Effect of sex and sub-zero storage temperature on the microbial and oxidative stability of beef packed in a high-oxygen atmosphere after different vacuum ageing times

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ABSTRACT

This study aimed to evaluate the effect of sex and sub-zero storage temperature on the microbial and oxidative stability of Belgian Blue beef packed in a high-oxygen atmosphere after different ageing times. *Longissimus thoracis et lumborum* from Belgian Blue young bulls and cull cows were aged at -1 or $4\,°C$ for 80 days in vacuum. Every 20 days, samples were repackaged in a high-oxygen atmosphere (70/30% O_2/CO_2) and stored for 7 days (2 days at $4\,°C$ + 5 days at $8\,°C$). Ageing at $-1\,°C$ had a protective effect against the growth of lactic acid bacteria and Enterobacteriaceae and myoglobin oxidation. *Brochothrix thermosphacta* was the limiting parameter for ageing longer than 20 days at $-1\,°C$, permitting a subsequent 7-day shelf-life in a high-oxygen atmosphere. Meat from young bulls was more sensitive to oxidation than meat from cull cows. Extending Belgian Blue meat ageing for > 20 days had a negative impact on retail shelf-life.

1. Introduction

Modes of processing, distribution and consumption of fresh meat have dramatically changed over the past decades, resulting in re-organisation of the meat industry. Chilling at sub-zero temperatures, above the freezing point of beef, associated with vacuum packaging (VP) has permitted the shelf-life of fresh meat to be extended to several weeks (Jeremiah & Gibson, 2001) without resorting to freezing, making it possible to centralise slaughtering and to trade this product world-wide. In some EU countries, VP is almost exclusively reserved for intermediate levels of the beef chain, while modified atmosphere packaging (MAP) is more common in the retail marketplace to give meat its appealing bright red colour.

The end of the shelf-life of food is considered to be the point beyond which it is no longer acceptable to the consumer, or it could be when a food safety issue emerges. In the case of fresh meat, the shelf-life is mainly limited by microbial growth and alteration phenomena. As

reviewed by Coombs, Holman, Friend, and Hopkins (2017), an increase in the spoilage bacteria population in meat, including lactic acid bacteria (LAB), Enterobacteriaceae and *Brochothrix thermosphacta*, is associated with the development of undesirable flavours, discolouration and reduced product safety. Contrariwise, oxidative processes are the primary non-microbiological factors implicated in quality deterioration of meat during chilled storage. Lipid oxidation results in the formation of several products, some of them being often associated with the development of off-flavours (Smith, Morgan, Sofos, & Tatum, 1996) even at low concentrations (Stetzer, Cadwallader, Singh, McKeith, & Brewer, 2008). Moreover, the oxidation of myoglobin turns this pigment to metmyoglobin (MMb), which gives a brown colour to the meat. An essential challenge for the meat industry is to maintain the fresh appearance of the product, which might be based on the assessment of microbial, pigment and lipid stability.

In Belgium, meat from bulls accounts for about 55% of the total volume of bovines slaughtered, and beef from young bulls represents

the most significant part of the bovine meat market for supermarkets. However, an increase in the consumption of meat from cull cows was observed in the past decades (SOGEPA, 2016). In fact, Belgian Blue cull cows are often slaughtered at a relatively young age, which implies that their carcase and meat are still of excellent quality (Fiems, De Campeneere, Van Caelenbergh, & Boucqué, 2001). Also, the Belgian meat sector often complains of the high sensitivity of Belgian Blue beef to alteration processes, in particular, high-oxygen modified atmosphere-packaged meat previously aged in vacuum. Limited literature is available looking at the differences between meat from male and female Belgian Blue animals, and the impact of extended vacuum ageing on beef quality during MAP display. In this context, the objective of this experiment was to study the effect of category (young bulls and cull cows) and sub-zero storage temperature (-1 and $4\,°C$) on the microbiological and oxidative stability of Belgian Blue beef packed in a high-oxygen atmosphere after different ageing times.

2. Material and methods

2.1. Samples

Twelve VP *longissimus thoracis et lumborum* muscles from four young bulls (1.8 ± 0.1 years, two of conformation class S and two of conformation class E, and all of fat-cover class 2 in the EUROP grid) and eight cull cows (6.0 ± 1.7 years, four of conformation class S and fat-cover class 3, and four of conformation class E and fat-cover class 2 in the EUROP grid) from the Belgian Blue breed were supplied 2 days after slaughter by two slaughterhouses located in the Walloon Region of Belgium. The samples from young bulls will be referred to as YB, and those from cull cows will be referred to as CC. In the laboratory, 20 steaks (2–3-cm thick) per muscle were cut, totalling 240 steaks. Each of the 20 steaks from each animal was randomly assigned to one of the 20 treatments (two ageing temperatures × five ageing times × two display times) described below.

