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**Sous vide processing of beef tournedos: thermal validation, proximate
composition prediction and sensory evaluation**

Débora Rezende Ferreira
Doctor Scientiae

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Sous vide processing of beef tournedos: thermal validation, proximate composition prediction and sensory evaluation

Thesis submitted to the Food Science and Technology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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ABSTRACT

FERREIRA, Débora Rezende, D.Sc., Universidade Federal de Viçosa, June, 2026. **Sous vide processing of beef tournedos: thermal validation, proximate composition prediction and sensory evaluation.** Adviser: Edimar Aparecida Filomeno Fontes. Co-advisers: Paulo Rogerio Fontes and Bruno Ricardo de Castro Leite Junior.

This study investigated the processing of *sous-vide* beef tenderloin medallions, integrating microbiological safety, physicochemical quality, and sensory acceptance. In Chapter 2, equivalent heat treatments were validated based on USDA guidelines. The process at 58°C for 32 minutes showed the best performance, ensuring the complete inactivation of *Salmonella* spp. (reduction 6.5 log CFU/g). Furthermore, it resulted in lower exudate loss (3.8%), low lipid oxidation (TBARS < 0.25 mg MDA/kg), higher soluble collagen retention (28%), and reduced shear force (28 N), indicating more tender and juicy meat. In Chapter 3, a predictive model for proximate composition was developed using portable NIR spectroscopy combined with PLS regression and jack-knife selection. Prediction errors from external validation (RMSEP) for the compact InnoSpectra and NeoSpectra instruments evaluated were, respectively, 0.92% and 0.65% for moisture, 0.72% and 0.93% for fat, 0.93% and 0.60% for protein, and 0.12% and 0.03% for ash, demonstrating the potential to replace destructive laboratory methods with a rapid, sustainable approach applicable in real-time within the industry. In Chapter 4, sensory analysis using the RATA method revealed that the 58°C/32 min treatment was associated with higher scores for good appearance (1.9/3), juiciness (1.9/3), and characteristic meat aroma (1.5/3), resulting in the highest purchase intention (82% of consumers). The sensory results corroborated the physicochemical and predictive data, confirming the robustness of the proposed integrated system. It is concluded that it is possible to combine food safety and gastronomic quality through a system that integrates cooking process validation, methods for predicting meat composition, and sensory evaluation. Thus, the sous-vide beef tournedos emerges as a safe, high-quality product with significant market acceptance potential.

Keywords: low-temperature cooking; meat quality; near-infrared spectroscopy; chemometrics; sensory analysis

RESUMO

FERREIRA, Débora Rezende, D.Sc., Universidade Federal de Viçosa, junho de 2026. **Processamento sous vide de tornedor bovino: validação térmica, predição da composição centesimal e avaliação sensorial.** Orientadora: Edimar Aparecida Filomeno Fontes. Coorientadores: Paulo Rogerio Fontes e Bruno Ricardo de Castro Leite Junior.

Este trabalho investigou o processamento do tornedor de filé mignon bovino 'sous vide', integrando segurança microbiológica, qualidade físico-química e aceitação sensorial. No Capítulo 2, foram validados tratamentos térmicos equivalentes com base em guias do USDA. O processo a 58 por 32 minutos apresentou o melhor desempenho, garantindo a completa inativação de *Salmonella* spp. (redução 6,5 log UFC/g). Além disso, resultou em menor perda de exsudato (3,8%), baixa oxidação lipídica (TBARS < 0,25 mg MDA/kg), maior retenção de colágeno solúvel (28%) e força de cisalhamento reduzida (28 N), indicando carne mais macia e succulenta. No Capítulo 3, foi desenvolvido um modelo preditivo da composição centesimal utilizando espectroscopia NIR portátil associada à regressão PLS e seleção jack-knife. Os erros de previsão resultantes da validação externa (RMSEP) para os instrumentos compactos InnoSpectra e NeoSpectra avaliados foram, respectivamente, 0,92% e 0,65% para umidade, 0,72% e 0,93% para gordura, 0,93% e 0,60% para proteína e 0,12% e 0,03% para cinzas, demonstrando potencial para substituir métodos laboratoriais destrutivos por uma abordagem rápida, sustentável e aplicável em tempo real na indústria. No Capítulo 4, a análise sensorial pelo método RATA revelou que o tratamento a 58 /32 min foi associado a maiores escores de boa aparência (1,9/3), succulência (1,9/3) e aroma característico de carne (1,5/3), culminando na maior intenção de compra (82% dos consumidores). Os resultados sensoriais corroboraram os dados físico-químicos e preditivos, confirmando a robustez do sistema integrado proposto. Conclui-se que é possível unir segurança alimentar e qualidade gastronômica por meio de um sistema que combina validação do processo de cozimento, métodos de previsão da composição da carne e avaliação sensorial. Dessa forma, o tornedor bovino sous vide se apresenta como um produto seguro, de boa qualidade e com grande potencial de aceitação pelo mercado.

Palavras-chave: baixa temperatura de cozimento; qualidade da carne; espectroscopia NIR; quimiometria; análise sensorial

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1. GENERAL INTRODUCTION

The sous vide method has emerged as a cooking technology capable of yielding meat that is more tender and succulent, with better color retention and reduced moisture loss. Despite its technological and sensory advantages, its industrial-scale application still raises concerns regarding microbiological safety, as heat treatments are conducted at milder temperatures over extended periods, without clearly established regulatory criteria for thermal lethality indicators.

Chapter 1 highlights this scientific and regulatory gap, demonstrating the lack of consistent parameters for defining thermal lethality indicators in sous vide meat products. This absence of criteria underscores the need for rigorous validation and interim guidelines to ensure food safety.

Chapter 2 moves into experimental practice, proposing and validating equivalent heat treatments for beef tenderloin tournedos. In addition to microbiological validation, physicochemical attributes—such as exudate, lipid oxidation, collagen content, and texture—are evaluated, providing insight into how different cooking conditions affect the product's technologic

In Chapter 3, attention shifts to analytical innovation. Near-infrared (NIR) spectroscopy—combined with multivariate regression and variable selection via the jack-knife method—is applied as a rapid, sustainable alternative for predicting proximate composition, as opposed to the traditional, destructive, and time-consuming laboratory methods. This approach offers a fast, sustainable alternative aligned with Industry 4.0 principles, enabling the real-time prediction of parameters such as moisture, protein, fat, and ash content in beef tenderloin tournedos.

Finally, in Chapter 4, sensory analysis using the RATA method links microbiological and physicochemical data to consumer perception, revealing which cooking conditions maximize attributes such as tenderness, juiciness, and purchase intent.

Thus, this work is structured as an integrated response to a central problem: how to establish a 'sous-vide' processing system for beef *tournedos* that ensures microbiological safety, allows for the rapid and sustainable prediction of proximate composition, and preserves sensory attributes valued by consumers?

The scientific justification lies in the need to address regulatory and methodological gaps, while the practical justification lies in contributing to the development of safe, high-quality products with greater market acceptance potential.

2. GENERAL OBJECTIVE

To establish and validate ‘sous vide’ thermal protocols for beef tenderloin tournedos, ensuring microbiological safety and characterizing their physicochemical and sensory attributes, as well as to develop a predictive model for proximate composition using NIR spectroscopy, in order to identify the treatment that balances food safety, technological quality, and consumer acceptance.

3. SPECIFIC OBJECTIVES

- Review selection criteria for thermal lethality indicators in ‘sous vide’ meat products, identifying regulatory gaps and proposing interim risk-reduction guidelines.
- Validate equivalent heat treatments for beef tenderloin medallions, considering heat penetration curves, lethality parameters (F_0), and microbiological profile.
- Evaluate the impact of heat treatments on physicochemical characteristics, including proximate composition, water activity, pH, exudate release, lipid oxidation, shear force, soluble and insoluble collagen, myofibrillar fragmentation, and instrumental color.
- Develop and validate predictive models for proximate composition (moisture, protein, fat, and ash) using portable NIR spectroscopy combined with PLS regression and variable selection via the jack-knife algorithm, comparing the performance of different instruments.
- Develop and validate predictive models for the proximate composition of ‘sous vide’ beef tenderloin medallions using portable NIR spectroscopy.
- Conduct sensory analysis using the RATA (Rate-All-That-Apply) method, correlating attributes perceived by consumers (tenderness, juiciness, flavor, purchase intention) with physicochemical and predictive results.
- Determine the optimal heat treatment for sous-vide beef tenderloin tournedos that balances microbiological safety, physicochemical quality, and sensory acceptance, establishing an integrated protocol for their industrial processing.

CHAPTER 1: INDICATORS OF THERMAL LETHALITY IN SOUS VIDE MEAT PRODUCTS: a review

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ABSTRACT

This study presents the sensory characterization of beef tournedos processed by the sous vide method at different temperature–time combinations (56.1 °C/62 min, 58 °C/32 min, 62 °C/12 min, and 72 °C/no dwell time). One hundred consumers evaluated appearance, aroma, flavor, texture, and purchase intent using the Rate All That Apply (RATA) test and hedonic scales. Significant differences were observed among treatments: samples cooked at 58 °C for 32 minutes showed the most pleasant appearance, balanced aroma and flavor, and higher scores for tenderness, juiciness, and overall acceptability. The 72 °C treatment resulted in darker color, firmer texture, and oxidized flavor, while the 56.1 °C treatment retained redness but exhibited a bloody taste. The 58 °C/32 min condition achieved the highest mean hedonic score and purchase intent, confirming it as the optimal sous vide processing parameter for sensory quality and consumer acceptance of beef tournedos.

Keywords: meat quality; food safety, cooking method.

1. INTRODUCTION

It is well established that meat is a significant component of the human diet. However, over time, significant changes in meat quality have occurred, such as those resulting from leaner animals due to the demand for healthier foods. Traditional thermal treatments used in meat processing, due to the high temperatures employed, lead to moisture loss which, combined with lower fat content, results in less succulent meats (Cropotova et al., 2019).

'Sous vide' (SV) technology is a cooking method where food is vacuum-sealed in impermeable packaging and cooked in circulating water at a controlled temperature for an extended period. The cooking temperature for meat is generally around 60 °C for varying times depending on the meat cut (Dominguez-Hernandez & Erthbjerg, 2021). Long-term heating at lower temperatures compared to those used for conventional foods results in meat tenderization and facilitates the production of particularly flavorful and nutritious products, even from cheaper cuts (Ayub & Ahmad, 2019).

Although 'sous vide' cooking can improve meat quality, such as tenderness with a significant reduction in cooking loss and color preservation, and prevents post-cooking recontamination, the handling of mild temperatures becomes a major concern regarding the safety aspect associated with microbial risk. Therefore, the selection of correct temperatures and times is crucial in 'sous vide' preparation, as it affects not only the physicochemical properties but also the microbiological properties of the meat (Baldwin, 2012).

Microbial safety assessment is an important benchmark for determining the efficiency of 'sous vide' cooking. SV cooked meat products are highly dependent on the pathogens present in the raw material and those that survive after cooking (Kathuria et al., 2022). However, it is observed in the literature that various thermal lethality indicators are used in meat processing, often without proper justification or basis for their selection. As there is no specific regulation for 'sous vide' cooking, this becomes a food safety issue leading to public health risks.

Therefore, the objective of the present study was to examine, among publications on 'sous vide' meat product cooking, the criteria established for electing the thermal lethality indicator, evaluating the foundation based on the relevance attributed to the safety of the processed food.

2. METHOD

This study is a literature review with a qualitative approach, aiming to gather and analyze scientific publications related to the use of 'sous vide' technology as a thermal treatment for meat products, with an emphasis on the thermal lethality indicator microorganisms adopted in these studies. The bibliographic search was conducted in the PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar databases, covering the period from 2000 to 2026.

The search terms used were “meat sous vide conditions”, “meat pasteurization”, “thermal lethality”, and “meat quality”, combined using the Boolean operators AND and OR.

3. RESULTS

A total of 340 articles were analyzed in the relevant databases; 37 were considered eligible studies and 12 were included in the study (Figure 1).

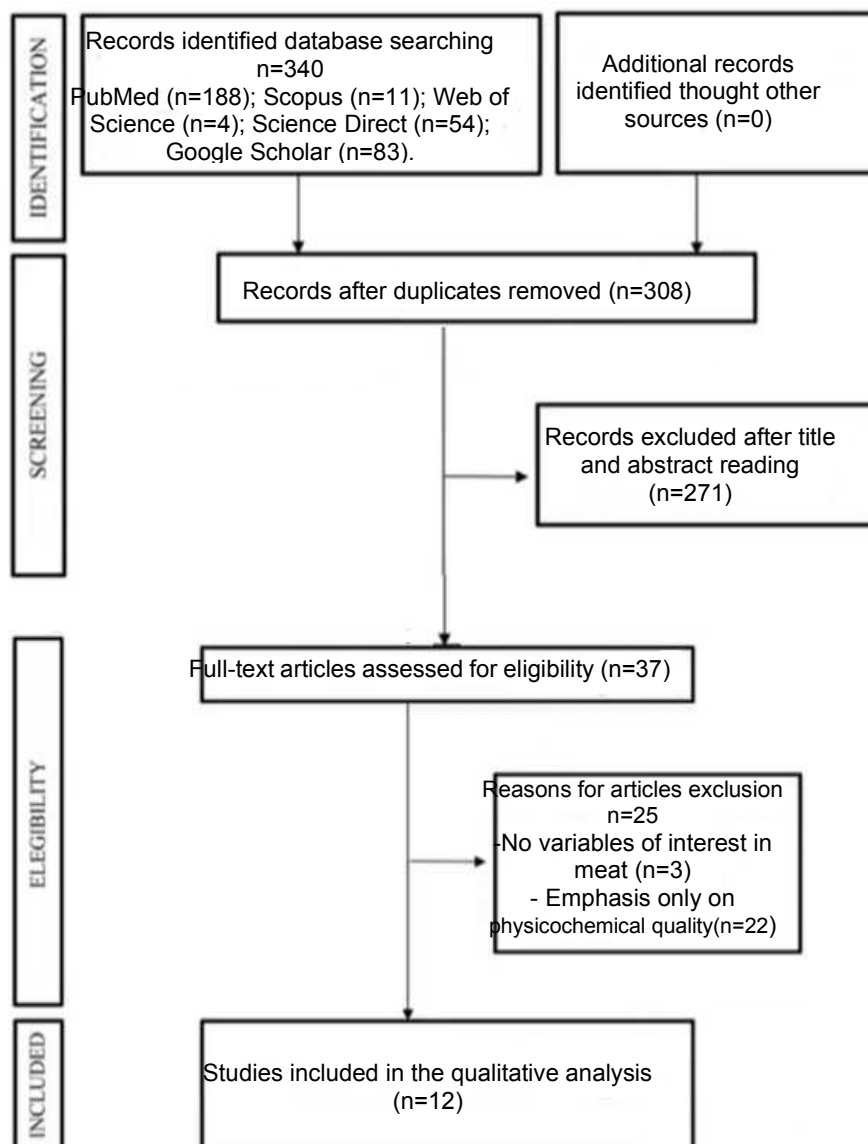


FIGURA 1: Diagram of records identified database searching.
Fonte: FERREIRA D.R et. al., 2026.

4. DISCUSSION

The 'sous-vide' processing of meat (beef, pork, and poultry) has been studied by various researchers using a wide range of temperatures (50 – 96 °C) and times (4 - 360 min) (Table 1).

'Sous vide' cooking has become the focus of much research due to its versatility in manipulating cooking conditions, prioritizing low temperatures and long times. These are less severe thermal treatments that result in different degrees of meat tenderness, color, succulence, and palatability by avoiding moisture and flavor loss through evaporation during cooking, in addition to inhibiting cross-contamination after cooking. These factors are of utmost importance in determining the sensory, physical, and chemical quality of meat (Baldwin, 2012)

TABLE 1 - Thermal lethality indicators cited in 'sous vide' meat processing studies.

Product	'Sous vide' conditions	Reference microorganism	Key findings	Reference
Marinated chicken breast	55, 57.5, 60°C in a period of one hour.	<i>Salmonella</i> e <i>L. monocytogenes</i>	D values for <i>Salmonella</i> : ranged from 47.65 min at 55°C to 7.48 min at 60°C. D values for <i>L. monocytogenes</i> : ranged from 54.81 min at 55°C to 10.39 min at 60°C.	Karyotis et al. (2017)
Chicken breasts.	two-step temperature treatment: 1*- 50° C / 40min and 60° C / 80min; 2*- 50° C / 80min and 60° C / 40min *total cooking time 1 and 2=120 min compared with the one-step temperature treatment (60° C for 120 min).	<i>Enterococcus faecalis</i>	The one-step sous vide treatment performed at 60°C/120 minsuccesfully inactivated <i>Enterococcus faecalis</i> and 120min at two temperatures, 50°C. The two-step sous vide treatment performed at 60°C, achieved the target pasteurization performance criterion of a 3 log reduction in <i>Enterococcus faecalis</i>	Hasani et al. (2023)
Game meat: both loins (Musculus longissimus thoracis et lumborum) of roe deer and wild boar.	50 °C: 60 min, 120 min, 180 min, 240 min, 300 min, 360 min; 55 °C: 20 min, 40 min, 60 min, 80 min, 100 min, 120 min; 60 °C: 4 min, 8 min, 12 min, 16 min, 20 min	<i>L. monocytogenes</i>	Sous-vide cooking for several hours is able to destroy large amounts of vegetative pathogens. The results showed that the decimal reduction values (D values) were largely dependent on the surrounding matrix and the inactivation temperatures.	Abel et al. (2020)
Pork fillet	55°C: 30, 60 and 90 min	<i>L. monocytogenes</i>	Treatments at 55 °C do not guarantee complete inactivation of <i>L. monocytogenes</i>	Vila-Brugalla et al. (2026)
Beef tournedos	56.1 °C/62 min; 58 °C/32 min; 62 °C/12 min; 72 °C/no dwell time	<i>Salmonella</i>	All treatments were effective in inactivating <i>Salmonella</i> .	Ferreira et al. (2026)

pork meat marinated in tomatillo beef marinated on a red chili sauce	55, 57.5, 60, and 62.58°C for different periods of time	<i>L. monocytogenes</i>	The quantitative assessment of heat resistance of <i>L. monocytogenes</i> , as defined by D- and z values, should enable food processors to design thermal treatments with lower processing times and temperatures to inactivate this pathogen in sous-vide processed Mexican meat products.	Valenzuela-Melendres et al. (2016)
Beef Steaks	62.5 °C / 25min; 60.0 °C / 35 min; 57.5 °C / 29min; and 52.5 °C / 36min.	<i>S. enterica</i> and <i>L. monocytogenes</i> .	The time required for a 6.5 log ₁₀ cfu/g reduction of <i>S. enterica</i> : 13.90, 21.13, 28.47, and 86.74 min, while <i>L. monocytogenes</i> required significantly longer times of 24.12, 36.87, 74.54, and 347.45 min at 62.5 °C, 60.0 °C, 57.5 °C, and 52.5 °C, respectively.	Manzoor et al. (2026)
Spiced turkey breast meat	65, 70, 75 °C x 20, 40, 60 min	Total mesophilic aerobic bacteria, total Enterobacteriaceae, <i>Escherichia coli</i> , <i>Listeria</i> spp. and <i>Salmonella</i> spp.	The count of total mesophilic aerobic bacteria decreased approximately by 2 log CFU/g for the samples prepared in each cooking temperature/time combinations. Only in the samples cooked at 65 °C for 20 min, the presence of <i>Listeria</i> spp. was detected. Except the cooking at 65 °C for 20 min, all temperature-time combinations used for cooking turkey cutlets provided inactivation for pathogenic microorganisms	Biyikli et al. (2020)
Pork hams	(61°C or 71°C), time (45 or 90 min)	total viable and coliform count	No microbial growth was detected for samples subjected to sous-vide cooking in any treatment groups.	Jeong et al. (2018)

Turkey breast	96°C / 30 min	<i>L. monocytogenes</i>	The effectiveness of heat treatment for inactivating the pathogen was affected by product surface roughness. About 50 min of heating time was needed to achieve a thermal kill of 7 log ₁₀ CFU/cm ² on products with surface roughness up to 15 mm in depth.	Murphy et al. (2003)
Ready-to-eat meat products: turkey, ham, and roast beef.	90,6°C, 93,8°C or 96,1°C / 2 to 10 min	<i>L. monocytogenes</i>	Minimal heating regimens of 2 min at 90,6 to 96,1°C can readily provide 2-log reductions in most RTE deli meats we processed and suggest that this process may be an effective microbial intervention against <i>L. monocytogenes</i> on RTE deli-style meats.	Muriana et al. (2002)
Beef semitendinosus muscles	50°C: 90min; 150min; 270min; 390min. 55°C: 390min 60°C: 90min; 150min; 270min. 65°C: 90min	Total viable count (TVC), total viable psychrotroph (Ps), Enterobacteriaceae and <i>Bochothrix thermosphacta</i> counts. <i>Lactobacillus</i> spp.	The pasteurization values obtained were enough to inactivate vegetative cells but were insufficient to achieve a significant reduction of <i>Clostridium botulinum</i> spores. All the heat treatments reduced both counts below the detection limit of total viable count (TVC), total viable psychrotroph, <i>Lactobacillus</i> and <i>B. thermosphacta</i> .	Vaudagna et al. (2002)
Source: FERREIRA DR, et al., 2026; data extracted from databases PubMed, Scopus, Web of Science, ScienceDirect e Google Scholar				

Many publications aim to study the parameters that impact the sensory quality of meat and disregard microbiological quality, whereas the primary concern in the 'sous-vide' cooking method is the safety aspect associated with microbial risk (Ismail et al., 2022).

There are also those dedicated to the study of microbiological risk control without concern for damage to sensory properties by using high cooking temperatures, such as the research conducted by Murphy et al. (2003) which verified the thermal inactivation of *Listeria monocytogenes* in ready-to-eat turkey breast meat products during 'sous vide' cooking. For this, a temperature of 96 °C for 50 minutes was used, which was the time the authors reported as

necessary to achieve a thermal elimination of 7 log₁₀ CFU/cm² in products with surface roughness up to 15 mm deep.

