Shelf-life of sheep *arrosticini* packaged in protective atmosphere

Simone Stella,[1] Cristian Bernardi,[1] Massimo Fioretti,[2] Lodovico Lorenzini,[1] Erica Tirloni[1]

[1]Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi; [2]Health Protection Agency (ATS), Milan, Italy

Abstract

The aim of this study was to evaluate the shelf-life of pre-packaged sheep's *arrosticini* produced in a factory located in northern Italy. Samples were divided into two series and packaged in modified atmosphere with specific gas mixtures: conventional (C: 35% O_2/15% CO_2/50% N_2) and experimental (E: 30% CO_2/70% N_2). All the samples were stored at 4°C for 10 days and subjected, after 5, 8 and 10 days (t5, t8, t10), to triplicate microbiological and chemical-physical (pH, total volatile basic nitrogen, thiobarbituric acid reactive substances) analyses. At the same time, the colorimetric analysis and a sensorial evaluation were carried out (pack tightness, presence of color or odor alterations), assigning a discrete score from 0 to 5. The samples showed initial total bacterial counts close to 5 Log CFU/g: these values gradually increased during storage, exceeding the threshold of 8 Log CFU/g in the C series; lower values were detected in the E series. A similar trend was highlighted for *Enterobacteriaceae*, with initial loads of about 3 Log CFU/g and an increase until t10, reaching values higher than 6 Log CFU/g in the C series and close to 5 Log CFU/g in the E series (P=0.002). *E. coli* also showed a similar trend, although with values approximately 1 Log lower than *Enterobacteriaceae*. *Pseudomonas* spp. showed initial counts close to 4.5 Log CFU/g, with a different increase in the C series (6.5 Log CFU/g at t10) and the E series (4.95 Log CFU/g) (P= 0.006). The higher growth in the C series was also observed for lactic acid bacteria, with an increase from 3 to 5 Log CFU/g (3.8 Log CFU/g in the E series P=0.016). The other microbiological parameters showed very low counts and, in most cases undetectable counts (<2 Log CFU/g) for the entire period considered. The initial values obtained from the measurement of the colorimetric indices were within the norm for this type of product: however, starting from t5, lower values of red index and lightness in the E series were measured, with an evident greying of meat surface. The results of the sensory evaluation indicated that the product showed optimal sensorial characteristics up to 8 days of shelf-life in the C series, while the use of an oxygen-free atmosphere, despite having a moderate inhibiting effect on the microbial populations, has led to an early modification of the product (5 days of storage), due to the presence of superficial greyish areas. The microbiological characteristics of *arrosticini* strictly depend on the hygienic conditions of slaughtering and production; even in optimal situations, the product is particularly perishable, and requires careful management of storage temperatures and times, to maintain its quality characteristics.

Correspondence: Erica Tirloni, Department of Veterinary Medicine and Animal Sciences, University of Milan, Via dell'Università 6, IT-26900, Lodi, Italy.
Tel: 02.50334333. E-mail: erica.tirloni@unimi.it

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Introduction

Arrosticini are a typical product of the Italian region Abruzzo and are included in the list of Italian Traditional Agro-food Products (MIPAAF, 2022). They are usually obtained from the slaughter of adult sheep aged between 3 and 6 years. The product seems to be born around the 1930s, following the intention of some shepherds of the Abruzzo Apennines, dedicated to transhumance, to avoid the waste of any component of their flocks; therefore, the slaughter of the older animals was followed by the separation of the meat in small pieces to reduce the consistency of meat due to advanced age. Over time, *arrosticini* became increasingly common on the tables of the Abruzzo people first, and then of the Italians, finding its maximum diffusion in the post-war period. Today *arrosticini* are made up of different cuts, also coming from different animals, which are reduced to small cubes of about 1 cm^3 and inserted on a skewer. As raw meat is submitted only to fragmentation, this product must be considered just as fresh meat and cannot be included in the category of meat preparations as defined by Regulation (EC) 853/04 (European Parliament, 2004). Thus, no mandatory microbiological parameters are set by Regulation (EC) 2073/2005 (European Commission, 2005a). Nevertheless, process hygiene criteria set for minced meat and meat preparations can be a useful guideline for the evaluation of this type of meat.

