EFFECT OF MARINATION ON THE QUALITY CHARACTERISTICS AND MICROSTRUCTURE OF CHICKEN BREAST MEAT COOKED BY DIFFERENT METHODS

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Abstract
Marination is a simple technological treatment used to improve the tenderness and flavor of meat by soaking it in an aqueous solution which composed of different ingredients. The effect of marination and different cooking methods (microwave, roasting, boiling, and frying) on the chemical composition, quality characteristics (i.e., PH value, meat color (L*, a*, and b*), cooking loss, water holding capacity(WHC), and Microstructure characteristics (i.e., myofibril of pectoralis major) of chicken breast meat were analysed. The present study demonstrated that the use of marination process together with cooking methods significantly modified the chemical composition of chicken breast meat. Also quality characteristics were evaluated and they were significantly different \( P \leq 0.05 \) between the marinated cooked chicken breast and the other unmarinated cooked (control). In addition transmission electron microscopy (TEM) observations illustrated changes in myofibril units (sarcomeres) of pectoralis major of chicken muscle Fragmentation, physical disruptions, and gaps or splitting occurring within these myofibril, especially in the regions adjacent to the Z-lines and within the A-band and I-band myosin/actin are associated with tenderization. Finally our results indicate that marination process is a good technique to improve the quality of chicken breast meat cooked by different methods.

Key words: Marination, cooking methods, Chemical composition, quality, Microstructure

INTRODUCTION

Poultry breast meat is of particular interest. However, while birds with increased growth rates have generally heavier muscles, they also have thick fiber [11]. For instance, the metabolic type of muscle fiber is associated with the color of meat, its tenderness which is also partly a function of the diameter of the muscle fibers and its flavor and juiciness. The biochemical properties and the microstructure [42] of these fibers also influence some of the parameters of the muscle such as PH decline, drip loss and meat color.

Meat tenderness is a function of production, processing, value adding, and cooking method used to prepare the meat for consumption by the consumer. It can be related principally to the connective tissues and myofibrillar protein components of muscle [21]. Tenderness of meat products together with juiciness, flavor and color are the main eating quality characteristics that do influence the consumer’s overall judgement of quality [47]. They can be influenced by several production factors (Genetics, feeding system, etc) and processing techniques (chilling, marination, and cooking) [34].

Marination is a traditional culinary technique that is used to tenderize and to improve flavor, juiciness of poultry meat [23]. Sodium chloride, polyphosphates and sugars are considered important ingredients of marinades and they improve meat tenderness and flavor [43], [41]. Acidic marination involves immersion of meat in an acidic solution of vinegar, wine or fruit juice [8]. Marinating also increases water binding capacity of meat, thus reducing cooking losses and improving meat juiciness. Marinades are incorporated into meat by soaking texture and moisture retention [48]; to enrich the meat flavor [10]; to tenderize the fibers of muscle foods; and to preserve the product over a longer time [36].

The pH of meat has a great impact on three sensory quality characteristics of muscle foods: appearance/color, texture/tenderness, and flavour, all of which affect the consumer acceptance of meat[32], [27]. Water-holding capacity (WHC) of meat is of great importance in meat industry, as it
affects both economic and sensory attributes of meat [31]. The structural organization of the muscle proteins is exisive for the distribution of the water [17].

Defined cooking as the heating of meat to a sufficiently high temperature to denature proteins [12]. Temperature and cooking time have a large effect on physical properties of meat and eating quality. The components of muscle that control toughness are the myofibrillar proteins and the connective tissues proteins. During heating, the different meat proteins denature and they cause structural changes in the meat, such as the destruction of cell membrane, shrinkage of meat fibers, the aggregation and gel formation of myofibrillar and sarcoplasmic proteins shrinkage and solubilisation of the connective tissue [34], [44]. The measurement of changes occurring during cooking may be carried out by a wide range of analytical methods including textural and microstructural evaluation [48] protein fragmentation [14] cooking loss or colour evaluation [30].