Muriana et al. (2002) also evaluated the reduction of *Listeria monocytogenes* in ready-to-eat 'sous vide' meats using temperatures of 90.8 °C, 93.38 °C, and 96.11 °C for times varying between 2 and 10 min. They suggest that processing for 2 min at 90.8 °C to 96.11 °C was effective in this study.

Higher temperatures (above 70 °C), such as those used by Murphy et al. (2003) and Muriana et al. (2002) lead to greater microbiological safety but may result in the generation of umami taste from nucleotides and also volatile flavors from the degradation of amino acids by the Strecker reaction, which suppresses the advantages of 'sous vide' cooking (Ismail et al., 2022).

These authors justify the use of *Listeria monocytogenes* as a thermal lethality indicator due to its potential as a contaminant in food processing environments because it is heat-resistant, has the ability to grow at refrigeration temperatures, and the capacity to form biofilms on all types of surfaces. Furthermore, they state that the potential contamination of *L. monocytogenes* in ready-to-eat meat products represents a threat to food safety.

However, Abel et al. (2020) state that although cooking duration depends on the type of meat product and the processing environment, temperatures of 60 °C are sufficient to inactivate *Listeria monocytogenes*. Yet, for food safety reasons, it is recommended to pan-fry after 'sous-vide' treatment or to cool rapidly. The authors further justify the use of the model microorganism for the heat inactivation experiment because it is a pathogen with high tolerance to pH and salt, in addition to having relatively high heat resistance. Additionally, listeriosis presents the highest mortality rate (16.2%) among all zoonoses monitored in Europe (Fernández-Martínez et al., 2022).

In another study, Vila-Brugalla et al. (2026) limited the study to a single temperature of 55 °C for 30, 60, and 90 min to propose a predictive model for survival and recovery of *Listeria monocytogenes*. The authors mention that *Listeria monocytogenes* is concerning due to its high hospitalization and mortality rates. Listeriosis outbreaks are predominantly associated with refrigerated ready-to-eat (RTE) foods, such as the one involving RTE pork and salmon in Spain in 2019 with 207 confirmed cases, resulting in 3 deaths and 141 hospitalizations (Fernández-Martínez et al., 2022). International regulatory approaches differ, with tolerance limits ranging from 100 CFU/g (which may occur throughout the declared shelf life) as in Canada, to a strict zero-tolerance policy for RTE meat products in the United States (Farber et al., 2021) and Brazil (BRASIL, 2022).

A study conducted by Valenzuela-Melendres et al. (2016) also used *Listeria monocytogenes* as a thermal lethality indicator when performing 'sous vide' cooking of pork marinated in tomatillo sauce and beef marinated in red chili sauce. They also used as justification for this choice the fact that the microorganism has high thermal resistance, in addition to the need to control this pathogen in 'sous vide' products.

Karyotis et al. (2017)) described the inactivation kinetics for *L. monocytogenes* and *Salmonella* in marinated chicken breast heated at temperatures of 55 °C, 57.5 °C, and 60 °C for 1 hour. These authors justify the use of these microorganisms as thermal lethality indicators based on the possibility that 'sous vide' processing may not be able to eliminate *Listeria monocytogenes* and non-viable populations of *Salmonella* spp. These pathogens can grow during refrigerated storage, especially if subjected to inadequate temperature conditions, commonly observed in retail and domestic environments.

Salmonella was used as a thermal lethality indicator by (Ferreira et al., 2026) when studying the impact of the 'sous vide' process on the quality of beef tournedos. The researchers based their work on Appendix A of the United States Food and Drug Administration Food Code (USDA & FSIS, 2021) which establishes time and temperature combinations for thermal treatments of 'sous vide' meat products targeting the inactivation of *Salmonella*. In all thermal treatments of the experiment, they successfully inactivated the microorganism. In addition to the cooking guideline, they also based the cooling on Appendix B of the same code (FSIS & USDA, 2021) with the objective of adding to the barrier of mild thermal treatment, refrigeration, and proper maintenance of the refrigerated product.

Similarly to Ferreira et al. (2026), Manzoor et al. (2026) used the justification that *Salmonella* is considered a lethality indicator by the Food Safety and Inspection Service of the United States Department of Agriculture (USDA & FSIS, 2021) to study its inactivation dynamics in 'sous vide' cooking of beef under the following conditions: 62.5 °C/25 min; 60.0 °C/35 min; 57.5 °C/29 min; and 52.5 °C/36 min. However, in addition to *Salmonella*, the authors also evaluated the inactivation of *Listeria monocytogenes* with the justification of it being a thermotolerant microorganism. As *L. monocytogenes* exhibited greater thermal resistance than *S. enterica* under the evaluated 'sous vide' cooking conditions, the researchers highlight the need for a comprehensive risk assessment to understand if *L. monocytogenes* should also be considered a lethality indicator organism during 'sous vide' processing by regulatory bodies.

In addition to *Listeria* spp. and *Salmonella* spp., Bıyıklı et al. (2020) analyzed total mesophilic aerobic bacteria, Enterobacteriaceae, and *Escherichia coli*. Although the study

evaluated the effect of 'sous vide' cooking using the binomials 65, 70, 75 °C x 20, 40, 60 min in the cooking of seasoned turkey breast, the authors did not justify the choice of these microorganisms as thermal lethality indicators. They only described the results, emphasizing only those related to pathogenic microbiota, which does not support the purpose of performing microbiological analyses in this study.

Other microorganisms were also evaluated by Vaudagna et al. (2002) as thermal lethality indicators with the justification that the treatment must be based on heat sensitivity and microbiological risk, without specifying the risks for each evaluated microorganism. In this experiment, beef was subjected to 'sous vide' cooking under the conditions of 50 °C (90 min; 150 min; 270 min; 390 min), 55 °C - 390 min; 60 °C (90 min; 150 min; 270 min), and 65 °C - 90 min, and total viable count (TVC), total viable psychrotrophs (Ps), Enterobacteriaceae, *Lactobacillus* spp., and *Bochothrix thermosphacta* counts were performed. The results indicate that the treatments were sufficient to inactivate vegetative cells but were insufficient to achieve a significant reduction of *Clostridium botulinum* spores. However, they justify this reduction inefficiency by the fact that the contamination level of *C. botulinum* spores is higher in fish than in beef, thus not being a potential hazard for products such as vacuum-packaged beef muscles. So why analyze this microorganism for 'sous vide' meat products? The authors only mention that counts of *Lactobacillus* and *B. thermosphacta* were performed because they are frequently isolated from meat; they do not base the choice of these as thermal lethality indicators.

Based on the 'sous vide' pasteurization performance criterion for poultry meat, *Enterococcus faecalis* was selected as the target microorganism in the work developed by Hasani et al. (2023) which compared the effect of one- and two-step 'sous vide' cooking on the microbiological and oxidative stability of chicken breast. For the pasteurization of food products, a 6-log reduction of pathogenic bacteria is defined as a pasteurization performance criterion (NACMCF, 2006), and the proposed treatments achieved this criterion.

When studying the effect of the 'sous-vide' method at different temperatures, times, and vacuum levels on the quality, structural, and microbiological properties of pork ham, Joung et al. (2018) used coliform counts as a thermal lethality indicator. The authors cite the study's objective to demonstrate cooking effectiveness through pathogen inactivation. However, no evidence was found that pathogenic microorganisms were evaluated in this work, which confirms the need for a regulation that establishes criteria for the use of thermal lethality indicators based on cooking conditions and food matrix, aiming at the safety of the processed food.

4. CONCLUSION

Understanding the public health implications of the survival of pathogenic microorganisms during cooking and the choice of an appropriate thermal lethality indicator are crucial for assessing the safety of 'sous vide' processing. However, it was observed in the evaluated works that the microbiological analysis after cooking was not based on a thermal lethality indicator that supported the safety of the food after SV cooking.

To ensure that treatments causing unacceptable thermal damage due to the use of high processing temperatures are not used, while simultaneously ensuring the microbiological safety of the product, there should be a labeling requirement to insert recommendations and instructions regarding cooking prior to consumption on the labels of ready-to-eat meat products. This is an interesting alternative to be adopted until a regulation is created that supports and establishes criteria for defining thermal lethality indicators and their detection limits, but it does not replace the latter. And the question arises: will the consumer have time to read? Simple and practical communication should be prioritized, highlighting explanatory images. It is not ideal, but until we have regulation for 'sous vide' cooking of meat products, we can minimize the impacts on food safety with this mitigation action.

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CHAPTER 2: IMPACT OF SOUS VIDE PROCESS ON THE QUALITY OF BEEF TOURNEDO

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ABSTRACT

This study evaluated the influence of different heat treatments (T1:56.1°C/62 min; T2:58°C/32 min; T3:62°C/12 min; T4:72°C/no dwell time) on the quality of sous vide cooked beef tournedos. Heating and lethality curves were constructed using *Salmonella* spp as the target microorganism, and F0 values were estimated using numerical models. Comprehensive assessments of chemical, physical, and microbiological quality were performed. No significant differences ($p>0.05$) were observed in the centesimal composition treatments. The maintenance stage was the primary contributor to thermal lethality across all treatments, with an average contribution of 81.88%. The heating stage contributed an average of 13.99%, demonstrating that its role in the cooking process should not be disregarded. Treatment T4 resulted in significantly greater exudation compared to T3 ($P < 0.05$) and also exhibited higher lipid oxidation and myofibrillar fragmentation index values than T1 and T2 ($P < 0.05$). The internal color was used to classify the treatments by their degree of doneness: T1 (very rare), T2 (rare), T3 (well done), and T4 (well done). Given that all the treatments complied with microbiological safety parameters, the physical and chemical characteristics had a greater impact on overall product quality. Treatment T2, which showed a more consumer-acceptable internal color and less exudate release than T4, was concluded to be the most suitable for the industrial processing of sous vide beef tournedos due to its impact on key sensory characteristics.

Keywords: lethality; meat quality; color in cooked meat; pasteurization.

1. INTRODUCTION

The changing lifestyle of consumers, who increasingly seek healthy and practical food options, have driven the industry to invest in processing techniques that provide ready-to-eat foods.

Food safety is a major public health issue, as microbiological contamination is one of the primary causes of foodborne illness and food spoilage. Food processing technologies must therefore ensure that process parameters are sufficient to inactivate pathogenic microorganisms and their spores. Consequently, preservation techniques are necessary for microbial control and for mitigating their effects on food products (Onyeaka et al., 2022a).

Among various preservation techniques, sous vide (SV) is considered an emerging French culinary technology that involves minimal heat treatment of food (Kathuria et al., 2022). This method consists of cooking vacuum-packed food by immersion in water bath (Sun et al., 2017) at low temperatures (50°C - 80°C) for extended periods (1-7 h), depending on the type of food (Zavadlav et al., 2020). For meat products, temperatures below 70°C are typically used for times that vary according to the meat type (Baldwin, 2012; Yang et al., 2020). The product is

then rapidly cooled (0 to 4°C) so it can be stored and marketed under refrigeration, or frozen (-18°C).

The low temperatures used in SV technology have attracted criticism regarding microbiological safety (Onyeaka et al., 2022b), making it imperative to assess the quality of products processed using this method. Although existing data in the literature support the thermal lethality of *Salmonella* in meat products subjected to equivalent thermal processes, this study is essential for sous vide beef rendering due to the peculiar characteristics of this food matrix. The absence of chemical preservatives in its composition is one such peculiarity, with preservation being attributed exclusively to the heat treatment applied during cooking and the subsequent cooling or freezing maintained for storage and marketing. Another factor to consider is that literature data often only account for the maintenance temperature used in heat treatment, disregarding the temperatures reached during the heating and cooling stages, which are specific to each process and contribute to the overall thermal lethality.

The heating rate is another factor that influences thermal lethality and is not consistently considered in the literature that provides equivalent thermal processes for meat products. Different technologies and equipment achieve different heating rates that can significantly interfere with thermal lethality, increasing the relevance of this specific validation study. Furthermore, this study addresses an industrial production product, which prompted an investigation into whether data available in literature on the thermal lethality of *Salmonella* can be safely applied to sous vide beef processing on a production line, as bench studies may have limitations that make their results inapplicable to an industrial process.

The different regulatory requirements applied in various countries also justify this study, since it is a product that the industry intends to commercialize internationally, and it is necessary to have a study that provides a basis for the applied heat treatment. Studying equivalent thermal processes is valuable, as it allows industry to choose the binomial time-temperature that maximizes the physical and chemical characteristics it considers important. This flexibility also makes it possible to adjust process capacity and cost, since cooking time has a direct impact on industrial productivity. Moreover, the literature lacks data on the effects of equivalent thermal processes on the physical and chemical quality of meat products in association with microbial inactivation.

Among the attributes related to the physical quality of meat, color is prominent, as it influences the consumer's decision to purchase and consume the product. This attribute is influenced by the thermal process applied. The cooking process results in the characteristic brown coloration of cooked meat products due to the denaturation of myoglobin. However, the

denaturation temperature depends on the redox forms of myoglobin present in the meat, with deoxymyoglobin (at $\approx$ pH 5.6) being the most heat-resistant. Therefore, the brown coloration inside a cooked product is not necessarily a reliable indicator that the meat has been cooked sufficiently to guarantee its safety (AMSA, 2012).

On the other hand, the SV technique provides several benefits that have been extensively studied by various researchers. These include an attractive texture, minimization of lipid oxidation and losses of taste and flavor due to the use of low temperatures, and inhibition of cross-contamination during processing and storage, since the product is cooked inside its packaging (Thathsarani et al., 2022).

Consumers generally relate meat quality to tenderness, juiciness, and flavor, as well as color (Zavadlav et al., 2020). SV cooking of meat prevents the loss of moisture and nutrients and preserves intramuscular fat (Kathuria et al., 2022). Moisture and fat content increase the palatability of meat, specifically contributing to flavor, juiciness, and tenderness (Hopkins & Mortimer, 2014).

In general, the quality of the product obtained by the sous vide technique is influenced by the temperature and time used (Kathuria et al., 2022). Identifying the ideal time and temperature for processing meat products is one of the biggest challenges of this technology. Increasing the temperature provides greater microbiological safety but also causes greater changes in the physicochemical and sensory characteristics. Lower temperatures, on the other hand, have the advantage of maintaining the product's characteristics, but it must be ensured that the processing time is sufficient to eliminate the pathogenic microbiota to a safe level (Ismail et al., 2019b).

Therefore, this study aimed to determine and validate the ideal temperature/time binomial for the industrial sous vide processing of beef tournedos based on the impact on their microbiological and physicochemical quality characteristics, namely color, pH, water activity, TBARS (mg MAD/kg), exudate released (% m/m), total, soluble and insoluble collagen (%), myofibrillar fragmentation index, and Warner-Bratzler shear force (WBSF).

2. MATERIAL AND METHODS

2.1 Preparation of packaged beef tenderloin tournedos

Chilled beef tournedos cuts (temperature between 0 and 4°C; pH between 5.7 and 5.8), purchased from a slaughterhouse approved and inspected by the Federal Inspection Service (Serviço de Inspeção Federal – SIF), were transported in refrigerated trucks and kept in a

refrigerated chamber at 4°C until sample preparation.

In a tumbler, cold water (0–4°C), modified cassava starch, maltodextrin, salt, and black pepper were added to form a brine (temperature between 0°C and 4°C). The filet mignon pieces were added to the tumbler after the ingredients had been homogenized and were tumbled for 40 minutes to incorporate the brine. With the tumbling process, the temperature of the meat increases and therefore the pieces were then stored in a cold room at 4°C until they reached a temperature between 10°C and 12°C to ensure that the meat's retention capacity is not lost due to denaturation of the myofibrillar proteins. They were then rolled, wrapped in PVC film, and frozen in a cold room at -12°C. After the pieces reached a temperature between -2°C and -5°C, the plastic film was removed. Cross-sections (diameter between 57 and 60 mm and thickness between 38 and 40 mm) were hand-cut and weighed individually on a digital scale to a mass of $125 \text{ g} \pm 5 \text{ g}$, yielding approximately thirteen slices of tenderloin from each piece, referred to as a tournedo.

The tournedo was seared on a hotplate (temperature close to 215°C) to form a golden-brown color on the surface. The product reached a surface temperature of approximately 40°C and was then stored in a cold room at 4°C before packaging. Once cooled (between 4°C and 10°C), the tournedos were packed in heat-moldable, vacuum-sealed containers (Multivac).

2.2. *Experimental design and cooking treatments*

Cooking treatments equivalent in terms of *Salmonella* thermal lethality (T1: 56.1°C/62 min; T2: 58°C/32 min; T3: 62°C/12 min; and T4: 72°C/no dwell time) were applied to samples of vacuum-packed beef tournedos to record temperature readings during the heating, maintenance and cooling stages of sous vide cooking.

These temperature-time combinations were determined based on the 'Guideline for Meat and Poultry Products - Appendix A' (USDA & FSIS, 2021). The Food Safety and Inspection Service has established temperature/time binomials to assist the industry in cooking ready-to-eat products based on the required levels of *Salmonella* spp. lethality. The minimum internal temperatures must be maintained for the times indicated in this guide. The minimum temperature used for cooling, 4.4°C, was based on the 'Stabilization Guideline for Meat and Poultry Products - Appendix B' (USDA, FSIS, 2021), which establishes standards for preventing or limiting the growth of spore-forming microorganisms and other pathogens during cooling.

The experiment was arranged in a randomized block design with 3 batches of beef tenderloin for the 4 heat treatments studied. For each batch, thirteen samples of packaged beef tournedos were used. Four of these samples had a thermocouple inserted into the center of the

product to take temperature readings during heating, maintenance, and cooling. Five samples were used for microbiological analysis, and the remaining four were used for the analysis of quality parameters (Figure 1). To prevent air or water from entering the bag pierced by the thermocouple, it was inserted through an adhesive foam strip placed on the packaging (Figure 2).

In the heat treatment studies, two 630-liter tanks (PPIENK, model BM-100E, Brazil) were used, each with a capacity for 30 samples, a cage, and 3 trays. A datalogger (Testo 176 T4) with four thermocouples inserted in the geometric center of the product was used to monitor and record the internal temperature every minute throughout the process in all treatments. The thirteen samples were distributed across three trays. In tray 1 (bottom), 6 samples were placed, 2 of which had thermocouples. In tray 2 (middle), 7 samples were positioned, 1 of which had a thermocouple. In tray 3 (top), 4 samples were positioned, 1 of which had a thermocouple (Figure 2). A pre-heating tank (Tank 1 - Figure 3) was maintained at 85°C, and the cage containing the samples was immersed for 3 minutes. The inlet temperature of the product in Tank 1 varied between 7°C and 10°C, and the outlet temperature after 3 minutes at 85°C was approximately 30°C.

Immediately thereafter, the cage was transferred to Tank 2, which had been adjusted to the respective temperature of the heat treatment under study (Figure 3). The temperature was monitored during the heating, maintenance, and cooling stages. After reaching the reference temperatures for each heat treatment and being maintained for the established dwell times, the cage was transferred to a cooling tank for pre-cooling for 15 minutes in a room-temperature water bath. Subsequently, the cage was moved to a second cooling tank (0 to 2°C) until the product reached a minimum internal temperature of 4.4°C. The samples were then quick-frozen in a nitrogen tunnel and stored in a freezer at -18°C until microbiological, chemical, and physical quality analyses were conducted. The samples containing thermocouples were discarded.

2.2.1 Calculating of thermal lethality and estimating of F_0

Thermal lethality (L) was calculated (Equation 1) for the cooking stages (heating, maintenance, and cooling), considering *Salmonella* spp as the reference pathogenic microorganism, with a z-value of 6.6°C (Horn et al., 2016) for each reference temperature (T_{ref}) used (56.1°C; 58°C; 62°C and 72°C).

$$L = 10^{\frac{T - T_{ref}}{z}} \quad (1)$$

Where T is the temperature ($^{\circ}\text{C}$) at a specific time during the cooking stages of the packaged beef tournedo samples.

The calculation of F_0 (heat treatment time, min) for each cooking stage was estimated using the Simpson (Equation 2) and Trapezoidal (Equation 3) numerical models (Praharasti et al., 2020).

$$\frac{1}{3} \cdot [L_0 + L_f + 4 \left(\sum_{i=1}^{\text{impar}} L_1 + L_3 + \dots + L_{\text{impar}} \right) + 2 \left(\sum_{i=2}^{\text{par}} L_2 + L_4 + \dots + L_{\text{par}} \right)] \quad (2)$$

$$\frac{1}{2} \cdot [L_0 + L_f + 2 \left(\sum_{i=1}^f L_1 + L_2 + \dots + L_{f-1} \right)] \quad (3)$$

Where L_0 is the initial lethality value and L_f is the final lethality value.

2.3 Microbiological analysis

Coagulase-positive staphylococci (ISO 6888-1, 2021), staphylococcal enterotoxin (AOAC 2007.06, Vidas©; Biomerieux, France), *Escherichia coli* (ISO 16649-2, 2001), *Clostridium perfringens* (ISO 15213-2, 2023), *Listeria monocytogenes* (ISO 11290-2, 2017), and *Salmonella spp.* (ISO/TS 6579-2, 2012) were analyzed to determine the microbiological quality of both the raw and the cooked sous vide beef tournedos. Approximately 25 g of each sample was homogenized with 225 mL of 0.1% peptone water in a Stomacher (model 1204 M; Metroterm Ind. e Com., Porto Alegre, RS, Brazil) for 3 min and plated on the surface after successive decimal dilutions. *Clostridium perfringens* was cultured on Tryptose Sulfite Cycloserine (TSC) agar at 46–48 $^{\circ}\text{C}$ and incubated at $36 \pm 1^{\circ}\text{C}$ for 18 to 24 hours. *Escherichia coli* was cultured on Tryptone-bile-glucuronic (TBX) agar and incubated at $44^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 18 to 24 hours. The presence of *Listeria spp.* and *Salmonella spp.* was analyzed according to ISO 11290-1 (2017) and (ISO 6579-1 (2017) methods, respectively.

2.4 Evaluation of chemical and physical quality parameters

2.4.1. Centesimal composition

Moisture (ISO 1442, 2023), fixed mineral residue (ISO 936, 1998), total lipids (NMKL 181, 2005), and nitrogen content (ISO 1871, 2009) were determined according to the official methods for analyzing animal products. The value obtained for nitrogen was multiplied by 6.25 to obtain the protein content (Brasil, 2022). Carbohydrates were calculated by difference.

