IOP Conference Series: Earth and Environmental Science

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To cite this article: M Starcevic *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **854** 012094

Culinary preparation and processing of meat with wooden breast myopathy

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Abstract: Recently, poultry meat production and consumption has become increased worldwide. Decades of intensive selection in poultry breeding resulted in fast-growing broilers, improved food conversion, low production costs, and high yield of breast meat, as the most valuable part of the carcass. Some side-effects of intensive production and rapid growth of broilers include the appearance of myopathies in breast muscle. Increasing attention has been paid to the defect known as "wooden breast" (WB) due its incidence and severity of anomaly. WB is characterized by the hardness and pale colour of the fillet. These changes lower the consumer acceptance of the meat, and a pronounced WB is unsuitable for culinary and industrial processing. Different procedures can be used to tenderize the meat and include physical and chemical procedures, often combined in industry. Physical procedures comprise the application of heating, mechanical force, ultrasound, electric stimulation, hydrodynamic shock wave-pressure technology, high pressure processing, and pulsed electric field. Chemical procedures include marinating, exposure to the endogenous enzymes, and the use of exoenzymes. In the future, it is necessary to develop optimal tenderizing techniques or combinations of different tenderizing techniques to achieve better sensory quality and improved nutritional value of WB.

1. Poultry meat production

Over the last decades, the production of food has managed to meet the needs of increasing world population. The five-year average meat production (2016-2020) in the world was 323.25 million tons mt) and poultry meat had the biggest share (37.99%). From 2000 to 2020, world poultry meat production has increased from 40 mt to 132 mt, with an annual increase from 3.5% to 4.7%. The average annual consumption of poultry meat is 15 kg per capita, being the most widely consumed type of meat in the world [1]. The reasons for the increasing poultry meat production should be sought in its exceptional nutritional value (low fat content and high protein content), the perception that it is healthier than other meats, pleasant flavour, acceptance by most cultures and religions, quick and easy preparation, affordable price, and utilization of all carcass parts in industrial processing [2, 3]. One of the important features of poultry meat is its possibility to be used as a functional food by formulating diet for poultry nutrition. Thus, by adding selenium, especially organic, with or without vitamin E in poultry diet, higher content of Se and vitamin E can be achieved in meat. Moreover, use of feeds rich in polyunsaturated fatty acids (PUFA), n-3 fatty acids or conjugated linoleic acid (CLA), results in higher PUFA content, balanced ratio of n-6 to n-3 fatty acids and higher CLA content in poultry meat with beneficial health effects for humans [4, 5, 6].

2. Myopathies in poultry

Myopathies are defined as progressive and degenerating neuromuscular diseases that are characterized by hypotonia, muscle fibre damage and muscle atrophy [7]. So far, several causes of myopathies in poultry have been described, like genetics, nutrition, metabolic disorders, toxins, infectious agents, environmental factors and unknown aetiology [8]. In congenital myopathies, different genes have been identified as associated with the various phenotypic and histological changes in muscles. Causes for some poultry myopathies are still not known, and intensive work is being done on their identification [9].

Genetic selection of poultry has been carried out in order to shorten the fattening period, increase the growth rate, obtain higher final body weight and breast yield, and improve feed conversion. Better growth performance results have been accompanied by undesirable occurrence of myopathies in breast muscle [10, 11, 12]. To date, five myopathies associated with rapid growth of poultry (broilers and turkeys) are known and described: deep pectoral myopathy (DPM), pale, soft, exudative meat (PSE), white striping (WS), wooden breast (WB), and “spaghetti” meat (SM) [2]. WB myopathy, unlike the others mentioned, has not been found in turkey breast meat. Myopathies do not pose a safety risk to human health, since they are not biological, chemical or physical hazards. In effect, myopathies deteriorate meat quality (sensory, nutritional and functional properties) and reduce the economic value of meat. Economic losses can be remarkable and depend on the type of defect, the incidence of occurrence, as well as the intensity of its severity. Economic losses occur as a consequence of consumers’ refusal to buy meat with pronounced myopathy, and it must be used for the production of meat products [3, 13].

