

# Microbiological, rheological and physical-chemical characteristics of bovine meat subjected to a prolonged ageing period

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## Abstract

The aim of this study was to evaluate the effects of a long ageing period on the microbiological, rheological and physical-chemical characteristics of bovine beef. For the trial n. 3 Marchigiana bovine breed (live weight of 760 kg approximately), slaughtered at 34 months were chosen and the loin muscles were undergone to a prolonged ageing process. The analytical determinations performed were: pH and  $a_w$  values, texture profile analysis, Warner-Bratzler shear force, colour (CIE L\*a\*b\*), centesimal analysis, total bacterial count, *Enterobacteriaceae*, *Listeria monocytogenes*, yeasts and moulds. The results indicate that extended ageing has a negative effect on weight loss but, by the means of the standardization of dry aging parameters, reduce lipid oxidation and improve tenderness.

## Introduction

The consumer meat quality perception plays an important role in the marketing and important sensory attributes are taste, tenderness, juiciness, freshness, leanness, healthiness and nutrition (Grunert *et al.*, 2004) that are mostly influenced by breed, sex and age. These parameters are also correlated to techniques of handling, feeding, slaughtering and period of refrigeration-ageing post mortem.

The influence of ageing on beef quality in terms of tenderness is considered the most important trait affecting consumer beef-eating satisfaction (Beermann, 2009). Effects of ageing on beef tenderness have been well-documented (Bratcher *et al.*, 2005; Colle *et al.*, 2015; Dixon *et al.*, 2012; Eilers, *et al.*, 1996; Gruber *et al.*, 2006). It is

indeed demonstrated that ageing increase beef tenderness because of the decrease of shear force values during post-mortem storage (Field *et al.*, 1971; Jennings *et al.*, 1978) as a result of the calpains myofibrillar proteolysis (Koochmarie, 1996). Dry ageing is one of the ageing techniques commonly used where unpacked meat is exposed directly to controlled environmental conditions (temperature, humidity and ventilation).

Dry-aged beef show a particular taste and flavour; however, this process is expensive because of high drying up, weight loss, possible high bacterial load and economic investment in terms of equipment and space (Parrish *et al.*, 1991). However, consumers would be willing to pay more the ageing beef meat once familiarized with its peculiar rheological characteristics (De Geer *et al.*, 2009).

Most research (Lepper-Blilie *et al.*, 2012; Perry, 2012) on beef tenderness has focused on the effects of relatively short-term ageing (80 days or less); the effects of extended ageing on tenderness, colour and on other features of beef meat have not yet been investigated.

The aim of the work is the evaluation of the effect of a long ageing period (290 days) on the microbiological, rheological and physical-chemical characteristics of bovine beef.

## Materials and Methods

### Sampling

For the trial n. 3 Marchigiana bovine breed, slaughtered in an EU authorized slaughterhouse at 34 months and live weight of approximately 760 kg were chosen. The half-carcasses were cold stored ( $0\pm 3^\circ\text{C}$ ) for five days and then the sirloin steak muscles (SSM) from both sides of the animal was removed. Subsequently the SSM were placed during the period of dry ageing in a forced ventilation cell with an automatic extraction system set at a temperature of  $0^\circ\text{C}$  and at HR values ranging between 68 and 70%. The back wall of the ageing cell was characterized by a particular coating represented by rock salt tiles (Pink Himalaya Salt) of about 2 cm deep. The analytical determinations, performed in Food Chemistry and Food Microbiology laboratories of the Department of Veterinary Medicine and Animal Productions of the University of Naples Federico II, were performed on SSM at T0 (13 days post slaughter (dPS)), T1 (36-dPS), T2 (110-dPS), T3 (170-dPS) and T4 (290-dPS). WBSF test was performed in

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Key words: ageing process, texture, meat maturation, tenderness.

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Contributions: GS, AA and RM designed the overall study. GS and LV performed all chemical and rheological determination. MFP and RLA performed microbiological analysis. RM, CMAB and GS analysed the results and drafted the paper. RM, MFP and AA contributed to the ideas behind the study and the writing of the paper.

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Animal Science laboratory of Department of Agricultural Science of the University of Naples Federico II. From each SSM 3.00 cm-thick steaks were aseptically removed each time of sampling.

