Influência do pH final nos parâmetros físico-químicos, bioquímicos e sensoriais associados à qualidade da carne de bovinos *Bos indicus*

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2 PROTEOME OF HIGH AND NORMAL pH BEEF: FIRST INSIGHTS EMPHASIZING THE DYNAMIC PROTEIN CHANGES OF THE MUSCLE OVER DIFFERENT POST-MORTEM TIMES

2.1. Introduction

Brazil has the largest bovine herd in the world and is the largest beef exporter, with a beef livestock agribusiness movement of approximately US$ 169.29 billion. Most of the Brazilian herd has influence of zebu cattle (*Bos taurus indicus*), mainly Nellore breed, which combine productivity, disease resistance and heat tolerance once animals are predominantly finished on pasture (ABIEC, 2022; Carvalho et al., 2014). However, although beef cattle have adapted well to tropical conditions in Brazil (Carvalho et al., 2014; Rodrigues et al., 2017; Malheiros et al., 2020), beef cattle finished in extensive systems (pasture-based) is usually darker in color appearance and produces a less tender cooked product than those cattle finished in feedlot systems (grain-based) (Antonelo et al., 2022; Gómez et al., 2022) which may be directly related to the rate and extent of muscle pH decline. In fact, ultimate pH (pHu) is associated with muscle glycogen and *postmortem* energy metabolism. Several factors influence muscle pHu, including increased physical activity, feed deprivation, season, and pre-slaughter stress. These events are well-known to rapidly reduce muscle glycogen stores (Zhao et al., 2022; Terlouw et al., 2021; García-Torres et al., 2021) which leads to a high pHu (Poleti et al., 2018; Kiyimba et al., 2021) that negatively affect the final beef quality (Wu et al., 2020; England et al., 2016; Franco et al., 2015). As a result, carcasses with darker lean are generally downgraded during the process of grading, leading to significant economic losses globally for the beef sector (Gagaoua, Warner, et al., 2021; Ponnampalam et al., 2017). Thus, it is essential to avoid failures in the categorization of carcasses according to the pHu and develop early *postmortem* tools for their monitoring.
Gagaoua, Warner, et al. (2021) reported that proteomics techniques together with mass spectrometry (MS) and bioinformatics approaches have been widely used to show the mechanisms that drive the beef quality development in postmortem muscle, in addition to investigating the dynamic changes and modifications that occur in the muscle proteome and search for candidate biomarkers (Picard & Gagaoua, 2020). Proteomics was successfully applied to the discovery of biomarkers of dark-cutting beef, however a strong disparity among the studies was revealed using a meta-analysis integromics approach (Gagaoua, Warner, et al., 2021). Although certain consistent pathways were reported, the authors evidenced a very weak overlap in the putative biomarkers among the eight dark-cutting beef proteomics studies (< 8%) with only 10 common protein biomarkers reported across no more than two studies. From the ten common proteins, eight were related to dark-cutting beef conditions in different directions (positive and negative). It was suggested that the molecular signatures underpinning dark-cutting beef development are more complex than expected, and exploratory studies of the muscle proteomes of the same animals at both early postmortem and at the usual sampling times used to categorized carcasses based on their high pHu (24 or 48h) would allow further insights on the biochemical mechanisms and discovery of more appropriate biomarkers. Foreseeing the above, the current study used a shotgun proteomics approach to fill the gap in the early postmortem muscle metabolism in Bos indicus cattle, aiming to evaluate for the first time in these animals and by following the temporal dynamic changes of high and normal muscle pHu at three different times these being 30 min, 9 h and 44 h postmortem. The study further aimed to reveal the unknowns behind the protein changes and the possibility of proposing a more robust list of biomarkers of dark-cutting beef condition.

2.2. Conclusion

The temporal shotgun proteomics coupled with an in-depth bioinformatics analysis allowed for the first time to evidence the dynamic time-course changes and molecular signatures underpinning the dark-cutting beef condition
development. The pivotal role of energy metabolism, cellular response to stress, muscle system process and oxidoreductase activity were significantly revealed throughout the early postmortem metabolism. Twenty-three proteins overlap among postmortem times (30 min, 9 h and 44 h postmortem) and may be suggested as candidate biomarkers to monitor the proteome changes in the dark-cutting development.