2.4.2. Water activity and pH

A crushed portion of the tournedo was used to determine the water activity (a_w), using the Aqualab® CX2 apparatus (Decagon Devices Inc., Pullman, United States). The pH was determined in samples previously thawed at room temperature (25 °C), using an insertion electrode applied at three distinct locations (in triplicate), with a portable pH meter (model HI 99163; Hanna Instruments, Woonsocket, RI, USA), previously calibrated with standard buffer solutions (pH 4.01 and 7.01) at the same temperature.

2.4.3. Exudate release

The exudate released during the cooking processes was assessed by weighing the packaged pieces (in duplicate) after unpacking. The percentage of exudate released (EL, % m/m) was determined according to Equation 4.

$$EL (\%) = \frac{PCE - PCD}{PCE} \times 100 \quad (4)$$

where PCE is the mass (g), of the packaged product and PCD is the mass (g), of the unpackaged product.

2.4.4. Lipid Oxidation

Lipid oxidation was assessed by determining thiobarbituric acid reactive substances (TBARS) according to the method of Raharjo et al. (1993) adapted by Wang et al. (2002). Approximately 4 g of the crushed sample was weighed and homogenized (Turratrec TE 102; TECNAL, Piracicaba, SP, Brazil) in 15 mL of 5% trichloroacetic acid (TCA) and 1 mL of 0.15% hydroxybutyltoluene (BHT) antioxidant. The homogenate was centrifuged (Hettich- EBA 21; Nova Analítica Importação e Exportação Ltda., São Paulo, Brazil) at 3000×g for 5 min. Then, a 2mL aliquot of the homogenate was added to 2mL of 0.08M thiobarbituric acid (TBA; in 50% acetic acid) and homogenized in a vortex mixer.

The homogenates were heated in a water bath at 40°C for 90 min, centrifuged at 2,000g for 15 min and the absorbance was read in a spectrophotometer (Genesys 10 UV; ThermoScientific Varian, São Paulo, Brazil) at 532nm to determine malondialdehyde (MDA). To determine hexanal, the homogenates were kept in a water bath at 25°C for 4 hours. After centrifugation at 2,000g for 15 min, the reading was taken at 450nm. TBARS₅₃₂ values (mg MDA/Kg) were expressed from an analytical curve with 1,1,3,3-tetraethoxypropane (TEP), and TBARS₄₅₀ values (mg HEX/kg) from an analytical curve with hexanal (HEX).

2.4.5. Shear force

Shear force was determined according to the Warner-Bratzler shear (WBsSF) method (Silva et al., 2015). The cooked sample was cut into rectangles (1cm x 1cm x 2.5cm) in the direction of the muscle fibers. A Warner-Bratzler blade coupled to a texturometer (TAXTplus, Stable Micro Systems Ltd., Godalming, Surrey, UK) cross-sectioned the samples at 3.33 mm/s, and the maximum force (N) required to completely shear each sample was measured. The result was expressed as the average of the maximum forces (N) of six samples measured for each treatment and repetition (batch).

2.4.6. Soluble and Insoluble Collagen and Myofibrillar Fragmentation

The soluble and insoluble fractions of collagen were separated according to the procedure proposed by Hill (1966). Briefly, 4 g of sample was added to 50 ml centrifuge tubes containing 12 ml of 1/4 concentration Ringer's solution and suspended in a water bath at 77°C for 70 minutes with constant stirring. The heat treatment was terminated by transferring the tubes to a cold-water bath for 10 min. After centrifugation (3,000 g for 10 min), the supernatant was collected, and the residue in the tube was washed with 8 ml of Ringer's solution. After additional centrifugation, the supernatants were pooled, and each fraction (residue and supernatant) was hydrolyzed in 6 N HCl at 105°C for 16 hours. The collagen content of the individual fractions was quantified by determining the concentration of the amino acid hydroxyproline (Bergman & Loxley, 1963), as described by Ramos & Gomide (2017). The hydroxyproline content was converted into collagen content (mg/g) using the factors 7.52 for the soluble fraction and 7.25 for the insoluble fraction, as proposed by Cross et al. (1973). The total collagen content was the sum of the soluble and insoluble fractions, and the percentage of soluble collagen was calculated relative to the total collagen content.

The degree of myofibrillar fragmentation was determined using the fragmentation index (FI) described by Aroeira et al. (2020). The weight of the retained fragments was measured after homogenization (15,000 rpm in Turratrec TE 102; TECNAL, Piracicaba, SP, Brazil) of 5 g of sample in 50 ml of extraction solution (0.25 M sucrose and 2 mM KCl), cooled to 4°C, and vacuum filtration (NOF-650; Bomba Nova, São Paulo, Brazil) through a 250 µm nylon mesh. The results were expressed as the weight (g) of the fragments retained on the screen after 10 min at room temperature. Lower FI values indicate meat with greater myofibrillar

fragmentation.

2.4.7. Instrumental color

A CM-700 portable spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) with an 8 mm aperture port, illuminant A, and a 10° angle to the observer, in specular light excluded (SCE) mode, was used for the instrumental assessment of the color of both the outside and the inside (product cut in half) of the tournedo.

The lightness (L^*), yellow (b^*), and red (a^*) indices were obtained from the average of five readings taken on the surface of samples thawed at room temperature and kept for 30 min of exposure to atmospheric air for blooming (Ramos & Gomide, 2017). To calculate saturation (C^*) and hue angle (h° , in degrees), equations 5 and 6 were used, respectively:

$$(5) \quad C^* = \sqrt{(a^*)^2 + (b^*)^2}$$

$$(6) \quad h^* = \text{Arctang} \left(\frac{b^*}{a^*} \right)$$

2.5 Statistical analysis

Temperature data recorded during the heating, maintenance, and cooling stages of the sous vide cooking process, obtained from the application of different thermal treatments (T1, T2, T3, and T4) and monitored by four thermocouples in three experimental replicates, were analyzed using descriptive statistics, with presentation of means ($n = 3$) and corresponding standard errors (SE). Data related to the evaluation of chemical and physical quality parameters of beef tournedos prepared by the sous vide method were organized in a completely randomized design (CRD), with thermal treatments (T1, T2, T3, and T4) as the experimental factor, and three replicates ($n = 3$). Subsequently, the data were subjected to analysis of variance (ANOVA), and the estimated means, along with their respective standard errors for each chemical and physical quality parameter, were compared using Tukey's test at a 5% significance level ($P < 0.05$). Statistical analyses were performed using Statistica® 8.0 software (StatSoft Inc., Tulsa, USA).

3. RESULTS AND DISCUSSIONS

3.1 Cooking curves and Thermal Lethality

According to the temperatures reached in the different positions of the tank (Table 1), the heat distribution was uniform, with little variation in temperatures between the

thermocouples relative to the average. On the other hand, the average temperature of the maintenance stage of T4 showed greater variation from the proposed temperature, which was 72°C. This can be explained by the fact that the bath temperature was higher (75°C), which has a greater impact on the heating speed. Additionally, the products could only be cooled after all positions had reached the temperature proposed for each treatment. Therefore, when waiting for the temperature of 72°C to be reached in thermocouple 1, positioned in the lower right corner of the cooking tank (which had the lowest temperature), the other positions exceeded the proposed temperature more quickly, which justifies the observed temperatures above those established for this treatment.

Treatments T1 and T2 exceeded the proposed time for each treatment in the Maintenance stage by between 1 and 2 minutes (Table 1). In an industrial process, these small variations are common. Shorter times than those established are unacceptable because they would affect the thermal lethality achieved. The greatest influence of exceeding the cooking time at the programmed temperature is on the sensory characteristics, altering attributes valued by the consumer, such as color and texture.

From the average temperatures ($n=3$) reached by each thermocouple over time, cooking curves (Figure 4) and thermal lethality curves (Figure 5) were obtained for each heat treatment during the heating, maintenance, and cooling stages. Small variations in the programmed temperature were observed during cooking, which is expected in an industrial process (Figure 4a) with large volumes of water used in the water bath, as this affects the speed of heating and cooling. Despite these small variations, also visible in the average temperatures in Table 1, the minimum programmed temperature was guaranteed during the proposed cooking time. This was a key concern when monitoring temperature variations, since the principle of thermal lethality presented in the 'Cooking Guideline for Meat and Poultry Products, Appendix A' (USDA & FSIS, 2021) is to maintain the proposed temperature for the time determined for each treatment.

The thermal lethality values above 1.0 shown in Figure 5 indicate the points at which the temperature surpassed the reference temperature for each heat treatment (56.1°C, 58°C, 62°C, and 72°C, respectively). The F_0 values estimated by numerical calculations using the Simpson and Trapezoidal equations (Table 2) were very close. The areas under the curves for the longest treatments showed the greatest variation, which can be explained by the difficulty in stabilizing the maintenance temperature across all thermocouples for an extended period. A study by Onyeaka et al. (2022) suggests the need for developing standardized industrial equipment for future applications of SV technology, arguing that currently used equipment

often does not provide uniform cooking.

The maintenance stage made the greatest contribution to achieving thermal lethality in all heat treatments. In T1, it accounted for 85.15% (Simpson model) and 85.16% (Trapezoidal model) of the lethality. The heating stage contributed 13.14% (Simpson) and 13.21% (Trapezoidal) in T1. The cooling stage had the least influence on lethality, with its greatest contribution observed in T4 at 7.91% (Simpson) and 7.99% (Trapezoidal) of the total F_0 achieved.

It is noteworthy that the 'Guideline for meat and poultry products - Appendix A' (USDA & FSIS, 2021) proposes keeping the product at different time/temperature binomials to achieve thermal lethality. However, it disregards the lethality achieved in the heating and cooling stages, which justifies the provision of an exclusive guide for cooling 'Stabilization guideline for meat and poultry products - Appendix B' (FSIS & USDA, 2021).

However, when estimating the thermal lethality of the entire process, the total area under the curve (heating, maintenance, and cooling) should be considered, not just the Maintenance stage. This is because each industrial facility has different cooking equipment that achieves different heating rates, in addition to the influence of the meat matrix on lethality.

Thus, the heat treatment time calculated using the area under the lethality curves for all treatments was sufficient to achieve the target thermal lethality, considering *Salmonella* spp as the reference pathogenic microorganism. The industry can even base its total heat treatment time on the calculation of F_0 for each batch produced to reduce its processing time, which could be an interesting alternative for increasing its production capacity while maintaining quality and existing structures.

3.2 Validation of the thermal process by microbiological profile

The low temperatures used in SV technology are sometimes criticized for being potentially insufficient to guarantee the microbiological safety of food. As microbial contamination is a leading cause of food spoilage, foodborne disease outbreaks, and product recalls, it is essential to assess the safety of products cooked using the SV technique (Onyeaka et al., 2022).

The validation of the thermal process by microbiological profile was conducted using analyses of the finished product subjected to the four thermal treatments and comparing the results with current legislation and the raw product (Table 3).

All treatments showed the same microbiological results after cooking, which are in line with the Brazilian legislation that establishes food standards for ready-to-eat products, the

category in which sous vide beef tournedos fall (BRASIL, 2022). A similar result was presented by Jeong et al. (2018), where no microbial growth occurred in the microbiological assays performed in hams subjected to sous vide treatment at different times, temperatures, and degrees of vacuum.

In addition to the microbiological analysis of the cooked products, the raw product was also subjected to the same analyses to certify the initial microbial load before cooking. The objective of this analysis was to have a measure of microbial reduction with heat treatments.

Although SV processing can be applied as the sole heat treatment in a food process and ensure its microbiological safety, as presented in this study, there is concern about its use in isolation by individuals without technical knowledge.

This concern arises because the cost of the equipment initially limited the technique's use to restaurants, industrial kitchens, and factories. However, with the availability of more affordable thermal circulators, there has been an increase in domestic consumers experimenting with technology.

3.3 *Evaluation of chemical and physical quality characteristics.*

The results of the analysis of the centesimal composition of the sous vide beef tournedos cooked in the different treatments showed no significant difference ($P > 0.05$) in terms of moisture (65.54% to 69.64%), protein (21.19% to 23.65%), fat (2.77% to 5.63%), ash (1.94% to 2.04%), carbohydrates (2.24% to 4.27%), and chlorides (1.03% to 1.15%). According to the Brazilian Food Composition Table (NEPA, 2011), raw beef tenderloin has 71.9% moisture, 21.6% protein, and 5.6% fat, values that are close to those found for the SV beef tournedo. A study published by Hocquette et al. (2018) refers to beef containing 21% to 31% protein, which shows that the values found in this study are within the same range.

In line with Bıyıklı et al. (2020) and Del Pulgar et al. (2012), the effect of the different time/temperature binomials on moisture content was not significant ($P > 0.05$). The effect of heat treatments on the protein and fat content of sous vide filet mignon tournedos was also not notable ($P > 0.05$). At the same time, the amount of ash and chlorides present in the samples processed by the temperature/time binomials used in cooking in this study showed a statistically insignificant variation ($P > 0.05$).

Analyses of pH, water activity, released exudate, and TBARS of the sous vide beef tournedos were carried out for the different temperature and time combinations (Table 4). Increasing the cooking temperature led to an increase in TBARS values. The highest value (1.50 ± 0.10 mg MAD/kg) was observed in the treatment that raised the product's

center temperature to 72°C. Wang et al. (2002) applied the same modified methodology for TBARS analysis used in this study to assess lipid oxidation in cooked ground beef. Cooking was carried out in a water bath at 94°C until the meat reached an internal temperature of 70°C. The values found were between 2 and 10.8 mg/kg, consistent with this study, which increased as storage time increased.

Similar results were found by Bıyıklı et al. (2020), who obtained the highest BARS values for sous vide turkey meat processed at the highest temperature (75°C/ 20 and 40 min), with a significant difference between the highest and lowest temperature treatments (60°C/ 20 and 40 min). This can be explained by the fact that these thermal processes lead to the denaturation of meat proteins and the consequent release of iron ions. Thus, the iron released catalyzes the lipid oxidation reaction (Estévez & Heinonen, 2010).

The different temperature/time binomials applied were insufficient to cause a change in the water activity of the samples ($P > 0.05$). However, the effect of temperature/time on the percentage of exudate released and on pH was significant ($P < 0.05$), with a greater change for treatment T4 compared to T3. Approximately 80% of the water in meat is held within the myofibrils between the actin and myosin filaments. At temperatures above 60–65°C, the fibers shrink, causing them to pull apart and consequently release a substantial amount of water. This water loss tends to increase with increasing temperature (Baldwin, 2012), which explains the greater mass loss in T4. This same trend was observed in studies carried out by Ismail et al. (2019) in which increasing the temperature from 45 °C to 65°C when cooking beef sous vide also led to significant mass loss ($P < 0.05$).

Protein denaturation during meat cooking at temperatures between 45 and 80 °C promotes the release of ionic groups that can increase the pH of cooked meat compared to raw meat (De Moraes & Rodrigues, 2017), as occurred in the processed meats in this study (pH of raw meat between 5.7 and 5.8). As T4 used the highest processing temperature, there may have been a greater release of ionic groups, which justifies the significant difference ($P < 0.05$) in the pH of T4 compared to T2.

The texture of the cooked meat depends on the cooking method adopted, as well as the shear force and solubility of the collagen, which is why they were evaluated (Table 5). The proportion of intramuscular collagen is easily solubilized during cooking (soluble collagen), but another part is resistant (insoluble collagen), and its denaturation temperature is highly dependent on the heating rate (Purslow, 2018). The variation with the different temperature/time binomials applied was significant ($P < 0.05$) for insoluble collagen between T1 and the other treatments, for soluble collagen between T4 and T3 and T2, and for total collagen between T1

and T2. The denaturation of collagen occurs at around 65°C, and this explains why the highest value of soluble collagen was in treatment 4, the only one with a temperature above 65°C, and the highest value of insoluble collagen was in treatment 1, where the lowest cooking temperature was applied.

A study by Palka (1999) found that the percentage of soluble collagen in sous vide beef changed little up to an internal temperature of 60°C, but almost doubled at 70°C and decreased in the 80 to 121°C range. These results are like those found in the present study, in which a higher concentration of soluble collagen was found in samples cooked above 70°C, differing significantly ($P < 0.05$) from the others.

At cooking temperatures up to 65 °C, collagen makes an important contribution to the strength of cooked meat (Purslow, 2018), but at higher temperatures, shear strength is mainly due to myofibrillar proteins (Christensen et al., 2000). Therefore, when the Warner-Bratzler shear force is measured in meats cooked above 70 °C, the contribution to hardness is mainly from myofibrillar proteins, not reflecting the contribution of intramuscular collagen (Purslow, 2018).

The variation with the different temperature/time binomials applied was significant ($P < 0.05$) for the shear force between T1 and the other treatments. Meat tenderness is influenced by myofibrils and connective tissue proteins and their transformations during heat treatment, mainly the denaturation of myofibrillar proteins (myosin and actomyosin) and the solubilization of collagen (Ayub & Ahmad, 2019).

Contrary to expectations, the shear force in the treatment that applied the lowest temperature, and longest time was significantly lower than the others. In the study of Hwang et al. (2019) the shear force of the pork loin was affected by the process temperature, while the process time had a significant effect on the tenderness of the meat only at 50 °C. It was suggested that the tenderness of meat cooked for longer periods was favored by the activity of intrinsic proteases that remained active at low temperature. Thermal inactivation of proteases at temperatures above 56 °C may have been the reason why heat-induced structural changes in meat proteins had a significant influence on meat tenderness.

The correlation between cathepsinase activity and meat shear force was reported by Dominguez-Hernandez & Ertbjerg (2021) when studying the effect of LTLT heat treatment on the activities of cathepsin B and L and the denaturation of pork myofibrillar proteins. These authors also observed that increasing cooking time decreased shear force, especially at 58 °C, and no clear effect on tenderness was observed with increasing heating time, as in the present study.

The cooking temperature significantly affects the shear force (Jeoung et al., 2018), and Tornberg (2005) explains that the resistance observed for meat up to around 50 °C is because the stress generated with the application of the shear force is produced by the viscous flow in the fluid-filled channels between the fibers and fiber bundles. At temperatures around 65°C, the shear force tends to decrease due to the weakening of the collagen connective tissue. However, the opposite is observed in this investigation; in treatments 3 and 4, closer to 65°C, there is an increase in shear force compared to T1. It is suggested that the difference in heating rate may be the cause of this result and that there was contraction of the connective tissue, even at temperatures below 65°C, forming a denser material and therefore tougher meat.

Christensen et al. (2013), investigated the effect of cooking time and temperature on sous vide beef and found a decrease in shear force with an increase in cooking time from 3 to 12 hours at temperatures of 56 °C, 58 °C, and 60 °C. They showed that the contribution of collagen to the strength of meat decreases with longer cooking times. Therefore, the difference between these results and those of the present study may be due to the times at which the temperatures were maintained during cooking, which were quite different. One of the reasons why meat loses less exudate when cooked at low temperatures and for long periods of time is that collagen does not reach the temperature of 65°C at which it shrinks (Vaudagna et al., 2002).

Alahakoon et al. (2018) found that the shear force values for sous vide processed beef brisket at 70°C/24 hours were significantly higher ($P < 0.05$) than those treated at 65°C/72 hours and 60°C/72 hours, showing the contribution of myofibrillar proteins to the increase in meat strength at higher temperatures, above 70°C.

The fragmentation index showed a significant difference ($P < 0.05$) between T4 and the other treatments, indicating smaller fragmentation at the higher temperature used in this study. Karki et al. (2022) also reported an increase in the fragmentation index as the cooking temperature of sous vide beef ribs increased. According to Jia et al. (2022) cooked meats have higher fragmentation rates compared to raw meat due to the increased degradation of myofibrillar proteins during heating, and there may be an increase in the degradation of the actomyosin complex with an increase in the time-temperature conditions practiced in sous vide processing.

Color is a determining characteristic in the attractiveness of meat and meat products to consumers (Bıyıklı et al., 2020). Instrumental color parameters such as luminosity (L^*), saturation (C^*), and hue angle (h°) are shown in Table 6. The heat treatments applied did not significantly affect these parameters (internal and external) ($P > 0.05$).

The hue angle (h°) is affected by the chemical state of myoglobin and is inversely

related to the a^* (Del Pulgar et al., 2012). The results obtained for the h° values were between 47.40° and 51.03° for the external shade and 35.56° to 43.76° for the internal shade, both between 0° and 90° , characterizing the shade of the products between red and yellow (Ramos et al., 2016).

Christensen et al. (2013) reported a decrease in redness and an increase in yellowing in sous vide beef as a result of the denaturation and oxidation of oxymyoglobin (OxyMb) and deoxymyoglobin (DeoxyMb) to metmyoglobin (MetMb). The ratio between them determines the final color of the cooked meat. Lighter meat will have a higher concentration of metmyoglobin (Baldwin, 2012). Prill et al. (2019), when evaluating the perceptions and knowledge of consumers and chefs about the degrees of cooking of beef, correlated its internal coloration with the 'cooking point' using digital photos. When comparing the photos in this study with a standard cooking point chart (Appendix 1), T1 represents very rare beef, T2 represents rare, T3 medium-rare, and T4 well-done. Domestic consumers use this color pattern to determine the cooking point of beef at the time of preparation. Chefs, on the other hand, mainly use texture to determine the degree of cooking.

The work carried out by Del Pulgar et al. (2012) also showed higher h° results for samples cooked at 80°C compared to those cooked at 60°C ($P < 0.0001$) and those cooked in boiling water ($P < 0.05$). According to Bhat et al. (2020), sous vide processing of beef is characterized by redder colors than atmospheric cooking due to the protection against myoglobin oxidation promoted by vacuum packaging.

Denaturation of myoglobin begins at a temperature between 55°C and 65°C and is most extreme when the temperature reaches between 75°C and 80°C (Hunt et al., 1999). No treatment reached internal temperatures above 74°C , which may be why there was no statistical variation in luminosity (L^* value) for the treatments carried out. The color change is also related to the chemical state of myosin and actin (Tornberg, 2005), becoming lighter after heat treatment due to increased light scattering by denatured proteins (Christensen et al., 2011; Rinaldi et al., 2014).

4. CONCLUSION

This study represents an advance in scientific literature by associating microbiological validation with the physical and chemical quality of an industrial meat products from equivalent thermal processes.