Samples at display time 0 (D0) were prepared as follows. Ten steaks from each animal were put in vacuum bags, sealed and aged at -1 (five steaks/animal) or $4\,°C$ (five steaks/animal) for up to 80 days. Vacuum bags (Cryovac) were 60 μm thick, and oxygen permeability was $13\,cm^3/m^2 \times 24\,h \times bar$ at $23\,°C$ and 0% relative humidity (RH). One VP steak from each animal and each ageing temperature (-1 and $4\,°C$) was analysed after 0, 20, 40, 60 and 80 days of ageing (A0D0, A20D0, A40D0, A60D0 and A80D0).

Samples at display time 7 (D7) were prepared as described hereafter. The ten remaining steaks from each animal were VP and aged similarly to the samples at D0. Every 20 days, one VP steak from each animal and each ageing temperature was repackaged in PP/EVOH/PP trays containing a modified atmosphere of 70/30% O_2/CO_2 and sealed with a PET/PP film. Trays (ES-Plastic) were 187 × 137 × 50 mm, and the oxygen permeability was $4.0\,cm^3/m^2 \times 24\,h \times bar$ at $23\,°C$ and 0% RH. The oxygen permeability of the sealing film (Wipak) was $8.4\,cm^3/m^2 \times 24\,h \times bar$ at $23\,°C$ and 0% RH. MAP samples were then stored for 7 days. A temperature of $4\,°C$ was used during the first 2 days, and $8\,°C$ was applied during the last 5 days of storage. The latter temperature was chosen to simulate a break in the cold chain during distribution. One VP steak from each animal and each ageing temperature (-1 and $4\,°C$) was analysed after 0, 20, 40, 60 and 80 days of ageing and 7 days of MAP storage (A0D7, A20D7, A40D7, A60D7 and A80D7). The experimental scheme and the different treatments are represented in Fig. 1.

Microbiological analysis and evaluation of colour (CIE $L^*a^*b^*$), percentage metmyoglobin (MMb%) and thiobarbituric acid reactive substances (TBARS), as an indicator of lipid oxidation, were performed every 20 days, before and after MAP display. Determination of the fat and α-tocopherol content was done only at D0 (A0D0).

2.2. Microbial counts

Twenty-five square centimetres (1-cm thick) of meat were transferred to a sterile bag with 225 mL sterile peptone water and homogenised for 120 s using a stomacher. Serial decimal dilutions were prepared using the same dilutant. Total viable count (TVC), LAB and Enterobacteriaceae counts were performed using an automated bioMérieux TEMPO® for enumeration in food products. Cards containing the sample and culture media were incubated at $22\,°C$ for 48 h for TVC and LAB, and at $30\,°C$ for 24 h for Enterobacteriaceae. *Brochothrix thermosphacta* was determined according to the ISO 13722:1996(F) procedure (ISO, 1996a) by plating on STAA agar with STAA-selective supplement (Oxoid) and incubating at $22\,°C$ for 48 h. Colonies were confirmed by oxidase tests (Bactident). The following acceptability thresholds were considered: $6.7\,\log_{10}$ CFU/g for LAB (Picgirard, 2009), $5.0\,\log_{10}$ CFU/g for Enterobacteriaceae (FCD, 2016) and $6.0\,\log_{10}$ CFU/g for *B. thermosphacta* (Mills, Donnison, & Brightwell, 2014).

2.3. Colour and metmyoglobin%

Instrumental colour was evaluated 1.5 h after removal from the package using a Minolta CR-400 chromameter (11-mm aperture, D_{65} illuminant, 2° observation angle). Values for CIE L^*, a^* and b^* were measured in ten different zones of each sample and averaged. The colour difference between samples before and after MAP display (ΔE) was calculated using the equation $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$.

The method of Tang, Faustman, and Hoagland (2004) was used to determine MMb%, by measuring the absorbance of an aqueous meat extract at 503, 525, 557, 565 and 582 nm.

2.4. Free fat content and lipid oxidation measurement

The free fat content was determined on the dried residue ($103\,°C$) of 5 g of sample (ISO, 1997) by the Soxhlet method (ISO, 1996b). Briefly, the fat was extracted from samples with petroleum ether for 6 h and weighed following the removal of the solvent by evaporation at $103\,°C$.

To assess lipid oxidation, an aqueous acid extraction method was used to measure the amount of TBARS by spectrophotometry at 530 nm (Raharjo, Sofos, & Schmidt, 1992). 1,1,3,3-Tetraethoxypropane was used to prepare the standard curve and determine TBARS recovery. The results are expressed as the malondialdehyde (MDA) equivalent content in milligrams per kilogram of meat.

2.5. α-Tocopherol content

A protocol adapted from Liu, Scheller, and Schaefer (1996) was used to extract and quantify the α-tocopherol content in meat samples. HPLC analysis was carried out using a Model 600 E solvent delivery system, equipped with a Model 717 automatic injector, a Mistral™ oven and both 996 PDA and 2475 fluorescence detectors (all from Waters). HPLC conditions were: stationary phase – Waters Resolve 5-μm spherical silica column (3.9 × 150 mm), column temperature – $15\,°C$, mobile phase – 96/4% isooctane/tetrahydrofuran (v/v), flow rate – 1.0 mL/min, injection volume – 30 μL, detection – fluorescence (excitation wavelength 296 nm and emission wavelength 325 nm), calculation – external standard method based on the peak area.

