MICROBIOLOGICAL AND PHYSICOCHEMICAL QUALITY EVALUATION OF VACUUM-PACKED ARGENTINE BEEF IMPORTED INTO ITALY

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ABSTRACT

A total of 8 lots of vacuum-packed bovine rump hearts (*Gluteus medius* muscle) imported in Italy from Argentina were submitted to microbiological (total bacterial count, *Enterobacteriaceae*, *Pseudomonas* spp., Lactobacilli, sulfite-reducing Clostridia, *Listeria monocytogenes*) and physicochemical analyses (pH, total volatile basic nitrogen, color measurement and shear force) after different storage times (35, 75 and 100 days). Lactobacilli were the predominant microbial population (about 6 log cfu/cm^2), causing a microbial stabilization and acidification of meat. Seventy-three Lactobacilli isolates were submitted to random amplified polymorphic DNA-polymerase chain reaction and identified, showing a high prevalence of *Lactobacillus sakei* (in all the samples) and *Lactobacillus curvatus* (in samples stored for 75 or 100 days). We observed high total volatile basic nitrogen levels (>27 mgN/100 g) in all the samples and a discoloration of beef after the opening of the packs. Our results suggest the need for a higher standardization of production conditions.

PRACTICAL APPLICATIONS

Vacuum-packed raw beef from Argentina is globally commercialized, and it is frequently shipped to European markets. Considering the perishability of this product and the very long shelf life assigned, the availability of microbiological and physicochemical data could be useful for quality evaluations purposes. Our data indicate that a long shelf life (3–4 months) is potentially achievable, but the application of the best hygienic practices during meat production and an optimal stabilization of microflora by the selection (or addition) of lactic acid bacteria must be assured. As protein degradation and microbial population showed to be stable during the shelf life, quality characteristics that are perceived by the consumer (such as color indexes) become important parameters for a proper evaluation of meat quality.

INTRODUCTION

Argentina is one of the most important suppliers of high-quality beef around the world; data concerning the fresh beef trade indicate an export quota of 118.000 tons in 2011 (Ministerio de Agricultura, Ganaderia y Pesca, Republica Argentina 2012). This value includes also the high-grade "Hilton beef" (about 20.000 tons in 2011), defined as "selected beef cuts obtained from steers, young steers or heifers having been exclusively fed through pasture grazing since their weaning" and classified by the Secretariat of Agriculture, Livestock, Fisheries and Food in Argentina (EC 2008). Hilton quota guarantees certain suppliers access to the EU beef market at reduced tariffs; the annual quota settled for Argentina is actually 29.500 tons. For the production of Hilton beef, steers and heifers are slaughtered in authorized plants; carcasses are deboned and primal cuts are vacuum packaged and shipped in refrigerated containers until final marketing (Europe, North America, etc.).

In the international market, Argentine beef has an excellent reputation, due to technological, sensory and nutritional quality and to its symbolic associations (linked to extensive pasture rearing) (Champredonde 2008). The most important quality characteristics that are perceived by consumers and can influence their acceptance are the color and the tenderness of meat. Argentine beef is characterized by a pronounced red color, due to a higher concentration (compared with European beef) of myoglobin. This can be explained by the higher proportion of oxidative fibres within the muscles, due to genetic factors and grass-based feeding (Vestergaard *et al.* 2000; Raes *et al.* 2003; Schor *et al.* 2008).

Pasture-based diet also ensures the protection from myoglobin oxidation, as it supplies high concentrations of natural antioxidants, such as β-carotene and α-tocopherol (Insani *et al.* 2008). Considering beef tenderness, it has to be noted that Argentine beef undergoes a prolonged aging process that takes place during the long shipping period (Lee and Yoon 2001). During aging, proteolytic and lipolytic reactions take place, leading to a higher concentration of low molecular weight saturated and unsaturated aldehydes, which are the products of lipid oxidation, as well as branched and aromatic aldehydes. The results are a marked tenderization of beef and the development of typical flavor (Raes *et al.* 2003; Fadda *et al.* 2008; Schor *et al.* 2008).

