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Examination of indicator and pathogenic microbiota during the shelf-life of vacuum-packed *Longissimus dorsi* (sirloin)

Acompanhamento da microbiota indicadora e patogênica durante a vida útil de *Longissimus dorsi* (contrafilé) embalado a vácuo

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Highlights

Quantity of *E. coli* did not comply with the legal standard after 20 d of shelf-life.
Psychrotrophs, especially *Pseudomonas,* progressively increased during shelf-life.
Listeria monocytogenes was detected in all analyses.
EPEC, ETEC, STEC, and EIEC were identified.
Salmonella spp. was detected at day 20 of shelf-life period.

Abstract

Brazil is among the largest meat producers worldwide. Owing to the high productivity and concern regarding meat quality, slaughterhouses are looking for better ways to preserve meat. Vacuum packaging is the most widely used method to preserve meat. In this study, we aimed to monitor the indicator and pathogenic microbiota during the shelf-life of vacuum-packed bovine, *Longissimus dorsi.* Sirloin samples collected and conditioned in the boning section of a slaughterhouse under the Brazilian federal inspection were evaluated. Each sample was divided into four pieces, and each piece was used to make up a part of each pool, totaling four pools suspended at 7 ºC and analyzed from 0 to 60 d of primary packaging, with an interval of 20 d. Mesophilic aerobes, psychrotrophs, enterobacteria, coliforms at 30 °C, *Escherichia coli* and *Staphylococcus* spp. were quantified. Moreover, pathotypes of Shiga toxin-producing (STEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroaggregative (EAEC), enterotoxigenic (ETEC), and enteroinvasive (EIEC) *E. coli, Pseudomonas* spp. (psychrotrophs), *Salmonella* spp., and *Listeria monocytogenes* were characterized. Number of indicator microorganisms progressively increased at

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each analysis interval. Specifically, psychrotrophs increased from 5 × 10^1 CFU/g on day 0 to 4.2 × 10^8 CFU/g on day 60, and *Pseudomonas* spp. was the predominant species (48%). The limits for standard counts set by the current Brazilian legislation were exceeded, such as for *E. coli* since day 20 (7 × 10^2 CFU/g). EPEC, ETEC, STEC, and EIEC were identified in addition to *Listeria monocytogenes* and *Salmonella* spp.; the latter was not detected on the first day of shelf-life. Therefore, revisions to self-control plans and greater microbiological rigor in the production and processing of beef are necessary to improve its shelf-life and safety.
Key words: Beef. Conservation. Microbiological quality. Shelf-Life.

Resumo

O Brasil é um dos maiores produtores de carne do mundo. Tendo em vista essa grande produtividade e a preocupação com a qualidade da carne produzida, os estabelecimentos produtores estão buscando meios para maior conservação do produto, sendo a embalagem a vácuo um dos mais utilizados. O objetivo do trabalho foi acompanhar a microbiota indicadora e patogênica durante a *shelf life* de *Longissimus dorsi* bovino embalado a vácuo. Foram avaliadas amostras de contrafilé coletadas e acondicionadas na seção de desossa de um frigorífico sob inspeção federal. Cada amostra foi fracionada em quatro peças e cada peça foi utilizada para compor uma parte de cada um dos *pools,* sendo totalizado quatro *pools* mantidos à 7ºC e analisados de 0 até os 60 dias de embalagem primária, com intervalo de 20 dias. Foram quantificados aeróbios mesófilos, psicrotróficos, enterobactérias, coliformes a 30°C, *Escherichia coli* e *Staphylococcus* spp. Através de abordagens moleculares foram caracterizados os patótipos de E. coli produtora da toxina shiga (STEC), enteropatogênica (EPEC), enterohemorrágica (EHEC), enteroagregativa (EAEC), enterotoxigênica (ETEC) e enteroinvasiva (EIEC), *Pseudomonas* spp. entre os psicrotróficos, *Salmonella* spp. e *Listeria monocytogenes.* As quantificações dos micro-organismos indicadores foram aumentando progressivamente a cada intervalo de análise, com destaque para os psicrotróficos que aumentaram de 5 x 10^1 no dia 0 para 4,2 x 10^8 UFC/g no dia 60, predominando *Pseudomonas* spp. (48%). As contagens que possuem padrão determinado por legislações vigentes tiveram seus limites ultrapassados, como *E. coli*, desde o dia 20 (7 x 10^2 UFC/g). Foram identificadas EPEC, ETEC, STEC e EIEC, além de *L. monocytogenes* em todas as análises e *Salmonella* spp., essa última só não detectada no primeiro dia de *shelf life.* Fazem-se necessárias, portanto, revisões nos planos de autocontrole assim como maior rigor microbiológico na produção e processamento da carne bovina para melhoria da *shelf life* do produto e aumento da sua segurança.
Palavras-chave: Carne bovina resfriada. Conservação. Qualidade microbiológica. Vida útil.