Transmission electron microscopy (TEM) are closely to flesh firmness [50]. Longitudinal section were observed in order to characterize modifications induced by the marination and cooking methods on the myofibrils meat. Meat structure can be considered in its simplest form as a collection of parallel fibers, a myofibrillar structure, bound together by a connective tissue [7]. A muscle is a complex structure composed of muscular fibers, a cytoskeleton of extracellular matrix and water. The region of the Z disc is considered to be the main place of structural changes that occur during the ageing of meat [16]. The principal proteins responsible for meat texture include stromal and myofibrillar proteins [9].

The objective of this research was to: 1- investigate the influence of marination and different cooking methods on the chemical composition, main quality characteristics and microstructure of chicken breast meat. 2- To evaluated the modification brought about by these treatments on the myofibrillar structure and the texture of the meat by TEM.

MATERIAL AND METHODS

Chemicals: All chemicals used were analar grade and obtained from Sigma chemicals (St Louis, Mo, USA).

Muscle samples: Fresh deboned breasts broilers chicken (1.5±0.2 kg ) live weight and 38 days age obtained from a local processor Minia, Egypt. The samples (n=25, average weight 150 ± 50 gm) were transferred to Food Science Department Laboratory after one hour of slaughtering under cooling conditions and then divided into two groups: Unmarinated (control) and marinated (M). The samples were stored at 4°C.

Marinade: The marinade formula consisted of orange juice, 1.2% (final weight of product) and water, phosphate, salt, and spices. Marinade solution was prepared 1 day before use and were stored at 4°C. Marinade was formulated based on percentage of meat weight Table1.

Marination process: The experimental design consisted of two group, one group of chicken breast meat used as a control (no marination) n=12. The other group was weight and individually identified, Breasts meat n=12 were immersed for 24h in the marinade. The proportion between the meat and the marinade was fixed at 1: 2. The samples control and marinated were then enclosed in sealed plastic pouches and stored in a refrigerator over night before cooking process.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Meat Weight (%)</th>
<th>Control (g)</th>
<th>Marinated (1.2% orange juice) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>3000</td>
<td>3000</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>20</td>
<td></td>
<td>600</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.3</td>
<td></td>
<td>0.900</td>
</tr>
<tr>
<td>Salt</td>
<td>4</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Spices¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange Juice</td>
<td>1.2</td>
<td></td>
<td>36</td>
</tr>
</tbody>
</table>

¹ Spice blend contained 1.71% smoke flavor, 1.71% vinegar, 0.31% ginger, 0.03% thyme, 0.07% clove, 0.88% minced onion, 0.34% salt, 0.17% red pepper flakes, 0.13% pepper blend, 7.20% cooked diced green peppers.
Samples cooking: The unmarinated (control) and marinade samples were cooked by four different cooking methods. 1- Microwave cooking: The samples were individually exposed to microwaves and cooked until an internal temperature 78°C was reached (~3 min.) full power. Moulinex, Micro –Chef FM 2735, 850 W. Type 049. 2- Dry heat cooking: (Roasting) The samples were roasted in a conventional oven at 250°C. The samples were placed individually on mesh rocks in aluminum pans and two thermocouples (Omega Model 199, Engineering Standford, CN USA) were inserted into the geometric centre of each in order to monitor the internal temperature of 85°C ± 3°C nearly (~20 min.)3- Moist heat cooking : (Boiling) The samples were placed in cook bags and thermo couples were inserted in their geometric center then vacuum sealed and cooked in a water bath to an internal temperature 77°C ± 3°C.4- Frying cooking: The samples were at a time on a temperature regulated hotplate in a teflon-coated frying pan in rapeseed oil (olein) at 180°- 200°C for 6 min. on each side, turning every 2 min. core temperature of the sample at the end of cooking were 76°C ± 2°C.

Proximate analysis: Unmarinated cooked by boiling (Control) and marinated cooked by different methods: marinated microwave (MM)), marinated Roasting (MR), marinated Boiling (MB) and marinated frying (MF) were analysed in triplicate for the proximate composition following AOAC procedure [3]. Moisture content was determined on samples by the oven drying methods at 105°C for 6h. Total protein content was determined by the Kjeldhal method N X 6.25. Total lipids were evaluated by the soxhlet method and total ash content was determined by weight after heating samples in muffle furnace at 550°C for 4h.