The shelf life assigned to Argentine vacuum packed beef cuts is generally 120–150 days in refrigerated storage, a significantly longer period if compared with the standard shelf life of European beef obtained in similar conditions (30–45 days). Vacuum packaging is considered a protective factor, as it can extend its shelf life by a strong reduction of oxidative reactions and, after all, by a selection of anaerobic microflora on the surface of meat (Seideman *et al.* 1976; Fu *et al.* 1992). During the storage, the microbiota is so dominated by gram-positive psychrotrophs, especially lactic acid bacteria (LAB, mainly *Lactobacillus*, *Leuconostoc* and *Carnobacterium*), and other spoilage organisms as *Brochothrix thermosphacta* and *Enterobacteriaceae* (Bell and Garout 1994; Lücke 2000; Sakala *et al.* 2002).

Actually, there is little information about the quality of very long-shelf life vacuum-packed meat, as few data have been published (Fu *et al.* 1992; Bell and Garout 1994; Lee and Yoon 2001; Stella *et al.* 2005). In optimal production conditions, some authors reported a possible mean shelf life of at least 90–120 days (Bell and Garout 1994), but the presence of negative modification of sensory characters has been described (Lee and Yoon 2001). The qualitative problems that are occasionally reported consist of an excess in protein hydrolysis or a gradual development of microbial spoilage that can lead to the presence of off-odors and off-flavors (sulfurous or sour, due to the growth of *Enterobacteriaceae* or lactic acid bacteria; liver-like due to protein hydrolysis), meat surface discoloration or mushy texture (Pierson *et al.* 1970; Bell and Garout 1994; Katikou *et al.* 2005). In addition, some concomitant physical changes, i.e., drip loss and/or pH changes, during storage can reduce the overall appearance and acceptability of packaged meat (Doherty *et al.* 1996; Lee and Yoon 2001).

This study aims to evaluate the microbial and physico-chemical quality of beef primal cuts imported in Italy from Argentina, with particular attention to the composition of its microbial population.

MATERIALS AND METHODS

Beef Samples

For the study, samples of vacuum-packed boneless beef cuts (heart of rump, *gluteus medius* muscle) imported from Argentina, belonging to eight different lots, were purchased from an import company; the label reported a shelf life of 120 days from packaging (the cuts were packaged 5 days after slaughtering). The packages were shipped to Italy in refrigerated containers and then kept into the importer's refrigerated store; during transport and storage, a temperature of $0 \pm 2C$ was assured.

The lots were divided into three groups based on the storage duration: 35 days from packing (2 lots), 75 days from packing (2 lots) and 100 days from packing (4 lots). For each lot, three samples were submitted to the analyses that were performed in duplicate. Before sampling, each beef pack was checked in order to evaluate its integrity (presence of holes in the plastic bag or in the seal, leaks of liquids and air entry). The gas composition of the headspace was also measured by a portable O_2–CO_2 analyzer equipped with a pointed probe that was inserted into the closed packs (Oxybaby M–O_2/CO_2, Witt Gasetechnik, Witten, Germany).

Microbiological Analyses

Immediately after the opening of the packs, each beef cut was submitted to sampling for microbiological analysis using a nondestructive technique, by superficial swabbing (swab Cultiplast, LP Italiana, Milan, Italy) of a 100 cm^2 ($10 \times 10 \text{ cm}$) area. Each swab was put into a tube containing 10 mL of saline solution (NaCl-tryptone, 0.9%) and shaken; then, serial dilutions were prepared. Microbiological analyses were performed using ISO (International Organization for Standardization) and AFNOR (Association Française de Normalisation) methods, when available. The following microbiological parameters were considered:
• Total bacterial count (TBC) by the ISO 4833:2003 method (ISO, 2003a);