These products are extremely perishable from a microbiological point of view, and the evaluation of their shelf-life is of great

value to managing the production process and as a support in risk assessment. In the last years, most attention was focused on the determination of beef shelf-life by food business operators, while very little information on the hygienic quality of sheep meats is present in the literature (Teixeira *et al*., 2020). A previous Australian study investigated microbiological loads on sheep carcasses and boneless sheep meat highlighting that *Escherichia coli* was detected on 75% and on 47.7% of these samples, respectively (Vanderlinde *et al*., 1999); while a study by Phillips *et al.* (2001) showed microbial loads above 3 Log CFU/cm^2 or g on carcasses and frozen boneless meat, respectively, and a presence of *E. coli* on 29.2% of carcass samples and 24.5% of frozen meat samples.

The present study aimed to establish the proper shelf-life of refrigerated, pre-packaged *arrosticini* produced in a factory located in northern Italy, comparing two typologies of modified atmosphere packaging.

Materials and Methods

Meat and packaging conditions

The study considered pre-packaged *arrosticini*, a traditional foodstuff made only from sheep meat. The package contained 500 g of product consisting of small cubes of sheep meat of about 1 cm^3 each, arranged in 6/8 for each wooden skewer. 20 skewers were contained in a polystyrene tray sealed with a transparent film. For conservation, two modified atmospheres were considered in parallel: a conventional series (C) consisting of a mixture of gases that included O_2 (~40%), CO_2 (~10%) and N_2 (~50%) and an experimental series (E) consisting of a mixture of CO_2 (~25%) and N_2 (~75%). Samples were stored at 4°C for 10 days, and analyzed at time 0 (t0), and after 5, 8 and 10 days of storage (t5, t8 and t10). At each sampling time, 3 samples for each series were submitted to the analyses described below.

Evaluation of microbial populations during the storage

For microbial counts, 10 g of each sample from the two series were homogenized in 90 mL of sterile diluent solution (0.85% NaCl and 0.1% tryptone), and serial 10-fold dilutions were prepared. The total viable count (TVC) was determined according to the ISO 4833-2 method (2013). Lactic acid bacteria (LAB) were enumerated according to the ISO 15214 method (1998). The number of *Enterobacteriaceae* was determined by the ISO 21528-2 method (2017). *Pseudomonas* spp. were enumerated by the ISO 13720 method (2010). Yeasts and molds were enumerated according to the ISO 21527-1 method (2008). *E. coli* were enumerated according to the ISO 16649-1 method (2018). Coagulase Positive Staphylococci were enumerated according to the ISO 6888-1 method (2021). Finally, spores of sulfite-reducing Clostridia were enumerated according to the ISO 15213 method (2003), after treating the sample dilutions for 10 minutes at 80°C.

Chemical-physical analyses

pH

The pH of the products was determined by a pHmeter (Ghiaroni, mod. XS pH 6, Buccinasco, Italy), following the MFHPB-03 method (Health Canada, 2014). Before measurement, the samples were minced and mixed with distilled water (max 1:2 w/w) to obtain a fluid consistency.

Color parameters

The measurement of color parameters was performed after a 45-minute oxygenation (samples were exposed to air at refrigeration temperature) by a Minolta Chroma Meter CR-400 (Minolta Camera Co., Ltd., Osaka, Japan). The CIE L*, a* and b* values, which describe the intensity of whiteness/brightness, red color (a*>0), and yellowness (b*>0), respectively, were taken at 6 locations of each sample.

Total volatile basic nitrogen

Total volatile basic nitrogen (TVBN) [Regulation (EC) N. 2074/2005; European Commission, 2005b] was determined on each sample.

Thiobarbituric acid reactive substances

Lipid oxidation was determined by defining the thiobarbituric acid reactive substances (TBARS), following the method described by Lorenzo *et al*. (2014). The TBARS values were calculated from a standard curve of malonaldehyde (MDA) and expressed as mg MDA per kg sample.

Sensorial characteristics

At each sampling time, the samples from the two series were subjected to a subjective evaluation of the following characteristics: i) tightness of the package (possible penetration of air or leakage of liquid); ii) formation of gas inside the package (presence of gas bubbles or blowing); iii) presence and quality of exudate on the surface of the meat or in the package; iv) presence of color alterations, assessed upon opening the package and after a brief exposure to air; iv) odor (normal/acid/sulfur), assessed upon opening the package and after exposure to air to evaluate its persistence; v) texture of the meat. To provide a general evaluation of the sensory acceptability of the product, a numerical scale was used, assigning a score from 0 to 5: grade 5 indicates an optimal condition of the product, 4 indicates a good condition, 3 indicates an acceptable condition, 2 indicates an initial modification of the product (which can be noticed by the consumer), 0 and 1 indicate an evident modification/alteration of the product (the product is not acceptable to the consumer).