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3 DECLÍNIO DO pHf E SUA RELAÇÃO COM A MACIEZ DA CARNE EM BOVINOS DA RAÇA NELORE (*Bos taurus indicus*)

3.1. Introdução

A taxa e extensão do declínio do pH *postmortem* é um importante fator da qualidade da carne bovina, influenciando sobretudo a maciez. Alguns estudos, como Pereira et al. (2015) demonstraram que bovinos (*Bos taurus indicus*), principalmente raça Nelore, têm apresentado carne com grande variabilidade de maciez atribuída a diferentes enzimas proteolíticas encontradas nos músculos dos animais.

A maciez é uma das principais características da qualidade da carne bovina, sendo considerada prioridade pelo consumidor. A inconsistência na maciez pode influenciar as decisões de compra dos consumidores (Ferrinho et al., 2021). É conhecido que a maciez da carne é afetada por diferentes fatores, como sistema de produção, dieta, idade do animal no momento do abate, efeito do pH, entre outros. Segundo Gagaoua et al. (2021), essas mudanças são inicialmente acompanhadas por uma queda do pH e temperatura, descritos como os primeiros fatores do amaciamento da carne.

Assim, o pH merece atenção especial, visto que, a degradação de proteínas musculares está ligada ao metabolismo energético (aparecimento de fragmentos de enzimas glicolíticas e oxidativas), morte celular programada, proteínas de choque térmico, estresse oxidativo, etc. Os estudos recentes que abordaram esses aspectos sugeriram que os mecanismos subjacentes estão ligados ao pH inicial e à extensão do declínio do pH durante o processo de glicólise (Gagaoua et al., 2021). Em um estudo anterior, Lomiwes et al. (2014) utilizaram técnicas sofisticadas acoplada à espectrometria de massas e indicaram que o amaciamento da carne bovina provavelmente será categorizado pelo pH final, sendo pH final baixo ou normal (≤ 5,79), intermediário (5,80-6,19) e alto (≥ 6,20).

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Portanto, o objetivo desse estudo foi avaliar o declínio do pH em diferentes faixas de pH versus tempo postmortem, e quantificar através de SDS-PAGE e Western Blotting a intensidade relativa de proteínas envolvidas na proteólise do músculo postmortem, bem como avaliar os parâmetros de maciez da carne bovina.

3.2. Conclusão

O presente estudo evidenciou que o declínio nas faixas de pH normal e intermediário foram significativos (P<0,01), mostrando redução dos valores de pH ao longo do tempo postmortem. O estudo quantificou por meio de SDS-PAGE os pesos moleculares das proteínas com destaque para 0,5 h; 6 h e 44 h postmortem que apresentaram diferença significativa (P<0,05) entre as faixas de pHf. Além disso, o pHf alto apresentou o menor valor de força de cisalhamento e parece que a associação da desmina com menores valores de força de cisalhamento no pHf alto às 44 h postmortem podem contribuir para melhorar a maciez da carne bovina.

Para HSP90, o pH normal e intermediário foram associados à níveis de expressão maiores de HSP90, especificadamente às 44 h postmortem no pHf intermediário. Na HSP27 houve interação entre os tempos postmortem com maior percentual de intensidade relativa para 9 h postmortem. Em nossas observações, o equilíbrio entre Bax e Bcl-2 ainda não foram totalmente compreendidos, mas os resultados sugerem que uma das vias representativas para induzir a apoptose pode ser a liberação de citocromo C da mitocôndria que pode ter sido promovida por Bcl-2 no pH normal em 0,5 h e 9 h postmortem. Em relação a caspase-3, foi observado que a intensidade relativa das bandas foi maior em pH normal, tempo 0,5 h, seguido de pH alto, tempo 6 h e suportam a teoria que as caspases são ativadas imediatamente após a sangria do animal, apresentando níveis de expressão mais elevados nos primeiros momentos do período postmortem e diminuindo ao longo do tempo.