Introduction

Brazil is among the largest cattle meat producers, being the second largest producer and largest exporter of cattle meat worldwide (Instituto Brasileiro de Geografia e Estatística [IBGE], 2023). In 2022, estimated 9.7 million tons of cattle meat were produced in Brazil, generating over 1.3 billion dollars in export revenue (Associação Brasileira das Indústrias Exportadoras de Carnes [ABIEC], 2023).

Considering its significant demand and growing consumer interest in high-quality products, all production chains are required to prepare food according to the international and national legal standards and meet the consumer expectations while preserving the meat quality during logistical processes of distribution and sale (Bezerra & Martins, 2008).

Food and water are the sources of disease transmission, mostly via infections and poisoning. Therefore, animal-derived food is important as several pathogens are part of the autochthonous microbiota of production animals. Cattle meat is contaminated by several human pathogens from animal microbiota, such as *Salmonella* spp. and *Escherichia coli* enteropathogens, and other microbes due to improper or unsanitary/unhygienic processing, such as *Listeria monocytogens* and *Staphylococcus aureus* (Carhuallanqui-Pérez, 2020).

To preserve the quality and safety of meat and minimize the product losses due to short shelf-life, modified atmosphere packaging, vacuum packaging, and refrigeration are used to prevent quality alterations and microbial deterioration of the product (McMillin, 2008). These methods combined with temperature control inhibit microbial multiplication (Redondo-Solano et al., 2020).

Brazilian legislation does not provide any expiration date for meat products; it is at the discretion of each production establishment to define them as long as they are based on studies and guarantee the physicochemical, microbiological, and sensory stability of the product (Furlanetto, 2020). Vacuum packaging is mostly used for meat products as deboning is the only factor to enhance the shelf-life of a product (Millset al., 2014).

In this study, we aimed to monitor the indicator and pathogenic microbiota during the shelf-life of vacuum-packed *Longissimus dorsi* (sirloin) via microbiological analysis and biomolecular characterization. Moreover, we identified the predominant microbiota under refrigeration and primary partial vacuum packaging conditions.

Materials and Methods

In this study, five samples were collected from sirloin beef cuts (*L. dorsi*) and subjected to vacuum packaging. The cut was chosen in response to industry demands for establishing an operational sanitary procedure using predictive microbiology. The samples were collected from an abattoir registered with the Federal Inspection Service (FIS) in Araguaína city in the northern region of Tocantins State, Brazil. The number of meat cuts evaluated was also

based on the FIS-determined shelf-life. Each sample was divided into four pieces for each collaborator in the establishment according to the ordinary operational flow in the slaughterhouse. Each of the four pieces was then used as a part of the pools, totaling four pools with five fractions each, with one for each meat cut. The pools were constructed on-site and packaged in standard vacuum-sealed packaging.

The samples were refrigerated at the Food Microbiology Laboratory of the North Federal University of Tocantins in the same city and immediately evaluated. The samples were stored under refrigeration (≤7 °C) in a biochemical oxygen demand booth, as per specific indications in the fabricant's label. Initial analysis was conducted on the same day of fractionation (D-0) and successive analyses were conducted, one for each pool, after 20 (D-20), 40 (D-40), and 60 (D-60) d of storage at 7 ºC.