Quality parameters:
- pH measurement: Meat sample (10g) were homogenized in 90 ml distilled water. The pH of homogenized sample were measured using a glass pH electrode (ICM Digital pH, Model 41250, Hillsboro, OR, USA).
- Color: The color of cooked samples in the anterior location was determined in three replicate of each smaple (n=3) using TLunterlab colorimeter and reported as the complete international commission on Illumination (CIE) system color of lightness (L*), redness (a*), and yellowness (b*).
- Cooking loss: Cooking losses were calculated from differences in the weight of raw and cooked meat breast.

Water holding capacity (WHC): The samples wrapped with a nylon net and 3 pieces of filter paper (Whatman No.44). The wrapped samples were centrifuged at 3000 Xg for 20 min. The percentage ratio of sample weight difference between, before and after centrifuge, to sample weight before centrifuge provided free water content. The difference between moisture content and free water content was described as the water-holding capacity index [51].

Microstructure of muscle: Breast muscles (Pectoralis major) from raw (R) and marinated. Also unmarinated control (C) and marinated (M) cooked individually by the four different cooking methods were subjected to 24h aging at 4°C prior further study. Samples were cut into small pieces (about 1mm²) were fixed as soon as possible in 3% cacodylate buffered glutaraldehyde (pH 7.3) according to [48]. After two rinses in the buffer ,the tissues were postfixed in 1% buffered osmium tetroxide, followed by washing twice in cacodylate buffer for 30 min. The specimen were dehydrated in ascending grades of ethanol (50-100%), then cleared in toluene for 10 minutes and embedded in epoxy resin as indicated by [25]. Semithin sections of 1.5 mµ were cut from each block and stained with toluidine blue for light microscopy examination. Ultrathin sections (700Å) were cut with glass knives on NOVA ultramicrotome. These sections were stained with saturated uranyl acetate in 70% alcohol for 20 minutes followed by lead acetate for 5 minutes. The sections were examined using a JEOL-100 CXII electron microscope, in the Central Lab. Of Microanalysis, Minia University, Egypt and representative areas were photographed.

Statistical analysis: Data were analyzed with the GLM (General Liner Model) program using Statistical analysis system [39]. Mean values were compared by Duncan’s Multiple Rang Test.
RESULTS AND DISCUSSION

Proximate sample analysis: Proximate analysis of Unmarinated cooked by boiling (control) and marinated chicken breast meat cooked by different methods are shown in table 2. The marinating treatment and cooking methods significantly modified the chemical composition of chicken breast meat. Significant differences (P≤0.05) were found among the control and marinated cooked breast meat. Values for moisture ranged between 58.94% and 72.33%. The lowest value was for the (MF) samples table 2. Control and bioling exhibited similar moisture content. In comparison this results agree with the results of [51] that the moisture content was decreased when samples were heated above 60°C. The frying samples showed significantly lower moisture content than the others. Statically significant differences (P≤0.05) in the total protein content were always observed between marinated cooked and the other control samples. Values for protein ranged between 18.42% and 29.50%. Significant differences (P≤0.05) in the total lipid content were found between the control and the others samples. Values for lipids ranged between (4.79 to 9.59). The MR meat had the highest lipids values content, and the MM meat had the lowest values content. The different total ash content were found only between the frying and the samples cooked by microwave method. It ranged between 2.20 to 3.30. The proximate composition differences showed in table 2. were the result of the marinating effect in addition to the effect of the whole cooking loss, due to mainly water evaporation melting of fats and loss of soluble proteins, hence, it was quite difficult to fully account for each component in an accurate mass balance calculation, however, proximate composition data from our study were in agreement with those found by authors [1], [2].