• Count of *Enterobacteriaceae*, by the ISO 21528-2:2004 method (ISO, 2004);
• Count of *Escherichia coli*, by the ISO 16649-2:2001 method (ISO, 2001);
• Count of *Pseudomonas* spp., by the ISO 13720:2010 method (ISO, 2010);
• Count of Lactobacilli on deMan, Rogosa, Sharpe (MRS) agar (Oxoid, Basingstoke, UK); plates were incubated in hermetic jars (AnaeroJar, Oxoid) with anaerobiosis generators (AnaeroGen, Oxoid) at 30C for 48 h, according to the manufacturer's instructions (Oxoid 2006); and
• Count of spores of sulfite-reducing Clostridia, by the ISO 15213:2003 method (ISO 2003b), after pasteurization of the dilutions.

The presence of *Listeria monocytogenes* was also investigated, considering the possible persistence and slow development of this pathogen in vacuum packaged beef. The analysis was performed following the AFNOR BRD 07/4–09/98 method (AFNOR 1998).

Identification of Lactobacilli Isolated from the Samples. The population of Lactobacilli from beef samples was submitted to molecular identification, as they represent the main bacterial population in vacuum-packed meat, and could play a critical role as potential competitors toward spoilage microorganisms. Colonies grown on the plates were randomly picked (5–10 colonies for each sample), subcultured in deMan, Rogosa, Sharpe (MRS) broth (VWR International PBI, Milan, Italy) and plated again on MRS agar in order to obtain pure cultures. Then, the pure strains were stored at –25C in cryovials (Microbank, Pro-Lab Diagnostics, Round Rock, TX) until molecular identification, which was performed by VenetoAgricoltura Laboratory (Thiene, Italy).

A total of 74 LAB strains were classified by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). For the analysis, primers M13 and D11344 were used, applying the method reported by Andrighetto *et al.* (2002). Such primers had already shown optimal reproduciblity and discrimination capability with lactic acid bacteria (Andrighetto *et al.* 2009; Menz *et al.* 2010).

Grouping of the RAPD-PCR profiles was obtained with the Gel Compar 4.1 software package (Applied Maths, Kortrjik, Belgium), using the Pearson product moment correlation coefficient and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis; a similarity threshold value of 0.85 was considered. One selected strain for each genetic profile obtained was then identified by DNA sequencing of V3–V8 region of 16S rDNA. DNA was amplified using primer pair P1 (Muyzer *et al.* 1993) and L1401 (Zoetendal *et al.* 1998); after purification, PCR products were sequenced. The species attribution was performed after comparison of the obtained sequences with the public database available from the National Centre for Biotechnology Information (NCBI 2012), using the Basic Local Alignment Search Tool (BLAST) software (BlastN algorithm).

Physical and Chemical Analyses

When the packs were opened, the quantity of liquid within the packs was evaluated by measuring the gross weight and subtracting the weight of the cut and that of the plastic bag (previously dried). Meat pH was measured using a pH meter HD 2105.2 (Delta Ohm, Caselle di Selvazzano, Italy) equipped with a probe that was inserted into the muscular tissue; pH of the liquid taken from the pack was also measured using the same equipment. Total volatile basic nitrogen (TVBN) was determined (EC 2005) for estimation of spoilage in duplicate.

In order to evaluate beef tenderness, beef slices (3-cm thick) were removed parallel to the muscle fibre orientation, vacuum packed and frozen; the samples were stored at –20C for a maximum of 1 week before the analysis. The slices were then thawed at 4C until thermal equilibration, and cooked following the method by Honikel (1998) with minor modifications. In brief, the slices were put into 190×300 mm, 65-μm thick Polysilk bags (thermal resistance: -40 ± 80C; Baglight, Interscience, Saint Nom, France) and placed in a thermostatic water bath at 75C, until they reached a targeted peak internal temperature of 72C. Temperature measurements were obtained by a thermometer (735-2, Testo, Settimo Milanese, Italy) equipped with a probe (PT-100, Testo) inserted into the core of each steak. When the end-point temperature was reached, the bags were removed from the water bath and rapidly cooled under tap water and then chilled in refrigerator until equilibrated (+4C). The samples were submitted to the measurement of Warner–Bratzler shear force (WBSF) evaluation by an Instron Universal Testing Machine (model 5542, Instron Engineering Corp., Canton, MA); the analysis was performed on 6 shares (1.27 cm in diameter) from each sample. The shares were cut parallel to the longitudinal orientation of muscle fibres; the peak shear force was measured (Warner-Bratzler blade speed 200 mm/min), and mean values were recorded.

