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MeatCulture: microbiological, hygienic and qualitative profile of bovine fifth quarter viscera

Giuliana Siddi, Francesca Piras, Maria Pina Meloni, Mattia Migoni, Mario Cuccu, Roberta Lai, Fabrizio Simbula, Daniela Cabras, Marco Manca, Enrico Pietro Luigi De Santis, Christian Scarano

Department of Veterinary Medicine, University of Sassari, Italy

Correspondence: Francesca Piras, Department of Veterinary Medicine, University of Sassari, via Vienna 2, 07100, Sassari, Italy.
Tel.: +39.079 229447.
E-mail: fpiras1@uniss.it

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Abstract

The MeatCulture project aimed to improve the quality and safety of bovine fifth quarter viscera (tripe, intestine, lung, and trachea), which are traditional dish ingredients in many communities. Stabilization studies were conducted on tripe and intestine samples, and preservability studies were conducted on lungs and trachea. Tripe samples (24 across two batches) underwent washing and bleaching treatments with different temperature-time combinations, then they were vacuum-packed, stored at 2±2°C, and analyzed at various timepoints. The pH was determined, and the *Enterobacteriaceae* count and the presence of *Salmonella*, *Listeria monocytogenes,* and *Yersinia enterocolitica* were investigated. Results indicated that applying a washing step at a temperature range between 55°C and 65°C for 2 minutes, followed by bleaching at a temperature range between 58°C and 70°C for 2 minutes, preserved stable pH (T_{10}: 7.24±0.06) and controlled *Enterobacteriaceae* counts, which decreased at T_7 (2.78±0.48 CFU/g) before slightly increasing at T_{10} (3.24±1.55). Pathogens were never detected. Part of the small intestine (jejunum) and colon samples were vacuum-packed (30 samples overall), subjected to four types of heat treatments, stored at 2±2°C, and analyzed at various timepoints. pH and microbial profile were evaluated as previously described. The treatment with water at 70°C for 15' showed the best results with *Enterobacteriaceae* counts below the detection limits in most samples at T_{10}. The lungs (153 samples) and trachea (81 samples) samples were packaged with CO_2 and N (ratios of 50/50 and 70/30) modified atmosphere packaging (MAP). The samples were stored at two different temperatures (2±2°C and 8±2°C) and analyzed at various timepoints. On each sample, pH, aerobic colony count, *Enterobacteriaceae* count, mesophilic lactic acid bacteria count, and *Pseudomonas* spp. count were evaluated. The microbial profile of the samples stored at 2±2°C and with the 50/50 MAP showed lower average values.

Introduction

The global population is estimated to grow from 8 billion in 2020 to about 9.9 billion by 2050 (United Nations, 2024), and it will be critical to guarantee that the population has access to food, with a distinct focus on meat availability, as it provides the proteins with the highest biological value. To meet this rising demand, global meat production should increase by 200 million tons by 2050 (Lynch *et al.,* 2018). However, this strategy has a very poor sustainability as it implies high environmental consequences and therefore alternative solutions should be considered by adopting a circular economy that maximizes the use of existing resources (Henchion *et al*., 2016). In this context, one potential approach is to utilize the "fifth quarter", starting from the many traditional

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dishes that use this raw material for their production. The "fifth quarter" comprises all the parts of food animals not directly used as meat, including visceral organs, fat, and bones, and is a necessary and abundant product during slaughter, which can account for up to 20% of the beefing performance (Lynch *et al.,* 2018). Despite its abundance, the fifth quarter is considered waste in most slaughterhouses, and its valorization could improve the economic sustainability of the meat industry (Soladoye *et al*., 2021). Furthermore, the nutritional profile of these products is comparable to lean meat, offering high-quality proteins, essential amino acids, vitamins, minerals, and low-fat content (Toldra *et al*., 2021).

