, chap. 5). A salt concentration of 1.0 to1.6% can be considered the most widely used in poultry meat formulations. Our data revealed that even low concentration (i.e. 0.5%) of salt can significantly improve WHC of poultry meat (Petracci *et al.*, 2013d).

Different strategies have been developed worldwide to alleviate the excess of sodium intake such as increase the awareness of public by education or by individual dietary counseling, improving food labeling, coordinated and voluntary sodium reduction by the industry, government and private sector food procurement policies, and setting of regulations (Laura *at al.*, 2012; Busch e*t al.*, 2013).

In this context, Finland and UK had successfully achieved a reduction in salt consumption by adopting some strategies (He *et al.*, 2013). In general, processed meat products contribute about 10-21% of dietary sodium daily intake (FSA, 2009). In particular, in some countries like U.K and Ireland, processed meat products contribute more than 20% of daily dietary sodium intake (Engstrom *et al.*, 1997) which can be considered a vital portion in comparison with other foods. Replacing sodium chloride directly by some of common salt replacers (potassium, magnesium, and calcium chloride, salt of lactate, citrate, ascorbate, and sulphate) is one of the less sophisticated strategies.

Potassium chloride is most commonly used in meat formulations. Nevertheless, inclusion of salt replacers at certain levels could add bitter and metallic aftertaste to meat products. Bitter/metallic aftertaste could be usually managed in two approaches, either by using of salt replacers in certain levels under the threshold value of bitter aftertaste detection, or by using additional components or ingredients that can mask the metallic/bitter aftertaste. The first

approach has been extensively evaluated and several studies revealed that there is a possibility to use potassium chloride up to 30 to 40% as a salt replacer without impairing the functional and sensory properties of processed meat products, but the 15% increase in the amount of potassium chloride in comparison to sodium chloride must be added to achieve the same strength of protein solubility due to the differences in molecular mass between potassium and sodium chloride. Replacing sodium chloride with potassium chloride could have some consequences for the microbiological stability of the product at certain levels. Excess of potassium chloride intake could be a serious problem for people who are suffering from heart problems (Li *et al.*, 2009; Lee *et al.*, 2012; Sinopoli and Lawless, 2012).

In the second approach, masking the metallic/bitter aftertaste can be achieved by modulation of processing techniques or tuning the formulation or addition of some flavors (pepper, onion, garlic, sweet pepper) (Koliandris *et al.*, 2010; Toldra and Reig, 2011). Another strategy for achieving a salt reduction in the meat products; is represented by the use of flavor or salt enhancers (alapyridain, alkyldienamides, high ribonucleotide yeast extract, and dehydrated protolyzed milk or cereal proteins) which increase the perception of salt in the finished products. Salt enhancers can achieve up to 20% of salt reduction (Desmond, 2006; Busch *et al.*, 2013).

Finally, some attempts have been done to enhance the taste bioavailability of salt by modifying its physical status (i.e. lowering particle size by means of micronization/encapsulation) (Broadway *et al.*, 2011; Busch *et al.*, 2013). However, this approach is more effective for product in which the salt maintains its original physical form (i.e. sprinkled on the surface), whereas it is less effective when salt is solubilized in water such in meat system.

6.3.2 Phosphates

Phosphates are salts of phosphoric acid and are available in different chemical forms (orthophosphates, pyrophosphates, tripolyphosphates, and polyphosphates). Phosphates are used to improve the quality of many foodstuffs, but specifically in meat and seafood, phosphates work as water binding, antioxidant, antimicrobial and buffering agents. In the last decades, some EU countries, such as Italy and France, showed a huge drop of phosphate usage due to the bad consumer perception towards this type of additive. In meat related applications, different phosphate blends which are available in the market showed better functionality than single phosphates. The most popular phosphates are alkaline polyphosphates such as tripolyphosphate

which represents more than 50% of the phosphates used by the meat industry. However, the usage of phosphate has been restricted in dosages and in some countries; they have been banned in meat products (Feiner, 2006a, chap. 5).

Phosphates improve the functionality of meat proteins in different ways: the first, phosphate dissociates the acto-myosin complex, which forms during rigor mortis by sequestrate calcium (Ca^{2+}) and magnesium (Mg^{2+}) cations. Calcium makes bridges between actin and myosin during the contraction of the muscle where phosphates have the ability to break down these bridges (Feiner, 2006a, chap. 5). The second, phosphates cause a slight raise in pH of meat products which increases the gap between ultimate pH and isoelectric point. Third, phosphates increase the electrostatic repulsive forces which expand the spaces between actin and myosin allowing for more water to entrap in these gaps (Barbut, 2002a, chap. 9). Finally, phosphates increase the ionic strength of the meat which leads to more severe swelling of muscle fibers and activation of protein. Only phosphates are able to exert all these functions which justify their worldwide use (Feiner, 2006a, chap. 5).

Sodium chloride in combination with phosphate can improve protein functionality. When acto-myosin complexes separate from the effect of phosphate, addition of sodium chloride increases ionic strength and as a consequence the solubility of muscular proteins improves. Solubilized proteins have higher ability to immobilize high levels of added water as well as emulsify a large amount of fat. This synergistic effect between phosphates and sodium chloride gives more flexibility for each one to replace by the other (Xiong *et al.*, 2000). Therefore, phosphates can be used in the development of low salt meat product (Ruusunen and Puolanne, 2005). However, recently there are also some nutritional concerns about the use of phosphates in foods. Some researchers have shown that phosphates form insoluble salts with calcium, iron and other metal ions which might result in lowering the absorption of these minerals inside the intestinal tract and also as a result increase risk of bone diseases (Sherman and Metha, 2009). Moreover, high phosphorus intake increases the potential risk of chronic kidney diseases (Uribarri, 2009). Apart from these nutritional drawbacks, nowadays term “phosphate” sometimes has negative connotations. To address natural and clean label trends, processors are interesting in phosphate replacers that are natural (natural animal and plant derivatives) and easy to understand for consumers.

Phosphates are considered as master additives in improving WHC, the yield and eating quality of meat. A big challenge for the meat industry is to find ingredients that have equivalent functionality as phosphates. In response to the nutritional drawbacks and negative connotation of phosphates, the current trends now are to evaluate different food ingredients as phosphate replacers, optimizing processing techniques and improving the formulation to reduce or eliminate phosphates in meat products.

6.3.3 Citrates and carbonates

Citrates are widely used in poultry meat product formulation to improve water binding capacity by increasing the ionic strength and swelling the muscle fiber structure. Alkaline citrates (e.g. trisodium citrate) are the most common salts used in the meat industry to improve WHC by raising the pH value (Feiner, 2006a, chap. 5). Citrates also reduce the oxidative processes by chelating the oxidizing metals. Alkaline salts of carbonates minimized the problem of pale, soft and exudative in meat products (Alvarado and Sams, 2003). But more recent studies revealed that sodium bicarbonate is able to reduce shear force and improves the yield of poultry marinated meat (Sen *et al.*, 2005; Petracci *et al.*, 2012b).

Sodium bicarbonate could be used in meat formulations as phosphate replacers. It was found that the quality traits (color, WHC, texture, and yield) of meat treated with sodium bicarbonate were similar to some extent to meat treated with phosphates. Meat treated with bicarbonate showed more coarse structure which could be explained by generation of carbon dioxide during cooking (Sen *et al.*, 2005; Sheard and Tali, 2010). Due to differences in buffering capacity and ionic strength, bicarbonate showed a greater ability to increase meat pH (0.7 *vs.* 0.3 pH units) and higher yield in comparison with sodium tripolyphosphate. The alkalinization effect of bicarbonate moves pH of meat away from isoelectric point of myofibrillar proteins and increases net negative charge. Electrostatic repulsion forces cause expansion of muscle fibers which allow more water to be immobilized in the myofibrillar lattice. Low-resolution nuclear magnetic resonance technique showed that the combined use of bicarbonate with sodium chloride determined a remarkable increase of the proportion of entrapped water into myofibrillar spaces (Petracci *et al.*, 2012b).

6.4 Ingredients with indirect functional effect on the meat system

A second group of functional ingredients includes a large variety of compounds of different origins like extenders (non-meat compounds with considerable protein content), fillers (plant substances with high carbohydrate content) and binders (substances with high-protein content able to bind both water and fat) (Table 6.4).

6.4.1 Starches and cereal flours

In processed poultry meat products, starches are commonly used as a thickener as well as gelling, water retention and bulking agents to improve texture characteristics (Table 6.4). Moreover, particular modified starches can be used to provide the desired simulated fat properties (termed as “fat mimetic”) by improving the mouth-feel properties arising from bulking and moisture retention, and also can provide freeze/thaw stability of the gelled water in meat products. Native starches from various botanical origins contain different ratios of amylose and amylopectin which impart specific functional properties for each type of starch. Even amylose and amylopectin are composed from the same monomer (D-glucopyranose), but due to the differences in the type of bonds, molecular weights, and the shape of polymers (linear *vs.* branched), they exhibit different functional properties.

Starches containing higher amylose content impart higher gel strength. Linear structure of amylose molecules can easily dissolve in a solution and during heating, arrange themselves with one another, associating hydrogen bonding in gel matrix design which builds texture of the meat products during processing. During storage, amylose molecules start to re-associate together, leaving less space which pushes water molecules out of gel matrix, then starch starts to recrystallize or retrograde. This process determines the quantity of purge loss of cooked meat products during storage, especially those products that are packaged under vacuum, as a result affecting the long-term stability of products. The branched amylopectin molecules cannot align as easily as amylose, and thus, give weaker hydrogen bonding and gel strength. Amylopectin is responsible about the elasticity and viscosity of a starch gel. Starches high in amylopectin (i.e. waxy starches) are easier to cook and generally gelatinise at lower temperatures than starches with high in amylose content (Feiner, 2006b, chap. 6).

Table 6.4 Classification, dosage, functional properties and main product applications of the main functional organic compounds used in poultry meat processing.

Compounds	E-number	Current dosage	Functional properties			Main product applications			
			Water gelling	Texture Modulator	Fat stabilization	Whole-Muscle	Formed/ Restructured	Coarse Ground	Emulsified
PLANT COMPOUNDS									
Starches	No/Yes	< 4%	+++	+++	+ ++[1]	+++	+++	+++	+++
Cereal flours	No	< 4%	++	++	+	O	+	+++	o/+
Proteins	No	< 2%	+++	+++	+++	+++	+++	++	+++
Fibres	No/Yes	< 2%	+++	+++	+++	o/+	++	+++	+++
HYDROCOLLOIDS	Yes	< 0.5%	+++	+++	o	+++	+++	+	+++
MEAT COMPOUNDS									
Collagen derivates:									
- Low content	No	< 3.5%	+	Yes	+	o	o	+	++
- High content			+++	Yes	+++	+++	+++	+	+++
Blood proteins[2]:									
- Plasma fraction	No	< 2.0%	+++	Yes	+	+++	+++	+	+
- Globin fraction			+	No	+++	O	O	O	+++
Sodium caseinate	No	< 2.0%	+	No	+++	++	++	+	+++
Whey proteins	No	< 3.5%	+	No	+	++	++	+	++
Albumen	No	<10%	+++	Yes	+	O	++	O	+

[1] OSA-starch; [2] Available in the market only of porcine origin

+++ = high; ++ = medium; + = low; o = none

Potato and tapioca starches are the most widely used in poultry meat products. Potato starch has some privileges like low gelatinization temperature (60-65°C), high water binding capacity

and high viscosity which make it favorable to the meat industry. Tapioca starch is usually used to impart shiny, smooth texture and neutral taste for meat products.

Each type of starch undergoes to different structural transformation patterns (granules swelling, viscosity, loss of birefringence, heat adsorption, and gelling temperature) when introduced to meat products and subjected to heat treatment. Integrated approaches employing these different structural transformation patterns combined with optimizing product formulations and processing techniques from one side, and meeting the consumer expectations and satisfactions from the other side could be useful tools in exploring novel modified starches or starch mixtures. In this regards, several physical and chemical modifications had been implemented on native starches to improve gelling properties, swelling, viscosity, heat adsorption, texture properties and water binding capacity; such as oxidization (Pietrzyk *et al.*, 2012), phosphorylation (Lu *et al.*, 2012), acetylation and oxidation (Chibuzo, 2012), hydroxypropylation (Moghaddam *et al.*, 2013), treatment with octenyl succinic anhydride (OSA) and heat treatment (Song *et al.*, 2010; Timgren *et al.*, 2013), and heat-moisture treatment (Puncha-arnon and Uttapap, 2013).

Modified food starches are considered as food additives which make them non favorable for clean label products. In order to meet market requirements for clean label, special “native functional” starches (i.e. potato, tapioca, waxy maize) produced by means of physical treatments have been introduced in the markets (i.e. Novation, from National Starch) (Anonymous, 2012). These starches are available in the market in two forms. The first, cook-up starches (pre-gelatinized) are mostly used in cooked products because being able to bind water during heat treatment, and the second is instant or cold swelling starches which are able to bind water already in meat batters before cooking. It was found that the use of different starches (regular and modified potato and tapioca) reduced cooking loss about 5% in PSE-like meat (Zhang and Barbut, 2005; Feiner, 2006b, chap. 6). Due to the increase of demand on low-fat frankfurters, modified starches were utilized for this purpose as “fat mimic” agents (Keeton, 2001). Reduction of fat content usually coincides with the increase in meat content, which lead to increase in redness values, firmness, and decrease in WHC. In this context, number of hydrocolloid compounds were developed to improve water binding capacity in the gel matrix to minimize the effect of fat reduction (Weiss *et al.*, 2010; Kwang Nam *et al.*, 2013).

Native and pre-gelatinized cereal flours (obtained mainly from wheat and rice) are commonly used in minced meat products (1 to 3%) in order to retain water in both cold and warm conditions, bind meat particles, and impart desired textures as well as enhance the forming properties of comminute meat batters (i.e. during the production of patties or nuggets) (Barbut, 2002d, chap. 10, Talukder *et al.*, 2013). Pre-jellification of cereal flours enhanced the swelling and hydration properties of starch and proteins under cold conditions as well as giving a complete gelation of starch/ protein matrix even in products to be cooked at low endpoint temperatures (i.e. 68-70°C) (Keeton, 2001).

6.4.2 Vegetable proteins

Proteins derived from various plant origins are used as binders and extenders to increase water retention in meat products (Table 6.4). Rehydrated flaked textured vegetable proteins (i.e. from soy, wheat and pea) are widely used in meat products such as burgers, patties, pies and salami to optimize the cost of formulation by reducing the lean meat content (Sadler, 2004; Asgar *et al.*, 2010). The most common plant proteins that are used in meat products derived from soybeans or wheat. However, pea proteins are becoming popular in Europe because they are currently produced by none genetically modified organisms (non-GMO) (Feiner, 2006b, chap. 6).

Soy and pea proteins are categorized as flours, concentrates, and isolates on the basis of their dry-weight protein content (50, 70, and 90%, respectively) as well as textured materials. The mechanism of action of soy protein can be interpreted by protein-water interaction (wet ability, solubility, and swelling) which increase the viscosity and create a gel matrix during heating. (Moure *et al.*, 2006).

Soy isolates (90% protein) are mainly used to increase the level of protein content, optimize the cost (by replacing the lean meat in restructured and emulsified meat products), enhance the texture profile and fat emulsification capacity (improve sliceability in meat sausage rolls and consistency in Bologna-type products), and increase moisture retention (higher yield, reduce the purge loss during storage, and more juiciness for marinated injected/tumbled whole-muscle or restructure products).

Soy protein concentrate (70% protein) is rich in fibers (contains most of the fibers in original soybean), it is widely used in meat formulation to improve water and fat holding capacity and also it can add nutritional value to meat products due to its fiber content. Soy flour has less

protein content (50%) and it is used as filler and extender in meat products and also it could improve the water binding capacity depending on the commercial forms (natural or full fat, defatted, and lecithinated soy flours), but it may impart “bean taste” to meat products (Feiner, 2006b, chap. 6). In some cases, soy is added for the purpose of protein enrichment in the finished product (Asgar *et al.*, 2010). Specialized soy proteins with high gelling properties were used to manufacture low-cost (low lean meat content) emulsified sausages, burgers, meatballs and nuggets.