Color Parameters Measurement

Samples stored for 35 and 100 days were submitted to the color measurement, using a Minolta CR-300 Chroma Meter (Minolta, Osaka, Japan) working as a CIELab system. After calibration using a standard white plate, the chromameter instrument was positioned perpendicular to the slice surface, after removing the superficial fat. Measurements were performed on 2-cm thick meat slices, at different time intervals: (a) immediately after opening of the pack; (b)

45 min after opening, in order to allow blooming (deoxy-myoglobin oxygenation); (c) 24 h after opening (aiming to evaluate the myoglobin stability). During this time, beef slices were put into Polysilk bags (Baglight), in order to permit the contact with air but avoiding superficial drying, and maintained at refrigeration temperature (2 ± 1C) to maintain optimal storage condition. The L^*, a^* and b^* values, which describe the intensity of whiteness/brightness, red color ($a^* > 0$) and yellowness ($b^* > 0$), respectively, were taken at ten locations on the upper layer of each sample. The respective mean of 10 such measurements was expressed as the final value. The hue angle (h) was calculated as $h = \arctan(b^*/a^*)$, where $h = 0°$ for red hue and $h = 90°$ for yellowish hue.

Statistical Analysis

Data concerning bacterial counts (TBC, Lactobacilli and *Enterobacteriaceae*), physicochemical analyses and color parameters measurements were grouped based on the storage duration (35, 75 or 100 days), and submitted to one-way analysis of variance (ANOVA) by SAS/stat package version 8.0 (SAS Inst. Inc., Cary, NC). A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

All the packs analyzed were intact at an external control (no holes, air into the pack or liquid spilling were observed). The analysis of internal gas composition confirmed the integrity of vacuum packages; the concentration of carbon dioxide was >90% in all the packs, whereas oxygen content was <1%.

Microbiological Analyses

The results of the microbial populations performed on vacuum packed cuts are reported in Table 1. The samples analyzed showed a low level of bacterial contamination, taking into account the long-term storage of these cuts. These data suggest that, when a low initial contamination level of beef is assured, the storage conditions applied (vacuum packaging associated with optimal refrigeration

temperatures) give a sufficient stability for long-distance marketing.

TBC was about 6 log cfu/cm², and was not associated to any evident sensorial alteration of beef. Almost the whole bacterial population was formed by Lactobacilli, which are naturally selected in vacuum packaging environmental conditions, especially when the packs are stored for a long time. These results confirm the small number of data obtained from similar samples (Seideman *et al.* 1976; Bell and Garout 1994; Lee and Yoon 2001). The counts of potential spoilage bacteria were very low; as expected, the number of *Pseudomonas* spp. on meat surface was under the limit of detection (10 cfu/cm²), due to the anaerobic atmosphere. A low level of contamination by *Enterobacteriaceae* was observed; these bacteria represent the main spoilage agents in vacuum-packed meats, as some members of this family are psychrotrophic and proteolytic spoilage agents (Fu *et al.* 1992; Borch *et al.* 1996), but the presence of high counts of Lactobacilli could inhibit their growth, as reported by Lee and Yoon (2001). The presence of *Escherichia coli* and sulfite-reducing Clostridia, which can be considered as contamination indicators and potential pathogens, was not detected; also, the pathogenic *Li. monocytogenes* was absent in all the samples analyzed.