Currently, the market distribution of fifth quarter viscera is inconsistent and linked to regional delicacies or traditional recipes (Strong, 2006). Enriching these markets is a way to promote these products that, in addition, are central to many ethnic cuisines, such as African and Oriental, where they are main ingredients in traditional meals (Edwards *et al*., 2013). For example, offal, including the lungs and the trachea, is used, among others, in the "offal stew", "tripe and trachea soup", and other traditional African dishes. Moreover, the intestines are also considered a delicacy in many traditional cuisines. Oriental dishes like *makchang* and *kushiyaki horumon*, or African *mala mogodu* are a few examples (Edwards *et al*., 2013; Erasmus *et al*., 2017). With increasing multiculturalism, valorizing these products could therefore open new market opportunities.

The MeatCulture project was conducted in partnership with a leading company in the meat sector in Sardinia (Italy) and had the valorization of products belonging to the fifth quarter of the bovines (tripe, intestine, lung, and trachea) among its objectives.

At the time of the study, the slaughterhouse plant that partnered with the MeatCulture project used specific equipment to apply cold washing to tripe, though without controlled temperature or duration, while other fifth quarter cuts were discarded. To the best of our knowledge, scarce information is available in the literature regarding the hygiene characteristics of the bovine fifth quarter. Within this framework, the following rationale underpinned the studies on the tripe, intestine, lung, and trachea: on the tripe and intestines the study aimed to identify a stabilization treatment that would control the product contamination levels and enhance the final hygienic quality of the product; on lungs and tracheas, a shelf-life study was conducted, following portioning and subsequent packaging in a modified atmosphere (MAP).

Materials and Methods

Tripes

A total of 24 tripe samples from a combination of rumen, reticulum, and omasum were randomly collected at the slaughterhouse. Samples were divided into two batches (12 samples for each batch). The samples underwent stabilization treatments consisting of washing and bleaching using water through a machine similar to the one currently used at the slaughterhouse, but specifically constructed and adapted for research purposes in order to replicate the test in a small setting. The washing and bleaching treatment was performed in the same machine, using only hot water and avoiding the use of additional additives that would require labeling. Specifically, combinations of temperature, duration time, and washing speed were assessed. The following parameters, defined by the machine employed and available at the slaughterhouse, were applied: for batch 1, washing temperatures in a range between 58°C and 62°C for 1-2' were applied; for batch 2, washing temperatures in a range between 55°C and 65°C for 2' followed by a bleaching treatment of 58-70° C for 2' were applied. For each batch, a control sample was subjected to washing according to the procedure used at the slaughterhouse (parameters not known). At the end of the stabilization treatment, all the samples were vacuum-packaged with polyamide/polyethylene bags (30×40 cm, 90 µm thickness), stored at temperatures of 0-4°C, and analyzed at the following times: within 24 hours after treatment (T_0), after 7 days (T_7), and after 10 days (T_{10}). For each sample, the potentiometric measurement of pH was carried out by inserting a pH-meter GLP 21 (Crison, Carpi, MO, Italy) in 10 g of sample homogenized with 10 mL of distilled water. The centesimal chemical composition was evaluated using FoodScan (FOSS, Analytic, Hillerød, Denmark) on a 100g pool of the samples. The microbiological analyses included *Enterobacteriaceae* count (ISO, 2017a), *Salmonella* (ISO, 2020), *Listeria monocytogenes* (ISO, 2017b), and *Yersinia enterocolitica* (ISO, 2017c) detection.

Intestines

A total of 30 intestinal samples were analyzed, divided into 15 samples of small intestine (jejunum) and 15 samples of colon; the samples were washed at the slaughterhouse according to the procedure used at the slaughterhouse (hot water, temperature unknown) and then vacuum-packed with the same bags previously described. The samples were subjected to 4 types of heat treatment using water at 70°C for different times, ranging from 5' to 30'. Following the heat treatments, the samples were stored at refrigeration temperature (2±2°C) and analyzed on the day of packaging (T_0), 7 (T_7), and 10 (T_{10}) days after packaging.

Furthermore, control samples of small intestine and colon, not subjected to heat treatment but vacuum-packed and stored at refrigeration temperature, were also analyzed at the same timepoints. All the samples were tested for the pH value and the composition as previously described; *Enterobacteriaceae* count and detection of *Salmonella, L. monocytogenes,* and *Y. enterocolitica* were performed according to the aforementioned ISO standards. *Escherichia coli* was tested according to the ISO standard (ISO, 2018).

