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Is there a Possibility of Meat Tenderness Protein-Biomarkers on the Horizon?

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Abstract – **Over the years, meat scientists have shown that tenderness is the most important meat quality attribute that determine consumer satisfaction. The challenge of achieving consistency in meat tenderness is a thorn in the industry worldwide. This is mainly due to the amalgamation of many factors involved in the tenderisation process. Even though these factors are known and well-studied, there is still a lack of distinct tenderness biomarkers. Up to date, several studies proposed different biomolecules such as proteins as biomarkers for tenderness, but no single biomarker that fulfil the necessary requirements for a tenderness biomarker has been proposed. The lack of tenderness biomarkers is a threat to achieving consistency in meat tenderness grading worldwide.**

Keywords – **Biomarkers, Proteomics, Tenderness, Ageing, pH and Temperature.**

I. Introduction

Originally, meat producers refrigerated meat in caves or under cool water. Meat has been hung and dry aged throughout history after butchers discovered that this method made beef more tender and flavourful than meat eaten immediately after its preparation. Ageing of meat at a temperature of about 1-3 ºC for a period ranging from two days to several weeks allowed the actions of enzymatic tenderisation to proceed. Temperature must be controlled very well because the meat will spoil if the room is too hot and the process of dry ageing stops if the water in the meat freezes. Water needs to evaporate slowly, thus the room humidity must be kept at approximately 85% and the room should be well ventilated to prevent bacterial meat spoilage. Since the 1960s, new ageing technologies such as wet ageing in vacuumed bags were developed because of the meat hanging's expense (weight loss) [1, 2]. Meat hanging is still popular and dry aged beef is continuously being sold in high-end restaurants around the world.

Consistency of meat tenderness remains a challenging factor facing the meat industry worldwide. Studies showed that meat tenderness is one of the quality attributes driving consumer decision on repurchase [3]. There has been advances in trying to understand the mechanisms involved in tenderisation processes but not one defining mechanism has ever been elucidated. Meat tenderisation is a multivariate phenomenon influenced by factors including live animal production, slaughtering, storage, preservation and cooking.

The global meat industry is advancing the efforts to search and identify factors and mechanisms affecting tender-ness so that a proper practice can be developed to control the process profitably. Scientists believe that identifying certain molecules as tenderness biomarkers would be an achievement that will help alleviate meat tenderness inconsistencies. A tenderness biomarker can be any molecular indicator of a particular biological property or function that can be measured in blood or tissues or a biochemical feature that can be used to propose a particular pattern in relation to tenderness.

So far, the effect of temperature and pH on proteases and protease inhibitors, such as the calpain proteolytic system, structural muscle proteins, and metabolic proteins have been studied to further understand and to link them with tenderisation process [4]. Meat tenderness can be assessed by measuring the force needed to shear muscles mechanically or by the force of biting (trained sensory panel). The more force needed to shear through meat the tougher the meat. Warner-Bratzler shear force technology was developed in the 1930s; it is an instrumental force measured in Newton or kilograms required to shear e.g. a one cubic centimetre meat sample using an Instron instrument fitted with a Warner Bratzler shear force tool [5, 6].

This review will briefly highlight all the known factors affecting meat tenderness, the pit falls regarding tenderness prediction as well as the progress thus far of searching for biomarkers to enable predicting meat tenderness.

II. Effect of Carcass Temperature and pH on Meat Tenderness

The effect of temperature play an important role in the meat tenderisation process. In a study of cold shortening effects on beef muscles by [7], it has been found that low temperatures can play a role in beef muscle shortening with a consequence of yielding tougher meat. Very cold chilling temperatures causes muscles to contract, causing sarcomeres to shorten before the onset of *rigor mortis*. This phenomenon is called 'cold shortening'. This is mainly caused by the failure of the release of sarcoplasmic reticulum calcium ions. Red muscles experience more cold shortening than white muscles because they possess more sarcoplasmic reticula that are delicate. As studied by [8] the effect of different temperatures during rigor on meat quality and found that rigor temperature at roughly about 15 °C resulted in the longest sarcomere length and shear force values. Studies have associated temperatures of around 14–20°C with minimal sarcomere shortening while

temperatures of about 0–10°C were associated with sarcomere shortening. When a pre-rigor muscle goes to rigor at temperatures around 15–20 °C, the lowest amount of sarcomere shortening is achieved. As described by [9], there is an optimal pH/temperature decline to avoid cold and hot shortening as shown in Figure 1. The feasibility of holding beef carcasses during production at the optimal temperatures of 15–20 °C to minimise cold shortening is not advisable because of possible microbial contamination.