The analysis of data showed the absence of differences among the samples with increasing storage time. Such results reflected a selection of Lactobacilli population, which overgrew the other classes of bacteria during the prolonged storage and could represent a protective factor for the hygienic status of beef for the whole shelf life.

Identification of Lactobacilli

The results of Lactobacilli genotypic classification and identification are shown in Figure 1. A total of 73 isolates were identified: *Lactobacillus sakei* was the dominating species (56 isolates), followed by *Leuconostoc mesenteroides* (9 isolates) and *Lactobacillus curvatus* (8 isolates). These bacterial species are often isolated from vacuum-packed meat, and represent the natural microflora selected by long-time storage of meat in refrigerated anaerobic environment. In particular, *La. sakei* and *La. curvatus* are the most widespread species of Lactobacilli in vacuum-packed beef (Yost and Nattress 2002; Fontana *et al.* 2006). Between the two

TABLE 1. BACTERIAL POPULATIONS OBTAINED FROM BEEF CUT SAMPLES (LOG CFU/CM²)

Days of storage	Total bacterial count	Lactobacilli	*Enterobacteriaceae*	*Pseudomonas* spp.	*Escherichia coli*	Sulfite-reducing Clostridia
35	6.2 ± 0.2	6.0 ± 0.5	3.2 ± 0.5	<1	<1	<1
75	5.7 ± 0.8	5.5 ± 0.2	3.3 ± 1.4	<1	<1	<1
100	6.4 ± 0.7	6.2 ± 0.8	2.4 ± 1.6	<1	<1	<1

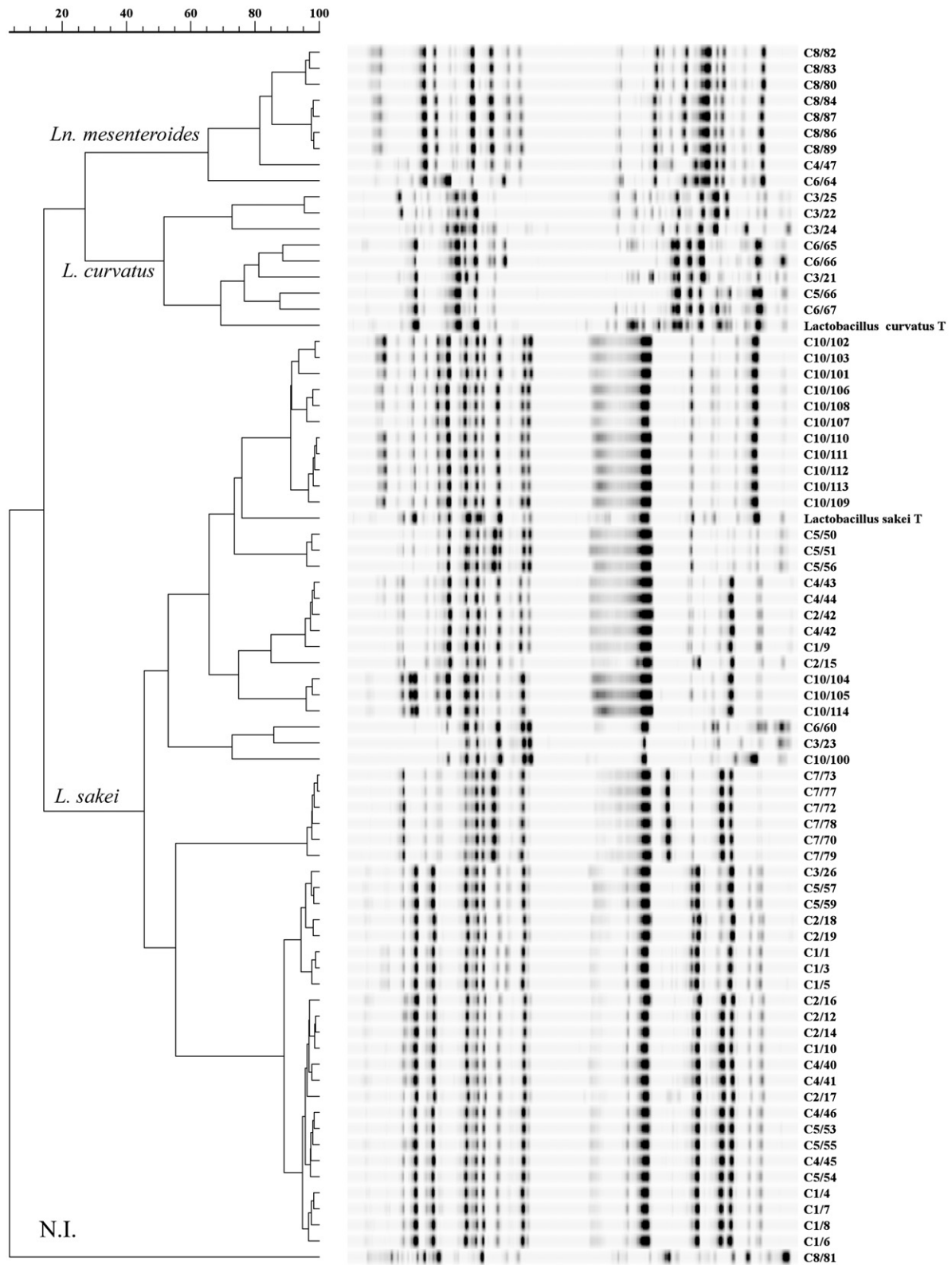N = 24 samples. The values are expressed as means ± standard deviations.