Lungs and trachea

A total of 153 lung samples were collected and divided into three batches. For each batch, three control samples were analyzed on the day of slaughter (T_0). The remaining 48 samples in each batch were processed as follows: 24 samples were MAP with a gas mixture of 50% CO_2 and 50% N and in polystyrene rigid trays (2 mm thickness) that were closed by heat-sealing in a high-barrier film (oxygen transmission rate of 1.8 $cm^3/m^2/24$ h/bar). Of these, 12 samples were stored at refrigeration temperature, while the other 12 were stored under thermal abuse conditions (8±2°C). The other 24 samples were packaged in MAP using a mixture of CO_2 and N_2 in a 70/30 ratio and stored at the above-mentioned temperatures. All samples were analyzed 3, 5, 7, and 10 days after packaging (T_3, T_5, T_7, T_{10}).

As regards trachea samples, a total of 81 samples were analyzed in three batches. For each batch, 27 samples were collected, of which three were analyzed on the day of slaughter (T_0) and considered as control samples. The remaining were subdivided, packed, and stored according to the methods previously described for lung samples and analyzed 5 and 10 days after packaging (T_5 and T_{10}).

In total, 153 lung and 81 trachea samples were analyzed. pH and composition evaluation were carried out on each sample as

previously described, and the following microbiological analyses were performed: aerobic colony count (ACC) (ISO, 2013), *Enterobacteriaceae* count (ISO, 2017a), mesophilic lactic acid bacteria (LAB) (ISO, 1998), and *Pseudomonas* spp. (ISO, 2010).

Statistical analysis

Differences between samples were evaluated using one-way analysis of variance with post-hoc Tukey honestly significant difference with Statgraphics-Centurion XIX software (Stat Point Technologies, Warrenton, VA, USA). The significance level was defined as $p<0.05$.

Results and Discussion

Although preliminary, the results of this investigation offer insights into the microbial profile and the physical-chemical characteristics of some parts of the fifth quarter. These results can be leveraged to determine the most suitable stabilization treatments, ensuring their safety and allowing their valorization. Tables 1-3 show the results relating to the pH (mean ± standard deviation) and microbial profile (log CFU/g mean ± standard deviation), respectively, of the tripe, intestines, lungs, and trachea samples analyzed during the shelf-life.

Table 1. pH values, mean *Enterobacteriaceae* counts (log CFU/g; mean±s.d.) and pathogens (presence/absence in 25g) detected in tripes samples analyzed during shelf-life. No statistically significant differences were observed among the samples.

Batch	Sample	Time	pH	*Enterobacteriaceae*	*Salmonella*	*Yersinia enterocolitica*	*Listeria*
1	Control	0	7.06	1.85	-	-	*L. spp*
	Treated	0	6.96±0.27	1.62±0.58	-	-	*L. spp*
		7	7.08±0.19	4.95±0.46	-	-	*L. spp*
		10	6.94±0.33	5.36±0.46	-	-	-
2	Control	0	6.78	3.85	-	-	-
	Treated	0	7.07±0.10	3.22±1.94	-	-	-
		7	7.24±0.03	2.78±0.48	-	-	-
		10	7.24±0.06	3.24±1.55	-	-	-

-, not detected in 25 g.

Table 2. pH, *Enterobacteriaceae* count (log CFU/g; mean±s.d.) and pathogens (presence/absence in 25g) detection of the small intestine and colon samples subjected to four types of treatments. No statistically significant differences were observed among the samples.

