UNIVERSITÉ DE LIÈGE

FACULTÉ DE MÉDECINE VÉTÉRINAIRE

Département des Sciences des denrées alimentaires

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Study of microbial and physicochemical factors determining the long-term preservation of chilled fresh beef at subzero temperatures

Étude des facteurs microbiens et physico-chimiques déterminant la conservation longue durée de la viande bovine fraîche réfrigérée à températures sous zéro

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THÈSE PRESENTÉE EN VUE DE L'OBTENTION DU GRADE DE DOCTEUR EN SCIENCES VÉTÉRINAIRES

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Some hae meat and canna eat,

And some wad eat that want it,

But we hae meat and we can eat,

Sae let the Lord be Thanket!

— Robert Burns

Beef is the king of meat.

— Jonathan Swift

Abstract

Fresh meat is a highly nutritious but extremely perishable food, and its preservation is a significant challenge since early civilisations. In the past decades, food trade has become more globalised, forcing supply chains to become larger and more complex. As such, meat, which typically has a short shelf life, is required to stay fresh for longer. Extended shelf life in beef may be achieved through strict control of the product hygiene associated with vacuum packaging and subzero chilling. The research presented in this thesis was designed to investigate the effect of microbial and physicochemical factors, namely the growth of spoilage bacteria and oxidation of myoglobin and lipids, on the long-term preservation of chilled fresh beef at temperatures above the freezing point of meat (−2°C) but below 0°C.

This research was divided into the following main topics: (1) the study of the preservation of chilled fresh meat with extremely long shelf life, (2) the study of the preservation of chilled fresh meat from the Belgian Blue breed and (3) the study of *Carnobacterium maltaromaticum* as a protective culture for meat.

This study provided evidence that the use of sub-zero temperatures during vacuum storage is essential in meat shelf life extension since it retards meat deterioration, preventing the oxidation of myoglobin (discolouration) and growth of spoilage bacteria. Intrinsic factors including the metmyoglobin reducing activity as well as the activity of antioxidant enzymes, such as catalase, superoxide dismutase and

glutathione peroxidase, can be associated, in varying degrees of importance, with the retardation of oxidation reactions in meat. *Carnobacterium maltaromaticum* was the dominant bacteria in meats with extremely long shelf life. Selected *C. maltaromaticum* isolates obtained from these meats showed an antilisterial activity *in vitro*, which was optimised at low temperatures. When studied directly in raw meat with the natural indigenous microbiota, *C. maltaromaticum* showed an antimicrobial effect against Enterobacteriaceae.

Further research on the antioxidant mechanisms and microbial ecosystems associated with meats with extremely long shelf life will be a key to understand and improve the extension of the shelf life of fresh meat.

Résumé

La viande fraîche est un aliment très nutritif mais extrêmement périssable, et sa conservation est un défi majeur depuis les premières civilisations. Au cours des dernières décennies, le commerce alimentaire s'est mondialisé, rendant les chaînes d'approvisionnement plus étendues et plus complexes. De cette manière, la viande, qui a généralement une durée de vie très courte, doit rester fraîche pendant plus longtemps. En effet, une durée de conservation prolongée pour la viande bovine peut être obtenue par un contrôle strict de l'hygiène du produit associé à un conditionnement sous vide et à un refroidissement à des températures sous zéro. La présente recherche vise à étudier l'effet des facteurs microbiens et physicochimiques, notamment la croissance des bactéries d'altération et l'oxydation de la myoglobine et des lipides, sur la conservation à long terme de la viande fraîche à des températures au dessus du point de congélation de la viande (−2°C) mais en dessous de 0°C.

Cette recherche a été divisée en trois axes principaux: (1) l'étude de la conservation des viandes fraîches réfrigérées avec une durée de conservation extrêmement longue; (2) l'étude de la conservation des viandes fraîches réfrigérées de la race Blanc Bleue Belge; (3) l'étude de *Carnobacterium maltaromaticum* en tant que culture protectrice pour la viande.

Cette étude a démontré que l'utilisation des températures sous zéro pendant la conservation sous vide est essentielle pour prolonger la durée de conservation de la

viande puisqu'elle retarde la détérioration, en prévenant l'oxydation de la myoglobine (décoloration) et la prolifération des bactéries d'altération. Des facteurs intrinsèques, y compris l'activité réductrice de la metmyoglobine et l'activité des enzymes antioxydantes, à savoir la catalase, la superoxyde dismutase et la glutathion peroxydase, peuvent être associés, avec des degrés d'importance variables, au ralentissement des réactions d'oxydation dans la viande. *Carnobacterium maltaromaticum* était la bactérie dominante dans des viandes avec une durée de conservation extrêmement longue. Des isolats sélectionnés de *C. maltaromaticum* obtenus à partir de ces viandes ont montré une activité anti-*Listeria in vitro*, qui a été optimisée à basses températures. Lorsque *C. maltaromaticum* a été étudiée directement dans la viande fraîche avec le microbiote indigène naturel, elle a montré un effet antimicrobien sur les Enterobacteriaceae.

Des recherches ultérieures sur les mécanismes antioxydants et les écosystèmes microbiens associés aux viandes à très longue durée de conservation constitueront une clé pour la compréhension et l'amélioration de l'extension de la durée de conservation de la viande fraîche.

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Abbreviations

AMSA	American Meat Science Association
ATP	adenosine triphosphate
a_w	water activity
B.	*Brochothrix*
C.	*Carnobacterium*
CAT	catalase
DMb	deoxymyoglobin
E.	*Escherichia*
EDTA	ethylenediaminetetraacetic acid
EU	European Union
FASFC	Federal Agency for the Safety of the Food Chain
FAO	Food and Agriculture Organisation
GSH-Px	glutathione peroxidase
L.	*Listeria*
LAB	lactic acid bacteria
Lb.	*Lactobacillus*
MAP	modified atmosphere packaging
Mb	myoglobin

MDA malondialdehyde

MMb metmyoglobin

MRA metmyoglobin reducing activity

NADH nicotinamide adenine dinucleotide (reduced form)

OECD Organisation for Economic Co-operation and Development

OMb oxymyoglobin

pO_2 partial pressure of oxygen

ROS reactive oxygen species

SOD superoxide dismutase

SOGEPA Société wallonne de gestion et de participations

US United States

UK United Kingdom

Introduction

Fresh meat is a highly nutritious but extremely perishable food, and its preservation is a significant challenge since early civilisations. Meat frequently has to be stored, transported, prepared and distributed, all of which is considerably time-consuming. To safeguard fresh meat during this extended time, specific methods of preservation must be applied.

Humans have always known that cold temperatures are a natural method for preserving food. In Palaeolithic times, the depths of caves made a smart hiding place as well as an excellent location for cold storage. The use of cold environments on a large scale as a means of preserving food dates back to the 19th century, with the invention of mechanical refrigeration. At the time, Europe and especially Great Britain needed to bring frozen meat over from Argentina, Australia and New Zealand to feed its ever-growing population. In the 1960s, vacuum packaging has become popular, permitting to extend the shelf life of fresh meat without freezing it.

Food trade is becoming progressively more globalised for most countries, as more and more food manufacturers attempt to reach different markets, inevitably forcing supply chains to become larger and more complex. As such, vulnerable produce including meat, which typically has a short shelf life, is required to stay fresh for longer. At this time, it is not rare to find imported chilled raw meat in the European market with several-months-long shelf lives. At first sight, this might appear excessive or make

suspect the use of illegal technologies. In fact, extended shelf life in beef[1] may be achieved by strict control of the product hygiene associated with two hurdles[2]: vacuum packaging and chilling at temperatures above the freezing point of meat (−2°C) but below 0°C. In this way, the present research was designed to investigate the microbial and physicochemical factors determining the long-term preservation of chilled fresh beef at subzero temperatures.

[1] The shelf life of vacuum-packaged fresh beef primals and sub-primals is generally reported as approximately 35 to 45 days.

[2] Hurdle technology advocates the deliberate combination of existing and novel preservation techniques to establish a series of preservative factors (hurdles) to improve the microbial stability and the sensory quality of foods as well as their nutritional and economic properties. These hurdles may be temperature, water activity, pH, redox potential, preservatives and so on.

Section I

Literature review and objectives

The first section of this work is divided into two chapters. **Chapter 1** involves a thorough review of the scientific literature concerning meat and the meat sector, the preservation of this commodity and the evaluation of its shelf life. This chapter will emphasise beef and the meat sector in the European Union (EU) and Belgium more specifically. **Chapter 2** presents the general and specific objectives of this thesis.

Chapter 1
Literature review

1.1 Meat – the product

The Oxford English Dictionary defines meat as the flesh of animals used as food. The word developed from the Old and Middle English *mete*, which referred to food in general, not specifically animal flesh (Walker, 2010). In the Regulation (EU) No 1169/2011 on the provision of food information to consumers (European Parliament and Council of the European Union, 2011), meat is defined as skeletal muscles of mammalian and bird species recognised as fit for human consumption, where the total fat and connective tissue content does not exceed 25% each (for mammals other than rabbits and porcine). The Regulation (EC) No 853/2004 on the hygiene of foodstuffs (European Parliament and Council of the European Union, 2004) provides a broader definition of meat as it also includes the blood. According to the same regulation, fresh meat refers only to meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere.

Meat has been an essential part of the diet of hominins for over hundreds of thousands of years (McPherron *et al*., 2010) and has exerted a crucial role in human evolution (de Castro Cardoso Pereira and dos Reis Baltazar Vicente, 2013; Williams and Hills, 2017; Mann, 2018). There is evidence that meat consumption has influenced

cranial-dental and intestinal morphologic changes, erect human posture, reproductive characteristics, longer lifespan, and, most importantly, brain and intellectual development (Aiello and Wheeler, 1995; Hladik and Pasquet, 2001; Wang and Crompton, 2004; Psouni *et al.*, 2012).

Protein is the primary nutritional component of meat, and it contains all eight essential amino acids needed for the growth and maintenance of the human body (Wyness *et al.*, 2011). Then, fat is the second nutritional component of meat (mainly saturated and monounsaturated fatty acids), which contributes substantially to its energy content in fatty cuts. Specifically, fresh beef is rich in various vitamins and minerals, especially iron[3] and zinc, and is therefore recommended as part of a healthy diet (Gidding *et al.*, 2009). **Table 1** presents information on selected nutrients in fresh lean beef in the Belgian market according to Nubel (2017).

1.2 The meat sector

The meat sector in developed countries usually consists of farmers, abattoirs and companies involved in various aspects of distribution and marketing of meat. As an example, **Figure 1** presents a commented infographic on the beef supply chain worldwide.

1.2.1 Livestock production

The world food economy is being increasingly driven by the shift of diets and food consumption patterns towards livestock products. Growth in the demand for meat will stem mostly from an increase in population, income and urbanisation, especially in countries with large middle classes in Asia, Latin America and the Middle East (Organisation for Economic Co-operation and Development [OECD], 2016). In developing countries, where almost all world population increases take place, consumption of all kinds of meat has grown at an average rate of 5.1%/yr since 1970 (Alexandratos and Bruinsma, 2012). However, meat production continues to be concentrated in a few countries including Australia, Brazil, China, and the United States (US). In 2016, Brazil and the EU led the expansion in world meat exports (OECD, 2017).

[3] Iron in beef is mostly in the heme form, which is absorbed very efficiently.

Table 1 Nutrient composition of fresh lean beef.

	Amount per 100 g	% Daily value*
Calories (kcal)	101.0	5.1
Fat (g)	2.0	4.0
of which		
Saturates (g)	0.8	4.0
Mono-unsaturates (g)	0.7	n/a
Polyunsaturates (g)	0.2	n/a
Carbohydrate (g)	0.0	0.0
Protein (g)	20.8	41.6
Salt (g)	0.1	2.3
Vitamin B12 (µg)	1.5	60.0
Phosphorus	166.0	23.7
Iron (mg)	1.6	11.4
Zinc (mg)	3.2	32.0
Selenium (µg)	17.0	30.9

*Based on the Regulation (EU) No 1169/2011 (European Parliament and Council of the European Union, 2011).
n/a = not applicable

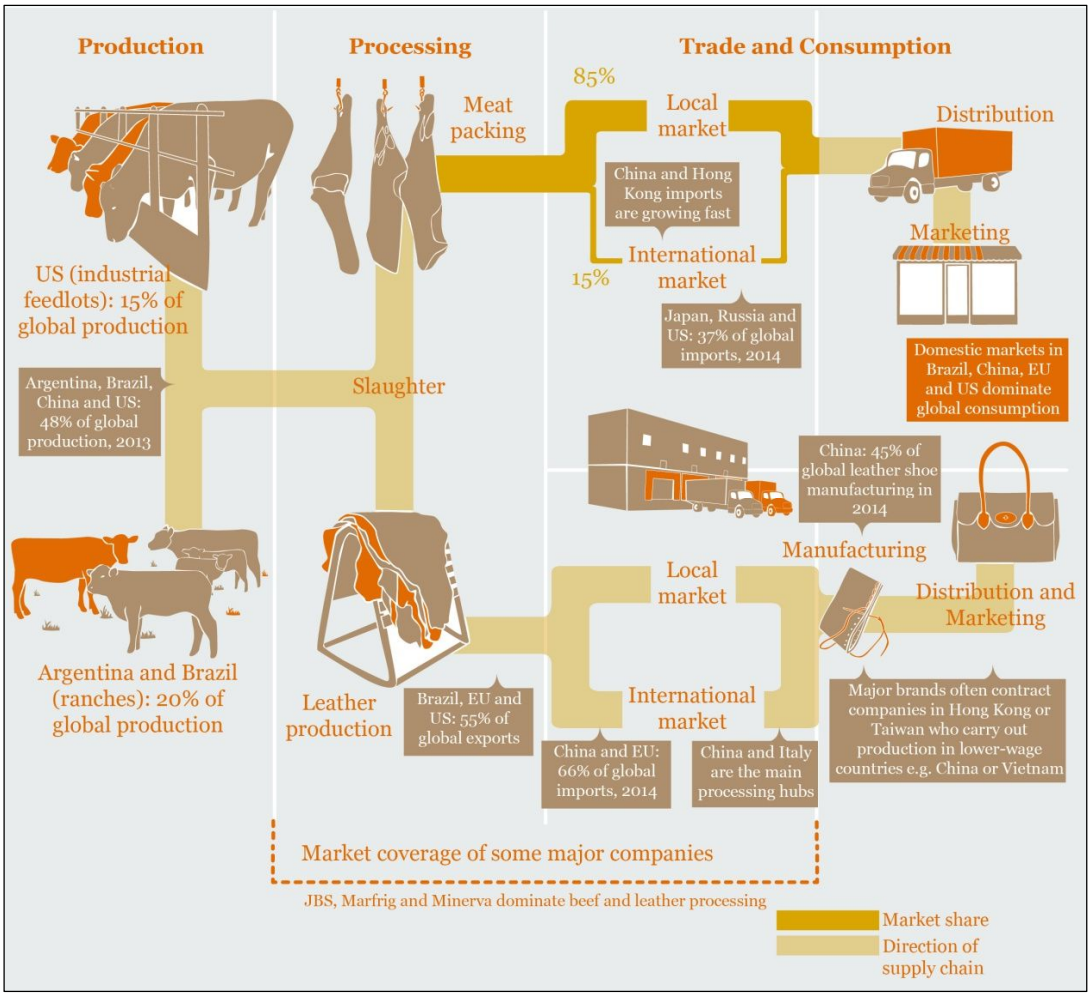

Figure 1 The beef and leather supply chain (Webb, 2016).

Still in 2016, Spain, Germany, France, the United Kingdom (UK) and Italy held the most significant populations of livestock in the EU[4]. The highest numbers of bovines were recorded in France (19.0 million head), Germany (12.5 million head) and the UK (9.8 million head). Belgium held 2.5 million head of bovines. Regarding value, bovine animals represented 8.1% of total agricultural output[5] and 18.8% of animal output[6], without taking animal products into account (Eurostat, 2017).

The size of beef farms is increasing in all the western European countries (Sarzeaud *et al.*, 2008). Overall, at the EU, while slightly more than a quarter (27.5%) of agricultural holdings disappeared between 2003 and 2013, the total utilised agriculture area remained stable. As a result, the average area per holding, in general, grew from 11.7 ha in 2003 to 16.1 ha in 2013. In 2010, the highest average utilised agricultural areas per holding were registered in the Czech Republic (133.0 ha), the UK (93.6 ha) and Slovakia (80.7 ha). In contrast, average areas below 10 ha per holding were recorded in Malta (1.2 ha), Cyprus (3.1 ha) and Romania (3.6 ha)[7]. The average area per holding in Belgium was 31.7 ha. To compare, the average area per holding in Luxemburg, Germany, France and the Netherlands was 59.6, 55.8, 53.9 and 25.9 ha, respectively (Eurostat, 2013).

Beef farms are classified into three groups from the beef production system practised: (1) breeders are farmers with suckler cows not fattening their calves, (2) breeders and fatteners fatten the calves born on their farms and (3) fatteners purchase young male animals and then finish fattening them (European Commission, 2013b).

Based on the degree of integration with crops and its relation to land, farming can be classified into three production systems: (1) grazing, (2) mixed farming and (3) industrial systems. Grazing systems are based almost exclusively on livestock production on native grassland and are commonly found in the pastoral regions of Europe where cereals are hard to grow, notably the western fringes of the continent (in Ireland, Great Britain and the Atlantic arc) and mountainous areas throughout Europe.

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[4] At the time of writing this thesis, the Brexit negotiations were taking place between the UK and the EU for the prospective withdrawal of the UK from the EU, following the UK's referendum on EU membership in June 2016.

[5] Agricultural output comprises the value of all agricultural good (crops and animals) and agricultural services.

[6] Animal output includes the value of both animals (sales and changes in stock levels) and animal products (e.g., milk, eggs and wool).

[7] In 2013, one in three agricultural holdings in the EU was located in Romania (3.7 million holdings or 33.5% of EU total).

In Belgium, grazing farms represent 75.9% of the total livestock-producing farms[8] and 43.4% of the total agriculture holdings. In mixed farming systems, crops and livestock production are integrated on the same farm. These systems are typical in the southern regions of Europe, where the hotter climate halts grass growth in the summer, and also in areas where cereals grow abundantly (e.g., central Europe). In Belgium, some regions specialised in arable crops (e.g., the Loamy Region) and others in animal husbandry (e.g., the Ardennes), resulting in a progressive decrease in mixed farming. Finally, industrial systems are defined as production systems in which less than 10% of the feed is produced within the production unit. Typical examples are large-scale feedlots in the US and the ex-Soviet Union (European Comission, 2004; de Haan *et al.*, 2006; Peeters, 2010).

Bulls account for about 55% of the total volume of bovines slaughtered in Belgium, 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 (Société wallonne de gestion et de participations [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 *et al.*, 2003).

1.2.2 Slaughtering

Slaughtering is the action of killing domesticated animals raised to produce commodities. The objective of the slaughter process is to convert live animals into carcases efficiently and to harvest dress-off items and offals. Cattle slaughter requires several distinct stages: (1) cattle receiving and animal handling, (2) antemortem inspection, (3) stunning, (4) bleeding, (5) skinning, (6) evisceration, (7) splitting, (8) postmortem inspection, (9) classification[9] and (10) chilling (Food and Agriculture Organisation [FAO], 1991; Woerner *et al.*, 2014).

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[8] Dairy cows, cattle, sheep and other ruminants.

[9] In the EU, carcases are classified according to their category (A = carcase of uncastrated male animal aged from 12 to 24 months, B = carcase of uncastrated male animal aged from 24 months, C = carcase of castrated male animal aged from 12 months, D = carcase of female animal that has calved, E = carcase of other female animal aged from 12 months and Z = carcase of animal [male or female] aged from 8 to 12 months), conformation (S = superior, E = excellent, U = very good, R = good, O = fair and P = poor) and fat coverage (1 = low, 2 = slight, 3 = average, 4 = high and 5 = very high).

The different stages of slaughtering may affect the quality of beef. For instance, short-term acute stress, such as excitement immediately before slaughter, produces lactic acid from the breakdown of glycogen, resulting in meat which has a lower pH, lighter colour, reduced water binding capacity, and is possibly tougher (Grandin, 1980). Moreover, the primary sources of microbiological contamination of beef carcases along the slaughter line are the leakage of intestinal contents and cross-contamination with dirty hides of slaughtered animals (Madden *et al.*, 2004; Blagojevic *et al.*, 2012). In both cases, the contamination is of faecal origin (Barco *et al.*, 2015) and compromises the shelf life of meat. Finally, efficient postmortem chilling processes of beef carcases ensures food safety, maximising shelf life (Savell *et al.*, 2005).

1.2.3 The conversion of muscle to meat

During the 24 to 48 h postmortem, bovine muscle undergoes a complex biochemical and physiological mechanisms in the process of becoming meat. After slaughtering, blood is no longer transferred to tissues to support metabolic functions. In consequence, when there is no more O_2 available from the circulatory system, the myofibres use remaining O_2 bound to myoglobin (Mb) to maintain aerobic metabolism generating adenosine triphosphate (ATP). That O_2 is rapidly depleted, and ATP is subsequently derived from creatine phosphate and adenosine diphosphate by the enzyme creatine kinase (Strasburg and Xiong, 2017). Then, glycolysis proceeds without O_2 and produces lactic acid as a result of anaerobic glycolysis, creating a lactic acid build-up and therefore a decrease in pH. Usually, the pH in the bovine muscle decreases from 7.0 upon slaughter to approximately 5.3-5.8 in 18-40 h when chilling is applied (Savell *et al.*, 2005).

Additionally, after death, the concentration of Ca^{2+} in the cytosol increases due to the deterioration of the sarcoplasmic reticulum, and, with the depletion of ATP, the activity of Ca^{2+} ion pumps, which remove calcium from the cytoplasm, stops (Strasburg and Xiong, 2017). Once Ca^{2+} is introduced into the cytosol, it binds to the troponin of thin filaments, which causes the troponin-tropomyosin complex to change shape and allow the myosin heads to link to the active sites of actin proteins (Cooper, 2000) (**Figure 2**). When there is little ATP available to break down the actin and myosin bonds (**Figure 3**), muscles cannot relax and therefore become inextensible (Savell *et al.*, 2005). This state is called *rigor mortis*.

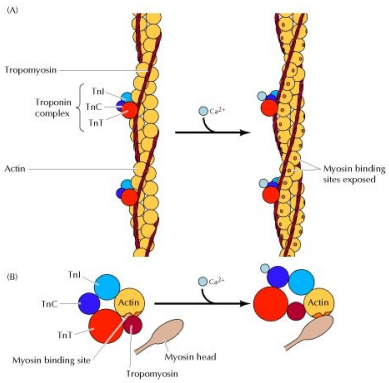


Figure 2 (A) In the absence of Ca^{2+}, the troponin-tropomyosin complex blocks the binding of myosin to actin. (B) Cross-sectional view (Cooper, 2000).

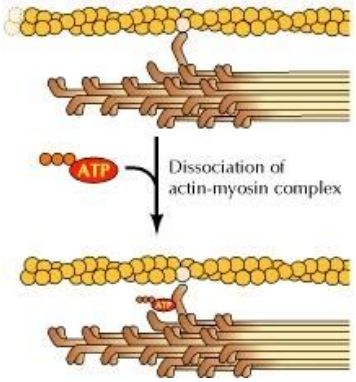


Figure 3 The binding of adenosine triphosphate (ATP) dissociates myosin from actin (Cooper, 2000).

In the last stage of the conversion of muscle into meat, enzymatic degradation caused by proteolytic enzymes including cytoplasmic calpains and lysosomal proteases increases eating quality regarding meat tenderness (Savell *et al.*, 2005). Furthermore, enzymatic reaction will also break proteins into savoury amino acids and fats and fat-like membrane molecules into aromatic fatty acids (Perry, 2012). This process, called ageing, usually takes several days for beef at chilling temperature and may be performed (1) by hanging half-carcases or quarters in a chill room (dry-ageing on the carcase), (2) by placing primal or sub-primal cuts on a rack to dry at chill temperatures (dry-ageing) or (3) by sealing meat in vacuum bags and storing them in a refrigerator unit (wet-ageing) (Perry, 2012; Imazaki *et al.*, 2018).

1.2.4 Processing and distribution

Traditionally, beef used to be distributed between abattoirs to retailers outlets as half carcases or quarters without protective packaging at refrigeration temperatures. In the 1960s, a combination of costs related to meat hanging, the trend toward centralised processing and the development of food-grade impermeable packaging films have contributed to the specialisation of cutting plants and the expansion of the marketing of primal and sub-primal cuts in vacuum bags (Hodges *et al.*, 1974). In fact, more than 80% of the beef in Europe and North America is shipped from meat packers to retailers and hotel/restaurant/institutional (catering) operation in the form of vacuum-packaged cuts (Brody, 1999). At the retail level, modified atmosphere

packaging (MAP) systems with 70-80% O_2 and 20-30% CO_2 are widely used because O_2 favours the bright red colour of meat which is appealing to consumers. Besides, CO_2 aids in preventing microbial growth (Kim *et al.*, 2010). Further information on meat packaging will be presented in **Subsection 1.4.2**.

1.3 Factors limiting the shelf life of meat

The factors that affect the shelf life and stability of meat are numerous, complex and interconnected. The manner in which meat is produced will ultimately affect its shelf life and stability. Maintaining the appropriate level of hygiene pre- and postproduction is essential for ensuring low levels of pathogenic and spoilage micro-organisms (O'Sullivan, 2016). Moreover, the control of the oxidation of Mb and lipids can prevent the development of discolouration and off-flavours, respectively. The microbial factors limiting the shelf life of meat will be discussed in **Subsection 1.3.1.**, while the physicochemical factors will be considered in **Subsection 1.3.2**.

1.3.1 Microbial

Fresh meat is, from a microbiological point of view, a highly perishable product due to its biological composition, pH and water activity (a_w), which are usually compatible with microbial development. Although deterioration of meat can occur in the absence of micro-organisms (e.g., proteolysis, lipolysis and oxidation), microbial growth is by far the most critical factor about keeping the quality of fresh meat (Lambert *et al.*, 1991). Meat is contaminated with the microbiota originating initially from the animal and abattoir facilities (Pothakos *et al.*, 2015), and then from the processing environment, transportation and distribution (McMillin, 2008; Nychas *et al.*, 2008; O'Sullivan, 2016). In this subsection, pathogenic and indicator micro-organisms involved with public health will be presented briefly. Then, a more in-depth description of spoilage micro-organisms associated with beef, which are subject of this thesis, will be done.

The presence and the development of pathogenic bacteria may limit the shelf life of fresh meat. The Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (European Commission, 2005) present specific tolerance limits for

pathogenic and indicator micro-organisms loads for which food must comply. In this regulation, hygiene process criteria are established for beef. These criteria indicate the acceptable functioning of the production process and are not applicable to products placed on the market for the final consumer. Furthermore, in Belgium, the Federal Agency for the Safety of the Food Chain (FASFC) has published an inventory of action limits and proposals for microbial hazards that can be used in the context of official controls or by operators as part of self-checking (FASFC, 2018). **Table 2** presents the microbiological hygiene process criteria and action limits for cattle carcases and fresh beef.

Odour, colour and even texture defects may result from the synthesis of specific molecules by spoilage bacteria through metabolic pathways, such as those involved with energy production or synthesis of molecules essential for bacterial growth. As a consequence of primals and sub-primals packaging and storage conditions (i.e., low temperature and low-O_2 atmospheres), mainly psychrotrophic and facultative anaerobic species present the capacity to grow in such environments, leading to the development of bacteria including Proteobacteria (e.g., *Acinetobacter*, *Enterobacter*, *Hafnia*, *Proteus*, *Serratia*, *Aeromonas*, *Alcaligenes* and *Providencia*) and Firmicutes (e.g., *Brochothrix*, *Carnobacterium*, *Enterococcus*, *Leuconostoc*, *Weissella*, *Lactobacillus* and *Lactococcus*) (Zagorec and Champomier-Vergès, 2017). Four major groups related to fresh meat spoilage (lactic acid bacteria [LAB], Enterobacteriaceae, *Pseudomonas* and *Brochothrix thermosphacta*) will be presented below.

Table 2 Microbiological criteria and action limits for cattle carcases and beef.

Food category	Micro-organism	Sampling		Limits		Stage where the criterion applies
		n	c	m	M	
Carcases of cattle[a]	Aerobic colony count			3.5 log CFU/cm²[b]	5.0 log CFU/cm²[b]	Carcases after dressing but before chilling
	Enterobacteriaceae			1.5 log CFU/cm²[b]	2.5 log CFU/cm²[b]	
	Salmonella	50	2	Absence[c]		
Fresh beef[d]	*Escherichia coli*	1	0	100 CFU/g		Cutting plant
	STEC[e]	1	0	Absence/25 g		

n = number of units comprising the sample.
c = maximum number of sample units giving values between m and M.
m = microbiological level that separates good quality from defective, or in a three-class plan good from marginally acceptable quality.
M = microbiological level in a three-class plan that separates marginally acceptable quality from unacceptable (defective). In a two-class plan M is assimilated to m.
[a] European Commission, 2005
[b] daily mean
[c] area tested per carcase
[d] FASFC, 2018
[e] Shiga-toxin-producing *Escherichia coli*

Lactic acid bacteria (LAB) make part of a vast group that comprises more than ten genera with the largest one, *Lactobacillus*, including over 100 species (Zagorec and Champomier-Vergès, 2017). They are facultative anaerobes or microaerophilic, and their fermentative metabolism produces lactate as at least half of the end products (Kraft, 1992). A large number of LAB have been described in meat products (Ercolini *et al.*, 2006; Doulgeraki *et al.*, 2012). In fact, psychrotrophic LAB are the dominant component of the microbial flora of vacuum-packaged fresh chilled meats (Egan, 1983). Even though the presence of LAB ensures that shelf life is maximal (Egan, 1983), their role in meat spoilage is still very controversial (Pothakos *et al.*, 2015). Indeed, most of LAB are considered as beneficial for the product and lots of them, including *Lactobacillus sakei* and *Carnobacterium maltaromaticum* have been proposed as biopreservative agents for meat and meat products (Leisner *et al.*, 2007; Jones *et al.*, 2010; Chaillou *et al.*, 2014; Koné *et al.*, 2018). Nevertheless, *Lactococcus piscium* has been reported as a meat spoiler responsible for buttery off-odours (Sakala *et al.*, 2002) and *Leuconostoc gasicomitatum* has been associated to gas and slime formation, greening of beef and development of pungent acidic and buttery off-odours (Johansson *et al.*, 2011). The concomitant role of specifically *C. maltaromaticum* as a spoiler or protective culture in meat has also been documented (Laursen *et al.*, 2005; Leisner *et al.*, 2007).

Although most attention is paid to the pathogenic properties of particular genera of Enterobacteriaceae including *Escherichia coli* and *Salmonella*, some members of the family constitute a critical spoilage group for meat and meat products (Kraft, 1992). This group includes *Serratia liquefaciens*, *Hafnia alvei* and *Pantoea agglomerans*. They use glucose as a source of energy, but can also degrade amino acids after carbohydrates with the release of amines, organic sulphides and H_2S. Moreover, Enterobacteriaceae are also associated with blown pack-type spoilage of vacuum-packaged beef (Chaves *et al.*, 2012). Additionally, DFD[10] meat is not suitable for vacuum packaging. In fact, spoilage becomes more apparent when bacteria attack amino acids. This does not occur until bacteria exhaust the glucose at the meat surface. However, since glucose is absent in DFD meat, amino acids are utilised without delay and spoilage becomes evident at lower cell densities than in normal meat. Additionally, the high pH of DFD meat allows growth of potent spoilage organisms which would be inhibited at the normal ultimate pH of meat (Newton and Gill, 1981). Finally, the H_2S produced by

[10] Acronym for dark, firm and dry used to describe meat whose pH remains high 24 h after slaughter. Dark, firm and dry meat is the result of an exhaustion of the muscle's glycogen stores before slaughtering notably resulting from an intense physical effort.

Enterobacteriaceae combines with Mb to form sulphmyoglobin, causing the greening of meat (García-López *et al.*, 1998).

Pseudomonas ludensis, *Pseudomonas fragi* and *Pseudomonas fluorescens* are the main species of the genus *Pseudomonas* that are encountered in meat and meat products (Nychas *et al.*, 2008). Originating from the environment, they are highly dominant on the carcase after slaughtering (Zagorec and Champomier-Vergès, 2017). *Pseudomonas* play a significant role in limiting the shelf life of fresh foods stored aerobically at cold temperatures (Remenant *et al.*, 2015) via the activity of heat-stable extracellular proteases and lipases (Tryfinopoulou *et al.*, 2002; Ercolini *et al.*, 2007; De Jonghe *et al.*, 2011; Quigley *et al.*, 2013). In the case of meat, their growth, utilisation of food nutrients such as sugars and amino acids, and release of both volatile and non-volatile metabolites such as esters, ketones, alcohols, aldehydes organic acids, sulphur compounds and amines cause organoleptic changes to the meat resulting in spoilage (Montel *et al.*, 1998; Ercolini *et al.*, 2006; Samelis, 2006; Stanborough *et al.*, 2018).

Brochothrix thermosphacta is a facultative anaerobe that can grow on chilled meats and fish stored under low O_2 and vacuum packaging (Borch *et al.*, 1996; Ercolini *et al.*, 2006). *Brochothrix thermosphacta* can cause severe economic losses in the food industry due to its ability to produce metabolites associated with off-odours (Illikoud *et al.*, 2018). In beef, for example, it has been shown that *B. thermosphacta* produce cheesy and creamy dairy off-odours associated with the production of 3-hydroxy-2-butanone (acetoin), 2,3-butanedione, and 3-methyl-1-butanol (Dainty and Mackey, 1992; Casaburi *et al.*, 2014). Despite their widespread occurrence within spoiling species, only a few studies have addressed their genomic characteristics (Papadopoulou *et al.*, 2012; Illikoud *et al.*, 2018) and little is known about their metabolic pathways leading to spoilage.

1.3.2 Physicochemical

Oxidative processes are the primary non-microbiological factor implicated in quality deterioration of meat during chilled storage. This subsection emphasises three major oxidation processes in beef: Mb, lipid and protein (other than Mb) oxidation. The conversion of oxymyoglobin (OMb) to metmyoglobin (MMb) provokes meat discolouration and compromises its appearance. The oxidation of unsaturated fatty acids from phospholipids and triacylglycerols contributes to off-flavours (Faustman *et al.*, 2010a). Recent studies also highlighted the potential impact of specific protein

carbonyls derived from protein oxidation in particular meat quality traits such as water-holding capacity, texture, flavour and nutritional value (Estévez, 2011).

1.3.2.1 Myoglobin (Mb) oxidation

The colour of meat and meat products is an essential aspect for consumer acceptability (Faustman and Cassens, 1990). Consumers consider the bright cherry-red colour of raw beef as an indicator of freshness and wholesomeness (Hood and Riordan, 1973). According to Clydesdale and Ahmed (1978), colour is perhaps the most important sensory attribute of food because if it is deemed unacceptable, the food will not be purchased and eaten, and consequently, all other sensory attributes lose significance. It is clear that colour is, therefore, a critical factor capable of influencing customers when buying. Contrariwise, the tenderness and juiciness of the meat remain the determining factors of their satisfaction at the moment of consumption (Grunert, 1997; Monson *et al.*, 2005; Grobbel *et al.*, 2008).