2.6. Statistical analysis

Treatments were arranged in a split-plot design with category (YB and CC) as the whole plot, and ageing temperature (-1 and $4\,°C$), ageing time (A0, A20, A40, A60 and A80) and display time (D0 and D7) as split plots. *Longissimus thoracis et lumborum* muscles were the whole plot while the obtained steaks were the split-plot experimental units.
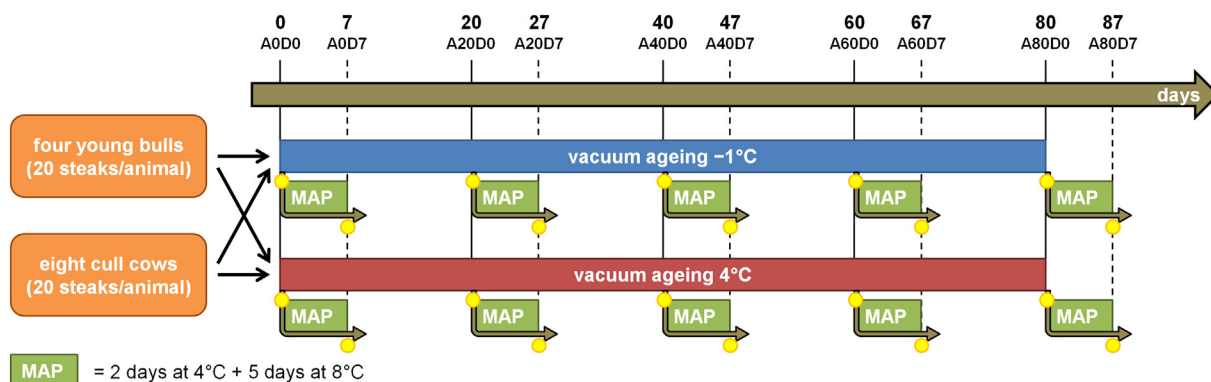
Fig. 1. Experimental scheme. Each yellow circle represents one of the 20 treatments applied to steaks of each animal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The model statement included the measured trait and all possible interactions (combined effects of factors on the dependent measure) among category, ageing temperature and ageing time as well as display time when applicable. This model was used to analyse TVC, LAB, Enterobacteriaceae, *B. thermosphacta*, ΔE, MMb% and TBARS.

Student's *t*-test was performed to evaluate the effect of the category on the initial values of L^*, a^*, b^* and free fat and α-tocopherol content at D0. Tests were conducted at a significance level of $P < 0.05$.

Pearson correlation coefficients were calculated between MMb% and TBARS.

All statistical analysis was performed using the computing environment R (R Core Team, 2016).

3. Results and discussion

3.1. Microbial profile

The effect of category (YB and CC), ageing temperature (−1 and 4 °C), ageing time (A0, A20, A40, A60 and A80) and display time (D0 and D7) and all their various combinations was evaluated for TVC, LAB, Enterobacteriaceae and *B. thermosphacta*. Ageing temperature × ageing time had an impact on Enterobacteriaceae, while an effect was noticed for ageing temperature × display time and ageing time × display time on TVC, Enterobacteriaceae and *B. thermosphacta*. Finally, an effect of temperature × ageing time × display time was observed for LAB

($P < 0.05$) (Table 1). For practical purposes, the microbiological results are presented as temperature × ageing time × display time means (Fig. 2). Table 2 shows the maximum Belgian Blue beef vacuum ageing period permitting subsequent 7-day modified atmosphere storage for the different microbiological parameters, taking into account the thresholds indicated in Subsection 2.2.

The initial (A0D0) TVC was 3.2 $\log_{10}$ CFU/g, and it increased ($P < 0.05$) to 6.6 $\log_{10}$ CFU/g after a 7-day MAP display (A0D7). During vacuum ageing, TVC growth was slower at −1 °C. At 4 °C, there was a considerable increase ($P < 0.05$) in this parameter during the first 20 days of ageing, and, after the 40th day of ageing (A40D0), it remained stable (Fig. 2). However, the temperature of the previous ageing did not have an impact on TVC during display. As explained before, in several EU countries, wholesale cuts are usually vacuum-packed and sent to retailers where the meat is cut into portion sizes, and placed onto trays and then overwrapped with an air-permeable film, or onto trays that are filled with a modified atmosphere usually containing 70–80% O_2 and 20–30% CO_2. The greatest advantage of MAP at the retail market is the retardation of the growth of spoilage bacteria and hence an extended shelf-life when compared to meat exposed to air (Xiong, 2017). Nevertheless, when compared to vacuum storage, high-oxygen modified atmospheres tend to promote the growth of aerobic bacteria, reducing the shelf-life (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007; McMillin, Huang, Ho, & Smith, 1999).