Statistical analysis

The data were submitted to statistical analysis (one-way ANOVA) by SAS/stat package version 8.0 (SAS Inst. Inc., Cary, USA), comparing the two sample series. A significance threshold of P=0.05 was considered.

Results and Discussion

Microbiological analyses

Microbiological results are reported in Figure 1A-E. The hygiene of the *arrosticini* evaluated was good, as all the microbiological parameters showed a low to moderate level of initial contamination. Also if *arrosticini* cannot be defined as minced meat or meat preparations, as defined by Regulation (EC) 853/2004 (European Parliament, 2004), a comparison with the process hygiene criteria thresholds set by Regulation (EC) 2073/2005 (European Commission, 2005a) for these two food categories can be useful: TVC showed values close to 5 Log CFU/g at t0 (a lower threshold of 5.70 Log CFU/g is defined by the Regulation for minced meat), and *E. coli* counts were not detectable (<2 Log CFU/g, with lower thresholds set at 1.70 and 2.70 Log CFU/g for

minced meat and meat preparations, respectively). *Pseudomonas* represented the main part of the initial microbial population (4.5 Log CFU/g), while low or moderate contamination was detected for *Enterobacteriaceae* (mean count near 3 Log CFU/g), Lactic Acid Bacteria and yeasts (counts <3 Log CFU/g), with undetectable counts for the other parameters (counts <2 Log CFU/g for coagulase-positive Staphylococci and molds, and <1 Log CFU/g for spores of sulfite-reducing Clostridia).

During the storage for 10 days at 4°C, an increase in microbial counts was observed, as generally expected. TVC showed a gradual increase in both series through the sampling times, reaching counts above 8 Log CFU/g and above 7,5 Log CFU/g in C and E series, respectively, after 10 days. The values detected at the last sampling time were associated with a moderate deterioration of the product. The same trend was observed for *Enterobacteriaceae* that showed a gradual increase in both series and especially in the C series, reaching after 10 days of storage values above 6 Log CFU/g: these values were higher than those detected in the E series (Δ=1,10 Log CFU/g at t10). Among *Enterobacteriaceae*, *E. coli* showed an increase during the trial, reaching counts above 4 Log CFU/g in the C series, whereas lower values (around 3.5 Log CFU/g) were detected. Also *Pseudomonas* spp. showed the same trend with a gradual increase in both series from t0 reaching values >6 Log CFU/g after 10 days of storage in the C series; these values were significantly higher if compared to those obtained for the E series (Δ=1,61 Log CFU/g; P= 0.01). Finally, LAB showed the same trend with an evident increase in both series from t0 to t10; C series samples reached counts close to 6 Log CFU/g, significantly higher values if compared to E series (Δ=2,02 Log CFU/g at t10; P=0.02). Yeasts showed only a limited increase during the storage, reaching values close to 4 Log CFU/g in both series.

The other contaminants were detected only in low counts in single samples (coagulase-positive Staphylococci) or were not detected in any of the samples (sulfite-reducing Clostridia, molds), showing a limited role in this product.

The modified atmosphere containing about 10% of CO_2 (C series), certainly exerted a certain inhibiting effect on the microbial development but did not prevent the progressive increase of the counts of aerobic bacteria, especially *Pseudomonas* spp. and *Enterobacteriaceae* that after 10 days of storage were above the threshold conventionally considered for fresh meat (respectively 6 and 5 Log CFU/g), suggesting a proper shelf-life of 8 days, as these levels were exceeded after 10 days of storage. Apparently, the experimental packaging exerted an inhibiting effect, that was evident towards all the microbial groups considered, both aerobic (*Pseudomonas*), facultative anaerobic (*Enterobacteriaceae*), or strict anaerobic (LAB).

The data referred to the pH of *arrosticini* showed evident acidification of the product during the test while maintaining values within the normal range for this type of product. Samples packaged with the two different preservation atmospheres did not show any statistically significant difference.