Myoglobin (Mb) is the heme protein (**Figure 4**) responsible for meat colour, and the three different redox forms of this protein define the colour of fresh meat. The reduced Mb or deoxymyoglobin (DMb) is the dark purplish-red or purplish-pink pigment typical of raw vacuum-packaged beef. Deoxymyoglobin (DMb) contains ferrous iron (Fe^{2+}) with a vacant sixth coordination site. Exposed to air, DMb combines with O_2 to form the bright red pigment OMb, which has O_2 connected to the sixth coordination site of Fe^{2+}. Beyond a certain time, influenced by the intrinsic properties and the preservation conditions of the meat, the surface OMb layer gradually disappears in favour of the oxidised Mb form or MMb. Metmyoglobin (MMb) is the oxidised brown coloured form of Mb, and it contains ferric iron (Fe^{3+}). Water is the ligand at the sixth position of Fe^{3+} in MMb (Mancini and Hunt, 2005; Mancini *et al.*, 2005; Kim *et al.*, 2006; Suman *et al.*, 2010; American Meat Science Association [AMSA], 2012; Suman *et al.*, 2014).

Myoglobin (Mb) oxidation is favoured by high temperatures (Brown and Mebine, 1969), low pH values (Gotoh and Shikama, 1974) and the presence of non-heme iron (Allen and Cornforth, 2006). Low non-zero partial pressures of oxygen (pO_2) favour MMb formation (George and Stratmann, 1952; Ledward, 1970). Conversely, pO_2 in which a complete vacuum exists ($pO_2 = 0$) or in which O_2 saturation is attained (high pO_2) favour ferrous Mb forms (Faustman *et al.*, 2010a). In bloomed meat, after storage, OMb, MMb and DMb co-exist, and their concentration depends on the freshness and pO_2

throughout the meat. The primary pigment in the most superficial layer is OMb (high pO_2), while a thin layer of MMb will exist at a certain depth in the meat (low non-zero pO_2) followed by DMb in the most profound layer ($pO_2 = 0$). The colour of different Mb forms is shown in **Figure 5**, and the Mb redox interconversions are shown in **Figure 6**.

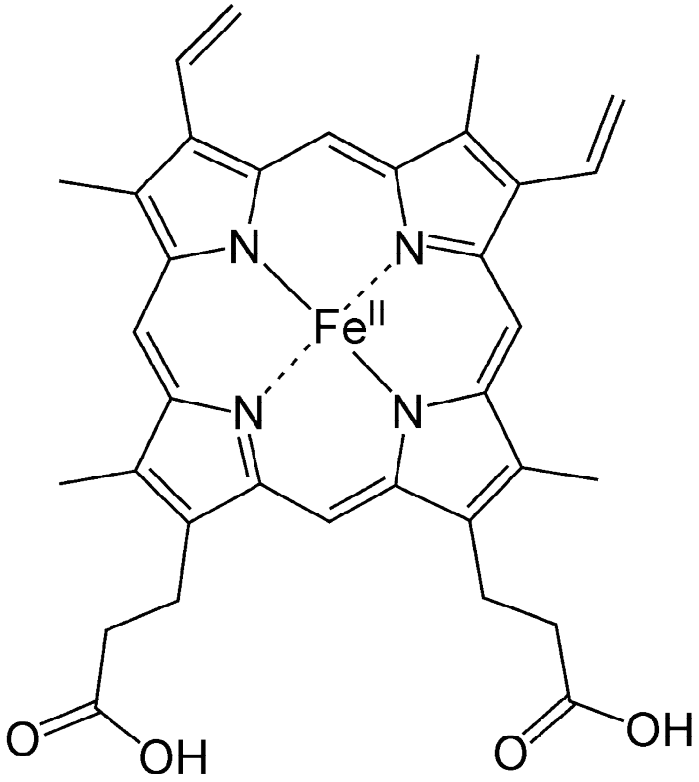


Figure 4 Chemical structure of heme B.

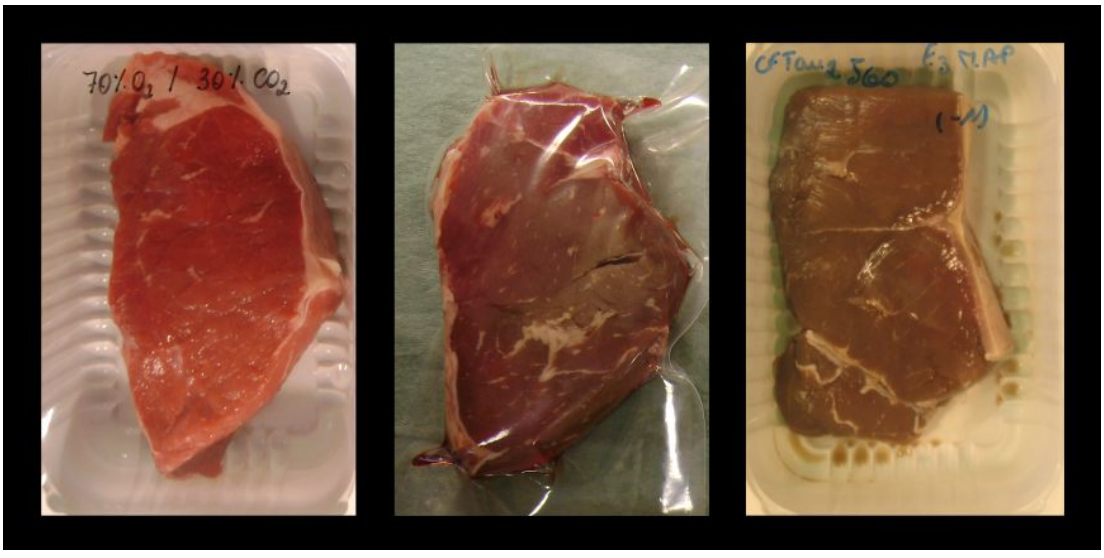
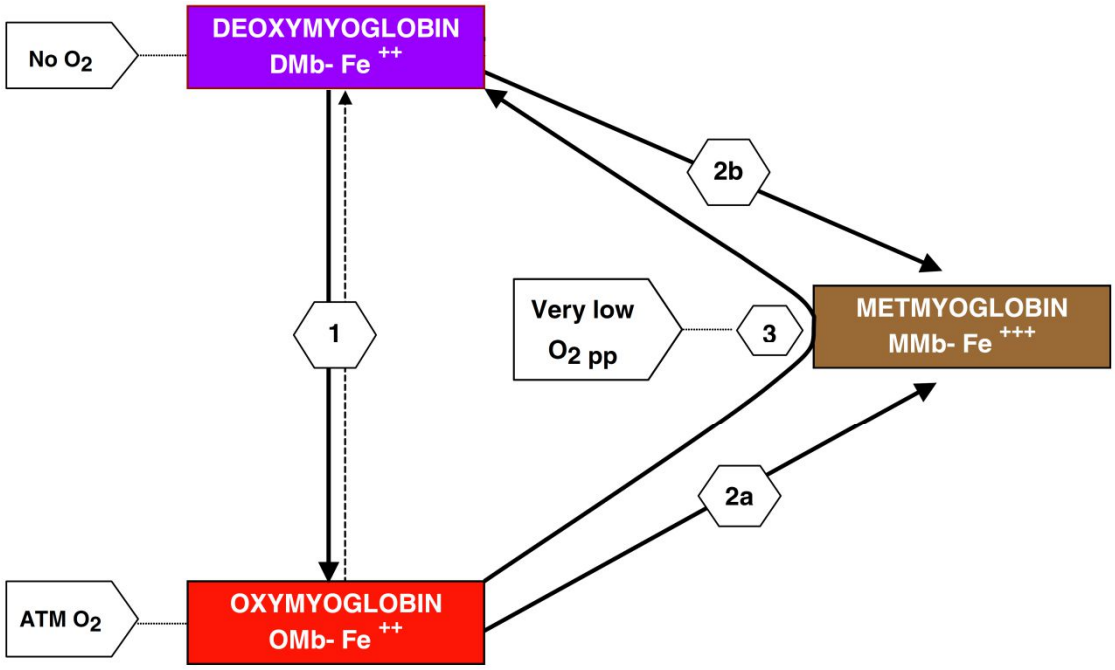


Figure 5 Beef containing high proportions of oxymyoglobin (left), deoxymyoglobin (middle) and metmyoglobin (right) on surface.



Rx 1 (Oxygenation): $DMb + O_2 \rightarrow OMb$

Rx 2a (Oxidation): $OMb + [\text{oxygen consumption or low } O_2 \text{ partial pressure}] - e^- \rightarrow MMb$

Rx 2b (Oxidation): $[DMb - \text{hydroxyl ion} - \text{Hydrogen ion complex}] + O_2 \rightarrow MMb + O_2^-$

Rx 3 (Reduction): $MMb + \text{Oxygen consumption} + \text{metmyoglobin reducing activty} \rightarrow DMb$

Figure 6 Visible myoglobin redox interconversions, adapted from Mancini and Hunt (2005). Rx: reaction.

The formation of MMb can be limited in postmortem muscle by the transfer of an electron to heme Fe^{3+} to form DMb. This phenomenon is called metmyoglobin reducing activity (MRA) and involves a series of enzymatic and non-enzymatic mechanisms (Bekhit and Faustman, 2005). The enzyme NADH-cytochrome b_5 reductase is the best-characterised enzyme involved in the reduction of oxidised heme proteins. The major components required for the enzymatic reduction of MMb by that system are the enzyme NADH-cytochrome b_5 reductase itself, the intermediate cytochrome b_5 and the cofactor nicotinamide adenine dinucleotide in its reduced form (NADH). Moreover, researchers have also reported non-enzymatic reduction for MMb by high concentrations of NADH or nicotinamide adenine dinucleotide phosphate (reduced form) in the presence of ethylenediaminetetraacetic acid (EDTA) (Brown and Snyder, 1969; Bekhit *et al.*, 2001).

1.3.2.2 Lipid oxidation

Lipid oxidation represents a significant cause of deterioration in meat and meat products. Several products of lipid oxidation are responsible for rancid odours and

flavours, and some are very reactive (Pearson *et al.*, 1977; Faustman *et al.*, 2010a). Substrates necessary for lipid oxidation in meat include unsaturated fatty acids, O_2 and chemical species that accelerate oxidation (e.g., iron) (Kanner *et al.*, 1988; Sohaib *et al.*, 2017). The intake of oxidised lipids raises blood oxidation markers, leads to cell damage, and increases the risk of suffering health disorders such as coronary heart diseases, neurodegenerative disorders, and certain types of cancer (Esterbauer *et al.*, 1992, 1993; Sies *et al.*, 2005; Awada *et al.*, 2012). The role played by lipid oxidation products in the pathogenesis is usually linked to the cytotoxicity and mutagenicity potential of these species on the gastrointestinal tract or internal organs upon absorption (Esterbauer *et al.*, 1993)

Lipid oxidation is the result of chain reactions that occur in three simultaneous phases: initiation, propagation and completion. The two first phases lead to the formation of radicals, which are rapidly transformed into non-radical compounds such as conjugated dienes and hydroperoxides, which are both considered primary products of lipid oxidation. These compounds decompose further and give rise to carbonyl compounds, ketones, alcohols, and aldehydes, which are considered secondary products of lipid oxidation (Guyon *et al.*, 2016). The most well-known secondary product of lipid oxidation in red meat is malondialdehyde (MDA) (Faustman *et al.*, 2010b). Other examples of secondary products include hexanal, propanal (Siu and Draper, 1978; Sakai *et al.*, 1998) and 4-hydroxynonenal (Sakai *et al.*, 1995). The nature of formed primary and secondary oxidation products reflects the degree and location of unsaturation in the fatty acid substrates (Belitz *et al.*, 2009; Faustman *et al.*, 2010b). A general overview of the different steps of lipid oxidation in meat is shown in **Figure 7**.

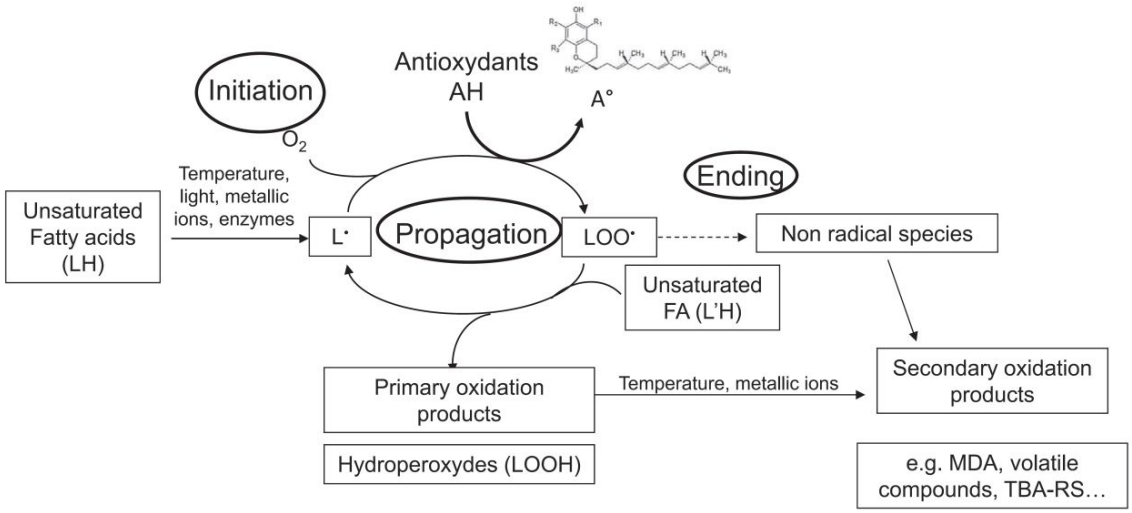


Figure 7 General overview of the different steps of lipid oxidation in meat (Guyon et al., 2016).

A variety of factors can predispose meat to lipid oxidation. For example, meat from non-ruminants contains higher relative concentrations of unsaturated fatty acids within triacylglycerols (Enser *et al.*, 1996) and displays more rapid lipid oxidation than that of ruminants (Tichivangana and Morrissey, 1985). Furthermore, muscles with higher proportions of red fibres are more susceptible to oxidation because they contain more iron and phospholipids than muscles containing predominantly white fibres (Wood *et al.*, 2004). Lipid oxidation and Mb oxidation often appear to be linked, and the oxidation of one of these leads to the formation of chemical species that can exacerbate oxidation of the other (Faustman *et al.*, 2010a). **Figure 8** summarises the potential interacting oxidation reactions between OMb and unsaturated fatty acids in lipid bilayers.

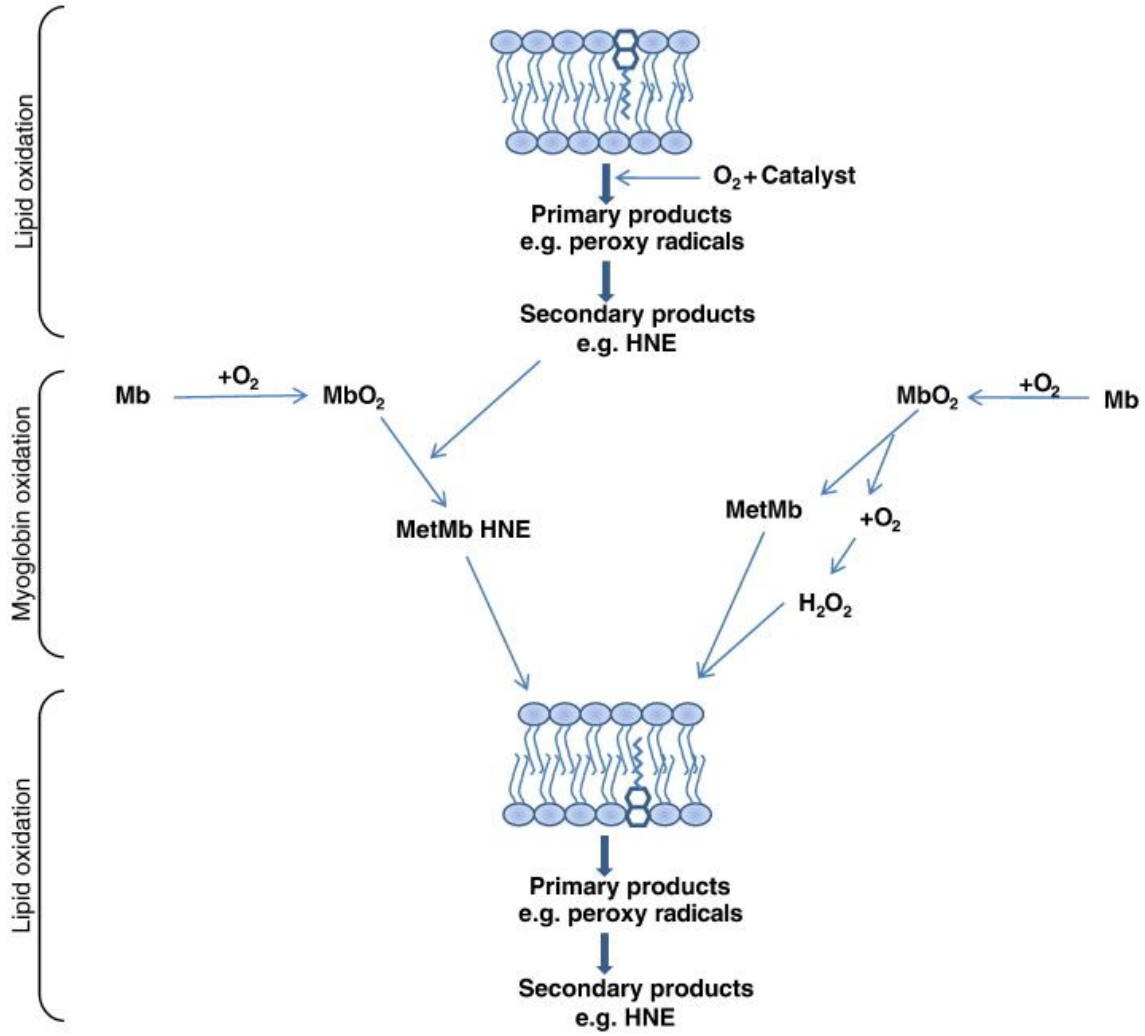


Figure 8 Summary of potential interacting oxidation reactions between oxymyoglobin and unsaturated fatty acids in lipid bilayers (Faustman *et al.*, 2010a). HNE: 4-hydroxynonenal; MbO_2: oxymyoglobin; MetMb: metmyoglobin; MetMb HNE: covalent attachment of MetMb and HNE.

1.3.2.3 Protein oxidation

Protein oxidation is a relatively new topic of increasing interest among meat researchers. This process can lead to denaturation and loss of functionality, impacting meat quality traits such as water-holding capacity, texture, flavour and its nutritional value (Estévez, 2011; Jongberg *et al.*, 2017) (**Figure 9**). Recent reports have indicated the involvement of dietary protein oxidation species on particular health disorders (Estévez and Luna, 2017; Jongberg *et al.*, 2017). In fact, the oxidation of food proteins during processing and storage leads to the inexorable accumulation of oxidation products that will be primarily exposed to the gastrointestinal tract. Scientific evidence supports the impact of dietary oxidised proteins on intestinal flora disturbance, the redox state of intestinal tissues and the onset of local pathological conditions (Keshavarzian *et al.*, 2003; Fang *et al.*, 2012; Xie *et al.*, 2014). The occurrence of oxidised proteins in the gut may also have consequences on internal organs upon intestinal uptake of oxidised amino acids/small peptides. Gurer-Orhan *et al.* (2006) already hypothesised that oxidised amino acids might be misincorporated into proteins such as enzymes and structural elements in cells, potentially contributing to malfunction, cell apoptosis and disease.

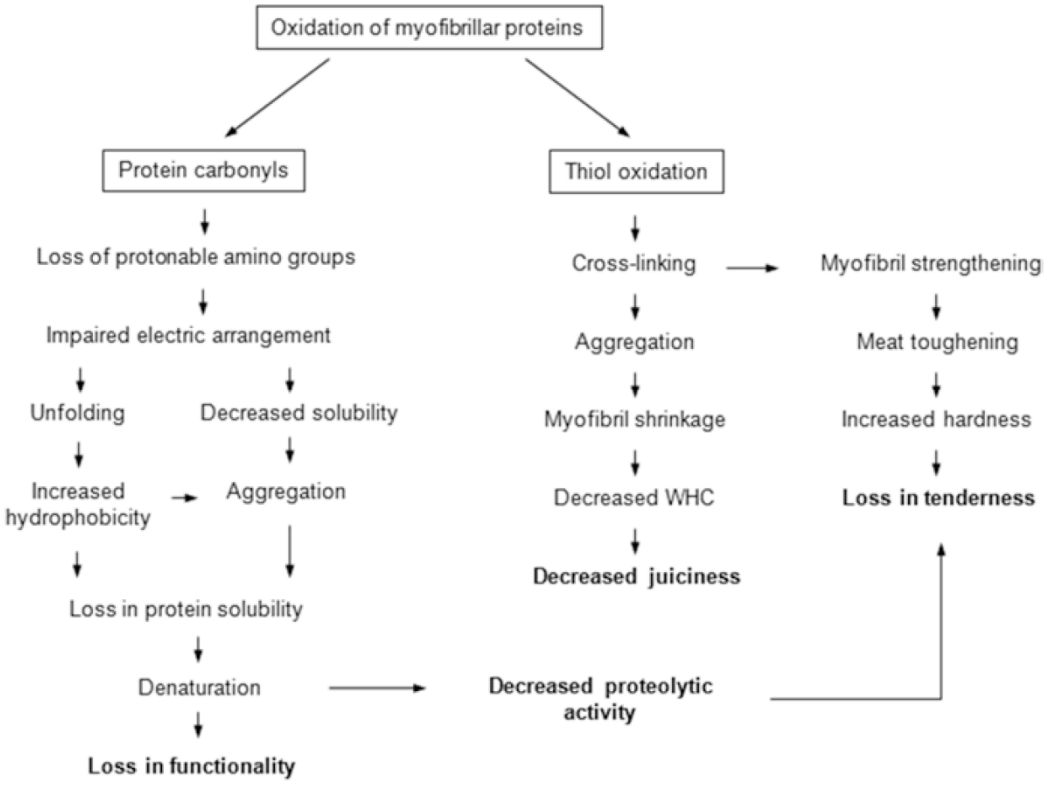


Figure 9 Altered meat protein functionalities due to oxidation of myofibrillar proteins (Jongberg *et al.*, 2017). WHC: water holding capacity.

Oxidation of proteins is believed to progress through a free radical chain reaction similar to that of lipid oxidation although the oxidation of proteins seems to be more complicated and to produce an even higher multiplicity of reaction products (Dean *et al.*, 1997; Jongberg *et al.*, 2017). The abstraction of a hydrogen atom by reactive oxygen species (ROS) leads to the generation of a protein carbon-centred radical (P•) which is consecutively converted into a peroxyl radical (POO•) in the presence of O_2, and an alkyl peroxide (POOH) by abstraction of a hydrogen atom from another molecule. Further reactions with HO_2• lead to the generation of an alkoxy radical (PO•) and its hydroxyl derivative (POH) (Lund *et al.*, 2011).

Myofibrillar proteins are susceptible to oxidative reactions with myosin being the most sensitive, followed by troponin T (Martinaud *et al.*, 1997). Among amino acids, cysteine, tyrosine, phenylalanine, tryptophan, histidine, proline, arginine, lysine and methionine have been described as particularly susceptible to ROS (Dean *et al.*, 1997). The nature of the protein oxidation products formed is highly dependent on the amino acids involved and how the oxidation process is initiated. The side chains of some particular amino acids such as arginine, lysine and proline are oxidised through metal-catalysed reactions into carbonyl residues while others such as cysteine or methionine are involved in cross-linking or yield sulfur-containing derivatives (Lund *et al.*, 2011).

Lastly, oxidation processes related to lipids, proteins, and heme proteins are individually relatively well understood. However, the coupling between degradation processes in meat has not been investigated in detail.

1.3.2.4 Endogenous antioxidants in meat

Meat has a complex physical structure and chemical composition that is very susceptible to oxidation. The oxidative stability of meats depends upon the balance and the interaction between endogenous anti- and pro-oxidant substances and the composition of substrates prone to oxidation including polyunsaturated fatty acids, cholesterol, proteins and pigments (Decker and Xu, 1998).

Endogenous antioxidant systems are composed of non-enzymatic compounds such as vitamin E, vitamin C, carotenoids, ubiquinols, polyphenols and cellular thiols, and enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). Together, enzymatic and non-enzymatic antioxidant systems operate to counteract the action of pro-oxidants in muscle tissues (Chan and Decker,

1994; Decker *et al.*, 2000; Pastsart *et al.*, 2013; Imazaki *et al.*, 2018) both in living animals and after slaughter (Serpen *et al.*, 2012).

1.4. Preservation techniques for fresh meat

Fresh meat is a highly perishable product due to its biological composition. Many interrelated factors influence the shelf life and freshness of meat such as holding temperature, O_2, endogenous enzymes, moisture, light and micro-organisms (Zhou *et al.*, 2010). This subsection is divided into four parts. The first two parts (**Subsections 1.4.1** and **1.4.2**) describe the two techniques authorised for fresh meat preservation in the EU: refrigeration and vacuum packaging/MAP. **Subsection 1.4.3** gives a brief overview of unauthorised preservation techniques in the EU and **Subsection 1.4.4** explores the estimation of the maximum shelf life possible for fresh meat.

1.4.1 Refrigeration

Refrigeration retards spoilage as it will primarily hinder the development of micro-organisms. However, refrigeration cannot improve the initial quality of the product, hence the importance of assuring high microbial quality in the starting material. Unlike thermal sterilisation, refrigeration is not a method of permanent preservation. Thus, refrigerated foods have a definite shelf life (Berk, 2018). For fresh meat, refrigeration, above or below the freezing point, has been the traditional preservation method for centuries[11]. During meat storage, transport and retail display, maintaining the cold chain and therefore the temperature of the product is a primary aim. Within the cold chain, two processes often used by the meat industry are chilling and freezing (James and James, 2004; Zhou *et al.*, 2010).

[11] Recognition by early civilisations of the preservative effects of cold temperature storage of perishable products such as meat led to storage of such products in natural caves where temperatures were relatively low throughout the year.

1.4.1.1 Chilling

Chilling can be defined as the fundamental operation in applying cold to meat (or other foods) to reduce its temperature (Cano-Muñoz, 1991). According to the Regulation (EC) No 853/2004 on the hygiene of foodstuffs (European Parliament and Council of the European Union, 2004), beef chilling should start in the slaughterhouse immediately after postmortem inspection to ensure a temperature throughout the meat of not more than 7°C along a chilling curve that provides a continuous decrease of the temperature. A limiting factor, however, is the difficulty in removing heat quickly from the more deep tissues of carcases (Zhou *et al.*, 2010), while avoiding cold shortening. Cold shortening can often be seen in beef and mutton, when the meat, still in its *pre-rigor* phase, reaches temperatures of 10°C or lower. These conditions cause irreversible immobilisation of the muscle tissue which toughens the meat even after prolonged ageing (Cano-Muñoz, 1991).

Stored chilled meat is mainly intended to serve as buffer stock between production, transport and consumption (Cano-Muñoz, 1991). In general, as meat moves along the cold chain it becomes increasingly difficult to control and maintain its temperature: temperatures of bulk packs of meat in vast storerooms are far less sensitive to small heat inputs than single consumer packs in transport or open display cases (Aidani *et al.*, 2014).

1.4.1.2 Freezing

Freezing consists of lowering the temperature of meat or other foods to −18°C and keeping it at this temperature for storage. Modern commercial meat freezing has a surprisingly long history. It is believed that the first contemporary meat freezing works were established at Darling Harbour in Sydney (Australia) in 1861, and the first entirely successful frozen meat shipment was that of the SS *Paraguay* from Buenos Aires (Argentina) to Le Havre (France) in 1877[12] (Troubridge Critchell and Raymond, 1912). Freezing is usually limited to meat intended for export or later processing. Indeed, when the preservation period is more extended than that acceptable for chilled meat, freezing must be used to minimise any physical, biochemical and microbiological changes affecting quality in storage. During freezing most of the water content of the

[12] Due to a collision, the ship took seven months(!) to complete her journey; however, the 5,500 mutton carcases were still reported to be in “tip-top condition” when the ship arrived at Le Havre.

meat, about 80%, solidifies into pure ice crystals, accompanied by a separation of dissolved solids (Cano-Muñoz, 1991). In consequence, the preserving action of freezing is explained primarily by the depression of a_w (Berk, 2018).

The preservation capacity of frozen meat is limited because the physical, chemical or biochemical reactions that take place in animal tissues after slaughtering do not stop entirely after cold treatment (Rosmini *et al.*, 2004). Microbial growth stops at −12°C, and total inhibition of the cellular metabolism in animal tissues occurs below −18°C (Perez-Chabela and Mateo-Oyague, 2004). Complete quality changes of meat can be prevented at a temperature of −55°C (Hansen *et al.*, 2004), which is currently economically unfeasible. In this manner, during freezing at −18°C, enzymatic reactions, oxidative rancidity and ice crystallisation still play an essential part in spoilage (Zhou *et al.*, 2010).

Frozen meat also suffers from a perception that its eating quality is not as good as that of “fresh” chilled meat. Studies have shown that the formation of ice crystals during freezing damages the fibre structure and concentrates the solutes in the meat which, in turn, leads to alterations in biochemical reactions that occur at the cellular level, influencing the physicochemical quality parameters of the product (Leygonie *et al.*, 2012; Li *et al.*, 2018). Nonetheless, many inconsistencies exist in the literature regarding the combined effect of freezing and thawing on quality attributes of meat including colour, oxidation susceptibility, tenderness, juiciness, the degree of liking and the microbiological shelf life post freeze/thaw (Lagerstedt *et al.*, 2008; Leygonie *et al.*, 2012; Muela *et al.*, 2012; Kim *et al.*, 2017; Bogdanowicz *et al.*, 2018).

1.4.2 Packaging

The primary function of food packaging is to protect products against deteriorative effects, which may include discolouration, off-flavour and off-odour development, nutrient loss and texture changes (Zhou *et al.*, 2010). However, packaging and packaging-related product traits also influence purchase intentions and decisions by consumers at the retail level (Verbeke *et al.*, 2005; Grobbel *et al.*, 2008). In this manner, it is required that the design of packaging maintain and inhibit deterioration of quality attributes, providing consumers convenience and information about the product (Yam *et al.*, 2005). In consequence, packaging functions have been categorised into four roles: protection, preservation, handling facilitation and communication (Lindh *et al.*, 2016).

As already discussed in **Subsection 1.2.4**, significant changes in raw chilled meat packaging were observed in the middle of the 20th century. The economies of carcase breaking in processing plants and shipment of primal cuts rather than carcases, sides or quarters to retail stores for cutting into retail portions propelled advances in vacuum packaging materials and equipment. Moreover, butcher cutting and wrapping of meat in paper or waxed paper upon demand by the purchaser has been gradually, but not totally, replaced by centralised cutting operations and display of the packages in refrigerated self-service display cases in many developed countries (McMillin, 2008). Many of the case-ready systems incorporate modification of the atmosphere because system cost economies and efficiencies coupled with the developments in packaging materials, processing and equipment have made vacuum packaging and MAP practical and economically competitive for meat (Zhao *et al.*, 1994).

Today, popular packaging options for raw chilled meat are air-permeable packaging (stretch wrap), vacuum (low-O_2) and high-O_2 modified atmosphere (Zhou *et al.*, 2010). The choices of packaging for specific cuts or products are usually dependent upon the characteristics desired for storage and display and the expectations of purchasers. Air-permeable packaging is used for short-term storage (3 to 4 days) and retail display of raw chilled meat. Conversely, vacuum and high-O_2 modified atmosphere are used for long-term storage or display of raw chilled products, depending upon the specific desired attributes and applications (Lindh *et al.*, 2016; McMillin, 2017). Vacuum and high-O_2 modified atmosphere will be described in the next subsections.

1.4.2.1 Vacuum packaging

Vacuum packaging is the process of removing gases (with particular emphasis on the removal of O_2) from within the packaging environment (Kerry and Tyuftin, 2017). The lack of O_2 in vacuum packaging minimises the oxidative deteriorative reactions and reduces aerobic bacteria growth (McMillin *et al.*, 1999; Lund *et al.*, 2007; Imazaki *et al.*, 2018). Indeed, a significant benefit of using vacuum packaging is that it creates an O_2-less environment. Thus, rapid aerobic meat spoilers such as *Pseudomonas* species are controlled, and the product shelf life significantly extended. Spoilage of vacuum packaging red meat usually develops over several weeks and is brought about by the presence of Enterobacteriaceae, *B. thermosphacta* or LAB (Kerry and Tyuftin, 2017).

Vacuum packaging causes Mb to be in DMb state (Imazaki *et al.*, 2018), which gives to meat the dark purplish/brown colour of freshly slaughtered muscle. While this has always been relayed to be a negative owing to consumers not liking this form of meat colour, retailing organisations have bucked this widely accepted view by successfully retailing red meat sub-primal cuts in essentially vacuum-packed formats in certain countries (Robertson, 2012; Kerry and Tyuftin, 2017).

A relatively new low-O_2 vacuum packaging for retail meat cuts is vacuum skin packaging systems. In this kind of packaging, meat is placed in a styrene or polypropylene tray, and vacuum sealing barrier films that are heat shrunk conform to the shape of the product (Belcher, 2006). **Figure 10** shows an example of vacuum-packaged sub-primal and a vacuum skin packaged retail cut.

1.4.2.2 Modified atmosphere packaging (MAP)

Modified atmosphere packaging (MAP) is a reliable retail packaging method used to increase product shelf life of commodities such as burgers, meat medallions, sausages, minced meat and steaks in a consumer-friendly presentation format. Modified atmosphere packaging (MAP) preserves food by manipulating the gaseous environment immediately around the product. The gaseous atmosphere has a different composition from that of regular atmospheric air. Depending on the product being packaged, a suitable gas mixture must be selected for that product based on its chemical composition and on the spoilage factors that need to be controlled and which are unique to that product.

Figure 10 Vacuum-packaged sub-primal (left) and vacuum skin packaged retail cut (right).

In general, the gaseous environment created is based on the manipulation of CO_2, O_2, and N_2[13] (Kerry and Tyuftin, 2017). Carbon dioxide is used due to its particular bacteriostatic properties, which prevents or delays the growth of fast spoiling aerobic bacteria such as *Pseudomonas*, which dislikes the presence of this gas, even at low concentrations (Hintlian and Hotchkiss, 1987; McMillin, 2008; Vermeulen *et al.*, 2013). Gram-negative bacteria are more sensitive to CO_2 than Gram-positive bacteria (Church, 1994) because most Gram-positive bacteria are facultative or strict anaerobes (Gill and Tan, 1980), but individual bacteria vary in sensitivity to CO_2 (Farber, 1991). Excessive CO_2 absorption into a product such as meat can cause moisture loss and negatively impact on texture, discolouration and taste (Kerry and Tyuftin, 2017). Dioxygen is used to maintain the fresh red colour of meat. However, it must be noted that while O_2 is delivering this commercially desired effect, it is also driving negative oxidative reactions leading to lipid oxidation, protein oxidation, microbial growth, and vitamin degradation. Dinitrogen is an inert gas, and its primary functions are to displace O_2 and to counter-balance CO_2 levels in packs to keep the shape of the package by preventing pack collapse (Kerry and Tyuftin, 2017). Beef repackaged in a high-O_2 atmosphere can be seen in **Figure 5 (left)**.