The samples comprising each pool were gathered in a sterile plastic bag and homogenized in Stomacher for 180 s (Alnajrani et al., 2018). Serial decimal dilutions were performed in a saline solution (0.85%).

Number of coagulase-positive *Staphylococci* was determined according to the ISO 6888-1:1999/Amd 1:2003 method (International Organization for Standardization 6888-1 [ISO], 1999). Surface seeding was performed on Baird-Parker agar plates. After colony isolation, a catalase test was conducted. The coagulase test yielded positive results (Costa et al., 2011).

For psychrotrophs, serial decimal dilutions were inoculated on the surface of standard agar plates for counting and subsequently incubated at ± 7 °C for 10 d (Frank & Yousef, 2004).

The counts of mesophilic aerobes, enterobacteria, total coliforms (30 ºC), and *Escherichia coli* were determined using Compact Dry TC, ETB, and EC (Nissui Pharmaceutical, Tokyo, Japan), according to the manufacturer's instructions.

Since D-40, predominance of psychrotrophs was observed, and *Pseudomonas* spp. were distinguished from other psychrotrophs to verify the evolution of microorganisms relevant for quality assessment due to known deterioration potential.

E. coli isolates and psychrotrophs distinguished from *Pseudomonas* spp. were recovered by brain heart infusion (BHI) and subjected to DNA extraction as described by Ribeiro et al. (2016). In *E. coli* isolates, virulence factors *eaeA* and *CVD432* and *LT* e *ST, stx1, stx2,* and *ipaH* genes (Aranda et al., 2004) were investigated via polymerase chain reaction (PCR), as presented in Table 1. For confirmation of enteropathogenic (EPEC), Shiga toxin-producing (STEC), enteroaggregative (EAEC), enteroinvasive (EIEC), enterotoxigenic (ETEC), and enterohemorrhagic (EHEC) *E. coli,* characterization was performed by determining the positivity of genes encoding STEC and EPEC. *stx1, stx2, LT, ST,* and *ipaH* genes were analyzed using a multiplex essay with five pairs of primers. The same assay was also used for gene *eaeA* and target *CVD432,* but with two pairs of primers at the same concentration and volume optimized for 25 µL. Moreover, *Pseudomonas* spp isolates were subjected to *16S rRNA* analysis (Spiker et al., 2004), as presented in Table 1.

Salmonella spp. were detected according to the ISO 6579 (2005) method, and *Listeria* spp. were detected according to the ISO 11290 (2004) method, with some modifications. After isolation on plates, different strains were recovered in BHI and subjected to DNA extraction (Ribeiro et al., 2016) and genus- and species-specific PCR (Table 1).

For all PCR essays, DNA extraction products (approximately 50 ng) were used at a final volume of 25 µL and the PCR conditions were the same as those described by Ribeiro et al. (2019).

Results and Discussions

In this study, the number of indicator microorganisms progressively increased at each interval between analyses (Table 2 and Figure 1).

The results of the research on mesophilic aerobic microorganisms were non-standard according to the current legislation after D-40 (Figure 1). The Normative Instruction n° 161 since 2022 (Agência Nacional de Vigilância Sanitária [ANVISA], 2022) sets the standard at or below 1.0×10^6 UFC/g for raw cattle meat. Here, the evaluated samples showed mesophilic aerobes of 1.2×10^8 and 7.0×10^8 UFC/g at D-40 and D-60, respectively (Table 1).

Bomar (1985) proposed that meat exhibiting a mesophilic aerobic count of 3.5×10^7 UFC/g is unfit for consumption. Based on this assumption, *L. dorsi* evaluated in this study was considered to be unfit for consumption from D-40.