Proteins obtained by non-GMO pea represent a good alternative of genetically modified (GM) soy. Pea protein isolates are soluble proteins with good gelling, emulsification and water binding properties which make them convenient for applications in processed meat products. However, pea protein isolate is still not plain in flavor as it is in soy proteins. This defect in pea protein isolate could be representing a challenge in formulating of mild flavor meat products where detection of this flavor becomes easier.

6.4.3 Vegetable fibers

From a technological viewpoint, the use of vegetable fibers from different botanical origins to develop the quality of meat products is a promising trend. Fibers have multifunctional properties: enhance WHC, modulate texture, stabilize fat in emulsified products, exert a fat mimetic behavior in reduced-fat products, and so on. Fibers could also be exploited as a way for nutritional enrichment of processed meats (Fernandez-Gines *et al.*, 2007; Bodner and Sieg, 2009; Toldra and Reig, 2011).

Bamboo, oat and wheat bran are a good sources of insoluble fibers (cellulose, hemicelluloses and lignin), while psyllium, fruit (citrus, apple) and chicory are considered a good sources of soluble fibres (gums, pectins and mucilages). Both types are available in the market in different processing levels (native or modified) and in various particle sizes and shapes (Bodner and Sieg, 2009). Various types of fibres have been studied as single or in combination with other ingredients to formulate reduced-fat meat products, coarse ground and restructured products, and meat emulsions (Table 6.5)

Table 6.5 The main applications of vegetable fibers used in poultry meat processing.

Type	Dosage	Fiber size	Main applications
Insoluble (i.e. bamboo, wheat, oat, etc.)	Up to 0.5%	30-40 µm	Injected products
	Up to 1.5%	90-200 µm	Restructured, Coarse/Ground or Emulsified products
Soluble (i.e. inuline)	Up to 1%	Not relevant	All categories
Soluble rich (i.e. lemon, orange)	Up to 1%	40-100 µm	Injected products (40 um)
			Restructured, Coarse/Ground or Emulsified products
Intermediate (i.e. pea, potato)	Up to 1.5%	100-400 µm	Restructured, Coarse/Ground or Emulsified products

The functional properties of fibers depend on plant origin (i.e. pea, carrot), botanical part utilized to extract the fibers (i.e. pea husk *vs.* pea inner part), physical status of fiber particles (i.e. fiber length or fiber "expansion"), and the technology used to extract the fibers (i.e. separation of desired fibers, chemical/physical treatment to obtain desired characteristics in the product). Each commercial product has, to some extent a special functional behavior, and so it is not easy to find different commercial products have the same fingerprint of functional properties.

6.4.4 Hydrocolloids

Hydrocolloids are hydrophilic polymers mainly derived from plant or marine sources that have the ability to spread throughout water forming either gel or viscous solution (reversible or irreversible) (Table 6.6). Gums exhibited gelling properties in meat products, reduced the cooking loss, improved the texture, and prevented syneresis without interfering meat protein functionality (Feiner, 2006b, chap. 6). Among many hydrocolloids that are available in the market, carrageenans and alginates are the most commonly used in poultry meat product formulations.

Carrageenans are widely used at a low active dosage (i.e. 0.1 to 0.3%) for injecting and/or tumbled meat parts (i.e. for injected turkey breast) to improve yield, control purge loss, improve finished product sliceability, and enhance the juiciness. There are three main types of

carrageenans, namely k-, i- and l-carrageenans having different functional properties. However, only k- and i-carrageenans are incorporated in poultry meat products. k-carrageenans produce a very firm but brittle gel (a strongest gel is obtained by the presence of potassium ions) which tends to exhibit syneresis, while i-carrageenan produces an elastic gel which is resistant to syneresis. This is why k- and i- fractions of carrageenans are always commercially available in blends in order to modulate texture and control syneresis and purge loss in the final product (Feiner, 2006b, chap. 6).

Consumer interest is currently shifting towards low-fat/leaner meat products and at the same time with reasonable prices. Therefore, this trend generated a great interest in the meat industry to use polysaccharide gums (such as carrageenans and alginates) in meat formulation to reduce the cost and the fat content in lean meat products by improving the WHC. The effect of carrageenans on the functional properties of poultry meat products has been the subject of numerous studies (Verbeken *et al.*, 2005).

Several studies have evaluated the different functional aspect of carrageenan in meat products such as: the carrageenan function in relation to the muscle fiber type (Amako and Xiong, 2001), impact of carrageenan on technological and sensory properties of sausages (Ayadi *et al.*, 2009), effect of carrageenans in combination with whey proteins on texture properties (Barbut, 2010), impact of carrageenans on the cooking and storage losses (Cierach *et al.*, 2009), synergistic effect of k-carrageenans and locust beans gum in presence of potassium and calcium chloride (Garcia-Garcia and Totosaus, 2008), and the effect of different ratios of whey powder, i-carrageenan, and fat on texture sensory traits (Nacim *et al.*, 2012).

It was found that alginate had the ability to form a heat stable gel in the presence of calcium ions, enhance and improve the binding of small meat particles in poultry products (Barbut, 2002d, chap. 10; Keeton, 2001). Alginates can also be used for the production of meat replacers where a gel like meat-alginate matrix is formulated from a little amount of meat, high amount of water, flavors and colorants. This mix is used as meat replacers to produce different varieties of low-value meat products. In addition, special alginate blends were prepared to produce stable cold fat emulsions (i.e. with chicken skin) such as chicken nuggets or sausages.

Table 6.6 Origins, functional properties and main functions of hydrocolloids used in poultry meat processing.

Hydrocolloid	Origin	Thickening properties	Gelling Properties	Main functions
Carrageenan	Marine Algae	No/Low	Yes	Gelling agent for medium (20-40%) to high (60-100%) extended meat products
Alginate	Marine Algae	Yes	Yes	Mainly used for cold meat binding, fat emulsions or as a meat extender
Locust bean gum	Seed	Yes	No	Mainly used for its synergistic behavior with k-carrageenans to increase gel elasticity and resistance to syneresis
Xanthan gum	Bio-fermentation	Yes	No	Generally used to increase brine viscosity in order to avoid starch precipitation and improve marinade retention
Guar Gum	Seed	Yes	No	Cold swelling hydrocolloid with thickening capacity. Mainly used with other ingredients (starches, vegetable fibers, etc.)

6.4.5 Proteins from animal sources

Numerous types of animal proteins derived from animal itself (meat, skin, blood) or by its products (egg and milk) are available in the market as functional ingredients (collagen, gelatine, whey protein, casein, albumen, dehydrated beef protein, and so on) to add various technological and sensory properties to meat products (Xiong, 2009). Gelatine, collagen and blood derived proteins are widely used in meat formulations. Recently, the interest in gelatin has started to grow due to the manifestation of broad new applications in the food industry. Collagen and gelatin showed more favor water binding capacity in comparison with starches and other hydrocolloids in formulation of meat products (Cheng and Sun, 2008; Gomez-Guillen *et al.*, 2011).

Wide range of gelatine products derived from pork and beef are more available and favorite in use than poultry, this could be attributed to that pork and beef proteins are more functional (i.e. gelling ability) and less expensive. The commercial collagen derivatives have different abilities to swell in cold or hot conditions and this allows meeting wide industrial applications. Cold

swelling products are suitable for the use of raw minced meat products (i.e. hamburgers and sausages), whereas a hot swelling products are useful in preparation of brines to be injected in whole meat parts to be subsequently cooked.

Protein derivatives of blood are also available to use as functional ingredients in poultry meat products. Plasma proteins possess featured functional properties: excellent solubility, low viscosity and the ability to form strong, elastic and irreversible gels which increase the gel strength as the temperature increases (Prabhu, 2002). These characteristics make plasma proteins very useful for incorporation in a brine to be injected, and during cooking plasma proteins produce strong stable gelled matrix inside injected meat. In addition, plasma proteins are also good emulsifiers and as a consequence, they are used in emulsified sausages to improve yield, knack (plasma gel is also thermal irreversible) and stability of fat when low quality meat is used, or to replace some of the lean meat fractions.

Poultry protein isolates (PPI) are promising functional ingredients usually used in processed poultry products to improve the texture, sensorial properties, reduce the cost of formulation (by reduction of lean meat content), and decrease the fat content. Poultry protein isolates are usually prepared from low quality meat (mechanically separated poultry meat MSPM). Low-fat Turkey Bologna formulated from PPI showed the same quality characteristics (cooking yield, purge loss, and fat stability) of that one formulated with soy protein isolate (Silva *et al.*, 2011; Omana *et al.*, 2012).

Transglutaminase is a food-grade commercially available enzyme which has the ability to make cross linking between protein forming gel network, which retains big quantity of water and thus improving the water binding capacity. Transglutaminase was used to improve gelling properties of heat-induced myofibrillar protein isolates and low-value meat products (Sun and Arntfield, 2011), the texture properties of chicken breast patties (Uran *et al.*, 2013), and water holding capacity (Xian-bao and Wei, 2010).

Sodium caseinate and whey proteins and their derivative are used in comminute and emulsified meats (frankfurters and bologna), and coarse ground products (fresh sausage, meat patties and meatballs), and marinated or injected meats to improve moisture retention, fat binding and textural characteristics of cooked meats (Barbut 2006; Xiong, 2009). In general, sodium caseinates contribute to the overall firmness of meat products (i.e. hams). On the other hand, functionality of whey protein was improved with sodium tripolyphosphate (Smith and

Rose, 1995) or by addition of biopolymers prepared from soybean proteins and caseins (Muguruma *et al.*, 2003).

It was found that skim milk powders were the most beneficial in improving yield and texture in comparison to caseinate and modified whey proteins (Barbut, 2010). Finally, Hongsprabhas and Barbut (1999) evidenced that the cold setting of whey protein isolate improved the binding of raw and cooked meat batters, particularly at low salt level. Egg albumen proteins are often used in poultry meat products. Egg white is used in cooked sausages such as frankfurters because of its ability to form a stable and heat-irreversible gel, thus positively contributing to the firmness of low-cost emulsified sausages (Alleoni, 2006; Teye *et al.*, 2012).

6.5 Conclusions

Innovative meat formulations as well as improving the health image of processed meat products can be achieved by adopting integrated strategies that balance between using different types of functional ingredients derived from both animal or plant origin or also by using some of inorganic salts and innovative processing technologies. These integrated approaches could be useful during formulation of processed meat products taking into consideration: the market demands (i.e. national, ethnic and traditional values) and policies, legislation restrictions, food allergy and intolerance issue (i.e. coeliac disease, protein allergens), clean label requests (i.e. short list of ingredients, absence of additive, etc.), available processing technologies and most important economic feasibility (i.e. least cost formula). Additional challenge for poultry meat industry is to address consumer demand for healthier meat products that are low in sodium, fat, cholesterol, calories, free phosphate, and incorporate vegetable proteins and dietary fibers. For this reason, part B of Ph.D research project was dedicated for these purposes and in particular, to study the possibility to formulate phosphate marinated chicken breast meat and low sodium marinated rabbit meat products.

CHAPTER 7

Comparison between the quality traits of phosphate and bicarbonate-marinated chicken breast fillets cooked under different heat treatments

7.1 Abstract

The use of phosphates has recently diminished in meat industry due to the nutritional drawbacks of phosphates, some researchers started to evaluate sodium bicarbonate as a phosphate replacer in cooked meat products. Several studies evaluated the differences in quality properties between phosphate and bicarbonate-marinated chicken fillets under similar processing conditions; however, there is very little information about the differences in quality traits between phosphate and bicarbonate-marinated chicken fillets cooked under different heat treatments. The aim of this study is to evaluate the effect of a different temperature-combination of dry air-heat treatments (Air and Core temperatures: 160-76°C, 160-80°C, 200-76°C, 200-80°C, respectively) on quality characteristics of phosphate and bicarbonate-marinated chicken breast. A batch of 24h *post-mortem* broiler breast meat of 80 fillets was divided into two groups of marination treatments (0.3% sodium bicarbonate n=40, 0.3% sodium tripolyphosphate n=40) and were vacuum tumbled (45 min, -0.95 mbar, 20 rpm). Different temperature-combinations of heating treatments showed significant differences in the chemical composition. The most severe heat treatment (200-80°C) exhibited significantly ($P<0.05$) lower moisture content and the higher protein content for both types of marinated fillets in comparison with other heat treatments. Bicarbonate marinated fillets showed higher ability to retain water (67.3 *vs.* 65.7%, $P<0.05$) during severe heat treatment and lower cook losses (30.7 *vs.* 33.4%, $P<0.05$) when compared with phosphate-marinated fillets. The effect of changing the cooking temperatures on Texture Profile Analysis (hardness, cohesiveness, gumminess, springiness, and chewiness) was more significant in phosphate marinated fillets than bicarbonate. Bicarbonate-marinated fillets showed significant differences in the percentage of bound water, latent heat, and water activity after cooking in comparison to phosphate-marinated fillets. The results of this study revealed that phosphate-marinated fillets interact with heat treatments in a different pattern in comparison with bicarbonate-marinated fillets.

Keywords: Marination, sodium bicarbonate, heat treatments, chicken fillets, quality traits.

7.2 Introduction

Marination is one of the most common techniques that are usually used to improve the flavour, tenderness, succulence, stability and safety of meat from an aspect and enhance the yield from another aspect (Alavardo *et al.*, 2007). Several studies have implemented on marinated meat to evaluate the processing conditions: time and type of marination, salt concentration, polyphosphate concentration, cooking methods, and other processing parameters by employing several quality measures such as marinade uptake, water retention, water binding capacity, cooking loss, texture and sensorial properties (Xiong and Kuspski, 1999; Zheng *et al.*, 2000).

Phosphates that are used in meat formulation; made from sodium or potassium salt of phosphoric acid. Particularly, pyrophosphate and tripolyphosphate are frequently used to increase the water binding capacity of the meat. Sodium tripolyphosphate (STPP) accounts for approximately 80% of the phosphates used in further-processed meat products. In this context, phosphates offer a wide range of functional properties to the processed meat products which render them as a preferable choice for meat producers. The maximum dose of phosphates according to the EC regulations must be less than 0.5% (w/w) expressed as P_2O_5. Phosphates can impart the functional properties to meat products in several synchronized ways: by shifting the pH far away from isoelectric point, increasing the ionic strength, and improving the solubilization of myosin and actin by sequestering Mg and Ca ions which involve during the formation of actomyosins complex. Dissociation of actomyosins enhances the solubilization and the functional properties of proteins during processing (Smith and Young, 2007). Phosphates also have a strong synergistic effect in the presence of sodium chloride. Sodium chloride is used in combination of phosphate in marinades to improve the texture and yield of muscle meat products (Petracci *et al.*, 2012b). Beside to the former unique characteristics, phosphates improve the oxidative stability, flavour and retard the microbial growth in meat products (Barbut, 2002c).

Increase of phosphate dietary intakes could have an impact on the health aspects: first, some people cannot adopt high level of phosphate, which leads to an allergic reaction and Attention Deficit Disorder (ADD) in children (Hafer, 2002). Second, increase the levels of phosphates in the blood over the limits of metabolic process could affect the function of some organ like kidneys or in some cases it causes damage or even organ failure (Dikeman *et al.*, 2003). Third, diarrhoea and sometimes hardening of soft tissues and organs are also

symptoms of increase dietary phosphate (Berner and Shike, 1988). Finally, high consumption of phosphates reduces the absorption of calcium and as a result it can lead to osteoporosis in postmenopausal women and the development of brittle bones in renal patients (Anderson, 1996). Moreover, it may interfere with utilization of iron, calcium, magnesium, and zinc by the body.

With all of the foregoing, the use of phosphates have been recently diminished in meat industry due to some nutritional drawbacks of phosphates which previously mentioned as well as for their ability to interfere the absorption of some minerals in the gut by forming insoluble complexes with calcium and magnesium. Several countries have banned their use in raw meat production (Sebranek, 2009). In response to these nutritional drawbacks, many studies started to evaluate some functional ingredients to replace the use of phosphate in meat products. The current consumption trends shift towards low-sodium and phosphate-free meat products. Sodium citrate, carageenans, non-meat proteins employed to meet these purposes (Petracci *et al.*, 2013a).