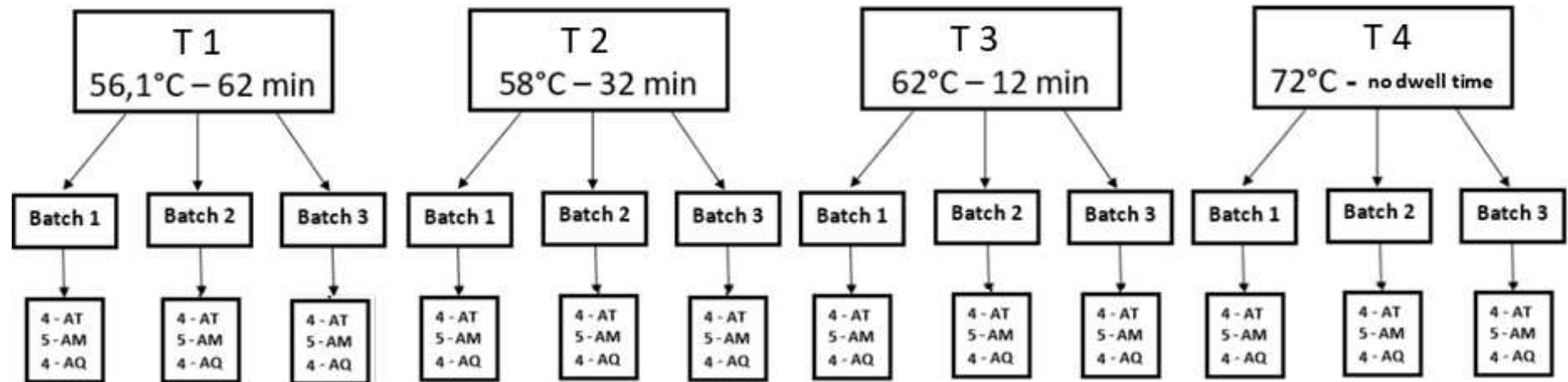
Considering that all the treatments were safe from a microbiological point of view, ideal temperature/time binomial for the industrial sous vide processing of beef tournedo was based on the impact on its physical and chemical characteristics. The 58°C / 32 minutes

heat treatment (T2) showed less lipid oxidation and exudate release, as well as greater intensity of the red color on the inside compared to the higher temperature treatment (T4). Therefore, it was considered the most suitable for industrial processing of sous vide beef tournedos.

As a future study, we suggest complementing the evaluation with a comparative sensory analysis between the 58°C / 32 minutes treatment and the 72°C treatment in the center of the product and, perform storage stability analysis in order to understand how each treatment will affect quality retention throughout its useful life.

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Caption:

AT - Samples for taking temperature readings during applied heat treatment

AM - Samples for microbiological analysis

AQ - Samples for analysis of quality parameters of the sous vide meat product

T1 = treatment 1

T2 = treatment 2

T3 = treatment 3

T4 = treatment 4

Figure 1: Experimental design

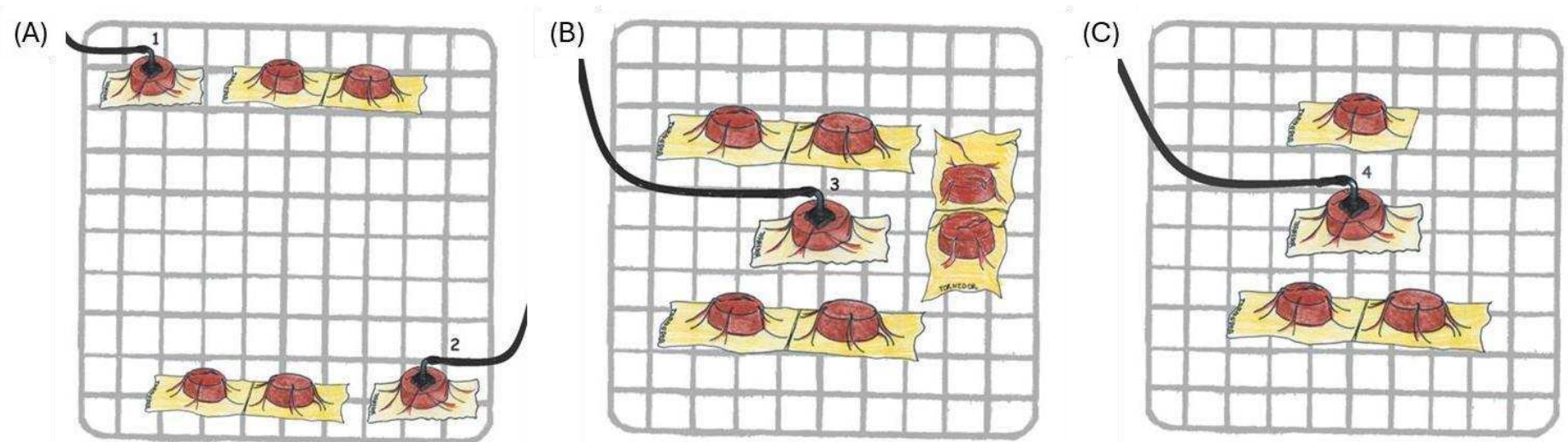
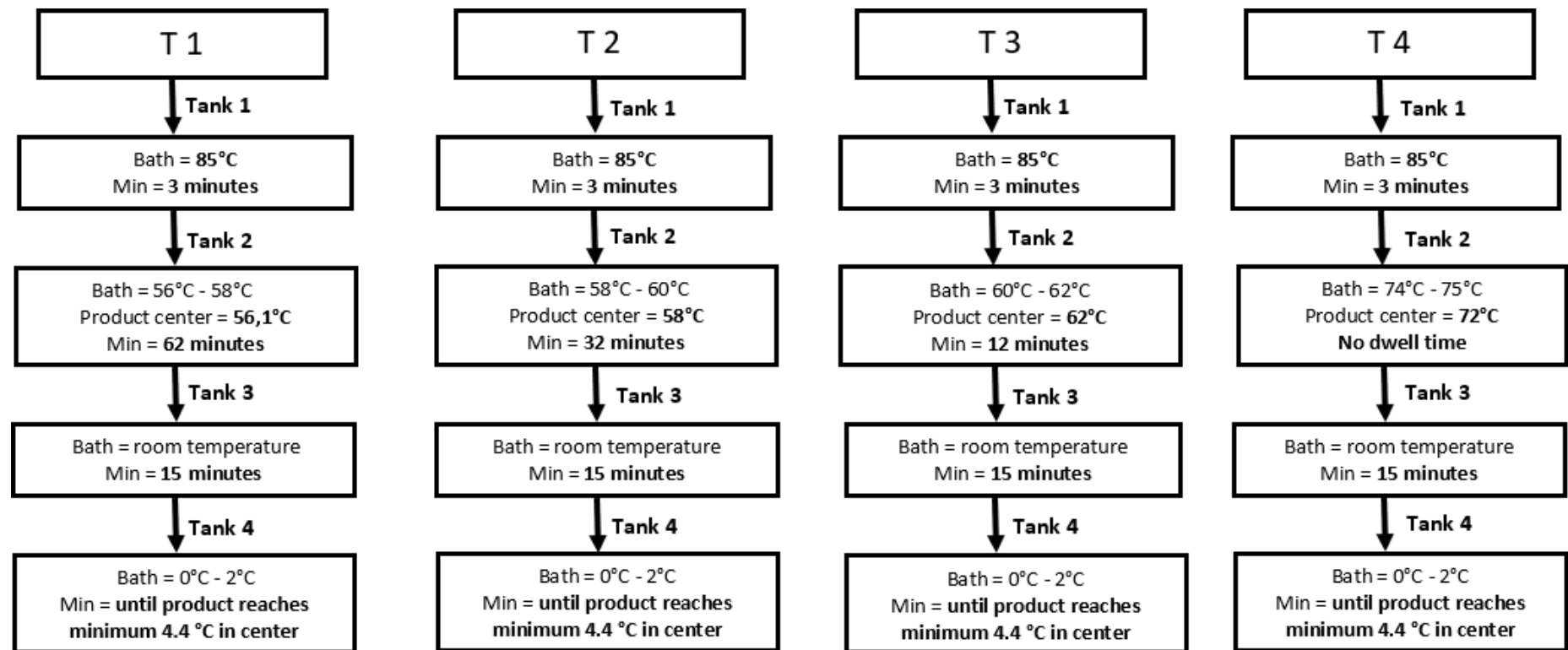


Figure 2: Trays used for sous vide cooking with positioning of the 13 samples and thermocouples (A) tray 1 - bottom - with six samples, two with thermocouples 1 and 2; (B) tray 2 - middle - with seven samples, one with thermocouple 3; (C) tray 3 - top - with four samples, one with thermocouple 4.



*T1 = treatment 1 / T2 = treatment 2 / T3 = treatment 3 / T4 = treatment 4

Figure 3: Experimental diagram of the application of different heat treatments in the sous vide processing of the meat product.

Table 1 - Mean values (n=3) of the temperature measurements of each thermocouple and retention time during the heating, maintenance, and cooling stages of the sous vide beef tenderloin tournedo for the different heat treatments applied.

Heat treatment ⁽¹⁾	TC*	Average heating temperature (°C)	Average heating time (min)	Average maintenance temperature (°C)	Average maintenance time (min)	Average FINAL cooling temperature (°C)	Average cooling time (min)
T1	1	40.86	44.00	56.67	63.00	3.10	78.00
	2	40.98	42.67	56.74	65.00	2.80	77.83
	3	42.70	43.67	56.72	63.67	2.97	77.67
	4	41.25	43.67	56.58	63.67	2.63	77.67
	Average	41.45	43.50	56.68	63.23	2.88	77.67
	(SEM)	(2.38)	(4.69)	(0.13)	(1.47)	(0.69)	(7.86)
T2	1	40.24	42.00	58.70	34.67	3.67	67.67
	2	41.90	42.00	58.70	34.67	3.90	67.67
	3	40.82	42.67	58.63	34.00	3.53	67.67
	4	41.16	42.00	58.57	34.33	3.43	68.00
	Average	41.03	42.17	58.65	34.42	3.63	67.75
	(SEM)	(2.14)	(6.35)	(0.18)	(1.12)	(0.44)	(2.43)
T3	1	34.20	32.00	62.30	12.00	3.4	64.00
	2	36.30	31.33	62.00	12.33	3.57	64.67
	3	35.39	29.67	62.65	12.33	3.13	64.00
	4	33.63	30.33	62.31	12.00	1.90	64.00
	Average	34.88	30.83	62.32	12.17	3.01	64.17
	(SEM)	(2.41)	(4.13)	(0.22)	(0.17)	(0.74)	(3.94)
T4	1	33.67	26.67	73.14	-	3.87	64.00
	2	34.6	25.33	73.43	-	3.67	64.00
	3	34.86	24.00	74.66	-	3.43	64.00
	4	34.19	24.67	74.92	-	3.00	63.6
	Average	34.34	25.17	74.04	-	3.49	63.92
	(SEM)	(0.93)	(1.08)	(0.66)		(0.24)	(9.80)

⁽¹⁾Reference heat treatments: T1 (56.1°C / 62 min); T2 (58°C / 32 min); T3 (62°C / 12 min); T4 (72°C / no dwell time).

* TC = thermocouple: 1 and 2 (bottom tray); 3 (middle tray); 4 (top tray); 1 and 2 (bottom tray); 3 (middle tray); 4 (top tray). SEM = standard error of the mean (n = 12).

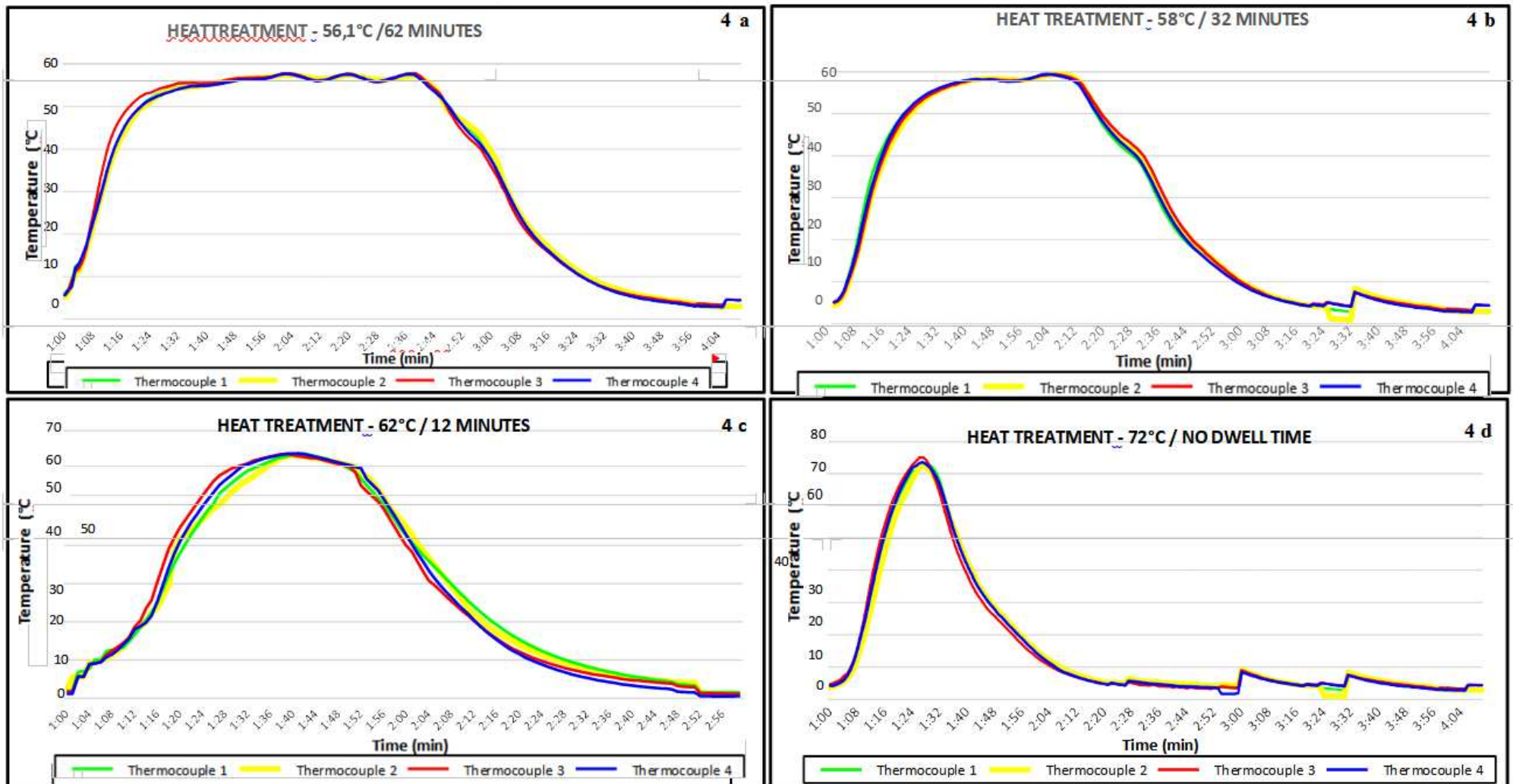


Figure 4: Variation of the cooking curves of the sous vide beef tenderloin tournedo by thermocouple for the different heat treatments applied: a) 56.1°C / 62 minutes; b) 58°C / 32 minutes; c) 62°C / 12 minutes; d) 72°C / no dwell time.

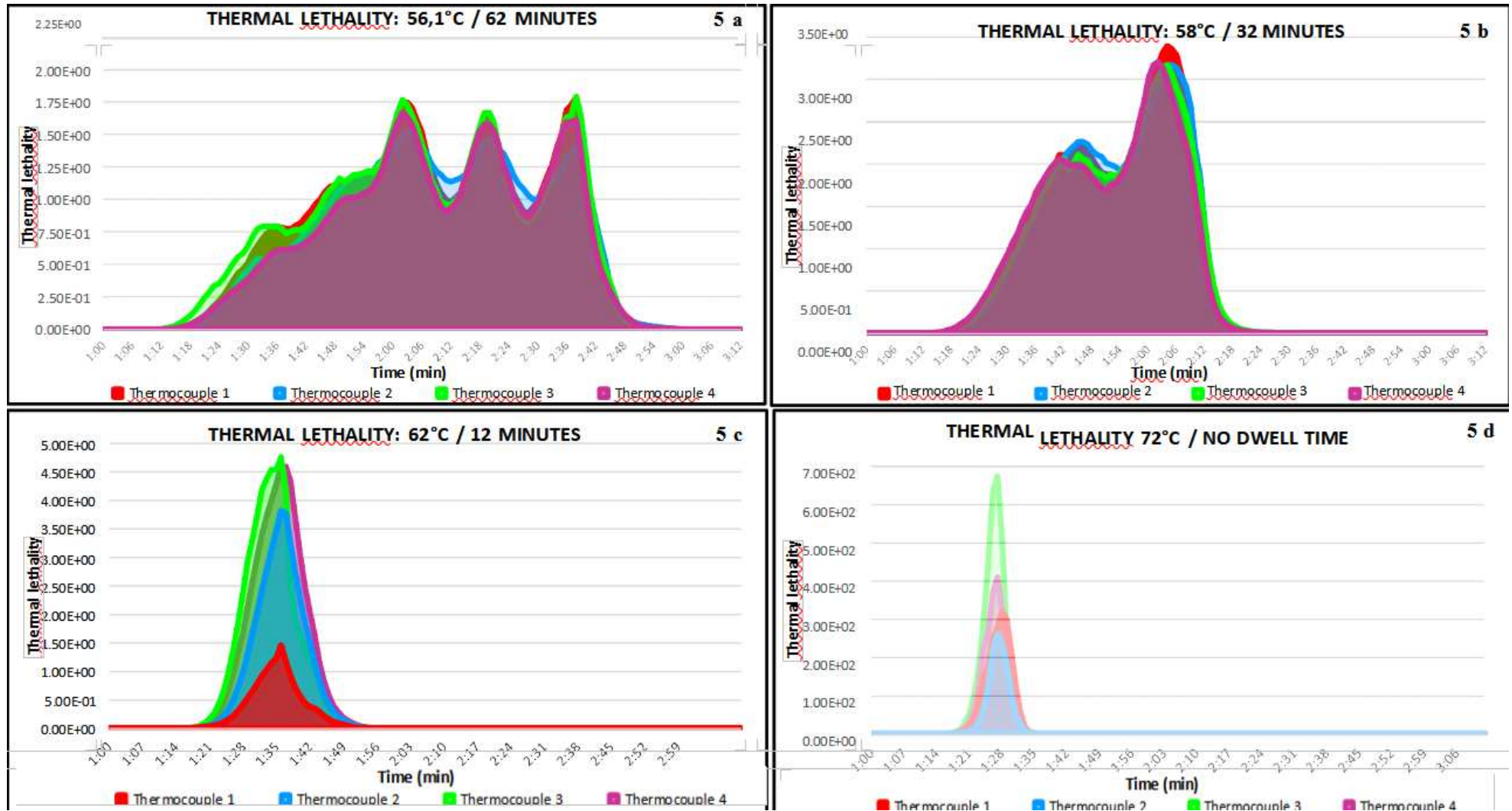


Figure 5: Lethality obtained from the average temperatures reached over time for each heat treatment applied in the sous vide cooking of the beef tenderloin tournedo: a) 56.1°C / 62 minutes; b) 58°C / 32 minutes; c) 62°C / 12 minutes; d) 72°C / no dwell time.

Table 2 – Estimated mean values of F_0 (n=3) and percentage contribution to thermal lethality of the heating, maintenance, and cooling stages for the different heat treatments applied by thermocouple.

Treatment	TC	F ₀ Simpson						F ₀ Trapezoid					
		Heating min	%	Maintenance min	%	Cooling min	%	Heating min	%	Maintenance min	%	Cooling min	%
T1	1	12.94		77.11		1.84		13.03		77.26		1.45	
	2	12.52		77.77		1.33		12.62		78.48		1.37	
	3	10.16		73.87		1.44		10.47		74.13		1.53	
	4	11.88		79.02		1.56		11.89		79.65		1.57	
	Average (SEM)	11.88 (2.76)	13.14	76.94 (3.07)	85.15	1.54 (0.23)	1.71	12.00 (2.76)	13.21	77.38 (3.19)	85.16	1.48 (0.24)	1.63
T2	1	9.05		43.92		2.13		9.37		43.90		1.42	
	2	10.03		44.62		1.01		10.44		45.26		1.05	
	3	9.65		42.74		1.55		10.00		45.29		1.49	
	4	10.15		43.53		1.21		10.20		43.79		1.29	
	Average (SEM)	9.72 (1.94)	17.71	43.70 (3.05)	79.61	1.47 (0.12)	2.69	10.00 (1.91)	18.15	44.56 (3.12)	79.48	1.39 (0.08)	2.37
T3	1	1.13		14.08		1.40		1.19		14.31		0.81	
	2	1.10		14.52		0.53		1.15		14.97		0.59	
	3	1.41		17.84		0.61		1.15		15.87		0.92	
	4	1.88		15.82		0.63		1.98		16.24		0.66	
	Average (SEM)	1.38 (0.22)	8.01	15.56 (0.96)	87.52	0.79 (0.18)	4.47	1.37 (0.21)	8.05	15.35 (0.88)	87.69	0.74 (0.12)	4.26
T4	1	1.29		4.95		1.12		1.49		3.88		0.77	
	2	1.57		7.11		0.70		1.61		7.54		0.88	
	3	1.29		6.84		0.36		1.46		6.16		0.36	
	4	1.39		7.65		0.58		1.58		8.10		0.76	
	Average (SEM)	1.39 (0.06)	15.89	6.64 (0.76)	76.20	0.69 (0.07)	7.91	1.53 (0.10)	17.78	6.42 (0.60)	74.23	0.69 (0.09)	7.99
Average thermal lethality contribution (%)			13.69		82.12		4.19		14.29		81.64		4.06

Reference heat treatments: T1 (56.1°C / 62 min); T2 (58°C / 32 min); T3 (62°C / 12 min); T4 (72°C / no dwell time). Guideline for meat and poultry products – Appendix A (USDA & FSIS, 2021b). SEM = standard error of the mean (n = 12). ¹TC = thermocouple: 1 and 2 (bottom tray); 3 (middle tray); 4 (top tray) Average thermal lethality contribution between Simpson and Trapezoid model: Heating = 13.99%; Maintenance: 81.88%; Cooling: 4.12%.