Additionally, temperature plays an important role especially in the first 24 h after slaughter because of variations in proteolytic activity and resultant variation in tenderness. The chilling temperature and pH decline affects the enzymatic degradation and resultant muscle fibre fragmentation, and amount and extent of cross-linking of connective tissue. On the other hand in a study to evaluate the effect of high pre-rigor temperature on meat tenderness, [10] found that high pre-rigor temperature of 38 °C enhanced the commencement of rigor resulting in more protein denaturation because of the increased pH decline at high temperature. In their observation, they also found reduced activity of calpain-1 (μ-calpain) autolysis and desmin degradation. This observation suggests that high temperatures before the commencement of *rigor mortis*, negatively affect meat tenderness defined as heat toughening/shortening. The rate of pH decline has an effect on the ultimate tenderness and declines from about 7.1 at slaughter to almost 5.8–5.3; typically, the pH decline takes about 18–40 h in beef. Overall, carcasses exposed to low pH and high temperature experiences low water-holding capacity, poor tenderisation, a pale colour, early browning through retail display and sarcomere shortening. Study by [11] attributed low pH and high temperature exposure to the aggravating denaturation of myofibrils at high ATP levels. In summary, various studies have found collectively that *post mortem* temperature and pH have an effect on the meat quality attributes such as water holding capacity, higher drip-loss, sarcomere shortening, calpain proteolytic system, and protein denaturation. All these effects are ultimately because of the shift in energy metabolism of the muscle after slaughter and the changes in the expression of several glycolytic enzymes as well as Krebs citric acid cycle enzymes involved in the energy metabolism. One of the sources of energy *post mortem* is the degradation of glycogen to lactic acid, which builds up in the muscle. This increase the hydrogen ions and inversely lowers the muscle pH [12]. While the degradation of glycogen continues, the muscle pH continues to drop and the activity of the glycolytic enzymes drop. The activity of the glycolytic enzymes may remain stable up to a pH of 5.5. The main enzymes regulating the rate of glycolysis are glycogen phosphorylase and phosphofructokinase [13]. Glycolysis is sped up by applying electrical stimulation to the carcass or muscle. According to [14], electrical stimulation is more beneficial if there was sufficient muscle glycogen before animal exsanguination. Electrical stimulation depletes the muscle energy quicker by accelerating the natural process resulting in early *rigor mortis*. Although electrical stimulation speeds up the rate of energy metabolism, this process must be optimally controlled because as it has been noted by [15], the faster the rate of *post mortem* metabolism, the higher the rate of denaturation of sarcoplasmic proteins.

III. Other Interactive Factors Affecting Meat Tenderness

Apart from muscle temperature and pH variables, other variable factors such as sarcomere length, myofibrillar fraction length (MFL), water holding capacity, collagen content and collagen solubility, the activity of the calpain proteolytic system and protein denaturation also play a role in meat tenderness an already a complicated process. As reported by [16] that longer muscle sarcomere lengths preferentially lowered shear force values compared to shorter sarcomere lengths. This report is supported by the study of [17] who found that there is a greater negative association between sarcomere length and meat toughness with sarcomere lengths of less than 2 μm. ATP levels play a role in the shortening of the sarcomere, and sarcomere shortening is also caused by cold exposure immediately after slaughter, a phenomenon called 'cold shortening'. The action of *post mortem* proteinases such as the calpain proteinase system is another factor affecting meat tenderness. Calpains belong to a large family of intracellular Ca^{2+}-dependent cysteine neutral proteinases [18]. Recent studies on the regulation of the calpain system yielded conflicting results. The resultant myofibril fragmentation length (MFL) affects tenderness as the degradation of myofibrillar proteins such as nebulin, titin and desmin make them susceptible to degradation by proteasomes and lysosomes, resulting in shorter myofibril fraction lengths and sometimes complete degradation to amino acids. The shrinkage of myofibrils also have a negative effect on the water holding capacity of meat. Several studies have investigated factors influencing water-holding capacity [19, 20, 21, 22,]. Reviews by [23, 24, 25] discuss the factors influencing in depth. Another tenderness base-line factor is collagen. Collagen content causes a certain percentage on the overall tenderness (called background tenderness) which may vary depending on genetic characteristics of a breed or age of the animal. Collagen turnover may be affected by oxidative stress, which affects the balance between its degradation by the enzyme matrix metalloproteinase-2 (MMP-2) and its synthesis by intramuscular fibroblasts. The effect of stress on meat tenderness is an immeasurable and unpredictable factor contributing to variability in meat tenderness [26]. Given all the known mechanisms/factors affecting meat tenderness, a factor cannot be used in isolation to predict tenderness.