Carbonate and bicarbonate compounds are considered as a new promising agent as phosphate replacer. Some recent studies showed that bicarbonate compounds can reduce the drip loss and shear force; improve the yield, and other characteristics as well as phosphates (Wynveen *et al.*, 2001; Bertram *et al.*, 2008; Shread and Tali, 2010). This effect could be explained that bicarbonates have the higher buffering capacity and ionic strength than phosphates (Shread and Tali, 2010; Bertram *et al.*, 2008). But in general, the exact mechanism that stands behind this effect is still not fully understood. However, introduction of sodium bicarbonate to marinated meat is not easy applicable task because the high pH condition increases the risk of bacterial growth. Therefore, there is a need to add some food preservatives to the marinade solution or changing the processing operations to improve the microbiological stability of meat products. But from the health aspect viewpoint, the addition of food preservative may impart negative connotation to the product which might reduce the willingness of consumers to accept it.

The impact of different heat treatments on the quality traits of marinated poultry meat had been subjected for evaluation and investigation by several authors. Air-steam treatment was one of the best methods for obtaining more tender chicken slices. It was found that the effect of heat treatment time on cooking loss was more than heating temperature (Barbanti and Pasquini, 2005). Low relative humidity-heat treatment showed higher quality traits for cooked turkey meat when compared to high steam treatment (Mora *et al.*, 2011). Cooking conditions (temperature and cooking time) have a massive impact on the physical

characteristics of meat and eating quality. The types and the quantity of meat muscle proteins (myofibrillar and the connective tissue proteins) have a major role in the toughness of meat. Upon cooking, these proteins undergo to different structural changes: denaturation, destruction of cell membrane, shrinkage of meat fibres, the aggregation and gel formation of myofibrillar and sarcoplasmic proteins shrinkage and solublisation of the connective tissue (Brake and Fennema, 1999).

Bicarbonate compounds have been evaluated as a phosphate replacer under the same conditions of heat treatment. There were no studies that evaluated the effect of bicarbonate in comparison to phosphates under different conditions of heat treatments. The aim of this study to evaluate the effect of different heat treatments on the quality traits of bicarbonate-marinated breast fillets in comparison to phosphates-marinated fillets.

7.3 Materials and methods

7.3.1 Samples preparation and classification

A batch of 80 skinless chicken breasts was obtained from commercial plant after 24 h *postmortem* from the same flock. The breast fillets were trimmed and adjusted to have raw weight mean ± SEM (141.03 g ± 0.93 g) to exclude the interaction between the effect of weight and the effect of the heat treatment. The colour values (CIE: L*, a*, b*) have been measured for each fillet according to the procedure described in 3.3.2.1 Colour measurements. The samples were reorganized in two groups (n=40) have the similar average (without statistical difference) lightness values (L*) ± SEM (51.02 ± 0.25) and (50.94 ± 0.20) for bicarbonate and phosphate marinating treatments respectively. There was no significant difference due to groups' assignment. pH was measured according to the Jeacocke (1977) procedure described 3.3.2.3 pH measurements. Color values (L*, a*, b*) were measured before and after marination and after cooking as described in 3.3.2.1 Color measurements.

7.3.2 Water Holding Capacity (WHC)

The WHC of the raw breast cuts was measured by modifying Van Laack method (2000). 30 g of minced meat was stirred with 90 ml of 1% sodium chloride in a 250 ml Byrex bottle. The samples were homogenized by using a high speed blender (ultra-turrax®, T 25 basic) for 1 min at 9500 rpm. Each 20 g of homogenized solution was centrifuged for 1 min at speed 22000 rpm and temperature at 6-7°C. The supernatant was removed to calculate the moisture uptake as follows:

$$Moisture\ uptake\ (\%) = \left(\frac{meat\ weight\ after\ centrifuge - meat\ weight\ before\ centrifuge}{Weight\ of\ \ meat\ before\ centrifuge} \right) \times 100$$

7.3.3 Water activity (a_w)

a_w was measured at a constant temperature (25 ± 1°C) by a water activity meter mod Aqualab (Decagon Devices Inc., Pullman, WA) that bases its measure on the chilled-mirror dew-point technique. For each marination treatment, the Aw was detected in 3 samples before tumbling, after tumbling, and after cooking.

7.3.4 Marination and cooking process

The first group was marinated with sodium tripolyphosphate (P) and the second group marinated with sodium bicarbonate (B) by vacuum tumbling (45 min, -0.95 bar, and 20 rpm) with target marination level 20% and 0.3% for each salt. Marinade uptake was measured according to 3.3.2.4.2 Marinated meat cuts. Each type of marination treatments was divided into four groups (n=10) and subjected to different heat treatments by air oven (oven-core temperatures: 160-76°C (A), 160-80°C (C), 200-76°C (D), and 200-80°C (E)). Each group of samples was distributed over metal trays and each two groups were cooked as one batch at similar oven temperature while the cooking process was ceased at a different core temperature (76 and 80°C). Cooking loss was determined as described in 3.3.2.4.2 Marinated meat cuts (c).

7.3.5 Textural analysis

Samples for Texture Profile Analysis (TPA) were excised as described in Figure 7.1. TPA has been assessed according to 5.3.6 Textural traits. On the other hand, shear force samples (Figure 7.1) were measured by Allo-kramer method as described in 3.3.2.4.1 Non-marinated meat cuts (c).

7.3.6 Proximate chemical composition

The chemical composition has been determined according to AOAC before and after cooking. All samples in each group were used to assess moisture, protein, fat, and ash as described in 3.3.2.5.1 Moisture, 3.3.2.5.3 Total crude protein content, 3.3.2.5.2 Total crude fat content, and 3.3.2.5.4 Total ash content, respectively.

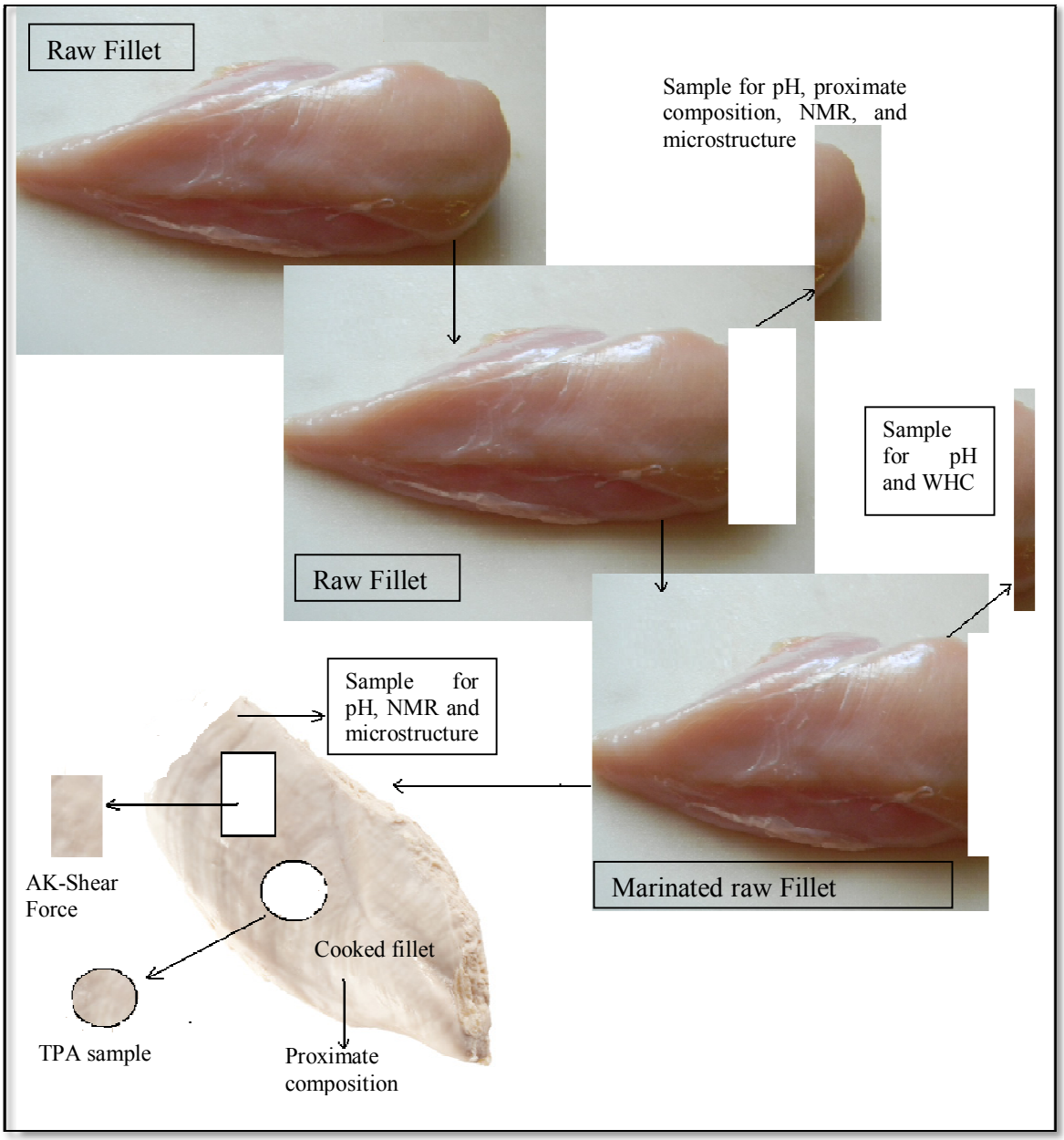


Figure 7.1 Sampling protocol during processing

7.3.7 Freezable water (FW)

The quantity of freezable water was determined in triplicate using raw, marinated and cooked breast meat samples by a Pyris 6 Differential scanning calorimeter (DSC; Perkin Elmer Corporation, Wellesley, USA). The DSC was provided with a low-temperature cooling unit Intacooler II (Perkin Elmer Corporation, Wellesley, USA). The temperature was calibrated using a standard ion exchanged distilled water (m.p. 0.0°C), indium (m.p. 156.60°C) and zinc (m.p. 419.47°C). In order to calibrate the heat flow, it was used the heat of fusion of indium (Δh = 28.71 J/g). During the calibration the identical heating rate utilized

for sample measurements was used and a flux of dry nitrogen gas (20 mL/min) was applied. Approximately 25-30 mg of meat sample was accurately weighted in 50 µL aluminium pans using a small spatula. The pan was hermetically sealed and moved to the DSC instrument at room temperature together with an identical empty pan used as a reference. The breast samples were cooled until -60°C, using 5°C/min of heating rate, then held for 1h at the final temperature of -60°C, scanned by increasing the temperature until 20°C at 5°C/min of heating rate according with Brake and Fennema (1999). FW was determined as:

$$FW = \frac{\Delta H_m}{\Delta H_w}$$

Where ΔH_w (325 J/g) is the latent heat needed to melt one gram of pure water at 0°C (Roos, 1986) and ΔH_m (J/g) is the measure latent heat of melting of water for gram of sample, obtained by the integration of the melting endothermic peak.
FW amount was expressed as gram per gram of fresh sample weight.

7.4 Statistical analysis

The effect of marination and heat treatments on quality traits of chicken breasts were evaluated by ANOVA option of the GLM procedure (Statistica 6). Means were separated using Tukey's honestly significant difference multiple range test with $P \leq 0.05$ considered as significant.

7.5 Results and discussion

7.5.1 Effect of heat treatment on proximate composition

The results of proximate analysis of raw and marinated cooked chicken breast are shown in Table 7.1, where they were pooled taking in the consideration just the effect of the marination process on the chemical composition. As expected due to the evaporation effect of cooking process, the chemical composition was significantly modified after cooking for both types of marinating treatments (bicarbonate and phosphate). In general, protein content significantly ($p<0.05$) increased and moisture content decreased.

Bicarbonate-marinated fillets exhibited significant differences in chemical composition in comparison to phosphate-marinated fillets when the results were pooled just according to marination treatment. In general, cooked chicken breasts treated with bicarbonate showed significantly ($P<0.05$) higher moisture (69.66 *vs.* 68.75%) and lower ash contents (1.58 *vs.* 2.01%) than phosphate-marinating treatment. There were no differences in lipids and protein contents among the two treatments. The difference in chemical composition (particularly

moisture content) denoted that the water binding capacity in meat treated with bicarbonate was higher than phosphate treatment and this also could explain the difference in ash content due to the lost moisture Table 7.1.

Table 7.1 Proximate composition (± standard error) for raw and cooked treated breast with bicarbonate and polyphosphate (pooled according to marination treatment).

Status of chicken breasts	Proximate chemical composition			
	Total moisture (g/100 g)	Total proteins (g/100 g)	Total lipids (g/100 g)	Total ash (g/100 g)
Fresh or raw	74.47± 0.10 [a]	22.82 ± 0.67 [b]	1.49 ± 0.23	1.37 ± 0.01[b]
Cooked and treated with bicarbonate	69.66 ± 0.25 [b]	28.07 ± 0.28 [a]	1.55 ± 0.06	1.58 ± 0.06 [b]
Cooked and treated with polyphosphate	68.75 ± 0.37 [c]	28.04 ± 0.42 [a]	1.46 ± 0.09	2.01 ± 0.08 [a]

[a-c] Different superscript letters within a column mean significant difference ($P < 0.05$).

The effect of heat treatment and marination process together in proximate composition was shown in Table 7.2. It was found that there were slight differences in the ash and lipid contents in chicken breasts marinated with bicarbonate and cooked under different heat treatments (BA, BC, BD, BE). Low variability in the moisture change between different heat treatments, could also explain the slight differences in the ash and fat contents (Table 7.2), while the lowest moisture content and the highest protein content were observed in the most severe heat treatments for both bicarbonate and phosphate treatments (BE and PE). Treatment BA and BD had no significant differences in moisture and protein contents. The highest moisture content (71.36%) has been observed in BC treatment if compared with all other treatments. In general, the most severe heat treatment (highest set and core temperature) caused higher significant changes in proximate composition for both types of marinating treatments. The results also showed that breast fillets treated with bicarbonate and cooked at the highest severe heat treatment (E) had higher ability to retain water than phosphate treatment (67.28 *vs.* 65.68%, $P<0.05$). The effect of heat treatments on the chemical composition of bicarbonate-marinated fillets were different from phosphate-marinated fillets. In general, both marination and cooking treatments have resulted in significant differences in proximate composition which could be explained by different factors: water evaporation, fats melting and loss of soluble proteins (Bertam *et al.*, 2004).

Table 7.2 Proximate composition (± standard error) for raw and treated breast with bicarbonate and polyphosphate under different heat treatments.

	Proximate chemical composition			
Type of treatment*	**Total moisture (g/100 g)**	**Total proteins (g/100 g)**	**Total lipids (g/100 g)**	**Total ash (g/100 g)**
BA	69.98 ± 0.44 ^{b}	26.82 ± 0.56 ^{cd}	1.60 ± 0.13 ^{ab}	1.44 ± 0.07 ^{d}
BC	71.36 ± 0.3 ^{a}	28.37 ± 0.7 ^{b}	1.70 ± 0.2 ^{a}	1.54 ± 0.1 ^{cd}
BD	70.02 ± 0.41^{b}	26.67 ± 0.67 ^{cd}	1.31 ± 0.22 ^{abc}	1.60 ± 0.20 ^{cd}
BE	67.28 ± 0.31^{c}	30.44 ± 0.59 ^{a}	1.59 ± 0.19 ^{ab}	1.76 ± 0.10 ^{bcd}
PA	70.16 ± 0.39 ^{b}	26.97 ± 0.50 ^{bcd}	1.60 ± 0.30 ^{ab}	2.10 ± 0.07 ^{ab}
PC	69.92 ± 0.41 ^{b}	27.27 ± 0.46 ^{bc}	1.37 ± 0.12 ^{abc}	1.79 ± 0.09 ^{cb}
PD	69.22 ± 0.43 ^{b}	26.85 ± 0.88 ^{bcd}	1.39 ± 0.09 ^{abc}	1.79 ± 0.06 ^{cb}
PE	65.68 ± 0.20 ^{d}	31.06 ± 0.62 ^{a}	1.48 ± 0.17 ^{ab}	2.36 ± 0.26 ^{c}

* B and P represent bicarbonate, phosphate respectively, while A, C, D, E represent heat treatment at different core and oven temperatures: 76-160°C, 80-160°C, 76-200°C, 80-200°C respectively.

$^{a-d}$ Different superscript letters within column for each marination treatment mean a significant difference ($P<0.05$).