Table 3 – Microbial count of beef tenderloin tournedos cooked sous vide at different combinations of temperature and time, and of the raw product.

Microbiological tests	Treatments (n= 5)				Brazilian legislation ¹			Raw product ²
	T1	T2	T3	T4	c	m	M	
<i>Clostridium perfringens</i> (CFU/g)	<10×10 ¹	<10×10 ¹	<10×10 ¹	<10×10 ¹	1	10 ²	5×10 ²	<10×10 ³
Staphylococcal enterotoxin (ng/g)	Negative	Negative	Negative	Negative	0	Negative	Negative	Negative
<i>Escherichia coli</i> (CFU/g)	<10×10 ¹	<10×10 ¹	<10×10 ¹	<10×10 ¹	2	10	20	<10×10 ²
Coagulase-positive staphylococci (CFU/g)	<10×10 ¹	<10×10 ¹	<10×10 ¹	<10×10 ¹	2	10 ²	10 ³	<10×10 ²
<i>Listeria monocytogenes</i> (CFU/g)	Absence	Absence	Absence	Absence	0	10 ²	-	Absence
<i>Salmonella</i> spp. (CFU in 25 g)	Absence	Absence	Absence	Absence	0	Absence	Absence	Absence

Reference heat treatments: T1 (56.1°C / 62 min); T2 (58°C / 32 min); T3 (62°C / 12 min); T4 (72°C / no dwell time).

¹ IN 161/ 2022 (BRASIL, 2022b).

n: number of sampling units to be analyzed.

c: maximum permitted number of sampling units that can present results between the limits "m" and "M". m: maximum acceptable limit for the microorganism in a sampling unit, considered satisfactory.

M: maximum tolerable limit for the microorganism in a sampling unit, above which the result is considered unsatisfactory.

² Without heat treatments

Table 4 – Mean values of pH, water activity, mass loss, and TBARS of beef tenderloin tournedos cooked sous vide at different combinations of temperature and time.

Characteristics	T1	T2	T3	T4	SEM	Pr > F
pH	5.97 ^{ab}	5.87 ^b	6.06 ^{ab}	6.10 ^a	0.04	0.040
Water activity	0.976	0.975	0.974	0.976	0.001	0.686
Exudate released (%m/m)	34.43 ^{ab}	35.26 ^{ab}	30.89 ^b	39.03 ^a	1.07	0.031
TBARS, mg MAD/kg	0.75 ^b	0.90 ^b	1.16 ^{ab}	1.50 ^a	0.10	0.011

Reference heat treatments: T1 (56.1°C / 62 min); T2 (58°C / 32 min); T3 (62°C / 12 min); T4 (72°C / no dwell time). SEM = standard error of the mean (n = 12). / ^(a,b) Averages followed by different letters in the row differ by the Tukey test ($P < 0.05$).

Table 5 – Mean value for shear force, fragmentation index, and collagen of beef tenderloin tournedos cooked sous vide at different temperature and time combinations.

Characteristics	T1	T2	T3	T4	SEM	Pr > F
Shear force, N	17.06 ^b	26.46 ^a	24.20 ^a	23.26 ^a	2.03	0.005
Fragmentation index	526.56 ^c	578.78 ^b	573.00 ^b	638.67 ^a	8.61	0.002
Soluble collagen, mg/g	0.25 ^a	0.19 ^b	0.18 ^b	0.32 ^a	0.03	0.005
Insoluble collagen, mg/g	1.71 ^a	1.42 ^b	1.41 ^b	1.47 ^b	0.10	0.007
Total collagen, mg/g	1.89 ^a	1.60 ^b	1.67 ^{ab}	1.82 ^{ab}	0.12	0.012

Reference heat treatments: T1 (56.1°C / 62 min); T2 (58°C / 32 min); T3 (62°C / 12 min); T4 (72°C / no dwell time). SEM = standard error of the mean (n = 12).

^(a-c) Averages followed by different letters in the row differ by the Tukey test ($P < 0.05$).

Table 6 – Mean values of internal and external color measurements of beef tenderloin tournedos cooked sous vide at different temperature and time combinations.

Color indices	T1	T2	T3	T4	SEM	Pr > F
External						
Lightness, L^*	40.01	41.96	41.02	44.09	0.77	0.068
Chroma, C^*	17.26	17.09	17.54	17.41	0.36	0.958
Hue angle, h (°)	47.40	49.03	49.17	51.03	0.57	0.057
Internal						
Lightness, L^*	50.24	51.27	54.52	53.86	0.97	0.327
Chroma, C^*	29.80	27.53	28.18	23.77	0.92	0.187
Hue angle, h (°)	35.56 ^{ab}	34.66 ^b	36.41 ^{ab}	43.76 ^a	1.28	0.033

Reference heat treatments: T1 (56.1°C / 62 min); T2 (58°C / 32 min); T3 (62°C / 12 min); T4 (72°C / no dwell time). SEM = standard error of the mean (n = 12).

^(a-c) Averages followed by different letters in the row differ by the Tukey test ($P < 0.05$).

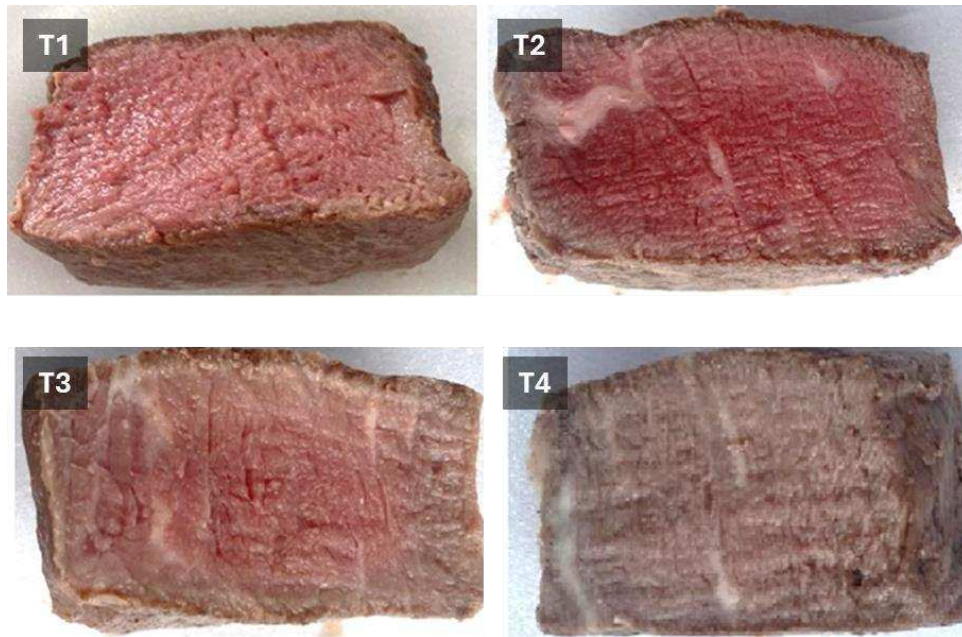


Figure 6 – Internal coloration of beef tenderloin sous vide tournedos after completion of each heat treatment: T1 = 56.1°C / 62 min; T2 = 58°C / 32 min; T3 = 62°C / 12 min; T4 = 72°C / no dwell time.

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Chapter 3: Composition assessment of sous vide beef meat by near-infrared spectroscopy based on compact spectrophotometers, multivariate regression, and jack-knife variable selection

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ABSTRACT

'Sous vide' is a cooking process that uses long time and low temperatures, affecting the quality and composition of the product. This study aimed to develop and validate prediction models for the proximate composition of sous vide beef tenderloin using near-infrared reflectance spectroscopy (NIRS) and to compare the performance of two portable NIR spectrometers. Original spectra were pretreated using the Standard Normal Variate (SNV) method, followed by the 1st derivative. Models using partial least squares regression (PLS) were constructed with all spectral variables, and after the jack-knife algorithm performed wavelength selection. The centesimal composition of the ground samples was accurately determined by the models, except for the carbohydrate content. The prediction errors resulting from external validation (RMSEP) for the InnoSpectra and NeoSpectra evaluated compact instruments were, respectively, 0.92% and 0.65% for moisture, 0.72% and 0.93% for fat, 0.93% and 0.60% for protein, and 0.12% and 0.03% for ash. Conversely, poor results were obtained for samples where readings were taken from whole roasters with or without packaging. A new method for assessing the quality and usefulness of multivariate models was proposed based on RMSEP and comparing the distributions of the reference and validation data. The NIRS-based method is fast, requires simple sample preparation, does not require the use of chemicals, and employs low-cost instruments.

Keywords: sous vide meat; near-infrared spectroscopy; chemometrics; variable selection; quality characteristics.

1. INTRODUCTION

'Sous vide' is a French technique, meaning 'under vacuum' (Baldwin, 2012), characterized by cooking vacuum-sealed food for long periods at low temperatures (Kathuria et al., 2022). Food is cooked in thermostable packaging, followed by cooling and storage at low temperatures (Yang et al., 2020). This technique has been used for various raw materials, such as fruits, vegetables, seafood, and meats (Kathuria et al., 2022).

Several studies demonstrate the advantages of applying the 'sous vide' technique for meats and meat products, including reduced cooking loss and improved nutrient retention. (Baldwin, 2012; Joung et al. 2018; Zhu et al. 2018; Ismail et al. 2019; Bıyıklı et al. 2020; Lee et al. 2021). These advantages are reflected in the proximate composition of the processed product (Roldan et al., 2014).

The chemical composition of 'sous vide' beef tenderloins is predominantly composed of water, protein, lipids, and ash (NEPA, 2011). Understanding the product's composition is crucial for meeting the requirements of official inspection and regulatory bodies (USDA, 2025; BRASIL, 2018), as well as ensuring the contracted quality standard demanded by customers (Weeranantanaphan et al., 2011).

According to the Food and Drug Administration (FDA), which sets the rules for nutritional labeling in the United States, detailed in the Code of Federal Regulations (CFR), the content of naturally occurring nutrients in food must be at least 80% of the declared value on the label (USDA, 2025). A similar percentage variation is established by Brazilian legislation, through Collegiate Board Resolution (RDC) N° 429 of the National Health Surveillance Agency, which allows, for inspection purposes, up to 20% above the declared value on the label for various components, including fat and carbohydrate, and the same percentage below for protein and minerals (BRASIL, 2020). Considering legal tolerance, the food industry typically creates nutritional tables during the development of a new product and often does not account for possible variations in processed raw materials, especially those of animal origin.

Conversely, consumers are increasingly concerned about food composition, and nutritional labeling is a key source of information that influences their purchasing decisions (Duarte et al., 2021). Thus, accurate information is essential for consumers to make informed purchasing decisions (Weeranantanaphan et al., 2011).

North American legislation mandates that the proximate composition of a product be determined using official AOAC analytical methods or other reliable and appropriate methods (USDA, 2025). Although RDC N° 429 (BRASIL, 2020) permits the use of direct and indirect calculations based on the constituents used in product formulation to determine its proximate composition, the most precise methodology for this determination is laboratory analysis. However, given the existing legal requirements that allow for a 20% variation from the declared value and the cost of establishing and maintaining an in-house laboratory, many industries opt to outsource their physical-chemical analyses. These analyses are often performed only during the development of a new product or when facing an official audit.

Another challenge in using laboratory analysis to determine the proximate composition of food is the requirement for exclusive use of official reference methods. Most of these methods are not suitable for meeting the demands of production in a global market, as they require complex sample preparation, long processing times, and are expensive and polluting (Prieto et al., 2017a). Consequently, the results are time-consuming, rendering official methods

inadequate for industrial use because they do not provide the rapid and reproducible results necessary to continuously and representatively certify product quality (Cozzolino et al., 2011). Therefore, to meet practical industrial demands, instrumental methods must be objective, fast, economical, and precise. Near-infrared (NIR) spectroscopy possesses these characteristics and has proven highly suitable for food products (Aleixandre-Tudó et al., 2020). This technique emerges as an alternative to traditional analytical methods for determining various properties or constituents of agricultural matrices (Williams & Sobering, 1993). NIRS is a widely recognized technique, based on the interaction of electromagnetic radiation in the wavelength range of 750 to 2500 nm with the vibrational bonds of the molecular species constituting various types of samples (Pasquini, 2018).

NIR radiation interacts with matter in various ways, with the absorption of radiation by the sample's constituents being the most relevant source of information for analytical applications. This is because the interaction of NIR radiation with the substances present in the sample stimulates vibrational energy transitions in the chemical bonds between atoms, generating absorption spectra that provide qualitative and quantitative analytical information associated with various sample properties (Pasquini, 2003).

Near-infrared spectroscopy offers advantages such as speed, minimal or no sample preparation, ease of use (Cozzolino et al., 2011), ecological friendliness, the ability to quantify various components from measurements taken in a few seconds, and applicability to molecules containing CH, NH, SH, or OH bonds (Qu et al., 2015). Disadvantages include the difficulty in assigning chemical groups, as information is often obscured in complex spectra characterized by weak signals (Olinger & Griffiths, 1988), broad bands, and severe overlaps (Porep et al., 2015). Other sources of variability, such as instrumental noise, scattering, environmental effects, and sample heterogeneity, also contribute to the complexity of a NIR spectrum (Dos Santos et al., 2017).

To remove uninformative sources of variability and enhance spectral characteristics, NIR spectral data undergo some form of pre-treatment before being used for qualitative or quantitative purposes. The primary source of this type of variability in solid samples originates from the scattering of radiation by pulverized solid samples (Pasquini, 2018).

Analytical information from pre-treated NIR spectra is extracted using chemometric tools for the appropriate multivariate analysis of the data, enabling both qualitative and quantitative purposes. Chemometrics has enabled significant advances in applying spectroscopic techniques to food and other commodity analysis (Cozzolino et al., 2011).

Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression are commonly used multivariate analytical techniques for exploratory analysis and regression of multivariate data.

Considering regression models, after the calibration process is complete, the precision and robustness of the model must be tested with an independent sample set (validation set) (Ziegel, 2004). The predictive capacity of multivariate models is typically evaluated by calculating the error associated with their estimates. The determination of the Root Mean Square Error of Prediction (RMSEP) and/or the Standard Error of Prediction (SEP) is an indicator of prediction accuracy and is the most common way to assess the quality of regression models (Bro et al., 2005).

Continuous advancements in chemometric methods, technology, and instrumentation have enabled NIR spectroscopy to achieve increasingly robust models for identification and quantification. NIR spectrometers basically include a light source, a wavelength selector, a sample detector, an optical detector, and a data processing/analysis system (Pasquini, 2018; Prieto et al., 2017a). The development of compact spectrophotometers operating in the near-infrared region has made it possible to monitor, characterize, and identify products, as well as reduce the time and cost of analyses in the pharmaceutical and food industries, for example (Savoia et al., 2020).

The advancement of the NIR technique has expanded its versatility, enabling the replacement of some slow, expensive, and labor-intensive instrumental analysis methods with vibrational spectrophotometric methods. This technique can perform real-time analyses at the production site with a similar level of precision and accuracy (Dos Santos et al., 2017). This versatility was reaffirmed by Patel et al. (2021) when they highlighted portable NIR instruments as a fast and practical option in meat processing plants for collecting spectra and developing prediction models, and by Porep et al. (2015), who stated that it is a suitable technique for implementation as an analytical tool in industrial processing.

Therefore, the objective of this study was to compare the performance of two low-cost portable NIR spectrometers for the construction and validation of regression models aimed at determining the proximate composition of 'sous vide' beef tenderloins during their industrial processing, on an individualized basis, with an evaluation of the possibility of printing the nutritional table, displaying its composition in a more representative way.

2. MATERIAL AND METHODS

2.1 *Preparation of packaged beef tenderloins*

The packaged beef tenderloins were prepared according to the flowchart shown in Figure 1.

A set of 132 samples of 'sous vide' beef tenderloins was used for the construction and validation of the regression models. The sample set had its composition identified and quantified by reference methods, representing the samples industrially processed between

August 2023 and May 2024. The chilled beef tenderloin was sampled according to their production by the industry, with 4 samples being collected per production batch. These different times made it possible to cover greater variability in the composition of the raw material in order to build a more accurate predictive model.

2.2 *Description of the portable NIR spectrophotometers*

Two spectrophotometers were used throughout this study: the InnoSpectra (Texas Instruments Inc.) and the NeoSpectra (Si-Ware, Egypt). These instruments are portable and compact, capable of monitoring the near-infrared spectral ranges of 921–1683nm and 1350–2550 nm, respectively, with a nominal resolution of 10 nm.

The NeoSpectra consists of an illumination unit containing three tungsten filament sources and an optical coupling system, designed for reflection measurements. The instrument is based on a complete Michelson interferometer implemented on a MEMS chip. The manufacturer reports a signal-to-noise ratio (SNR) of 2,000 at a wavelength of 2350 nm with a 2 s integration time. In turn, the manufacturer of the InnoSpectra reports an SNR of 6000:1 and a scan time of 0.3 seconds, with Grade-MEMS technology.

The instruments were connected to the USB port of a notebook (Dell Technologies, 16 GB DDR4, 512 GB SSD) and powered on for 30 minutes to allow for stabilization before the measurements began. The radiation source was activated only during the acquisition of the spectra (Figure 2).

2.3 *Protocol for obtaining sample spectra*

The acquisition of representative NIR spectra of the samples was performed in three

ways: on the whole tenderloin covered by its packaging; on the whole tenderloin covered by a glass Petri dish (diameter of 7.2 cm and height of 1.3 cm); and with the ground tenderloin placed in the glass Petri dish, filling its entire extension up to its maximum height.

For the measurements of the whole product, its original packaging was opened, and its surface was gently blotted with a paper towel to standardize the surface moisture. The part of the packaging without graphic printing was placed over the product, and the equipment was positioned to obtain the spectra. A Spectralon® reference tile (corresponding to 100% reflection of NIR radiation) covered by the product packaging or by the glass of the Petri dish was used for spectral reference measurements necessary for calculating the reflectance spectra. The reference was measured before each sample was taken.

The readings on the whole tenderloin, covered by a glass Petri dish, were performed in the same manner as the measurements of the product in its packaging, with the only difference being the replacement of the plastic packaging with the glass Petri dish.

For the ground product, a domestic food processor (Walita Philips, 600W) was used, where the product, cut into approximately 1cm slices, was minced for 3 minutes at speed 2 of the equipment. The resulting ground meat mass was spread evenly on a 7.2 cm diameter glass Petri dish, avoiding the presence of air bubbles, to produce a layer with a thickness of 1.3 cm. With the dish covered, it was turned upside down and the spectrophotometer was placed on the original bottom of the dish to perform the readings. The Spectralon (reference corresponding to 100% reflection of NIR radiation) covered by the lid of the Petri dish was used for spectral reference measurements, which are necessary for calculating the reflectance spectra.

The samples in all their forms were measured in quintuplicate, obtaining a representative average spectrum with an improved signal-to-noise ratio. Figure 3 shows, for a ground sample, the position of the reading points.

The spectra of the samples were obtained at ambient temperature ($25 \pm 1^\circ\text{C}$) over a period of thirteen days at the GAES Chemistry Laboratory (Group for Analysis and Education for Sustainability) at the Federal University of Viçosa. Three samples were measured five times according to the procedure described above to estimate the repeatability of the validated NIR models showing the best performance.

2.4. Proximate composition

The determination of total water (ISO 1442:2023 (E), 2023), fixed mineral residue (ash) (ISO 936:1998 (E), 1998), total fats (NMKL 181, 2005), and nitrogen content (ISO 1871:2009

(E), 2009) were performed according to official methods for the analysis of products of animal origin. The obtained nitrogen value was multiplied by 6.25 to determine the protein content (Brasil, 2022). Carbohydrate content was calculated by difference from the proximate composition.

2.5 Multivariate analysis and construction of regression models

The obtained spectral datasets were processed using the chemometric software Unscrambler 10.5. Preliminary regression models based on Partial Least Squares (PLS) Regression were constructed with all samples in the set and for each of the studied properties, aiming to estimate the levels of protein, water, fat, ash, and carbohydrates present in the samples.

With preliminary models constructed, the Kennard-Stone algorithm (Kennard & Stone, 1969) was used to select the calibration set (typically comprising 90 samples) and the validation set (comprising 42 samples). This procedure guarantees the selection of a uniform and representative subset (of calibration) of the samples.

The spectra were pre-treated using Standard Normal Variate (SNV) followed by the 1st derivative calculated using the Savitzky-Golay algorithm, employing 11 points in the smoothing window and a 2nd-degree polynomial fit, to minimize the effect of radiation scattering, highlighting the relevant information. Models were constructed using the entire useful spectral range of the spectrophotometers and using only the variables selected by the jack-knife algorithm available in the Unscrambler 10.5 software. The objective of the variable selection performed by this algorithm is to use in the regression process only the variables that produce, in a first analysis, stable regression coefficients identified during the cross-validation (employing 20 subsets of samples randomly selected) process that accompanies the construction of the regression models (Esbensen & Swarbrick, 2018).

3. RESULTS AND DISCUSSION

3.1. Spectral data

The original average spectra obtained from the five readings of the intact tenderloins, using both spectrophotometers, with and without packaging, and for the ground samples, are shown in Figure 4 (A and C).

For analysis by conventional methods, the product must be grounded and homogenized.

This implies that in developing the regression models, the NIR spectra of the intact product (with and without packaging) must be compared with the results of analyses performed using traditional methods on the ground product. It should be anticipated that the spectra of the intact samples, despite efforts to minimize the effect of the packaging and the glass Petri dish, better reflect their surface composition than their average composition as determined by the reference methods. The intact product undergoes a searing step, which results in a darkening of its surface, primarily due to the Maillard reaction. Thus, the spectra obtained under these conditions are not representative of the sample as a whole (bulk).

The obtained spectra were transformed to minimize the effects of radiation scattering by applying the standard normal variate (SNV) followed by a 1st derivative calculated using the Savitzky-Golay algorithm with 11 points in the smoothing window and a 2nd degree polynomial fit. Figure 4 shows the spectra before (A and C) and after the pre-treatments (B and D) for the ground "sous vide" tenderloin samples.