### Physical-chemical analyses

Physical-chemical analyses were evaluated by: pH, measured with a digital pH-meter (Crison-Micro TT 2022, Crison Instruments, Barcelona),  $a_w$  (Aqualab 4 TE - Decagon Devices Inc., USA), Moisture (%) determined by oven drying for 24-h at  $105^\circ\text{C}$  (AOAC, 1990). Fat alteration index was evaluated by Thiobarbituric acid test (AOAC, 2000).

For the determination of nutritional value and for labelling purpose, according to Reg. EU 1169/11, total fat content, total saturated fat, salt content (% NaCl), sugars, carbohydrates, proteins and energy were determined (AOAC, 2000). All tests were done on n. 3 different samples in duplicate.

### Microbiological analyses

Ten grams of each sample and 90 mL (1:10 (W/W)) of sterilized Peptone Water (PW, CM0009, OXOID, Basingstoke, UK) were placed in a sterile stomacher bag and homogenized for three minutes at 230 rpm using a peristaltic homogenizer (BagMixer®400 P, Interscience, Saint Nom, France).

Subsequently, ten-fold serial dilutions of each homogenate were prepared in PW, followed by streaking in duplicate for total bacterial counts (TBC) performed according to ISO 4833-2:2013 on Plate Count Agar (PCA; CM0325, Oxoid) incubated at 30°C for 48/72-h and for the count of the Enterobacteriaceae (EB) performed according to ISO 21528-2:2017 on Violet Red Bile Glucose Agar (VRBG, CM1082, Oxoid) incubated at 37°C for 24-h. The enumeration of yeasts and moulds was performed according to ISO 21527-1 on Dichloran Rose-Bengal Chloramphenicol Agar (DRBC, CM0727, Oxoid) incubated at 25°C for 120/168-hours.

The isolation of *L. monocytogenes* was performed using the microbiological isolation methods ISO 11290-1:2017. In brief, 25 grams portions of each sample were homogenized into 225 ml (1:10 (W/W)) of Half Fraser broth (HF, CM1053, Oxoid) and incubated at 30°C for 24-h. Subsequently, a loopful of the enrichment both was transferred to the surface of ALOA and PALCAM plates and incubated at 37°C for 24-h. Moreover, 0.1 ml of the Half Fraser broth was also transferred to 10 ml of Fraser broth and incubated at 37°C for 24/48-h. Afterward, the subculture of Fraser broth was transferred in PALCAM and ALOA medium and incubated at 37°C for 24/48-h. To confirm the presence of *L. monocytogenes*, morphological, physiological and biochemical test were carried out.

### Rheological analysis

On all samples were determined: a) Texture Profile Analysis (TPA) that measures the compression force developed by the texturometer (Shimadzu EZ test) when compressing a piece of meat (Ruiz de Huidobro, 2003). A cylindrical 10 mm-diameter probe of ebonite was used for all TPA tests in this study.; b) Warner-Bratzler shear force (WBSF), that measures the force necessary to shear a piece of meat (1.27 cm of diameter) was determined using a Instron universal testing machine (Model 4201; Instron, Corp., Canton, MA ) with a 50 kg compression load cell operating at a crosshead speed of 250 mm/min. The parameter recorded was the maximum shear force which is the maximum resistance of the sample to shearing (Xiong *et al.*, 2006);

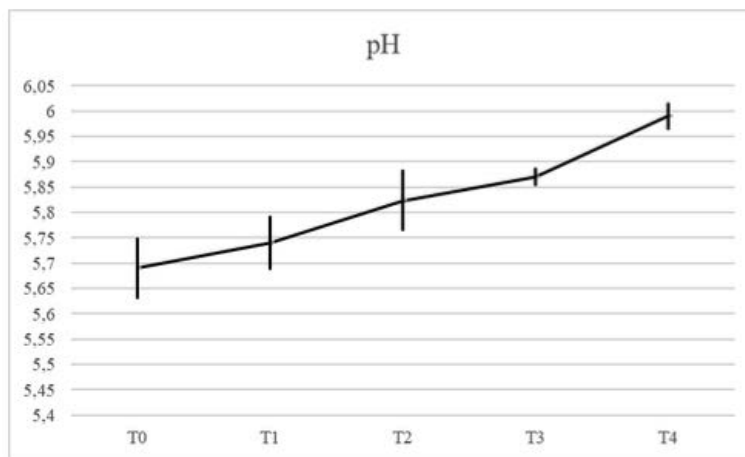


Figure 1. Trend of pH of the meat samples during ageing process (average ± SD).