7.5.2 Effect of heat treatments on texture properties

Changing of heat treatments had a significant effect on the texture profile of polyphosphate-marinated meat, while this effect was not clear in the fillets treated with bicarbonate (Table 7.3 and Table 7.4). Both types of marination treatment did not show any change in hardness (resistance to deformation) at different heat treatment conditions (Table 7.3 and Table 7.4). The effect of heat treatments was significant in PE treatments where cohesiveness (the strength of the internal bonds making up the product), gumminess (the energy required to disintegrate a semisolid food to a state ready for swallowing), and chewiness (a low resistance to break-down on mastication) values were significantly ($P<0.05$) increased (Table 7.3). It is known that during the marination process and by using phosphates, myofibrillar proteins are extracted on the surface of meat. The extracted proteins have two functions during the cooking process. First, they improve the binding properties by coagulation. The second, they facilitate the retention of moisture in the meat tissue by sealing the micro-capillaries with coagulated proteins (Smith, 2001). It is not known that marinated meat with bicarbonate shows the same behaviour in comparison with phosphate-marinated meat. But in general, the higher water holding capacity and swelling of myofibrils are responsible about the mechanism of increased tenderness and juiciness (Offer and Knight,

1988). This part of the results that belong to effect of bicarbonate on the texture properties needs further investigations.

Both types of marination treatments showed higher shear values in the most severe heat treatments (PE and BE). Heat treatments changed the elasticity of fillets treated with bicarbonate as represented by springiness values (Table 7.3). The effect of heat treatments on the texture profile of bicarbonate-marinated fillets were less than phosphate-marinated fillets; this could be explained due to the generation of carbon dioxide produced during cooking and formation of air-filled pockets which could dilute the load-bearing material during the texture analysis (Sorheim *et al.*, 2004). Moreover, the lower hardness values (softness) could be attributed to the large amount of water retained in the meat. The role of phosphate in improving the tenderness of meat is well known. Polyphosphates promote the weakening of the myosin heads to actin, and thus promote the dissociation of actomyosin, increase the electrostatic charge and therefore, they could allow more water to be retained or taken up by the meat. The increased tenderness might be attributed directly to the higher water content and weakened muscle structure (Xiong, 2004).

Table 7.3 Shear force and texture analysis profile (± standard mean error) for breast treated bicarbonate under different heat treatments

	Heat treatment conditions*			
Quality traits	**76-160°C (BA)**	**80-160°C (BC)**	**76-200°C (BD)**	**80-200°C (BE)**
Shear force (kg/g)	2.52 ± 0.09 ^{ab}	2.41 ± 0.13 ^{b}	2.59 ± 0.14 ^{ab}	2.85 ± 0.09 ^{a}
Hardness (kg/g)	2.44 ± 0.13	2.25 ± 0.27	2.03 ± 0.12	1.88 ± 0.11
Cohesiveness	2.87 ± 0.09	3.16 ± 0.13	2.94 ± 0.06	3.11 ± 0.11
Gumminess (kg/g)	6.92 ± 0.22	7.01 ± 0.78	5.97 ± 0.35	5.79 ± 0.32
Springiness	1.48 ± 0.04 ^{ab}	1.44 ± 0.03 ^{b}	1.54 ± 0.04 ^{ab}	1.56 ± 0.02 ^{a}
Chewiness	10.25 ± 0.39	10.04 ± 1.00	9.14 ± 0.53	9.05 ± 0.48

$^{a-b}$ Different superscript letters within a row mean a significant difference ($P<0.05$).

* B represents bicarbonate while A, C, D, E represents heat treatment at different core and oven temperatures: 76-160°C, 80-160°C, 76-200°C, 80-200°C respectively

Table 7.4 Shear force and texture analysis profile (± standard mean error) for breast treated phosphates under different heat treatments

Quality traits	Heat treatment conditions*			
	76-160°C (PA)	**80-160°C (PC)**	**76-200°C (PD)**	**80-200°C (PE)**
Shear force (kg/g)	2.11 ± 0.10 [c]	2.08 ± 0.11 [c]	2.39 ± 0.09 [b]	2.71 ± 0.10 [a]
Hardness (kg/g)	2.26 ± 0.20	2.25 ± 0.17	1.81± 0.31	2.44 ± 0.11
Cohesiveness	2.78 ± 0.07 [b]	2.83 ± 0.11 [b]	2.73 ± 0.11 [b]	3.12 ± 0.05 [a]
Gumminess (kg/g)	6.21 ± 0.43 [ab]	6.30 ± 0.36 [ab]	4.91 ± 0.78 [b]	7.60 ± 0.29 [a]
Springiness	1.64 ± 0.05	1.60 ± 0.04	1.65 ± 0.04	1.60 ± 0.04
Chewiness	10.12 ± 0.53 [ab]	10.03 ± 0.47 [ab]	8.05 ± 1.23 [b]	12.10 ± 0.32 [a]

[a-c] Different superscript letters within a row mean a significant difference (P<0.05).

* P represent phosphate while A, C, D, E represent heat treatment at different core and oven temperatures: 76-160°C, 80-160°C, 76-200°C, 80-200°C respectively

7.5.3 Water Activity, freezable and bound Water

There are three different forms of water inside the meat tissues. The first one is the major part of the water (more than 80%) retained in meat as free which can be expressed by water activity (a_w). The second part of water (10-15%) is immobilized and entrapped under the effect of net charge attraction. The third part of water is a minor part (around 4%) usually bound to the ionizable groups of amino acids of the proteins and other groups able to form H bonds. The first two parts of water are affected or lost during processing like cooking, cutting, grinding, and storage (Pearce *et al.*, 2011). In our study, Water activity (a_w) was used to estimate mainly the first two parts.

Water activity (a_w) was significantly higher in bicarbonate treated fillets when compared to phosphate marinated fillets (0.998 *vs.* 0.995, P<0.05) (the result was not mentioned in the figures and tables). Water activity was reduced after cooking in all types of heat treatments in comparison with marinated meat (Figure 7.2 and 7.3) which may be explained by the loss of major amounts of free water due to evaporation by dry cooking. The most severe heat treatment (80-200°C) exhibited the lowest a_w for both types of marination treatments. For bicarbonate treatment, there was no significant difference in water activity between group D and E. On the contrary, the group A showed significantly higher water activity (0.991 *vs.* 0.989) than group C. Moreover, there was no significant difference between raw and marinated meat in water activity, this result may be explained due to the high moisture content of raw meat.

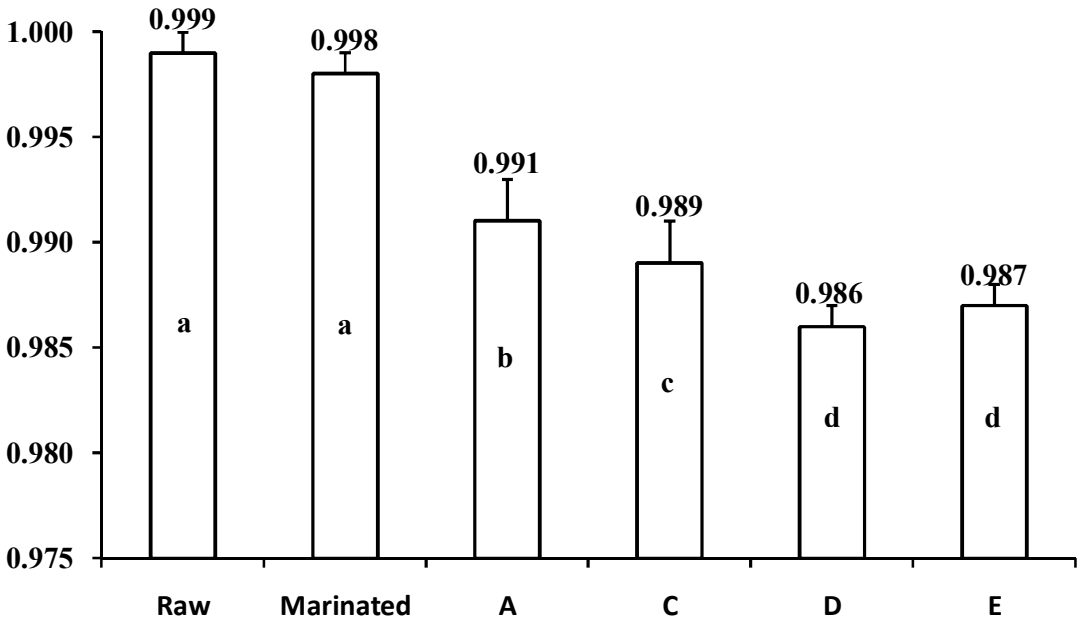


Figure 7.2 Water activities (*a*w) (mean ± standard mean error) for chicken breast meat raw and marinated with bicarbonate (B) and cooked at different heat treatments (A, C, D and E represent different core and oven temperatures: 76-160, 80-160, 76-200 and 80- 200ºC, respectively).
[a-d] Different superscript letters mean a significant difference (P <0.05).

Similarly, there were no significant differences in water activity after marination process for phosphate treatment in comparison to raw meat, which may explain as mentioned for bicarbonate treatment. Phosphate-marinated fillets showed the same trend in the change of water activity during different cooking treatments. The effect of core temperature on water activity was stronger than the effect of oven temperature in both of marination treatments. It seems that water activity was highly affected by oven temperature which at the same time affected highly the moisture content and also cooking loss. There was no significant difference in water activity between group D and E while group A and C showed intermediate values. Both group D and E had significantly higher values of water activity (0.987 and 0.985 *vs.* 0.990, P<0.05) than group A.

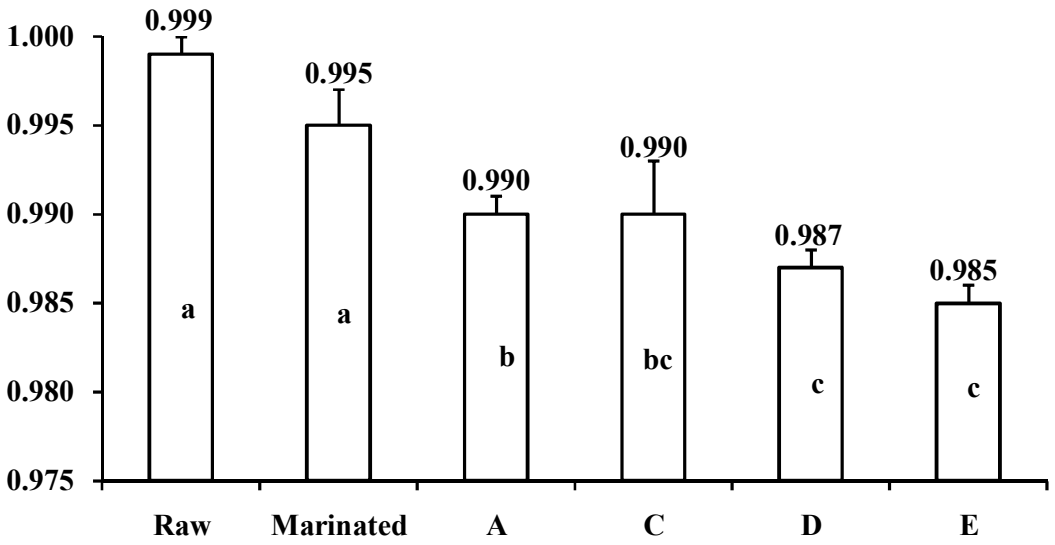


Figure 7.3 Water activity (a_w) (mean ± standard mean error) for raw and marinated chicken breast meat with phosphate (p) and cooked at different heat treatments (A, C, D and E represent different core and oven temperatures: 76-160, 80-160, 76-200 and 80- 200ºC, respectively).

[a-c] Different superscript letters mean a significant difference ($P < 0.05$).

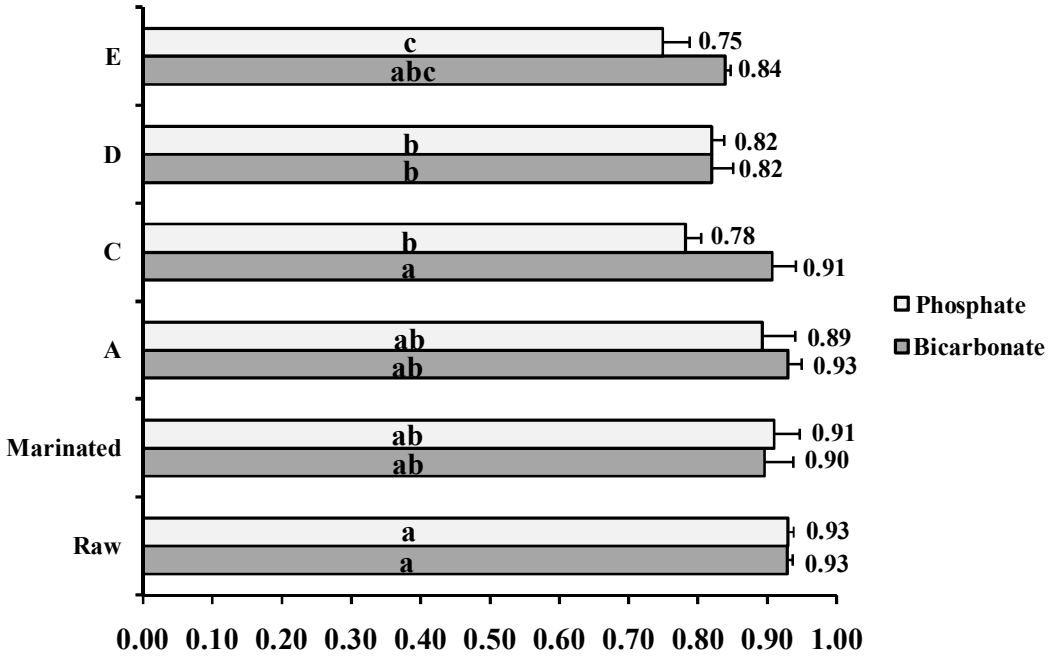
There were significant differences in total latent heat and bound water among bicarbonate-marinated and phosphate-marinated fillets (Table 7.5). Bicarbonate-marinated fillets cooked at 76-200°C and 80-200°C exhibited significantly higher percentage of bound water (18.10 and 16.08 *vs.* 7.02%, $P<0.05$) than fillets cooked at 76-160°C. Fillets after marination, group A and group C showed intermediate values of bound water. Group E and D had significantly higher values of latent than all other treatments while there were no significant differences between other groups. In general, marination process had a minor effect (intermediate values) on bound water of bicarbonate-marinated (10.43 *vs.* 7.16%) and phosphate-marinated (9.07 *vs.* 7.16%) fillets in comparison to raw fillets. In addition, there were no significant changes in latent heat before and after marination for both phosphate and bicarbonate treatments. Cooking treatments C, D and E for phosphate-marinated fillets showed slight significant differences in bound water. Phosphate-marinated fillets cooked by heat treatment (E: 80-200°C) showed the lowest latent heat value (159.98 J/g) and highest bound water percentage (25.05%) in comparison with other groups. Fillets after phosphate marination exhibited higher latent heat than all groups (A, C, D, and E) after heat treatments.

Table 7.5 The results of enthalpy and bound water for both types of marination treatments

	Bicarbonate		Phosphate	
	Bound water (%)	Latent heat (J/g)	Bound water (%)	Latent heat (J/g)
Before marination	7.16±0.75^{c}	224.69±1.82^{a}	7.16±0.75^{c}	224.69±1.82^{ab}
After marination	10.43±4.11^{abc}	227.64±10.45^{a}	9.07±3.71^{bc}	230.19±9.39^{a}
A (76-160˚C)	7.02±1.91^{c}	211.47±4.34^{a}	10.81±4.74^{bc}	203.36±10.82^{bc}
C (80-160˚C)	9.2±3.36^{bc}	210.24±7.81^{a}	21.81±2.21^{a}	177.68±5.04^{de}
D (76-200˚C)	18.10±3.07^{a}	186.85±7.00^{b}	18.09±1.76^{ab}	184.27±3.97^{cd}
E (80-200˚C)	16.08±0.73^{ab}	183.50±1.60^{b}	25.04±3.81^{a}	159.98±8.13^{e}

$^{a-c}$ Different superscript letters within a column mean a significant difference (P<0.05).

The freezable water content for raw fillets was about 93% of the total amount while after marination it was 91% and 90% for phosphate and bicarbonate-marinated fillets respectively. The change in freezable water content after marination was slightly significant for both types of treatments. There were significant differences in freezable water after cooking in all groups of treatment, but in different degrees (Figure 7.4).