At A0D0, the concentration of LAB was < 2.0 $\log_{10}$ CFU/g, and the

Table 1

Analysis of variance (*F*-values) on the effect of category (young bull and cull cow), ageing temperature (−1 and 4 °C), ageing time (0, 20, 40, 60 and 80 days), display time (0 and 7 days) and all their combinations on total viable count (TVC), lactic acid bacteria (LAB), Enterobacteriaceae, *B. thermosphacta*, ΔE, percentage met-myoglobin (MMb%) and thiobarbituric acid reactive substances (TBARS).

Effect	TVC	LAB	Enterobacteriaceae	*B. thermosphacta*	ΔE	MMb%	TBARS
Category (C)	**4.30***	**13.59***	0.40	1.39	**15.78***	**27.98***	0.87
Ageing temperature (T)	**6.55***	**88.10***	**102.03***	**8.94***	0.00	**15.13***	**36.32***
Ageing time (A)	**163.1***	**80.18***	**59.84***	**7.47***	**10.78***	**151.03***	**48.60***
Display time (D)	**112.2***	**117.75***	**17.27***	**50.13***	n/a	**449.74***	**133.15***
C × T	0.51	0.05	1.33	0.10	**4.23***	**4.99***	**9.14***
C × A	0.19	0.44	1.39	**14.53***	**7.42***	0.63	0.59
C × D	0.92	0.28	2.29	**6.99***	n/a	**9.35***	**18.39***
T × A	0.08	**5.37***	**6.55***	2.18	0.77	1.11	0.02
T × D	**17.73***	**30.62***	**10.46***	**12.00***	n/a	**12.40***	0.14
A × D	**63.96***	**8.07***	**50.63***	**81.10***	n/a	**156.66***	**93.61***
C × T × A	0.21	0.01	0.20	1.87	0.06	0.00	0.05
C × T × D	0.37	0.04	2.38	0.22	n/a	0.38	0.17
C × A × D	0.00	1.95	**4.39***	**12.01***	n/a	3.35	**6.07***
T × A × D	0.16	**21.49***	0.00	0.70	n/a	0.34	0.34
C × T × A × D	0.64	0.60	0.44	0.83	n/a	0.04	0.03

Significant probabilities are in bold; n/a: not applicable.

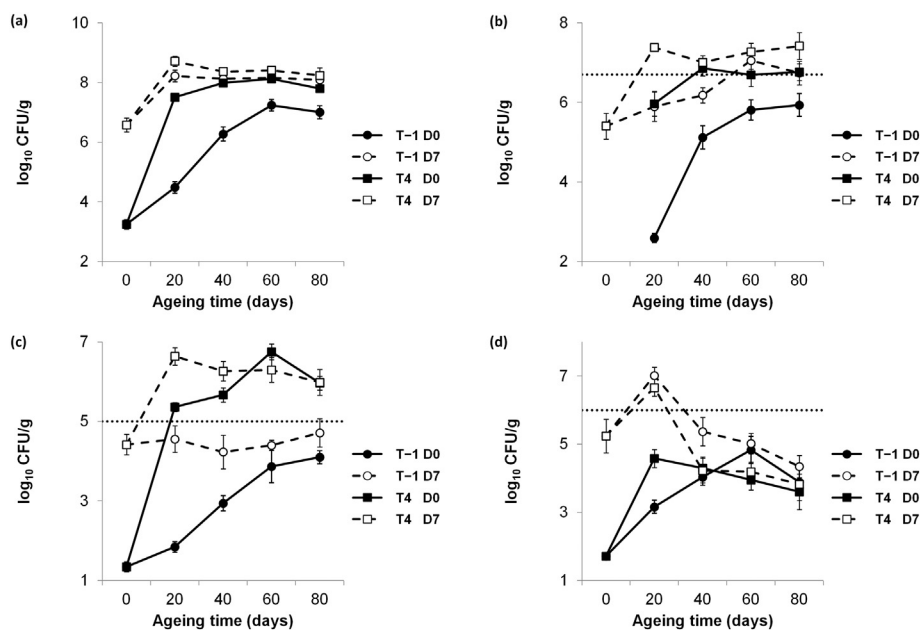* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Fig. 2. (a) Total viable count, (b) lactic acid bacteria, (c) Enterobacteriaceae and (d) *Brochothrix thermosphacta* in *longissimus thoracis et lumborum* from animals for the combination ageing temperature × ageing time × display time. Samples aged at −1 °C (●) and 4 °C (■), before MAP display (D0), are shown linked by full lines. Samples aged at −1 °C (○) and 4 °C (□), after MAP display (D7), are shown linked by dashed lines. The horizontal dotted lines represent the acceptability threshold. The lowest y-axis value indicates the lowest limit for counting. Bars represent standard error.

Table 2

Maximum Belgian Blue beef vacuum ageing period permitting subsequent 7-day modified atmosphere storage at 4 °C for 2 days and 8 °C for 5 days for different microbiological and physicochemical parameters.