IV. Recent Developments

In recent years, meat scientists ventured into the field of proteomics with the sole aim of identifying protein biomarkers related to meat tenderness. Proteomics is still a promising field in this regard. Since its inception in Meat Sci, several discoveries have been accomplished. A review by [27] highlights proteomics in Meat Sci, whereby proteins

involved in post-mortem glycolytic rates affected meat tenderness. The integration of 2DE-based comparative proteomics, mass spectrometry and bioinformatics data is important in paving a way to achieve the markers related to meat tenderness. A comprehensive review by [28] has summarised biomarkers related to meat tenderness. It was reported that most of the proposed meat tenderness biomarkers belongs to various pathways such as glycolytic energy metabolism pathway, oxidative energy metabolism pathway, cell detoxification and Heat Shock Protein family. With all the information that a classical and proteomics approach have achieved, there is still one important shortfall being the biomarker for meat tenderness. This lack of a distinct biomarker for meat tenderness emphasises the complexity of the meat tenderisation process. At this point, the available information suggest that it is still not possible to have one single representative biomarker for meat tenderness. This is mainly as a result of the multiple processes involved in meat tenderisation as suggested in a study by [29] highlighting the protein changes immediately *post mortem*. In a recent study by Moloto *et al*. (unpublished), it was found that expression of myosin light chain changes with ageing and is correlated with lower Warner-Bratzler shear force measurements in different cattle breeds. Moloto concluded that myosin light chain expression can be used as one of the meat tenderness markers as demonstrated in Figure 2. Other studies [30, 31, 32] reported and proposed different proteins as meat tenderness biomarkers (see Table 1). Therefore, it is difficult to find a representative biomarker. Some proposed biomarkers include the family of heat shock proteins, e.g. Hsp70, Hsp27 and α-crystallin. These proteins were proposed to have an influence on the regulation of meat tenderness and quality [33, 34]. Furthermore, as heat shock proteins, they are influenced by the ultimate pH (pHu) [32] that in turn depends on the muscle glycogen content at slaughter [35]. In a study by [36], rapid tenderisation of high pHu beef was attributed to early degradation of larger myofibrillar proteins such as titin and filamin because of instant activation of μ-calpain.

Owing to the massive number of the proposed biomarkers for meat tenderness in literature as reported in [37, 28, 38] and keeping in mind that multiple processes are involved in meat tenderisation, the main question remains: Is the meat scientist ever going to achieve a representative biomarker that can be precisely measured to predict meat tenderness? Table 1 emphasises the degree of complexity of tenderness biomarkers. So far, the different potential biomarkers belong to different metabolic pathways. These entire pathways operate interdependently and makes it difficult to synchronise them in order to map a proper way so that proteins can be measurable distinctively.

The complexity of meat tenderness through biological progression led to several studies investigating mechanical tenderization methods such as blade tenderization, electrical stimulation, flaking, mincing, stretching, alternative hanging, wrapping and rapid crust freezing [43]. Other studies dealt with chemical tenderisation whereby myofibrillar proteins are degraded to yield a more fragmented myofibril structure [44]. As reviewed in [45], such methods include marination, injection of exogenous enzymes from plant proteases (e.g ficin, bromelain, papain, actinidin, zingibain) and microbial proteases.

V. Conclusion

This review posed the question of whether it is possible to find unique biomarkers for meat tenderness or whether the complexity of tenderisation precludes the discovery of such biomarkers. Based on current information, the prospect of a distinctive meat tenderness protein biomarker seems unlikely. Proposed meat tenderness biomarkers are subjective and cannot be employed as general biomarkers. This review will try to bring up-to-date the findings of the biomarkers of meat tenderness identified so far, as meat tenderness guarantee remains a challenge in the meat industry sector worldwide. Due to the lack of a distinctive meat tenderness biomarker, a meat tenderness guarantee continues to be a threat to the industry and consumer satisfaction is uncertain.