Carbon monoxide (CO) has a history in the meat industry due to its colour stabilising effects coupled with antioxidant abilities. However, CO is prohibited in the EU mainly due to fears it may mask spoilage, therefore, misleading consumers. In fact, CO can readily bind to Mb to form the highly-stable red pigment carboxymyoglobin, maintaining an acceptable colour beyond the spoilage shelf life (Van Rooyen *et al.*, 2017).

1.4.3 Unauthorised technologies in the EU for the preservation of fresh meat

1.4.3.1 Ionising radiation

Ionising radiation has been proposed as a suitable method for the prolongation of storage life of meat and meat products (Millar *et al.*, 2000). The advantages of ionising radiation for food preservation include their highly efficient inactivation of bacteria, the low total chemical change they cause and the substantial thickness of material which can be treated after packing in containers (Ahn *et al.*, 2017). Radiation energies are

[13] The Regulation (EC) No 1333/2008 on food additives allows the use of other gases in food packaging including argon and helium.

classified into three categories: electromagnetic radiation (γ and X-rays), charged particle radiation (α- and β-rays and electron beam) and uncharged particles (photons and neutrons). Among the ionising radiation, only high-energy electrons from linear accelerators, X-rays produced by the collision of high-energy electrons with a metal (e.g., tungsten) target and γ-rays from radioactive sources (e.g., ^{60}Co) are useful for treating foodstuffs (Brynjolfsson, 1989). Due to the high penetrating capabilities of X-rays and particles, micro-organisms present on food surfaces and deep layers of food are inactivated (Lawrie and Ledward, 2006).

Ionising radiation is more efficient than thermal treatments due to the low energy needed to reduce microbial counts (Aymerich *et al.*, 2008). Nonetheless, the application of irradiation may accelerate lipid and protein oxidation and off-odour production (Ahn *et al.*, 2017). However, Graham *et al.* (1998) demonstrated that if radiation dosage level is correctly applied (2 to 5 KGy), then even the most sensitive nutrients would not be affected during irradiation of muscle foods. Irradiation technology has been accepted in 50 countries (Aymerich *et al.*, 2008; Zhou *et al.*, 2010). The radionuclides approved for food irradiation include ^{137}Cs and ^{60}Co (Zhou *et al.*, 2010). The consumer resistance to its use in foods, especially within the EU, continues to dampen the widespread usage of this preservation technique (Kerry and Tyuftin, 2017).

1.4.3.2 Chemical preservatives

For millennia, humans have used a wide range of naturally occurring substances derived from organic and inorganic sources for addition to foodstuffs in an attempt to make them safer to eat and to allow consumption of these food products over a more extended period (Kerry and Tyuftin, 2017).

Lactates, for example, have been a frequently active inhibitory agent used in fresh meat preservation (Zhou *et al.*, 2010) through fermentation processes. The antimicrobial effects of lactates are due to their ability to lower a_w and the direct inhibitory effect of the undissociated form lactic acid[14], which penetrates the cytoplasmic membrane, resulting in reduced intracellular pH and disruption of the transmembrane proton motive force (Ray and Sandine, 1992; Houtsma *et al.*, 1993; Koos and Jansener, 1995). In 2013, the use of lactic acid to reduce microbiological

[14] At pH 5.5, only 2.2% of the total lactic acid is in its undissociated form.

surface contamination on bovine carcases only was approved in the EU (European Commission, 2013a). The antimicrobial activity of other acid salts including sodium diacetate in meat products has also been studied (Koo *et al.*, 2012).

Natural compounds, such as essential oils, chitosan, nisin and lysozyme, have been investigated to replace chemical preservatives and to obtain 'green label' products. Various spices and essential oils have preservative properties and have been used to extend the storage life of meat products. These include eugenol in cloves and allyl isothiocyanate in mustard seed (Zhou *et al.*, 2010).

1.4.3.3 Biopreservation

Biopreservation is the general term for methods of food preservation that use microbial activities to inhibit the growth of spoilage and pathogenic microbes (Yost, 2014). Actually, storage life may be extended and safety increased by using natural or controlled microflora, of which LAB and their antimicrobial products such as lactic acid and bacteriocins have been studied extensively (Leisner *et al.*, 1995; Laursen *et al.*, 2005; Castellano *et al.*, 2008). Most of the LAB species are considered as beneficial, and lots of them have been proposed as biopreservative agents for meat products (Zagorec and Champomier-Vergès, 2017).

Among LAB of meat products, *Lb. sakei* is undoubtedly one of the most studied species due to its role in the fermentation of sausage and its prevalence during cold storage of raw meat products. This species exhibits a wide genomic diversity that can be observed when studying different strains and on which probably rely its multiple facets in meat products: starter, spoiler, or protective culture (Zagorec and Champomier-Vergès, 2017). Biopreservation trials in fresh meat products have been directed either against pathogens (e.g., *Listeria monocytogenes*, Salmonella Typhimurium and *E. coli* O157:H7) or against spoilers (e.g., *B. thermosphacta* and *Clostridium estherteticum*) aiming at extending shelf life (Jones *et al.*, 2008; Chaillou *et al.*, 2012).

Castellano *et al.* (2008) proposed the use of *Lactobacillus curvatus* as a protective culture in fresh beef since it is useful in inhibiting *Listeria innocua* and *B. thermosphacta* as well as the indigenous contaminant LAB. Its inhibitory effect is observed at low temperatures, and it has a negligible effect on meat pH. Nevertheless, Pereira *et al.* (2001) and Pachlová *et al.* (2018) reported the production of biogenic amines by *Lb. curvatus*.

Carnobacterium divergens, *C. maltaromaticum* and *Leuconostoc* spp. have been extensively studied as protective cultures capable of inhibiting the growth of *L. monocytogenes* in fish and meat products (Hastings *et al.* 1994; Leisner *et al.*, 2007; Koné *et al.*, 2018). Furthermore, the genus *Carnobacterium* predominates in vacuum-packaged beef primal with an extremely long shelf life (Imazaki *et al.*, 2011; Imazaki *et al.*, 2012; Kaur *et al.*, 2017).

The bacteriocins produced by LAB are a heterogeneous group of antibacterial peptides that vary in the spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties (Stiles and Hastings, 1991), and the use of bacteriocins themselves as additives is now being considered (Yost, 2014). Class I bacteriocins are small (< 5 kDa) membrane-active peptides called lantibiotics. Class II bacteriocins are small (< 10 kDa) heat-stable non-lantibiotics that can be grouped into three subclasses: subclass IIa comprises *Listeria*-active peptides that contain the N-terminal consensus sequence Tyr-Gly-Asn-Gly-Val-Xaa-Cys-, subclass IIb comprises poration complexes consisting of two peptides for activity and subclass IIc comprises thiol-activated peptides requiring reduced cysteine residues. Class III comprises large (> 30 kDa) heat-labile bacteriocins. Class IV comprises complex proteins composed of one or more chemical moieties (Klaenhammer, 1993). Nisin belongs to the class I lantibiotics and is the most widely exploited and applied bacteriocin (Zacharof and Lovitt, 2012). The nisin produced by *Lactococcus lactis* subsp. *lactis* is active against Gram-positive organisms, including bacterial spores, but is not active against Gram-negative bacteria, yeasts and fungi (Economou *et al.*, 2009). Nisin is virtually nontoxic to humans and is degraded without damage to the intestinal tract (Bernbom *et al.*, 2006; Joo *et al.*, 2012). The high specificity of class IIa bacteriocins against the psychrophilic food pathogen *L. monocytogenes* has brought this group to prominence in its potential use against this lethal pathogen. It should be considered as an essential advantage that class IIa bacteriocins do not undergo further post-translational modifications than the cleavage of a leader peptide from the precursor (Ríos Colombo *et al.*, 2018).

1.4.3.4 High hydrostatic pressure

Derived from material sciences, high-pressure technology (100–1000 MPa) is of increasing interest to biological and food systems (Cheftel and Culioli, 1997). The usefulness of high hydrostatic pressure as a food preservation technology is based on the destruction of foodborne pathogens, spoilage organisms, and inactivation of specific enzymes at low temperatures (Farkas and Hoover, 2000). Pressure processing is

usually carried out in a steel cylinder containing a liquid pressure-transmitting medium such as water, with the sample being protected from direct contact by using sealed flexible packaging. Maintaining the sample under pressure for an extended period does not require any additional energy apart from that required to maintain the chosen temperature (Cheftel and Culioli, 1997).

Several sites in the microbial cell can be damaged by high pressure, depending on the level of pressure applied. Protein synthesis is inhibited at 50 MPa, while partial denaturation of proteins occurs at 100 MPa. The increase in pressure to 200 MPa causes damage to the bacterial cell membrane, and a further increase in pressure to about 300 MPa causes irreparable damage to enzymes and proteins. Those cellular lesions result in disruption of the cytoplasmic membrane, leakage of cellular material, and subsequent death of the microbial cell (Abe, 2007). High hydrostatic pressure has been shown to result in changes in the mechanical properties leading to an improved tenderness of meat (Cheftel and Culioli, 1997; Ma and Ledward, 2004; Sikes *et al.*, 2010). However, high hydrostatic pressure, even at low temperatures, may have an undesirable effect on fresh meat colour (Carlez *et al.*, 1995; Jung *et al.*, 2003) as a result of denaturation of globin in Mb with haem displacement or release and ferrous oxidation (Mor-Mur and Yuste, 2003).

1.4.4 The maximum shelf life of chilled fresh beef

The extension of the shelf life of raw chilled meat must be based on both the retardation of the onset of bacterial spoilage and the delay of the deterioration of the appearance of the product. Bacterial spoilage is delayed by packaging under anaerobic conditions or aerobic atmospheres containing CO_2. The control of bacterial spoilage also requires that product temperature be maintained close to the optimum for chilled storage and attention to the hygienic condition of the product before it is packaged. Preserving the product appearance is mostly a matter of slowing or preventing the formation of brown MMb at muscle surfaces. Browning is slowed in atmospheres which are rich in O_2, and persistent browning is entirely prevented when meat is packaged under O_2-depleted atmospheres (Gill, 1996).

Considerable gains in shelf life can be made when chilling temperatures are close to the freezing point of meat (−2°C) (Jeremiah and Gibson, 2001; Small *et al.*, 2012). In a study by Jeremiah and Gibson (2001), storage life was more than doubled for retail-ready beef steaks by storage at a subzero temperature (−1.5°C) when compared

to storage at 2 and 5°C. Beef stored at −1.5°C presented a shelf life superior to 24 weeks. Moreover, samples stored at −1.5°C maintained a retail case-life of 30 h when stored for up to 17 weeks, while samples stored at 2 and 5°C kept a retail case-life of 30 h when stored for only eight and seven weeks, respectively. In a study by Small *et al.* (2012), vacuum-packaged strip loins and cube rolls stored at −0.5°C also had a shelf life superior to 26 weeks. However, the effect of repackaging in retail cases and display was not evaluated in the last study.

1.5. Evaluation of the shelf life of meat

In the EU, the responsibility for determining shelf life lies with the manufacturer or the packer. Thus, conducting storage trials of the product under defined storage conditions is crucial for determining its shelf life. Factors involved in the evaluation of shelf life of meat must consider microbiological safety, spoilage issues and sensory and physicochemical parameters (O'Sullivan, 2016). Microbial, physicochemical and sensory analysis used to evaluate the shelf life of meat will be discussed in **Subsections 1.5.1**, **1.5.2** and **1.5.3**, respectively.

1.5.1 Microbial analysis

A detailed description of the microbial factors limiting the shelf life of meat was given in **Subsection 1.3.1**. In this subsection, thresholds are proposed for each of these factors when evaluating the shelf life of fresh beef.

Many groups of organisms contain members that potentially contribute to meat spoilage under specific conditions, making the microbial ecology of spoiling raw meat very complex and the spoilage thus very difficult to prevent (Ercolini *et al.*, 2006). Meat spoilage is most frequently caused by the following groups of bacteria *Pseudomonas* spp., Enterobacteriaceae, *B. thermosphacta* and LAB (Pennacchia *et al.*, 2011). Under aerobic conditions, a few species of the genus *Pseudomonas* are recognised to dominate the meat system and to actively contribute to spoilage owing to their capability for glucose and amino acid degradation, even at refrigeration temperatures (Labadie, 1999; Ellis and Goodacre, 2001; Koutsoumanis *et al.*, 2006). It is believed that the deterioration process starts when *Pseudomonas* reach a

concentration of 5.0 $\log_{10}$ CFU/cm² (Dias dos Anjos Gonçalves *et al.*, 2017). *Brochothrix thermosphacta* is a microorganism for which meat is considered an ecological niche, even though it can also occur in spoiled fish. The capability of *B. thermosphacta* to grow on meat during both aerobiosis and anaerobiosis makes it a significant meat coloniser and a critical member of the spoilage-related flora due to off-odour production (Dainty and Mackey, 1992; Pin *et al.*, 2002) at 6.0 $\log_{10}$ CFU/cm² (Mills *et al.*, 2014). Many members of the Enterobacteriaceae, belonging to the genera *Serratia*, *Enterobacter*, *Pantoea*, *Proteus* and *Hafnia*, often contribute to meat spoilage (Borch *et al.*, 1996; Labadie, 1999; Gram *et al.*, 2002; Jay *et al.*, 2003) at concentrations higher than 5.0 $\log_{10}$ CFU/cm² (Ercolini *et al.*, 2009). Finally, LAB can play an essential role in the spoilage of refrigerated raw meat when they reach a concentration of 6.7 $\log_{10}$ CFU/cm² (Picgirard, 2009).

1.5.2 Physicochemical analysis

Consumers routinely use product colour and appearance to select or reject products, and suppliers of muscle food products must also create and maintain the desired colour attributes (AMSA, 2012). One of the most common methods for instrumental colour evaluation is the measurement of tristimulus values (CIE $L^*a^*b^*$[15]) with either a colourimeter or a spectrophotometer (Tapp *et al.*, 2011). The lack of standardisation of this method (Brewer *et al.*, 2001) makes the comparison of results obtained by different laboratories and instruments demanding.

Since the colour of muscle foods revolves around Mb redox forms (AMSA, 2012), quantifying the redox forms of Mb can give a good indication of meat colour. The method of Tang *et al.* (2004) to quantify Mb redox forms is based on the absorbance measurement of an aqueous meat extract at selected wavelengths with a spectrophotometer. There are also two reflectance methodologies for quantifying Mb redox forms, which have the advantage of being non-destructive and evaluating the aspect of the surface of the meat only. One involves using surface reflectance to

[15] The CIE $L^*a^*b^*$ colour space allows expressing colour in a three-dimensional space. In the centre of the colour space is neutral grey. Along the X axis, a positive a^* represents red, and a negative a^* represents green (scale from +60 for red to −60 for green). Along the Y axis, a positive b^* represents yellow, and a negative b^* represents blue (scale from +60 for yellow to −60 for blue). The third dimension L^* is represented numerically where 100 is white, and 0 is black.

calculate K/S[16] ratios at isosbestic wavelengths for each Mb redox form (Francis and Clydesdale, 1975). The other method uses selected wavelengths with a correction factor (Krzywicki, 1979) to calculate percentages of DMb and MMb, and determines OMb by difference from 100%. The value of 40% MMb was reported by Greene *et al.* (1971) as the threshold for consumers to reject meat.

Lipid oxidation in meat and meat products can lead to quality deterioration which has the potential of limiting shelf life from a flavour perspective (O'Sullivan, 2016). Malondialdehyde (MDA) is a significant carbonyl decomposition product of oxidised polyunsaturated lipids (Crawford *et al.*, 1966). The tissue distillation-thiobarbituric acid (TBA) assay method of Tarladgis *et al.* (1960) is regarded as the standard method for MDA analysis. More recently, Raharjo *et al.* (1992) proposed a faster method for the determination of MDA replacing the distillation by an acid extraction. Both methods are based on the reaction of one molecule of MDA with two molecules of TBA to form a pink complex which can be quantified spectrophotometrically. Thiobarbituric acid (TBA)-reactive substances have been correlated to sensory-determined rancidity. Greene and Cumuze (1982) and Campo *et al.* (2006) stated that the general population of meat consumers would not detect oxidation flavours until oxidation products reached levels of at least 2.0 mg/kg tissue. This value can be considered as the limiting threshold for acceptability of oxidation in beef.

1.5.3 Sensory analysis

Sensory evaluation has been used extensively over the past decades to assess the acceptability and quality of food products including meat (Torrico *et al.*, 2018). Changes in sensory properties during storage will affect the appeal of the product to the consumer in the case of colour and repeat purchase in the case of flavour and texture (O'Sullivan, 2016). Thus, it is crucial to validate the length of time that a product will remain the same acceptable quality level or have no change in desired sensory characteristics.

There are two types of methods that can be employed in the sensory evaluation of the shelf life meat: difference testing and descriptive attribute testing. Difference testing can be categorised into (1) overall difference tests, to determine whether a

[16] Absorption coefficient (K)/scattering coefficient (S).

sensory difference is detectable between samples and (2) attribute-specific directional difference tests, which ask whether a specified attribute is perceived as different between samples. Differently, descriptive analyses are methods in which panellists quantify specific sensory attributes (e.g., appearance, flavour, texture, and aftertaste). In these cases, the final sensory profile can display quantifiable sensory changes in meat over the course of preservation, which is an essential tool in defining sensory shelf life changes over time, but does not reflect consumer sentiment or acceptance of the product (O'Sullivan, 2016).

Chapter 2
Objectives

2.1 General objective

The primary objective of this work was to study how microbial and physicochemical factors, namely spoilage bacteria growth and Mb and lipid oxidation, determine the long-term preservation of chilled fresh beef at temperatures above the freezing point of meat (−2°C) but below 0°C.

2.2 Specific objectives

The three principal topics of the present research are presented below with their specifics objectives:

a) Study of the preservation of chilled fresh meat with extremely long shelf life. This topic aimed to:

- evaluate the shelf life of vacuum-packaged fresh beef with extremely long shelf life at a subzero temperature (−1°C), and to which extent storage at 4°C would influence the shelf life;

- evaluate the shelf life of these meats during simulated retail display under a high-O_2 atmosphere;
- investigate the ecosystems associated with meats with an extremely long shelf life;
- identify and isolate LAB with potential for biopreservation.

b) Study of the preservation of chilled fresh meat from the Belgian Blue breed. This topic aimed to understand the influence of the following factors on the shelf life of meat:

- ageing time – up to 80 days;
- ageing temperature – −1 and 4°C;
- ageing technique – carcase ageing and vacuum ageing;
- animal category – young bull and cull cow;
- muscle – *longissimus thoracis et lumborum* and *rectus femoris*;
- packaging – vacuum packaging and MAP.

c) Study the potential of *C. maltaromaticum* as protective culture for meat. The objectives of this topic were to:

- characterise *C. maltaromaticum* isolated from meat with extremely long shelf life;
- evaluate *in situ* the effect of *C. maltaromaticum* on spoilage bacteria of meat;
- estimate the impact of *C. maltaromaticum* on the sensory quality of a meat product;
- evaluate *in vitro* the effect of *C. maltaromaticum* on pathogen bacteria of meat.

Section II
Experimental studies

Section II presents the experimental studies of this research, and is divided into three chapters. **Chapter 3** is dedicated to the preservation of fresh meat with extremely long shelf life. Then, the preservation of fresh meat from the Belgian Blue breed will be discussed in **Chapter 4**. Finally, **Chapter 5** is about biopreservation in fresh meat.

Chapter 3

Preservation of fresh meat with extremely long shelf life

Over the past decades, Australia, Brazil, Argentina, US and other beef producing countries have consolidated an export market for chilled, vacuum-packaged beef primals to a large number of countries. For many years, shipping and cold storage at −1°C permitted to achieve a shelf life superior to 100 days for chilled fresh beef. In a global market, the shelf life of fresh meat can be the decisive factor that determines the trade of this commodity. Therefore, long shelf life is of high value.

The evaluation of the pigment and lipid stability, as well as the investigation of the microbial ecology in beef with extremely long shelf life, was the subject of the study presented in this chapter and entitled “Oxidative stability and microbial ecology of beef with extremely long shelf life”. The manuscript of this study is currently under review by *Meat Science*.

OXIDATIVE STABILITY AND MICROBIAL ECOLOGY OF BEEF WITH EXTREMELY LONG SHELF-LIFE

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Abstract: **This study aimed to evaluate the pigment and lipid stability, and the microbial ecology, by classical methods and metagenetics, in beef with an extremely long shelf-life. The *longissimus thoracis et lumborum* from beef of different origins (Australia, Brazil, Ireland, United Kingdom) were aged at −1 or −1/+4 °C two-level stepwise scheme (simulated sub-zero and conventional storage, respectively) until the end of their shelf-life. At different times, samples were repackaged under a high-oxygen atmosphere (70/30% O_2/CO_2) and stored at 4 °C for 2 days, then 8 °C for 5 days (simulated retail display). Sub-zero ageing had a protective effect against the myoglobin oxidation and also the growth of total aerobic mesophilic flora and Enterobacteriaceae. Metmyoglobin formation and lactic acid bacteria growth limited the shelf-life of beef during simulated retail display. Lactic acid bacteria were underestimated by classical microbiological methods. Metagenetics permitted to evidence high proportions of *Carnobacterium maltaromaticum* in Australian beef, which could have contributed to its extremely long shelf-life.**

Keywords: **Bioprotection, *Carnobacterium maltaromaticum*, Metagenetic analyses, Oxidation, Sub-zero chilling**

Highlights:

- **Culture-dependent methods underestimate psychrotrophic lactic acid bacteria**
- **Sub-zero chilling ensures the optimal shelf-life of vacuum-packaged beef**
- **Ageing meat for extended periods is not recommended for retail display**
- **Metagenetics is a powerful tool to evaluate the microbial ecosystem of meat**
- ***Carnobacterium maltaromaticum* is associated with meat with extremely long shelf-life**

1 Introduction

The production of living animals is increasingly driven by a shift in diet and food consumption patterns towards livestock products (Food and Agricultural Organisation of the United Nations [FAO], 2013). Growth in the demand for meat will stem mostly from an increase in population, income and urbanisation, especially in regions with large middle classes, such as Asia, Latin America and the Middle East (the Organisation for Economic Co-operation and Development [OECD], 2016). However, beef production continues to be concentrated in a few countries, including Australia, Brazil, China and the United States; in 2016, Brazil and the European Union led the expansion in world meat exports (OECD, 2017). One of the critical challenges for today's meat export industries is to deliver a fresh product of superior quality to distant markets (Mills, Donnison, & Brightwell, 2014), considering several weeks of chilled transportation must be anticipated to service these markets by sea (Bell, Penney, Gilbert, Moorhead, & Scott, 1996). Vacuum packaging (Gill, 1996) and storage at sub-zero temperatures (Bell et al., 1996; Jeremiah & Gibson, 2001; Tewari, Jayas, & Holley, 1999) prevent the growth of oxygen-requiring spoilage bacteria and provide oxidative stability and the necessary product-life without recourse to freezing. In the past years, the arrival of chilled beef with extremely long shelf-life in the European market has triggered intense reactions by the meat sector, who mistakenly suspected the use of unauthorised technologies in the European Union to maintain the stability of these meats.

The shelf-life of fresh meat is mainly limited by microbial growth and oxidation phenomena. As reviewed by Coombs, Holman, Friend, and Hopkins (2017), an increase in the spoilage bacteria population of meat, including lactic acid bacteria (LAB), Enterobacteriaceae (EB) and *Brochothrix thermosphacta* (BT), is associated with the development of undesirable flavours, discolouration and possibly reduced product safety. Contrariwise, oxidative processes are the primary non-microbiological factor implicated in quality deterioration of meat during chilled storage. Lipid oxidation results in the formation of several products leading to the development of off-flavours and off-odours (Shahidi, 2016). Moreover, the oxidation of myoglobin to metmyoglobin (MMb) induces a brown appearance to the meat. Hence, the control of microbial, pigment and lipid stability by the meat industry is essential to maintaining the safety and fresh appearance of the product.

Several studies investigated the stability of beef with long shelf-life (Bell et al., 1996; Jeremiah & Gibson, 2001; Rodas-González et al., 2011) and the microbiota associated with these meats, either by classical microbiological methods (Small, Jenson, Kiermeier, & Sumner, 2012; Youssef, Gill, Tran, & Yang, 2014) or by 16S rRNA gene sequencing from isolated colonies (Youssef, Gill, & Yang, 2014). Also, culture-independent high-throughput (CIHT) sequencing methods have been used to characterise

the microbial populations of several types of meat and meat products (Cauchie et al., 2017; Delhalle et al., 2016; Kergourlay, Taminiau, Daube, & Champomier-Vergès, 2015; Korsak et al., 2017). Nevertheless, the scientific literature lacks studies combining the assessment of the stability and microbial communities by CIHT sequencing methods in beef with extended shelf-life. In this context, this study aimed to evaluate the oxidative and microbial stability, as well as the microbial diversity by metagenetics, of beef with extended shelf-life at two ageing temperatures (−1 °C and two-level stepwise −1/+4 °C) and in two kinds of packaging (under vacuum and in high-oxygen atmosphere).

2 Material and methods

2.1 Samples

Eighteen vacuum-packaged *longissimus thoracis et lumborum* (LTL) from four different origins (six from Australia, three from Brazil, three from Ireland and six from the United Kingdom [UK]) with large differences in shelf-life were supplied by a Belgian food wholesaler. Three samples of the same origin were transported to our laboratory each time, over a period of one year, as soon as a load with imported meat was delivered to the food wholesaler. The shelf-life was labelled 140 days at <2 °C for Australian, 120 days at 0 °C for Brazilian, 35 days at 0–3 °C for Irish and 45 days for British meat. There was no storage temperature set by the British meat cutting plant. Our Belgian supplier reported storage at maximum 0 °C throughout distribution for all different meats. Samples arrived at our laboratory at between ⅓ and ⅔ of their shelf-life and were stored at −1 °C until ⅔ of their shelf-life ($SL_{⅔}$) when they were cut into six 2–3 cm-thick steaks.

Three steaks were used to evaluate meat packaged in vacuum (wholesale market simulation), and were handled as follows. One steak was analysed immediately after LTL were cut into steaks ($SL_{⅔}$). Two steaks were vacuum packaged and stored at –1 or 4 °C until the end of the shelf-life ($SL_{-1°C}$ and $SL_{-1/+4°C}$, respectively) to simulate sub-zero (–1 °C) and conventional storage (4 °C). Vacuum bags (Cryovac) were 60 µm thick, and the oxygen permeability was 13 cm^3/m^2 24 h bar at 23 °C and 0% relative humidity (RH).

The three remaining steaks from each LTL were used to evaluate meat packaged in a modified atmosphere (retail display simulation), and were, as a first step, vacuum packaged as described above. Then, at $SL_{⅔}$, $SL_{-1°C}$ and $SL_{-1/+4°C}$, they were repackaged in PP/EVOH/PP trays (ES-Plastic, dimensions: 187 × 137 × 50 mm, oxygen permeability: 4 cm^3/m^2 at 24 h bar, 23 °C and 0% RH) containing a modified atmosphere of 70/30% O_2/CO_2 and sealed with a PET/PP film (Wipak, oxygen permeability: 8.4 cm^3/m^2 at 24 h bar, 23 °C and 0% RH) with a gas headspace-to-meat ratio of approximately 4:1. Modified atmosphere packaged (MAP) samples were stored for seven days ($SL_{⅔/MAP}$,

$SL_{-1°C/MAP}$ and $SL_{-1/+4°C/MAP}$). A temperature of 4 °C was used during the first two days and 8 °C was applied during the five last days of storage. The latter temperature was chosen to simulate a break in the cold chain during distribution, according to the *Association Française de Normalisation* (AFNOR) NF V01-003 standard (AFNOR, 2004). Three climatic chambers (Model MIR 254, Sanyo) were set at −1, 4 and 8 °C, and samples were transferred from on climatic chamber to another each time a temperature change had to be applied.

In total, six treatments were applied to steaks of each origin (**Figure 1**), and each steak was assigned randomly to a different treatment. Analyses were performed on samples at $SL_{⅔}$, $SL_{⅔/MAP}$, $SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$ and $SL_{-1/+4°C/MAP}$, except for α-tocopherol content (only at $SL_{⅔}$) and metagenetics (only at $SL_{⅔}$ and $SL_{-1/+4°C}$). The corresponding time in days for the six treatments applied to samples is shown in **Table 1**.

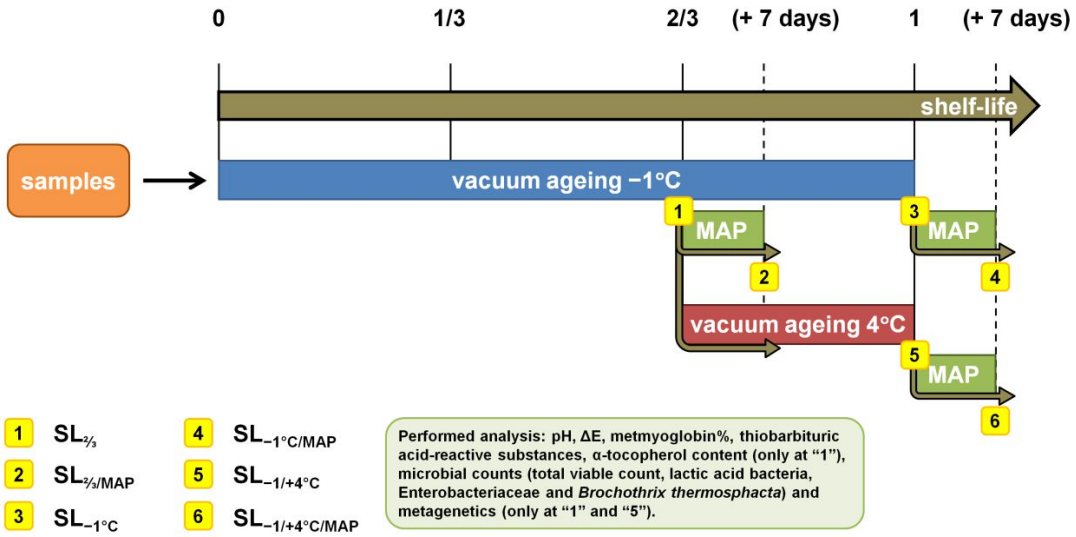

Figure 1 Experimental scheme. The yellow rounded squares show the six different treatments applied to samples. MAP = modified atmosphere packaging (70/30% O_2/CO_2) for 2 days at 4 °C + 5 days at 8 °C.

Table 1 Corresponding time in days for the six treatments applied to samples of different origins.

Treatment	Origin			
	Australia	Brazil	Ireland	United Kingdom
$SL_{⅔}$	93	80	23	30
$SL_{⅔/MAP}$	93 + 7	80 + 7	23 + 7	30 + 7
$SL_{-1°C}$	140	120	35	45
$SL_{-1°C/MAP}$	140 + 7	120 + 7	35 + 7	45 + 7
$SL_{-1/+4°C}$	140	120	35	45
$SL_{-1/+4°C/MAP}$	140 + 7	120 + 7	35 + 7	45 + 7

2.2 pH measurement

pH was measured using a Knick 765 pH meter equipped with a pH electrode (Model 104063123, Ingold), according to the International Organisation for Standardisation (ISO) 2917 procedure (ISO, 1999). Measurements were performed in five different zones of each sample, and the values were averaged.

2.3 Colour measurement and MMb%

Instrumental colour was evaluated at 1.5 h after removal from vacuum-packed bags and exposure to atmospheric air (bloom time) at 4 °C, using a Labscan II spectrophotometer (HunterLab). Measurement conditions were 25 mm diameter aperture, D65 illuminant and 10° observation angle. Values for CIE L^*, a^* and b^* were measured in five different zones of each sample and averaged. The colour difference between samples before and after MAP display (ΔE) was calculated using the equation $\sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$. Higher ΔE values mean greater total colour changes over time.

The MMb% was determined as described elsewhere (Tang, Faustman, & Hoagland, 2004), by the spectrophotometric absorbance measurement of an aqueous meat extract at 503, 525, 557, 565 and 582 nm (Spectronic Genesys 2PC UV-Vis, Thermo Fisher Scientific).

2.4 Lipid oxidation measurement

To assess lipid oxidation, an aqueous acid extraction method was undertaken before spectrophotometric measurement of the thiobarbituric acid-reactive substances (TBARS) at 530 nm (Raharjo, Sofos, & Schmidt, 1992). Five grams of sample were used for the extraction. 1,1,3,3-Tetraethoxypropane was used to prepare the standard curve and determine the TBARS recovery. The results were expressed as mg malondialdehyde (MDA) equivalents per kg of meat.

2.5 α-Tocopherol content

A protocol adapted from Liu, Scheller, and Schaefer (1996) was implemented to extract and quantify the α-tocopherol content in meat samples. The method consists of a saponification step, followed by a double isooctane extraction of the saponified samples. α-Tocopherol in the extracts was separated by normal phase chromatography and quantified by fluorescence detection. High-pressure liquid chromatography (HPLC) analysis was performed using a Model 600 E solvent delivery system equipped with a

Model 717 automatic injector, a Mistral oven, a 2475 fluorescence detector (excitation at 296 nm wavelength and emission at 325 nm wavelength) and a Resolve spherical silica column (3.9 × 150 mm, 5 μm) set at 15 °C (all from Waters). The mobile phase was isooctane/tetrahydrofuran (96:4 v/v) at 1.0 mL/min flow rate, and the injection volume was 30 μL. Quantification was by an external standard method, based on peak area.

2.6 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 (primary suspension). Serial decimal dilutions were prepared using the same dilutant. Total viable count (TVC) and LAB and EB counts were performed using the TEMPO® system (bioMérieux) 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 EB. *Brochothrix thermosphacta* (BT) was enumerated by plating on streptomycin–sulphate, thallous–acetate and actidione (STAA) agar with STAA-selective supplement and incubating at 22 °C for 48 h, as per ISO 13722 (ISO, 1996). Colonies were confirmed by oxidase tests. The acceptability thresholds were set at LAB = 6.7 $\log_{10}$ CFU/cm^2 (Picgirard, 2009), EB = 5.0 $\log_{10}$ CFU/cm^2 (Féderation du Commerce et de la Distribution [FCD], 2016) and BT = 6.0 $\log_{10}$ CFU/cm^2 (Mills et al., 2014). Furthermore, the primary suspensions prepared in this step were inoculated on plate count agar, and the obtained colonies were stored for DNA extraction and 16S rRNA genes sequencing (section 2.8).