Parameter	Category	Temperature (°C)	Maximum vacuum ageing period (days)
Lactic acid bacteria	Both	−1	< 60
		4	< 20
Enterobacteriaceae	Both	−1	> 80
		4	< 20
Brochothrix thermosphacta	Both	−1	< 20
		4	< 20
TBARS	YB	Both	< 40
	CC	Both	> 80
MMb%	YB	−1	< 40
		4	< 20
	CC	−1	< 60
		4	< 40

TBARS: thiobarbituric acid reactive substances, MMb%: metmyoglobin percentage.

threshold of 6.7 $\log_{10}$ CFU/g was not exceeded during 80 days of vacuum ageing at −1 °C. Conversely, at 4 °C, the threshold for LAB was exceeded between 20 and 40 days of ageing (Fig. 2). Furthermore, the growth of LAB in samples previously aged at 4 °C during MAP display was more significant ($P < 0.05$) than in samples previously aged at −1 °C. Currently, the role of LAB in fresh meat is still controversial, as they represent a heterogeneous group of species that either contribute to spoilage through the generation of offensive metabolites or serve as bioprotective agents, with particular strains causing unperceivable or no alterations (Pothakos, Devlieghere, Villani, Björkroth, & Ercolini, 2015). Nonetheless, the enumeration technique used in the present study does not permit distinction of the specific LAB species present in the samples.

Ageing temperature affected the growth of Enterobacteriaceae during the vacuum ageing itself and MAP display as well; the concentration of Enterobacteriaceae was higher ($P < 0.05$) in samples aged at 4 °C. The threshold of 5.0 $\log_{10}$ CFU/g for Enterobacteriaceae was not exceeded in samples aged at −1 °C both during 80 days of vacuum ageing and after a 7-day MAP display (Table 2 and Fig. 2). This result confirms the advantage of combining sub-zero ageing with subsequent display in an atmosphere containing CO_2, which is a gas that

has an antimicrobial potential against Enterobacteriaceae (Milijasevic, Babic, & Veskovic-Moracanin, 2015).

Ageing at −1 °C favoured ($P < 0.05$) *B. thermosphacta* growth during MAP display when compared to ageing at 4 °C. *Brochothrix thermosphacta* is psychrotolerant (Leroi, Fall, Pilet, Chevalier, & Baron, 2012) and may have been selected during ageing at −1 °C. Moreover, after 20 days of ageing, the concentration of *B. thermosphacta* during display reached the highest value and then decreased as ageing time, at both temperatures, increased ($P < 0.05$) (Fig. 2). Pennacchia, Ercolini, and Villani (2011) observed that *B. thermosphacta* is one of the predominant species in chilled beef at the beginning of chilled storage. This could explain the initial growth of *B. thermosphacta* in our samples. Moreover, Russo, Ercolini, Mauriello, and Villani (2006) evidenced *in vitro* a decrease in the growth of *B. thermosphacta* in the presence of LAB, which could explain the reduction of *B. thermosphacta* in our samples over time, when the LAB population started to increase.

An effect of category alone or in combination with other factors was observed ($P < 0.05$) for all microbial parameters (Table 1). Since YB and CC were slaughtered and processed in different plants, the difference in microbial growth between categories is likely related to diverse hygienic conditions in both structures.

Finally, meat is a complex environment with physicochemical properties that allow the colonisation and development of a variety of microorganisms (Stellato et al., 2016). The observations of the present study suggest that the nature of germs changes during vacuum ageing and influences the microbiological profile of the meat when repacked in MAP. Nevertheless, the study of the microbiota of chilled beef can be laborious since some of its members may be missed or not identified by cultivation-based methods. In fact, current microbiological standards, which are based mainly on total viable mesophilic counts, lack the discriminatory capacity to detect psychrotrophic bacteria (Pothakos, Samapundo, & Devlieghere, 2012). According to Simmons, Tamplin, Jenson, and Sumner (2008), reducing incubation temperature may improve the accuracy of counting methods for chilled beef. In this study, the incubation temperatures used to evaluate TVC, LAB and Enterobacteriaceae were lower than those recommended by the enumeration kit supplier, to reduce eventual underestimation of bacteria that might have been selected during chilled storage. Lastly, the use of culture-independent high-throughput sequencing methods could circumvent the constraints related to the culture of psychrotrophic bacteria. These methods have shown to be a useful tool for a depth

Table 3

Initial physicochemical attributes in *longissimus thoracis et lumborum* from Belgian Blue young bulls (YB) and cull cows (CC).

|	Category		*P*	SEM
	YB	CC		
L_*	39.4	36.8	NS	0.880
a_*	22.1	18.9	*	0.763
b_*	12.0	12.3	NS	0.699
Free fat (%)	1.1	1.7	NS	0.209
α-Tocopherol (µg/g)	3.0	3.6	NS	0.212

NS: non-significant.

* $P < 0.05$.

assessment of the changes and interactions within microbial populations during meat storage under different conditions (Delhalle et al., 2016; Imazaki et al., 2011).