WB is the most common myopathy in broilers. It is manifested by the hardness of the pectoral muscle, especially in the cranial part. The muscle is pale, covered with viscous contents and petechial lesions [2, 13]. Other muscles of the carcass are unaffected. Histological analysis determines fibre atrophy, variations in fibre shape and size, multifocal degeneration and necrosis, loss of striation, mononuclear cell infiltration, interstitial inflammation, oedema, deposition of extracellular collagen, and thickening of the perimysial connective tissue [3]. The changes are very similar to the WS. These two defects often occur simultaneously [11, 14]. The defect is usually graded as mild (focally diffused and light firmness), moderate (focally diffused with extensive firmness), severe (more than 75% of breast is firm), and extremely severe (firm breast) [3]. From normal breast WB differs in colour (bright red, blurred), higher pH value and lower water holding content (WHC), higher drip loss, higher protein content in exudate, and higher MDA value and carbonyl content [15]. The aetiology of WB is still unknown, but it is mainly associated with the genetics, age, higher final weight, higher dressing percentage and breast weight, and nutrition [3, 12]. WB myopathy has been found in many countries around the world, and data on the incidence of this defect show it is becoming more common [15]. In Italy, WB was found in 53.2% of broiler carcasses [16], and in the USA in 50% of cases [17]. Tijare *et al*. [18] identified this defect in 90.1% of the samples. The WB defect is, according to the findings of Xing *et al*. [15], found in 30.8% of broilers in China. This defect remains pronounced even after heat treatment.

WB is the subject of intensive research in the world. Research refers to examination of morphometric parameters of breasts (weight, yield, length, width, height of different parts of breast), chemical composition (the content of moisture, protein, fat, ash, collagen, fatty acids, malondialdehyde, protein carbonyls), functional properties (L, a, b values, pH, shear force, hardness, cooking loss, thawing loss, salt-induced water uptake, drip loss), sensory properties (appearance, colour, texture, odour, and taste), nutritional value and consumer acceptability. Important conditions for the appearance of this defect were examined, as well as the application of different meat processing procedures with WB [3].

3. Meat tenderization

Skeletal muscles consist of two fractions of proteins, myofibrils, involved in muscle contraction and sensitive to endogenous enzymes, and collagen, the main constituent of connective tissue responsible for fixed toughness [19]. The purpose of meat tenderization, both in culinary preparation and processing, is to disrupt the structure of myofibrils, sarcoplasmic proteins and collagen, and consequently to facilitate and improve the digestibility of nutrients. Meat tenderization procedures can be divided into premortal and *postmortem*. Premortal procedures were applied in cattle and largely abandoned. Premortal procedures included oral administration or injection of vitamin D that elevated calcium serum level and in turn activated *postmortem* tenderization process in meat [20]. *Postmortem* tenderizing procedures include physical and chemical methods, in practice often combined.

3.1. Physical procedures

Physical procedures include the application of thermal treatment, mechanical force, ultrasound, high-voltage electric stimulation, hydrodynamic shock wave-pressure technology, high pressure processing, and pulsed electric field [20, 21, 22].

One of the physical procedures most often used is heating at different temperatures, with or without the addition of water (cooking, in pressure cooking, microwave cooking, baking, stewing, frying, grilling). Heat treatment of meat contributes to its safety in terms of the absence of biological hazards, reduces the nutritional value, and contributes to the formation of odour and taste. During heating, the texture of the meat changes due to the denaturation of myofibrillar proteins and sarcoplasmic proteins. Hydrolysis of collagen induced by heating leads to meat tenderization, since collagen is degraded to smaller molecules of gelatin. Gelatin has good solubility and the ability to bind water. Meat tenderization sharply increases with exposing meat to temperatures higher than 70 °C [23]. Heat treatment of meat can be applied at lower temperature and for a longer time (low-temperature long-time method and sous vide cooking – processing in vacuum) [21, 24].