Psychrotrophs are the best indicators of refrigerated food contamination (Wei et al., 2019); proteolytic and lipolytic microorganisms are the most predominant psychrotrophs (Ribeiro et al., 2018; Wei et al., 2019), mainly responsible for meat deterioration (Djordjević et al., 2018; Wickramasinghe et al., 2019). However, Brazilian legislation does not establish standards for psychrotrophs in meat or other foods. Considering the standard 1.0×10^7 UFC/g, stipulated by the International Commission on Microbiological Specifications for Foods [ICMSF] (1986a; 1986b), the samples evaluated by the present study were also in disagreement starting from D-40, in which was observed count of 1.4×10^7 UFC/g.

In the present study, the psychrotrophs were increasing according to the intervals between the analysis, ranging from 5 × 101 at D-0 to $>10^5$, 1.4×10^7 and 4.2×10^8 at D-20, D-40 and D-60, respectively, as presented in Table 2, progressive increase practically linear (Figure 1). This increase in psychrotrophic bacteria was also observed by Marquezini et al. (2016), considering that the sample evaluated on day 60 was beyond the standard parameter stipulated by the ICMSF. According to these authors, this occurred because of the temperature conditions conducive to the development of these microorganisms and the limitations of the multiplication of strict mesophilic microorganisms.

Table 1
Genes and amplifications conditions of PCRs

Microorganism	Gene	Primers (5′ - 3′)	Sizes (bp)	Amplification conditions	Reference
Salmonella spp.	*invA*	GTGAAATTATCGCCACGTTCGGGCAA	284	94ºC-1m 35x (94ºC-1m, 64ºC-30s, 72ºC-30s) 72ºC-7m	Shanmugasamy et al. (2011)
		TCATCGCACCGTCAAAGGAACC			
Listeria monocytogenes	*lmo0733*	CGCAAGAAGAAATTGCCATC	465	95ºC-5m 40x (94ºC-45s, 52ºC-45s, 72ºC-2m) 72ºC-10m	Chen and Knabel (2007)
		TCCGCGTTAGAAAAATTCCA			
Escherichia coli	*eaeA*	CTGAACGGCGATTACGCGAA	917	95ºC-5m 40x (95º-40s, 56ºC-1m, 72ºC-2m) 72ºC-7m	
		CCAGACGATACGATCCAG			
	CVD432	CTGGCGAAAGACTGTATCAT	630		
		CAATGTATAGAAATCCGCTGTT			
	stx1	ATAAATCGCCATTCGTTGACTAC	180		
		AGAACGCCCACTGAGATCATC			
	stx2	GGCACTGTCTGAAACTGCTCC	255		
		TCGCCAGTTATCTGACATTCTG			
	LT	GGCGACAGATTATACCGTGC	450	95ºC-5m 40x (95ºC-45s, 50ºC-1m, 72ºC- 1m) 72ºC-7m	Aranda et al. (2004)
		CGGTCTCTATATTCCCTGTT			
	ST	ATTTTTMTTTCTGTATTRTCTT	190		
		CACCCGGTACARGCAGGATT			
	ipaH	GTTCCTTGACCGCCTTTCCGATACCGT	600		
		GCCGGTCAGCCACCCTCTGAGAGTAC			
Pseudomonas spp.	*16S rRNA*	GACGGGTGAGTAATGCCTA	618	94ºC-1m 35x (94ºC-1m, 58ºC-1m, 72ºC-1m) 72ºC-10m	Spiker et al. (2004)
		CACTGGTGTTCCTTCCTATA			

Table 2
Microbiological counts (CFU/g) during each stage of *Longissimus dorsi's* shelf life collected in a slaughterhouse at North of Tocantins, Brazil, from September 25th to December 6th of 2021

Repetition *Shelf life*	Mesophilic aerobes	Psichrotrophics	Coliforms at 30°C	*Escherichia coli*	Enterobacteria	Coagulase-negative *Staphylococcus*
D-0	$1.8x10^4$	$5x10^1$	$2.0x10^1$	$1.0x10^1$	$2.3x10^2$	$4.0x10^2$
D-20	$3.8x10^5$	$>10^5$	$1.7x10^4$	$7.0x10^2$	$3.5x10^4$	$1.6x10^3$
D-40	$1.2x10^8$	$1.4x10^7$	$5.2x10^6$	$2.5x10^5$	$1.9x10^6$	$3.1x10^5$
D-60	$7.0x10^8$	$4.2x10^8$	$3.1x10^6$	$3.0x10^4$	$1.1x10^7$	$>10^6$