* A, C, D, E represent heat treatment at different core and oven temperatures: 76-160°C, 80-160°C, 76-200°C, 80-200°C respectively.

$^{a-c}$ different superscript letters mean a significant difference (P<0.05).

Figure 7.4 Freezable water for fresh, phosphate, and bicarbonate marinated chicken breast fillets cooked at different heat treatments.

Phosphate-marinated fillet cooked by the most severe heat treatment (80-200°C) showed the lowest value of freezable water. Bicarbonate fillets of group C showed higher content of freezable water (0.91 *vs.* 0.82, $P<0.05$) than group D. There was no significant difference in freezable water between C and D group of phosphate treatment while at the same time they were significantly higher than raw. According to these results there was no relation between freezable water and water activity values. The changing in the percentage of freezable and bound water during different cooking treatments could be attributed to the loss of water by evaporation, loss of some soluble proteins due to cooking loss, and denaturation of proteins in which the type or the forms of bonds with water change**.**

7.5.4 Color, pH, marinade uptake, drip and cooking loss and WHC (Pooled as Marination)

There were no significant differences in L* (Lightness), a* (redness) and b* (yellowness) values of raw chicken meat fillets which were dedicated for bicarbonate and phosphate treatments. The samples were dispersed in a systematic way to obtain consistency of color between treatment groups. The consistency in the color is important because it was found that meat color extremes may affect marination uptake, cook yield, and shear force (Qiao *et al.*, 2002). After the marination process, L* (Lightness) values for bicarbonate (55.30 *vs.* 51.02) and phosphate (56.51 *vs.* 50.94) treatments were significantly ($P<0.5$) higher than raw fillets while there were no significant differences in a* (redness) and b* (yellowness) values (Table 7.6). This increase in lightness could be due to the increase in extracellular water as a result of the marination process.

In general, there was no consensus between the previous studies on the effect of the marination process on colour values. Young *et al*. (2005) found similar L*, a* and lower b* values in marinated fillets in comparison to non-marinated fillets while Lyon *et al*. (1998) pointed out that marinated poultry muscles had lower red (a*) and yellow (b*) values when compared to non-marinated meat. In another study, marinated meat showed slight significant decrease in L* and a* values (Smith and Young, 2007). It was found that both a* and b* values decreased in marinated fillets (Young and Lyon, 1997; Allen *et al.*, 1998). It is not easy to resolve the changes in the color values of poultry muscle after marination because they depend on different factors, but the most important is the pH (Young and Lyon, 1994). It was found also that vacuum tumble marination has resulted to increase cooked meat lightness and decrease cooked meat redness (Young and West, 2001). After marination, phosphate

marinated fillet exhibited higher a* (0.84 *vs.* 0.33, P<0.05) and b* (10.19 *vs.* 9.05, P<0.05) than bicarbonate marinated fillets.

As expected, meat pH was increased after both types of marination treatment because both marinating ingredients are alkaling agents. Phosphate alone increased significantly (P<0.05) the pH of meat by approximately 0.15 units, whilst bicarbonate alone increased the pH by 0.34 units (Table 7.6). After cooking, bicarbonate marinated fillets exhibited higher pH value (6.24 *vs.* 6.15, P<0.05) than phosphate marinated fillets. This difference may explain due to the fact that phosphate marinated fillets before cooking had lower pH value (6.07 *vs.* 6.26) than bicarbonate marinated fillets. Bicarbonate showed a higher effect on the pH than phosphate and these results are consistent with the previous studies (Sen *et al.*, 2005). The differences in buffering capacity and ionic strength between phosphate and bicarbonate may be explained by the difference in the ultimate pH (Sindelar *et al.*, 2003). The greater effect of bicarbonates may be more due to a higher buffering capacity and ionic strength than phosphates.

Table 7.6 Color, pH, marinade uptake, drip loss, WHC and cooking loss (± standard error) for raw and cooked breast treated with bicarbonate and polyphosphate (pooled as cooking treatment).

Quality traits		Bicarbonate treatment	Phosphate treatment
L*	Fresh	51.02 ± 0.25^{x}	50.94 ± 0.25^{x}
	Marinade	55.30± 0.26$^{b;y}$	56.51 ± 0.37$^{a;y}$
a*	Fresh	0.46± 0.08	0.77 ± 0.08
	Marinade	0.33± 0.09^{b}	0.84 ± 0.10^{a}
b*	Fresh	9.50 ± 0.24	9.55 ± 0.24
	Marinade	9.05± 0.28^{b}	10.19 ±0.32^{a}
pH	Fresh	5.92± 0.02^{x}	5.92 ± 0.02^{x}
	Marinade	6.26± 0.02^{a,y}	6.07 ± 0.01^{b,y}
	Cooked	6.24± 0.01^{a,y}	6.15 ± 0.01^{b,y}
Marinade uptake%		17.27± 0.30^{a}	16.00 ± 0.41^{b}
Drip loss%*		3.24 ± 0.11^{a}	2.74 ± 0.10^{b}
WHC%		-2.43± 1.05	-2,38 ±1.61
Cooking loss		26.33± 0.49	27.03 ± 0.82

$^{a-b}$ Different superscript letters within a row mean significant difference (P<0.05).

^{x,y} Different superscript letters within a column mean significant difference (P<0.05).

* Water holding capacity estimated by the quantity of absorbed moisture using Van lack method

The pH of meat treated with bicarbonate and phosphate was not affected after cooking. This result was not in agreement with Sindelar *et al* (2003) who found that the pH of marinated sow loins treated with bicarbonate and polyphosphate increased after cooking. Bicarbonate-marinated fillets had significantly ($P<0.05$) higher values of marinade uptake (17.3 *vs.* 16.0%) and drip loss (3.24 *vs.* 2.74%) in comparison with phosphate-marinated fillets, respectively. Joo *et al.* (1999) found that drip loss was correlated with protein solubility, increase the solubility of myofibrillar, sarcoplasmic, and total proteins reduced the drip loss. The causes of the difference in the drip loss between phosphate and bicarbonate are not known, and so the protein solubility should be evaluated when bicarbonate uses in comparison to phosphate. The increase in marinade uptake can be attributed to the increased net negative charge associated to bicarbonate as well as due to the difference in pH. Water holding capacity and cooking loss did not show any significant differences among the treatments (Table 7.6).

Because the pH has great impact on tenderness, color, WHC and meat protein binding ability. The raw chicken fillets were distributed in a way to obtain no significant differences in the initial pH and lightness (L*) among different groups of treatments (Table 7.7). Lightness significantly ($P<0.05$) increased after marination and after cooking in all treatment groups of bicarbonate-marinated and phosphate-marinated fillets. Even all groups of each treatment (bicarbonate or phosphate) were separately marinated in one batch, but they showed different lightness values between groups within the same batch after marination and after cooking. In general, bicarbonate-marinated fillets cooked under different heat treatments exhibited slightly higher lightness values than phosphate-marinated fillets; in spite of that bicarbonate shifts the pH higher than phosphate and also it is well known that meat with high ultimate pH exhibits more dark color (less lightness) because its surface scatters less light than meat with a low ultimate pH (Lawrie, 1998). Nevertheless, the color of bicarbonate-marinated fillets became lighter.

Redness (a*) and yellowness (b) values after marination did not change in all groups of treatments. On the other hand, after cooking redness (a*) and yellowness (b) values were significantly ($P<0.05$) increased in all groups (Table 7.7). These results were in agreement with Resurrección (2003) who found that the marinated cooked samples were generally lighter (higher L*) and more yellow (higher b*) whereas a* (red color) increased as temperature and cooking time increased. The increase in lightness (L*) values after cooking could be explained by meat proteins denaturation during heating process which leads to

increased the reflection and scattering of light giving more lighter meat (Alvarado and Sams, 2003).

Table 7.7 Colour and pH values (± standard error) marinated breast with bicarbonate and polyphosphate cooked under different heat treatments.

Quality traits	Type of heat treatment*							
	76-160°C (PA)	80-160°C (PC)	76-200°C (PD)	80-200°C (PE)	76-160°C (BA)	80-160°C (BC)	76-200°C (BD)	80-200°C (BE)
L* raw	50.99 ± 0.87^{x}	50.45 ± 0.92^{x}	51.75 ±0.67^{x}	50.57 ±0.87^{x}	49.82 ± 1.13^{x}	52.22 ± 0.81^{x}	51.56 ± 0.43^{x}	50.47 ± 0.75^{x}
L*marinade	56.68±0.69^{abcy}	54.52± 0.68^{cy}	57.34±0.40^{aby}	57.48± 0.73^{ay}	55.24±0.88^{bcy}	54.56± 0.61^{cy}	55.62±0.65^{abcy}	55.76±0.70^{abcy}
L* cooked	76.97± 0.47^{cz}	78.69±0.66^{abcz}	77.11 ± 0.76^{cz}	78.01±0.77^{bcz}	77.46±0.58^{bcz}	79.26±0.61^{abz}	79.27±0.53^{abz}	80.01 ±0.35^{az}
a* raw	0.91 ± 0.34^{x}	,0.82 ± 0.10	0.72 ± 0.25^{x}	0.62 ± 0.21^{x}	0.98 ± 0.27^{x}	-0.06 ± 0.34^{x}	0.65 ± 0.21^{x}	0.28 ± 0.20^{x}
a* marinade	0.77 ± 0.27^{abx}	0.96 ± 0.12^{ax}	0.91 ± 0.19^{ax}	0.71±0.18^{abx}	0.50±0.23^{abcx}	0.10 ± 0.32^{bcx}	0.89 ± 0.32^{ax}	-0.19 ± 0.14^{cx}
a* cooked	2.13±0.16 ^{cdey}	2.39±0.14 ^{bcdy}	2.73± 0.14 ^{aby}	1.69 ± 0.15^{ey}	2.65± 0.24^{abcy}	2.2±0.24 ^{bcdey}	2.93 ± 0.16^{ay}	1.85 ± 0.20^{dey}
b* raw	10.46 ± 0.88^{x}	8.42 ± 0.73^{x}	9.77 ± 0.62^{x}	9.53 ± 0.64^{x}	8.19 ± 0.79^{x}	10.88 ±0.66^{x}	8.84 ± 0.86^{x}	10.07 ± 0.95^{x}
b* marinade	11.02±0.98^{axy}	8.20 ± 0.18^{bx}	10.77± 0.40^{ax}	10.77± 0.39^{ax}	8.10 ± 0.86^{bx}	10.07±0.61^{abx}	9.04 ± 0.78^{abx}	8.99 ± 0.83^{abx}
b* cooked	13.31 ± 0.81^{y}	13.98 ± 0.30^{y}	14.58 ± 0.38^{y}	14.62 ± 0.63^{y}	13.22 ± 0.57^{y}	14.94 ± 0.59^{y}	15.30± 0.49^{y}	14.92 ± 0.47^{y}
pH raw	5.92± 0.02^{x}	5.92± 0.02^{x}	5.92± 0.02^{x}	5.92± 0.02^{x}	5.92± 0.02^{x}	5.92± 0.02^{x}	5.92± 0.02^{x}	5.92± 0.02^{x}
pH marinade	6.05 ±0.02^{cxy}	6.09 ± 0.03^{bcy}	6.07 ± 0.03^{cy}	6.05 ± 0.02^{cy}	6.21±0.087^{aby}	6.34 ± 0.05^{ay}	6.21 ± 0.02^{aby}	6.26 ± .02^{ay}
pH cooked	6.16 ± 0.02^{dy}	6.17 ± 0.02^{bdy}	6.14 ± 0.02^{dy}	6.14 ± 0.01^{dy}	6.23± 0.03^{abcy}	6.28± 0.04^{ay}	6.21± 0.02^{bcdy}	6.24 ±0.02^{abcy}

* B and P represent bicarbonate, phosphate respectively while A, C, D, E represent heat treatment at different core and oven temperatures: 76-160°C, 80-160 °C, 76-200°C, 80-200°C respectively.

$^{a-e}$ Different superscript letters within a row mean a significant difference ($P<0.05$).

$^{x-z}$ Different superscript letters within a column mean a significant difference ($P<0.05$).

The increase in redness (a*) and yellowness (b) values in all groups could be explained by sugar-amine browning reaction that occurs on the dehydrated surface due to the dry heat, amine groups in the muscle proteins react with any available reducing sugars, such as free glucose, giving brown color derivatives. Browning occurs normally at high temperatures (more than 90°C); in our experiment the surface temperature was higher than 160°C. Another cause which may change redness (a*) and yellowness (b) values is formation of cooked meat pigments which show the brown color of metmyoglobin because of oxidation and denaturation of globular protein from heat (Hedrick *et al.*, 1994). Redness (a*) values for bicarbonate-marinated fillet were slightly higher than phosphate-marinated fillets. Trout (1989) pointed out that increase of the pH of the meat decreased heat denaturation of myoglobin during cooking, therefore resulting in an increase of pinkness or redness value. He observed also that the phosphate ion increases the susceptibility of myoglobin to heat denaturation, but the increase of pH due to the addition of tripolyphosphate compensates the effect of susceptibility to denaturation.

There were no significant differences in marinade uptake and drip loss between the groups assigned for different cooking conditions for both of bicarbonate and phosphate-marinated

treatments. The most severe heat treatment (80-200°C) showed the highest cooking loss (30.68 and 33.43%; P<0.05) and the lowest yield (69.32 and 66.57%; P<0.05) for both type of bicarbonate and phosphate-marinated treatments respectively (Table 7.8). At this type of heat treatment bicarbonate-marinated fillets showed higher ability to retain the moisture than phosphate, which can be seen by the results of cooking loss and yield. The rest of the heat treatment did not show any effect on the cooking loss and the yield in all of the groups.

By and large, Fillets treated with bicarbonate showed higher ability to retain water in comparison of phosphates. Actin (thin filament), myosin (thick filament), and their combined structure actomyosin are the most important protein, which play a major role in water binding capacity. Phosphates solubilize and unfold myofibrillar proteins due to electrostatic repulsion, and so more the amount of water that can be retained by the muscle due to increase the size of the space between the filaments. Therefore, anything that changes the spaces between the thick and thin filaments or the ability of the proteins to bind water can affect water-holding properties of the meat (Rust, 1987; Lawrie, 1998). The roles of phosphates in improving the water binding are well known and they work in different ways: due to their buffering capacity phosphates are able to shift pH far away from the isoelectric point of the myofibrillar proteins, unfolding muscle proteins which lead to more charged sites for water binding, and cleavage actomyosin bonds that formed in *post-rigor*, thereby increasing the potential for swelling of the filaments (Xiong, 2004). On the other hand, the exact mechanisms and the role of bicarbonate in improving the water binding-holding capacity are not well known.

Table 7.8 Marinade uptakes, Drip loss, cooking loss and yield (± standard error) for marinated breast with bicarbonate and polyphosphate cooked under different heat treatments.

	Type of heat treatment*							
Quality traits	76-160°C (PA)	80-160°C (PC)	76-200°C (PD)	80-200°C (PE)	76-160°C (BA)	80-160°C (BC)	76-200°C (BD)	80-200°C (BE)
Marinade uptake	16.16±0.77	14.66±0.72	16.68±0.87	16.50±0.85	16.20±0.57	17.18±0.83	18.06±0.93	17.65±0.93
Drip loss	2.64±0.09	2.35±0.15	3.10±0.23	2.87±0.19	3.52±0.39	2.74±0.61	3.60±0.20	3.10±0.13
Cooking loss	25.20±0.63^{c}	24.44±0.64^{c}	25.05±1.77^{c}	33.43±0.47^{a}	24.57±0.48^{c}	25.43±0.80^{c}	24.65±0.69^{c}	30.68±0.61^{b}
Yield	74.79±0.63^{a}	75.56±0.64^{a}	74.95±1.77^{a}	66.57±0.47^{c}	75.43±0.48^{a}	74.57±0.80^{a}	75.34±0.69^{a}	69.32±0.61^{b}

***** B and P represent bicarbonate, phosphate respectively while A, C, D, E represent heat treatment at different core and oven temperatures: 76-160°C, 80-160°C, 76-200°C, 80-200°C respectively.

$^{a-c}$ Different superscript letters within a row mean a significant difference (P<0.05)

In our results, bicarbonate exhibited a higher water binding capacity than phosphates which could be explained because bicarbonate increased more the pH and showed higher

ionic strength (Shread and Tali, 2010) and therefore it could increase the spaces between the thick and thin filaments more than phosphate. Sodium bicarbonate also produced holes during cooking due to the generation of carbon dioxide leading to coarser microstructure which could also improve the physical entrapment of water (Shread and Tali, 2010).