FIG. 1. CLASSIFICATION AND IDENTIFICATION OF LACTOBACILLI ISOLATES BY RANDOM AMPLIFIED POLYMORPHIC DNA-POLYMERASE CHAIN REACTION (RAPD-PCR) AND SEQUENCING OF THE V3-V8 REGION OF 16S rDNA.

The 0 to 100 scale indicates the percentage of genetical similarity between the strains. N.I., not identified.

species, *La. sakei* was isolated from samples with all the three storage times, whereas *La. curvatus* was isolated only from samples with 75 or 100 days of storage. An evident clonal variability was detected, as different patterns were observed for each species, but there was a high predominance of one *La. sakei* pattern (24 isolates). It could be due to long-time bacterial selection, but we can also suppose an addition of this strain to meat before packaging. In fact, some studies have previously shown the utility of using *La. sakei* and *La. curvatus* as bioprotective cultures for meat, and an application to vacuum-packed Argentine beef has been already described (Hugas *et al.* 1998; Castellano and Vignolo 2006; Castellano *et al.* 2010). These two species are able to produce bacteriocins (sakacins, lactocins, curvacin and curvaticin) that are active against gram-positive bacteria (*Li. monocytogenes* and *B. thermosphacta*), but they also produce organic acids that can cause a significant decrease also in gram-negative bacterial numbers (Lücke 2000; Katikou *et al.* 2005; Castellano *et al.* 2008). The role of these microorganisms in the storage of vacuum-packed beef must be further clarified, as in some cases, *La. sakei* was identified as spoilage agent. Some authors have evidenced its role in causing souring, production of sulfur compounds in strictly anaerobic conditions, gas production and slime (Egan *et al.* 1989; Castellano *et al.* 2008). Such alterations were not detectable in the samples analyzed in our study.

Physical and Chemical Analyses

On opening of the packs, a marked aroma was present; this organoleptic property is strongly demanded by several consumers, and it can be considered typical for Argentine beef. The mean content of liquid into the packs was 4.9%; an evident variability was observed between the packs, but it was not related to the duration of the storage. The high liquid content within meat vacuum packs has been already reported by other authors, and can be explained by the action of the negative pressure operated by vacuum pump during packaging (Seideman *et al.* 1979; Fu *et al.* 1992; Doherty *et al.* 1996; Lee and Yoon 2001).