2.7 Metagenetics

Total DNA was directly extracted from each primary suspension obtained in section 2.6, using a DNeasy Blood and Tissue kit for DNA extraction (Qiagen) by following the manufacturer's recommendations. Equal amounts of the extracted DNA were mixed into pools containing the DNA of three samples of each origin (Australia, Brazil, Ireland, UK) within the same treatment ($SL_{⅔}$, $SL_{-1/+4°C}$). Thus, 12 pools were obtained: termed $AU1_{⅔}$, $AU1_{-1/+4°C}$, $AU2_{⅔}$ and $AU2_{-1/+4°C}$, (Australian); $BR_{⅔}$ and $BR_{-1/+4°C}$ (Brazilian); $IE_{⅔}$ and $IE_{-1/+4°C}$ (Irish), and $GB1_{⅔}$, $GB1_{-1/+4°C}$, $GB2_{⅔}$ and $GB2_{-1/+4°C}$ (British). 16S rRNA PCR libraries targeting the V5–V6 hypervariable region were generated. Primers E783–797 and E1063–1081 (Brosius, Dull, Sleeter, & Noller, 1981), specific for bacteria, were selected for their ability to generate the lowest amplification bias among the various bacterial phyla. The oligonucleotide design included titanium A or B adapters (454 Life Sciences) and multiplex identifiers fused to the 5′ end of each primer. The amplification mix contained 5 U of FastStart high fidelity polymerase, 1 × enzyme reaction buffer (Eurogentec), 200 μM deoxynucleotide triphosphates (dNTPs; Eurogentec), 0.2 μM of each primer and 100 ng of genomic DNA, in a final volume of 100 μL. Thermocycling

conditions consisted of a denaturation step at 94 °C for 15 min, followed by 30 cycles of 40 s at 94 °C, 40 s at 56 °C, and 1 min at 72 °C, with a final elongation step at 72 °C for 7 min. These amplifications were performed on an EP Mastercycler Gradient System (Eppendorf). DNA fragments were purified using an SV PCR purification kit (Wizard). Pyrosequencing was performed by Quality Partner (Herstal, Belgium) with a FLX Genome Sequencer (Roche). The 16S rDNA sequence reads were filtered as described by Huse, Huber, Morrison, Sogin, and Welch (2007), processed using the Mothur program (Schloss et al., 2009) and compared to a reference dataset of aligned sequences of the corresponding region derived from the Silva database of full-length rDNA sequences (Pruesse et al., 2007). The final reads were clustered into operational taxonomic units (OTU) using the nearest neighbour algorithm and a Mothur with a 0.01 distance-unit cut-off. A taxonomic identity was attributed to each OTU by comparison to the Silva database using a 60% homogeneity cut-off.

2.8 16S rRNA genes sequencing

The genomic DNA of colonies obtained in section 2.6 was extracted, as described in section 2.7 and was used to amplify the entire 16S rRNA genes with the universal primers 16S1500F (5′-GAGTTTGATCMTGGCTCAG-3′) and 16S1500R (5′-TACGGTTACCTTGATTACGAC-3′) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C, 1 min at 72 °C and a final extension step at 72 °C for 5 min. All PCR reactions contained 1 U Diamond Taq polymerase buffer (Eurogentec), 1 U Diamond Taq polymerase (Eurogentec), 2 mM $MgCl_2$, 0.2 mM dNTPs (Eurogentec), 0.4 µM of each primer and 10–100 ng DNA, for a total volume of 20 µL. PCR products were purified with a Wizard SV PCR purification kit (Promega). The sequencing of the 16S fragments was performed by GIGA (Liège, Belgium), using an ABI3730 sequencing machine (Applied Biosystem). The resulting sequences were assembled and the identification made by BLAST comparison (Altschul, Gish, Miller, Myers, & Lipman, 1990).

2.9 Statistical analysis

Results for physicochemical parameters (pH, ΔE, MMb%, TBARS) and microbial counts (TVC, LAB, EB, BT) measured at ⅔ of the shelf-life ($SL_{⅔}$ and $SL_{⅔/MAP}$) are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to calculate the effect of origin (Australia, Brazil, Ireland, UK) within each display time (0 and 7 days) and the effect of display time within each origin. When necessary, Tukey's test was used to assess differences between test groups.

Data relating to physicochemical parameters (pH, ΔE, MMb%, TBARS) and microbial counts (TVC, LAB, EB, BT) measured at the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, $SL_{-1/+4°C/MAP}$) was arranged in a split-plot design in which origin (Australia, Brazil, Ireland, UK) was allocated in the whole plot, while the sub-plots were ageing temperature (−1 and stepwise −1/+4 °C) and display time (0 and 7 days). Considering ΔE combines *L**, *a** and *b** values before and after MAP display, display time was not included in the model for ΔE. When a post hoc test was suitable, Tukey's test was performed. All statistical analysis was performed using the R computing environment (R Core Team, 2016).

A summary of the tested effects for each group of treatments is shown in **Table 2**.

3 Results and discussion

3.1 Physicochemical parameters and oxidative stability

The pH of samples varied between 5.50 and 5.55 at $SL_{⅔}$ and between 5.45 and 5.50 at $SL_{⅔/MAP}$, respectively. No effect of origin or display time was observed at ⅔ of the shelf-life (**Table 3**). At the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, $SL_{-1/+4°C/MAP}$), the effect of origin (Australia, Brazil, Ireland, UK), ageing temperature (−1 and stepwise −1/+4 °C), display time (0 and 7 days) and all their interactions was evaluated for pH (**Table 4**). At this stage, pH decreased during the display time (P<0.05). However, the difference in pH between samples before and after MAP display was inferior to 0.05 (data not shown in graphical form). These minimal pH variations observed may not have influenced the other physicochemical and microbial parameters in our experiment.

Initial colour attributes (CIE *L*a*b**) of beef from different origins at $SL_{⅔}$ and $SL_{⅔/MAP}$ are shown in **Table 3**. Irish meat presented the lowest *a** at $SL_{⅔}$, while Brazilian

Table 2 Summary of the tested effects for each group of treatments.

Treatment	Effect		
	Origin*	Ageing temperature**	Display time***
$SL_{⅔}$ and $SL_{⅔/MAP}$	X		X****
$SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$ and $SL_{-1/+4°C/MAP}$	X	X	X

* Australia, Brazil, Ireland and the United Kingdom
** −1 °C and stepwise −1/+4 °C
*** 0 and 7 days
**** Except for α-tocopherol content

Table 3 Physicochemical parameters and microbial counts (mean ± standard deviation) in the *longissimus thoracis et lumborum* from different origins at ⅔ of the shelf-life, before and after a 7-day modified atmosphere packaging display. MMb% = metmyoglobin%, TBARS = thiobarbituric acid-reactive substances, TVC = total viable count, LAB = lactic acid bacteria, EB = Enterobacteriaceae, BT = *Brochothrix thermosphacta*.

Parameter	Display time (days)	Origin			
		Australia	Brazil	Ireland	United Kingdom
pH	0	5.50 ± 0.08	5.50 ± 0.02	5.55 ± 0.08	5.50 ± 0.03
	7	5.50 ± 0.07	5.45 ± 0.02	5.50 ± 0.02	5.50 ± 0.04
*L**	0	38.2 ± 1.7	41.6 ± 0.8	38.2 ± 5.2	36.7 ± 2.6
	7	39.0 ± 2.8^{AB}	41.7 ± 0.9^{B}	36.4 ± 0.5^{A}	37.4 ± 1.9^{A}
*a**	0	24.2 ± 1.6^{A}	24.3 ± 1.4^{aA}	19.6 ± 3.1^{B}	24.8 ± 0.7^{A}
	7	22.2 ± 2.2	21.3 ± 0.6^{b}	20.2 ± 2.1	22.9 ± 2.3
*b**	0	18.9 ± 2.1	19.6 ± 0.6^{a}	15.8 ± 2.5	18.1 ± 0.7
	7	17.5 ± 1.6	17.7 ± 0.5^{b}	15.8 ± 1.3	17.1 ± 1.2
ΔE	n/a	3.7 ± 1.0	4.0 ± 1.9	5.9 ± 2.3	3.0 ± 2.1
MMb %	0	11.3 ± 9.5^{a}	2.9 ± 1.1^{a}	5.1 ± 5.3	5.0 ± 3.8^{a}
	7	37.0 ± 10.7^{b}	21.5 ± 11.0^{b}	16.5 ± 12.4	34.8 ± 12.5^{b}
TBARS*	0	0.3 ± 0.2^{a}	< LOQ**	< LOQ**	0.3 ± 0.2^{a}
	7	1.5 ± 0.4^{b}	< LOQ**	1.1 ± 0.9	1.1 ± 0.7^{b}
TVC***	0	5.3 ± 0.9^{aB}	5.8 ± 0.6^{aB}	3.1 ± 0.5^{aA}	5.1 ± 0.5^{aB}
	7	6.0 ± 1.1^{bA}	8.3 ± 0.5^{bB}	7.4 ± 0.4^{bAB}	7.2 ± 0.4^{bAB}
LAB***	0	< 2.0	< 2.0	< 2.0	4.0 ± 1.7
	7	< 2.0	5.4 ± 0.3	6.4 ± 0.1	5.8 ± 0.8
EB***	0	< 1.0	< 1.0	< 1.0	2.6 ± 1.5
	7	< 1.0	< 1.0	2.5 ± 1.4	2.5 ± 1.4
BT***	0	< 2.0	< 2.0	< 2.0	3.2 ± 1.2^{a}
	7	< 2.0	< 2.0	6.5 ± 0.2	5.2 ± 1.1^{b}

* Expressed as mg malondialdehyde equivalents/kg
** Limit of quantification (LOQ) = 0.2 mg malondialdehyde equivalent/kg
*** Expressed as $\log_{10}$ CFU/cm^2
^{ab}Different lowercase letters in a column (display time effect) within each variable indicate statistical difference (P<0.05). No lowercase letter indicate no statistical difference.
^{AB}Different uppercase letters in a row (origin effect) indicate statistical difference (P<0.05). No lowercase letter indicate no statistical difference.

presented the highest L^* at $SL_{⅔/MAP}$ (P<0.05). A decrease of a^* and b^* was observed for Brazilian meat during simulated MAP display (P<0.05). No effect of origin was observed for ΔE at ⅔ of the shelf-life (**Table 3**). At the end of the shelf-life, the effect of origin (Australia, Brazil, Ireland, UK), ageing temperature (−1 and stepwise −1/+4 °C) and all

Table 4 Analysis of variance (F-values) on the effect of origin (Australia, Brazil, Ireland and the United Kingdom), ageing temperature (−1 and stepwise −1/+4 °C) and display time (0 and 7 days) on the pH, discoloration during modified atmosphere packaging display (ΔE), metmyoglobin% (MMb%), lipid oxidation (thiobarbituric acid-reactive substances [TBARS]), total viable count (TVC), lactic acid bacteria (LAB), Enterobacteriaceae (EB) and *Brochothrix thermosphacta* (BT) in beef at the end of the shelf-life.

Effects	pH	ΔE	MMb%	TBARS	TVC	LAB	EB	BT
Origin (O)	0.68	0.09	**16.01*****	**7.07*****	**13.02*****	**5.91****	**6.72****	0.98
Ageing temperature (T)	0.32	0.02	**8.94****	1.74	**18.86*****	3.16	**16.60*****	0.75
Display time (D)	**4.26***	n/a	**98.27*****	**28.41*****	**13.28*****	**20.66*****	3.40	0.38
O×T	1.76	1.22	**3.55***	0.79	0.93	0.31	2.41	0.86
O×D	1.38	n/a	1.19	**3.09***	0.14	0.12	1.02	0.04
T×D	1.50	n/a	0.16	0.22	**7.19****	0.25	2.44	0.19
O×T×D	0.77	n/a	1.64	0.07	0.77	**4.04***	1.34	0.55

Significant probabilities (P<0.05) are in bold.
*Significant at P<0.05.
**Significant at P<0.01.
***Significant at P<0.001.
n/a = not applicable.

their interactions was evaluated for ΔE (**Table 4**). ΔE values varied from 2.0 to 7.1 and from 2.0 to 10.7 between origins for the ageing temperatures of −1 and stepwise −1/+4 °C, respectively. Numerically, British meat presented the lowest and Brazilian meat the highest ΔE (data not shown in graphical form). Nevertheless, no statistically significant difference was observed between samples.

At ⅔ of the shelf-life ($SL_{⅔}$ and $SL_{⅔/MAP}$), an effect of display time (0 and 7 days) was observed for MMb% in Australian, Brazilian and British samples (P<0.05) (**Table 3**), whereas, at the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, $SL_{-1/+4°C/MAP}$), the effects of display time and the interaction origin (Australia, Brazil, Ireland, UK) × ageing temperature (−1 and stepwise −1/+4 °C) were significant for MMb% (P<0.05) (**Table 4**). In the present study, a 7-day simulated MAP display favoured pigment oxidation (P<0.05) (**Table 3** and **Figure 2.a**). Previous authors have also reported that pigment oxidation increases as display time increases (Mancini & Ramanathan, 2014; McKenna et al., 2005). According to Garner, Unruh, Hunt, Boyle, and Houser (2014), the metmyoglobin reducing activity (MRA), which represents properties that help minimise MMb, decreases over display time, contributing to an increase in MMb%. In regards to the interaction origin × display temperature, an ageing temperature of 4 °C during the last ⅓ of the shelf-life did not have an effect on Australian, Brazilian and Irish samples; however, pigment oxidation was higher at −1 than at 4 °C in British samples (**Figure 2.b**). As reviewed by Bekhit and Faustman (2005), MMb reduction by the MRA may be accelerated with

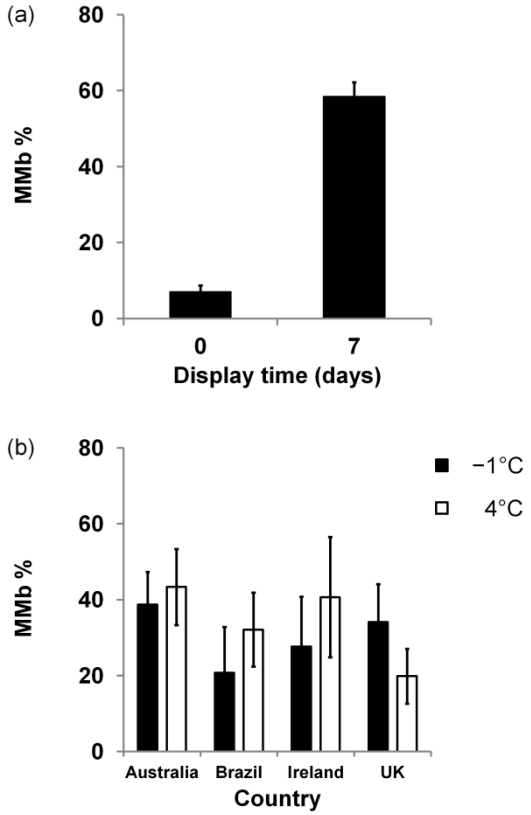

Figure 2 Effect of display and interaction origin × temperature for metmyoglobin (MMb, %) in the *longissimus thoracis et lumborum* from different origins at the end of the shelf-life. Bars represent standard error.

increased temperature, which could explain the decreased pigment oxidation in British meat at 4 °C, though it does not justify the higher pigment stability of meat from other origins at −1 °C. The value of 40% MMb, reported by Greene Hsin, and Zipser (1971) as the threshold for consumers to reject meat, was not exceeded by any sample at $SL_{⅔}$, $SL_{⅔/MAP}$, $SL_{-1°C}$ and $SL_{-1/4°C}$. Conversely, the limit was exceeded by all samples at $SL_{-1°C/MAP}$ and $SL_{-1/4°C/MAP}$, except for British meat previously aged at 4 °C, during the last ⅓ of the shelf-life (data not shown in graphical form).

At $SL_{⅔}$, the TBARS of samples did not exceed 0.3 mg MDA equivalent/kg, and, after a 7-day simulated MAP display ($SL_{⅔/MAP}$), there was an increase in the TBARS in samples of all origins (P<0.05), except for Brazilian (**Table 3**). At the end of the shelf-life, the interaction of origin (Australia, Brazil, Ireland, UK) × display time (0 and 7 days) was significant for lipid oxidation (P<0.05) (**Table 4**). At the end of the shelf-life before simulated MAP display, British meat presented the highest lipid oxidation (P<0.05), while Brazilian and Irish meat showed TBARS values inferior to the limit of quantification (LOQ; 0.2 mg MDA equivalent/kg). After 7 days of display, the lipid oxidation in Brazilian meat remained inferior to the LOQ, while Australian, Irish and British meat

presented equivalent TBARS values (**Figure 3**). As TBARS values correlate well with sensory testing (Fernández, Pérez-Álvarez, & Fernández-López, 1997), they can be a good indicator of rancidity in meat (Irwin & Hedges, 2004). Campo et al. (2006) proposed that the TBARS value of 2.0 mg MDA equivalent/kg in raw beef could be considered the threshold at which rancid flavour overpowers beef flavour when it is cooked, and consequentially, is the maximum level for a positive sensory perception of beef. This threshold was not exceeded in any sample for any treatment (data not shown in tabular form). High-oxygen MAP is typically used for beef cuts to promote pigment oxygenation, which prolongs the period before MMb is visible on the muscle surface (Mancini & Hunt, 2005). According to Jayasingh, Cornforth, Brennand, Carpenter, and Whittier (2002), although high-oxygen atmospheres maintain redness during storage, rancidity often develops while colour is still desirable. Nonetheless, in the present study, pigment oxidation reached undesirable levels before lipid oxidation. In fact, there is some evidence that myoglobin oxidation initiates prior to lipid oxidation. A possible mechanism was reviewed by Baron & Andersen (2002) and Richards (2013): the reaction of MMb with endogenous hydrogen peroxide results in the formation of ferrylmyoglobin, a strong pro-oxidant which is able to abstract a hydrogen atom from fatty acids.

Table 5 shows the α-tocopherol content in samples at $SL_{⅔}$. Brazilian meat α-tocopherol content was 3.2 µg/g, which was the lowest among the tested samples. Australian, Irish and British meat presented α-tocopherol contents equivalent to each other, ranging from 5.0 to 6.1 µg/g. Liu et al. (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 MetMb formation in fresh beef. Only Brazilian samples remained below this threshold; however the α-tocopherol content was not measured in muscles immediately after slaughter and may have decreased during storage. Dietary vitamin E supplementation

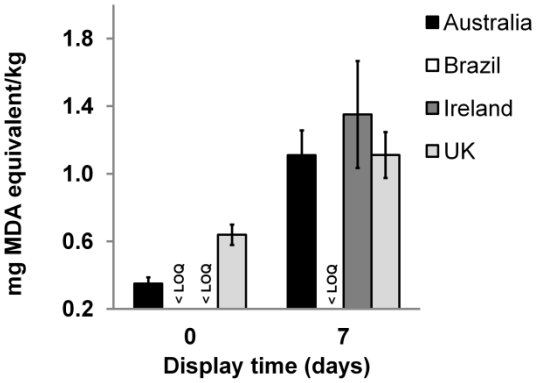

Figure 3 Interaction of origin × display time for lipid oxidation (thiobarbituric acid-reactive substances [TBARS]) in the *longissimus thoracis et lumborum* from different origins at the end of the shelf-life. Bars represent standard error.

Table 5 α-Tocopherol content (mean ± standard deviation) in the *longissimus thoracis et lumborum* from different origins at ⅔ of the shelf-life.

Origin	α-Tocopherol (μg/g meat)
Australia	6.1 ± 0.7^{b}
Brazil	3.2 ± 0.3^{a}
Ireland	5.1 ± 0.3^{b}
United Kingdom	5.0 ± 1.0^{b}

[ab]Different lowercase letters in a column (origin effect) indicate statistical difference (P<0.05).

to beef cattle increases the concentration of α-tocopherol in skeletal muscle. The increased α-tocopherol concentration allows this antioxidant to protect membranal lipids and MMb formation (Arnold, Arp, Scheller, Williams, & Schaefer, 1993; Liu, Lanari, & Schaefer, 1995; Lynch, Kerry, Buckley, Faustman, & Morrissey, 1999). However, meat with high content of polyunsaturated fatty acids may have an increased requirement for endogenous anti-oxidants to maintain colour and lipid stability (Yang, Lanari, Brewster, & Tume, 2002). Brazilian cattle production is primarily based on *Bos indicus* breeds and their crosses (FAO, 2007), whereas *Bos taurus* is the dominant species in Europe and Australia (O'Neill, Swain, & Kadarmideen, 2010). Recent studies highlighted that there are small differences in fat content and fatty acid composition of beef from *B. indicus* and *B. taurus* cattle, but diet and time of feed are much more important determinants of these parameters (Bressan et al., 2011; Bressan, Rodrigues, Rossato, Ramos, & da Gama, 2011; Smith, Gill, Lunt, & Brooks, 2009). As the proportion of peroxidisable lipids does not seem to be linked to the subspecies of cattle, the evaluation of other mechanisms involved in pigment and lipid oxidation, including antioxidant (Imazaki, Douny, Elansary, Scippo, & Clinquart, 2018; Pastsart, De Boever, Claeys, & De Smet, 2013) and glycolytic (Canto et al., 2015) enzymes could have provided additional clues to better understand the differences in oxidation profiles observed.

3.2 Microbial stability and microbial profile

At ⅔ of the shelf-life ($SL_{⅔}$ and $SL_{⅔/MAP}$), the effect of origin (Australia, Brazil, Ireland, UK) was significant for TVC, and the effect of display time (0 and 7 days) was observed for TVC and BT in British meat (P<0.05) (**Table 3**). At the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, $SL_{-1/+4°C/MAP}$), the effect of origin was significant for TVC, LAB and EB (P<0.05); the effect of ageing temperature (−1 and stepwise −1/+4 °C) was significant for EB (P<0.05); the effect of display time was significant for LAB (P<0.05); the interaction ageing temperature × display time was significant for TVC (P<0.05), and

the interaction origin × ageing temperature × display time was influential for LAB (P<0.05) (**Table 4**). For practical purposes, the microbial counts are presented as origin × ageing temperature × display time means.

The TVC ranged from 3.1 to 5.8 $\log_{10}$ CFU/cm² at $SL_{⅔}$ and from 6.0 to 8.3 $\log_{10}$ CFU/cm² at $SL_{⅔/MAP}$ (**Table 3**). At the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, $SL_{-1/+4°C/MAP}$), Brazilian meat presented the highest while Irish meat presented the lowest TVC (P<0.05), which might be simply related to their respective shelf-life (120 and 35 days). Furthermore, an increase of TVC was observed during MAP display for samples aged at −1 °C (P<0.05). This result was expected given that a high-oxygen modified atmosphere tends to promote bacterial growth when compared to vacuum storage (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007; McMillin, Huang, Ho, & Smith, 1999). However, there was no difference in TVC before and after MAP display for samples having undergone a stepwise –1/+4 °C ageing, maybe because these meats reached high TVC during vacuum storage (**Figure 4**).

The LAB counts ranged from <2.0 to 6.4 $\log_{10}$ CFU/cm² at ⅔ of the shelf-life ($SL_{⅔}$ and $SL_{⅔/MAP}$), and the acceptability LAB threshold of 6.7 $\log_{10}$ CFU/cm² was not exceeded

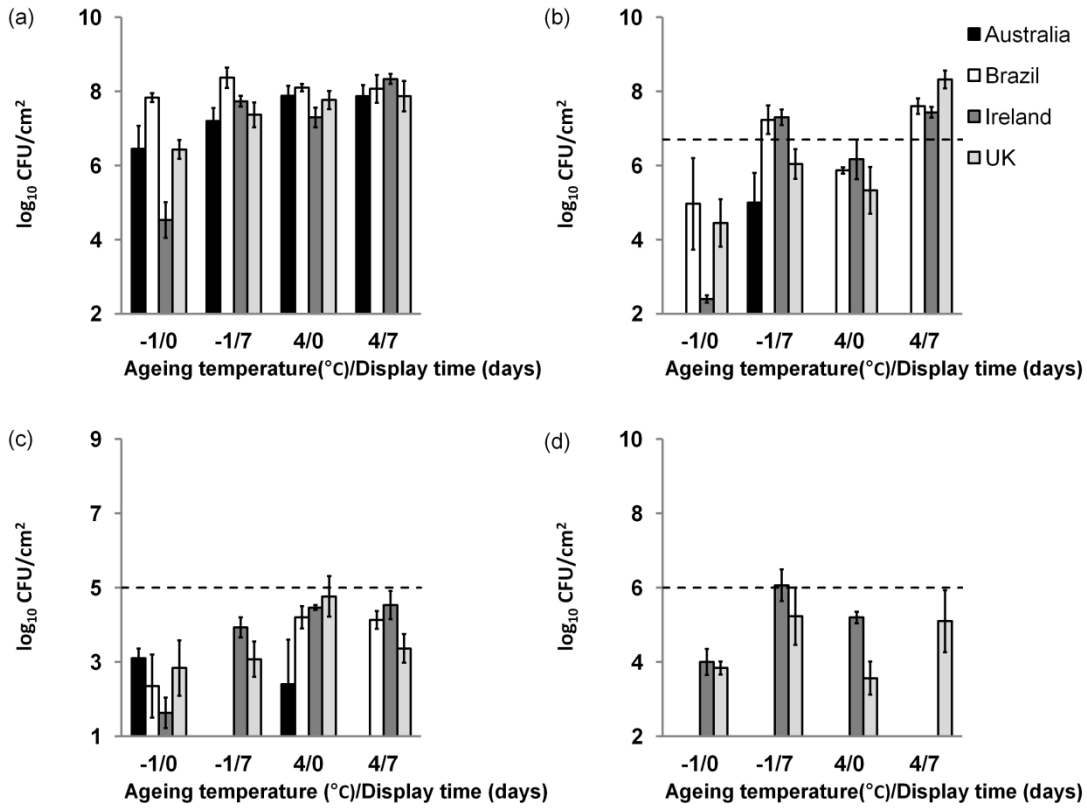

Figure 4 Interaction of origin × temperature × display time for (a) total viable count, (b) lactic acid bacteria, (c) Enterobacteriaceae and (d) *Brochothrix thermosphacta* in the *longissimus thoracis et lumborum* from different origins at the end of the shelf-life. Bars represent standard error.

by any sample (**Table 3**). At the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, $SL_{-1/+4°C/MAP}$), the acceptability threshold for LAB was exceeded in Brazilian and Irish samples at $SL_{-1°C/MAP}$ and in all samples at $SL_{-1/4°C/MAP}$, except for Australians. Surprisingly, LAB counts in Australian meat remained below 2.0 $\log_{10}$ CFU/cm² for treatments $SL_{-1°C}$, $SL_{-1/+4°C}$ and $SL_{-1/+4°C/MAP}$ (**Figure 4**). This result was unexpected at $SL_{-1°C}$ and $SL_{-1/+4°C}$, as when the growth of aerobic spoilage bacteria is inhibited by vacuum packaging, LAB become the dominant species of the microbial flora of chilled meats, and their presence usually ensures that shelf-life is maximal (Egan, 1983). Small et al. (2012) have previously reported unusual microbial counts in beef primals with extremely long shelf-life. According to Pothakos, Samapundo, and Devlieghere (2012), the current microbiological techniques, based on incubation at 30 °C, lack the discriminatory capacity to detect psychrotrophic LAB, whose growth was probably favoured by sub-zero ageing under vacuum for extended periods. Even if an incubation temperature of 22 °C was used, the psychrotrophic LAB might have been underestimated by the enumeration technique used in the present study.

Low levels of EB were found in samples at ⅔ of the shelf-life ($SL_{⅔}$ and $SL_{⅔/MAP}$) (**Table 3**). At the end of the shelf-life ($SL_{-1°C}$, $SL_{-1°C/MAP}$, $SL_{-1/+4°C}$, $SL_{-1/+4°C/MAP}$), Irish and British meats presented the highest EB counts, and a stepwise −1/+4 °C ageing promoted EB growth (P<0.05) (**Figure 4**). This result confirms the advantage of combining sub-zero ageing and strict temperature control with subsequent display in an atmosphere containing CO_2, which has an antimicrobial potential against EB (Milijasevic, Babic, & Veskovic-Moracanin, 2015). In all cases, the acceptability threshold for EB was not exceeded by any sample for any treatment.

All samples presented BT counts below the threshold of acceptability at ⅔ of the shelf-life ($SL_{⅔}$ and $SL_{⅔/MAP}$), except for British and Irish (only at $SL_{⅔/MAP}$ for Irish) (**Table 3**). Moreover, in Irish meat, BT counts at $SL_{-1°C/MAP}$ (6.1 $\log_{10}$ CFU/cm²) and $SL_{-1°C/4/MAP}$ (<2.0 $\log_{10}$ CFU/cm²) were lower than at $SL_{⅔/MAP}$ (6.5 $\log_{10}$ CFU/cm²) (P<0.05). Vacuum packaging limits the growth of BT in meat (Pennacchia, Ercolini, & Villani, 2011). The fact that British meat presented higher BT count at $SL_{⅔}$ than the other samples leads to the hypothesis of a delayed packaging or presence of residual oxygen in vacuum bags. However, since the initial handling conditions of samples are unknown, this hypothesis could not be verified. Russo, Ercolini, Mauriello, & Villani (2006) evidenced a decrease in the in vivo growth of BT in the presence of LAB, which could explain the reduction of BT in Irish samples over time. Furthermore, BT is psychrotolerant (Leroi, Fall, Pilet, Chevalier, & Baron, 2012) and may have been selected during ageing at −1 °C, which may explain the differences in BT count between $SL_{-1°C/MAP}$ and $SL_{-1°C/4/MAP}$.

Figure 5 presents the microbial diversity of samples evaluated by metagenetics. At first glance, Australian beef presented a lower bacterial diversity when compared to beef from other origins. Brazilian and Irish meats presented high bacterial diversity, which is evidenced by the size of the bars representing genus counting for less than 5% of relative abundance ("others"). In all cases, ageing time contributed to the selection of fewer genera in the beef microbial environment. The microbial ecology of raw meat is very complex, and its storage conditions will influence how different groups of organisms will colonise and eventually dominate the meat system (Ercolini, Russo, Torrieri, Masi, & Villani, 2006). As stated above, when meat is packaged under vacuum, psychrotrophic LAB become the dominant bacterial group (Egan, 1983), which is confirmed by the significant proportions of Lactobacillales, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus* and *Leuconostoc* found at the end of the shelf-life ($SL_{-1/+4°C}$). Moreover, the presence of members of the EB in certain meats, including the genus *Serratia*, may have contributed to meat spoilage. Surprisingly, the presence of *Pseudomonas*, which is often associated with meat spoilage under aerobic conditions, was detected in Brazilian and Irish beef at the end of the shelf-life. However, Motoyama, Kobayashi, Sasaki, Nomura, and Mitsumoto (2010) observed that *Pseudomonas fragi* could convert MMb into deoxymyoglobin and inhibit meat lipid oxidation. In this way, the interaction between the different bacterial groups found in meat and their effect on the oxidative stability should be further investigated. The relative abundance of *Carnobacterium*, specifically in Australian samples, reached up to 98%. This LAB can play a significant role in the spoilage of refrigerated raw meat, and, controversially, are also recognised as important competitors of other spoilage-related bacteria (Ercolini et al., 2006). Additionally, their presence in food is frequently underestimated with culture-depend methods (Huys, Leisner, & Björkroth, 2012). It is known that factors such as the presence of acetate or low pH values can inhibit the growth of *Carnobacterium* (Edima et al., 2007). However, the composition of the media

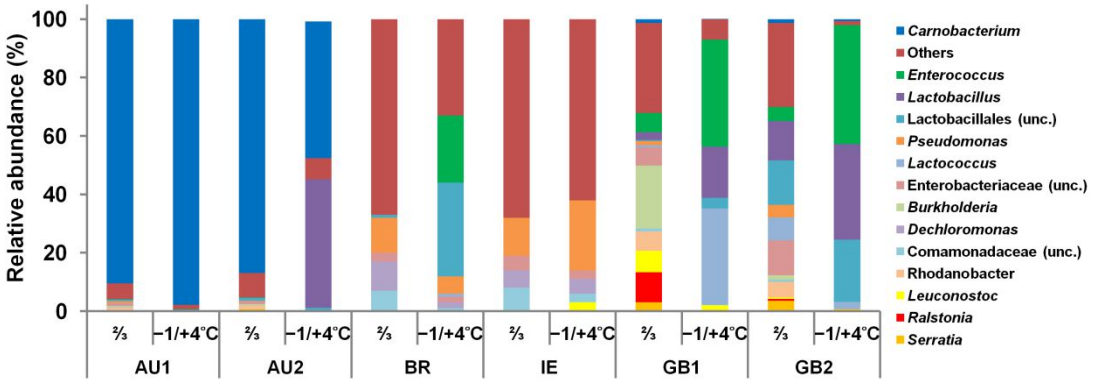

Figure 5 Distribution of reads percentages for major bacterial genera in beef from different origins at ⅔ and at the end of the shelf-life (for a stepwise −1/+4 °C ageing). Other genera represented, individually, less than 5% relative abundance in all samples. AU = Australia, BR = Brazil, IE = Ireland, GB = the United Kingdom, unc. = unclassified genus.

used in the TEMPO® system for automated enumeration is not available. Therefore, it was not possible to verify if the formulation of TEMPO® LAB test kits could explain the extremely low results obtained for LAB counts in Australian meat.

Sequencing the 16S rRNA genes of 11 isolates from different Australian meat samples revealed that all the 11 isolates belonged to the species *Carnobacterium maltaromaticum*. These LAB have been extensively studied as protective cultures in fish and meat products, and some strains can inhibit the growth of pathogenic bacteria, including *Listeria monocytogenes* (Leisner, Laursen, Prévost, Drider, & Dalgaard, 2007). In this manner, the presence of high proportions of *C. maltaromaticum* in Australian samples may have contributed positively to the extremely long shelf-life of these meats.

4 Conclusions

The use of sub-zero temperatures during vacuum storage is useful in meat shelf-life extension because it prevents myoglobin oxidation and TVC and EB growth. However, ageing at −1 °C did not prevent MMb formation and bacterial growth in meat, once it was repacked in a high-oxygen atmosphere (70/30% O_2/CO_2) at the end of the shelf-life, suggesting that extended beef ageing is more suitable for hotel/restaurant/catering (HORECA) operators, for whom high-oxygen MAP is not used. Differences in physicochemical and microbial parameters highlighted between origins should be considered carefully due to the low number of samples used in this research. Moreover, the lack of information related to the background of the samples hinders further evaluation of the possible effect of production conditions (e.g., finishing system, fat score and carcase handling) on the quality of meat.

Metagenetics proved to be a powerful and revolutionary tool for the evaluation of the bacterial diversity of fresh meat, as it could detect the presence of bacteria, notably psychrotrophic LAB, which are usually missed or underestimated by culture-dependent method assays.

Carnobacterium maltaromaticum was the dominant bacteria in Australian meat with 140 days of shelf-life. This species has been used for bioprotection and may have played an essential role in the extremely long shelf-life of these samples. The next step of this study will be characterising the obtained *C. maltaromaticum* isolates to better understand their role in food preservation.

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References

AFNOR. (2004). Hygiène et sécurité des produits alimentaires—Lignes directrices pour l'élaboration d'un protocole de test de vieillissement pour la validation de la durée de vie microbiologique—Denrées périssables, réfrigérées (2004), NF V01-003. La Plaine Saint-Denis: Association Française de Normalisation.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*, 403–410.