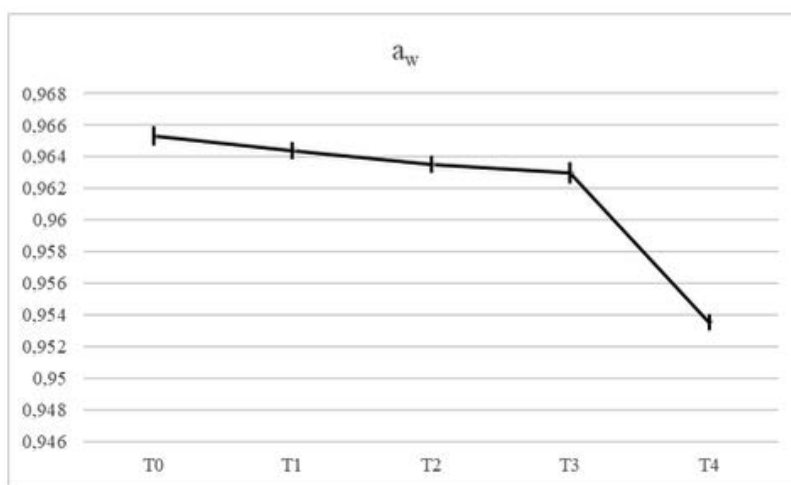


Figure 2. Trend of aw of the meat samples during ageing process (average ± SD).

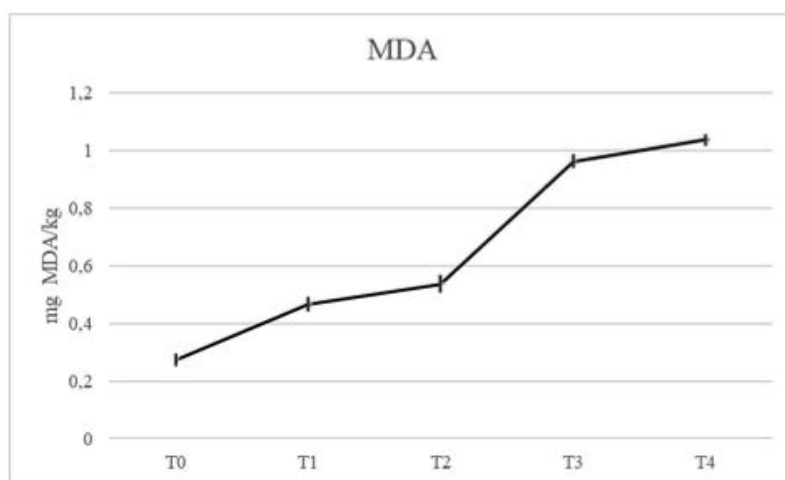


Figure 3. Trend of MDA during ageing process (average ± SD).

For all rheological analysis the steak cores were collected parallel to the muscle fibres, using a hand-held steel cork borer; c) Colorimetric assessment was performed by a Konica Minolta CR300 colorimeter (Minolta, Osaka, Japan) by CIE L\*a\* b\* colour scale.

## Results

### Physical-chemical analyses

PH increased during ageing process (Figure 1). On the other hand,  $a_w$  slowly decrease during the ageing process until T3: a sudden decline from 0.9629 to 0.9535 was observed (Figure 2). Figure 3 shows the trend of malondialdehyde (MDA) during ageing process.

Lipid oxidation increased slowly during the first sampling time of ageing process showing a sudden rise from T2 with values ranging between 0.273 and 1.036 mg MDA/kg (Figure 3).

Table 1, according to Reg. UE 1169/11, show the nutritional values of beef during the ageing period.

### Microbiological analyses

TBC and EB, showed a progressive decrease during ageing from 6.82  $\log_{10}$  CFU/g at T0 to 6.13  $\log_{10}$  CFU/g at T4 (Figure 4) and from 2.58  $\log_{10}$  CFU/g at T0 to 2.08  $\log_{10}$  CFU/g at T4 (Figure 5), respectively. The yeast slightly increased during ageing from 4.6  $\log_{10}$  CFU/g at T0 to 4.81  $\log_{10}$  CFU/g at T4 (Figure 6). Only moulds showed a stable trend (2  $\log_{10}$  CFU/g from T0 to T4 – Figure 7).