The results of pH, TVBN and WBSF measurements are shown in Table 2. pH values of the cuts showed a decreasing trend during the prolonged storage, and a significant differ-ence ($P = 0.024$) was observed between samples stored for 35 and 100 days. These values agree with other studies conducted on long-shelf life vacuum-packed beef (Fu *et al.* 1992; Stella *et al.* 2005), while some authors observed a higher pH stability (Bell and Garout 1994; Lee and Yoon 2001). We can suppose that a gradual acidification occurred during the storage, due to the growth of lactic-acid producing microflora, which is favored by vacuum conditions and became the prevalent microbial population during the long shelf life of the meat. This action was evident in particular in the liquid, which represent the best environment for the proliferation of Lactobacilli.

Total volatile basic nitrogen values measured in beef samples were very high in all the samples, with a significant increase ($P = 0.028$–0.030) in cuts stored for longer periods (75–100 days). These values were higher than that measured by other authors (Fu *et al.* 1992; Bell and Garout 1994; Lee and Yoon 2001), and exceeded the limit (20 mg N/100 g) indicated to define the deterioration of raw meat (Pearson 1973), but it has to be noted that high TVBN levels could also be measured in meat without evident odor or flavor deterioration. The formation of TVBN is due to the prolonged action of muscle proteolytic enzymes, but also the high number of Lactobacilli could contribute to this reaction (Castellano *et al.* 2008). Several LAB strains, including *La. sakei* and *La. curvatus*, have been shown to possess *in vitro* proteolytic abilities, as they contain several peptidases and proteases that can attack both myofibrillar and sarcoplasmic proteins (Fadda *et al.* 1999; Sanz *et al.* 1999; Katikou *et al.* 2005). The proteolytic reactions lead to the release of small peptides and free amino acids that will positively impact on meat taste and flavor (Fadda *et al.* 2008), but an excessive rate of this process results in a deterioration of its sensory properties.

The long storage/maturation period that characterizes Argentine beef had also an evident influence on meat tenderness; WBSF values were very low in all the samples, due to the prolonged action of proteolytic enzymes. Such values fall into the category of "very tender" meat (<2.54 kg), as indicated by Platter *et al.* (2005), and are evidently lower than those observed in European and North American beef, aged for shorter periods (Raes *et al.* 2003; Gruber *et al.* 2006).

Days of storage	pH		TVBN (mgN/100 g)	WBSF (kgf/cm²)
	Muscle	Liquid		
35	5.73 ± 0.1^y	5.70 ± 0.2	27.8 ± 2.2^z	2.08 ± 0.4
75	5.59 ± 0.1	5.49 ± 0.1	32.0 ± 0.9^y	1.57 ± 0.4
100	5.47 ± 0.1^z	5.38 ± 0.1	33.3 ± 0.8^y	2.02 ± 0.6

TABLE 2. RESULTS OF PHYSICOCHEMICAL ANALYSES

$N = 24$ samples. The values are expressed as means ± standard deviations.
Values in the same column with different letters ($^{y, z}$) are significantly different ($P < 0.05$).
TVBN, total volatile basic nitrogen; WBSF, Warner–Bratzler shear force.

TABLE 3. MEAT COLOR PARAMETERS

Days of storage	Exposition time	L*	a*	b*	Hue angle (°)
35	0 min	36.2 ± 2.7	19.9 ± 3.1	3.8 ± 1.2	10.7 ± 2.1
	45 min	36.9 ± 3.0	21.8 ± 2.2	8.2 ± 2.1^z	20.5 ± 3.1^z
	24 h	35.8 ± 1.9^z	16.3 ± 1.7	7.7 ± 2.5	24.8 ± 6.6
100	0 min	37.1 ± 1.6	21.3 ± 2.2	4.2 ± 0.9	11.2 ± 2.1
	45 min	39.3 ± 2.4	25.4 ± 1.8	12.6 ± 1.5^y	26.4 ± 1.4^y
	24 h	40.3 ± 1.5^y	16.0 ± 3.1	9.8 ± 1.1	31.9 ± 3.2

$N = 18$ samples. The values are expressed as means ± standard deviations.
Values in the same column and at the same exposition time with different letters ($^{y, z}$) are significantly different ($P < 0.05$).