VI. Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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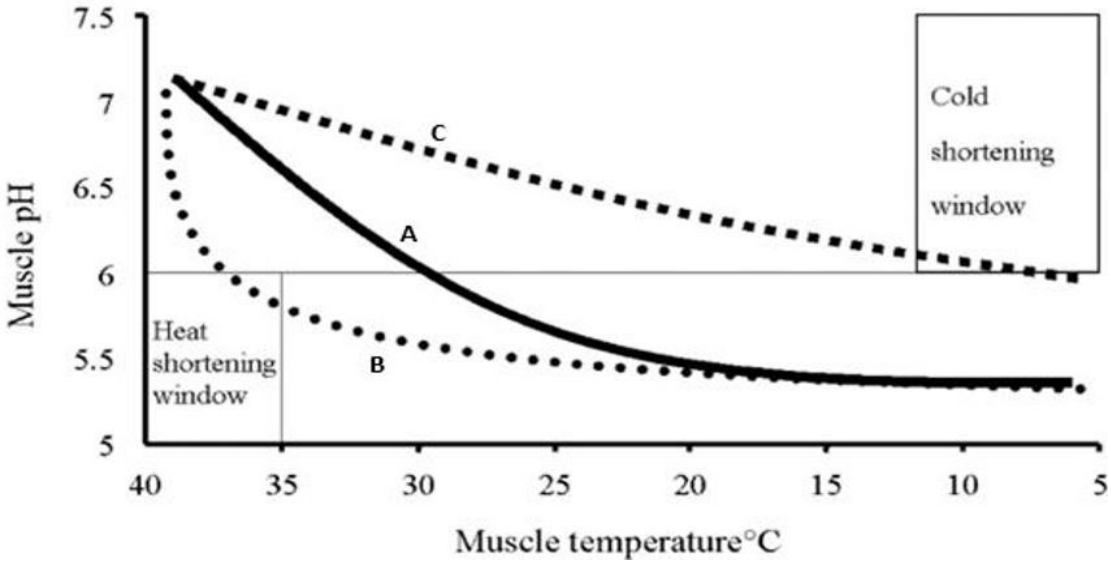

Fig. 1. Illustration of an ideal optimal pH/temperature decline (A), cold shortening circumstances (C) and hot shortening circumstances (B) [9](Thompson, 2002).

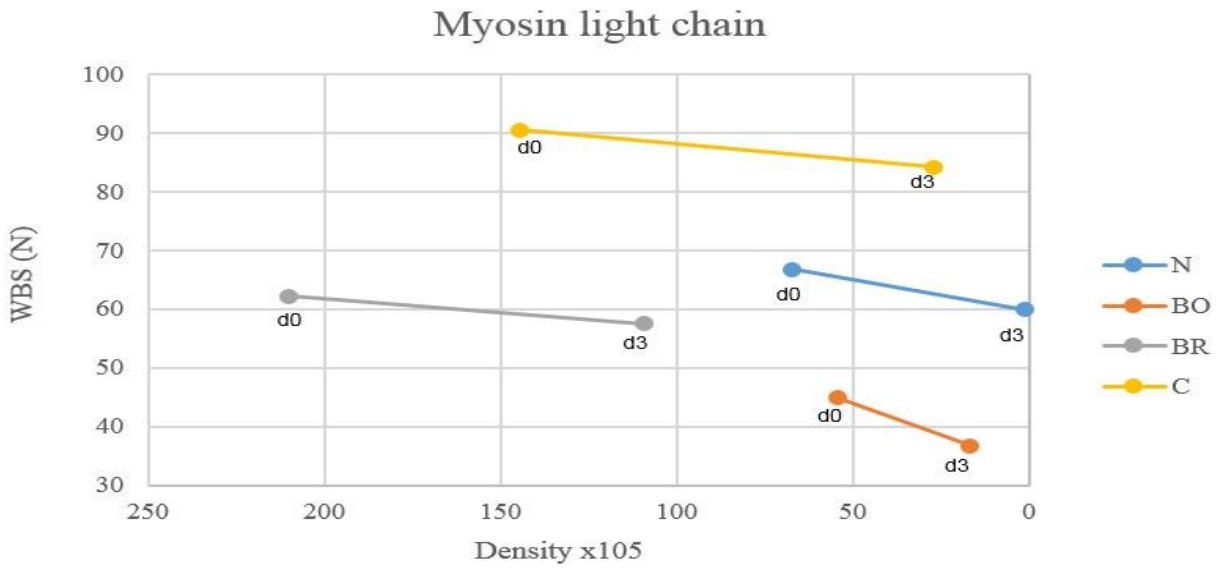

Fig. 2. The relationship between Warner-Bratzler shear force measurements and the relative abundance of myosin light chain in Nguni (N), Bonsmara (BO), Brahman (BR) and Charolais (C) meat over an aging period of 3 days.

Table 1. Identified meat tenderness biomarkers grouped according to metabolic pathway.

Pathways	Examples	References
Aerobic pathway	Succinate dehydrogenase ATP synthase Succinyl Co-A synthase, Isocitrate dehydrogenase	[39] [31];
Glycolytic pathway	Glyceraldehyde-3 phosphate dehydrogenase (GAPDH) Phosphoglucomutase β-enolase	[40] [30, 41]
Cell detoxification	Carbonic anhydrase Aldehyde dehydrogenases	 [40]
Heat Shock Proteins	HSP 70 Hsp27 β -Crystallin	[30] [30, 42] [32]
Structural proteins	Myosin light chain 1F F-actin-capping protein subunit β	[30]