No pathogens were found.

### Rheological analysis

Coefficient of variation (CV) for instrumental parameters of TPA and WBSF profile during the meat maturation are showed in Table 2 (average values of n. 10 test on n. 3 different steak).

Among TPA variables measured, friability and springiness showed the highest (148.75 %) and lowest (11.43%) coefficient of variation respectively.

Particularly, the adhesiveness and the hardness showed the same trend: the values increased up to T4, with a low decrease at T3. Elasticity, cohesion and resilience result constant throughout the meat ageing. Cohesion and gumminess increased slowly until T3 showing only in the last interval values 3-4 times higher than the initial ones.

WBSF parameters decreased during ageing period, due to an increase in tenderness.

Concerning colour analysis L\* decreased, reaching final values of 10.41

confirming meat darkening. The coordinates  $a^*$  and  $b^*$  showed the same behaviour:  $a^*$  and  $b^*$  values increased up to T2 and then showed a fluctuating trend. Considering the colour space CIE L\*a\*b\* meat sample during maturation moved to

the colour area coinciding with the red ( $a^*$ ) and yellow ( $b^*$ ) becoming significantly darker and slightly redder. This higher  $b^*$  value could result from faster oxygenation on the fresh aged cut before a measurement was taken (Mac Dougall, 1977).

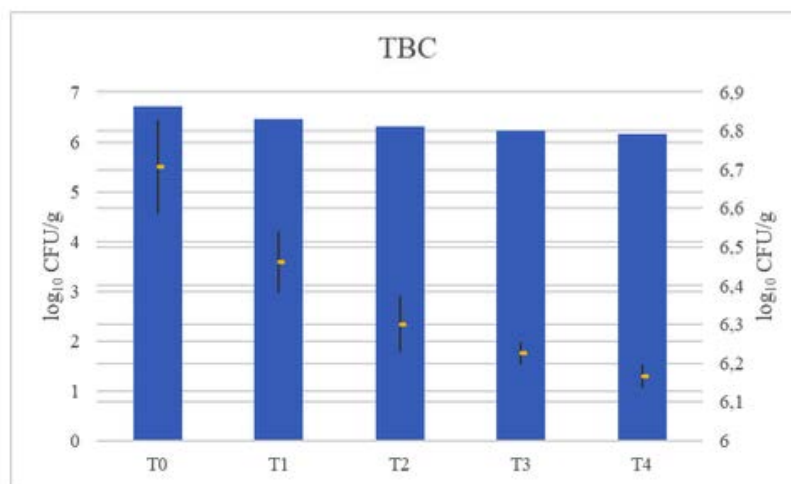


Figure 4. Total bacterial count ( $\log_{10}$  CFU/g  $\pm$  SD) of beef during the ageing period.

Table 1. Nutritional values of beef during the ageing period.

Parameters	Average values - 100 g				
	T0	T1	T2	T3	T4
Caloreis (Kcal)	171,68	175,1	185,69	192,84	192,98
Proteins	17,92	19,54	23,33	29,54	33,09
Carbohydrates	0,25	0,52	0,66	0,76	0,81
Sugars	/	/	/	/	/
Fat	11	10,54	9,97	7,96	6,38
Saturated fat	4,66	4,36	3,52	2,57	2,36
Sodium	0,66	0,72	1,33	1,45	1,56

Table 2. TPA and WBSF profile and coefficient of variation of during the meat maturation.

Parameters	T0	T4	CV (%)
Adhesiveness - TPA	-1.251	-13.566	106.45
Hardness - TPA	10.025	41.478	70.03
Springiness - TPA	0.653	0.660	11.43
Cohesiveness - TPA	0.425	0.295	20.11
Resilience - TPA	0.110	0.114	40.68
Cohesion - TPA	1.910	5.390	57.13
Gumminess - TPA	4.00	12.164	51.90
Chewiness - TPA	2.598	8.072	49.07
Friability - TPA	0	18.856	148.75
Peak force - WBSF	29.4	5.64	72.45
Initial force - WBSF	29.4	2.09	145.40
Work - WBSF	140.91	25.5	50.47

## Discussion

The initial pH can affect the improvement in tenderness: values detected in our work confirm result of Meat Export Federation of USA that found carcasses more tender with pH ranging between 5.4 and 5.7 (USMEF, 2014).