Mechanical methods of tenderization (hitting, pressure, cutting, stabbing, tumbling, massaging) disrupt the structure of meat, resulting in the extraction of soluble proteins, but on the other hand reduce cooking time, hardness and chewiness of meat [19]. Mechanical procedures for tenderizing meat also include hitting with a serrated blunt object (culinary processing) and passing through two rotating slitting blades, where the distance between the blades is adjusted to the thickness of the meat [23]. Mechanical processes most often precede marinating processes [25]. For tenderizing poultry meat with WB defect, Tasoniero *et al*. [26] recommended a “blade tenderization” procedure that involved cutting meat with a set of blades that can be used in the household and catering, but also in the meat industry. Blades disrupt the structure of muscle and connective tissue, and in combination with marinating and the application of enzymes, the penetration of the marinade and enzymes is enabled into deeper layers of meat, increasing the tenderness.

Ultrasound has been investigated as a method for meat tenderization [27] and, applied on carcasses, provokes lysosomal rupture and fragmentation of myofibrils and connective tissue [20]. Ultrasound applied to WB myopathy decreased the hardness of breast, confirmed by increased myofibrillar protein degradation [28]. High-voltage electric stimulation (ES) of pre-rigor carcasses has been applied for decades for meat tenderization [20]. ES increases the rate of *postmortem* glycolysis, leading to rapid pH decline and depletion of intracellular calcium, and thus preventing the cold-shortening during carcass chilling. Hydrodynamic shock wave-pressure technology is applied to packaged meat placed in water. Shock waves generated by explosion break down sarcomere proteins, resulting in higher tenderness [20]. The use of high hydrostatic pressure (high pressure processing) in the range of 350-600 MPa for a few minutes destructs the quaternary structure, and even the tertiary structure of proteins. High pressure disrupts ionic and hydrogen bonds in proteins, induces denaturation of proteins and forms gel consistency. After high pressure treatment, the elasticity of meat increases and it becomes more tender [21, 29].

3.2. Chemical procedures

Chemical procedures include marinating, the activity of endogenous enzymes, and the use of exoenzymes. Marinades usually contain sodium chloride, organic acids (citric, acetic, tartaric acid), phosphates, citrus juices, then oil, vinegar, wine, spices, and sugar. Meat tenderization occurs as a consequence of various physicochemical mechanisms that increase protein hydration, leading to "swelling and weakening" of muscles and water binding. During marination, an increase in proteolysis caused by higher activity of cathepsins and an increase of collagen transformation to gelatin that both contribute to tenderization of meat are observed [21]. Better effects are achieved when marinade is applied by injection technology than by immersion of poultry meat in marinade [30]. Chicken breasts exposed to combined effects of marinade with ultrasound achieved larger myofibril fragmentation and higher tenderness than breasts immersed only in marinade [25].

Enzymatic meat tenderization involves exposing the meat to endoenzymes and exoenzymes. In *postmortem* muscles, endoenzymes have significant roles in meat tenderization during aging. Several endogenous proteolytic enzymes, including calpains, lysosomal proteases and cathepsins, are involved in degrading myofibrillar and cytoskeletal proteins. A large number of studies indicate the calpain system as responsible for *postmortem* proteolysis and tenderization of meat. The role of calpain enzyme system (m-calpain, μ-calpain, and calpain 3) and their calcium-activated inhibitor (calpastatin) in meat tenderization was revealed [22]. Calpains are cysteine proteases that degrade myofibrillar proteins (tropomyosin, roponin T, troponin I, C-protein, connectin, titin, vinculin, and desmin) [31]. Aging of meat is often applied to beef, and it can last up to 55 days in controlled conditions (temperature and humidity) [21].