Arnold, R. N., Scheller, K. K., Arp, S. C., Williams, S. N., & Schaefer, D. M. (1993). Dietary alpha-tocopheryl acetate enhances beef quality in Holstein and beef breed steers. *Journal of Food Science, 58*, 28–33.

Baron, C. P., & Andersen, H. J. (2002). Myoglobin-induced lipid oxidation. A Review. *Journal of Agricultural and Food Chemistry*, *50*, 3887–3897.

Bekhit, A. E. D., & Faustman, C. (2005). Metmyoglobin reducing activity. *Meat Science, 71*, 407–439.

Bell, R. G., Penney, N., Gilbert, K. V., Moorhead, S. M., & Scott, S. M. (1996). The chilled storage life and retail display performance of vacuum and carbon dioxide packed hot deboned beef striploins. *Meat Science, 42*, 371–386.

Bressan, M. C., Rodrigues, E. C., Rossato, L. V., Ramos, E. M., & da Gama, L. T. (2011). Physicochemical properties of meat from *Bos taurus* and *Bos indicus*. *Revista Brasileira de Zootecnia, 40*, 1250–1259.

Bressan, M. C., Rossato, L. V., Rodrigues, E. C., Alves, S. P., Bessa, R. J. B., Ramos, E. M., & Gama, L. T. (2011). Genotype × environment interactions for fatty acid profiles in *Bos indicus* and *Bos taurus* finished on pasture or grain. *Journal of Animal Science, 89*, 221–232.

Brosius, J., Dull, T. J., Sleeter, D. D., & Noller, H. F. (1981). Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *Journal of Molecular Biology, 148*, 107–127.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*, 303–311.

Canto, A. C. V. C. S, Suman, S. P., Nair, M. N., Li, S., Rentfrow, G., Beach, C. M., . . . King, D. A. (2015). Differential abundance of sarcoplasmic proteome explains animal effect on beef *Longissimus lumborum* color stability. *Meat Science, 102*, 90–98.

Cauchie, E., Gand, M., Kergourlay, G., Taminiau, B., Delhalle, L., Korsak, N., & Daube, G. (2017). The use of 16S rRNA gene metagenetic monitoring of refrigerated food products for understanding the

kinetics of microbial subpopulations at different storage temperatures: the example of white pudding. *International Journal of Food Microbiology, 247*, 70–78.

Coombs, C. E. O., Holman, B. W. B., Friend, M. A., & Hopkins, D. L. (2017). Long-term red meat preservation using chilled and frozen storage combinations: a review. *Meat Science, 125*, 84–94.

Delhalle, L., Korsak, N., Taminiau, B., Nezer, C., Burteau, S., Delcenserie, V., . . . Daube, G. (2016). Exploring the bacterial diversity of Belgian steak tartare using metagenetics and quantitative real-time PCR analysis. *Journal of Food Protection, 79*, 220–229.

Edima, H. C., Cailliez-Grimal, C., Revol-Junelles, A.-M., Tonti, L., Linder, M., & Millière, J.-B. (2007). A selective enumeration medium for *Carnobacterium maltaromaticum*. *Journal of Microbiological Methods*, *68*, 516–521.

Egan, A. F. (1983). Lactic acid bacteria of meat and meat products. *Antonie van Leeuwenhoek, 49*, 327–336.

Ercolini, D., Russo, F., Torrieri, E., Masi, P., & Villani, F. (2006). Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Applied and Environmental Microbiology, 72*, 4663–4671.

FAO. (2007). *The state of the world's animal genetic resources for food and agriculture*. Rome (Italy): Food and Agriculture Organization.

FAO. (2013). Feeding the world. In FAO (Ed.), *Statistical yearbook 2013*. Rome: Food and Agriculture Organization.

FCD. (2016). *Critères microbiologiques applicables à partir de 2015 aux marques de distributeurs, marques premiers prix et matières premières dans leur conditionnement initial industriel*. Paris (France): Féderation du Commerce et de la Distribution.

Fernández, J., Pérez-Álvarez, J. A., & Fernández-López, J. A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chemistry, 59*, 345–353.

Garner, C. M., Unruh, J. A., Hunt, M. C., Boyle, E. A. E., & Houser, T. A. (2014). Effects of subprimal type, quality grade, and aging time on display color of ground beef patties. *Meat Science, 98*, 301–309.

Gill, C. O. (1996). Extending the storage life of raw chilled meats. *Meat Science, 43*, S99–S109.

Greene, B. E., Hsin, I. M., & Zipser, M. Y. W. (1971). Retardation of oxidative color changes in raw ground beef. *Journal of Food Science, 36*, 940–942.

Huse, S. M., Huber, J. A., Morrison, H. G., Sogin, M. L., & Welch, D. M. (2007). Accuracy and quality of massively parallel DNA pyrosequencing. *Genome Biology, 8*, R143.

Huys, G., Leisner, J., & Björkroth, J. (2012). The lesser LAB gods: *Pediococcus*, *Leuconostoc*, *Weissella*, *Carnobacterium* and affiliated genera. In S. Lahtinen, A. C. Ouwehand, S. Salminen & A. von Wright (Eds.)*, Lactic acid bacteria: microbiological and functional aspects* (pp. 93–122). Boca Raton: CRC Press.

Imazaki, P. H., Douny, C., Elansary, M., Scippo, M. L., & Clinquart, A. (2018). Effect of muscle type, aging technique, and aging time on oxidative stability and antioxidant capacity of beef packed in high-oxygen atmosphere. *Journal of Food Processing and Preservation*, *42*, e13603.

Irwin, J. W., & Hedges, N. (2004). Measuring lipid oxidation. In Steele, R (Ed.), *Understanding and measuring the shelf-life of food* (pp. 289–316). Cambridge: Woodhead Publishing.

ISO. (1996). *Viande et produits à base de viande—Dénombrement de Brochothrix thermosphacta—Technique par comptage des colonies obtenues, ISO 13722*. Genève: Organisation internationale de normalisation.

ISO. (1999). *Viande et produits à base de viande—Mesurage du pH—Méthode de référence, ISO 2917*. Genève: Organisation internationale de normalisation.

Jayasingh, P., Cornforth, D. P., Brennand, C. P., Carpenter, C. E., & Whittier, D. R. (2002). Sensory evaluation of ground beef stored in high-oxygen modified atmosphere packaging. *Journal of Food Science, 67*, 3493–3496.

Jeremiah, L. E., & Gibson, L. L. (2001). The influence of storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef. *Food Research International, 34*, 815–826.

Kergourlay, G., Taminiau, B., Daube, G., & Champomier-Vergès, M. C. C. (2015). Metagenomic insights into the dynamics of microbial communities in food. *International Journal of Food Microbiology, 213*, 31–39.

Korsak, N., Taminiau, B., Hupperts, C., Delhalle, L., Nezer, C., Delcenserie, V., & Daube, G. (2017). Assessment of bacterial superficial contamination in classical or ritually slaughtered cattle using metagenetics and microbiological analysis. *International Journal of Food Microbiology, 247*, 79–86.

Leisner, J. J., Laursen, B. G., Prévost, H., Drider, D., & Dalgaard, P. (2007). *Carnobacterium*: positive and negative effects in the environment and in foods. *FEMS Microbiology Reviews*, *31*, 592–613.

Leroi, F., Fall, P. A., Pilet, M. F., Chevalier, F., & Baron, R. (2012). Influence of temperature, pH and NaCl concentration on the maximal growth rate of *Brochothrix thermosphacta* and a bioprotective bacteria *Lactococcus piscium* CNCM I-4031. *Food Microbiology, 31*, 222–228.

Liu, Q., Lanari, M. C., & Schaefer, D. M. (1995). A review of dietary vitamin E supplementation for improvement of beef quality. *Journal of Animal Science*, *73*, 3131–3140.

Liu, Q. P., Scheller, K. K., & Schaefer, D. M. (1996). Technical note: a simplified procedure for vitamin E determination in beef muscle. *Journal of Animal Science, 74*, 2406–2410.

Lund, M. N., Lametsch, R., Hviid, M. S., Jensen, O. N., & Skibsted, L. H. (2007). High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage. *Meat Science, 77*, 295–303.

Lynch, M. P., Kerry, J. P., Buckley, D. J., Faustman, C., & Morrissey, P. A. (1999). Effect of dietary vitamin E supplementation on the colour and lipid stability of fresh, frozen and vacuum-packaged beef. *Meat Science, 52*, 95–99.

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science, 71*, 100–121.

Mancini, R. A., & Ramanathan, R. (2014). Effects of postmortem storage time on color and mitochondria in beef. *Meat Science, 98*, 65–70.

McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W., & Savell, J. W. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science, 70*, 665–682.

McMillin, K. W., Huang, N. Y., Ho, C. P., & Smith, B. S. (1999). Quality and shelf-life of meat in case-ready modified atmosphere packaging. In Y. L. Xiong, C. T. Ho & F. Shahidi (Eds.)*, Quality attributes of muscle foods* (pp. 73–93). New York: Kluwer Academic/Plenum Publishers.

Milijasevic, M., Babic, J., & Veskovic-Moracanin, S. (2015). Effect of vacuum and modified atmosphere on *Enterobacteriaceae* count determined in rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) steaks. *Procedia Food Science, 5*, 195–198.

Mills, J., Donnison, A., & Brightwell, G. (2014). Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: a review. *Meat Science, 98*, 71–80.

Motoyama, M., Kobayashi, M., Sasaki, K., Nomura, M., & Mitsumoto, M. (2010). *Pseudomonas* spp. convert metmyoglobin into deoxymyoglobin. *Meat Science*, 84, 202–207.

OECD. (2016). Meat. In OECD & FAO (Eds.), *Agricultural outlook 2016–2025*. Paris: Organisation for Economic Co-operation and Development.

OECD. (2017). Meat. In OECD & FAO (Eds.), *Agricultural outlook 2017–2026*. Paris: Organisation for Economic Co-operation and Development.

O'Neill, C. J., Swain, D. L., & Kadarmideen, H. N. (2010). Evolutionary process of *Bos taurus* cattle in favourable versus unfavourable environments and its implications for genetic selection. *Evolutionary Applications, 3*, 422–433.

Pastsart, U., De Boever, M., Claeys, E., & De Smet, S. (2013). Effect of muscle and post-mortem rate of pH and temperature fall on antioxidant enzyme activities in beef. *Meat Science, 93*, 681–686.

Pennacchia, C., Ercolini, D., & Villani, F. (2011). Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. *Food Microbiology*, *28*, 84–93.

Picgirard, L. (2009). Comparaison de muscles d'origine française et sud-américaine. *Viandes et Produits Carnés, 27*, 171–177.

Pothakos, V., Samapundo, S., & Devlieghere, F. (2012). Total mesophilic counts underestimate in many cases the contamination levels of psychrotrophic lactic acid bacteria (LAB) in chilled-stored food products at the end of their shelf-life. *Food Microbiology, 32*, 437–443.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research, 35*, 7188–7196.

R Core Team. (2016). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.R-project.org/

Raharjo, S., Sofos, J. N., & Schmidt, G. R. (1992). Improved speed, specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid–C_{18} method for measuring lipid peroxidation in beef. *Journal of Agricultural and Food Chemistry, 40*, 2182–2185.

Richards, M. P. (2013). Redox reactions of myoglobin. *Antioxidants & Redox Signaling*, *18*, 2342–2351.

Rodas-González, A., Narváez-Bravo, C., Brashears, M. M., Rogers, H. B., Tedford, J. L., Clark, G. O., . . . Miller, M. F. (2011). Evaluation of the storage life of vacuum packaged Australian beef. *Meat Science, 88*, 128–138.

Russo, F., Ercolini, D., Mauriello, G., & Villani, F. (2006). Behaviour of *Brochothrix thermosphacta* in presence of other meat spoilage microbial groups. *Food Microbiology, 23*, 797–802.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., . . . Weber, C. F. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology, 75*, 7537–7541.

Shahidi, F. (2016). Oxidative stability and shelf life of meat and meat products. In M. Hu & C. Jacobsen (Eds.)*, Oxidative stability and shelf life of foods containing oils and fats* (pp. 373–389). London: AOCS Press.

Small, A. H., Jenson, I., Kiermeier, A., & Sumner, J. (2012). Vacuum-packed beef primals with extremely long shelf life have unusual microbiological counts. *Journal of Food Protection, 75*, 1524–1527.

Smith, S. B., Gill, C. A., Lunt, D. K., & Brooks, M. A. (2009). Regulation of fat and fatty acid composition in beef cattle. *Asian-Australasian Journal of Animal Sciences, 22*, 1225–1233.

Tang, J., Faustman, C., & Hoagland, T. A. (2004). Krzywicki revisited: equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. *Journal of Food Science, 69*, C717–C720.

Tewari, G., Jayas, D. S., & Holley, R. A. (1999). Centralized packaging of retail meat cuts: a review. *Journal of Food Protection, 62*, 418–425.

Yang, A., Lanari, M. C., Brewster, M., & Tume, R. K. (2002). Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement. *Meat Science, 60*, 41–50.

Youssef, M. K., Gill, C. O., Tran, F., & Yang, X. (2014). Unusual compositions of microflora of vacuum-packaged beef primal cuts of very long storage life. *Journal of Food Protection, 77*, 2161–2167.

Youssef, M. K., Gill, C. O., & Yang, X. (2014). Storage life at 2 °C or −1.5 °C of vacuum-packaged boneless and bone-in cuts from decontaminated beef carcasses. *Journal of the Science of Food and Agriculture, 94*, 3118–3124.

Chapter 4

Preservation of fresh meat from the Belgian Blue breed

As discussed in **Chapter 3**, long shelf life is of high value in meat trading. Vacuum-packaged primals produced in Belgium, mainly from the Belgian Blue breed, have a maximum shelf life of 30 days, which is extremely short if there would be any interest to reach distant markets. However, the arrival of imported meat with a shelf life superior to three months in the Belgian market gave rise to extreme reactions from the meat sector. Some even suspected the use of illegal treatments in the EU. Furthermore, the Belgian meat industry regularly complains about the abnormally rapid discolouration of certain meats packaged under high O_2 atmosphere and believes in a particular sensitivity of Belgian Blue meat to oxidation.

This chapter is entirely dedicated to the preservation of fresh meat from the Belgian Blue breed and presents two studies on this subject.

The first study entitled “Effect of sex and sub-zero storage temperature on the microbial and oxidative stability of beef repackaged in high-oxygen atmosphere after different vacuum ageing times” investigated the effect of the category (young bull and cull cow) and sub-zero storage temperature on the microbial and oxidative stability of Belgian Blue beef repackaged in a high-O_2 atmosphere after different ageing times. The manuscript of this study has been accepted for publication in *Meat Science* and the copy-editing and production are in progress.

In the second study, entitled “Effect of muscle type, aging technique, and aging time on oxidative stability and antioxidant capacity of beef repackaged in high-oxygen atmosphere”, a comparison between two muscles muscle types (*longissimus thoracis et lumborum* and *rectus femoris*), two ageing techniques (carcase and vacuum ageing) and different ageing times (7, 21 and 35 days) on the oxidative stability of beef repackaged in a high-O_2 atmosphere was made. This study was published in the *Journal of Food Processing and Preservation* (Imazaki *et al.*, 2018).

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EFFECT OF SEX AND SUB-ZERO STORAGE TEMPERATURE ON THE MICROBIAL AND OXIDATIVE STABILITY OF BEEF PACKAGED IN 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 repackaged 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 more than 20 days had a negative impact on retail shelf-life.**

Keywords: **Belgian Blue, microbiological quality, spoilage bacteria, oxidation, color, lipid.**

Highlights:

- **Microbial and oxidative stability during display decreases as ageing time increases.**
- **Ageing at −1 °C can improve microbial and colour stability of meat.**
- **Meat from young bulls is more sensitive to oxidation than meat from cull cows.**
- ***Brochothrix thermosphacta* growth limits the length of beef ageing at −1 °C.**
- **Ageing Belgian Blue meat for more than 20 days is not recommended for retail display.**

1 Introduction

Modes of processing, distribution and consumption of fresh meat have dramatically changed over the past decades, resulting in reorganisation 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 worldwide. 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 bright red appealing 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). 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 °C and 4 °C) on the microbiological and oxidative stability of Belgian Blue beef repackaged 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 (2 ageing temperatures × 5 ageing times × 2 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 (5 steaks/animal) or 4 °C (5 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 $cm^3/m^2 \times 24\ h \times bar$ 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 two days and 8 °C was applied during the five last 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 **Figure 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 d_0 (A0D0).

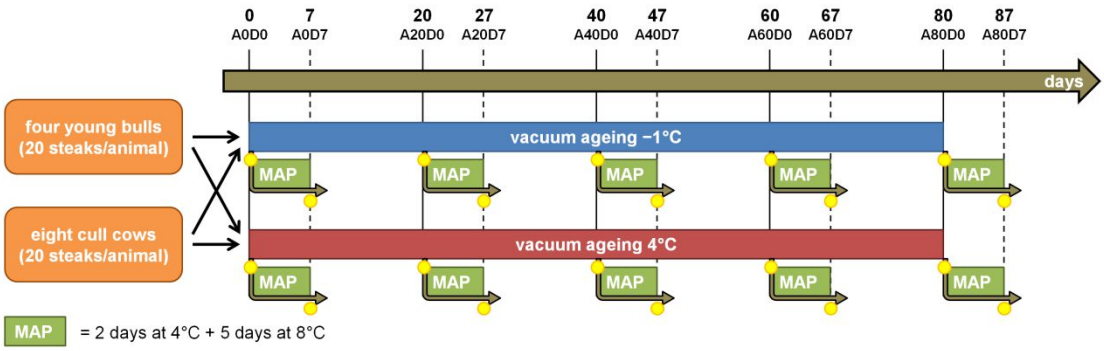

Figure 1 Experimental scheme. Each yellow circle represents one of the 20 treatments applied to steaks of each animal.

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 .

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 milligram 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^{TM} 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 12 replicates (each animal was used as replicate), 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. 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 *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 d_0. 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 will be presented as temperature × ageing time × display time means (**Figure 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 (**Figure 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-packaged 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 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 (**Figure 2**).

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 (EB), *Brochothrix thermosphacta* (BT), ΔE, percentage metmyoglobin (MMb%) and thiobarbituric acid reactive substances TBARS.

Effects	TVC	LAB	EB	BT	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)	**163.13*****	**80.18*****	**59.84*****	**7.47****	**10.78****	**151.03*****	**48.60*****
Display time (D)	**112.21*****	**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.
*Significant at $P < 0.05$
**Significant at $P < 0.01$
***Significant at $P < 0.001$
n/a: not applicable

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

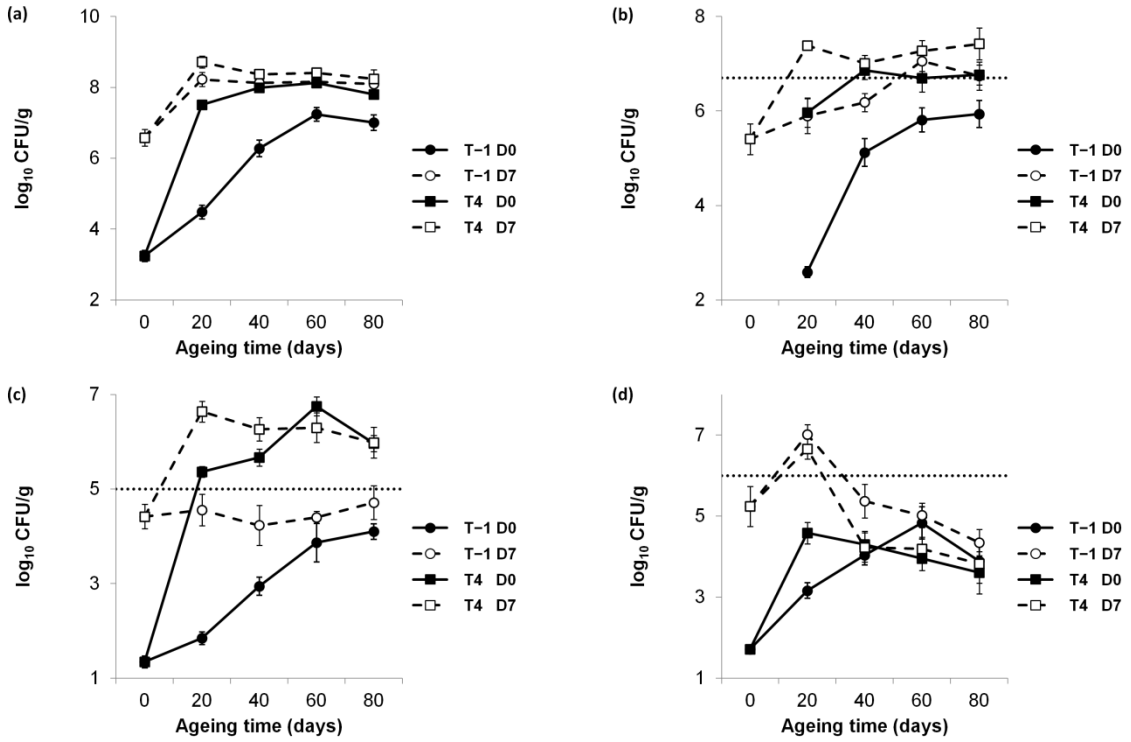

Figure 2 (a) Total viable count, (b) lactic acid bacteria, (c) Enterobacteriaceae and (d) *Brochothrix thermosphacta* in *longissimus thoracis et lumborum* from Belgian Blue animals for the combination ageing temperature × ageing time × display time. Samples aged at −1 °C (●) and 4 °C (■), before MAP 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.

vacuum ageing and after a 7-day MAP display (**Table 2** and **Figure 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 ($P < 0.05$) (**Figure 2**). Pennachia, Ercolini and Villani (2011) observed that *B. thermosphacta* is one of the predominant species in chilled beef in 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.

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

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 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 higher ($P < 0.05$) 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 (**Figure 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 combination 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

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, *: $P < 0.05$.

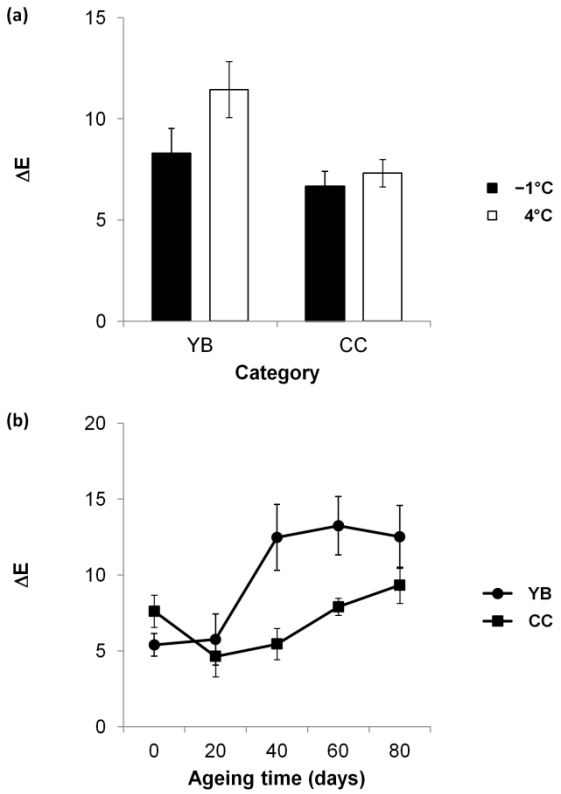

Figure 3 ΔE during a 7-day MAP display in *longissimus thoracis et lumborum* from Belgian Blue young bulls (YB) and cull cows (CC) previously aged at −1 and 4 °C, for 0, 20, 40, 60 and 80 days, for the combinations (a) category × ageing temperature and (b) category × ageing time. Bars represent standard error.

higher ($P < 0.05$) with increasing ageing time (**Figure 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 formation (Ben Abdallah, Marchello, & Ahmad, 1999; Robach & Costilow, 1961).

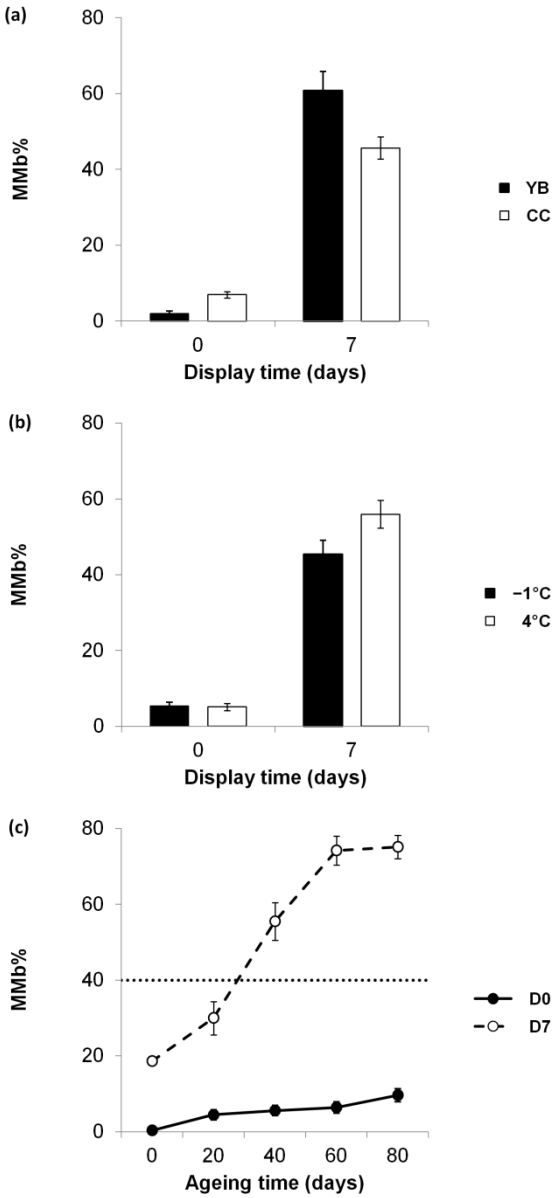

Figure 4 MMb% in *longissimus thoracis et lumborum* from Belgian Blue young bulls (YB) and cull cows (CC) aged for 0, 20, 40, 60 and 80 days at −1 and 4 °C, and stored for 7 days in a modified atmosphere of 70/30% O_2/CO_2 for the combinations (a) category × display time, (b) ageing temperature × display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

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 like 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 ($P < 0.05$) (**Figure 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/kg is usually considered as the 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

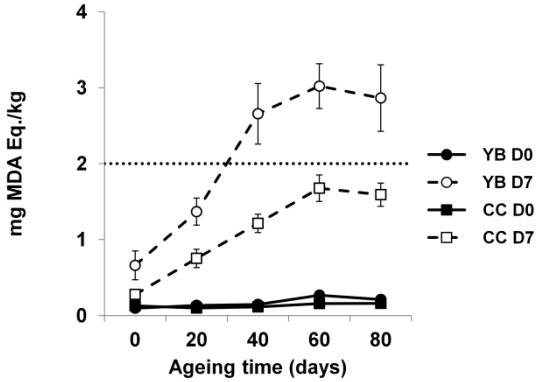

Figure 5 TBARS in *longissimus thoracis et lumborum* from Belgian Blue young bulls (YB) and cull cows (CC) for the combination category × ageing time × display time. YB (●) and CC (■) before MAP display (D0) are shown linked by full lines. YB (○) and CC (□) after MAP display (D7) are shown linked by dashed lines. The horizontal dotted line represents the acceptability threshold. Bars represent standard error.

YB (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. Nevertheless, the hypothesis of the influence of different PUFA content between 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, Morgan, Sofos, & Tatum, 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 et al. (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 MetMb formation in fresh beef. In our study, meat from YB remained below the threshold of 3.5 µg/g. This fact could explain that 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-packaged storage influenced 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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5 References

Bekhit, A. E., & Faustman, C. (2005). Metmyoglobin reducing activity. *Meat Science, 71*, 407–439.

Bekhit, A. E., Geesink, G. H., Ilian, M. A., Morton, J. D., & Bickerstaffe, R. (2003). The effects of natural antioxidants on oxidative processes and metmyoglobin reducing activity in beef patties. *Food Chemistry, 81*, 175–187.

Bekhit, A. E., Geesink, G. H., Morton, J. D., & Bickerstaffe, R. (2001). Metmyoglobin reducing activity and colour stability of ovine *longissimus* muscle. *Meat Science, 57*, 427–435.

Ben Abdallah, M., Marchello, J. A., & Ahmad, H. A. (1999). Effect of freezing and microbial growth on myoglobin derivatives of beef. *Journal of Agricultural and Food Chemistry, 47*, 4093–4099.

Boles, J. A., & Swan, J. E. (2002). Processing and sensory characteristics of cooked roast beef: effect of breed, age, gender and storage conditions. *Meat Science, 62*, 419–427.

Cabaraux, J. F., Hornick, J. L., Dotreppe, O., Dufrasne, I., Clinquart, A., & Istasse, L. (2004). Effects of the calving number on animal performance, carcass and meat composition in finishing Belgian Blue double-muscled culled females. *Livestock Production Science, 87*, 161–169.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science*, *72*, 303–311.

Coombs, C. E. O., Holman, B. W. B., Friend, M. A., & Hopkins, D. L. (2017). Long-term red meat preservation using chilled and frozen storage combinations: a review. *Meat Science, 125*, 84–94.

Delhalle, L., Korsak, N., Taminiau, B., Nezer, C., Burteau, S., Delcenserie, V., Poullet, J. B., & Daube, G. (2016). Exploring the bacterial diversity of Belgian steak tartare using metagenetics and quantitative real-time PCR analysis. *Journal of Food Protection, 79*, 220–229.

Dufrasne, I., Marche, C., Clinquart, A., Hornick, J. L., Van Eenaeme, C., & Istasse, L. (2000). Effects of dietary vitamin E supplementation on performance and meat characteristics in fattening bulls from the Belgian Blue breed. *Livestock Production Science, 65*, 197–201.

Faustman, C., Sun, Q., Mancini, R., & Suman, S. P. (2010). Myoglobin and lipid oxidation interactions: mechanistic bases and control. *Meat Science, 86*, 86–94.

FCD. (2016). *Critères microbiologiques applicables à partir de 2015 aux marques de distributeurs, marques premiers prix et matières premières dans leur conditionnement initial industriel*. Paris (France): Féderation du Commerce et de la Distribution.

Fiems, L. O., De Campeneere, S., Van Caelenbergh, W., & Boucqué, C. V. (2001). Relationship between dam and calf characteristics with regard to dystocia in Belgian Blue double-muscled cows. *Animal Science, 72*, 389–394.

Fiems, L. O., De Campeneere, S., Van Caelenbergh, W., De Boever, J. L., & Vanacker, J. M. (2003). Carcass and meat quality in double-muscled Belgian Blue bulls and cows. *Meat Science, 63*, 345–352.

Greene, B. E., Hsin, I. M., & Zipser, M. W. (1971). Retardation of oxidative color changes in raw ground beef. *Journal of Food Science, 36*, 940–942.

Imazaki, P. H., Maréchal, A., Nezer, C., Taminiau, B., Daube, G., & Clinquart, A. (2011, August). *Influence of temperature on conservability of chilled vacuum packed beef from different origins*. Paper presented at the 57th International Congress of Meat Science and Technology, Ghent (Belgium).

Imazaki, P. H., Teixeira Gonçalves, A., Krantz, M., Thimister, J., & Clinquart, A. (2016, December). *Effect of aging time, aging technique (dry- vs. wet-aging) and packaging on tenderness, pigment and lipid stability of Belgian blue beef*. Paper presented at the 3rd FARAH Day, Liège (Belgium).

ISO. (1996a). *Viande et produits à base de viande, dénombrement de Brochothrix thermosphacta, technique par comptage des colonies obtenues, ISO 13722*. Geneva (Switzerland): International Organization for Standardization.

ISO. (1996b). *Viande et produits à base de viande, détermination de la teneur en matière grasse libre, ISO 1444*. Geneva (Switzerland): International Organization for Standardization.

ISO. (1997). *Viande et produits à base de viande – détermination de l'humidité, ISO 1442*. Geneva (Switzerland): International Organization for Standardization.

Jeremiah, L. E., & Gibson, L. L. (2001). The influence of storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef. *Food Research International, 34*, 815–826.

Leroi, F., Fall, P. A., Pilet, M. F., Chevalier, F., & Baron, R. (2012). Influence of temperature, pH and NaCl concentration on the maximal growth rate of *Brochothrix thermosphacta* and a bioprotective bacteria *Lactococcus piscium* CNCM I-4031. *Food Microbiology, 31*, 222–228.

Liu, Q., Lanari, M. C., & Schaefer, D. M. (1995). A review of dietary vitamin E supplementation for improvement of beef quality. *Journal of Animal Science*, *73*, 3131–3140.

Liu, Q. P., Scheller, K. K., & Schaefer, D. M. (1996). Technical note: a simplified procedure for vitamin E determination in beef muscle. *Journal of Animal Science, 74*, 2406–2410.

Lund, M. N., Lametsch, R., Hviid, M. S., Jensen, O. N., & Skibsted, L. H. (2007). High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage. *Meat Science, 77*, 295–303.

McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W., & Savell, J. W. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science, 70*, 665–682.

McMillin, K. W., Huang, N. Y., Ho, C. P., & Smith, B. S. (1999). Quality and shelf-life of meat in case-ready modified atmosphere packaging. In Y. L. Xiong, C.-T. Ho, & F. Shahidi (Eds.), *Quality attributes of muscle foods* (pp. 73–93). New York (United States): Kluwer Academic/Plenum Publishers.

Milijasevic, M., Babic, J., & Veskovic-Moracanin, S. (2015). Effect of vacuum and modified atmosphere on *Enterobacteriaceae* count determined in rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) steaks. *Procedia Food Science, 5*, 195–198.

Mills, J., Donnison, A., & Brightwell, G. (2014). Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: a review. *Meat Science, 98*, 71–80.

Pennacchia, C., Ercolini, D., & Villani, F. (2011). Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. Food Microbiology, *28*, 84–9.

Picgirard, L. (2009). Viandes sous vide à longue DLC: comparaison de muscles d'origine française et sud-américaine. *Viandes et Produits Carnés, 27*, 171–177.

Pothakos, V., Devlieghere, F., Villani, F., Björkroth, J., & Ercolini, D. (2015). Lactic acid bacteria and their controversial role in fresh meat spoilage. *Meat Science, 109*, 66–74.

Pothakos, V., Samapundo, S., & Devlieghere, F. (2012) Total mesophilic counts underestimate in many cases the contamination levels of psychrotrophic lactic acid bacteria (LAB) in chilled-stored food products at the end of their shelf-life. *Food Microbiology, 32*, 437–443.

Pouzo, L. B., Descalzo, A. M., Zaritzky, N. E., Rossetti, L., & Pavan, E. (2016). Antioxidant status, lipid and color stability of aged beef from grazing steers supplemented with corn grain and increasing levels of flaxseed. *Meat Science, 111*, 1–8.

R Core Team. (2016). *R: a language and environment for statistical computing*. Retrieved from: https://www.R-project.org/

Raharjo, S., Sofos, J. N., & Schmidt, G. R. (1992). Improved speed, specificity, and limit of determination of an aqueous acid-extraction thiobarbituric acid-C_{18} method for measuring lipid-peroxidation in beef. *Journal of Agricultural and Food Chemistry, 40*, 2182–2185.