Regarding TVBN, the results obtained show considerable variability (Table 1). Considering a value of 30 mg/100 g as a general threshold for meat excessive proteolytic reactions, all samples resulted below this value, with the exception of sample E at t8 (30.78 mg/100 g). As no clear trends were observed during the trial, this value seems to be due to an initial high level, owing to the variability of meat pieces used for the production. No statistically significant difference could be observed between the two series. The data relating to TBARS (Table 1) showed very low values (close to 0 mg/kg), much lower than the maximum threshold value of 3 mg/kg, indicating that no lipid oxidation occurred during the shelf-life.

The values related to the lightness (L*) of the meat showed a decrease with increasing storage time, from an initial mean value of 41.82 to a mean value of 34.48 (C series) and 35.59 (E series) at t10 (Table 2). The values reflected an effective modification of the conditions of the product, with a clear decrease in brilliance. Between t8 and t10, a slight increase in the values was detected, but in any case, not enough to improve the appearance of the product.

The values of the red index (a*) reflected what has been described for the lightness, with a decrease from t0 (mean value of 21.88) to final mean values of 11.32 and 9.86 for the C and E series, respectively. C series showed significantly higher values at t8 if compared to the E series (P=0.05). an evident difference between the two series was also found during the evaluation of the yellowness (b*) at t8, with the C series showing significantly lower values if compared to the E series (P=0.01).

The scores assigned for the sensorial characteristics of the product allowed us to observe how the product inevitably deteriorates over time, starting from excellent initial values and reaching unacceptable values after 10 days (Table 3). In particular, the color parameter showed values below the acceptability threshold at t10 in both sample series; a brown discoloration was observed in the C samples, while the color was clearer in the E series. These data confirmed the values of color parameters measured on the samples. Experimental packaging resulted in an earlier deterioration of the color, with an evident modification from t5, also maintaining an acceptable appearance until t8. The odor and liquid parameters showed a deterioration in the final part of the trial; values just beyond the acceptability threshold were found at t10, indicating that the deterioration of the *arrosticini* was clear, in agreement

Table 1. Total volatile basic nitrogen and thiobarbituric acid reactive substances values detected during the trial.

	TBARS	t0	t5	t8	t10
C	Mean (mg/kg)	0.00	0.07	0.22	0.18
	SD	0.00	0.05	0.14	0.02
E	Mean (mg/kg)	0.00	0.23	0.18	0.13
	SD	0.00	0.12	0.04	0.13
	TVBN	t0	t5	t8	t10
C	Mean (mg/100 g)	16.98	17.89	14.18	21.37
	SD	6.69	3.62	2.67	2.01
E	Mean (mg/100 g)	16.98	24.77	30.78	24.47
	SD	6.69	0.54	12.85	1.99

TBARS, thiobarbituric acid reactive substances; TVBN, total volatile basic nitrogen; C, conventional packaging; E, experimental packaging; t0, time 0; t5, after 5 days; t8, after 8 days; t10, after 10 days; SD, standard deviation.

with the findings previously reported regarding microbial contamination. Meat texture showed a slight modification at the last sampling time, due to drying. No modifications were observed considering the other parameters (gas formation or problems linked to packaging).

Conclusions

Arrosticini have great commercial value because they allow the use of many sheep cuts, both of young and adult animals, and are very suitable for the modern market because they are easy and quick to prepare. However, this product is characterized by an unavoidable high perishability, owing to its composition (only fresh meat, without additives) and the presence of a very large exposed surface. Therefore, the shelf-life of this product is mainly entrusted to two hurdles, namely storage temperature and protective atmosphere. In the conditions tested during the trial (conventional atmosphere and maximum temperature allowed for product storage), a shelf-life of 8 days maximum can be set, based on microbiological, colorimetric, and sensorial data. In light of the microbial growth observed, it is essential to ensure a good starting quality of the product, through compliance with good slaughtering

Table 2. Color parameters detected during the trial.