The pH and  $a_w$  behaviour were similar to previous studies (Abram and Gaš, 2001; Dashdorj *et al.*, 2016; Jayasooriya *et al.*, 2007; Silva *et al.*, 1999; Tapp *et al.*, 2017). Final pH was different from values found by Velotto *et al.* (2015) in dry aged Marchigiana beef with a short period of maturation (25 days).

Aging method affected weight loss: in this work aged meat loose about 85 % of its weight from T0 to T4 considering losing in moisture and trimming waste, higher than those found by Velotto *et al.* (2015).

Lipid oxidation generally increased during ageing period as already reported by Colle *et al.*, (2015). However, in the present study MDA values never reached level higher than 1.03 mg MDA/kg of meat, below the threshold value for rancidity of 2 mg MDA/kg (Watts, 1962). Since oxidation decreases calpain activity (Huff-Lonergan and Lonergan, 2005) the low levels of lipid oxidation detected may allow the calpains to stay active longer, so increasing tenderness thanks to the breakdown of muscle fibres caused by enzyme activity. According consumers opinion, the main characteristic to choose meat, besides tenderness, is colour (Renner and Labas, 1987); unfortunately, meat subjected ageing process becomes darker. The darkening can be caused not only by lipid oxidation (Farouk *et al.*, 1998), but also by changes in reducing ability, oxygen consumption rate, oxygen penetration depth, and myoglobin content (McKenna *et al.*, 2005). In our study, even if oxidative processes are associated with discolouration of meat producing metamyoglobin by oxymyoglobin oxidation, low levels MDA reached at the end of process, contribute to a lower discoloration than natural meat ageing.

CBT and *EB* are bacteria used to evaluate process hygiene criteria during food production process (Reg. EC 2073/05); The initial counts reflect the general meat microbiological status in relation to carcass handling, time of chilled storage and temperature control (Reid *et al.*, 2017; Savell, 2008). In meat stored at refrigerated temperatures are mainly composed by *Pseudomonas*, *Brochothrix* and *Carnobacterium* (Peruzy *et al.*, 2019), common spoilage microorganism. In the present trial, the average TBC and *EB* levels of the meat was lower to those report in other

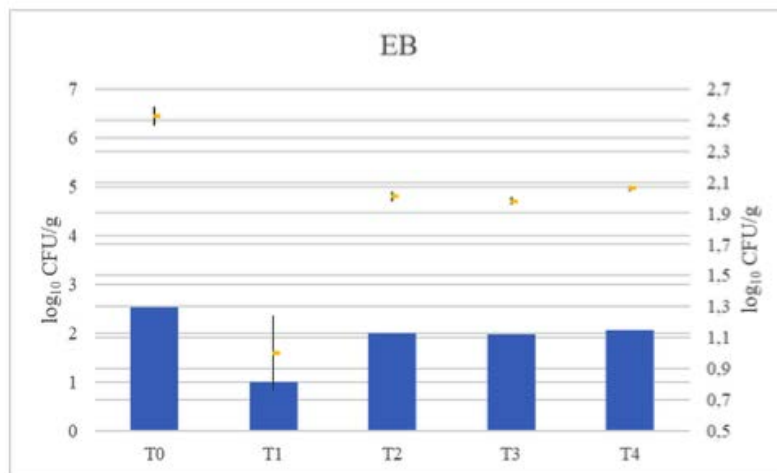


Figure 5. Enterobacteriaceae ( $\log_{10}$  CFU/g  $\pm$  SD) of beef during the ageing period.