Table 2. chemical Composition of control and marinated chicken breast muscle cooked by different methods (g/100g on wet basis )

<table>
<thead>
<tr>
<th>Cooking methods Component</th>
<th>Control*</th>
<th>Microwave(MM)</th>
<th>Roasting(MR)</th>
<th>Bioling(MB)</th>
<th>Frying(MF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>72.33±0.17</td>
<td>65.71±0.09</td>
<td>69.94±0.57</td>
<td>72.07±0.02</td>
<td>58.94±0.06</td>
</tr>
<tr>
<td>Total Protein</td>
<td>18.65±0.42</td>
<td>21.13±0.15</td>
<td>22.60±0.55</td>
<td>18.42±0.21</td>
<td>29.50±0.49</td>
</tr>
<tr>
<td>Total Lipids</td>
<td>6.56±0.5</td>
<td>9.59±0.21</td>
<td>4.79±0.18</td>
<td>7.54±0.23</td>
<td>8.20±0.3</td>
</tr>
<tr>
<td>Total ash</td>
<td>2.34±0.21</td>
<td>3.30±0.29</td>
<td>2.24±0.04</td>
<td>2.20±0.08</td>
<td>3.18±0.19</td>
</tr>
</tbody>
</table>

Each value is the mean of three determination ± standard error. Duncan’s test for independent samples: different super script letters within a raw mean significant differences (Ps0.05).

* Unmarinated cooked by boiling

The average results of pH, color (L*, a*, b*), cooking loss and water holding capacity (WHC) are presented in table 3

PH value: The pH value of the samples increased significantly (P<0.05) due to the marination and cooking methods. The pH value ranged from 5.63 to 5.95. Similar values were obtained for control and MB samples. MM and MR samples pH were increased by marinades, significantly increased (P<0.05) by approximately 0.3 unit, compared to the control samples, while MF samples pH alone increased by 0.12 unit. Similar increase in PH have been reported by others [41].

Color: The sample color values (L*, a*, and b*) had significant differences between the control and the others marinated cooked samples. Changes in color of control and marinated cooked chicken breast meat are shown in table 3. The system values of lightness (L*) and yellowness (b*) of chicken samples showed that the L* values decreased and the b* values increased significantly with the cooking methods and marination treatment (P≤ 0.05). The redness (a*) increased significantly in MM samples and MF samples and the same trend for other samples compared to the control. Color
Myoglobin is one of the more heat-stable of the sarcoplasmic protein, which is almost completely denatured between 80 and 85°C [20]. According to the authors [49] the compound involved in increasing redness of muscles should be globin hemochrome, in which the iron is in the Fe\(^{2+}\) state. Its color is typically dull red. Globin hemichrome, with the iron in the Fe\(^{3+}\) state, is largely responsible for the brown-grey hue. The balance between hemochromes and hemichromes is affected by the state of meat before cooking and other factors, including species, animal and maturity and muscle type.

**Cooking loss**: Cooking losses, shown in table 3, covered quite a large range, from 25.95% to 39.84%. Cooking losses from MF and MM samples were greater than the control. There were significant differences between treatments (P≤0.05). Cooking losses, from MR and MB samples were not significantly compared to the control (P≥0.05). These data are not in agreement with those reported in the literature [23] where it was generally recognized that marinated chicken samples show lower cooking losses. This difference is, most likely, due to our marinating treatment and different cooking methods. However, cooking losses data from our study were in agreement with those found by other authors [4].

**WHC**: The treatment effect on WHC are shown in table 3. A higher WHC was observed in MR, and MB chicken breasts 40.96% and 37.69% respectively. The slightly lower WHC of the MM samples were 30.78%. The lowest WHC in the MF samples were 21.17%. However no significant WHC differences were found for control samples and those MR and MB. Thus, the water holding capacity of the marinated chicken samples was not dramatically enhanced. The differences due to the cooking methods of the samples showed that the frying and Microwave increased the cooking loss. The absence of crust, clearly evident on the sample's surface allowing water evaporation and melted fats to escape from the breast sample is a likely explanation of our cooking loss results. These values are comparable to those obtained by [30]. The cooking loss was always influenced by the heat treatment time (P≤0.05).

**Analysis of myofibril microstructure**: Microstructure of raw (R), and marinated chicken
Fig. 1-2 Electron micrographs of longitudinal section of chicken breast (pectoralis) muscle: a (20000x) b (18000x). 1-Raw muscle 2- Marinated muscle Legends: Myosin rich – A bands (A), actin rich I-bands (I), Z-lines (Z), M-lines (M), H-zone (H) and sarcomere (SR)., breast meat present a standard preserved ultrastructural with thin Z-line, I-bands, A-band, M-line and H-zone can be seen, and alignments of Z-discs without apparent fragmentation (Figs. 1a,b).