Treatment	Sample	Days	pH	*Enterobacteriaceae*	*Salmonella*	*Yersinia enterocolitica*	*Escherichia coli*
Control	SI	0	6.69±0.04	3.36	-	-	+
		7	6.63±0.01	2.38	-	-	+
		10	6.65±0.03	3.28	-	-	+
	CS	0	6.86±0.05	4.31	-	-	+
		7	6.87±0.03	3.28	-	-	+
		10	6.88±0.03	3.78	-	-	+
Treatment 1 (70°C for 5')	SI	0	6.75±0.01	<LOD	-	-	-
		7	6.74±0.07	2.48	-	-	+
		10	6.68±0.02	<LOD	-	-	-
	C	0	6.87±0.01	<LOD	-	-	+
		7	6.86±0.01	2.30	-	-	-
		10	6.87±0.03	4.12	-	-	-
Treatment 2 (70°C for 10')	SI	0	6.72±0.02	<LOD	-	-	-
		7	6.67±0.03	2.46	-	-	+
		10	6.65±0.01	<LOD	-	-	+
	C	0	6.87±0.04	<LOD	-	-	-
		7	6.92±0.01	2.30	-	-	-
		10	6.87±0.01	<LOD	-	-	-
Treatment 3 (70°C for 30')	SI	0	6.72±0.01	<LOD	-	-	-
		7	6.69±0.01	2.04	-	-	+
		10	6.64±0.04	<LOD	-	-	-
	C	0	6.88±0.01	<LOD	-	-	-
		7	6.95±0.01	2.32	-	-	-
		10	6.86±0.01	<LOD	-	-	-
Treatment 4 (70°C for 15')	SI	0	6.76±0.02	<LOD	-	-	+
		7	6.66±0.01	<LOD	-	-	-
		10	6.66±0.04	2.11	-	-	-
	C	0	6.84±0.01	<LOD	-	-	-
		7	6.93±0.04	<LOD	-	-	-
		10	6.88±0.01	<LOD	-	-	-

SI, small intestine samples; C, colon samples; <LOD, below limit of detection.

Tripes

As shown in Table 1, in batch 1 (washing at 58-62°C for 2'), the pH mean values remained stable, with slight variations during the shelf-life showing no significant differences (p>0.05). At T_0, mean values of 6.96±0.27 were observed, at T_7 7.06±0.19, and at T_{10} pH was 6.94±0.33. The control sample had a pH of 7.06±0.14. *Enterobacteriaceae* counts rose from 1.62±0.58 at T_0 to 5.36±0.46 log CFU/g at T_{10}. Batch 2 (washing at 55-65°C for 2' followed by bleaching at 58-70°C for 2') pH values were 7.07±0.10 at T_0, increased slightly to 7.24±0.03 at T_7 and remained stable at T_{10} (7.24±0.06) at the end of the shelf-life, without significant differences (p>0.05). *Enterobacteriaceae* counts were 3.22±1.94 log CFU/g at T0, showed a decrease at T_7 (2.78±0.48), while a rise in the mean counts was observed at T_{10} (3.24±1.55). *Salmonella* and *L. monocytogenes* were never detected in tripes samples; *Listeria* spp. was detected in batch 1 at T_0 and T_7. Although no statistically significant differences were observed across batches (p>0.05), washing temperatures between 55°C and 65°C, combined with bleaching at 58°C to 70°C and vacuum-packaging, seemed to provide a better control of *Enterobacteriaceae* growth in tripe samples.

Tripe has a high bacterial load coming from the intestinal microflora of ruminants (Gill, 1988), and although it is cleaned to a certain extent before being sold to customers, most of these bacteria are still present in the finished product (Parry-Hanson *et al.,* 2008). Tripe is therefore a particularly perishable product because of its high bacterial load and autolytic enzyme content (Bensink *et al.,* 2002). Vacuum-packaging and refrigerated storage have proven to be feasible technologies for reducing bacterial levels in fresh and cooked tripe (Giaccone *et al*., 1994); pairing this with washing and bleaching treatments, employing hurdle technology, has proven to be useful to control *Enterobacteriaceae* growth and extend the shelf-life. These results serve as a foundation for further analysis of multiple batches of products.

Intestines

The pH values in the small intestine products ranged from 6.68±0.03 in treatment 2 samples to 6.69±0.06 in treatment 4 samples, while the colon samples had a consistent mean pH of 6.88±0.03 across all treatments.

Heat treatments applied to the small intestine samples for 5, 10, and 30 minutes initially resulted in counts below the detection limit. However, a rise to 2.3±0.12 log CFU/g was observed, followed by a drop back below detection at T_{10}. Inversely, a rise in counts was seen in the samples when the treatment was applied for 15', with values at T_{10} equal to 2.11 log CFU/g.