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4 ASSESSMENT OF BEEF SENSORY ATTRIBUTES AND PHYSICOCHEMICAL CHARACTERISTICS: A COMPARATIVE STUDY OF INTERMEDIATE VERSUS NORMAL ULTIMATE pH STRIPLOIN CUTS

4.1. Introduction

Animal protein, particularly bovine meat, is a fundamental part of the human diet, especially in Western countries where beef is a major source of protein. Consumer preferences for beef vary across different markets and are influenced by diverse factors including (i) cultural and psychological aspects of consumers (Troy & Kerry, 2010), (ii) the sensory properties of the products (Saldaña et al., 2020a), and (iii) marketing considerations such as price, regulation, quality standards, and distribution (Font-i-Furnols & Guerrero, 2014).

Texture is a key sensory attribute of beef, offering a range of mouthfeel experiences during consumption, such as tenderness, juiciness, and chewiness (Zhu et al., 2021). The aroma and flavour are also critical, arising from chemical compounds like amino acids, peptides, organic acids, and adenine nucleotide metabolism by-products (Spanier et al., 1997). The Maillard reaction and lipid degradation in meat generate various volatile compounds, contributing to the distinctive aromas (Kosowska et al., 2017). Recent research suggests that the chemical properties of muscle fibres (type I and II) vary and significantly influence the formation of volatiles and beef flavour (Li et al., 2023).

The sensory quality of beef is largely determined by its ultimate pH (pHu), which is linked to the animal's ability to store glycogen in muscles and the differing amounts of mitochondria in muscle fibres (Picard & Gagaoua, 2020; Poleti et al., 2018). Variations in pHu between intermediate and normal beef can result from factors like animal diet, exercise, pre-slaughter stress, and processing conditions, all impacting the final quality, particularly in terms of colour, tenderness, and flavour (Gagaoua et al., 2021a; Loudon et al., 2018; Ponnampalam et al., 2017).
This presents a challenge for food scientists and the industry in aligning consumer perception with variations in beef pHu (Warner et al., 2021).

The meat industry aims to consistently produce high-quality cuts while reducing consumer dissatisfaction (Gagaoua & Picard, 2020). Various sensory evaluation methods have been deployed, including both analytical (trained panels) and holistic (consumer) approaches (Saldaña et al., 2021). According to Silva et al. (2012), a product’s sensory profile can be established using the Optimised Descriptive Profile (ODP) by a trained panel, where product attributes are selected and agreed upon by the assessors (Aaslyng et al., 2014). Conversely, affective methods rely on consumer reactions, such as hedonic measures, and can be augmented with techniques like Check-all-that-apply (CATA) questions, a versatile tool for capturing perceptions, feelings, and attitudes (Jaeger et al., 2015).

Considering the prominence of beef in Brazilian diets and the preference for this animal protein, this study aimed to develop a comprehensive sensory profile and deepen our understanding of the acceptance and perception of beef cuts within two pHu ranges: intermediate and normal. This innovative study combined the expertise of trained assessors for consistent data, alongside the views of 135 regular beef consumers. Additionally, instrumental measurements taken at 3, 14, and 28 days post-mortem were analysed. The hypothesis was that individual reactions to sensory and physicochemical properties of beef could predict consumer perception of beef quality, providing valuable insights to the meat industry about consumer expectations and purchasing decisions.

4.2. Conclusion

This study provides valuable insights into assessing beef quality attributes and consumer perceptions based on variations in beef muscle pHu levels. We observed that beef with an intermediate pHu showed increased juiciness compared to normal pHu beef. This heightened juiciness is likely due to increased water-holding capacity. These findings align with consumer preference
assessments that identified steaks with intermediate pHu as tougher and those with normal pHu as drier. Consumer feedback indicates that attributes like tenderness and juiciness significantly influence overall beef appreciation. Other important factors include roast beef flavour, chewability, saltiness, and juiciness. The study also showed that steaks with intermediate pHu had higher deoxymyoglobin concentrations on the third day compared to the twenty-eighth day of ageing. Instrumental colour measurements, such as $L^*$, $b^*$, chroma, and oxymyoglobin, were significantly affected by both pHu category and ageing time (3, 14, and 28 days). This research enhances our understanding of pHu variations in beef muscle and their impact on sensory attributes and consumer preferences. Further studies with larger sample sizes are essential to support and expand these findings, aligning with consumer expectations for premium-quality beef.

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