After marination, the variety of appearances of the myofibrils (Figs. 2 a, b) depended on the marination applied. When samples were marinated the general aspect of myofibrils partly similar to the control with preserved Z-discs and I filaments disrupted. After treating, the myofibrillar proteins within the sarcomeres became more clearly defined (Figs. 2 a, b). There were changes in myofibrillar structure. In particular the H-zones tend to be most dispersed, Z- and M-line degradation I, and longitudinal fissure of myofibril. Observation of changes in protein’s structures as well as previous research [24], [18]. Ultrastructural marinated samples showed stretched sarcomere. As a consequence of the elongation of sarcomere, the intermyofibrillar spaces were enlarged and the Z-line altered (Figs. 2a, b). Our results, together with those of [5]. A similar result was obtained by [15].
Effect of cooking methods: Of all the treatment that can influence texture, cooking is probably the most important because it acts on all the tissue components, irrespective of identity, myofibrillar proteins are coagulated, collagen shrinks and is converted to gelatin, and water is released [22]. Weakening of myofibrils, with loss in the structure of Z-disks, occurred at temperature of 80 -100 °C for broiler muscles [46].

Effect of marination and microwave on the microstructure of chicken breast meat
Transmission electron micrographs is shown shrinkage of myofibril units sarcomere (Figs. 3 a, b) of the control samples. As a result of shrinkage of myosin and actin, the I-band was enlarged and A-band shrunk, producing gaps and discontinuity between sarcomere as well as disruption of the myofibrils, the gap filament, a set of thin filament, Z-disk thickening, fragmentation at the Z-discs, and the H-zone disappeared completely. loss of M-line distinctness and distortions in the arrangement of the sarcomere units in unmarinated breast meat (Figs. 3 a,b). When compered with marinated breast meat (fig. 4 a,b). It seems that during microwave cooking, muscles undergo disruption mainly in sarcomere, the physical disruption of muscles is generally known to cause tender texture. The typical structure of the sarcomere was still recognizable and the H-zone still visible (Figs. 4 a,b). Alarge gap was formed along M-line in the sarcomere, while this was not obvious in chicken breast control (Figs. 3 a,b). The Z-line appears more dispersed, I-band disappeared completely M-line completely lost and gap formed instead of both. These data not resemble any recent research regarding to use the marination and microwave cooking together.
Effect of marination and roasting on the microstructure of chicken breast meat:

![Electron micrographs of longitudinal section of chicken breast (pectoralis) muscle: a (20000x) b (18000x). 5-Unmarinated (Control) roasted cooked 6- Marinated roasted cooked. Legends: Myosin rich – A bands (A), actin rich I-bands (I), Z-lines (Z), M-lines (M), H-zone (H) and sarcomere (SR).](image)

Qualitative changes that took place in the microstructure of myofibrils of unmarinated and marinated chicken breast muscles during roasting are shown in (Fig 5,6). The myofibrils structure was less distinct, the gaps between myofibrils were visible and z-discs were less marked in the control sample (Fig. 5 a,b). On the micrographs was visible some granulation whose origin is difficult to explain. Similar results were obtained during the roasting of bovine muscle in a previous study [34]. In addition, a serious degradation of the sarcomere can be seen, (Fig 5, a,b) as well as the generation of many inter myofibrillar “gaps” in some occasions in the zone adjacent to Z-diss. I-band and A-band can not also be distinguished. After heating, denaturation and aggregation of myosin and action caused sarcomere shrinkage (Fig 5, a,b) and the cooked meat to appear grainy. As a result a shrinkage of myosin and action the I band was enlarged and A band shrunk producing gaps and discontinuity between sarcomere. The microstructural changes taking place during marination (Fig 6, a,b) appeared more pronounced in the MR meat than in the other control resulting in severe changes. The presence of holes in the control samples (Fig 5-b) were more than in the MR samples (Fig 6-b). This probably reflects that partially disintegrated fibre structures are more sensitive to marination and cooking upon which they disintegrate further as a consequence of further protein integrity as shown by [6]. During marination it was apparent that the individual fibres disintegrate at different rates. This is compatible with observation made by [29]. Our results, together with those of [28], bring evidence of the weakening of the myofibrils, thus contributing to the overall improvement in meat texture [16], [35].