As for the colon samples, the heat treatment applied for 5' resulted in a rise in counts, from below the detection limit to 4.12 log CFU/g at T_{10}. Heat treatments for longer times resulted in *Enterobacteriaceae* values below the detection limits at T_{10}. *Salmonella* and *Y. enterocolitica* were not detected in any samples. *E. coli* was present in all control samples and some treated samples. In the colon samples treated with water at 70°C for 15 minutes (treatment 4), *E. coli* was absent at all time points (T_0, T_7, and T_{10}), as it was in the small intestine products at T_7 and T_{10}. Similarly, treatment 3 (70°C for 30 minutes) resulted in *E. coli* absence in colon samples at all times, and in the small intestine at T_7 and T_{10}. Treatment 2 (70°C for 10 minutes) removed *E. coli*

Table 3. pH and microbial profile (mean ± standard deviation log CFU/g) of lung and trachea samples packaged with CO_2 and N (ratios of 50/50 and 70/30) modified atmosphere packaging. No statistically significant differences were observed among the samples.

	MAP	Temperature	Days	pH	ACC	*Enterobacteriaceae*	LAB	*Pseudomonas*
Lungs samples	Control		0	6.23±0.06	3.52±0.19	3.18±0.39	2.47±0.59	1.83±0.70
	50/50	2	3	6.11±0.20	2.86±0.50	2.59±0.15	2.38±0.57	2.27±0.78
			5	6.25±0.12	3.40±0.42	2.94±0.48	2.98±0.59	2.23±1.15
			7	6.18±0.19	3.46±1.15	3.81±1.58	2.43±0.97	2.40±1.17
			10	6.19±0.19	3.49±0.79	2.96±0.73	2.49±1.33	3.80±0.87
		8	3	6.12±0.18	3.09±0.73	2.31±0.92	2.72±0.94	2.51±1.05
			5	6.21±0.21	4.39±1.54	3.45±1.44	3.72±1.14	3.36±1.44
			7	6.12±0.11	6.12±1.03	5.28±1.33	4.95±1.32	3.84±1.26
			10	6.16±0.18	5.92±0.61	4.5±1.12	5.21±0.60	2.61±1.74
	30/70	2	3	6.28±0.10	3.06±0.99	2.18±0.75	1.73±0.45	2.32±0.36
			5	6.27±0.14	3.39±0.58	2.24±0.96	2.27±1.27	2.47±1.02
			7	6.25±0.15	4.31±1.07	3.33±1.07	3.10±1.40	3.72±0.96
			10	6.22±0.15	3.73±0.74	2.59±0.83	2.17±0.92	4.28±0.96
		8	3	6.38±0.18	3.60±1.21	2.61±0.54	2.98±0.50	3.11±1.17
			5	6.34±0.15	4.66±1.03	4.10±1.08	4.00±0.71	3.87±1.01
			7	6.22±0.29	6.63±0.62	5.95±1.21	5.12±1.01	4.87±0.82
			10	6.17±0.17	6.71±0.40	6.11±0.82	5.42±0.58	3.70±1.83
Trachea samples	Control		0	7.43±0.07	4.50±0.65	3.09±0.95	3.64±0.66	2.40±0.73
	50/50	2	5	7.35±0.35	3.24±0.73	3.07±1.20	2.92±1.57	2.84±1.02
			10	7.19±0.22	3.90±0.63	2.55±1.00	2.69±0.60	2.65±0.82
		8	5	7.09±0.32	4.42±0.65	3.17±1.53	3.90±0.86	2.40±1.24
			10	6.93±0.16	5.78±0.38	5.13±0.65	5.01±0.46	4.32±0.68
	30/70	2	5	7.06±0.33	4.04±1.10	3.22±0.41	3.25±0.69	2.63±0.69
			10	7.22±0.24	4.67±1.41	3.68±0.85	3.68±0.79	3.41±1.53
		8	5	7.20±0.42	4.99±0.68	4.37±1.21	4.34±1.26	3.58±1.16
			10	7.17±0.31	7.00±0.91	6.18±0.40	4.77±1.20	5.57±0.78

MAP, modified atmosphere packaging; ACC, aerobic colony count; LAB, lactic acid bacteria.

from the colon samples entirely. Overall, Treatment 4 (70°C for 15 minutes) was the most effective in reducing microbial counts. However, further research is needed to confirm these findings, given the limited data available.