7.6 Conclusions

Chicken breast fillets treated with phosphates exhibited different quality traits (texture profile analysis, shear force, water activity, freezable water and chemical compositions) when compared with fillets treated with bicarbonate. Bicarbonate-marinated fillet showed a better water binding capacity and texture properties. The exact roles that stand behind these differences between bicarbonate and phosphate are not well known, but the main discriminated features of bicarbonate its ability to raise the pH higher than phosphate and the generation of carbon dioxide gases during cooking. The findings of this study suggest that phosphate marinated fillets interact with heat treatments in a different way in comparison with bicarbonate marinated fillets. Overall, Bicarbonate could be a promising agent to replace phosphates in meat formulation, but there is a necessity to evaluate the use of bicarbonate under different processing conditions and formulations.

CHAPTER 8

Partial replacement of sodium chloride with potassium chloride in marinated rabbit meat

8. 1 Abstract

Rabbit meat has similar traits to poultry meat; especially in nutritional aspects. Therefore, the meat industry has recently started to introduce some of attractive and convenient rabbit meat products to the market such as hamburgers, stuffed rolls and baby foods. The aim of the study is to evaluate the possibility to produce low sodium marinated rabbit meat products. In order to achieve the purpose of the study, sodium chloride has been replaced by potassium chloride at different levels starting from 20% up to 50%. Technological, sensorial, and microbiological traits of marinated rabbit meat have been evaluated at different levels of sodium reduction. In total, 226 rabbit loin meat samples were obtained and subjected to vacuum tumbling using solutions with different NaCl: KCl ratios. Replacing of sodium chloride up to 30% by potassium chloride did not change microbiological traits (total aerobic mesophilic and lactic acid bacteria maximum cell loads), sensorial acceptability (perceived saltiness and overall liking) and technological traits (pH, colour, texture, cooking loss, and yield). Otherwise, reduction of sodium chloride to 50%, significantly decreased perceived saltiness (4.15 *vs.* 4.73; $P<0.05$) and reduced microbial shelf-life by 1 day when compared to control, even if there was still no effect on technological traits. In conclusion, it is feasible imparting an added value for processed rabbit meat products by a reduction of sodium content which could increase market interest.

Keywords: Rabbit meat; marination; salt replacement; potassium chloride; meat quality.

8.2 Introduction

In the last few years, high sodium dietary intake and its implications on hypertension and cardiovascular diseases has lead to several successful attempts to reduce sodium levels in meat products (Ruusunen and Puolanne, 2005; Desmond, 2006; Doyle and Glass, 2010). Nevertheless, dietary sodium intake is still underestimated and exceeds recommended dietary allowance (Newson *et al.*, 2013).

Particularly, reduction of sodium chloride in meat products adversely influences water binding capacity, texture, safety and microbial shelf-life (Lanciotti *et al.*, 2001; Desmond, 2006; Taormina, 2010). Moreover, sodium reduction at certain levels decreases the intensity of native and added flavours of meat products which could affect consumer acceptance of low-salt meat products (Ruusunen and Puolanne, 2005; Desmond, 2006).

There are actually different approaches to reduce sodium content in processed meat products. One of them is the use of salt substitutes (i.e. potassium, magnesium and calcium chloride and salt of lactate, citrate, ascorbate and sulphate) possibly together with masking agents. Another strategy is the use of flavour enhancers (i.e. yeast extracts and dehydrated proteolyzed milk or cereal proteins) which increases the perceived saltiness (Desmond, 2006; Petracci *et al.*, 2013a).

Although potassium chloride has been extensively evaluated as a salt replacer in different meat products (Gou *et al.*, 1996; Gelabert *et al.*, 2003; Campagnol *et al.*, 2011; Lee *et al.*, 2012; Jin *et al.*, 2013), no studies are available in the literature that evaluated the inclusion of potassium chloride in rabbit meat formulations.

Employing potassium chloride in meat formulations has well known limitations at certain concentrations such as bitter flavour. In addition, potassium chloride is less effective than sodium chloride in promoting meat protein functionality and some adjustments have to be done during formulation and processing when designing low-sodium products (Petracci *et al.*, 2013a). On the other hand, several studies revealed that potassium chloride had a similar effect against different spoilage and pathogenic species when used as salt replacer at certain conditions (Askar *et al.*, 1993; Bidlas and Lambert, 2008; Fuentes *et al.*, 2011; Harper and Getty, 2012).

Based on all former studies, technically potassium chloride can be suitable to reduce the level of sodium in processed rabbit meat products taking into consideration its limitations. In addition, the rabbit meat is considered as a unique white meat with functional and nutritional characteristics such as low-fat meat with a high unsaturation degree of fatty acids, low cholesterol and heme-iron levels, and high content of conjugated linoleic acid, vitamins and antioxidants (Dalle Zotte and Szendrő, 2011). However, the market share of rabbit meat is still limited to whole carcass or cut-up products

and until now, there are few processed rabbit meat products available in the market such as stuffed rolls, hamburgers and baby foods (Cavani *et al.*, 2009). Hence, production of processed rabbit meat products with added value (like low sodium contents) can alleviate the ethical implications which considered rabbit as a pet animal. Moreover, during processing as in marination, typical wild flavour of rabbit meat which negatively impact attitudes of young and unfamiliar consumers can be covered by introducing some flavoring agents (Petracci and Cavani, 2013a).

Marination can also improve the texture and juiciness and increase the yield, which could reduce the cost of production. All these processing aspects could impart most marketable features to rabbit meat. Thereupon, there were several studies which evaluated the effect of marination on other types of meat and also there are many products of marinated pork, beef, and poultry meat products available in the market (William, 2012), while similar rabbit meat products are not currently marketed nor well investigated.

The aim of this study was to evaluate the effect of sodium chloride replacement with potassium chloride (from 20 to 50%) on marination performances, technological and sensory properties as well as microbial shelf-life of marinated rabbit meat to be sold as an uncooked value-added product.

8.3 Materials and methods

8.3.1 Experimental design

The study was divided into two experiments: the first was dedicated to evaluate the effects of sodium chloride replacement with potassium chloride (from 20 to 50%) on marination performances and technological traits. In the second experiment, the effects of 30 and 50% salt replacement on sensory properties and microbial shelf-life were investigated. These levels were selected based on the results of the first experiment as well as on previous findings, which showed that sodium chloride replacement up to 30% can be achieved without impairing sensorial and microbiological properties, while 50% substitution level may determine some adverse effects (Ruusunen and Puolanne, 2005; Desmond, 2006; Doyle and Glass, 2010; Lee *et al.*, 2012).

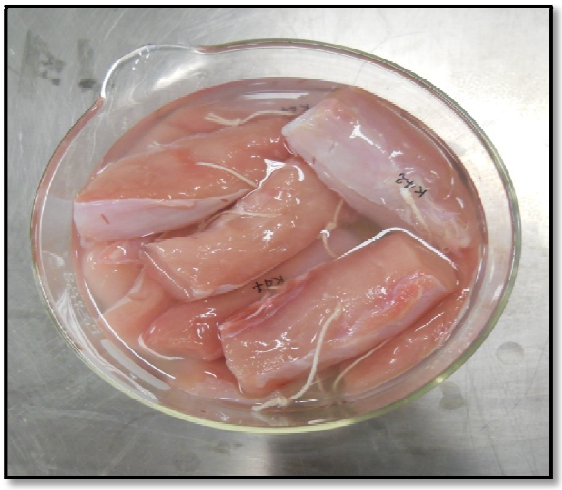
8.3.2 Experiment 1

A total of 100 *Longissimus lumborum* meat samples, belonging to the same rabbit batch (11 weeks-old, average weight of 2.7 kg), were obtained 24 hours *post-mortem* from a local commercial plant. The samples were trimmed in order to remove external connective tissue and fat. In two replications, the samples were divided into five groups (10 samples/group) having similar weight and pH. The colour was also measured on transversal section.

Each group was marinated under vacuum in a small-scale tumbler (model MGH-20, Vakona Qualitat, Lienen, Germany) (Figure 8.1b) using 20% (wt/wt) marinade solution (Figure 8.1a) formulated in order to achieve 1.8% as a final target concentration of salts in marinated meat and different NaCl:KCl ratios: 100:0, 80:20, 70:30, 60:40 and 50:50. Tumbling was performed in 46 min (4±1°C) at a speed of 20 rpm including three working cycles (13 min/cycle) and two pause-cycles (3 min/cycle). Marinade uptake, colour after marination, purge loss (Figure 8.1c), cooking loss, total yield, colour after cooking (Figure 8.1d and Figure 8.1e) and Allo-Kramer shear force (Figure 8.1f) were measured in each sample as described in 3.3.2 Quality traits analysis.

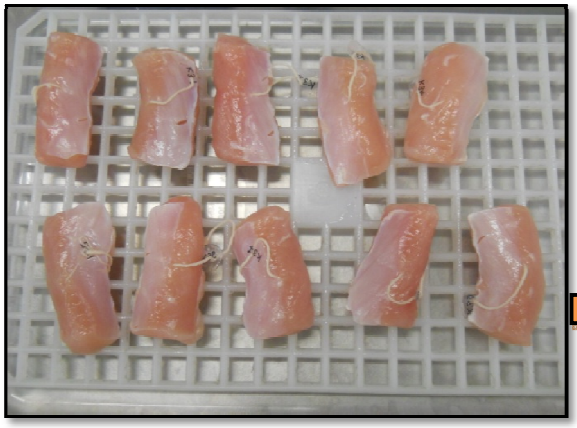
Colour measurement. The colour profile of lightness (L*), redness (a*), and yellowness (b*) was measured on a transversal section before and after marination and after cooking by a reflectance colorimeter.

Shear force measurement. The shear force test was performed using a "TA-Hdi® Texture Analyser" (StableMicro Systems, UK) equipped with an Allo-Kramer shear cell set with 25 kg load cell and crosshead speed of 500 mm/min. From each cooked loin, a 2-cm long sample was cut (removing both cranial and caudal parts), re-weighted and sheared while keeping blades perpendicular to the muscle fibres direction. Shear force was expressed as kilograms shear per gram of sample (Bianchi *et al.*, 2007b).



a. Sample preparation: labelling and soaking in marinade solution

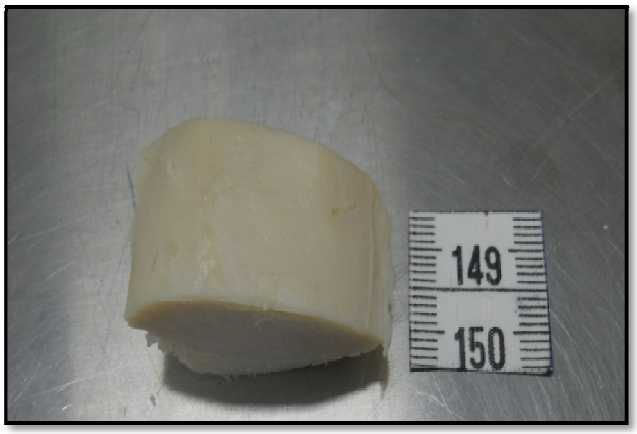
b. Marination by using small scale vacuum tumbler



c. Storage at refrigeration conditions for drip loss and purge loss

d. Cooking the samples in hot water bath at 80°C

e. Drying and weighting the samples for cooking loss



f. Sample for shear force

Figure 8.1 Schematic flowchart for experimental design

8.3.3 Experiment 2

Using the same procedures adopted for experiment 1, a total of 126 *Longissimus lumborum* meat samples were divided into three groups (42 samples/group) and tumbled using marinade solutions characterised by 100:00, 70:30 and 50:50 NaCl: KCl ratio, respectively. After tumbling, a half of samples from each group were used for assessing microbiological traits, while the remaining ones were used to perform sensory analysis.

Microbiological traits. After marination, samples were individually packed under vacuum and stored for 21 days at 4±1°C in a cooler. During this period, three samples (considered as replications) for each group were used to monitor the main microbial populations at 0, 3, 7, 10, 13, 17 and 21 days of storage. Aseptically, 10 g from each *Longissimus lumborum* were weighted into a sterile plastic bag, then 90 ml of saline/peptone water was added and homogenised using a Stomacher mixer (Lab Blender Seward, PBI International) for 120 sec. Suitable serial decimal dilutions were then prepared and aliquots (1 or 0.1 ml) used to inoculate plates in order to enumerate viable counts of total aerobic mesophilic bacteria, by using Plate Count Agar (Oxoid) incubated for 48-72 h at 30°C considering all colonies appeared in the petridishes, and lactic acid bacteria (LAB) evaluated with de Man

Rogosa, Sharpe (MRS) Agar (Oxoid); the plates were incubated under anaerobic conditions for 3 days at 37°C by using anaerobic jar.

Sensory analysis. Following marination, *Longissimus lumborum* samples were packaged under vacuum, cooked in a water bath (1 hour, 80°C) and allowed to equilibrate to room temperature. Afterwards, cranial and caudal parts were removed and central section was cut in order to obtain three samples from each loin with the same thickness. Consumer test was conducted in a certified sensory laboratory (UNI ISO 8589, 1989) using 40 untrained panellists demographically characterised as follows: 38% and 62% were male and females, respectively; 58% were from 20 to 30 years old, 22% were from 30 to 40 years old, and 20% were older than 40 years. Each panellist was offered three warm samples (1 for each group) according to a randomized block design and asked to score each sample for perceived saltiness, ranging from 1 (extremely unsalted) to 7 (extremely salted) and overall liking from 1 (extremely disliking) to 7 (extremely liking) by using the following questionnaire.1.

Questionnaire.1
CARD TESTER N.

NAME AND FAMILY :……..
Sex ☐ M ☐ F
Age : ……
are you regular consumer of meat? ☐ YES ☐ NO

INSTRUCTION OF TEST

Evaluate the samples from left to right. For each of the two samples, check the box on the judgments of Sapidity and Pleasantness.

Rabbit meat

Sample	LEVEL OF SALTNESS						
	Extremely bland	Very bland	Slightly bland	Neither salty nor bland	Moderately salt	Very salt	Extremely salt
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐

Sample	LEVEL OF ACCEPTANCE						
	Extremely unaccepted	Very unaccepted	Slightly unaccepted	Niether accepted nor unaccepted	Moderatley accepted	Very accepted	Extremely accepted
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐

NOTE:
……
……
……
……
……

8.4 Statistical analysis

Data about marination performances and technological traits were analysed by using two-ways ANOVA considering marinade solution, trial and their interactions as principal effects. Sensory data were analyzed using a two-way ANOVA, treating a marination type as a fixed effect, while panellist and panellist by marination-type interactions (if any) as random effects. Mean differences were separated by using Tukey's HSD post-hoc test at a significance level of 0.05. Differences in microbial loads at the same sampling time were assessed by using the t-test and significance of differences was defined at $P \leq 0.05$. Moreover, microbiological data of mesophilic and lactic acid bacteria collected over storage were modelled by using the Gompertz equation as modified by Zwietering *et al.* (1990).

$$y = K + A \exp\left\{-\exp\left[\left(\mu_{max}\, e/A\right)(\lambda - t) + 1\right]\right\}$$

Where y is the load cell data (Log CFU g^{-1}) at time t (days), K is the initial contamination level (Log CFU g^{-1}), A is the maximum cell increase attained at the stationary phase (Log CFU g^{-1}), μ_{max} is the maximum growth rate (Δ Log [CFU g^{-1}] per day) and λ is the lag phase length (days). The growth parameters derived from the Gompertz equation (i.e. A, that is the maximum cell increase, μ_{max} that is the maximum growth rate and λ the lag phase length) for the mesophilic bacteria at the different marinating conditions were then used to estimate the product shelf life, which was calculated as the time necessary to attain a threshold level of 7 Log CFU g^{-1} as a critical cell load for the mesophilic spoilage-associated microflora.

8.5 Results and discussion

8.5.1 Marination performances and technological properties (Experiment 1)

As a result of experimental design, pH and the colour of the samples before marinating did not differ among experimental groups (Table 8.1). It is noteworthy to highlight this aspect because of the well-known influence of pH and colour on water holding capacity of the meat (Hulot and Ouhayoun, 1999). In this way, it was possible to exclude any interference on subsequent measurements of water holding and binding capacities that are normally used to evaluate the ability of meat to absorb liquid during marinating and retain it during subsequent storage and cooking phases. In this regard, some

studies performed on poultry demonstrated that both raw meat pH and colour significantly influenced marinade solution adsorption and cooking performance (Qiao *et al.*, 2002).