3.2 Sample quality characteristics.

The results of the proximate composition analysis of the 'sous vide' beef tenderloins obtained by the reference method (Table 1) are close to those listed in the Brazilian Food Composition Table (NEPA, 2011) for raw beef tenderloin, which averages 71.9% water, 21.6% protein, and 5.6% fat. A study by Hocquette et al. (2018) mentions that beef contains 21% to 31% protein, which indicates that the values found in the present study fall within these ranges. Mitsumoto et al. (1991) Compared the quality of beef cuts using near-infrared (NIR) spectroscopy with reflectance, transmittance, and a fiber optic probe. The mean values found by the authors for protein, water, and fat were similar to those of the 'sous vide' beef tenderloin, at 18.8%, 69.7%, and 8.8%, respectively.

The most significant inverse correlations are observed between the protein and carbohydrates content ($r = -0.5403$) and fat and water ($r = -0.7556$) (Table 2), with the latter exhibiting the highest correlation coefficient among the property values determined by the reference methods.

3.3 Elaboration, optimization, and validation of regression models.

Preliminary PLS regression models were developed using the complete pre-treated spectra obtained from the two instruments for each of the three sample measurement forms.

The models constructed with the spectral data set from the intact samples, both with and without packaging for both instruments, did not yield satisfactory results. Coefficients of determination (R^2) below 0.300 and high root mean square error of cross-validation (RMSECV) values indicate that the representativeness of the spectra, obtained predominantly from the surface of the intact samples, is insufficient to establish a correlation between the spectral data and their properties, which are inferred from portions of the ground and homogenized samples processed by conventional methods.

Conversely, the results for the preliminary models built with the spectra from the ground and homogenized samples for both instruments show satisfactory R^2 and RMSECV values for all modeled parameters, except for carbohydrate content. Therefore, the results presented below pertain only to the models constructed with spectra from the ground samples.

The preliminary models were refined by eliminating anomalous samples (outliers) from the data sets, using leverage, the spectral residuals of the samples, and the X-Y relation of anomalous samples (X-Y relation outliers)(Esbensen & Swarbrick, 2018). Subsequently, the remaining samples were divided using the Kennard-Stone algorithm (Kennard & Stone, 1969) to produce a calibration set containing 90 samples and an external validation set constituted of the remaining samples.

Tables 3 and 4 present the preliminary calibration and validation results for the PLS models developed to determine the protein, fat, water, ash, and carbohydrate content using all spectral variables from the full spectral range accessible by both instruments. The same tables also display the results obtained using only the variables selected by the jack-knife algorithm in Unscrambler 10.5 software, which was used for chemometric data treatment. This algorithm analyzes the behavior of the regression coefficients for all variables used in the PLS model construction in relation to their stability during the cross-validation process. In this study, segmented cross-validation was employed with 20 groups, each containing 4 to 5 randomly selected samples. The behavior of the 20 sets of regression coefficients was statistically evaluated, and spectral variables associated with highly unstable regression coefficients were eliminated. The selected variables for each modeled property and for each of the two instruments are shown in Tables S1 and S2 in the supplementary material. New models were constructed using the selected variables, and the results are presented in Tables 3 and 4.

As observed in Tables 3 and 4, the models built with the selected variables perform better than those using all variables. The RMSECV values for the models with selected variables are invariably better than those obtained with all variables. A notable point is the

reduction in the number of PLS factors required by the models with selected variables, as seen in all models except for the water content model from the InnoSpectra data, which increased from 4 to 6, and the ash content model from the NeoSpectra data, which remained at 3. These results indicate that models based on selected variables are generally simpler, more robust, and utilize the most relevant spectral information for each modeled parameter.

The results for the models constructed with or without variable selection for determining carbohydrate content showed unsatisfactory performance. This result is likely due to the low carbohydrate content and its non-homogeneous distribution on the sample surface. Furthermore, its content is determined with lower precision and accuracy, as it is calculated indirectly by subtracting the sum of the other constituents.

The ash content of the samples refers to inorganic constituents present in the meat that should not exhibit spectral characteristics in the near-infrared (NIR) region. However, models for determining ash content exhibit acceptable performance. This performance likely results from the indirect association of other organic sample constituents with this property. However, due to the characteristics of this property, the results should be considered with caution and must be submitted to further evaluation.

The repeatability of the predicted results determined by the validated models employing jack-knife selection of variables was evaluated by the mean estimated standard deviation of the results obtained by 3 samples measured in quintuplicate as, 0.46, 0.43%; 0.52, 0.45%; 0.39, 0.35% and 0.05, 0.02% for protein, water, fat and ash contents and models obtained using the NeoSpectra and InnoSpectra, respectively. The repeatability of the results obtained by the NIR method is very good, shows values lower than the RMSEP of the validated models, and is, in general, somewhat better for the InnoSpectra instrument.

Figures S1-S4 in the supplementary material present the cross-validation results for each instrument during model construction for water, fat, ash, and protein content, using all spectral variables, selected variables, and the validation of models with variable selection.

The models built with the selected variables were successfully validated, yielding promising root mean square errors of prediction (RMSEP) for determining the main quality characteristics of the "sous vide" samples, as shown in Tables 3 and 4.

3.4. Considerations on the evaluation of model quality

Several criteria for classifying the quality of multivariate models are described in the literature. The Standard Practices for Infrared Multivariate Quantitative Analysis (ASTM) has

issued two guides recommending best practices for quantitative and qualitative multivariate analysis based on NIRS (ASTM E1655-05, 2012; ASTM E1790-04, 2016).

For multivariate regression, these ASTM guides do not recommend the coefficient of determination (R^2) as a model quality criterion, as it is heavily dependent on the data distribution. That is, R^2 depends on the variance of the concentration or reference values. Despite this, it is very common to find it expressed in NIR spectroscopy literature as a measure of the quality of multivariate models. Many of these present comparison plots between the values predicted by an NIR model and the reference values, where most values are, for example, close to the upper limit, while a few are near the lower limit, indicating an asymmetric distribution. In such cases, R^2 can approach 1, being considered excellent. However, if the few lower values are removed, R^2 would assume much smaller values (Pasquini, 2018). The R^2 values obtained in the validation of the models built in this work ranged from 0.258 to 0.784 for the protein and water content models, respectively, using data from NeoSpectra, and from 0.330 to 0.710 for the protein and fat content models, respectively, using InnoSpectra. It is noted that the protein content values are distributed in a narrow range in the calibration set and an even narrower range in the validation set. Considering the standard deviation of the reference method for this parameter, it is evident that the R^2 value could not reach much higher values.

However, the RMSEP of the models shows values of 0.93% and 0.96% for protein content obtained with models from the NeoSpectra and InnoSpectra data, respectively, despite R^2 values that are much lower than 1. These error values are acceptable for expressing product quality within Brazilian labeling standards.

The agricultural field, including food science, frequently uses the ratio of performance to deviation (RPD) as a quality criterion for a model (Prieto et al., 2017b; Leone et al., 2012). Its calculation considers the ratio between the model's validation error (RMSEP) and the standard deviation of the reference values. However, this quality criterion is susceptible to the presence of outliers in the validation set, which can significantly affect the calculation of the standard deviation of the reference data. Thus, this criterion is also subject to data distribution. The RPD-based criterion advocates values greater than 2 for models of satisfactory quality (Prieto et al., 2017b; Leone et al., 2012).

To minimize the effect of outliers on the calculation of a criterion similar to RPD, the ratio of performance to interquartile (RPIQ, the ratio between performance and interquartile) was proposed (Bellon-Maurel et al., 2010; Olivieri, 2018). This parameter is less susceptible to data distribution, as the presence of outliers has a lesser influence on it. However, the literature

does not mention a consensus critical value by which models could be considered of adequate quality. Thus, RPIQ is more useful for comparing the performance of different models than for quantifying their absolute quality.

Based on the foregoing considerations, it is suggested that the RMSEP value, analyzed according to the accuracy required to attest product quality, be preferentially used as a quality criterion that is less dependent on the reference data distribution and more suitable for real-world situations where this distribution does not always favor the calculation of R^2 or RPD. Thus, for example, if this work were repeated using InnoSpectra equipment, the average agreement between the reference method and the model should be 0.92% for moisture, 0.72% for fat, 0.93% for protein, and 0.12% for ash (Table 3), which are adequate values to express the product's quality.

On the other hand, to verify if the predicted values from the models maintain the original distribution of the reference data, we propose observing the variation in the maximum, minimum, median, and quartiles 1 and 3 between the results from the reference methods and those from the model validation. The criterion for verifying the distribution of results considers the distributions of the reference and validation results to be indistinct if the differences between the values are less than the model's RMSEP, considering that RMSEP values can be accepted to estimate quality parameters with the necessary accuracy. Tables 5 and 6 show the results of the evaluation of the differences between the quartiles for each of the models and for the InnoSpectra and NeoSpectra equipment, respectively.

Adopting this criterion, it is observed that for the InnoSpectra instrument, there is a distortion of the distribution for high predicted values of protein content. For fat content, the validation results using this equipment show a significant distortion in the distribution of predicted values relative to the reference values, with most of the differences being greater than the model's RMSEP.

Regarding the NeoSpectra instrument, the analysis of the distribution behavior of the reference results compared to the validation results shows minor differences for the models built for protein and fat determination, allowing for the attestation of the quality of all models built for the four quality parameters. In other words, with this equipment, acceptable prediction errors are obtained to attest to the product's quality, and simultaneously, the validation results of the models maintain their original distribution.

The quality analysis of the models proposed in this work is not based on a single parameter, such as R^2 , RPD, or RPIQ, to determine the model's quality. It employs the results

concerning the accuracy of the models (RMSEP) and, if these are satisfactory for the problem at hand, analyzes the non-parametric distribution of the results obtained by the reference method compared to those obtained by the model. The procedure is effective as it considers aspects inherent to the accuracy requirements of the model (RMSEP) while maintaining attention to the distribution of the validation results.

3.5. *Comparison of the performance of the InnoSpectra and NeoSpectra equipment*

Table 7 allows for a comparison of the external validation results for the models built with variables selected by the jack-knife algorithm for both instruments used in this work. Considering only the validation error values (RMSEP), both instruments demonstrated similar and adequate performance in determining the four quality parameters of "sous vide" meat, to express them on the product label. The NeoSpectra shows more accurate results for the model built to determine the water content. This is associated with the spectral information obtained by this instrument, which monitors a broader spectral range, which the InnoSpectra does not access (Wilson et al., 2015).

The performances of portable visible NIR (Vis-NIRS), NIRS, and a Micro-NIRS instrument were evaluated by Patel et al. (2021) by constructing and evaluating prediction models for beef characteristics from spectra obtained directly from its surface. As in the present study, the authors concluded that the prediction performances of the three instruments were similar, although the Micro-NIRS performed better for some characteristics.

If RPD or RPIQ values were adopted as the quality criterion for the models, it would indicate that only the models built for determining water and fat content obtained by the InnoSpectra could be accepted based on RPIQ values greater than 2. Likewise, for the NeoSpectra, only these two models would have their quality attested by the RPIQ values, with the water content model also being acceptable based on its RPD. However, adopting the criterion established in this work, which considers the accuracy (RMSEP) sufficient for the intended purpose of the models and the maintenance of the distribution of the predicted values when compared to the distribution of the reference results, only the model for fat and the protein model, considering high contents, obtained with the InnoSpectra would require caution for routine use. The models obtained by the NeoSpectra are deemed adequate according to this quality criterion.

Both instruments can operate completely autonomously, utilizing batteries, and allow for the remote transmission of the obtained spectra. However, the cost of the InnoSpectra is

about 5 times lower than the cost of the NeoSpectra.

3.6. *Comparison with similar results found in literature*

In assessing the intramuscular fat and protein content of lyophilized ground beef using near-infrared spectroscopy, Bailes et al. (2022) obtained validation error (RMSEP) values of 0.16% and 0.50% respectively, showing that prediction models can be used with precision for the evaluated product/characteristics. A good correlation was also found between the data obtained by the reference method and NIR for water content. Water has a high absorption in the near-infrared spectral region, which is why NIR spectroscopy is widely used to measure water content (Weeranantanaphan et al., 2011).

When comparing the prediction error results from the external validation of the beef characteristics measured by Patel et al. (2021) is observed that the results obtained for the 'sous vide' beef tenderloin were slightly better for water and lipids. For water, the validation error for the tenderloin varied between 0.69% - 0.92% and for the beef between 0.96% - 1.25%, depending on the instrument used. Lipids varied between 0.72% – 0.76% for the tenderloin and 0.80% - 1.06% for beef. For protein, a slightly better validation error was obtained for the beef, ranging from 0.51% to 0.62%, and for the tenderloin, from 0.93% to 0.96%. The validation error results for ash were very similar between the studies, varying between 0.12% – 0.13% for the tenderloin and 0.06% for beef, independent of the instrument used.

Dixit et al. (2017) explored the potential of near-infrared (NIRS) spectroscopy to predict the fat and water content of ground beef samples in both on-line and at-line modes. Although the performance of the PLSR models was measured by R^2 and RPD, the authors presented a table with SEP values, being 6.84 for fat and 4.72 for water for the at-line mode, and, 5.95 for fat and 4.33 for water for online mode. These values were reported without units hidden a more accurate comparison with the results of this work.

Considering the results provided by Dixit et al. (2017) express absolute values, the validation results obtained in this work are considerably better with being of 0.92% e 0.69% for water content, and 0.72% e 0.76% for fat employing the InnoSpectra e NeoSpectra, respectively. The results for the bias were also presented by those authors. For fat content are reported bias of 3.89 (at line) and 6.95 (on line). For water content -1.24 (at line) and -4.01. Both are higher than observed in the present work (Tables 3 and 4).

Although there is an intrinsic correlation between water and fat contents, as shown in Table 2, the regression coefficients of the models differ significantly, as illustrated in Figures

S5 (A and B) in the supplementary material. This means that the spectra provide distinct information used to estimate water and fat content. If the regression coefficients were equal, only inverted since the observed correlation is negative, it would not be viable to state that the NIR accessed information individually associated with the water and fat present in the sample. The only work found in the literature using NIR spectroscopy on 'sous vide' meat products (Perez-Palacios et al., 2019) reports the determination of characteristics related to the texture of pork loin cooked 'sous vide' at different cooking times. Among the parameters, the total water content was determined, but only the R^2 and the scaled mean absolute error (MASE) were presented as quality criteria for the obtained model, which were 0.83 and 0.054%, respectively. The authors classify models based on their correlation coefficients, distinguishing between those with high correlation coefficients ($R^2 > 0.75$), acceptable correlations ($R^2 = 0.5-0.75$), and low correlations ($R^2 = 0.25-0.5$). Using this metric, the model obtained for humidity in this work using the NeoSpectra spectrometer also had a high correlation coefficient ($R^2 = 0.784$), and the InnoSpectra resulted in an acceptable correlation model ($R^2 = 0.517$). The authors also evaluated the influence of sample presentation (ground and in 1cm wide pieces), finding no influence on the absorbance values obtained by NIR, suggesting that similar results could be obtained if the sample spectra had been obtained from the product before seared.

4. CONCLUSION

Near-infrared spectroscopy associated with multivariate calibration methods using PLS enabled the development of a rapid method for determining the water, protein, fat, and ash content of pre-processed 'sous vide' beef tenderloins. The results indicate that the models developed using variable selection via the jack-knife algorithm provide reliable results, demonstrating the potential for implementing NIR technology for process control and monitoring, for research in the field, and for meeting the requirements of regulatory agencies. The results obtained by the two instruments were similar, except for the determination of water content, as the NeoSpectra instrument covers a spectral region where water absorbs with greater intensity.

A new approach to certifying the quality and utility of the validated NIR models is proposed, based on the analysis of the adequacy of the errors (RMSEP) and the preservation of the data distribution, as assessed by the differences between the parameters of maximum, minimum, median, first quartile (Q1), and third quartile (Q3) between the reference results and the prediction results of the validation samples.

This study represents a significant contribution to the field of meat product processing, as studies involving 'sous vide' technology and NIR spectroscopy are scarce. The results demonstrate that the developed PLS-NIR multivariate calibration models enable the implementation of printing nutritional tables on the packaging of 'sous vide' beef tenderloins with greater frequency, on a per-production-lot basis, thereby generating more accurate and reliable information. Objective quality measurements of the products will also enable industrial managers to achieve higher levels of quality for their products.

A complementary future study is suggested to evaluate non-pre-processed (seared) products, that is, without the interference caused by surface alterations, as it is believed that good results could be obtained for the intact product without packaging, enabling the implementation of non-destructive in-line inspection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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FIGURAS E TABELAS

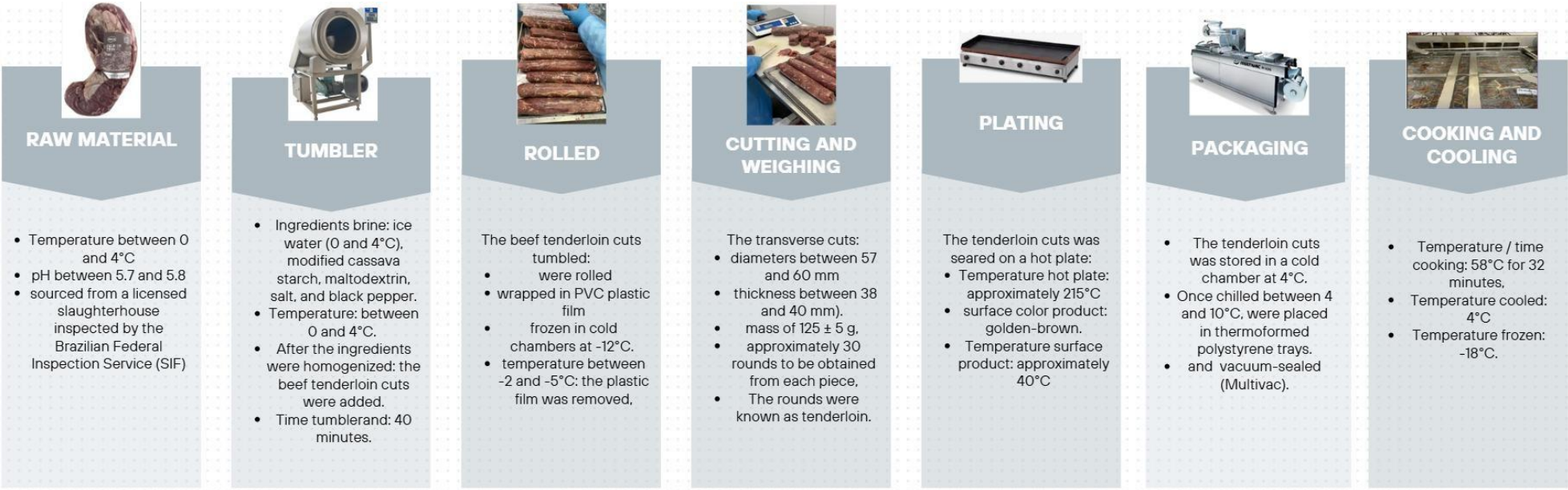


Fig. 1 Flowchart of preparation of packaged beef tenderloins.