Robach, D. L., & Costilow, R. N. (1961). Role of bacteria in the oxidation of myoglobin. *Applied Microbiology, 9*, 529–533.

Russo, F., Ercolini, D., Mauriello, G., & Villani, F. (2006). Behaviour of *Brochothrix thermosphacta* in presence of other meat spoilage microbial groups. *Food Microbiology, 23*, 797–802.

Seideman, S. C., Cross, H. R., Smith, G. C., & Durland, P. R. (1984). Factors associated with fresh meat color: a review. *Journal of Food Quality, 6*, 211–237.

Simmons, J., Tamplin, M. L., Jenson, I., & Sumner, J. (2008). Effect of incubation temperature on aerobic plate counts of beef and sheep carcasses. *Journal of Food Protection, 71*, 373–375.

Smith, G. C., Morgan, J. B., Sofos, J. N., & Tatum, J. D. (1996). Supplemental vitamin E in beef cattle diets to improve shelf-life of beef. *Animal Feed Science and Technology, 59*, 207–214.

SOGEPA. (2016). *Regards sur l'économie wallonne: Le secteur de la viande en Wallonie*. Liège (Belgium): Société Wallonne de Gestion et de Participations.

Stellato, G., La Storia, A., De Filippis, F., Borriello, G., Villani, F., & Ercolini, D. (2016). Overlap of spoilage-associated microbiota between meat and the meat processing environment in small-scale and large-scale retail distributions. *Applied and Environmental Microbiology, 82*, 4045–4054.

Stetzer, A. J., Cadwallader, K., Singh, T. K., McKeith, F. K., & Brewer, M. S. (2008). Effect of enhancement and ageing on flavor and volatile compounds in various beef muscles. *Meat Science, 79*, 13–19.

Tang, J., Faustman, C., & Hoagland, T. A. (2004). Krzywicki revisited: equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. *Journal of Food Science, 69*, C717–C720.

Tang, J. L., Faustman, C., Hoagland, T. A., Mancini, R. A., Seyfert, M., & Hunt, M. C. (2005). Interactions between mitochondrial lipid oxidation and oxymyoglobin oxidation and the effects of vitamin E. *Journal of Agricultural and Food Chemistry, 53*, 6073–6079.

Venkata Reddy, B., Sivakumar A. S., Jeong, D. W., Woo, Y.-B., Park, S.-J., Lee, S.-Y., Byun, J.-Y., Kim, C. H., Cho, S. H., & Hwang, I. (2015). Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environments. *Animal Science Journal, 86*, 1–16.

Vitale, M., Pérez-Juan, M., Lloret, E., Arnau, J., & Realini, C. E. (2014). Effect of aging time in vacuum on tenderness, and color and lipid stability of beef from mature cows during display in high oxygen atmosphere package. *Meat Science, 96*, 270–277.

Xiong, Y. L. (2017). The storage and preservation of meat: I—thermal technologies. In F. Toldrá (Ed.), *Lawrie's meat science* (pp. 205–230). Duxford: Woodhead Publishing.

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ORIGINAL ARTICLE

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Effect of muscle type, aging technique, and aging time on oxidative stability and antioxidant capacity of beef packed in high-oxygen atmosphere

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Abstract

This study aimed to compare the effect of muscle type, aging technique, and aging time on the oxidative stability of beef packed in high-oxygen atmosphere. *Longissimus thoracis et lumborum* (LTL) and *rectus femoris* (RF) muscles from Belgian Blue cows were vacuum aged (VA) or aged on the carcass (CA) for 7 days. Then, they were stored under vacuum at −1 °C for up to 28 days. At different times, samples were repackaged under a high-oxygen atmosphere and stored at 4 °C for 7 days. The following parameters were evaluated: color, metmyoglobin %, fat content, fatty acid profile, lipid oxidation, metmyoglobin reducing activity (MRA), α-tocopherol content, and antioxidant enzymes activity. The sensitivity of meat to oxidation was influenced by muscle type (RF > LTL), aging technique (VA > CA), aging time (35 > 21 > 7 days), and display time (7 > 0 days), and could be related to MRA and antioxidant enzymes activity.

Practical applications

High-oxygen modified atmosphere packaging is often used by the meat industry at the retail level to maintain an attractive color in fresh meat. Nevertheless, this kind of packaging can promote early discoloration, lipid oxidation, and sensory changes. In this way, the meat sector seeks solutions to prevent meat oxidation and to extend the shelf-life of beef packed in high-oxygen atmosphere. The results obtained in this study show that aging on the carcass before cutting and further vacuum storage can limit the oxidation of meat when it is repacked in a high-oxygen atmosphere. Moreover, this technique shows an additional advantage when handling muscles which are sensitive to oxidation, including *rectus femoris*. Therefore, the present work provides new knowledge that contributes to the understanding of the oxidative capacity of meat under different conditions and the development of appropriate strategies for beef aging and preservation.

1 | INTRODUCTION

Post-mortem aging is a natural process that consists of storing meat for an extended period to improve palatability attributes such as flavor and tenderness. Traditionally, beef used to be aged by hanging entire carcasses or half carcasses without protective packaging at refrigeration temperatures. In the 1960s, a combination of costs related to meat hanging, the trend toward centralized processing, and the development of food-grade impermeable packaging films have contributed to the expansion of vacuum-aging (Hodges, Cahill, & Ockerman, 1974). This technique consists of aging meat in a vacuum-sealed barrier package at refrigerated temperatures (Smith et al., 2008), and, today, most of the beef sold in food stores in industrialized countries is vacuum aged (Stenström, Li, Hunt, & Lundström, 2014).

The oxidation of lipids and pigments limits the shelf life of fresh meat, producing rancid flavors and surface discoloration. However, meat contains endogenous antioxidants and utilizes several cellular mechanisms of protection against oxidative processes. These mechanisms include the metmyoglobin (MMb) reducing system and antioxidant enzymes comprising catalase (CAT), glutathione peroxidase

(GSH-Px), and superoxide dismutase (SOD) (Hernandéz, Zomeño, Ariño, & Blasco, 2004). CAT and GSH-Px are major peroxide-removing enzymes, while SOD is an important antioxidant that catalyzes the dismutation of the superoxide radical (O_2^-) into either molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) (Chan, Decker, & Feustman, 1994).

The meat sector often complains of the sensitivity of beef to oxidation; in particular, the discoloration of individual vacuum-aged cuts when they are repackaged in a high-oxygen modified atmosphere (70–80% O_2), which is often used at the retail level to give fresh meat its appealing bright red color. Several studies comparing retail cutting yields and consumer sensory attribute evaluations of beef having undergone different aging techniques have been conducted (Obuz, Akkaya, Gök, & Dikeman, 2014; Smith et al., 2008, 2014), but the relationship between aging technique and oxidative stability during display needs further investigation. Moreover, previous studies have focused on color-stable muscles including *longissimus thoracis et lumborum* (LTL) (Beriain, Goni, Indurain, Sarries, & Insausti, 2009; Lindahl, 2011), while studies on color-labile muscles such as *rectus femoris* (RF) are limited.

The development of the Belgian Blue breed has been made following the demand of a meat industry very sensitive to the muscle conformation. Belgian Blue beef also responses the request of Belgian consumers for a tender and lean meat and, nowadays, it represents more than 80% of the total beef consumption in the Belgian market (SPW, 2014). Nevertheless, in recent years, very few studies specifically addressing the quality of Belgian Blue meat have been published.

In this context, the present study was conducted to evaluate the potential effect of muscle type (LTL and RF), aging technique (vacuum-aging and stepwise aging on the carcass/vacuum-aging), aging time (7, 21, and 35 days), and display time (0 and 7 days) on the oxidative stability of Belgian Blue meat packaged in high-oxygen atmosphere. The originality of this approach lies in the comparison of two aging techniques followed by different storage times in vacuum and a final storage in a high-oxygen atmosphere, to simulate wholesale and retail trade.

2 | MATERIAL AND METHODS

2.1 | Samples

Three days after slaughtering (d_0), LTL and RF muscles were cut from four half carcasses of four Belgian Blue cows (7.9 ± 1.4 yr, all of conformation class E and fat-cover class 2 in the EUROP grid), vacuum packaged, and wet aged at 1.5 °C for 7 days. In parallel, the other four half carcasses from the same animals were aged on the carcass at the same temperature at 99% RH for the same period, before deboning and cutting. After the 7-day vacuum or carcass-aging step (d_7), 2.5–3 cm thick steaks were cut, vacuum sealed individually and stored (vacuum aged) at −1 °C for up to 28 days (d_{35}). Vacuum bags (Cryovac) were 60-μm thick, and the oxygen permeability was 13 $cm^3/m^2 \cdot 24$ hr · bar at 23 °C and 0% RH. The samples that were vacuum aged from d_0 to d_7 will be referred to as VA, and those that were aged on the carcass from d_0 to d_7 will be referred to as CA. Evaluation of color (CIE $L^*a^*b^*$), percent metmyoglobin (MMb %), metmyoglobin reducing activity (MRA), lipid oxidation (using the 2-thiobarbituric acid reactive substances (TBARS) determination), and antioxidant enzymes activity (CAT, GSH-Px, and SOD) was performed on one VA and one CA steak of each muscle type from each animal at d_7, d_{21}, and d_{35}. At d_0, one steak of each muscle type from each animal was used for the analysis cited above and for the determination of the fat content and fatty acid profile. The α-tocopherol content was determined in another set of one VA and one CA steak from each animal at d_7.

Moreover, at d_7, d_{21}, and d_{35}, one VA and one CA steak of each muscle type from each animal were repackaged individually in PP/EVOH/PP trays (ES-Plastic, dimensions: 187 × 137 × 50 mm, oxygen permeability: 4 $cm^3/m^2 \cdot 24$ hr · bar at 23 °C and 0% RH) and sealed with a PET/PP film (Wipak, oxygen permeability: 8 $cm^3/m^2 \cdot 24$ hr · bar at 23 °C and 0% RH) containing a modified atmosphere of 70/30% O_2/CO_2. Modified atmosphere packaged (MAP) samples were stored for 7 days at 4 °C (simulated retail display) and analyzed (at d_{7+7}, d_{21+7}, and d_{35+7}) for the following parameters: color (CIE $L^*a^*b^*$), MMb %, MRA, TBARS, and antioxidant enzymes activity (CAT, GSH-Px, and SOD). Thirteen treatments were applied to each muscle type (Table 1), and each steak was assigned randomly to a different treatment. The experimental scheme is shown in Figure 1.

2.2 | pH measurement

pH was measured using a pH meter Knick type 765 and a combined pH electrode model 104063123 (Ingold) according to ISO (1999). Measurements were performed in five different zones of each sample, and the values were averaged.

2.3 | Color measurement and metmyoglobin %

Instrumental evaluation of color was performed according to American Meat Science Association (2012) 1.5 hr after removal from packaging and exposure to atmospheric air (bloom time) using a Minolta CM-600d spectrophotometer (11-mm aperture, D65 illuminant, 10° observation angle, color space CIE $L^*a^*b^*$). The color difference between samples after and before MAP display (ΔE) was calculated using the equation $\sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2}$. Higher ΔE values mean greater total color changes over time.

MMb % was determined using the method of Francis and Clydesdale (1975) based on the reflectance measurement to calculate the ratio between absorption coefficient and scattering coefficient (K/S) at isosbestic wavelengths (474, 525, 572, and 610 nm) for each myoglobin redox form. Reflectance was measured using the same equipment described for instrumental color evaluation. K/S ratios were calculated using the equation $(1-R)^2 \div 2R$, where R = reflectance (expressed as a decimal, not as a percentage). Reference standards for 100% of MMb and deoxymyoglobin (DMb) were created by chemical induction using 1.0% potassium ferricyanide and 0.15% dithionite, respectively, according to American Meat Science Association (2012). MMb % was then calculated using the

TABLE 1 Different treatments applied to *longissimus thoracis et lumborum* and *rectus femoris* samples

Treatment	Vacuum-aging at 1.5 °C (days)	Carcass-aging at 1.5 °C (days)	Vacuum-aging at −1.0 °C (days)	MAP display at 4 °C (days)
A0M0	0	0	0	0
V7M0	7	0	0	0
C7M0	0	7	0	0
V7M7	7	0	0	7
C7M7	0	7	0	7
V21M0	7	0	14	0
C21M0	0	7	14	0
V21M7	7	0	14	7
C21M7	0	7	14	7
V35M0	7	0	28	0
C35M0	0	7	28	0
V35M7	7	0	28	7
C35M7	0	7	28	7

equation $\left(\frac{K/S_{572}}{K/S_{525}}100\%_{DMb} - \frac{K/S_{572}}{K/S_{525}}_{sample}\right) \div \left(\frac{K/S_{572}}{K/S_{525}}100\%_{DMb} - \frac{K/S_{572}}{K/S_{525}}100\%_{MMb}\right)$. Color and reflectance measurements were performed at the same time on ten different zones of each sample, and the results were averaged.

2.4 | Fat content and fatty acid profile

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

The fatty acid profile was determined by analysis of the fatty acid methyl esters (FAME) by gas chromatography-mass spectrometry (GC-MS) according to Douny et al. (2015). The method consists of the saponification/methylation of the fat extracted from meat samples, in the presence of the internal standard nonadecanoic acid (C19:0), followed by a double hexane extraction. FAME in the extract were separated on a Focus GC gas chromatograph (Thermo Fisher Scientific) using a CP-Sil88 column for FAME (Varian, 100 m × 0.25 mm, 0.2 μm) and analyzed with an ion trap PolarisQ mass spectrometer (Thermo Fisher Scientific). The GC conditions were: inlet – 250 °C, splitless injection, helium as carrier gas at 1.5 ml/min; temperature program – 55 °C for 1 min, followed by an increase of 5 °C/min to 180 °C, then 10 °C/min to 200 °C, 200 °C for 15 min, then a rise of 10 °C/min to 225 °C, and 225 °C for 14 min; total run time – 59.50 min; injection volume – 1 μl. The peaks were identified by comparing their mass spectrum and retention times with those of the corresponding standards. The MS conditions were: transfer line – 250 °C; ion source – 220 °C; collision energy – 35 eV, positive ionization mode. FAME were detected using selected ion monitoring (SIM) mode in five segment windows. In each chromatographic run, different ions were monitored for each fatty acid

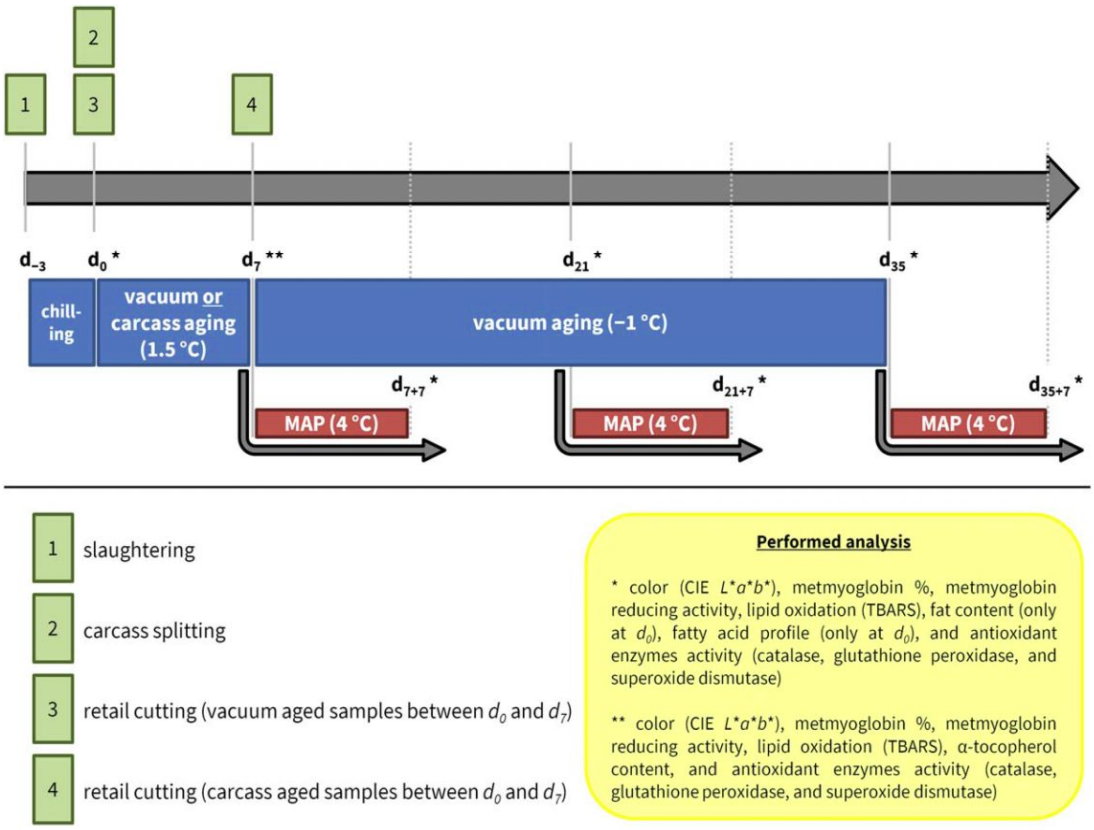

FIGURE 1 Experimental scheme. MAP = modified atmosphere packaging (70/30% O_2/CO_2)

analyzed, which allowed to perform detection and quantitative analysis: *m/z* 101 + 143 for saturated fatty acids (SFA), and 79 + 91 for monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The sum of SFA, MUFA, and PUFA were individually expressed as the percentage of total fatty acids and in mg per 100 g of meat.

2.5 | Lipid oxidation measurement

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

2.6 | Metmyoglobin reducing activity

The MRA was measured according to American Meat Science Association (2012). Surface pigments were initially oxidized to MMb by soaking the samples in 0.3% sodium nitrite for 20 min. The samples were vacuum packaged, and the MMb % was monitored for 2 hr at 30 °C by measuring reflectance *K/S* ratios (572/525 nm). Sample reducing ability was defined as the percentage decrease of MMb concentration in the surface during the incubation period.

2.7 | 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. The method consists of a saponification step, followed by a double isooctane extraction of the saponified samples. α-Tocopherol in the extracts was separated by normal phase chromatography and quantified by fluorescence detection. High-pressure liquid chromatography (HPLC) analysis was performed using a Model 600 E solvent delivery system equipped with a Model 717 automatic injector, a MistralTM oven and a 2475 Fluorescence detector (all from Waters). The following HPLC conditions were used: stationary phase – Waters Resolve 5 μm spherical silica column (3.9 × 150 mm); column temperature – 15 °C; mobile phase – isooctane/THF (96:4 vol/vol); flow rate – 1.0 ml/min; injection volume – 30 μl; detection – fluorescence (excitation wavelength 296 nm and emission wavelength 325 nm); quantification – external standard method based on peak area.

2.8 | Antioxidant enzymes activity

Samples were homogenized in phosphate buffer (0.05 M, pH 7) and centrifuged at 4 °C for 2 min at 7,000 rpm. The supernatant fraction was filtered (PET 0.45 μm, 25 mm) and used to determine CAT, GSH-Px, and SOD activity. CAT activity was measured by the rate of disappearance of 10 μM H_2O_2 for 30 s (Aebi, 1974) in 0.05 M phosphate buffer pH 7. The reduction of H_2O_2 was assessed by recording the decrease of the absorbance at 240 nm in a spectrophotometer Spectronic Genesys 2PC UV-VIS. One unit (U) of CAT was defined as the amount of extract required to decompose 1.0 μmole of hydrogen peroxide to oxygen and water per min. The GSH-Px activity assay was performed based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GSH-Px. GSSG was then reduced to GSH by glutathione reductase and NADPH (DeVore & Greene, 1982). NADPH consumption indicated GSH-Px activity. The concentration of NADPH was evaluated by absorbance at 340 nm using the same spectrophotometer described above. One unit (U) of GSH-Px was defined as the amount of extract required to oxidize 1 μmole of NADPH per min. SOD activity was determined following the method of Paoletti and Mocali (1990), based on the oxidation of NADH, mediated by superoxide radical generation from molecular oxygen, in the presence of EDTA, manganese(II) chloride, and mercaptoethanol. The presence of SOD in the medium causes a proportionate inhibition in the rate of NADH oxidation. The concentration of NADH was measured by absorbance at 340 nm using the spectrophotometer already described in this subsection. One unit (U) of SOD was defined as the amount of enzyme required to inhibit the rate of NADH oxidation of the control (blank) by 50%.

2.9 | Statistical analysis

The treatments were arranged in a split-plot design, with four replicates, in which muscle type (LTL and RF) and aging technique (VA and

TABLE 2 Physico-chemical parameters (mean ± SD) of Belgian Blue *longissimus thoracis et lumborum* (LTL) and *rectus femoris* (RF) samples at d_0

	LTL	RF
L^*	35.0 ± 1.2^b	43.8 ± 3.5^a
a^*	20.5 ± 1.9^a	22.4 ± 1.7^a
b^*	15.7 ± 1.4^b	20.6 ± 2.6^a
MMb %	0.0 ± 0.0^a	0.9 ± 1.3^a
TBARS	< LOQ	< LOQ
MRA (%)	57.5 ± 5.3^a	44.3 ± 6.3^b
Free fat (%)	2.1 ± 0.8^a	1.1 ± 0.3^b
SFA (%*)	53.5 ± 1.3^a	47.1 ± 0.8^b
MUFA (%*)	37.0 ± 4.6^a	32.7 ± 4.2^a
PUFA (%*)	9.5 ± 5.7^b	20.3 ± 4.3^a
SFA (mg/100 g meat)	1145 ± 431^a	529 ± 134^b
MUFA (mg/100 g meat)	809 ± 335^a	373 ± 134^a
PUFA (mg/100 g meat)	173 ± 44^a	220 ± 36^a
CAT (U/g)	269 ± 63^a	115 ± 30^b
GSH-Px (U/mg)	126 ± 35^a	95 ± 21^a
SOD (U/g)	190 ± 103^a	221 ± 82^a

[ab]Common lowercase letters in a row (muscle effect) indicate no statistical difference ($p < .05$).
*% of total fatty acids.
MMb % = metmyoglobin %; TBARS = thiobarbituric acid reactive substances; MRA = metmyoglobin reducing activity; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; CAT = catalase; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase.

CA) were allocated in the whole plots, while the subplots were aging time (7, 21, and 35 days) and display time (0 and 7 days). Each experimental unit consisted of a carcass. This model was used to analyze MMb %, MRA, and the activity of CAT, GSH-Px, and SOD. For ΔE and TBARS, display time was not included in the model. When a post hoc test was suitable, Tukey's test was performed.

Student *t*-test was performed to evaluate the effect of muscle type on L^*, a^*, b^*, MMb %, TBARS, MRA, free fat, the content of SFA, MUFA, and PUFA, and the activity of CAT, GSH-Px, and SOD at d_0, and to assess the effect of muscle type and aging technique on the α-tocopherol content at d_7.

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

3 | RESULTS AND DISCUSSION

3.1 | Color stability

The initial color parameters for LTL and RF at d_0 are shown in Table 2. RF presented higher lightness (L^*) and yellowness (b^*) than LTL ($p < .05$). However, initial redness (a^*) was similar between both types of muscle. The effect of muscle (LTL and RF), aging technique (VA and CA), aging time (7, 21, and 35 days), and all their interactions on ΔE are shown in Table 3. As ΔE combines L^*, a^*, and b^* values after and before MAP display, the effect of display time (0 and 7 days) could not be calculated for this parameter. The interaction aging technique × aging time was significant for ΔE ($p < .05$); vacuum-aging from d_0 to d_7 and total aging time favored the increase of ΔE, meaning a higher total color change over time (Figure 2).

pH-dependent changes in muscle structure determine the extent of light scattering at the meat surface (Hughes, Clarke, Purslow, & Warner, 2017) and thus affect meat color. The pH of LTL at d_0 was 5.50, and it did not vary during 35 days of aging. During MAP display, 5.50 was the lowest and 5.55 was the highest pH measured in LTL. For RF, the pH at d_0 was 5.55. During 35 days of aging, this value oscillated between 5.50 and 5.60. During MAP display of RF, the pH remained between 5.50 and 5.55 (data not shown in tabular form). These minimal pH variations observed over time may not have influenced color and color stability in our experiment.

MMb % at d_0 was 0.0 ± 0.0 and 0.9 ± 1.3 for LTL and RF, respectively (Table 2). The effect of muscle (LTL and RF), aging technique (VA and CA), aging time (7, 21, and 35 days), display time (0 and 7 days), and all their interactions on MMb % are shown in Table 3. The interactions muscle × aging technique × display time, muscle × aging time × display time, and aging technique × aging time × display time were significant for MMb % ($p<.05$). LTL had higher pigment stability than RF. Conversely, vacuum-aging from d_0 to d_7 and total aging time

TABLE 3 Analysis of variance (*F*-values) on the effect of muscle (*longissimus thoracis et lumborum* and *rectus femoris*), aging technique (vacuum-aging and stepwise carcass/vacuum-aging), aging time (7, 21, and 35 days), and display time (0 and 7 days) on the discoloration during MAP display (ΔE), metmyoglobin % (MMb %), lipid oxidation (TBARS), metmyoglobin reducing activity (MRA), and activity of catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) in beef

Effects	ΔE	MMb %	TBARS	MRA	CAT	GSH-Px	SOD
Muscle (M)	3.22	**50.71*****	**9.28***	**16.29****	0.11	1.34	2.95
Aging technique (T)	**4.86***	**34.30*****	0.58	3.14	0.16	1.97	**5.06***
Aging time (A)	**24.29*****	**68.51*****	**28.88*****	**4.16***	**20.01*****	**14.05*****	**36.62*****
Display time (D)	n/a	**421.77*****	n/a	**812.38*****	**4.86***	3.27	**28.10*****
M × T	0.04	4.62	0.14	0.19	1.72	0.20	1.86
M × A	2.60	**14.61*****	**9.72*****	0.81	3.72	2.07	2.43
M × D	n/a	**29.05*****	n/a	**19.57*****	1.49	0.73	0.83
T × A	**4.61***	**8.08*****	1.27	1.23	0.63	0.01	0.56
T × D	n/a	**49.18*****	n/a	**7.65****	0.07	1.53	**4.02***
A × D	n/a	**51.55*****	n/a	0.47	3.51	**42.59*****	**28.29*****
M × T × A	1.52	2.31	0.29	2.20	0.18	0.41	0.34
M × T × D	n/a	**4.94***	n/a	0.12	2.53	0.23	0.01
M × A × D	n/a	**5.14****	n/a	**3.87***	0.14	0.53	0.67
T × A × D	n/a	**10.46*****	n/a	1.41	0.42	0.49	0.44
M × T × A × D	n/a	0.46	n/a	0.36	0.26	1.44	0.01

Significant probabilities ($p < .05$) are in bold.
*Significant at $p < .05$.
**Significant at $p < .01$.
***Significant at $p < .001$.
n/a = not applicable.

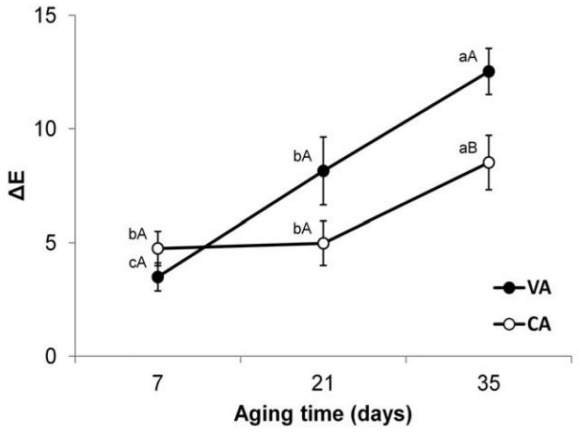

FIGURE 2 Total color change (ΔE) in beef during MAP display for the interaction aging technique × aging time. Bars represent standard error. Common lowercase letters (abc) for aging time within treatments and uppercase letters (AB) for treatments within each aging time indicate no statistical difference ($p < .05$). VA = vacuum-aging; CA = stepwise carcass/vacuum-aging

promoted early onset of pigment oxidation (Figure 3). The value of 40% MMb, reported by Greene, Hsin, and Zipser (1971) as the threshold value for consumers to reject meat, was exceeded in VALTL and VARF after 21 days of total aging and 7 days of MAP display. CARF exceeded the value of 40% after 35 days of total aging and 7 days of MAP display. By contrast, CALTL did not exceed this threshold in any condition (data not shown in tabular form).

The higher color stability of LTL compared to RF observed in the present study is consistent with the results from a study by McKenna et al. (2005), which classified LTL as a high color-stable muscle and RF as a moderate color-stable muscle. Color stability varies between muscles as their function in live animals will determine different metabolic patterns. Muscles associated with a glycolytic metabolism have a low oxygen consumption rate, which minimizes MMb accumulation. Additionally, muscles with a higher proportion of oxidative (β-red) fibers have faster rates of discoloration and increased MMb production under aerobic display (Kirchofer, Calkins, & Gwartney, 2002). Current data on the composition of muscle fiber types is variable. Ono, Solomon, Elsasser, Rumsey, and Moseley (1996) reported that LTL and RF from crossbred steers consist of 22.8% and 22.5% of β-red fibers, respectively. In another study by Kirchofer et al. (2002), a similar fiber-type composition was found in RF (23.9% β-red fibers), but a higher amount of β-red fibers (35.0%) was found in LTL from USDA Select carcasses. As the high myoglobin content of type β-red fibers results in a positive relationship between the proportion of these fibers and red color intensity (Listrat et al., 2016), and based on a^* only, our results suggest that there would be no significant difference in the β-red fiber composition between LTL and RF. In all cases, it is observed a lack of detailed studies involving the composition of muscle fiber types of Belgian Blue breed as well as other factors affecting discoloration of this meat including oxygen consumption rate, oxygen penetration depth, and myoglobin content. Finally, in a recent study by Canto et al. (2015), it was shown that

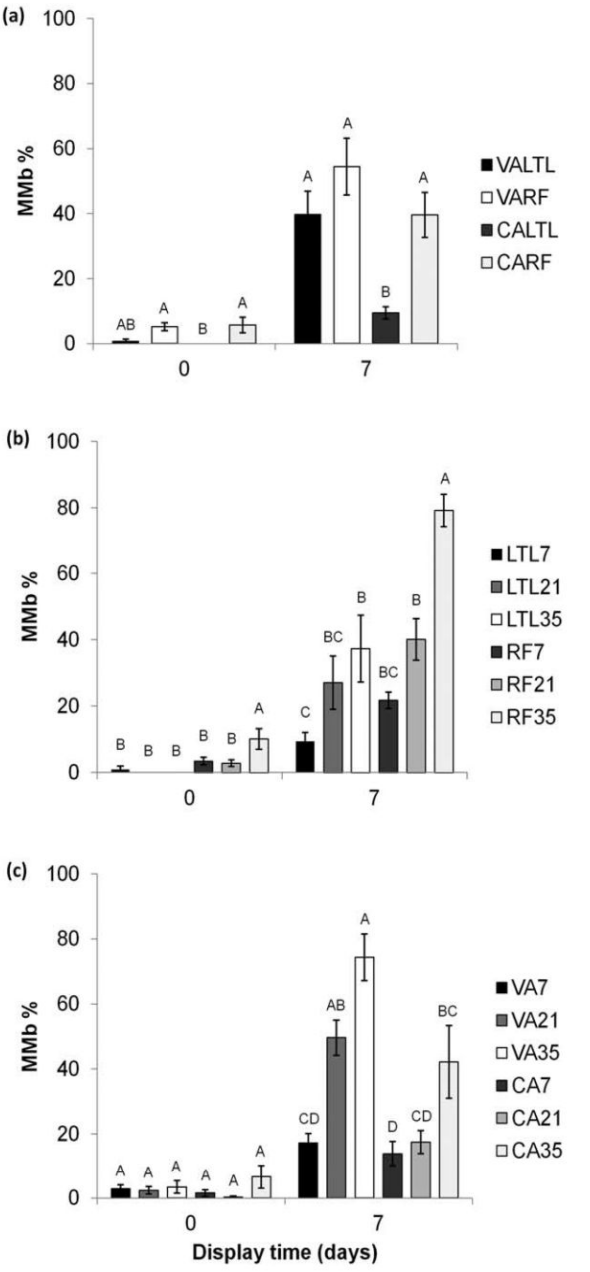

FIGURE 3 Myoglobin oxidation (MMb %) in beef for interactions (a) muscle × aging technique × display time, (b) muscle × aging time × display time and (c) aging technique × aging time × display time. Bars represent standard error. Display time effect within treatments was significant for all cases ($p < .05$). Common uppercase letters (ABC) for treatments within each display time indicate no statistical difference ($p < .05$). VA = vacuum-aging, CA = stepwise carcass/vacuum-aging; LTL = *longissimus thoracis et lumborum*; RF = *rectus femoris*

glycolytic enzymes including phosphoglucomutase-1, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase M2 were abundant in color-stable steaks and positively correlated with redness and color stability of *longissimus lumborum*. In this way, differential sarcoplasmic proteome may influence color and color stability and should be further investigated.

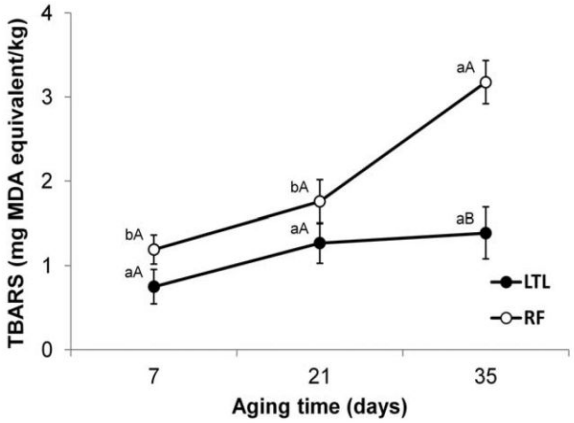

FIGURE 4 Lipid oxidation (TBARS) in beef during MAP display for the interaction muscle × aging time. Bars represent standard error. Common lowercase letters (ab) for aging time within treatments and uppercase letters (AB) for treatments within each aging time indicate no statistical difference ($p < .05$). LTL = *longissimus thoracis et lumborum*; RF = *rectus femoris*

The increase of discoloration and MMb % over time was also observed in a previous study by Vitale, Pérez-Juan, Lloret, Arnau, and Realini (2014) that found that color stability decreases during MAP display as aging time increases in meat types including LTL from mature cows. As reviewed by Suman, Hunt, Nair, and Rentfrow (2014), postmortem aging influences cellular mechanisms, including the mitochondrial reduction of MMb affecting the color stability of beef when it is subsequently retailed.

Lastly, differences in color stability between VA and CA can also be explained by our experimental scheme. LTL and RF are both entirely covered by superficial muscles from the back and thigh, respectively. In our study, VA samples were briefly in contact with air and light during retail cutting at d_0, while retail cutting of CA samples was performed at d_7 (Figure 1). It is known that exposure to atmospheric oxygen and light can lead to beef discoloration (Andersen, Bertelsen, & Skibsted, 1989; Lindahl, 2011). Thus, these factors may have contributed to the lower color stability of VA.