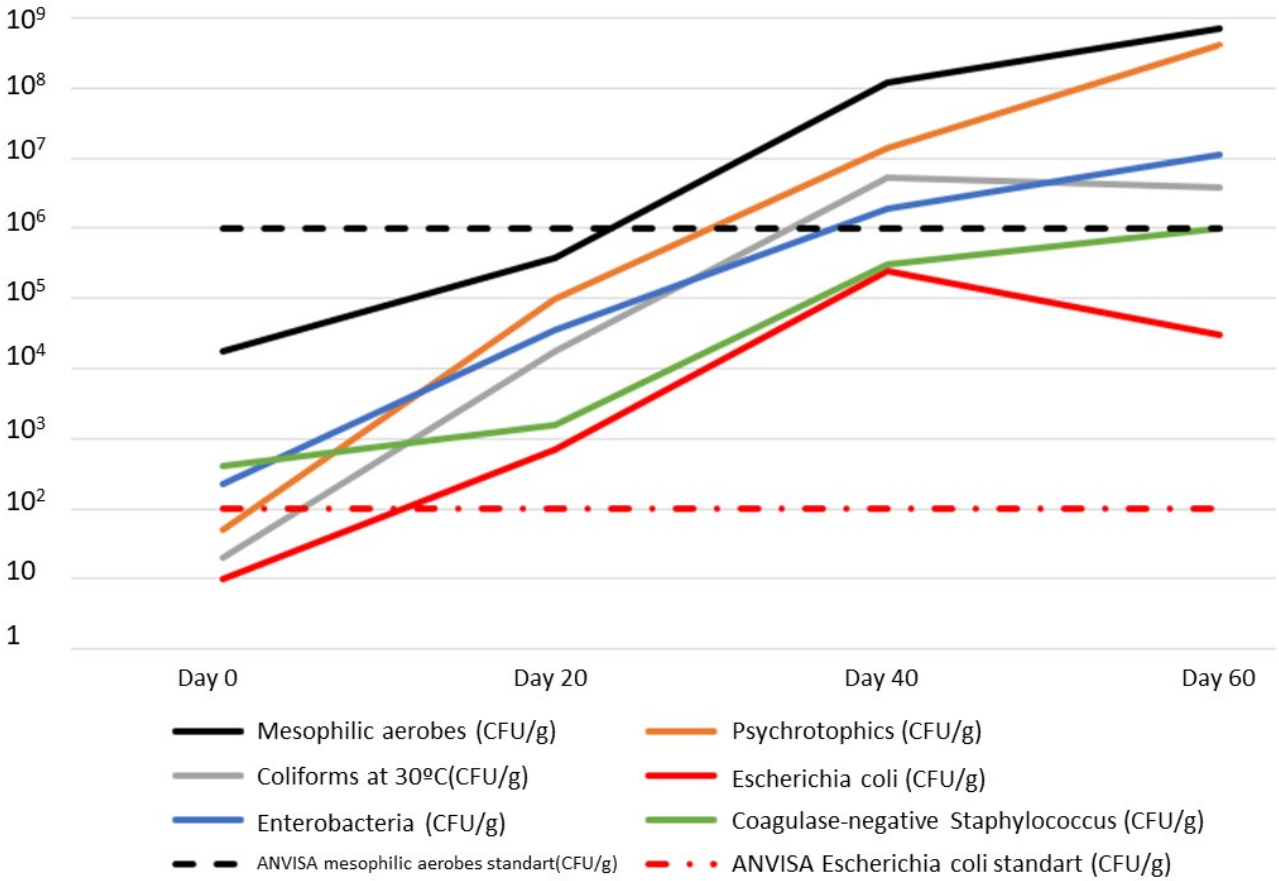

Figure 1. Evolution of indicators microorganisms counts in shelf-life of *Longissimus dorsi.*