Fig. 2 Instruments set-up used to obtain the spectra of sous vide samples: a) NeoSpectra; b) InnoSpectra



Fig. 3 Selected locations on the ground meat sample placed in a Petri dish for spectra acquisition. Center (1), top corner (2), bottom corner (3), left corner (4), and right corner (5)

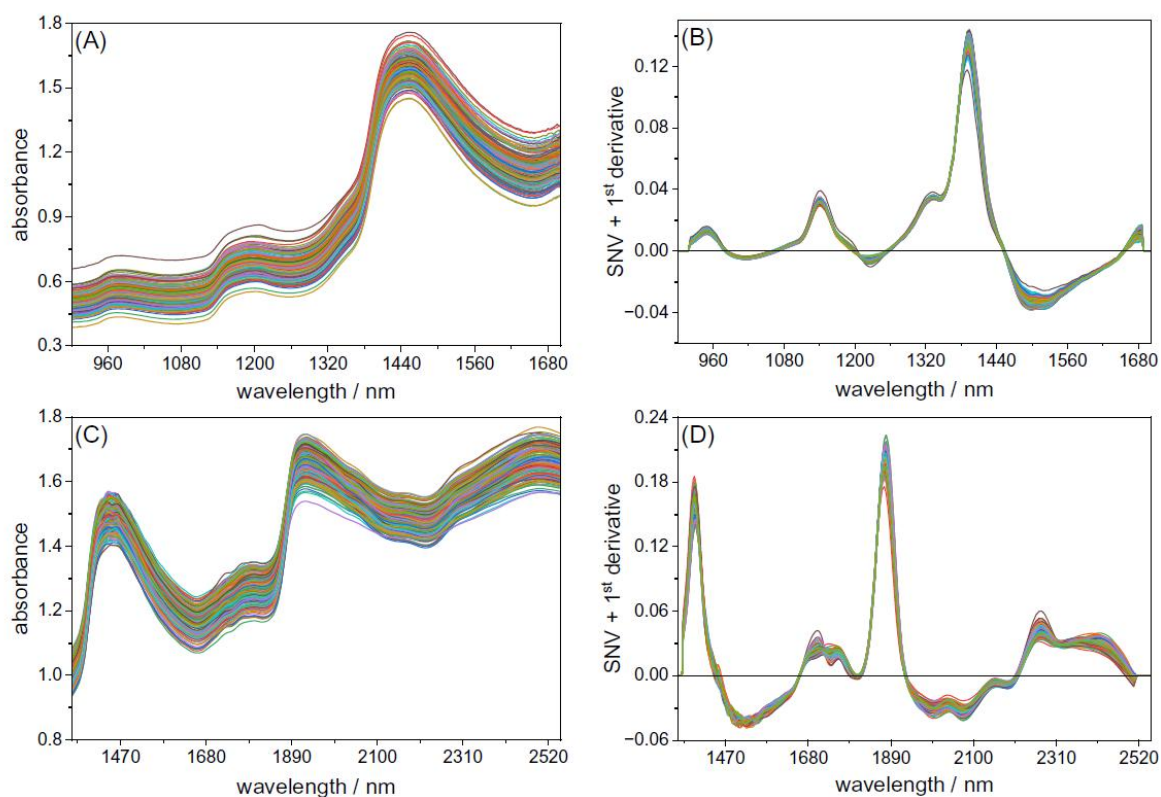


Fig. 4 (A) and (B) show the raw and pre-treated average spectra from the 132 samples obtained using the Nano_NIR spectrophotometer; (C) and (D) show the raw and pre-treated average spectra obtained by the NeoSpectra spectrophotometer

Table 1 – Mean values ($\pm$ standard deviation) and results for moisture, ash, protein, fat, and carbohydrate content of the 132 ground 'sous vide' beef tenderloin samples obtained by reference methods.

statistics	water content (%)	ash (%)	protein (%)	fat (%)	carbohydrates (%)
mean $\pm$ std deviation	68.96 $\pm$ 1.76	1.77 $\pm$ 0.19	24.14 $\pm$ 1.44	3.99 $\pm$ 1.78	1.14 $\pm$ 1.14
max	72.70	2.68	28.31	11.29	5.74
min	63.29	1.47	20.78	0.45	0.01
range	9.41	1.20	7.53	10.84	5.73
variance	3.10	0.04	2.08	3.18	1.31

Table 2 – Correlation analysis between the water, ash, protein, fat, and carbohydrate content of 'sous vide' beef tenderloins using values determined by reference methods.

statistics	water content (%)	ash (%)	protein (%)	fat (%)	carbohydrate (%)
water content (%)	1	0.3122	-0.2801	- 0.7556	- 0.0611
ash (%)	0.3122	1	0.4413	- 0.2597	0.3103
protein (%)	- 0.2801	- 0.4413	1	- 0.1369	- 0.5403
fat (%)	- 0.7556	- 0.2597	- 0.1369	1	- 0.1790
carbohydrates (%)	- 0.0611	0.3103	- 0.5403	- 0.1790	1

Table 3 - Results of the full spectrum models, with Jack-Knife variable selection, and validated (InnoSpectra) with external samples, developed for the components of the ground 'sous vide' beef tenderloin.

model	full spectrum					selected variables JK					validation (with variable selection)				
	Nc	F	R ²	RMSECV (%)	BIAS (%)	Nc	F	R ²	RMSECV (%)	BIAS (%)	Nv	F	R ²	RMSEP (%)	BIAS (%)
Protein	90	8	0.559	0.94	0	90	6	0.598	0.88	0.01	39	6	0.330	0.93	-0.15
Water content	90	4	0.706	0.94	0	90	6	0.856	0.67	0	42	6	0.517	0.92	0.09
Fat	90	3	0.785	0.84	0	90	2	0.795	0.82	0	42	2	0.710	0.72	0.05
Ash	90	10	0.428	0.13	0	90	4	0.589	0.11	0	38	4	0.453	0.12	0
Carboydrates	132	1	NA	1.2	0	132	1	0.002	1.15	0					

* Nc = number of samples; Nv = number of validation samples; F = number of factors; R² = coefficient of determination; RMSECV = cross-validation error; BIAS = systematic deviation; RMSEP = prediction error from external validation.

Table 4 - Results of the full spectrum models, with Jack-Knife variable selection, and validated (NeoSpectra) with external samples, developed for the components of the ground 'sous vide' beef tenderloin.

model	full spectrum					selected variables JK					validation (with variable selection)				
	Nc	F	R ²	RMSECV (%)	BIAS (%)	Nc	F	R ²	RMSECV (%)	BIAS (%)	Nv	F	R ²	RMSEP (%)	BIAS (%)
Protein	90	4	0.440	1.11	0.01	90	3	0.610	0.93	0	39	3	0.259	0.96	-0.42
Water content	90	5	0.865	0.65	0	90	4	0.879	0.61	0.01	40	4	0.784	0.69	-0.19
Fat	90	3	0.884	0.59	0	90	2	0.895	0.56	0	42	2	0.668	0.76	0.11
Ash	90	3	0.402	0.14	0	90	3	0.450	0.14	0	42	3	0.305	0.13	-0.02
Carboydrates	132	1	NA	1.11	0	144	1	0.006	1.14	0					

* Nc = number of calibration samples; Nv = number of validation samples; F = number of factors; R² = coefficient of determination; RMSECV = cross-validation error; BIAS = systematic deviation; RMSEP = prediction error from external validation.

Table 5 – Difference related to the distribution of results obtained by reference methods and by the validation of optimized models built with spectral data from the **InnoSpectra** equipment for ground samples.

model validation results with JK variable												
reference/validation values					selection				difference			
	water content (%)	ash (%)	protein (%)	fat (%)	water content (%)	ash (%)	protein (%)	fat (%)	Δ water content (%)	Δ ash (%)	Δ protein (%)	Δ fat (%)
max	72.2	2.2	27.6	6.6	71.6	2.1	25.9	7.8	0.6	0.1	1.7	-1.2
min	66.4	1.5	21.8	0.5	66.3	1.5	22.5	1.5	0.1	0.0	-0.7	-1.0
median	69.3	1.7	24.0	3.3	69.7	1.7	24.0	3.5	-0.4	0.0	0.0	-0.1
Q1	68.9	1.6	23.5	2.5	68.6	1.7	23.5	2.8	0.3	-0.1	0.0	-0.3
Q3	70.2	1.8	24.8	4.5	70.5	1.8	24.7	4.2	-0.3	0.0	0.1	0.3
RMSEP					0.92	0.12	0.93	0.72				
RPD					1.44	1.42	1.23	1.88				
RPIQ					1.41	1.60	1.39	2.70				

RPIQ = Ratio of Performance to InterQuartile; Q1 = quartile 1; Q3 = quartile 3

Table 6 - Difference related to the distribution of results obtained by reference methods and by the validation of optimized models built with spectral data from the **NeoSpectra** equipment for ground samples.

validation results of models with JK variable												
reference/validation values					selection				difference			
	water content (%)	ash (%)	protein (%)	fat (%)	water content (%)	ash (%)	protein (%)	fat (%)	Δ water content (%)	Δ ash (%)	Δ protein (%)	Δ fat (%)
max	71.6	2.2	26,9	6.8	71.5	2.0	25.7	6.4	0.1	0.2	1.2	0.3
min	65.2	1.5	22.0	0.5	65.6	1.6	22.9	1.5	-0.4	-0.1	-0.9	-1.0
median	69.4	1.7	24.5	3.3	69.3	1.8	24.2	3.1	0.1	-0.1	0.3	0.2
Q1	68.8	1.6	23.5	2.3	68.4	1.7	23.5	2.6	0.4	-0.1	0.0	-0.3
Q3	70.4	1.8	25.1	4.3	69.9	1.8	24.4	4.3	0.5	0.0	0.7	0.0
RMSEP					0.69	0.13	0.96	0.76				
RPD					2.17	0.80	1.16	1.75				
RPIQ					2.31	1.54	1.60	2.63				

RPIQ = Ratio of Performance to InterQuartile; Q1 = quartile 1; Q3 = quartile 3

Table 7 - Results of external validation of the models based on the selection of variables by jack-knife for the InnoSpectra and NeoSpectra equipment.

	validation InnoSpectra							validation NeoSpectra						
	Nv	F	R ²	RMSEP (%)	Bias(%)	RPD	RPIQ	Nv	F	R ²	RMSEP (%)	Bias(%)	RPD	RPIQ
protein	39	6	0.330	0.93	-0.15	1.23	1.39	39	3	0.259	0.96	-0.42	1.16	1.60
water content	42	6	0.517	0.92	0.09	1.44	1.41	40	4	0.784	0.69	-0.19	2.17	2.31
fat	42	2	0.710	0.72	0.05	1.88	2.70	42	2	0.668	0.76	0.11	1.75	2.63
ash	38	4	0.453	0.12	0.00	1.42	1.60	42	3	0.305	0.13	-0.02	0.80	1.54

Nv: number of validation samples; F: number of factors

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Chapter 4: Influence of the 'sous vide' process on the sensory quality of beef tournedo

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ABSTRACT

The effect of the 'sous vide' processing of beef tournedo subjected to four temperature/time combinations (56.1°C/62 min; 58°C/32 min; 62°C/12 min, and 72°C/no dwell time) was evaluated through selected sensory properties. The study assessed characteristics of appearance, aroma, flavor, and texture through different attributes for which the consumer judged the intensity of each, selecting the options of not applicable, weak, medium, or strong. The purchase intent of each sample was evaluated using a 5-point scale, and the degree to which the taster liked each sample was expressed through a nine-point hedonic scale. The PCA revealed three clear sensory clusters: one comprising treatments 1 and 2, another containing treatment 3, and a third encompassing treatment 4. There were no significant differences in the sensory scores for the attributes "characteristic meat flavor" and "glossy surface" ($p \geq 0.05$). Only treatment 2 differed ($p < 0.05$) from the others regarding overall acceptability and purchase intent, being evaluated as "liked very much" and "probably would buy," while the others were evaluated as "liked moderately" and "might buy; might not buy." Taking into consideration all the discussed sensory parameters, the ideal combination indicated by the consumers was 58°C / 32 min.

Keywords: sensory quality; low-temperature cooking; thermal treatment; meat

1. INTRODUCTION

Changes in lifestyle and the increasing number of women entering the labor market with full-time employment have led to a surge in the demand for semi-prepared foods and ready-to-eat meals (Kathuria et al., 2022). In addition to the convenience in meal preparation, there is a growing demand for a nutritionally balanced diet, prioritizing minimally processed foods that either do not contain synthetic additives or contain them in lower quantities (Can & Harun, 2015; Onyeaka et al., 2022).

This scenario presents challenges for the food industry, which must ensure microbiological safety and an extended shelf life without compromising composition or

nutritional value. One technique that meets these requirements is ‘sous vide’, which employs lower temperatures and prolonged cooking times, making it possible to offer ready-to-eat foods without the need for synthetic additives. (Tiban et al., 2024).

Sous-vide involves cooking vacuum-packed foods at moderate temperatures (50–85°C) for extended periods (2–48 hours), preserving nutritional and sensory properties. After cooking, the products are rapidly cooled and frozen until consumption. (Baldwin, 2012). This technique makes it possible to control attributes such as tenderness, color, juiciness, and palatability, in addition to reducing moisture loss and improving texture and digestibility (Thathsarani et al., 2022; Latoch et al., 2023; Karki et al., 2022).

Unlike conventional methods such as roasting, sous vide cooking takes place in airtight vacuum packaging, reducing oxidation and the loss of volatile compounds, which results in higher yield and superior sensory quality (Zavadlav et al., 2020; Fofanova, 2018). Studies also show greater retention of vitamins and minerals, with increased bioavailability of calcium, copper, iron, manganese, and zinc compared to traditional cooking methods (Ayub & Ahmad, 2019; Bhat et al., 2020; Modzelewska-Kapituła et al., 2019; da Silva et al., 2017).

Despite the reported advantages, it is not yet fully understood how different time-temperature combinations influence consumer sensory perception. This gap is significant, as attributes such as color, aroma, texture, and flavor are key determinants of the acceptance of ‘sous vide’ processed meat products (Dominguez-Hernandez et al., 2018; Mortensen et al., 2012).

To certify whether these improvements in sensory properties reported in the scientific literature regarding the ‘sous vide’ method are perceived by the consumer, sensory evaluation methods are applied. Rate-All-That-Apply (RATA) is one of them, serving as a sensory analysis tool that allows participants to evaluate the intensity or applicability of relevant attributes of a product (Ares et al., 2014).

Thus, this study aimed to evaluate the effect of different time/temperature combinations on the sensory properties and acceptance of sous-vide processed beef tournedos, complementing previously conducted physicochemical analyses (Ferreira et al., 2026). The goal was to identify cooking conditions that balance technological quality with consumer preference, thereby providing insights to enhance the flexibility and competitiveness of the industrial process.

2. MATERIAL AND METHODS

2.1 *Preparation of tournedos*

Chilled beef tournedos cuts (temperature between 0 and 4°C; pH between 5.7 and 5.8), purchased from a slaughterhouse approved and inspected by the Federal Inspection Service (Serviço de Inspeção Federal – SIF), were transported in refrigerated trucks and kept in a refrigerated chamber at 4°C until sample preparation.

In a tumbler, cold water (0–4°C), modified cassava starch, maltodextrin, salt, and black pepper were added to form a brine (temperature between 0°C and 4°C). The filet mignon pieces were added to the tumbler after the ingredients had been homogenized and were tumbled for 40 minutes to incorporate the brine. With the tumbling process, the temperature of the meat increases and therefore the pieces were then stored in a cold room at 4°C until they reached a temperature between 10°C and 12°C to ensure that the meat's retention capacity is not lost due to denaturation of the myofibrillar proteins. They were then rolled, wrapped in PVC film, and frozen in a cold room at -12°C. After the pieces reached a temperature between -2°C and -5°C, the plastic film was removed. Cross-sections (diameter between 57 and 60 mm and thickness between 38 and 40 mm) were hand-cut and weighed individually on a digital scale to a mass of $125 \text{ g} \pm 5 \text{ g}$, yielding approximately thirteen slices of tenderloin from each piece, referred to as a tournedo.

The tournedo was seared on a hotplate (temperature close to 215°C) to form a golden-brown color on the surface. The product reached a surface temperature of approximately 40°C and was then stored in a cold room at 4°C before packaging. Once cooled (between 4°C and 10°C), the tournedos were packed in heat-moldable, vacuum-sealed containers (Multivac).

2.2. *Cooking treatments*

Cooking treatments equivalent in terms of Salmonella thermal lethality (T1: 56.1°C/62 min; T2: 58°C/32 min; T3: 62°C/12 min; and T4: 72°C/no dwell time) were applied to samples of vacuum-packed beef tournedos. These temperature-time combinations were determined based on the 'Guideline for Meat and Poultry Products - Appendix A' (USDA & FSIS, 2021).

In the heat treatment studies, two 630-liter tanks (PPIENK, model BM-100E, Brazil) were used, each with a capacity for 30 samples. A datalogger (Testo 176 T4) with four thermocouples inserted in the geometric center of the product was used to monitor and record

the internal temperature. The inlet temperature of the product varied between 7°C and 10°C. After 3 minutes in a water bath at 85°C, the product reached a temperature of approximately 30°C.

Immediately, the bath temperature had been adjusted to the respective temperature of the heat treatment under study (T1: 56.1°C; T2: 58°C; T3: 62°C; and T4: 72°C).

After reaching the reference temperatures for each heat treatment, the residence times established for each treatment (T1: /62 min; T2: 32 min; T3: 12 min; and T4: 72°C/no dwell time) were recorded. Then, the product was pre-cooled for 15 minutes in a water bath at room temperature. Subsequently, the cage was moved to a second cooling tank (0 to 2°C) until the product reached a minimum internal temperature of 4.4°C. The samples were then quick-frozen in a nitrogen tunnel and stored in a freezer at -18°C until sensory analysis.

2.3 *Sensory analysis*

The samples previously cooked at 56.1°C / 62 minutes (T1); 58°C / 32 minutes (T2); 60°C / 12 minutes (T3), and 72°C / no dwell time (T4) were thawed under refrigeration (5 °C) and then prepared in a Rational Self Cooking Center oven using a temperature of 220 °C for 4 minutes, without humidity. Four samples were prepared at a time (one from each treatment), and while they were being heated, the recruited consumers received explanations on how to execute and complete the sensory evaluation form.

Each tournedo was divided into four equal pieces (halved twice); therefore, each sensory session was conducted with four panelists, ensuring that the sample (approximately 20 grams each) was served immediately after cutting without significant temperature loss. To transport the samples from the preparation site (Research and Development) to the break area, where the sensory analysis was performed, styrofoam boxes were used—one for each treatment—properly identified and lined with aluminum foil. The samples were served on a white disposable plate with a three-digit code (Figure 1).

The studied samples were evaluated for overall acceptability, purchase intent, and sensory characteristics by 100 consumers of 'sous vide' beef tenderloin tournedo (60 women and 40 men) aged between 18 and 63 years, all of whom were employees of the partner industry of this study, where the product is manufactured and sporadically offered as part of the meals in the company cafeteria. All evaluations were carried out in individual booths under white light. Acceptability was assessed for appearance, aroma, texture, and flavor using a 9-point hedonic scale (1 = "dislike extremely" to 9 = "like extremely"), while purchase intent was

evaluated on a 5-point scale (1 = "definitely would not buy" to 5 = "definitely would buy").

Sensory characterization was performed using the "Rate-All-That-Apply" (RATA) Test (Ares et al., 2014). Four coded samples were presented to the consumers in a monadic format and random order, along with an evaluation form containing 15 descriptive terms (red internal color; brown internal color; nice appearance; surface gloss; brown surface color; aroma of blood/raw meat; aroma of cooked meat; oxidized (rancid) aroma; characteristic meat flavor; oxidized (rancid) taste; taste of blood; strange flavor; soft; juicy/moist; firm/hard). The sensory terms were determined based on the literature. Consumers selected the applicable terms and assessed their intensity on a 3-point scale (0 = not applicable, 1 = weak, 2 = medium, 3 = strong). The order of the terms contained in the sensory form was randomized per consumer, while the sample order remained fixed. The form used for the sensory analysis is presented in Figure S1 of the Supplementary Materials.

Water and cracker biscuits were provided as palate cleansers between samples. This experiment was conducted in accordance with the ethical approval number CAAE: 73433123.7.0000.5588.

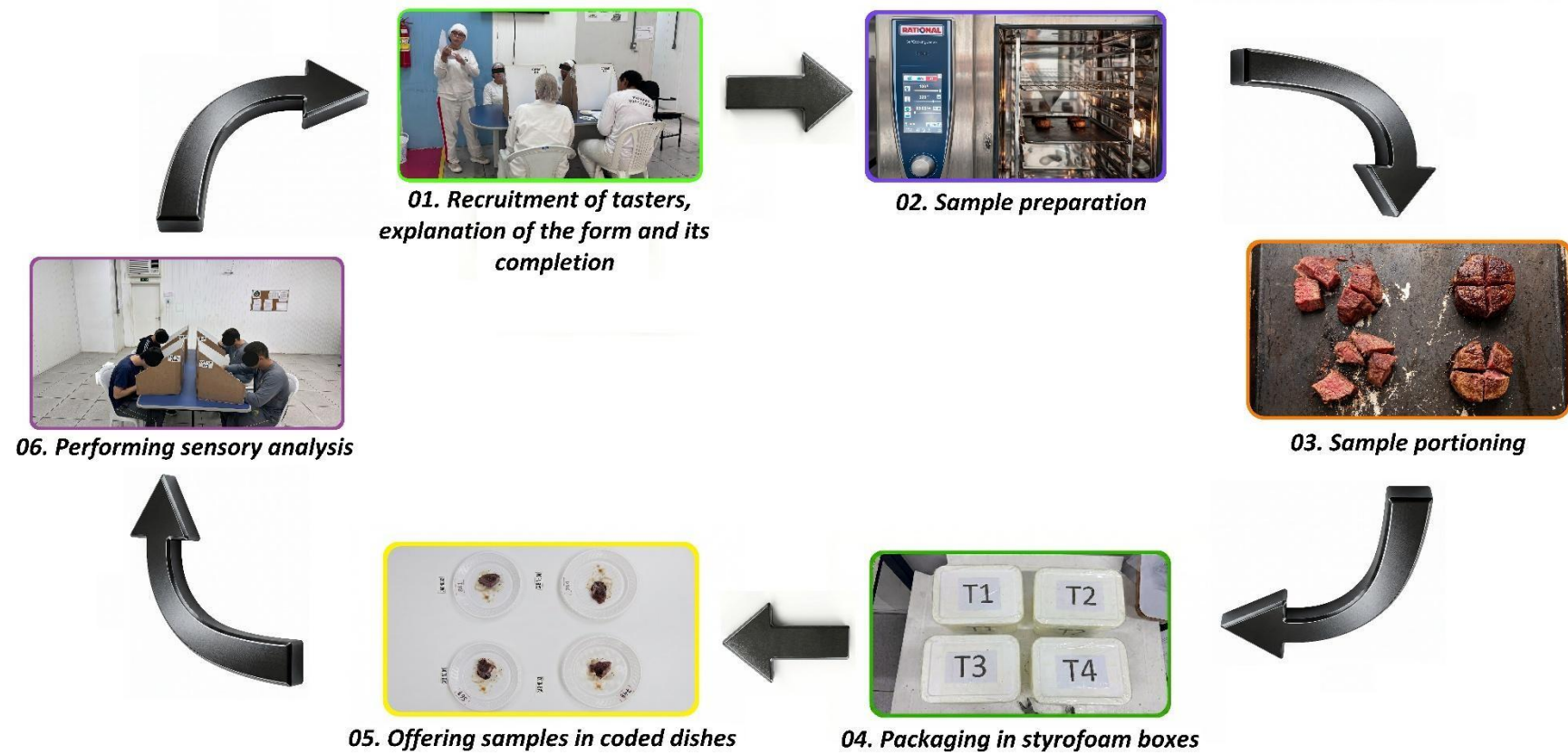


Figure 1: Sensory analysis diagram

2.4 Statistical Analysis

Data were analyzed using a one-way ANOVA and Tukey's test, with a significance level of 0.05, using R software (version 4.2.2). Furthermore, correlations among obtained data were conducted using the Pearson's correlation coefficient (r). Additionally, sensory data were analyzed using principal component analysis (PCA).

3. RESULTS AND DISCUSSION

Attributes such as appearance, flavor, aroma, and texture are key predictors of consumer acceptance of food products. These sensory parameters are crucial to a product's market success, as they directly influence quality perception and purchase intent. Consequently, the food industry needs to employ a variety of sensory tools during the development, reformulation, and evaluation of products, processes, and ingredients to ensure that technological modifications maintain or enhance desired characteristics. (de Kock & Magano, 2020).

Thus, the researchers Ferreira et al. (2026), when evaluating the impact of the 'sous vide' process on the quality of beef tournedo through microbiological, physical, and chemical analyses, suggested complementing the study with sensory evaluation as future work. The results regarding sensory characteristics, acceptability tests, and purchase intention for tournedos subjected to different time-temperature combinations during the sous-vide cooking process are presented in Table 1.