The effec of marination and boiling on the microstructure of chicken breast meat

TEM image of control and MB chicken muscle are shown in (Figs. 7,8). The actin and myosin can also be distinguished. After heating, denaturation and aggregation of actin and myosin caused sarcomere shrinkage (Figs. 7 a,b) and the boiled meat to appear grainy. As
a result of shrinkage, of myosin and actin, the I-band was enlarged, and A-band shrank, M-line disappear producing grany gaps and discontinuity between sarcomere. After marination (Figs. 8 a,b) were shown the typical structure of the sarcomere was still recognizable A-band, I-bands and Z-lines still visible. Cogulated sarcoplasmic protein can be seen in both the intracellular space. [33] showed that aggregated sarcoplasmic proteins and collagen can form a gel that glues the fibers and fiber bundles together, holding water and/ or plugging the intercellular capillaries to prevent water from being released. Compered with our study the cooking loss were lower 28.93% and water holding capacity were higher 37.69%. More work remains to be done. As stated earlierl [19], the muscle disintegration and fragmentation softened the texture of sample. Further study is needed to clarify the differences between studies.

The effect of marination and frying on the microstructure of chicken breast meat

Ultrastructure of unmarinated and marinated fried samples are displayed in (Figs. 9,10) respectivelt. These electron microscope observations illustrate that frying method caused considerable disruption of the myofibrillar lattice (lattice of filaments formed inside the myofibril) these alterations were largely wide spread. The most affected area was near and within the I-band region. The fracture zones where the Z-line is not fully attached to the A-band appear to have a fibrillar structure with a nonuniform density. Fragmented sarcomeres displayed A-band with uneven edges probably as a result of excessive stretching applied to the I-bands. Occasionally, a longitudinal gap or splitting of the myofibrils was observed (Figs. 9a,b). Ultrastructure of samples in (Figs. 10 a,b) showed that MF samples had a complete sarcomere disorganization and the typical dark and light banding pattern is not

Fig. 7-8 Electron micrographs of longitudinal section of chicken breast (pectoralis) muscle : a (20000x) b (18000x). 7-Unmarinated (Control) boiled cooked 8- Marinated boiled cooked Legends : Myosin rich – A bands (A), actin rich I-bands (I), Z-lines (Z), M-lines (M), H-zone (H) and sarcomere (SR).
Evident, and Z-lines appear to be more pronounced. The Z-discs appear as much darker lines than in the control (Figs. 9 a,b). Z-disc are partly disrupted, wider intermyofibrillar space observed. The muscle gradually lost its microstructure. M-line was completely lost. Disappearance of the M-line and broadening of I-bands, I-filaments were solubilized in the MF chicken breast meat as compared with that of the control. Most of actin filaments were solubilized, the myofibrils fused together and the Z-line were swollen and fragmented. This is compatible with observation made by [26] who considered that swollen myofibrils hold more water. The H zone disappeared completely. In fact several studies [38] suggested that the Z-line degradation is the main factor contributing to meat tenderization, which is the basis of the “Z-disk theory.”

CONCLUSIONS
The marination and cooking methods significantly changed the chemical composition of chicken breast meat. The suitable cooking methods for marinated chicken breast meats were roasting and boiling due to reducing the cooking loss and increasing the WHC. The microstructure of chicken breast meat has been changed dramatically upon marination and cooking. The marination was a beneficial technique to improve a both quality characteristics and texture of chicken breast.

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Fig. 9-10 Electron micrographs of longitudinal section of chicken breast (pectoralis) muscle: a (20000x) b (18000x), 9-Unmarinated (Control) fried cooked 10- Marinated fried cooked. Legends: Myosin rich–A bands (A), actin rich I-bands (I), Z-lines (Z), M-lines (M), H-zone (H) and sarcomere (SR).
assistance on preparing samples for transmission electron microscopy.

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