Exoenzymes used for tenderizing meat are of plant, bacterial or fungal origin. To date, five exogenous enzymes have been "generally regarded as safe" (GRAS) for use in the meat industry [32]. Exogenous proteases extracted from plants are papain (papaya), bromelain (pineapple), and ficin (fig), from bacteria (*Bacillus subtilis*) are subtilisin and neutral protease, and from fungi (*Aspergillus oryzae*) is aspartic protease [19]. Exogenous enzymes require optimal temperature and pH value. Their activities towards the hydrolysis of myofibrillar proteins and collagen are different, so in practice, the simultaneous use of two or more exoenzymes is recommended. The effect of different exoenzymes on degradation of myofibrillar proteins and collagen along with optimal pH and temperature for their activity are presented in Table 1 [33].

Table 1. Optimal pH and temperature for exoenzymes and their effectiveness on muscle proteins

Protease	**Active pH range**	**Optimal pH**	**Active temperature range (^{0}C)**	**Optimal temperature (^{0}C)**	**Hydrolysis of myofibrillar proteins**	**Hydrolysis of collagen**
Papain	4.0–9.0	4.0–6.0	50–80	65–75	Excellent	Moderate
Bromelain	4.0–7.0	5.0–6.0	50–80	65–75	Moderate	Excellent
Ficin	5.0–9.0	7.0	45–75	60–70	Moderate	Excellent
Protease of *A. oryzae*	5.0–9.0	7.0	50–65	55–60	Moderate	Poor
Proteases of *B. subtilis*	2.5–7.0	<6.5	40–60	55–60	Poor	Excellent

Papain is proteolytic enzyme derived from aqueous extract of papaya. The higher activity of enzyme is obtained from immature papaya fruit. It is capable of breaking down large protein molecules to small peptides and amino acids [31]. Papain cleaves lysine, phenylalanine and arginine peptide bonds, thus tenderizing meat [20]. Side-effects of papain use are “mushy” texture and off-flavours of meat [34]. Bromelain is obtained from fruits of pineapple family. Bromelain is effective in breaking down myofibrillar proteins and collagen, resulting in higher tenderness of meat. Bromelain and papain were used for tenderization of poultry meat (ducks and chickens) [35, 36]. Ficin is extracted from plant of genus *Ficus*. It is a proteolytic enzyme, a class of cysteine or sulfhydryl protease that enhances the solubility of muscle proteins. Although ficin is capable of degrading to some extent collagen and elastin, it is primarily effective in breaking down myofibrillar proteins [19, 31]. *Bacillus subtilis* contains subtilisin and neutral protease of high specific activity against collagen and elastin, but had no significant effect on myofibrillar proteins [31]. *Aspergillus oryzae* produces aspartic protease that preferentially affects myofibrillar proteins, with almost no effect on collagen [31].

Exoenzymes can also be used with other additives (acids, phosphates, salts) and mechanical processing procedures (tumbling, massaging, injection, blade tenderization) [19, 31]. Paddy oat fruit peel [37], kiwifruit [38] and ginger root [31] can also be used to tenderize meat. Actinidin derived from kiwifruit breaks down myofibrillar proteins and it is also involved in activation of m-calpain. Actinidin applied in high concentration moderately tenderize meat preventing the occurrence of mushy meat [31].

The efficiency of tenderizing procedures can be determined by various methods (sensory analysis, electrophoresis, electron microscopy, histological analysis, proteolysis index, pH value, WHC, peptide content, etc.) [39].

4. Conclusion

The WB defect is a novel condition to the poultry industry, and it is associated with huge economic losses. To alleviate negative consequences of WB occurrence, different strategies have been proposed. One of them is to include affected chicken breasts in various processed products. The other way to minimize adverse effects of genetic selection and improve sensory characteristics of WB is to apply different tenderizing techniques. Meat tenderization could allow the poultry industry to put on the market whole breast fillets. Therefore, further research is needed to develop an optimal tenderizing technique or combination of different tenderizing techniques to achieve better sensory quality and improved nutritional value of breasts with WB myopathy.

Acknowledgments

This paper was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract number 451-03-9/2021-14/200143).

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