No relevant differences were found in pH after cooking as well as in colour after marination and cooking with the exception for the groups with 40% and 50% sodium chloride replacement, which exhibited higher pH in respect to control group as well as increased redness (a*) values only if compared with the 80:20 NaCl: KCl group (Table 8.1). Overall, these results were in agreement with previous similar studies conducted on beef and pork in which no or slight differences were found when sodium chloride was replaced with potassium chloride up to 50% (Terrell *et al.*, 1981; Jin *et al.*, 2013). Moreover, also Petracci *et al.* (2012b) found that marinated cooked chicken meat with high ultimate pH was associated with higher a* values. This can be due to the protective effect exerted by high pH value against heat denaturation of myoglobin during cooking, which resulted in an increase of meat redness (Trout, 1989).

With regard to marination performances, partial sodium chloride replacement with potassium chloride up to 50% did not exhibit any negative effects on marinade uptake, cooking loss and total yield (Table 8.1). Significant differences were only found in purge losses which were higher in samples marinated by using a 40% sodium chloride substitution with respect to 20% ones, while other groups showed intermediate values.

In a similar study conducted on marinated chicken breast meat, Lee *et al.* (2012) found that sodium chloride replacement with potassium chloride up to 75% did not impair marinade uptake and retention, while the amount of juices lost during cooking increased when sodium chloride was partially replaced with potassium chloride. Recently, Jin *et al.* (2013) found that partial or complete replacement of sodium chloride by potassium chloride in sausages and cooked ham had no negative effect on water holding capacity. According to Hamm (1986) hypothesis, the role of sodium chloride depends mainly on Cl anions that penetrate into myofilaments leading to swelling them due to electrostatic force; this can explain why replacing sodium chloride with potassium chloride up to 50% in this study did not impair water holding capacity.

Finally, no significant differences were found in Allo-Kramer shear force values for all levels of salt replacement (Table 8.1). Lee *et al.* (2012) also ascertained that partial sodium chloride replacement with potassium chloride did not modify instrumental meat tenderness in marinated poultry meat, while Jin *et al.* (2013) evidenced a lower shear force in cooked ham formulated with potassium chloride. Furthermore, if the absolute Allo-Kramer shear force values that were obtained in the present study are compared to those found by Bianchi *et al.* (2007b) on non-marinated rabbit meat, it can be argued that the marination determines an increase in meat tenderness also in rabbits

as well as widely observed in different kinds of meat such as beef, pork and poultry (Scanga *et al.*, 2000; Sheard and Tali, 2004; Petracci *et al.*, 2012b).

Table 8.1 Effect of different levels of NaCl replacement with KCl (100:0, 80:20, 70:30, 60:40 and 50:50 NaCl: KCl ratio) on marination performances and quality traits of marinated rabbit meat (n=20/group).

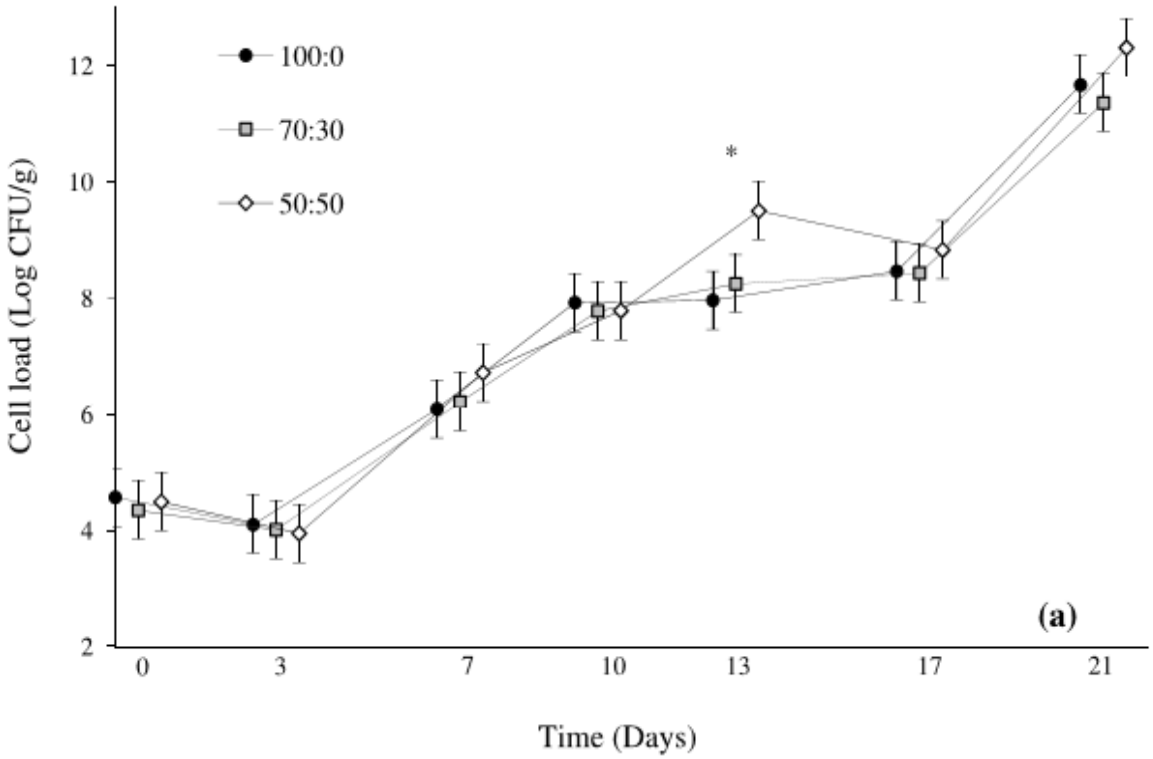
Parameter	Marinade solution (NaCl: KCl ratio)					RSE	Prob.
	100:0	80:20	70:30	60:40	50:50		
Non-marinated raw meat							
pHu	5.88	5.88	5.88	5.88	5.89	0.007	0.999
lightness (L*)	47.7	47.8	47.4	48.5	47.8	6.991	0.793
redness (a*)	2.79	2.56	2.58	2.63	2.81	0.416	0.618
yellowness (b*)	-1.06	-1.01	-1.41	-0.86	-0.92	1.189	0.545
Marinated raw meat							
uptake (%)	18.1	18.3	18.2	18.3	18.3	7.485	0.997
lightness (L*)	45.2	45.1	44.6	46.1	44.9	3.552	0.149
redness (a*)	3.76	3.68	3.53	3.60	3.70	0.470	0.851
yellowness (b*)	-0.45	-0.17	-0.64	-0.07	-0.68	1.165	0.274
purge loss (%)	2.27^{ab}	2.25^{b}	2.50^{ab}	2.58^{a}	2.34^{ab}	0.157	0.036
Marinated cooked meat							
cook loss (%)	20.7	20.2	20.8	20.7	20.9	5.008	0.892
total yield (%)	91.5	92.1	92.0	91.0	91.0	7.829	0.623
pH	6.08^{b}	6.10^{ab}	6.12^{ab}	6.15^{a}	6.14^{a}	0.004	0.010
lightness (L*)	73.5	74.2	74.5	74.7	74.6	2.285	0.106
redness (a*)	1.96^{ab}	1.70^{b}	1.92^{ab}	2.10^{a}	2.28^{a}	0.191	0.001
yellowness (b*)	7.46	7.07	7.06	7.18	7.38	0.381	0.157
Allo-Kramer shear force (kg/g)	1.91	2.07	2.04	1.90	2.03	0.067	0.128

RSE, residual square error.
$^{a, b}$ = $P < 0.05$

In conclusion, experiment 1 revealed that the replacement of sodium chloride up to 50% with potassium chloride did not determine any adverse effects on marination performances and appearance and tenderness of the rabbit meat.

8.5.2 Microbiological traits and sensory properties (Experiment 2)

As shown in figure 8.2, the total number of aerobic mesophilic bacteria, which is commonly used as a contamination index for meat products, and LAB were assessed to evaluate the microbial shelf-life. Although no significant differences (P>0.05) in total viable counts at the end of storage period (day 21) were detected among all marinating treatments, different growth patterns for lactic acid bacteria were observed when sodium chloride was replaced by different levels of potassium chloride. In this context, the gradual sodium chloride replacement resulted in a more rapid growth for the LAB as evidenced by the growth rate (μ_{max}). The values obtained by modelling the cell count data over storage with the Gompertz equation (Table 8.2). On the other hand, a lag phase extension of 1 and 3 days was detected in samples marinated by using 70:30 and 50:50 NaCl: KCl ratios, respectively; as shown by the λ values reported in Table 8.2. Evaluating the inhibitory effect of potassium chloride on microbial growth is of relevant importance in order to define its potentialities as sodium chloride replacer. In this context, Aliño *et al.* (2010) found that there were no significant differences in the counts of aerobic mesophiles and lactic acid bacteria when sodium chloride was partially replaced with mixtures of potassium, magnesium and calcium chloride in pork loins.



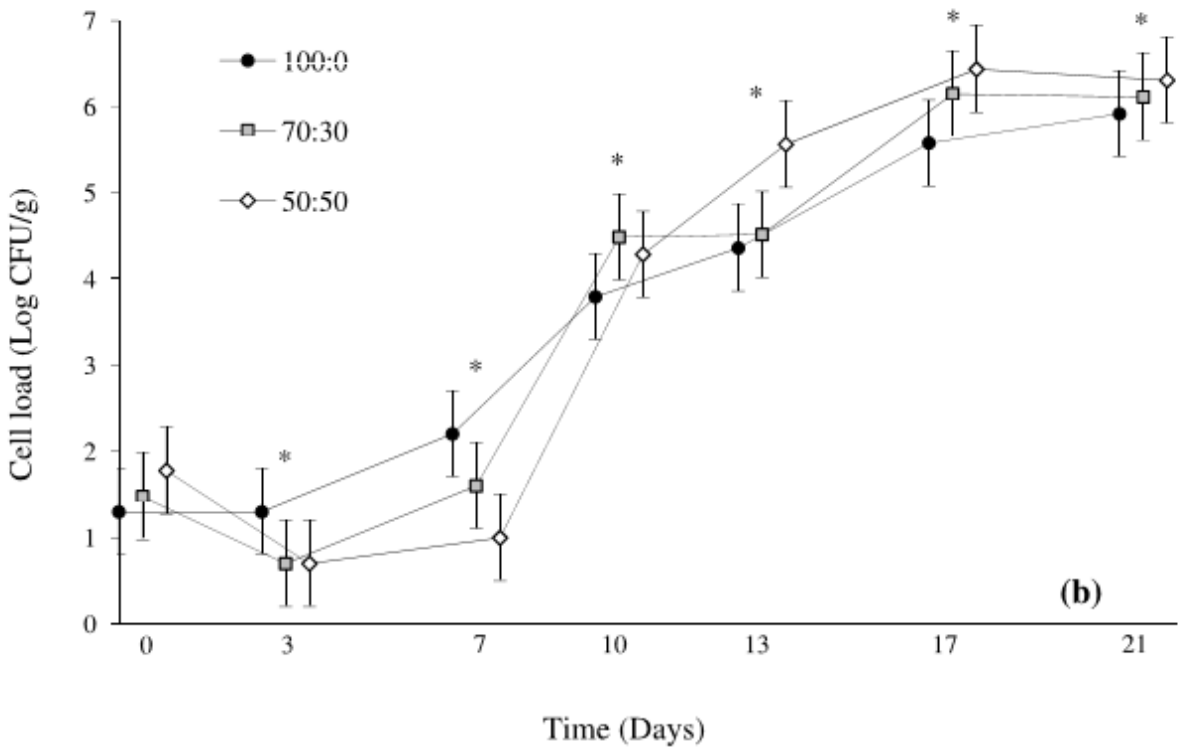


Figure 8.2 Effect of different levels of NaCl replacement with KCl (100:0, 70:30 and 50:50 NaCl:KCl ratio) on evolution over storage at 4°C of mesophilic bacteria (a) and lactic acid bacteria (b) of marinated rabbit meat packaged under vacuum (n=3/storage time/group) (* = P<0.05).

In a further study, a similar trend was also observed where different levels of NaCl replacement by KCl in fermented sausages showed that neither lactic acid bacteria nor *Micrococcaceae* were affected (Campagnol *et al.*, 2011).

On the other hand, it was demonstrated that equal molar concentrations of NaCl and KCl had a similar inhibitory effect against some food-borne pathogens (Gelabert *et al.*, 2003; Bidlas and Lambert, 2008). In this regards, Boziaris *et al.* (2007) found that potassium chloride maintained the same inhibitory activity as sodium chloride against *Listeria monocytogenes* Scott A in terms of lag phase duration, growth and death rate. Nevertheless, a study was carried out by Samapundo *et al.* (2010) indicated that the microbiological consequences of the full or partial replacement of NaCl on the growth of *Lactobacillus sakei* largely depend on the initial concentration of NaCl, level of its replacement and nature of the salt replacer. The same authors also reported that NaCl and KCl have approximately equal inhibiting effects on the estimated growth of *Lactobacillus sakei* in broth when added alone at equimolal concentrations. On the other hand, 50% replacement of NaCl (4.2%) by KCl resulted in a significantly faster growth rate, in addition to a 1 day extension of the lag phase.

Table 8.2 Predicted values of the Gompertz parameters obtained by modelling the viable count data of total mesophilic bacteria and lactic acid bacteria in marinated rabbit meat packaged under vacuum.

Marinade solution	Total mesophiilic bacteria			Lactic acid bacteria		
(NaCl:KCl ratio)	A[1]	μ_{max}[2]	λ[3]	A	μ_{max}	λ
100:0	4.14	0.76	3.41	4.96	0.42	4.55
70:30	4.53	0.73	2.53	5.02	0.72	5.93
50:50	4.82	0.88	3.13	5.09	1.26	7.55

[1] maximum cell increase (Log CFU/g)

[2] maximum growth rate (ΔLog CFU/g*day)

[3] lag phase length (day)

Considering mesophilic bacteria, the results demonstrated that up to 30% of the sodium chloride replacement did not affect their growth parameters, while a slightly higher growth rate was observed for the 50:50 NaCl: KCl ratio marinating group (Table 8.2). As a consequence, control and 70:30 NaCl: KCl groups presented a similar microbiological shelf-life (time necessary for the level of mesophilic bacteria to reach 7 Log CFU/g) of 8 days, while the 50:50 NaCl: KCl one had a one-day shorter shelf-life (Table 8.3). This result is in agreement with the work of Samapundo *et al*. (2010) who found that the growth rate of *L. sakei* was significantly lower when NaCl was used alone (4.2%) than that obtained at a replacement level of 50% with KCl. The findings of this study also revealed an extended shelf-life if compared with Rodriguez-Calleja *et al*. (2005) who estimated that aerobic counts of rabbit meat (overwrapped with oxygen-permeable film and refrigerated stored) achieved the critical value of 7 Log CFU/g after 5 days of storage. These results could be explained due to differences in the initial microbial load resulting from the hygienic conditions during sample collection, handling, and processing. Moreover, in our study vacuum packaging was used instead of overwrapped with an oxygen-permeable film thus imparting anaerobic conditions. It is well known that survival and growth of spoilage organisms are greatly affected by the gaseous composition of the atmosphere surrounding the meat. In particular, the aerobic storage can accelerate spoilage due to the fast growing of the pseudomonads, while vacuum and modified atmosphere packaging can favour the dominance of a facultative anaerobic population including lactic acid bacteria and *B. thermosphacta* (Doulgeraki *et al.*, 2012). As vacuum packaging can prevent the growth of some food-borne pathogens and spoilage bacteria commonly present on meat, it is widely used for packaging primal cuts for distribution to retailers.

Table 8.3 Effect of different levels of NaCl replacement with KCl (100:0, 70:30 and 50:50 NaCl: KCl ratio) on predicted microbial shelf-life of marinated rabbit meat packaged under vacuum.

Marinade solution (NaCl:KCl ratio)	Microbial shelf-life (days)[1]
100:0	8.3
70:30	8.2
50:50	7.3

[1] Time necessary to attain a mesophilic count of 7 Log CFU/g, calculated by using the predicted Gompertz parameters.