3.2. Colour stability

Initial colour attributes of YB and CC are shown in Table 3. $L*$ and $b*$ did not differ between categories. Conversely, $a*$ was higher in YB than in CC ($P < 0.05$). Boles and Swan (2002) reported a similar result; they found that the colour of raw meat from cows was significantly less red than meat from steers. However, our values conflict with a previous study from Fiems, De Campeneere, Van Caelenbergh, De Boever, and Vanacker (2003), which reported no difference in $b*$ between Belgian Blue bulls and cows, but higher $L*$ and $a*$ in cows than in bulls. According to Seideman, Cross, Smith, and Durland (1984), older animals have a more intense accumulation of myoglobin in the muscle and should logically be redder.

The effect of category (YB and CC), ageing temperature (−1 and 4 °C) and ageing time (A0, A20, A40, A60 and A80) and their various combinations was evaluated for ΔE. The effect of category × ageing temperature and category × ageing time was significant for this parameter ($P < 0.05$) (Table 1). Ageing at 4 °C had a greater ($P < 0.05$) effect on ΔE in YB than in CC. Moreover, a considerable increase ($P < 0.05$) in ΔE was observed between the 20th and 40th days of vacuum ageing in YB. In CC, a gradual increase ($P < 0.05$) in ΔE was observed after 20 days of vacuum ageing (Fig. 3).

The effect of category (YB and CC), ageing temperature (−1 and 4 °C), ageing time (A0, A20, A40, A60 and A80) and display time (D0 and D7) and all their combinations was evaluated for MMb%. Category × display time, ageing temperature × display time and ageing time × display time had an impact ($P < 0.05$) on MMb% (Table 1). After a 7-day MAP display, YB, as well as samples that were aged at 4 °C, presented a greater ($P < 0.05$) sensitivity to pigment oxidation. Furthermore, MMb% in MAP samples was higher ($P < 0.05$) with increasing ageing time (Fig. 4). An increase of MMb% over time was also observed by Vitale, Pérez-Juan, Lloret, Arnau, and Realini (2014) who reported that colour stability in meat types including *longissimus thoracis et lumborum* from mature cows decreases during MAP display as ageing time increases. In fact, meat contains endogenous antioxidants and several cellular mechanisms of protection against pigment oxidation such as the MMb reducing system (Bekhit & Faustman, 2005). In a study by McKenna et al. (2005), decreasing MMb reducing activity was observed with an increasing number of days on display. It is known that MMb reducing activity is dependent on several coenzymes, such as NADH, which can be degraded during display in MAP (Bekhit, Geesink, Ilian, Morton, & Bickerstaffe, 2003; Bekhit, Geesink, Morton, & Bickerstaffe, 2001). Moreover, the presence of oxygen in MAP reduces MMb reducing activity since the electron transport chain-dependent reduction of MMb requires low-oxygen or anaerobic conditions (Tang et al., 2005). Finally, microbial growth reduces O_2 tension on the surface of the meat, thus enhancing MMb
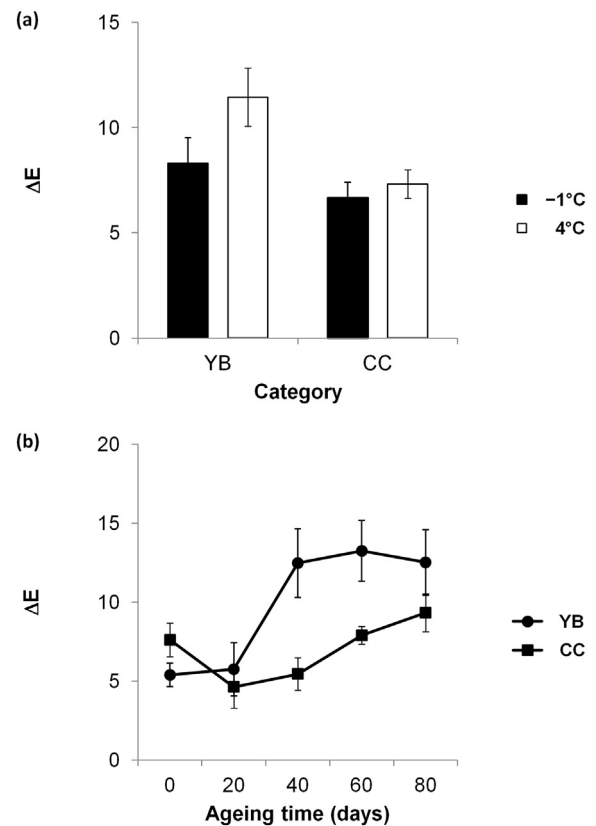
Fig. 3. ΔE during a 7-day MAP display in *longissimus thoracis et lumborum* from Belgian Blue young bulls (YB) and cull cows (CC) previously aged at −1 and 4 °C, for 0, 20, 40, 60 and 80 days, for the combinations (a) category × ageing temperature and (b) category × ageing time. Bars represent standard error.

formation (Ben Abdallah, Marchello, & Ahmad, 1999; Robach & Costilow, 1961).