3.2 | Lipid stability

LTL had a higher free fat content than RF (Table 2). RF, on the other hand, had a higher PUFA proportion (expressed as the percentage of total fatty acids) than LTL (20.3 ± 4.3% for RF and 9.5 ± 5.7% for LTL, $p < .05$). This result can be explained by the fact that RF had less intramuscular fat than LTL. As lean meat contains more muscle tissue, it should, therefore, contain a higher proportion of polyunsaturated lipids than fatter meat (Allen & Foegeding, 1981). Furthermore, cattle with low concentrations of fat in muscle overall have higher proportions of PUFA in total lipids (Wood et al., 2008). Our results are in agreement with previous studies (Cabaraux et al., 2004; Clinquart, Hornick, Van Eenaeme, & Istasse, 1998; De Smet, Webb, Claeys, Uytterhaegen, & Demeyer, 2000). Cabaraux et al. (2004) studied the meat composition of Belgian Blue double-muscled culled females and reported similar intramuscular fat level and PUFA content in *longissimus thoracis* as in LTL in our study. Clinquart et al. (1998) and De Smet et al. (2000) found a relationship between the unsaturated nature of intramuscular fat in Belgian Blue and low-fat content, which was also accompanied by a high phospholipids to triacylglycerols ratio in the fat fraction.

The TBARS values for all samples were below the LOQ (0.2 mg MDA equivalent/kg) before display in MAP (data not shown in tabular form). The effect of muscle (LTL and RF), aging technique (VA and CA), aging time (7, 21 and 35 days), and all their interactions on TBARS are shown in Table 3. As TBARS were systematically below the LOQ before MAP display, the effect of display time (0 and 7 days) could not be evaluated for this parameter. The interaction muscle × aging time was significant for TBARS ($p < .05$). RF was more sensitive than LTL to lipid oxidation, and lipid stability during MAP display decreased as aging time increased (Figure 4). Despite its higher fat content, LTL presented higher lipid stability than RF. In fact, lipid oxidation is not only related to fat content but also to fatty acid profile, as the lipid oxidation process is thought to be initiated at the membrane level due to the oxidation of highly unsaturated membrane lipids (Morrissey, Buckley, Sheehy, & Monahan, 1994). In this way, the fact that RF had a higher proportion of PUFA may have increased its susceptibility to lipid oxidation.

As TBARS correlate well with sensory testing (Fernández, Pérez-Álvarez, & Fernández-López, 1997), they can be a good indicator of rancidity, mainly in meat and fish products (Irwin & Hedges, 2004), and of warmed-over flavor in cooked meats (Wilson, Pearson, & Shorland, 1976). Campo et al. (2006) propose that the TBARS value of 2.0 mg MDA equivalent/kg in raw beef can be considered the threshold from where rancid flavor overpowers beef flavor when it is cooked, and, consequentially, as the maximum level for a positive sensory perception of beef. If a critical limit of acceptability of 2.0 mg MDA equivalent/kg were established, the maximum aging time that would permit a following 7-day display at the retail level under high-oxygen atmosphere would be less than 35 days after chilling for RF and more than 35 days after chilling for LTL.

Finally, lipid oxidation and pigment oxidation seem to be linked. The higher lipid oxidation observed in RF may have led to the formation of chemical species, including α,β-unsaturated aldehydes, that amplified pigment oxidation and vice-versa (Monahan, Skibsted, & Andersen, 2005). Utrera, Morcuende, and Estévez (2014) found that fat content had a significant impact on meat protein oxidation possibly by transfer of lipid radicals and other reactive oxygen species from oxidizing lipids to proteins. However, this relationship was not investigated in our study.

3.3 | Antioxidant capacity

The effect of muscle (LTL and RF), aging technique (VA and CA), aging time (7, 21, and 35 days), display time (0 and 7 days), and all their interactions on MRA are shown in Table 3. The interactions aging technique × display time and muscle × aging time × display time were significant for MRA ($p < .05$). VA and RF showed lower MRA when compared to CA and LTL, respectively (Figure 5). In this way, MRA results partially explain the higher color stability of LTL as well for CA. After 7 days of MAP display, the MRA of most of the samples was zero. McKenna

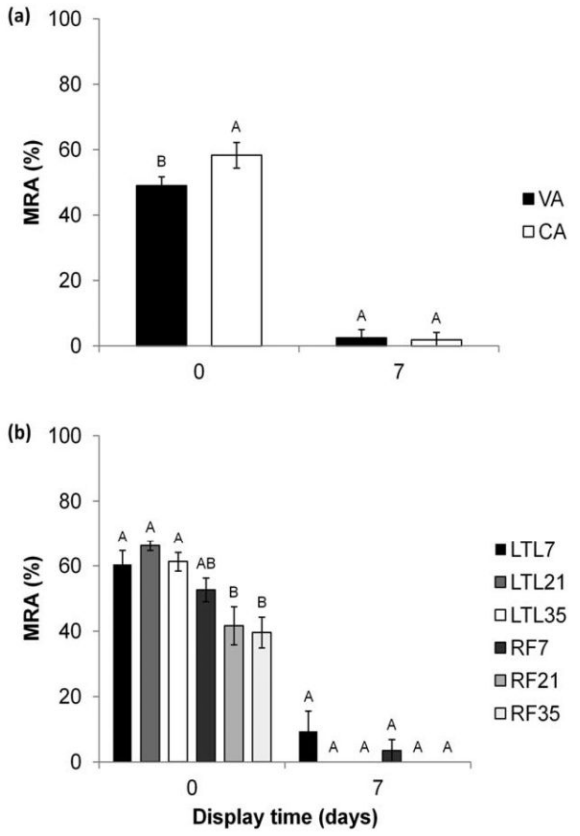

FIGURE 5 Metmyoglobin reducing activity (MRA) in beef for interactions (a) aging technique × display time and (b) muscle × aging time × display time. Bars represent standard error. Display time effect within treatments was significant for all cases ($p < .05$). Common uppercase letters (AB) for treatments within each display time indicate no statistical difference ($p < .05$). VA = vacuum-aging, CA = stepwise carcass/vacuum-aging; LTL = *longissimus thoracis et lumborum*; RF = *rectus femoris*

et al. (2005) also found that MRA was inversely correlated with days of display; specifically, decreasing MRA was observed with increasing days of display. As MRA is likely dependent on coenzymes such as NADH (Bekhit, Geesink, Morton, & Bickerstaffe, 2001), the enzymatic degradation of NADH could have been a reason for the reduction in MRA after 7 days of display in MAP (Bekhit, Geesink, Ilian, Morton, & Bickerstaffe, 2003). Tang, Faustman, Mancini, Seyfert, and Hunt (2005) highlighted that low oxygen or anaerobic conditions are required for electron transport chain dependent reduction of MMb confirming that the presence of oxygen in MAP reduces MRA.

RF had almost twofold higher α-tocopherol content than LTL ($p < .05$) (Table 4). The α-tocopherol content was not directly proportional to fat content but was directly proportional to PUFA %. Surprisingly, the higher α-tocopherol content observed in RF did not prevent oxidation in this muscle type, as shown by its higher sensitivity to oxidation than LTL. Research on the relationship between α-tocopherol content and oxidative stability is controversial. Lynch, Kerry, Buckley, Faustman, and Morrissey (1999) stated that dietary α-tocopherol supplementation appeared to delay MMb and TBARS formation and increase the color stability in LTL, *gluteus medius*, and *psoas major*

TABLE 4 α-Tocopherol content (mean ± SD) in Belgian Blue *longissimus thoracis et lumborum* (LTL) and *rectus femoris* (RF) samples at d_7

	α-Tocopherol (μg/g meat)	
Muscle	VA	CA
LTL	2.2 ± 0.6^{aB}	2.8 ± 0.3^{aB}
RF	4.4 ± 1.2^{aA}	4.1 ± 0.8^{aA}

[a]Common lowercase letters in a row (aging technique effect) indicate no statistical difference ($p < .05$).
[AB]Common uppercase letters in a column (muscle effect) indicate no statistical difference ($p < .05$).
CA = stepwise carcass/vacuum-aging; VA = vacuum-aging.

muscles. Other authors observed that α-tocopherol content played a minor role in lipid stability in beef with higher proportions of peroxidisable lipids (Yang, Lanari, Brewster, & Tume, 2002). Thus, α-tocopherol content alone cannot explain the difference in susceptibility to oxidation between muscles in our study.

The initial antioxidant enzymes (CAT, GSH-Px, and SOD) activity for LTL and RF at d_0 is shown in Table 2. Even though the activity of CAT and GSH-Px was numerically higher in LTL than in RF, only CAT activity was statistically significantly higher in LTL than in RF ($p < .05$). The difference in CAT activity between both muscle types can be partially related to the greater predisposition of RF to oxidation, as CAT limits oxidation in muscle tissues by decomposing hydrogen peroxide formed, for instance, after SOD scavenging of the superoxide anion (Terevinto, Ramos, Castroman, Cabrera, & Saadoun, 2010). The effect of muscle (LTL and RF), aging technique (VA and CA), aging time (7, 21, and 35 days), display time (0 and 7 days), and all their interactions on the activity of CAT, GSH-Px, and SOD are shown in Table 3. The interaction aging time × display time for the activity of the three antioxidant enzymes is represented in Table 5. As days of vacuum-aging between d_7 and d_{35} increased, the activity of GSH-Px and SOD decreased ($p < .05$). Furthermore, MAP display promoted a decrease of

TABLE 5 Catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) in beef for the interaction aging time × display time

		Display time			
Enzyme	Aging time	0	7	SEM	*p*
CAT (U/g)	7	210^{aA}	171^{bA}	7	.065
	21	174^{aA}	163^{aA}		
	35	224^{aA}	141^{bA}		
GSH-Px (U/mg)	7	108^{aA}	16^{bA}	4	<.001
	21	17^{aB}	12^{bA}		
	35	16^{aB}	10^{aA}		
SOD (U/g)	7	260^{aA}	61^{bA}	11	<.001
	21	215^{aA}	67^{bA}		
	35	97^{aB}	87^{aA}		

[ab]Common lowercase letters in a row (display time effect) indicate no statistical difference ($p < .05$).
[AB]Common uppercase letters in a column (aging time effect) within each enzyme indicate no statistical difference ($p < .05$).

the activity of CAT, GSH-Px and SOD in most of the samples ($p < .05$), which can explain pigment and lipid oxidation in samples during simulated retail display. Our results are in agreement with those from Pastsart, De Boever, Claeys, and De Smet (2013) who also observed a decrease in the activity of CAT and SOD in Belgian Blue *biceps femoris* and *longissimus dorsi* during MAP display. Finally, the SOD activity was higher in CA than in VA ($p < .05$) (data not shown in tabular form), which might have contributed to the oxidative stability of CA.

4 | CONCLUSIONS

Different muscles from the same animal have different oxidative patterns. RF presented a higher sensitivity to oxidation than LTL. Oxidative stability difference between muscles could be associated with MRA and CAT activity. A higher susceptibility to oxidation was also observed with VA when compared to CA. In this case, SOD activity could be related to the higher oxidative stability of CA samples. Moreover, increased aging time favored oxidation when meat was repackaged under a high-oxygen atmosphere, which could be explained by a decrease of MRA and the activity of CAT, GSH-Px, and SOD over time.

Despite the low number of animals used, this study contributes new knowledge about the oxidative status of beef when it is vacuum or carcass aged and subsequently stored in a high-oxygen atmosphere.

Further research should be conducted to understand the balance between pro-oxidants and antioxidants and their effect on lipid and pigment stability to explain differences between muscles. These studies will support the development of appropriate strategies for Belgian Blue beef aging and preservation.

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REFERENCES

Aebi, H. (1974). Catalase. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis* (2nd ed., pp. 673–684). Weinheim, Germany: Verlag Chemie GmbH.

Allen, C. E., & Foegeding, E. A. (1981). Some lipid characteristics and interactions in muscle foods. A review. *Food Technology*, *35*, 253–257.

American Meat Science Association. (2012). *Meat color measurement guidelines: Revised December 2012*. Champaign, IL: Author.

Andersen, H. J., Bertelsen, G., & Skibsted, L. H. (1989). Colour stability of minced beef. Ultraviolet barrier in packaging material reduces light-induced discoloration of frozen products during display. *Meat Science*, *25*(2), 155–159.

Bekhit, A. E., Geesink, G. H., Ilian, M. A., Morton, J. D., & Bickerstaffe, R. (2003). The effects of natural antioxidants on oxidative processes and metmyoglobin reducing activity in beef patties. *Food Chemistry*, *81*(2), 175–187.

Bekhit, A. E., Geesink, G. H., Morton, J. D., & Bickerstaffe, R. (2001). Metmyoglobin reducing activity and colour stability of ovine *longissimus* muscle. *Meat Science*, *57*(4), 427–435.

Beriain, M. J., Goni, M. V., Indurain, G., Sarries, M. V., & Insausti, K. (2009). Predicting *longissimus dorsi* myoglobin oxidation in aged beef based on early post-mortem colour measurements on the carcase as a colour stability index. *Meat Science*, *81*(3), 439–445.

Cabaraux, J. F., Hornick, J. L., Dotreppe, O., Dufrasne, I., Clinquart, A., & Istasse, L. (2004). Effects of the calving number on animal performance, carcase and meat composition in finishing Belgian Blue double-muscled culled females. *Livestock Production Science*, *87*(2–3), 161–169.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richardson, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science*, *72*(2), 303–311.

Canto, A. C., Suman, S. P., Nair, M. N., Li, S., Rentfrow, G., Beach, C. M., … King, D. A. (2015). Differential abundance of sarcoplasmic proteome explains animal effect on beef *Longissimus lumborum* color stability. *Meat Science*, *102*, 90–98.

Chan, K. M., Decker, E. A., & Feustman, C. (1994). Endogenous skeletal muscle antioxidants. *Critical Reviews in Food Science and Nutrition*, *34*(4), 403–426.

Clinquart, A., Hornick, J.-L., Van Eenaeme, C., & Istasse, L. (1998). Influence du caractère culard sur la production et la qualité de la viande des bovins Blanc Bleu Belge. *Productions Animales*, *11*, 285–297.

De Smet, S., Webb, E. C., Claeys, E., Uytterhaegen, L., & Demeyer, D. I. (2000). Effect of dietary energy and protein levels on fatty acid composition of intramuscular fat in double-muscled Belgian Blue bulls. *Meat Science*, *56*(1), 73–79.

DeVore, V. R., & Greene, B. E. (1982). Glutathione peroxidase in *post-rigor* bovine semitendinosus muscle. *Journal of Food Science*, *47*(5), 1406–1409.

Douny, C., El Khoury, R., Delmelle, J., Brose, F., Degand, G., Moula, N., … Scippo, M. L. (2015). Effect of storage and cooking on the fatty acid profile of omega-3 enriched eggs and pork meat marketed in Belgium. *Food Science and Nutrition*, *3*(2), 140–152.

Fernández, J., Pérez-Álvarez, J. A., & Fernández-López, J. A. (1997). Thiobarbituric acid test for monitoring lipid oxidation in meat. *Food Chemistry*, *59*(3), 345–353.

Francis, F. J., & Clydesdale, F. M. (1975). *Food colorimetry: Theory and applications*. Westport, CT: The Avi Publishing Company, Inc.

Greene, B. E., Hsin, I. M., & Zipser, M. W. (1971). Retardation of oxidative color changes in raw ground beef. *Journal of Food Science*, *36*(6), 940–942.

Hernandéz, P., Zomeño, L., Ariño, B., & Blasco, A. (2004). Antioxidant, lipolytic and proteolytic enzyme activities in pork meat from different genotypes. *Meat Science*, *66*(3), 525–529.

Hodges, J. H., Cahill, V. R., & Ockerman, H. W. (1974). Effect of vacuum packaging on weight loss, microbial growth and palatability of fresh beef wholesale cuts. *Journal of Food Science*, *39*(1), 143–146.

Hughes, J., Clarke, F., Purslow, P., & Warner, R. (2017). High pH in beef *longissimus thoracis* reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. *Food Research International*, *101*, 228–238.

Irwin, J. W., & Hedges, N. (2004). Measuring lipid oxidation. In R. Steele (Ed.), *Understanding and measuring the shelf-life of food* (pp. 289–316). Cambridge, UK: Woodhead Publishing.

ISO. (1996). *Viande et produits à base de viande – Détermination de la teneur en matière grasse libre, ISO 1444*. Geneva, Switzerland: Organisation Internationale de Normalisation.

ISO. (1997). *Viande et produits à base de viande – Détermination de l'humidité, ISO 1442*. Geneva, Switzerland: Organisation Internationale de Normalisation.

ISO. (1999). *Viande et produits à base de viande – Mesurage du pH, Méthode de référence, ISO 2917*. Geneva, Switzerland: Organisation Internationale de Normalisation.

Kirchofer, K. S., Calkins, C. B., & Gwartney, B. L. (2002). Fiber-type composition of muscles of the beef chuck and round. *Journal of Animal Science*, *80*(11), 2872–2878.

Lindahl, G. (2011). Colour stability of steaks from large beef cuts aged under vacuum or high-oxygen modified atmosphere. *Meat Science*, *87*(4), 428–435.

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., … Bugeon, J. (2016). How muscle structure and composition influence meat and flesh quality. *The Scientific World Journal*, *2016*, 1. Article ID 3182746, 14 pages.

Liu, Q. P., Scheller, K. K., & Schaefer, D. M. (1996). Technical note: A simplified procedure for vitamin E determination in beef muscle. *Journal of Animal Science*, *74*(10), 2406–2410.

Lynch, M. P., Kerry, J. P., Buckley, D. J., Faustman, C., & Morrissey, P. A. (1999). Effect of dietary vitamin E supplementation on the colour and lipid stability of fresh, frozen and vacuum-packaged beef. *Meat Science*, *52*(1), 95–99.

McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W., & Savell, J. W. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science*, *70*(4), 665–682.

Monahan, F. J., Skibsted, L. H., & Andersen, M. L. (2005). Mechanism of oxymyoglobin oxidation in the presence of oxidizing lipids in bovine muscle. *Journal of Agricultural and Food Chemistry*, *53*(14), 5734–5738.

Morrissey, P. A., Buckley, D. J., Sheehy, P. J., & Monahan, F. J. (1994). Vitamin E and meat quality. *The Proceedings of the Nutrition Society*, *53*(2), 289–295.

Obuz, E., Akkaya, L., Gök, V., & Dikeman, M. E. (2014). Effects of blade tenderization, ageing method and ageing time on meat quality characteristics of *Longissimus lumborum* steaks from cull Holstein cows. *Meat Science*, *96*(3), 1227–1232.

Ono, Y., Solomon, M. B., Elsasser, T. H., Rumsey, T. S., & Moseley, W. M. (1996). Effects of Synovex-S and recombinant bovine growth hormone (Somavubove) on growth responses of steers: II. Muscle morphology and proximate composition of muscles. *Journal of Animal Science*, *74*(12), 2929–2934.

Paoletti, F., & Mocali, A. (1990). Determination of superoxide dismutase activity by purely chemical system based on NAD(P)H oxidation. *Methods in Enzymology*, *186*, 209–220.

Pastsart, U., De Boever, M., Claeys, E., & De Smet, S. (2013). Effect of muscle and post-mortem rate of pH and temperature fall on antioxidant enzyme activities in beef. *Meat Science*, *93*(3), 681–686.

R Core Team. (2016). R: A language and environment for statistical computing. Retrieved from https://www.R-project.org/

Raharjo, S., Sofos, J. N., & Schmidt, G. R. (1992). Improved speed, specificity, and limit of determination of an aqueous acid-extraction thiobarbituric acid-C_{18} method for measuring lipid-peroxidation in beef. *Journal of Agricultural and Food Chemistry*, *40*(11), 2182–2185.

Smith, A. M., Harris, K. B., Griffin, D. B., Miller, R. K., Kerth, C. R., & Savell, J. W. (2014). Retail yields and palatability evaluations of individual muscles from wet-aged and dry-aged beef ribeyes and top sirloin butts that were merchandised innovatively. *Meat Science*, *97*(1), 21–26.

Smith, R. D., Nicholson, K. L., Nicholson, J. D., Harris, K. B., Miller, R. K., Griffin, D. B., & Savell, J. W. (2008). Dry versus wet ageing of beef: Retail cutting yields and consumer palatability evaluations of steaks from US Choice and US Select short loins. *Meat Science*, *79*(4), 631–639.

SPW. (2014). *Blanc-Bleu Belge, Blonde d'Aquitaine, Charolaise, Limousine, les quatre races viandeuses les plus répandues en Wallonie*. Jambes, Belgium: SPW Editions Bilans et perspectives.

Stenström, H., Li, X., Hunt, M. C., & Lundström, K. (2014). Consumer preference and effect of correct or misleading information after ageing beef *longissimus* muscle using vacuum, dry ageing, or a dry ageing bag. *Meat Science*, *96*(2 Pt A), 661–666.

Suman, S. P., Hunt, M. C., Nair, M. N., & Rentfrow, G. (2014). Improving beef color stability: Practical strategies and underlying mechanisms. *Meat Science*, *98*(3), 490–504.

Tang, J., Faustman, C., Mancini, R. A., Seyfert, M., & Hunt, M. C. (2005). Mitochondrial reduction of metmyoglobin: Dependence on the electron transport chain. *Journal of Agricultural and Food Chemistry*, *53*(13), 5449–5455.

Terevinto, A., Ramos, A., Castroman, G., Cabrera, M. C., & Saadoun, A. (2010). Oxidative status, *in vitro* iron-induced lipid oxidation and superoxide dismutase, catalase and glutathione peroxidase activities in rhea meat. *Meat Science*, *84*(4), 706–710.

Utrera, M., Morcuende, D., & Estévez, M. (2014). Fat content has a significant impact on protein oxidation occurred during frozen storage of beef patties. *LWT - Food Science and Technology*, *56*(1), 62–68.

Vitale, M., Pérez-Juan, M., Lloret, E., Arnau, J., & Realini, C. E. (2014). Effect of ageing time in vacuum on tenderness, and color and lipid stability of beef from mature cows during display in high-oxygen atmosphere package. *Meat Science*, *96*(1), 270–277.

Wilson, B. R., Pearson, A. M., & Shorland, F. B. (1976). Effect of total lipids and phospholipids on warmed-over flavor in red and white muscle from several species as measured by thiobarbituric acid analysis. *Journal of Agricultural and Food Chemistry*, *24*(1), 7–11.

Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., … Whittington, F. M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*, *78*(4), 343–358.

Yang, A., Lanari, M. C., Brewster, M., & Tume, R. K. (2002). Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement. *Meat Science*, *60*(1), 41–50.

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Chapter 5
Biopreservation of fresh meat

Biopreservation is the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life. Since ancient times, man has used the fermentation of meat as a mode of preservation.

If in fermented meat products the concept and applications of biopreservation appear natural, it is not the same for fresh products. Fermentation is, of course, an old method of biopreservation, but it results from a transformation of the product: the physicochemical parameters being modified, the ecosystem is affected, with positive consequences for the microbiological safety of these products. In the case of fresh meat, on the other hand, the aim is to preserve to the product all its initial organoleptic quality, modifying the microbial ecosystem in favour of the positive flora, without however initiating transformation by fermentation (Feurer *et al.*, 2013). At this time, there is no evidence that biopreservation is voluntarily used in fresh meat.

In **Chapter 5**, dedicated to the biopreservation in fresh meat, two studies will be included: “Phenotypic, genetic and functional characterisation of *Carnobacterium maltaromaticum* with potential as biopreservatives isolated from beef with extremely long shelf-life” and “*In vitro* evaluation of the competing effect of *Carnobacterium maltaromaticum* isolated from vacuum-packaged meat against food pathogens”. The manuscripts of these two studies are currently under preparation.

All *C. maltaromaticum* used in the studies of this chapter were isolated in the research presented in **Chapter 3**. The first study had as objective to perform a phenotypic, genetic and functional characterisation of *C. maltaromaticum*. The second study aimed to evaluate *in vitro* the effect of three *C. maltaromaticum* isolates against three major food pathogens, namely *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7.

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PHENOTYPIC, GENETIC AND FUNCTIONAL CHARACTERISATION OF *CARNOBACTERIUM MALTAROMATICUM* WITH POTENTIAL AS BIOPRESERVATIVES ISOLATED FROM BEEF WITH EXTREMELY LONG SHELF-LIFE

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Abstract: **This study aimed to perform a phenotypic, genetic and functional characterisation of *Carnobacterium* isolated from beef with extremely long shelf-life stored at −1 °C, and investigate their potential as protective cultures in meat. Eleven isolates were tested for their morphologic and metabolic characteristics, the presence of bacteriocin genes, their ability to grow under different atmospheres and their effect on the growth of spoilage bacteria in beef and sensory quality of beef patties. The phenotypic profiling revealed that all isolates belonged to the species *Carnobacterium maltaromaticum*. The genetic profiling showed the presence of gene *cbnBM1* coding for carnobacteriocin BM1 in all isolates and *cbnB2* coding for carnobacteriocin B2 in six isolates. *Carnobacterium maltaromaticum* displayed more significant growth in an atmosphere without oxygen, and its inhibition effect against Enterobacteriaceae was only observed in the absence of oxygen. Hence, the use of *C. maltaromaticum* as a protective culture should be associated with systems such as vacuum packaging. Finally, the negative impact of the inoculation with one of the isolates on the sensory quality of beef patties was not very intense. Further research should focus on the effect of *C. maltaromaticum* on the growth of pathogenic bacteria and sensory attributes of other meat products.**

Keywords: **biopreservation, carnobacteriocin, *Carnobacterium maltaromaticum*, meat, shelf-life**

Highlights:

- ***Carnobacterium maltaromaticum* was isolated from beef with extremely long shelf-life.**
- **Isolates possessed genes coding for bacteriocins.**
- ***C. maltaromaticum* showed an inhibition effect against Enterobacteriaceae in meat.**
- ***C. maltaromaticum* may have minimal impact on the sensory quality of meat.**

1 Introduction

The principal factors that must be addressed in the preservation of chilled meat are the retention of an attractive and fresh appearance of the product which is displayed and retardation of bacterial spoilage, while keeping food safety. The storage life of fresh meat can be extended to several weeks by proper control of the hygienic condition of the product and storage temperature, as well as the appropriate selection of packaging (Gill, 1996). The most commonly used method of preserving primal cuts is to vacuum packaging, thereby excluding oxygen and preventing the growth of oxygen-requiring spoilage bacteria (Gill, 1989; Mills, Donnison, & Brightwell, 2014). In order to further minimise a decrease in product quality, due either to spoilage by bacteria capable of anaerobic growth or to biochemical processes affecting oxidative stability, storage at subzero temperatures (−1.5 °C) has been recommended (Jeremiah & Gibson, 2001; Mills, et al., 2014). Currently, the European Union law allows only two preserving processes for fresh meat: refrigeration and packaging in vacuum and modified atmosphere (European Parliament and Council of the European Union, 2004). However, the development of novel preservation techniques, such as biopreservation, could constitute selective hurdles that the spoilage and pathogenic microorganisms should not be able to overcome.

Biopreservation is the general term for methods of food preservation that use microbial activities to inhibit the growth of spoilage and pathogenic microorganisms (Yost, 2014). Antagonistic cultures that are only added to inhibit pathogens and to extend the shelf-life, while changing the sensory properties of the product as little as possible, are termed protective cultures (Lücke, 2000). Lactic acid bacteria (LAB) have significant potential for use in biopreservation (Castro, et al., 2017; Orihuel, et al., 2018; Tirloni, et al., 2014), exerting their inhibitory effect against other microorganisms as a result of the competition for nutrients and the production of bacteriocins or other antagonistic compounds such as organic acids, hydrogen peroxide and enzymes (Castellano, Belfiore, Fadda, & Vignolo, 2008). Moreover, LAB are generally recognised as safe for consumption and, during storage, they naturally dominate the microbiota of meat and meat products (Castellano, et al., 2008).

Carnobacterium is a relatively recently described genus of the LAB group (Oluk & Karaca, 2018). The genus *Carnobacterium* is comprised of little more than ten species frequently isolated from foods of animal origin or cold environments (Cailliez-Grimal, Afzal, & Revol-Junelles, 2014), and *Carnobacterium divergens* and *Carnobacterium maltaromaticum* are frequently a predominating element of the microbiota of chilled vacuum- or modified atmosphere-packaged meat and seafood (Laursen, et al., 2005). The use of *C. maltaromaticum* as a protective culture against pathogenic (Brillet, Pilet, Prevost, Cardinal, & Leroi, 2005; dos Reis, et al., 2011; Koné, et al., 2018) and spoilage (Spanu, et al., 2018) microorganisms has been assessed in diverse food matrix. Nevertheless,

C. maltaromaticum may also be a potential spoilage organism in meat products (Laursen, et al., 2005).

In the present research, we studied *C. maltaromaticum* isolated from beef with extremely long shelf-life stored at −1 °C. We aimed to perform a phenotypic, genetic and functional characterisation of *C. maltaromaticum* isolates, and investigate their potential as protective cultures in meat and meat products.

2 Material and methods

2.1 Carnobacterium maltaromaticum *isolates*

Bacterial isolates of *C. maltaromaticum* from different vacuum-packaged beef cuts with extremely long shelf-life, in which previous metagenetic analysis had revealed the predominance of the genus *Carnobacterium* (Imazaki, et al., 2012), were selected for this study. Isolation of *C. maltaromaticum* was carried as follows. Six vacuum-packaged *longissimus thoracis et lumborum* from Australia were supplied by a Belgian food wholesaler. Three samples of the same batch were transported to our laboratory each time, over a period of three months, as soon as a load with imported meat was delivered to the food wholesaler. The shelf-life was labelled 140 days at < 2 °C, and our supplier reported storage at maximum 0 °C throughout distribution. Samples arrived at our laboratory approximately 60 days after packaging and were stored at −1 °C until ⅔ of their shelf-life (93 days) when they were cut, in a sterile way, into 2–3 cm-thick steaks. Part of the steaks was vacuum-packaged and stored at 4 °C until the end of the shelf-life (140 days). At ⅔ and the end of the shelf-life, 25 cm^2 (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 (primary suspension). Serial decimal dilutions were prepared using the same dilutant. Primary suspensions and their dilutions were plated on plate count agar (PCA). After incubation at 22 °C for 48 h, one colony out of each of the 32 plates showing bacterial growth was purified. The 32 pure colonies obtained were characterised using API 50 CHL galleries. Twenty-five isolates (78%) were identified as *C. maltaromaticum*, one isolate (3%) was identified as *C. divergens*, and six isolates (19%) could not be identified. Eleven isolates identified as *C. maltaromaticum* were selected for this research (CM_B820, CM_B821, ... and CM_B830), representing different meat samples and storage time/temperature (**Table 1**).

Table 1 Identification of *Carnobacterium maltaromaticum* isolates and storage conditions of Australian bovine vacuum-packaged *longissimus thoracis et lumborum* used to obtain the isolates.

Isolate code	Batch no.	Sample no.	Storage time at < 0 °C (days)	Storage time at 4 °C (days)	Total storage time (days)
CM_B820	1	1	93	47	140
CM_B821	1	2	93	47	140
CM_B822	1	3	93	47	140
CM_B823	1	1	93	0	93
CM_B824	1	2	93	0	93
CM_B825	1	3	93	0	93
CM_B826	2	4	93	0	93
CM_B827	2	6	93	0	93
CM_B828	2	4	93	47	140
CM_B829	2	5	93	47	140
CM_B830	2	6	93	47	140

2.2 Phenotypic characterisation of the isolates

The following analyses were performed on 11 isolates of *C. maltaromaticum* (CM_B820 to CM_B830) and two reference strains (LMG 11393 and LMG 22902). Cell morphology was determined by direct microscopic observation. Gram test and catalase activity were performed by standard methods. The activity of oxidase was assessed by Bactident oxidase tests (Merck), according to the supplier's instructions. The carbohydrate-fermenting capacity of 50 carbohydrates and the activity of 19 enzymes were examined using API 50 CH and API ZYM (Biomérieux), respectively. The testing tubes were inoculated with a pure culture suspension, previously grown overnight on PCA at 25 °C, according to the manufacturer's instructions. API 50 CH tubes were sealed using mineral oil and incubated at 30 °C for 48 h. API ZYM tubes were incubated at 30 °C for 4 h. After incubation, carbohydrate fermentation and enzyme activity were evaluated.

2.3 Genome sequencing and detection of bacteriocin genes

The eleven *C. maltaromaticum* isolates (CM_B820 to CM_B830) were cultured in brain heart infusion broth and harvested in the mid-logarithmic phase. The genomic DNA was extracted and purified using a DNeasy kit for DNA extraction (Qiagen) according to the manufacturer's instruction. Whole-genome sequencing was performed using a MiSeq sequencer (Illumina), and the genome was annotated using Subsystems Technology (RAST) version 2.0 (Meyer, et al., 2008). The genome of the 11 isolates was aligned using

the software tool Gegenees version 2.1 (Agren, Sundstrom, Hafstrom, & Segerman, 2012), and the results were visualised and analysed using the program Splits Tree version 4.13.1 (Huson, 1998). Subsequently, we focused on genes encoding for bacteriocins (*cbnBM1* for carnobacteriocin BM1 and *cbnB2* for carnobacteriocin B2). The identification of genes coding for bacteriocins was performed using the software BAGEL3 (van Heel, de Jong, Montalban-Lopez, Kok, & Kuipers, 2013).

2.4 Influence of different atmospheres on the growth of C. maltaromaticum

Irradiated minced pork meat, used as a model of sterile meat, was inoculated (1% v/w) with a suspension of the isolate CM_B820 (10^5 CFU/mL) in order to reach a theoretical concentration of 10^3 CFU/g. Meat and inoculum were homogenised in a stand mixer (model Kitchen Grand Chef, Kenwood) for 2.5 min. Eighty grams of inoculated meat were repackaged in PP/EVOH/PP trays (ES-Plastic, dimensions: 187 × 137 × 50 mm, oxygen permeability: 4 cm³/m² at 24 h bar, 23 °C and 0% RH) containing a modified atmosphere and sealed with a PET/PP film (Wipak, oxygen permeability: 8.4 cm³/m² at 24 h bar, 23 °C and 0% RH). Three atmospheres were tested: 100% N_2, 70/30% O_2/CO_2 and 70/30% CO_2/O_2. The trays were stored at 4, 8 and 12 °C for 7 days. Bacterial counting was performed on PCA at 25 °C after 0, 3 and 7 days of storage. Tests were performed in triplicate.