The microbial growth under refrigeration is associated to metabolic and structural psychrotrophic characteristics. When subjected to refrigeration, these microorganisms begin to synthesize enzymes with low activation energy, triggering secondary pathways, saccharolytic pathways, such as proteolytic and lipolytic pathways, which are catalyzed by enzymes that are activated at low temperatures (Ercolini et al., 2009; Wei et al., 2019). As the composition of sirloin and other beef cuts consists of 22% protein and 1-2% fat, technological and sensorial problems, such as proteolysis and rancidity, occur in refrigerated meat cuts with high counts of psychrotrophs (Franzetti & Scarpellini, 2007; Gowda et al., 2022; Wang et al., 2022).

Indeed, at D-60, these sensory alterations were perceived by researchers when the psychrotrophic count surpassed 10^8 CFU/g (Table 2). Changes in meat deterioration occur according to the type of dominant microorganism, storage conditions (such as temperature and packaging), composition, meat product properties (pH, lipids, and enzyme activity), and storage time (Sophos, 2014).

According to Cipriano et al. (2021), an unpleasant odor occurs due to lactic acid and protein decomposition that constitute meat, which are subproducts of psychrotrophic microbial metabolism. Maturation time also influences the odor intensity of meat (Nethra et al., 2023).

One of the major contributors to the decomposition of proteins in food are microorganisms belonging to the *Pseudomonas* spp. genus, which are known to be psychrotrophic and spoilage agents (Teider et al., 2019; Watson et al., 2023). Considering the significance of these microorganisms in the quality and shelf-life of meat, the psychrotrophs isolated from D-40, prevalence of *Pseudomonas* spp. was investigated. A total of 70 psychrotrophic colonies were recovered from the plates/dilutions used in the counts, 20 from D-40 and 50 from D-60, of which 55% (11) and 48% (24) were confirmed as *Pseudomonas* spp. by genus-specific PCR, consistent with previous studies demonstrating that this genus is primarily responsible for the degradation of refrigerated meat and its derivatives (Wickramasinghe et al., 2021; Chen et al., 2023; Watson et al., 2023) and is potentially the cause of sensory issues detected in D-60.

During food manufacturing, contamination can occur from various sources, the main ones being the hands of collaborators, the environment, poorly sanitized utensils, and equipment (Enciso-Martínez et al., 2022). Meat products are also susceptible to contamination from animal hides if sanitary procedures are not performed correctly during slaughter (Sagawa et al., 2022).

Staphylococcus spp. are microorganisms that can cause contamination through hand contact and failure in the production and preparation processes of the product (Savini et al., 2023). In the results obtained in the study, the counts of this microorganism ranged from 4.0×10^2 at day 0 to 1.6×10^3 on D-20, 3.1×10^5 on D-40 and $>10^6$ on D-60 (Table 2). All isolated microorganisms were coagulase negative despite the presence of the genus.

Coagulase-positive *Staphylococcus* are more associated with food poisoning (Savini et al., 2023). Miliotis and Bier (2003) emphasized that these microorganisms may have limited development in raw meat due to competition with mesophilic microorganisms, which were significantly quantified in the present study on D-40.

Enterobacteria are another group of microorganisms that indicate hygiene during manufacturing processes. These microorganisms are widely distributed in the environment and digestive tract of animals and can be inactivated using sanitizers (Hervert et al., 2016).

In this study, the counts of enterobacteria progressively varied between 2.3 × 10^2 (D-0) and 1.1 × 10^7 (D-40) UFC/g. The numbers found were considered high and non-compliant with the standards set by Normative Instruction 60 from 2018 (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2018), which defines the limit for the interpretation of *Enterobacteriaceae* results in bovine carcasses at 1.0 × 103 UFC/g.