Lungs and trachea

The pH of the lung samples stored in the 50/50 MAP decreased compared to control samples (T_0, values equal to 6.23±0.06) and during storage at both temperatures, with T_{10} values of 6.19±0.19 in 2°C samples and 6.16±0.18 in 8°C samples. For samples in 30/70 MAP, the pH initially increased at T_3, then decreased, especially under thermal abuse conditions (pH 6.17±0.17). The trachea samples also showed a pH decline during storage at both temperatures and MAP mixtures. The microbial profile (mean ± standard deviation log CFU/g) at T_0 was characterized by ACC, *Enterobacteriaceae*, and LAB values, respectively equal to 2.86±0.50, 2.59±0.15, and 2.38±0.57. At T_3, microbial counts generally decreased by 1 log, but *Pseudomonas* counts increased compared to T_0. After storage, ACC, *Enterobacteriaceae*, and LAB levels increased, with lower values observed in samples stored at +2°C and with both MAP mixtures compared to those stored at +8°C. *Pseudomonas* counts, however, reached higher levels in the samples stored at +2°C across both MAP mixtures. The trachea samples exhibited lower microbial levels overall, especially in the samples stored at +2°C in 50/50 MAP. Higher microbial levels were recorded in samples stored at +8°C. Interestingly, the samples packaged in 30/70 MAP and stored at +2°C had microbial levels comparable to those packed in 50/50 MAP and stored at +8°C, as shown in Table 3. Overall, 50/50 MAP showed superior control over microbial growth in both lungs and trachea, compared to 30/70 MAP. It also allowed for better maintenance of product color throughout the shelf life (Gill, 1998).

Centesimal composition

The centesimal composition of tripes showed 4.32% fat, 12.16% protein, and 80.58% humidity. The small intestine centesimal composition was 14.89% fat, 11.79% proteins, and 70.80% humidity, while the colon had 59.05% fat, 2.25% proteins, and 35.88% humidity. The lung samples contained 1.44% fat, 28.92% proteins, and 73.17% humidity. The trachea samples had 11.13% fat, 24.19% proteins, and 62.19% humidity. Compared to lean beef (fat 5-10%, proteins 26%, humidity 61%, Clarke *et al.*, 2004), fifth quarter cuts like trachea, lungs, and tripes provide optimal protein intake with lower fat percentages, making them valuable alternatives for protein consumption.

Conclusions

The main objective of this study was to verify the hygienic and sanitary conditions of the bovine fifth quarter. In fact, as already mentioned, the fifth quarter is considered waste in most slaughterhouses, and its valorization could improve the economic sustainability of the meat industry. The data we obtained demonstrate how the final qualitative and hygienic characteristics of the products depend on the type of processing carried out, which also influences the shelf-life, the risk for the consumer, and, last but not least, the acceptability by the consumer. The acquisition of data relating to the microbial and chemical-physical profile allowed us to identify the most appropriate stabilization treatments for the valorization of the fifth quarter product. Through the MeatCulture project, the partner production plant was able to take a step forward towards improving the quality and safety of products and cuts that are usually little marketed. The valorization of the viscera of the fifth quarter is a fundamental point in the economic balance of the production plant. In fact, the cost of disposal of these by-products can be transformed into profit, experimenting with new markets that take into account the evolution of local eating habits, but also the entry of multicultural consumers. This latter is certainly a market with a growing demand and a sure new source of income for the meat market. Furthermore, by embracing alternative solutions such as prioritizing resource efficiency and the use of animal by-products, we can significantly reduce waste and mitigate environmental damage. In conclusion, enriching these markets is a means to promote these products that are also central to many ethnic cuisines, such as African and Oriental, where they are main ingredients in traditional meals. With growing multiculturalism, the valorization of these products could therefore open new market opportunities.

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