The value of 40% MMb, reported by Greene, Hsin, and Zipser (1971) as the threshold value for consumers to reject meat, was exceeded in YB after 20 days of ageing, at both temperatures, and 7 days of MAP display (A20D7). By contrast, CC exceeded the value of 40% after 40 days of ageing (at both temperatures) and 7 days of MAP display (A40D7), confirming that YB was more sensitive to pigment oxidation than CC (Table 2).

3.3. Lipid stability

Fat content was 1.1% for YB and 1.7% for CC, but this difference was not statistically significant (Table 3). These values are similar to those reported in previous studies on the characteristics of Belgian Blue meat from *longissimus thoracis* from fattening bulls (Dufrasne et al., 2000) and finishing culled females (Cabaraux et al., 2004). However, Fiems et al. (2003) reported a total fat content twice as high in the *longissimus thoracis* of Belgian Blue cows as that in bulls.

The effect of category (YB and CC), ageing temperature (−1 and 4 °C), ageing time (A0, A20, A40, A60 and A80) and display time (D0 and D7) and their various combinations was evaluated for TBARS. The effect of category × ageing time × display time was noticed ($P < 0.05$) for TBARS (Table 1). All samples presented TBARS values below 0.3 mg MDA eq./kg during 80 days of vacuum ageing at −1 or 4 °C. YB in MAP showed a greater sensitivity to lipid oxidation than CC, and lipid stability during MAP display decreased for both categories as ageing time increased ($P < 0.05$) (Fig. 5). Ageing at 4 °C favoured ($P < 0.05$) lipid oxidation in YB but did not affect lipid oxidation in CC (data not shown in graphical form).

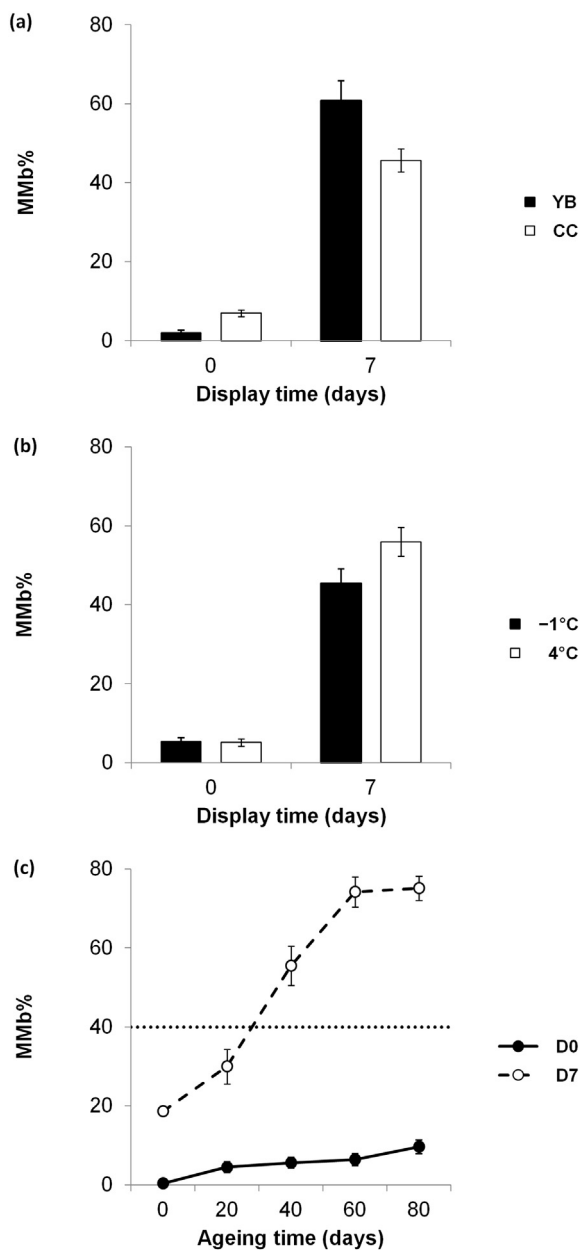A value of 2 mg MDA eq./kg is usually considered as the

Fig. 5. Thiobarbituric acid reactive substances (expressed as the malondialdehyde equivalent content in milligrams per kilogram of meat) in *longissimus thoracis et lumborum* from Belgian Blue young bulls (YB) and cull cows (CC) for the combination category × ageing time × display time. YB (●) and CC (■) before MAP display (D0) are shown linked by full lines. YB(○) and CC(□) after MAP display (D7) are shown linked by dashed lines. The horizontal dotted line represents the acceptability threshold. Bars represent standard error.

YB and CC on lipid stability was not verified in this study.

Lipid oxidation and myoglobin oxidation often appear to be linked, and oxidation of one of these leads to the formation of chemical species that can exacerbate oxidation of the other (Faustman, Sun, Mancini, & Suman, 2010). For the samples in this study, a high coefficient of correlation between lipid oxidation (TBARS) and myoglobin oxidation (MMb%) was observed ($r = 0.840$).