The elevated values observed may be attributed to the fact that the analyzed samples were products with more handling and contact with the surfaces during the processing stages than bovine carcasses. Those who underwent the entire slaughter process and stages in the deboning sector. As emphasized by Sophos (2014) and Mladenović et al. (2021), ground or processed meat products tend to have higher contamination than intact products or carcasses, due to utensil and grinder contamination, larger surface area and spread of *Enterobacteriaceae* contamination. However, even though the samples are manipulated products and undergo more processing than the carcasses, it cannot be asserted that they comply with the standards for the counts of this microorganism.

Furthermore, the presence of enterobacteria indicates favorable conditions for the presence and multiplication of pathogenic microorganisms, especially enteropathogens, raising questions regarding environmental sanitation (Ferreira, 2019).

More narrowly focused on enterobacteria, other subgroups of microorganisms, such as total and thermotolerant coliforms, have been used to determine hygienic and sanitary conditions for food production (Hervert et al., 2016). The investigation of *E. coli* in food provides, with greater certainty, information about the hygienic conditions of the product and a better indication of the possible presence of enteropathogens, as this microorganism is an indicator of fecal contamination and is found in the intestinal contents of humans and warm-blooded animals (Mendonça & Silva, 2012).

According to Normative Instruction n° 161 of 2022 (ANVISA, 2022), the limit for *E. coli* is 1.0 × 10^2 UFC/g in cattle meat. In this study, *E. coli* met the standard only at D-0 and not after D-20.

Decrease in *E. coli* counts from D-40 to D-60 (Figure 1) may have been due to the increased multiplication of other microorganisms, especially psychrotrophs, which compete for oxygen or hydrogen, nutrients, substances (e.g., bacteriocins), and volatile compounds, restricting the development of other microbes, thereby

causing competition among microorganisms (Tsigarida & Nychas, 2006). Moreover, sampling for microbiological analysis may not have fully determined the contamination of the product in this study.

Although *E. coli* is an indicator of hygienic-sanitary quality, it also acts as a pathogenic microorganism when it encounters virulence factors compromising its safety. Its presence is also indicative of the presence of other enteropathogens, such as *Salmonella* spp. (Cevallos-Almeida et al., 2021). Isolates suggestive of *E. coli* are shown in Table 3. Here, we identified the virulence factors *eaeA,* ST, *stx2,* and *ipaH,* characteristic of EPEC, ETEC, STEC, and EIEC, respectively. Genes encoding the virulence factors CVD432, LT, *stx1,* and *eaeA* + *stx1* or *2* of EAEC, ETEC, STEC, and EHEC were not identified during analyses.

Diarrheagenic *E. coli* pathotypes have been widely reported as causes of foodborne diseases in isolated cases or outbreaks, especially when products are consumed raw or undercooked (Castro et al., 2017; Jenkins et al., 2019; Tack et al., 2021). Brazilian legislation mandates the surveillance of STEC in bovine carcasses, considering this species as the main reservoir (MAPA, 2018). The large diversity of distinct pathotypes observed on different days of shelf-life is also concerning as it demonstrates widespread contamination, posing a risk to consumption and epidemiological importance, as meat can be a source of infection through cross-contamination.

Salmonella spp. was detected on D-20, D-40, and D-60, as presented in Table 3. Its isolation as an enteropathogen was consistent with *E. coli* isolated from D-20. *Salmonella* was not detected on day 0 possibly because the number of microorganisms in the samples was small, making isolation and detection impossible. As the shelf-life increased, *Salmonella* multiplied in the samples and was detected in subsequent analyses. These microorganisms have the ability to multiply at 5-48 °C (Autoridade de Segurança Alimentar e Econômica [FESA], 2023).