	L*	t0	t5	t8	t10
C	mean	41.82	41.13	33.04	34.48
	DS	1.84	1.63	0.48	2.57
E	mean	41.82	41.00	36.45	35,59
	DS	1.84	1.50	3.49	1.03
	a*	t0	t5	t8	t10
C	mean	21.88	18.77	14.12	11.32
	DS	0.95	0.37	1.10	1.46
E	mean	21.88	17.39	10.63	9.86
	DS	0.95	2.07	1.30	1.49
	b*	t0	t5	t8	t10
C	mean	16.71	14.71	8.96	9.29
	DS	0.77	0.43	0.14	1.06
E	mean	16.71	13.90	8.35	8.96
	DS	0.77	0.36	0.02	0.56

L*, lightness; a*, red index; b*, yellowness; C, conventional packaging; E, experimental packaging; t0, time 0; t5, after 5 days; t8, after 8 days; t10, after 10 days; SD, standard deviation.

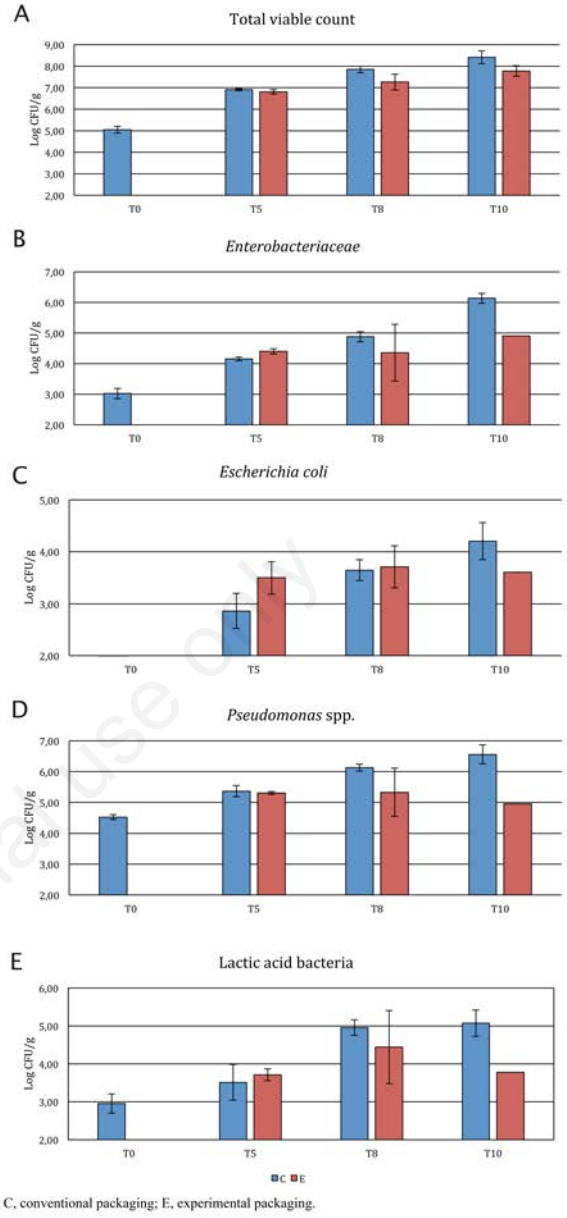

Figure 1. Results of microbiological analyses performed on the two sample series during the storage at 4°C. A) Total viable count; B) *Enterobacteriaceae*; C) *Escherichia coli*; D) *Pseudomonas* spp.; E) Lactic acid bacteria.

Table 3. Sensorial scores assigned during the trial.

C	Exudate	Color	Odor	Texture	Gas formation	Packaging	General score
t0	5	5	5	5	5	5	5
t5	5	5	4.5	5	5	5	5
t8	4	4.5	4	5	5	5	4
t10	3	2.5	3	3.5	5	5	3
E	**Exudate**	**Color**	**Odor**	**Texture**	**Gas formation**	**Packaging**	**General score**
t0	5	5	5	5	5	5	5
t5	5	3.5	4.5	5	5	5	4
t8	4.5	3.5	3.5	5	5	4.5	3.5
t10	3	2.5	3	3.5	5	5	3

C, conventional packaging; E, experimental packaging; t0, time 0; t5, after 5 days; t8, after 8 days; t10, after 10 days

and processing practices, to avoid the presence of pathogens and ensure low initial microbial loads.

The use of an oxygen-free experimental atmosphere (E series), determined a positive effect if considering strictly microbial population evolution, but its effect on the sensorial characteristics of the product (in particular the color) was not improved. Therefore, it was possible to confirm the best general performance of the product packaged using the conventional atmosphere.

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