Table 1. Mean hedonic scores and purchase intent of beef tournedo 'sous vide' processed at different temperatures/times.

Treatments	Appearance				Aroma			Flavor				Texture				Global impression	purchase intention
	Red internal color	Brown internal color	Nice appearance	Shine on the surface	Brown surface color	Blood Aroma	Cooked meat aroma	Oxidized (rancid) aroma	Characteristic meat flavor	Oxidized (rancid) taste	Taste of blood	Strange taste	Soft	Juicy / moist	Firm / hard		
T1	1.78 ^a	1.22 ^c	1.64 ^b	1.49 ^a	1.67 ^b	0.92 ^a	1.30 ^c	0.39 ^a	1.63 ^a	0.41 ^a	1.03 ^a	0.40 ^a	1.89 ^a	1.75 ^b	0.74 ^c	7.17 ^b	3.76 ^b
T2	1.52 ^b	1.53 ^b	1.87 ^a	1.55 ^a	1.81 ^a	0.66 ^b	1.51 ^b	0.21 ^b	1.69 ^a	0.20 ^b	0.79 ^b	0.22 ^b	1.89 ^a	1.89 ^a	0.47 ^d	8.31 ^a	4.59 ^a
T3	1.38 ^c	1.53 ^b	1.68 ^b	1.43 ^a	1.85 ^a	0.58 ^b	1.73 ^a	0.38 ^a	1.68 ^a	0.33 ^a	0.63 ^c	0.44 ^a	1.85 ^a	1.68 ^b	0.87 ^b	7.30 ^b	3.90 ^b
T4	0.94 ^d	1.91 ^a	1.60 ^b	1.30 ^a	1.84 ^a	0.36 ^d	1.79 ^a	0.42 ^a	1.64 ^a	0.45 ^a	0.40 ^d	0.49 ^a	1.66 ^b	1.53 ^c	1.07 ^a	7.00 ^b	3.58 ^b

Reference heat treatments: T1 (56.1 °C/62 min); T2 (58 °C/32 min); T3 (62 °C/12 min); T4 (72 °C/no dwell time).

Means with different lowercase letters in the column indicate a significant difference by Tukey's test ($p < 0.05$).

Tenderness, juiciness, flavor, and color are the primary factors affecting the choice and acceptance of cooked meat by consumers. Low-temperature cooking methods, such as 'sous vide', are sought to ensure these sensory characteristics. Thus, the overall acceptability of the beef tournedo processed at different 'sous vide' time/temperature binomials was evaluated, which did not differ significantly from one another ($p \geq 0.05$) and were rated as "liked moderately," with the exception of treatment 2, which differed from the others and was rated as "liked very much." This result expands the processing possibilities for the industry or even assists in decision-making regarding a product that may have undergone temperature variations throughout the process.

Regarding the "appearance" attribute, there was a significant difference ($p < 0.05$) across the five evaluated characteristics. Considering that the meat color change caused by heating is used as one of the indicators of the cooking stage, the internal coloration followed the expected trend of a higher intensity of red in samples subjected to milder thermal treatments, with treatment 1 having the reddest coloration and treatment 4 the least red.

The brown internal coloration and brown external coloration also met this expectation, being more intense in the harsher thermal treatments (T4) and less intense in the milder ones (T1). Mortensen et al. (2012) also reported a loss of red color on the cut surface and an increase in the intensity of the brown color with the increase in the 'sous vide' cooking temperature of beef. Regarding internal brown discoloration, the consumer did not perceive a significant difference among treatments 2, 3, and 4 ($p \geq 0.05$).

This loss of red color is due to the denaturation of myoglobin with the subsequent oxidation of Iron (II) in the denatured myoglobin to Iron (III) (Møller & Skibsted, 2006). Although myoglobin is stable up to approximately 60 °C, during thermal treatments at temperatures below 60 °C for prolonged periods, the onset or partial denaturation of myoglobin is expected, which can cause an increasing degree of cooking perceived visually. Not only the heating temperature but also the time period at that temperature affects the denaturation of myoglobin (Christensen et al., 2012). According to Davey & Gilbert (1974), even at 43°C, the color of cooked meat is modified. Vaudagna et al., (2002) observed a significant decrease in the red color of 'sous vide' beef at 50 °C, 60 °C, and 65 °C.

As for the characteristics of surface gloss and characteristic meat flavor, consumers did not perceive a significant difference between the samples.

The aroma attribute was evaluated regarding the aroma of blood, cooked meat, and oxidized (rancid) aroma. Regarding the latter, samples 1 and 2 differed significantly from the others ($p < 0.05$), with sample 2 showing a lower score for oxidation aroma. Regarding the blood aroma, all samples differed from one another ($p < 0.05$). However, the cooked meat aroma showed no significant difference between ($p \geq 0.05$) treatments 3 and 4. The treatment with the lowest temperature exhibited the highest aroma of blood and the lowest aroma of cooked meat. Following the same trend, as the temperature increased across the treatments, the aroma of blood decreased, and the aroma of cooked meat increased, with the exception of treatment 4. Similar to the aroma, the flavor was also evaluated regarding the taste of blood, characteristic cooked meat flavor, and oxidized taste.

The taste of blood received an evaluation very close to the aroma of blood, where all samples differed significantly from each other ($p < 0.05$), and the one subjected to the lowest thermal treatment received the highest score for these characteristics. However, in the evaluation of the oxidized taste, perceived mainly by the formation of aldehydes, ketones, and esters in the meat due to lipid oxidation (Latoch et al., 2023), only sample 2 differed from the others ($p < 0.05$), receiving the lowest score. All samples were evaluated with the same intensity

of characteristic meat flavor ($p \geq 0.05$). It would be expected that the oxidized aroma and taste, as well as the characteristic meat aroma and flavor, would exhibit the same behavior. However, orally evaluated descriptors (flavor) can sometimes be more precise than the corresponding aroma descriptors, which are evaluated by the nose (Mortensen et al., 2012).

Consumers also evaluated the perception of off-flavors in the analyzed samples; sample T2 differed significantly ($p < 0.05$) from the others and exhibited the lowest intensity for this characteristic.

The texture was evaluated according to the characteristics of softness, juiciness, and 'hardness'. The sample evaluated as the least soft (T4) was also rated as the firmest among the others ($p < 0.05$). Tenderness is a crucial characteristic of meat quality (Dominguez-Hernandez et al., 2018) and is associated with the amount of connective tissue, sarcomere length, and post-mortem protein degradation. Thermal treatment modifies the meat's structure, as the denaturation, aggregation, and degradation of myofibrillar and sarcoplasmic proteins and connective tissue depend primarily on the temperature used during cooking (Ismail et al., 2019). Muscle fibers begin to shrink between 35 and 40 °C and continue up to 80 °C, increasing the space between them, and actin and myosin denature at 40–50 °C (Tornberg, 2005). Additional heating of the meat causes fiber shrinkage, reducing water-holding capacity. This shrinkage increases with temperature (Baldwin, 2012), as does protein denaturation, increasing fluid loss (Li et al., 2013; Zhang & Wang, 2012) and rendering the meat firmer. Therefore, the meat will be softer, juicier, and less firm when cooked at lower temperatures, which justifies the consumer's perception of sample T4 as the least soft and the firmest among the others.

There was no significant difference ($p \geq 0.05$) among samples T1, T2, and T3 in the evaluation of softness, nor between samples T3 and T4 in the evaluation of juiciness. Sample T2 was evaluated as being less firm and juicier ($p < 0.05$). Heating at low temperatures reduces cooking losses compared to higher temperatures, resulting in a juicier meat product (Kathuria et al., 2022).

In terms of purchase intent, no significant differences were observed among the 'sous vide' beef tournedo samples ($p \geq 0.05$), being assigned the term "might buy; might not buy," with the exception of sample 2, which was assigned the term "probably would buy."

The preference map derived from the principal component analysis (PCA) based on the results of the overall impression acceptability test (Figure 2) was employed to obtain a broader understanding of the individual acceptability of consumers. Brito et al. (2025) emphasize that this analysis allows the visualization of subtle factors that influence the overall

acceptability of the samples, enabling a more detailed analysis of consumer preferences.

The first principal component (PC1) explained 47.08% of the total variability contained in the original variables, and the second (PC2) explained 29.94%, totaling 77.02% of the variance. (Rosenthal, 1999) states that an adequate result is one in which at least 70 to 80% of the variation among the treatments is explained in the first three principal components. Thus, in this study, the first two principal components were used, as they are in accordance with the aforementioned author; that is, PC1 and PC2 were considered representative of the sample dispersion, since they encompass most of the data variation.

In Figure 2, consumers are represented as dots on the scatter plot, and their proximity to the sample labels (T1, T2, T3, and T4) reflects a higher level of acceptance relative to the overall acceptability. This observation highlights a greater concentration of consumers closer to T2, which indicates a higher acceptance of the 'sous vide' beef tournedo sample processed at 58°C / 32 minutes.

It is important to note that the graph contains overlapping data points, making it difficult to perceive homogeneity. Furthermore, 8% of the consumers are located at the zero point of the graph, indicating a uniform acceptance of the samples by these consumers. This suggests that these consumers did not perceive differences among the 'sous vide' beef tournedo samples processed at different temperatures/times regarding overall acceptability.

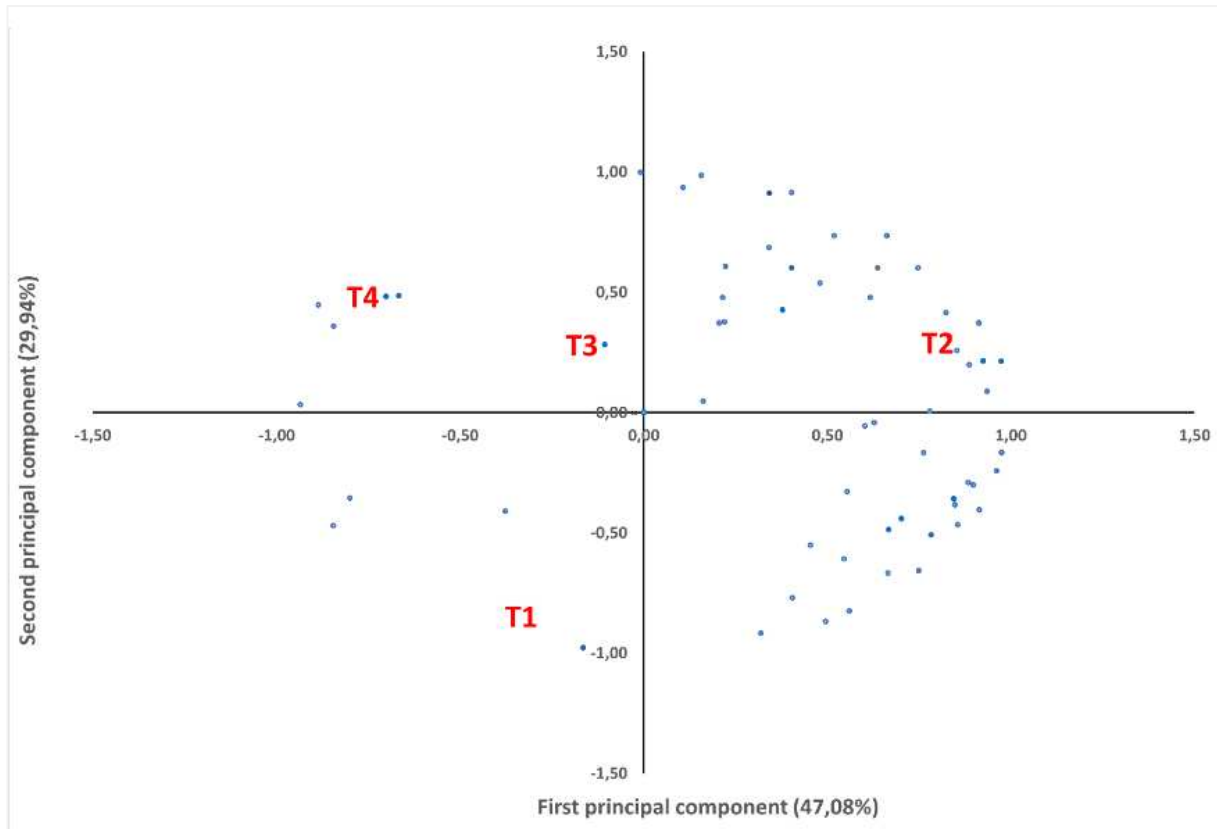


Figure 2. Preference map of the overall acceptability of beef tournedo ‘sous vide’ processed at different temperatures/times.

Reference heat treatments: T1 (56.1 °C/62 min); T2 (58 °C/32 min); T3 (62 °C/12 min); T4 (72 °C/no dwell time).

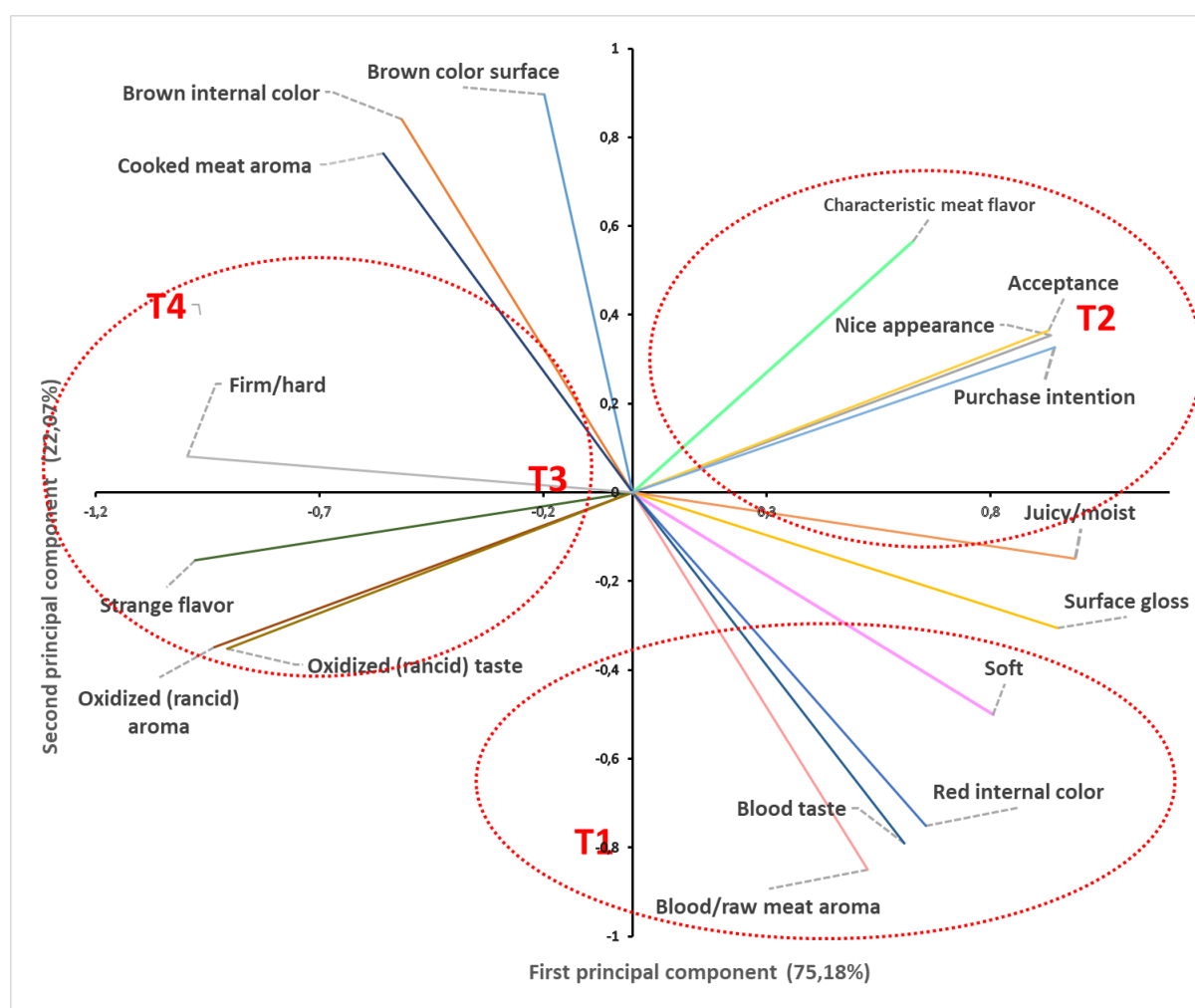
Descriptive sensory tests play a crucial role in improving product and process quality, comparing treatments, identifying development problems, and aligning with consumer preferences.

In this study, the "Rate-All-That-Apply" (RATA) test was employed, allowing consumers to identify and evaluate the intensity of the sensory attributes in the sample. The Principal Component Analysis (PCA) plot (Fig. 3) illustrates how each treatment relates to the main sensory descriptors, being a correspondence analysis derived from the RATA results.

The treatments of 'sous vide' beef tournedo processed at different temperatures/times did not show significant differences in the attributes "characteristic meat flavor" and "surface gloss" ($p \geq 0.05$). The PCA revealed clear sensory clusters that aligned with the results of the acceptability tests. Overall, the samples were grouped into three clusters: one group comprising treatments 3 and 4, another containing treatment 2, and a third group encompassing treatment 1. The first principal component alone explained the total variation among the treatments regarding the sensory attributes, accounting for 75.18% of the total variance, and the second

component (PC2) 22.07%. Together, these two components explained 97.25% of the total data variation, proving to be adequate for distinguishing the samples in terms of overall acceptability.

Figure 3. Descriptive sensory map obtained from treatments of beef tournedo ‘sous vide’ processed at different temperatures/times.



Reference heat treatments: T1 (56.1° C/62 min); T2 (58° C/32 min); T3 (62° C/12 min); T4 (72° C/no dwell time).

The main attributes associated with treatment 1 were "red internal color," "taste of blood," "soft," "blood/raw meat aroma". On the other hand, the main attributes associated with treatment 2 were "juicy/moist", "purchase intent", "nice appearance", "overall acceptability" and characteristic meat flavor. Sample T3 and T4, however, exhibited characteristics of "oxidized taste", "oxidized (rancid) aroma", "strange flavor", "firm/hard" and "cooked meat aroma." Based on the sensory attributes associated with the samples, it can be inferred that sample T2 presented higher levels of overall acceptability, nice appearance, and purchase intent compared to the other treatments.

Therefore, the RATA results contributed to the acceptability test results: the treatment 2 closest to the attributes of softness, juicy/moist, red internal color, and nice appearance were the most preferred, whereas the one linked to strange flavor, firm/hard, and oxidized aroma was less appreciated.

4. CONCLUSION

This study represents an enhancement of a published work and is considered an advancement in the scientific literature, as it complements the microbiological validation and physical and chemical quality already performed, with the sensory evaluation of industrial meat products originating from equivalent thermal processes.

The use of the RATA descriptive sensory technique revealed that the treatment at 58°C / 32 min was the most accepted and had the highest purchase intent compared to the others ($p < 0.05$). The evaluated sensory attributes remained consistent with this result, with emphasis on softness and juiciness, which were pointed out as preferred by consumers for the treatment in question.

Therefore, considering the results of the sensory attributes evaluated by the consumers, the most accepted and best-rated combination was 58°C / 32 min. This result confirms the evaluation resulting from the work of Ferreira et al. (2026), who considered the same treatment as the most appropriate, based on the microbiological, physical, and chemical characteristics of the 'sous vide' beef tournedo.

It is important to note that the quality over the shelf life was not evaluated in the present study. Future research should focus on stability analysis to understand how each treatment will affect quality retention throughout its shelf life.

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Supplementary material - S1



FEDERAL UNIVERSITY OF VIÇOSA
DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY



Sensory Assessment Form

BRASIL GOURMET

Age: _____ Gender: () F () M () I prefer not to identify myself

Sample Code: _____

You are receiving a sample of **beef tournedo**. Please **rate the sample** and indicate **your opinion** on the appearance and aroma. Then, **taste the sample** and **evaluate the flavor and texture** as you consume it. **Identify (mark with an X) your opinion** regarding the intensity of the attributes.

Feature	ATTRIBUTES	NOT APPLICABLE	WEAK	AVERAGE	STRONG
Appearance	Red internal color	()	()	()	()
	Brown internal color	()	()	()	()
	Nice appearance	()	()	()	()
	Surface gloss	()	()	()	()
	Surface gloss	()	()	()	()
Aroma	Aroma of blood/raw meat	()	()	()	()
	Aroma of cooked meat	()	()	()	()
	Oxidized (rancid) aroma	()	()	()	()
Flavor	Characteristic meat flavor	()	()	()	()
	Oxidized (rancid) taste	()	()	()	()
	Taste of blood	()	()	()	()
	Strange flavor	()	()	()	()
Texture	Macia	()	()	()	()
	Juicy/moist	()	()	()	()
	Firm/hard	()	()	()	()

► Mark with an X, using the scale below, **how much you liked or disliked** the product:

- () I really liked it
 () I liked it
 () I liked it moderately
 () I liked it slightly
 () I neither liked/disliked
 () I slightly disliked it
 () I moderately disliked it
 () I disliked it
 () I really disliked it

► What would be your **purchasing intention** in relation to this sample you just tried? Give your rating from 1 to 5, being:

- 1 = I certainly wouldn't buy it
 2 = I probably wouldn't buy
 3 = maybe I would buy it; maybe I wouldn't buy it
 4 = I would probably buy
 5 = I would certainly buy

Score: _____

FINAL CONCLUSION

‘Sous vide’ processing of beef tenderloin medallions can be conducted safely and efficiently by integrating thermal validation, physicochemical characterization, and sensory analysis.

Regarding microbiological safety, a thermal protocol ensuring product safety was established. Physicochemical characterization demonstrated that this treatment preserves key technological attributes, while predictive modeling using NIR spectroscopy proved to be a rapid, sustainable tool for estimating proximate composition—aligning with Industry 4.0 requirements. Sensory analysis confirmed that the same thermal protocol maximizes consumer-perceived quality attributes, such as tenderness and juiciness, resulting in higher purchase intent.

Collectively, the results establish an integrated methodological system that balances sanitary rigor, technological quality, and sensory acceptance. The scientific contribution of this thesis lies in proposing a model applicable to other meat products, while its practical contribution lies in providing validated protocols and modern analytical tools for the food industry.