Water activity (a_W) is a physical property that has a direct implication for microbiological safety and spoilage of food. Therefore, water activity a_W has been measured in parallel to microbiological analysis during the storage period (Figure 8.3). a_W is defined in terms of thermodynamic concepts such as the chemical potential and is related to the osmotic pressure of an aqueous solution. When a substance such as salt (sodium chloride) is dissolved in water, the water activity is reduced. For this reason, why a_W has been measured in this study. a_W of a food or solution is the ratio of water vapor pressure of the food or solution (p) to that of pure water (p_o) at the same temperature:

$a_W = p/p_o$

Overall, the results showed there was no significant change in a_W during storage for all treatments and also between treatments at each point of storage period. These results may be explained due to higher moisture content of meat and lower content of salt that may be not significant to detect.

In general, microorganisms grow best between a_W values 0.995-0.980, while most microbes cease growth at a_W <0.900. For this reason the effect of a_W on microbiological growth was not apparent.

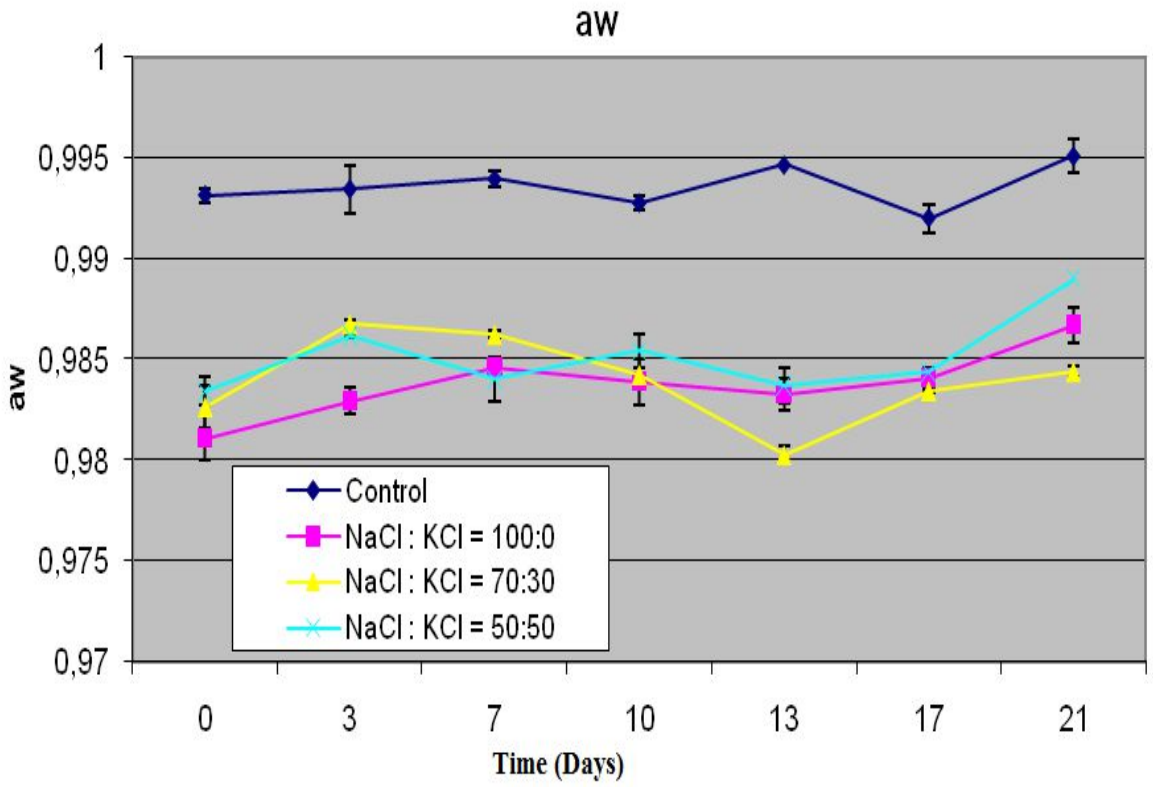


Figure 8.3 Effect of different levels of NaCl replacement with KCl (raw, 100:0, 70:30 and 50:50 NaCl: KCl ratio) on a_W.

Because pH has positive or detrimental effects on microbiological growth, it was also measured during the storage period (Figure 8.4). According to the chemiosmotic theory (Brock, 1969) pH has a profound effect on the growth and viability of microbial cells. Each species has an optimum and a range of pH for growth. The inhibition of growth at a low pH could arise from insufficient energy to shift protons outwardly through the cell membrane to establish a proton motive gradient (Garland, 1977). As it has also been accepted by many authors that membranes may not be impermeable to protons (Stouthamer, 1979).

The partial inhibition of growth could result from an additional expenditure of energy to maintain the membrane potential. Microbes, such as bacteria, are sensitive to the hydrogen ion concentration they find in their environment. Large proteins, such as enzymes, are affected by pH. Their shape changes (they denature) and very often brings about an alteration of the ionic charges on the molecule. Usually, the catalytic properties of the enzymes are lost and metabolism is halted.

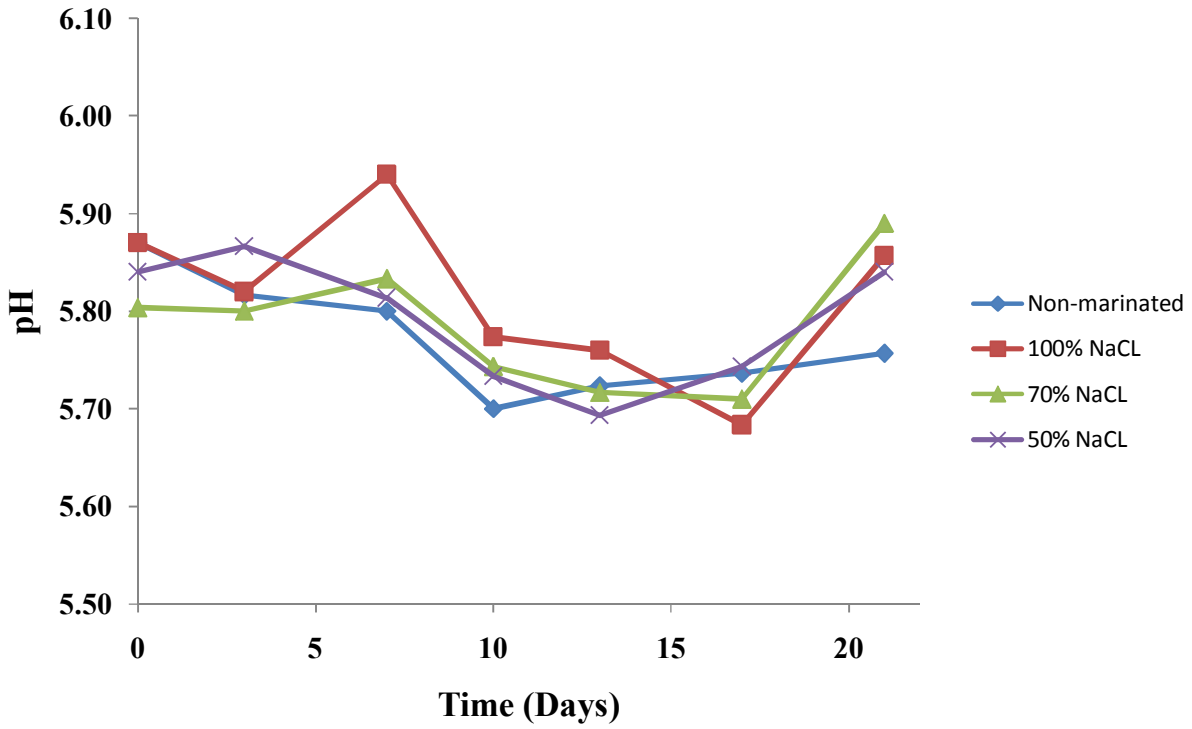


Figure 8.4 Effect of different levels of NaCl replacement with KCl (raw, 100:0, 70:30 and 50:50 NaCl: KCl ratio) on pH.

As for sensory evaluation, perceived saltiness and overall liking of cooked meat samples marinated with different NaCl: KCl ratios are presented in Figure 8.5. Overall, perceived saltiness was judged between score 4 (neither salty nor un-salty) and 5 (moderately salty) and overall liking very close to score 5 which corresponded to "moderately likely".

Panellist did not find any differences between control and 70:30 NaCl:KCl group for both perceived saltiness and overall liking, while panellists perceived lower saltiness in 50:50 NaCl: KCl group even if this result did not impair overall liking which did not differ if compared with control and 70:30 groups. Overall, reduced perceived saltiness can be likely the result of the lowered amount of sodium ions which were replaced with potassium ions and it could be also due to the change in chloride ion balance after replacement process. It is well-known that perceived saltiness and flavour are mainly related to sodium and chloride ions at certain concentration of salt concentration (Hutton, 2002).

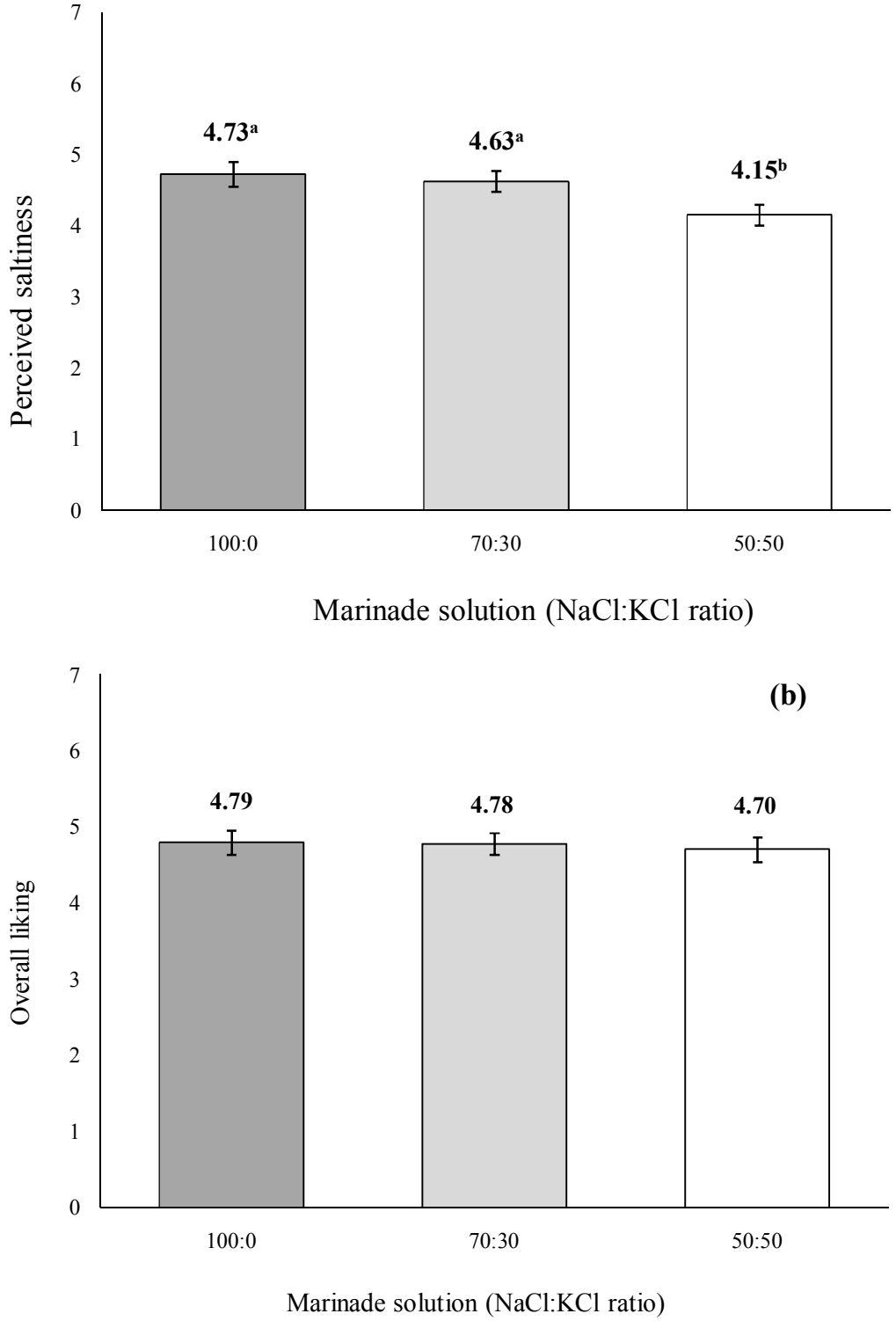


Figure 8.5 Effect of different levels of NaCl replacement with KCl (100:0, 70:30 and 50:50 NaCl: KCl ratio) on perceived saltiness (a) and overall liking (b) of marinated rabbit meat (a-b=P<0.05) (n. of panellists = 40).

The findings of the present study partially agree with those of Lee *et al.* (2012) who found that chicken breast meat samples with 75% replacement of NaCl had significantly perceived saltiness

lower than the control, while no significant differences were reported for 25% and 50% substitution levels. This different outcome may be attributed to the low concentrations of NaCl (0.5%) used in the marinating solution by Lee *et al.* (2012) with respect to the present study (1.8%). Several authors reported that replacement of NaCl with KCl can result in bitter or metallic off-flavours (Ruusunen and Poulanne, 2005; Desmond, 2006; Petracci *et al.*, 2013a). For example, Keeton *et al.* (1984) found that hams cured with one third substitutions with KCl exhibited a slight degree of bitterness without impairing acceptance, while higher substitution rates resulted in unacceptable products. Gou *et al.* (1996) reported taste defects in dry-cured pork loin formulated with 30% KCl replacement. On the other hand, Wheeler *et al.* (1990) reported that replacing 35% of the NaCl with KCl did not have any detrimental effect on sensory properties of restructured beef steaks. From the present study, sodium chloride in marinated rabbit meat can be replaced with KCl up to 30% without any detectable effect on sensory traits and in any case a substitution of up to 50% did not adversely affect product acceptability. This implies that it is not strictly needed to add masking agents in order to reduce bitterness delivered by KCl.

8.6 Conclusions

Overall, this study showed that raw rabbit meat has technological characteristics similar to the most common sources of meat (beef, pork and poultry) and it is suitable to manufacture uncooked value-added products. Thirty percent of sodium reduction of marinated products can be formulated by using potassium chloride as a salt replacer without negatively affecting microbiological shelf-life as well as sensory and technological characteristics of the meat. Otherwise, a 50% reduction of sodium chloride decreased perceived saltiness and microbial shelf-life. This implies that a more complex approach combining integrated formulation (i.e. flavour enhancers, antimicrobial agents) and processing solutions (i.e. high hydrostatic pressure) is needed for a replacement rates greater than 30%.

9. General conclusions

Recently, global meat market is facing several dramatic changes due to shifting in diet and life style, consumer demands, and economical considerations. Firstly, there was a tremendous increase in the poultry meat demand due to low cost, good nutritional profile, and suitability for further processing. Furthermore, current forecast and projection studies pointed out that the expansion of the poultry market will continue in future. In response to this demand, there was a great success to increase growth rate of meat-type chickens in the last few decades in order to optimize the production of poultry meat. Accordingly, the increase of growth rate induced the appearance of several muscle abnormalities such as pale-soft-exudative (PSE) syndrome and deep-pectoral-myopathy (DPM) and more recently white striping and wooden breast. Currently, there is growing interest in meat industry to understand how much the magnitude of the effect of these abnormalities on different quality traits for raw and processed meat. Therefore, the major part of the research activities during the PhD project was dedicated to evaluate the different implications of recent muscle abnormalities such as white striping and wooden breast on meat quality traits and their incidence under commercial conditions. Generally, our results showed that the incidence of these muscle abnormalities was very high under commercial conditions and had great adverse impact on meat quality traits. Secondly, there is growing market share of convenient, healthy, and functional processed meat products. Accordingly, the remaining part of research activities of the PhD project was dedicated to evaluate the possibility to formulate processed meat products with higher perceived healthy profile such as phosphate free-marinated chicken meat and low sodium-marinated rabbit meat products. Overall all findings showed that sodium bicarbonate can be considered as promising component to replace phosphates in meat products, while potassium chloride under certain conditions was successfully used to produce low marinated rabbit meat products.

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