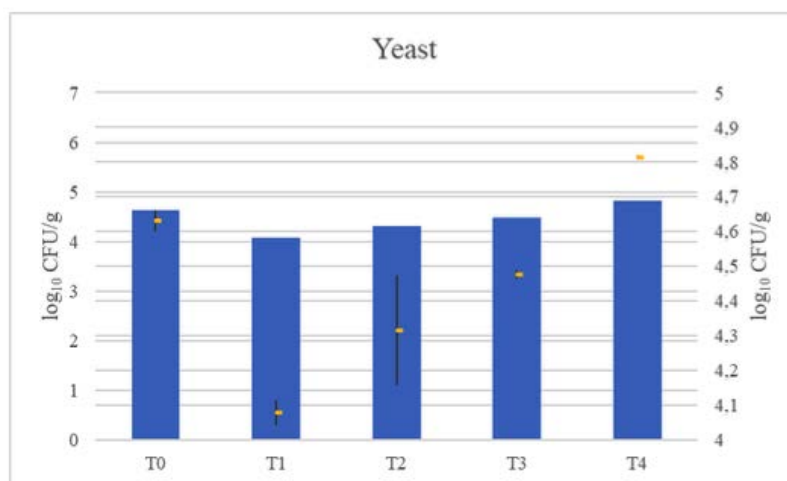


Figure 6. Yeast ( $\log_{10}$  CFU/g  $\pm$  SD) of beef during the ageing period.

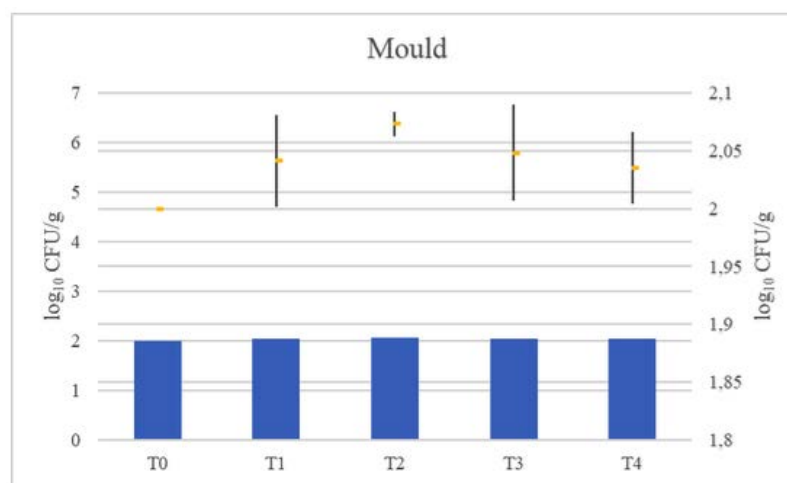


Figure 7. Mould ( $\log_{10}$  CFU/g  $\pm$  SD) of beef during the ageing period.

studies (Chuku *et al.*, 2016; Jahan and Siddique, 2015; Pennacchia *et al.*, 2011) and showed a progressive slightly decrease during ageing in agreement to ; this result could be due to the ageing protocol applied ( $T^{\circ}$  and  $U^R$ ) and to the subsequently decrease of  $a_w$  (Dashdorj *et al.*, 2016). Yeast and mould count had no significative changes because these microorganisms can tolerate the low temperature and  $a_w$ ; particularly, dry ageing encourages the growth of beneficial mould that could release proteases and create collagenolytic enzymes which break down the muscle and connective tissues bringing about tenderness and taste in the finished product (PrimeSafe).

Regarding the nutritional content, an increase in the content of proteins, NaCl and carbohydrates has been recorded, probably due to the weight-liquid loss during ageing with their concentration. Opposite behaviour for the fat content was recorded, probably due both to enzymatic lithic and oxidative processes (Dashdorj *et al.*, 2016).

WBSF values were ageing time and pH-dependent as showed in different studies (Savell, 2008; USMEF, 2014). The increase in tenderness was linked to enzymatic hydrolytic activities during post mortem ageing (Savell, 2008; Spanier *et al.*, 1997) by calcium-dependent hydrolases, calpain proteinases and cathepsins (Koohmaraie *et al.*, 2006). In this study peak force (WBSF), linked to meat tenderness, showed a progressive decrease, as described by George-Evins *et al.* (2004), to testify the greater tenderness achievement. Hardness had an increase during ageing period: this data demonstrates the surface drying.

## Conclusions

The results of the present study indicate that extended ageing cause a remarkable weight loss but a limited lipid oxidation and an improvement in tenderness.

Starting from meat with a low CBT counts and checking the protocol of maturation parameters, it's possible to get a more tender aged meat with a light darkening, more pleasing to the consumer.

Aged beef represents for its characteristics a niche product and as such, besides standardizing procedures in terms of  $U^R$ , ventilation and  $T^{\circ}$ , it is important to educate the consumer to this type of product.

Further research is needed to find a method to improve colour and to reduce the trimming waste of extended aged beef.

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