Color Parameters Measurement

The results of color parameters evaluation of meat cuts are presented in Table 3. After the "blooming" phase, the red color of beef ($a*$) was maintained also after a long storage period, as no significant differences ($P > 0.05$) were observed in samples stored for 35 and 100 days, but it was followed by a discoloration during the exposure to the aerobic environment. The hue angle measured after 1 h of exposition to air rose significantly ($P = 0.034$) during the storage, indicating a faster browning of beef stored for 100 days. Such difference was still evident, also if not statistically significant ($P > 0.05$), 24 h after pack opening, and must be considered as it represents a critical factor by consumers. Our results agree with those obtained by Lee and Yoon (2001) in similar conditions, while Rodas-Gonzalez *et al*. (2011) obtained a higher variation in $a*$ and $b*$ parameters, resulting in higher hue angle values after similar storage periods. The maintenance of red color is mainly due to the myoglobin reducing activity (MRA) of the meat, which is influenced by several factors. Argentine beef is generally characterized by high values of MRA, as it is obtained from animals fed on pasture during a long period of life, resulting in a natural richness in "red" oxidative muscle fibres and high concentrations of natural antioxidants (Raes *et al*. 2003; Descalzo and Sancho 2008; Insani *et al*. 2008; Schor *et al*. 2008). Moreover, vacuum-packaging represents a protective factor, as it removes oxygen from the environment and favours the growth of lactic acid bacteria. These microorganisms influence positively the MRA of meat by the production of lactate, which is known as a "color stabilizer", by different mechanisms that are only partially clarified (Amanatidou *et al*. 2001; Kim *et al*. 2006).

During the long period of meat ageing, MRA decreases gradually; the mechanism involved is not actually clear, and different factors could be involved (bacterial growth, deterioration of antioxidant compounds, formation of unsaturated aldehydes from the lipidic component), as explained by Mancini and Hunt (2005).

CONCLUSIONS

The quality of Argentine beef is well known, and it is important to evaluate the maintenance of its sensory and hygienic quality during the very long shelf life. Such a prolonged period of storage, if compared with European beef, leads to the formation of typical flavor and tenderness, which are in demand. However, it has to be considered that the shelf life of a metabolically active product as vacuum-packaged raw beef is necessarily variable, also if its microbiological status is quite stable. So, if in optimal production conditions, a long shelf life (3–5 months) has been frequently observed, the durability of the product cannot be constantly assured. In our study, the shelf life duration of beef was affected mainly by the degree of discoloration when submitted to the presence of air, while microbial populations were quite stable irrespective of the storage time.

Great effort is actually made by researchers and producers in finding technologies to ensure a constant quality for the whole shelf life, but it has to be considered that every potential treatment must join law requirements and consumers' acceptance. Raw meat cannot be submitted to "hard" conservative treatments, and the best approach is the synergic application of factors with slight single activity to modify the environment in order to limit the development of spoilage microflora and meat alterations.

In this field, a very promising approach is the use of "bioprotective" and "functional" LAB cultures (especially some *Lactobacillus* spp.), which pair an antagonistic action toward spoilage and pathogenic bacteria (by competition for nutrients, adhesion to the substrate and production of antibacterial compounds) with sensorial, technological, nutritional and/or health advantages, such as flavor and tenderness improvement and meat color stabilization. Considering the little number of scientific studies dealing with long-shelf life vacuum-packed meats, further research is needed in order to identify the microbiological and biochemical factors and their interrelationship that can influence this peculiar "ecosystem."

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