Table 3
Microbiological pathogens investigations during shelf life of *Longissimus dorsi* from a slaughterhouse in the North of Tocantins, Brazil, from September 25th to December 6th de 2021

Repetition *Shelf life*	*Salmonella* spp.		*Listeria monocytogenes*		*Escherichia coli*								
					Total	EPEC *(eaeA)*	STEC *(stx1)*	STEC *(stx2)*	EHEC	EAEC *(CVD432)*	EIEC *(ipaH)*	ETEC *(ST)*	ETEC *(LT)*
	n	Positives (%)	n	Positives (%)		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
D-0	37	0 (0)	30	2 (6.7)	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
D-20	40	2 (5)	30	6 (20)	7	1 (14.3)	0 (0)	2 (28.6)	0 (0)	0 (0)	1 (14.3)	2 (28.6)	0 (0)
D-40	40	1 (2.5)	20	1 (5)	25	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
D-60	35	2 (5.7)	35	3 (8.6)	3	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)

For *Salmonella* spp. research, the analysis was considered qualitative, with the result expressed based on the presence or absence the species, and only the absence result is tolerated, as defined in Normative Instruction n° 161/2022 (ANVISA, 2022). Thus, sources of contamination by enteropathogens from the carcasses to the final deboning phase need to be in an industrial environment to establish specific sanitary measures and procedures to reduce the occurrence of enteropathogens in meat cuts before primary packaging.

Salmonella spp. are the second most commonly reported group of bacteria causing foodborne diseases worldwide (Carrasco et al., 2012; He et al., 2023). Animal-origin foods, especially meat products, are particularly important for salmonellosis because the main habitat of these microorganisms is the gastrointestinal tract of the animals used for food production (Sophos, 2014; Thomas et al., 2020).

Miya et al. (2014) revealed that meat contamination by *Salmonella* can occur during slaughter, preparation, and deboning phases. Safety can only be compromised if the meat is consumed raw, undercooked, or mishandled during preparation, leading to transmission due to cross-contamination (Bucher et al., 2008).

Listeria monocytogenes is another pathogen expressed in terms of its presence and absence. All analyses conducted during the shelf-life of *L. dorsi* were positive for this pathogen, from D-0 to D-60 (Table 3). It is a psychrotrophic biofilm-forming pathogen of economic, sanitary, and environmental importance (Schoder et al., 2022), causing mild infections, such as gastrointestinal illness, severe and invasive syndromes, and meningeal infections, which can lead to premature birth and spontaneous miscarriage (Filipello et al., 2020; Jibo et al., 2022). Similar to the identification of enteropathogens, their presence in the samples evaluated in this study indicated operational failures during meat preparation.

Modified atmosphere packaging raises concerns regarding the multiplication of psychrotrophic pathogens, such as *Listeria monocytogenes* (Saraiva et al., 2016). As stated by Hugas et al. (1998), modified atmosphere packaging alone does not inhibit *Listeria monocytogenes* as this species is a facultatively anaerobe. The samples evaluated in this study may have been contaminated by this pathogen prior to or during the carcass deboning process.

Here, our results starting from the analysis at D-40 revealed high quantifications of indicator microorganisms, with values surpassing the limits established by current regulations. This timeframe coincides with the final period of meat maturation, which occurs under refrigeration for approximately 30 d after animal slaughter (Monsón et al., 2005). In this study, the samples underwent wet aging during shelf-life, and the meat moisture was preserved via packaging, which may have favored the maintenance of deteriorating and pathogenic microorganisms, as previously reported by Ribeiro et al. (2021).

Conclusion

In this study, our analyses revealed that the standard limits for beef set by the current Brazilian regulations (mesophilic aerobes and *E. coli*) were exceeded, with *E. coli* exceeding

the set limit at D-20 of shelf-life. Furthermore, pathogens compromising the safety of meat consumption were detected on the first day of primary packaging. Elevated counts of other quality indicators also indicated high initial contamination of the cuts and low shelf-life of the sirloins analyzed in this study. Therefore, measures for stricter control in the deboning area, such as adjustments to monitoring, preventive actions, and operational hygiene corrections, should be implemented to increase the shelf-life of vacuum-packed meat. Future studies should determine the origin of contamination in industrial environments to facilitate the establishment of self-controlled sanitary programs.

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