The α-tocopherol content was 3.0 μg/g in YB and 3.6 μg/g in CC, with no statistical difference between categories (Table 3). This result is not surprising since α-tocopherol tends to accumulate in fat tissues, and there was no difference in the fat content between YB and CC. Also, α-tocopherol content in meat is highly dependent on cattle diet (Smith et al., 1996). As no information about the nutritional background was provided, this hypothesis could not be verified in this research. Dufrasne et al. (2000) stated that α-tocopherol can significantly reduce lipid oxidation and tends to maintain the redness of Belgian Blue meat, and Liu, Lanari, and Schaefer (1995) suggest a value of 3.5 μg/g as the minimum muscle α-tocopherol concentration that provides for near-maximal suppression of lipid oxidation and MMb formation in fresh beef. In our study, meat from YB remained below the threshold of 3.5 μg/g. This fact could explain why meat from YB was more sensitive to colour and lipid oxidation than meat from CC. However, other cellular mechanisms of protection against oxidative processes including the MMb reducing system and antioxidant enzymes (Imazaki, Douny, Elansary, Scippo, & Clinquart, 2018) could be involved and need to be further investigated.

Overall, extended Belgian Blue meat ageing had a negative impact on retail shelf-life. Moreover, tenderness is one of the attributes most demanded by consumers, and its improvement is the primary reason for post-mortem ageing. Imazaki, Teixeira Gonçalves, Krantz, Thimister, and Clinquart (2016) did not observe any amelioration in the tenderness of meat from Belgian Blue cull cows after 21 days of ageing. Hence, improvements in organoleptic attributes associated with maturation would be in vain, as alterations related to microbial growth and oxidation reactions would make the product less attractive to consumers during retail commercialisation. Still, extended Belgian Blue ageing could be helpful for stock management by the foodservice industry sector, where high-oxygen atmosphere packaging is not used.

4. Conclusions

The duration and temperature of vacuum-packed storage influenced

Fig. 4. Percentage metmyoglobin (MMb%) in *longissimus thoracis et lumborum* from Belgian Blue young bulls (YB) and cull cows (CC) aged for 0, 20, 40, 60 and 80 days at −1 and 4 °C, and stored for 7 days in a modified atmosphere of 70/30% O_2/CO_2 for the combinations (a) category × display time, (b) ageing temperature × display time and (c) ageing time × display time. The horizontal dotted line represents the acceptability threshold. Bars represent standard error.

acceptability threshold for the rancidity of meat (Campo et al., 2006). Taking into account this parameter only, consumers would reject meat samples after 40 days of ageing and 7 days of display for YB (A40D7) and 80 days of ageing and 7 days of display for CC (A80D7), no matter the ageing temperature (Table 2). In reality, lipid oxidation is believed to be initiated at the membrane level in the highly unsaturated phospholipid fraction. Thus, increasing the muscle concentration of polyunsaturated fatty acids can result in a significant increase in TBARS levels (Pouzo, Descalzo, Zaritzky, Rossetti, & Pavan, 2016). As reviewed by Venkata Reddy et al. (2015), fat from the muscle of bulls and steers has a significantly higher sum of polyunsaturated fatty acids compared to fat from heifers and cows, which is attributable to the higher intramuscular fat content of meat from females. However, the hypothesis of the influence of different polyunsaturated fatty acid content between
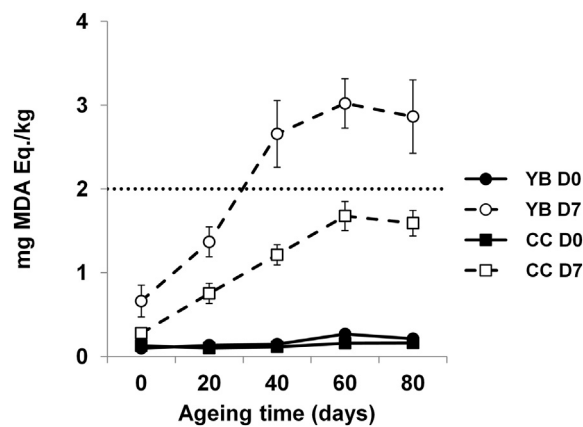
microbial growth and lipid and pigment oxidation of beef during subsequent high-oxygen MAP storage. In the conditions of the present experiment, *B. thermosphacta* was the limiting parameter for vacuum ageing longer than 20 days at −1 °C associated to a subsequent 7-day shelf-life in a high-oxygen atmosphere. More extended periods may be applied by HoReCa operators, where high-oxygen atmosphere packaging is not used. Ageing for > 20 days at 4 °C seems inappropriate for Belgian Blue beef due to high microbial growth at this temperature.

This study combined the evaluation of both microbiological and physicochemical parameters of meat, bringing new knowledge about beef ageing at sub-zero temperatures and subsequent storage in a high-oxygen atmosphere, and supporting the development of appropriate strategies for beef preservation. Differences in physicochemical parameters highlighted between YB and CC should be considered carefully due to the low number of animals used in this research.

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