2.5 Microbiological stability of commercial beef inoculated with C. maltaromaticum

Two bovine vacuum-packaged *psoas major* muscles from the same batch were supplied by a food wholesaler located in the Walloon Region of Belgium 16 days after slaughter. After the reception at our laboratory, 3 cm thick steaks were cut and inoculated on the surface (1% v/w) with a suspension of the isolate CM_B820 (10^5 CFU/mL) in order to reach a theoretical concentration of 10^3 CFU/g. Samples were repackaged in vacuum and stored at −1 °C for 7 days (large-scale market simulation). Vacuum bags (Cryovac) were 60 µm thick, and the oxygen permeability was 13 cm³/m² 24 h bar at 23 °C and 0% relative humidity (RH). Then, they were repackaged in PP/EVOH/PP trays containing a modified atmosphere and sealed with a PET/PP film. Two atmospheres were tested: 100% N_2 and 70/30% O_2/CO_2, and steaks from the same muscle were used for each tested atmosphere. The trays were stored at 4 °C for 7 days (display simulation). Blank was made by replacing the *C. maltaromaticum* suspension with sterile saline, and tests were performed in triplicate. Total viable count (TVC), LAB, Enterobacteriaceae (EB), *Pseudomonas* spp. (PS) and *Brochothrix thermosphacta* (BT) counts were performed immediately before inoculation with *C. maltaromaticum* (d_0) and at 0, 3 and 7 days of display (d_7, d_{10} and d_{14}) as follows. 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 (primary suspension). Serial decimal dilutions were prepared using the same dilutant. The concentration of TVC, LAB and EB was measured by plating on PCA, De Man, Rogosa and Sharpe agar and violet red bile dextrose agar, respectively. Plates were incubated at 22 °C for 48 h for TVC, 22 °C for 72 h for LAB and 30 °C for 24 h for EB. The enumeration of PS was performed by plating on cephalothin–sodium fusidate–cetrimide agar (CFC) agar with CFC-selective supplement and incubating at 25 °C for 48 h, as per ISO 13720 (ISO, 1995). Colonies were confirmed by oxidase tests and fermentation profiling on Kligler agar. Finally, BT was enumerated by plating on streptomycin–sulphate, thallous–acetate and actidione (STAA) agar with STAA-selective supplement and incubating at 22 °C for 48 h, as per ISO 13722 (ISO, 1996). Colonies were confirmed by oxidase tests.

2.6 Sensory quality of beef patties inoculated with C. maltaromaticum

Commercial bovine minced meat preparation (89% beef, water, 0.9% vegetal fibres, salt, silica dioxide, ascorbic acid, sodium acetate and sodium citrate) for the production of beef patties, displaying a shelf-life of 8 days, was supplied by a meat plant located in the Walloon region of Belgium on the same day of production. Three meat preparation batches were made, and each batch was inoculated (1% v/w) with a suspension of one of the three selected isolates of *C. maltaromaticum* (CM_B824, CM_B827 and CM_B829) at 10^6 or 10^8 CFU/mL in sterile physiological saline to achieve a final concentration of 10^4 and 10^6 CFU/g, respectively. After inoculation, portions of 90 g were moulded into 2 cm thick beef patties. The beef patties were packaged in PP/EVOH/PP trays containing a modified atmosphere (80/20% O_2/CO_2), sealed with a PET/PP film and stored at 4 °C for 5 days and then at 8 °C for 3 days, in order to simulate a break in the cold chain during distribution. Blank was performed with sterile physiological saline instead of a *C. maltaromaticum* suspension. After 8 days of storage, part of the samples was grilled on a frying top (model FTL35E/6/0, Tecnoinox) until they reached an internal temperature of 75 °C.

An untrained panel of 12 members was requested to make a sensory evaluation of raw and cooked samples after 8 days of storage, by scoring five descriptors (appearance, odour, colour, tenderness, flavour and juiciness) from 1 (= dislike) to 5 (= like). Tenderness, flavour and juiciness were evaluated only in cooked samples.

2.7 Statistical analysis

For the results concerning the influence of different atmospheres on the growth of *C. maltaromaticum*, two-way analysis of variance (ANOVA) was used to calculate the

effect of time (0, 3 and 7 days) and the atmosphere (100% N_2, 70/30% O_2/CO_2 and 70/30% CO_2/O_2), as well as their interaction, on the growth of *C. maltaromaticum* at each temperature (4, 8 and 12 °C). Data relating to the microbiological stability of commercial beef inoculated with *C. maltaromaticum* was analysed by two-way ANOVA. The effect of time (7, 10 and 14 days) and the inoculum (blank and *C. maltaromaticum*), as well as their interaction, on TVC, LAB, EB and BT were calculated. Concerning the results relating to the sensory quality of beef patties inoculated with *C. maltaromaticum*, two-way ANOVA was used to calculate the effect of the inoculum (blank, CM_B824, CM_B827 and CM_B829) and concentration of the inoculum (10^4 and 10^6 CFU/g meat) on different sensory attributes (appearance, odour, colour, tenderness, flavour and juiciness) in raw and cooked samples. When necessary, Tukey's test was used to assess differences between test groups. All statistical analyses were performed using VassarStats online.

3 Results

3.1 Phenotypic profiling

The colonies obtained from the eleven isolates (CM_B820 to CMB_830) and two reference strains (LMG 11393 and LMG 22902) presented the following characteristics: Gram-positive bacillus-shaped cells arranged in pairs, catalase and oxidase negative.

The API 50 CH system showed that the eleven isolates (CM_B820 to CMB_830) and two reference strains (LMG 11393 and LMG 22902) could ferment the following carbohydrates and derivates: glycerol, D-ribose, D-galactose (except CM_B828 and LMG 11393), D-glucose, D-fructose, D-mannose, D-mannitol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin ferric citrate, salicin, D-cellobiose, D-maltose (except CM_B823), D-lactose (only CM_B820, CM_B821, CM_B822, CM_B823, CM_B830, LMG 11393 and LMG 22902), D-melibiose (except CM_B826, CM_B827, CM_B828, CM_B830 and LMG 11393), D-saccharose, D-trehalose, inuline (except CM_B820, CM_B828 and LMG 22902), D-melezitose (except CM_B823 and LMG 22902), amidon (only CM_B821, CM_B826, CM_B829, CM_B830 and LMG 11393), gentiobiose, D-turanose and potassium gluconate. API 50 CH tests confirmed that all isolates belonged to the species *C. maltaromaticum*.

The API ZYM test revealed the activity of the following enzymes: alkaline phosphatase (only CM_B824), C4 esterase (except CM_B827), C8 esterase lipase, C14 lipase (only CM_B827), valine arylamidase (except CM_B822, CM_B823, CM_B825, CM_B826, CM_B828 and LMG 22902), cystine arylamidase (only CM_B821), trypsine (only CM_B826, CM_B828 and CM_B830), α-chrymotrypsine (only

CM_B829), acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, N-acetyl-α-D-glucosaminidase (only CM_B822 and LMG 22902) and α-mannosidase (only CM_B824, CM_B828 and CM_B830).

3.2 Genetic profiling

The interpretation of the phylogenetic tree resulting from the alignment of the genomes of the eleven isolates of *C. maltaromaticum* (CM_B820 to CM_B830), as well as one reference strain (CM_LMA29), revealed the existence of four distinct groups based on their genetic profile: (1) CM_LMA28, (2) CM_B820 to CM_B825, (3) CM_B826, CM_B828 and CM_B829 and (4) CM_B827 and CM_B830 (**Figure 1**). Based on this result, the isolate CM_B820 was selected for the study of the influence of different atmospheres on the growth of *C. maltaromaticum* and microbiological stability of commercial beef inoculated with *C. maltaromaticum* (**Section 3.3**) since it belonged to the group with the most isolates. Isolates CM_B824, CM_B827 and CM_B829 were chosen for the assessment of the sensory quality of beef patties inoculated with *C. maltaromaticum* (**Section 3.3**) because they belonged to three distinct genetic groups.

The detection of bacteriocin genes in the genome of the eleven *C. maltaromaticum* isolates (CM_B820 to CM_830) was performed to assess their bioprotective potential. All the isolates possessed the gene *cbnBM1* coding for carnobacteriocin BM1. However, only

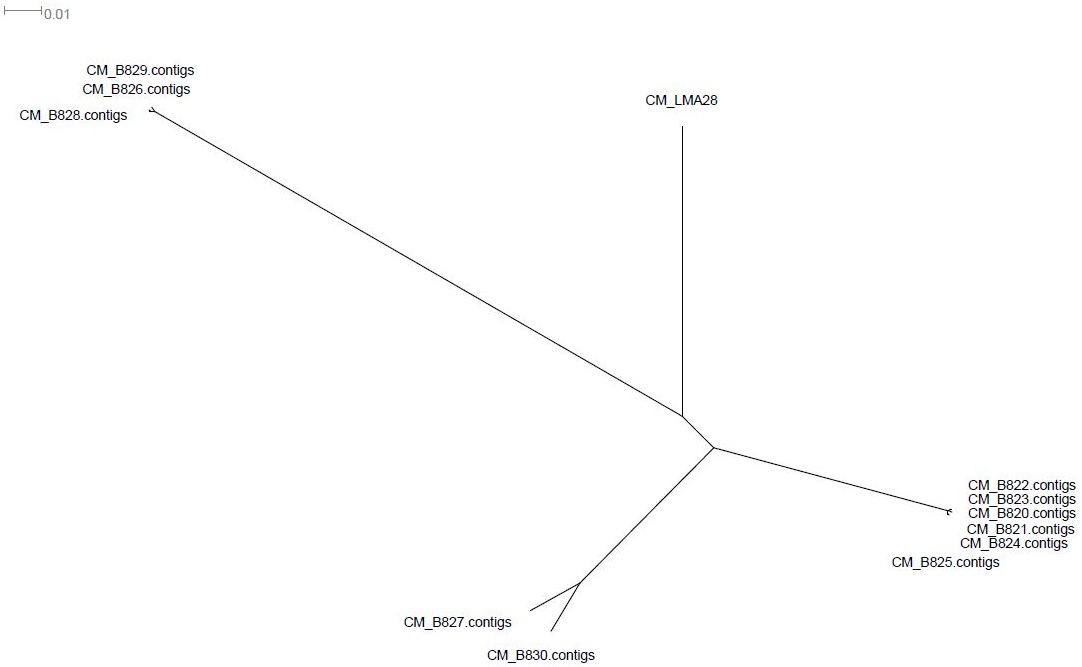

Figure 1 Graphical interpretation of the alignment of the genome of 11 isolates of *Carnobacterium maltaromaticum* (CM_B820 to CM_B830) and one reference strain (CM_LMA28).

isolates from batch no. 1 (CM_820 to CM_825) were positive for the gene *cbnB2* coding for carnobacteriocin B2.

3.3 Functional profiling

The influence of three different atmospheres (100% N_2, 70/30% O_2/CO_2 and 70/30% CO_2/O_2) on the growth of one isolate of *C. maltaromaticum* (CM_B820) at different temperatures (4, 8 and 12 °C) was evaluated in irradiated minced pork meat, used as model of sterile meat, after 0, 3 and 7 days of storage. Immediately after the inoculation of meat with a suspension of the isolate CM_B820 (d_0), the *C. maltaromaticum* load was 3.3 $\log_{10}$ CFU/g. After inoculation and incubation for 7 days, the interaction atmosphere × time was observed for the three storage temperatures ($P < 0.05$). At 4 and 8 °C, the atmosphere containing 100% N_2 promoted the highest growth of *C. maltaromaticum*. Furthermore, after 7 days of storage at 8 °C, *C. maltaromaticum* reached 7.7 $\log_{10}$ CFU/g (**Figures 2.a** and **2.b**). Contrariwise, at 4 and 8 °C, *C. maltaromaticum* presented a low growth in the atmospheres containing O_2 and CO_2. At 12 °C, the fastest growth rate was observed in the atmosphere with 100% N_2. However, after 7 days of incubation, *C. maltaromaticum* reached the same concentration in the atmospheres containing 100% N_2 and 70/30% O_2/CO_2. Finally, at 12 °C, the lowest growth of *C. maltaromaticum* was observed in the atmosphere containing 70/30% CO_2/O_2 (**Figure 2.c**).

Two vacuum-packaged *psoas major* samples were used to evaluate the microbial stability of commercial beef inoculated with one isolate of *C. maltaromaticum* (CM_B820) after 7 days of storage in vacuum at −1 °C (d_7) and a subsequent storage for 7 days in two modified atmospheres (100% N_2 and 70/30% O_2/CO_2) at 4 °C (d_{14}). **Table 2** shows the microbial counts for the steaks used to evaluate the atmosphere containing 100% N_2 immediately before inoculation with *C. maltaromaticum* (d_0). During the subsequent storage in 100% N_2, the interaction time (7, 10 and 14 days after inoculation) × inoculum (blank and *C. maltaromaticum*) was significant ($P < 0.05$). Total viable count (TVC) increased over time and was higher in inoculated samples than non-inoculated samples throughout the storage (**Figure 3.a**). Similarly, LAB increased in inoculated samples during 7 days of storage. However, they remained stable between the last 4 days of storage in non-inoculated samples (**Figure 3.b**). A growth of EB was observed during the last 4 days of storage in non-inoculated samples. By contrast, no growth of EB was observed in inoculated samples during 7 days of storage (**Figure 3.c**). The growth of BT was higher in inoculated samples than in non-inoculated samples (**Figure 3.d**). Finally, PS counts remained below the counting threshold (data not shown in graphical form). Regarding the evaluation of the microbial stability of beef inoculated with *C. maltaromaticum* in an atmosphere containing 70/30% O_2/CO_2, an effect of time was observed for TVC, LAB and

BT ($P < 0.05$). However, the effect of inoculum was not significant (data not shown in graphical form).

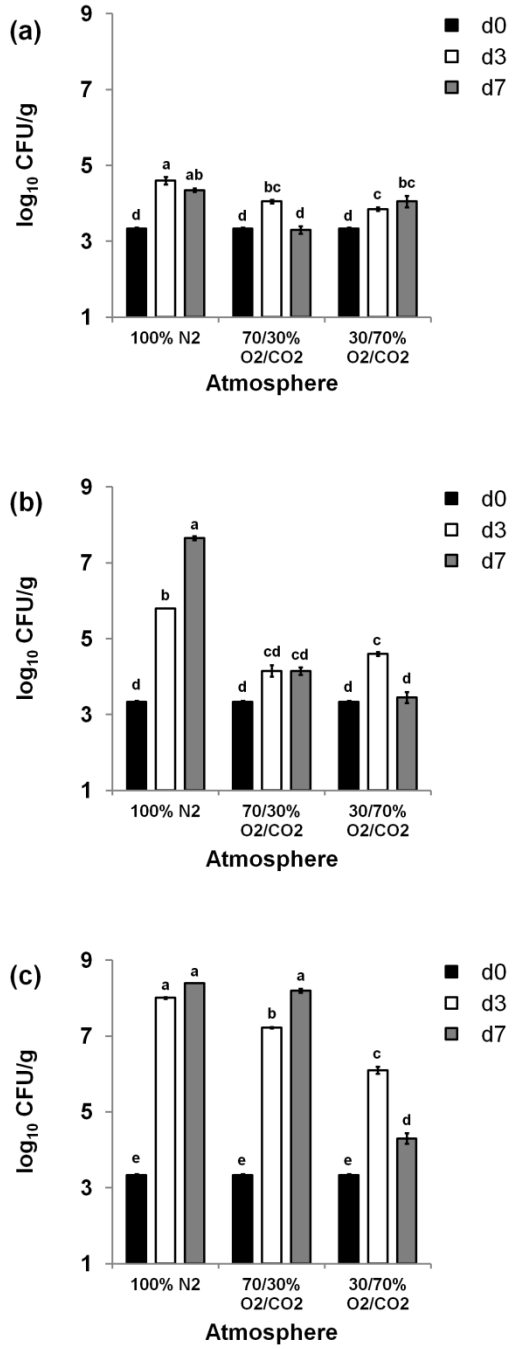

Figure 2 Growth of *Carnobacterium maltaromaticum* (isolate CM_B820) in sterilised minced pork repackaged in different atmospheres (100% N_2, 70/30% O_2/CO_2 and 30/70% O_2/CO_2) at (a) 4, (b) 8 and (c) 12 °C for 7 days. Bars represent standard error. Different letters within each temperature indicate significant differences ($P < 0.05$).

The impact of three isolates of *C. maltaromaticum* (CM_B824, CM_B827 and CM_B829) at two concentrations (10^4 and 10^6 CFU/g) on the sensory quality of meat was evaluated in raw and cooked beef patties prepared from a commercial bovine meat preparation. No effect of the concentration of *C. maltaromaticum* was observed for any

Table 2 Microbial counts in *psoas major* steaks before inoculation with *Carnobacterium maltaromaticum*.

Bacterial group	$\log_{10}$ CFU/g ± SD
Total viable count	5.6 ± 0.0
Lactic acid bacteria	3.1 ± 0.0
Enterobacteriaceae	2.5 ± 0.1
Pseudomonas spp.	2.5 ± 0.1
Brochothrix thermosphacta	2.1 ± 0.7

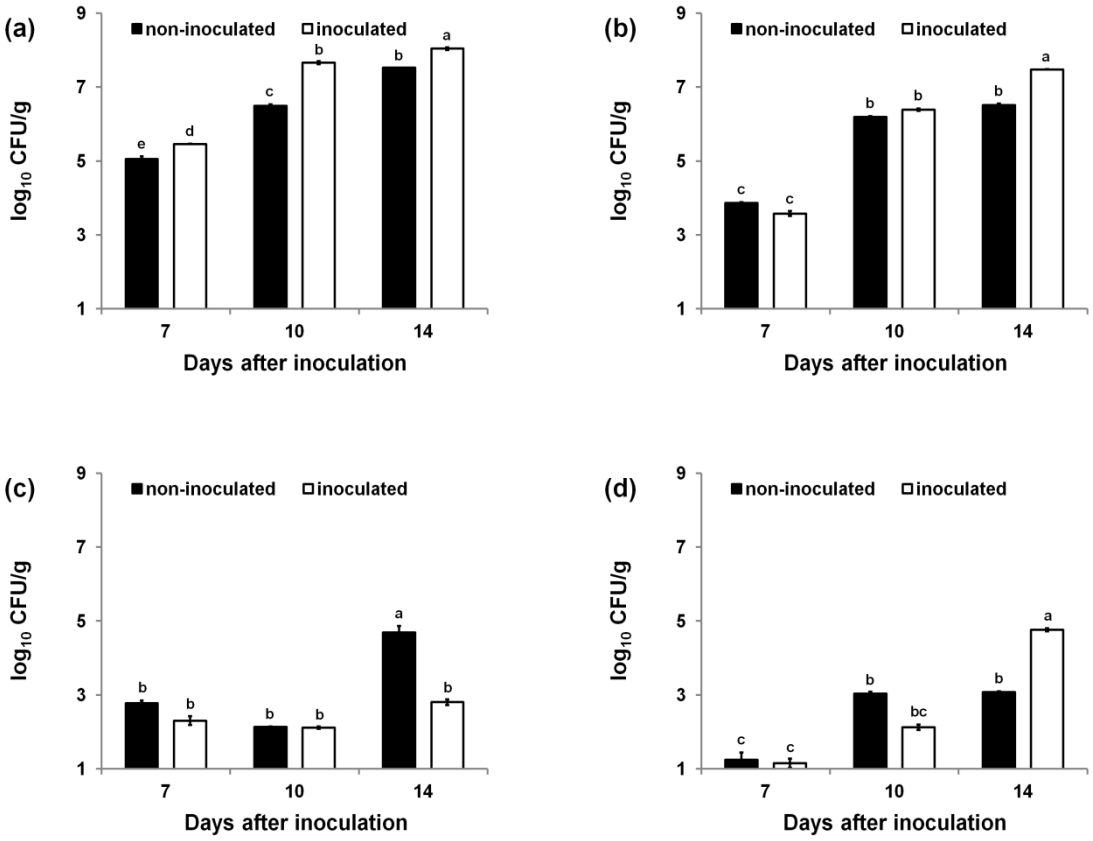

Figure 3 (a) Total viable count, (b) lactic acid bacteria, (c) Enterobacteriaceae and (d) *Brochothrix thermosphacta* in beef inoculated with *Carnobacterium maltaromaticum* (isolate CM_B820). Samples were stored in vacuum at −1 °C for 7 days and subsequently in a modified atmosphere (100% N_2) at 4 °C for 7 days. Bars represent standard error. Different letters within each bacterial group indicate significant differences ($P < 0.05$).

attribute in raw and cooked samples (data not shown in graphical form). Regarding raw beef patties, non-inoculated samples (blank) received the highest scores for appearance, odour and colour ($P < 0.05$). Raw samples inoculated with isolates CM_B827 and CM_B829 received the worst scores for appearance and odour ($P < 0.05$), while samples inoculated with isolate CM_B827 received intermediate scores. No significant difference was observed for colour between inoculated raw samples (**Figure 4**). As for raw beef patties, non-inoculated cooked samples received the best scores for all tested attributes (appearance, odour, colour, tenderness, flavour and juiciness), while samples inoculated with isolate CM_B829 received the worst scores ($P < 0.05$). Cooked beef patties inoculated with isolate CM_B824 received intermediate scores for appearance, tenderness, flavour and juiciness. However, they received the worst scores for odour and colour. Cooked beef patties inoculated with isolate CM_B827 received intermediate scores for all tested attributes (**Figure 5**).

4 Discussion

Raw, chilled meat has traditionally been regarded as a highly perishable product which must reach the consumer expeditiously if it is to be wholesome when it is prepared for consumption (Gill, 1996). It has been reported that subzero chilled storage is essential for the extension of the shelf-life of beef since it provides the greatest oxidative stability (Jeremiah & Gibson, 2001). However, little is known about the effects of extended ageing at subzero temperatures on the microbial ecosystem of beef. Previous studies have shown

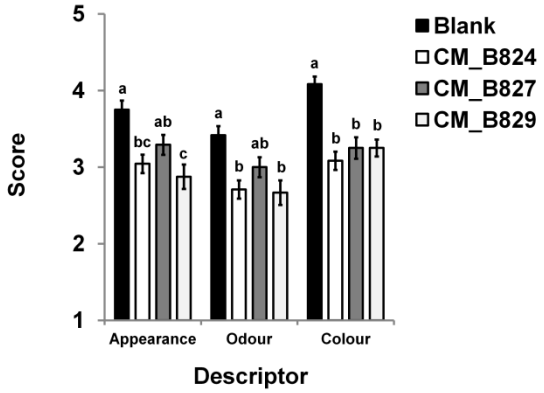

Figure 4 Sensory analysis of raw beef patties inoculated with three isolates of *Carnobacterium maltaromaticum* (CM_B824, CM_B827 and CM_B829) after 8 days of storage (5 days at 4 °C and 3 days at 8 °C). Bars represent standard error. Different letters within each descriptor indicate significant differences ($P < 0.05$).

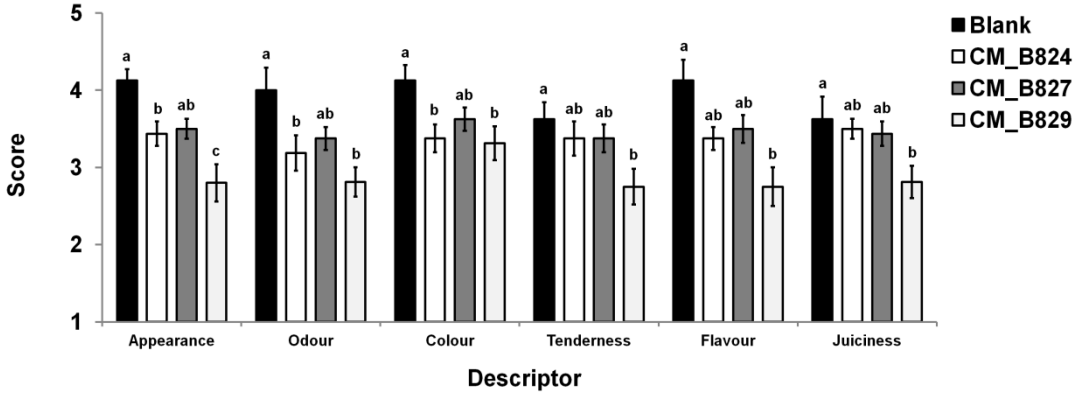

Figure 5 Sensory analysis of cooked beef patties inoculated with three isolates of *Carnobacterium maltaromaticum* (CM_B824, CM_B827 and CM_B829) after 8 days of storage (5 days at 4 °C and 3 days at 8 °C). Bars represent standard error. Different letters within each descriptor indicate significant differences ($P < 0.05$).

that *Carnobacterium* predominates on extended shelf-life vacuum-packaged beef primals, contributing to their long storage life (Kaur, Bowman, Porteus, Dann, & Tamplin, 2017; Zhang, Badoni, Gänzle, & Yang, 2018). Thus, *C. maltaromaticum* isolated in this study may have potential use as protective cultures in meat and meat products.

The phenotypic profiling revealed a biochemical and physiological heterogeneity of the isolates obtained in this study and the two reference strains (LMG 11393 and LMG 22902). Iskandar, et al. (2016) also observed a high heterogeneity in the metabolism of *C. maltaromaticum*, and speculates that this might be due to the ability of this species to adapt to a wide range of environments. As highlighted by Laursen, et al. (2005), phenotypic testing is time-consuming; however, it allows isolates of *Carnobacterium* to be identified at the species level with an acceptable degree of confidence.

The genetic profiling of *C. maltaromaticum* isolates revealed the presence of genes *cbnBM1* and *cbnB2* coding for the class IIa carnobacteriocins BM1 and B2, respectively. Class IIa bacteriocins identified so far are highly active against *Listeria* strains (Zou, Jiang, Cheng, Fang, & Huang, 2018). Nevertheless, class IIa bacteriocins could also have potential applications against spoilage microorganisms, since they display spectra of activity broad enough to encompass undesirable bacteria including other LAB, *Brochothrix* spp., *Clostridium* spp., *Bacillus* spp. and *Staphylococcus* spp (Ennahar, Sashihara, Sonomoto, & Ishizaki, 2000). In the present study, *C. maltaromaticum* showed an inhibition effect against EB, which are associated with spoilage of chilled meats. Hence, it could contribute to the extension of the shelf-life of meat and meat products. However, no inhibition effect was observed against BT.

The use of protective cultures must also take into account the physiological requirements of the biopreservative agent. Lactic acid bacteria (LAB) are facultative anaerobes with a preference of anaerobic conditions. The present study confirmed that

C. maltaromaticum displays more significant growth in atmospheres without oxygen. Furthermore, the inhibition effect of *C. maltaromaticum* against the spoilage group EB was only observed in the absence of oxygen. Therefore, the use of *C. maltaromaticum* as a biopreservative in chilled meat and meat products should be associated with systems such as vacuum packaging and vacuum skin packaging.

Despite its bioprotective properties, *C. maltaromaticum* has been found to possess a great potential for meat spoilage (Ercolini, Russo, Nasi, Ferranti, & Villani, 2009; Laursen, et al., 2005). Sensory analysis showed that the panellists could distinguish between non-inoculated and inoculated beef patties with *C. maltaromaticum*; however, the impact of inoculation with isolate CM_B827 was not very intense. Casaburi, et al. (2011) identified 3-methyl-1-butanol, 1-octen-3-ol, butanoic acid and aceteoin as the main spoilage molecules produced by *Carnobacterium* spp. Moreover, the same study reported that storage in air contributes to a higher perception of off-odours in meat inoculated with *Carnobacterium* spp. when compared to vacuum storage, confirming that the use of *C. maltaromaticum* as a protective culture could be more efficient in low-oxygen packaging systems.

5 Conclusions

In conclusion, we obtained in this study 11 isolates of *C. maltaromaticum* from vacuum-packaged beef with extremely long shelf-life. The major part of these isolates possessed genes coding for carnobacteriocins BM1 and B2. It was observed that *C. maltaromaticum* displays a higher growth rate and an inhibition effect against EB in low-oxygen atmospheres. Finally, isolate CM_B827 had a negligible negative impact on the sensory attributes of beef patties. Further research on these isolates should focus on their effect on the growth of pathogenic bacteria, including *L. monocytogenes*, and sensory attributes of other meat products.

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References

Agren, J., Sundstrom, A., Hafstrom, T., & Segerman, B. (2012). Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. *PLoS One, 7*, e39107.

Brillet, A., Pilet, M.-F., Prevost, H., Cardinal, M., & Leroi, F. (2005). Effect of inoculation of *Carnobacterium divergens* V41, a biopreservative strain against *Listeria monocytogenes* risk, on the microbiological, chemical and sensory quality of cold-smoked salmon. *International Journal of Food Microbiology, 104*, 309–324.

Cailliez-Grimal, C., Afzal, M. I., & Revol-Junelles, A. M. (2014). *Carnobacterium*. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology (Second Edition)* (pp. 379–383). Oxford: Academic Press.

Casaburi, A., Nasi, A., Ferrocino, I., Di Monaco, R., Mauriello, G., Villani, F., & Ercolini, D. (2011). Spoilage-related activity of *Carnobacterium maltaromaticum* strains in air-stored and vacuum-packed meat. *Applied and Environmental Microbiology, 77*, 7382–7393.

Castellano, P., Belfiore, C., Fadda, S., & Vignolo, G. (2008). A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. *Meat Science, 79*, 483–499.

Castro, S. M., Kolomeytseva, M., Casquete, R., Silva, J., Queirós, R., Saraiva, J. A., & Teixeira, P. (2017). Biopreservation strategies in combination with mild high pressure treatments in traditional Portuguese ready-to-eat meat sausage. *Food Bioscience, 19*, 65–72.

dos Reis, F. B., de Souza, V. M., Thomaz, M. R. S., Fernandes, L. P., de Oliveira, W. P., & De Martinis, E. C. P. (2011). Use of *Carnobacterium maltaromaticum* cultures and hydroalcoholic extract of *Lippia sidoides* Cham. against *Listeria monocytogenes* in fish model systems. *International Journal of Food Microbiology, 146*, 228–234.

Ennahar, S., Sashihara, T., Sonomoto, K., & Ishizaki, A. (2000). Class IIa bacteriocins: biosynthesis, structure and activity. *FEMS Microbiology Reviews, 24*, 85–106.

Ercolini, D., Russo, F., Nasi, A., Ferranti, P., & Villani, F. (2009). Mesophilic and psychrotrophic bacteria from meat and their spoilage potential *in vitro* and in beef. *Applied and Environmental Microbiology, 75*, 1990–2001.

European Parliament and Council of the European Union. (2004). Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Official Journal of the European Union, L 139*, 55–205.

Gill, C. O. (1989). Packaging meat for prolonged chilled storage: The CAPTECH process. *British Food Journal, 91*, 11–15.

Gill, C. O. (1996). Extending the storage life of raw chilled meats. *Meat Science, 43*, S99–S109.

Huson, D. H. (1998). SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics, 14*, 68–73.

Imazaki, P. H., Tahiri, A., Rodrigues, A., Taminiau, B., Nezer, C., Daube, G., & Clinquart, A. (2012). Vacuum packaged beef with long shelf life: isolation and characterization of *Carnobacterium* strains. In *14èmes Journées Sciences du Muscle et Technologies des Viandes*. Caen.

Iskandar, C. F., Cailliez-Grimal, C., Rahman, A., Rondags, E., Remenant, B., Zagorec, M., Leisner, J. J., Borges, F., & Revol-Junelles, A.-M. (2016). Genes associated to lactose metabolism illustrate the high diversity of *Carnobacterium maltaromaticum*. *Food Microbiology, 58*, 79–86.

ISO. (1995). *Viande et produits à base de viande, Dénombrement des Pseudomonas spp. présomptifs, ISO 13720*. Genève: Organisation internationale de normalisation.

ISO. (1996). *Viande et produits à base de viande, Dénombrement de Brochothrix thermosphacta, Technique par comptage des colonies obtenues, ISO 13722*. Genève: Organisation internationale de normalisation : Genève.

Jeremiah, L. E., & Gibson, L. L. (2001). The influence of storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef. *Food Research International, 34*, 815–826.

Kaur, M., Bowman, J. P., Porteus, B., Dann, A. L., & Tamplin, M. (2017). Effect of abattoir and cut on variations in microbial communities of vacuum-packaged beef. *Meat Science, 131*, 34–39.

Koné, A. P., Zea, J. M. V., Gagné, D., Cinq-Mars, D., Guay, F., & Saucier, L. (2018). Application of *Carnobacterium maltaromaticum* as a feed additive for weaned rabbits to improve meat microbial quality and safety. *Meat Science, 135*, 174–188.

Laursen, B. G., Bay, L., Cleenwerck, I., Vancanneyt, M., Swings, J., Dalgaard, P., & Leisner, J. J. (2005). *Carnobacterium divergens* and *Carnobacterium maltaromaticum* as spoilers or protective cultures in meat and seafood: phenotypic and genotypic characterization. *Systematic and Applied Microbiology, 28*, 151–164.

Lücke, F.-K. (2000). Utilization of microbes to process and preserve meat. *Meat Science, 56*, 105–115.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., Wilkening, J., & Edwards, R. A. (2008). The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics, 9*, 386.

Mills, J., Donnison, A., & Brightwell, G. (2014). Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: A review. *Meat Science, 98*, 71–80.

Oluk, C. A., & Karaca, O. B. (2018). The current approaches and challenges of biopreservation. In A. M. Grumezescu & A. M. Holban (Eds.), *Food Safety and Preservation* (pp. 565–597): Academic Press.

Orihuel, A., Bonacina, J., Vildoza, M. J., Bru, E., Vignolo, G., Saavedra, L., & Fadda, S. (2018). Biocontrol of *Listeria monocytogenes* in a meat model using a combination of a bacteriocinogenic strain with curing additives. *Food Research International, 107*, 289–296.

Spanu, C., Piras, F., Mocci, A. M., Nieddu, G., De Santis, E. P. L., & Scarano, C. (2018). Use of *Carnobacterium* spp protective culture in MAP packed Ricotta fresca cheese to control *Pseudomonas* spp. *Food Microbiology, 74*, 50–56.

Tirloni, E., Cattaneo, P., Ripamonti, B., Agazzi, A., Bersani, C., & Stella, S. (2014). *In vitro* evaluation of *Lactobacillus animalis* SB310, *Lactobacillus paracasei* subsp. *paracasei* SB137 and their mixtures as potential bioprotective agents for raw meat. *Food Control, 41*, 63–68.

van Heel, A. J., de Jong, A., Montalban-Lopez, M., Kok, J., & Kuipers, O. P. (2013). BAGEL3: Automated identification of genes encoding bacteriocins and (non-)bactericidal posttranslationally modified peptides. *Nucleic Acids Research, 41*, W448–453.

Yost, C. K. (2014). Biopreservation. In G. Smithers (Ed.), *Encyclopedia of Meat Sciences* (pp. 76–82). Oxford (United Kingdom): Academic Press.

Zhang, P., Badoni, M., Gänzle, M., & Yang, X. (2018). Growth of *Carnobacterium* spp. isolated from chilled vacuum-packaged meat under relevant acidic conditions. *International Journal of Food Microbiology, 286*, 120–127.

Zou, J., Jiang, H., Cheng, H., Fang, J., & Huang, G. (2018). Strategies for screening, purification and characterization of bacteriocins. *International Journal of Biological Macromolecules, 117*, 781–789.

IN VITRO EVALUATION OF THE COMPETING EFFECT OF CARNOBACTERIUM MALTAROMATICUM ISOLATED FROM VACUUM-PACKAGED MEAT AGAINST FOOD PATHOGENS

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Abstract: **Foodborne disease outbreaks are one of the leading causes of infections, hospitalizations and deaths provoked by pathogenic bacteria including *Listeria monocytogenes*, *Salmonella* spp and *Escherichia coli* O157:H7. *Carnobacterium maltaromaticum* is a lactic acid bacteria, which could prevent the growth of pathogens in refrigerated food. The aim of this study was to determine the bioprotective potential of three strains of *C. maltaromaticum* isolated from chilled vacuum-packaged beef. They were tested *in vitro* against the pathogens cited above. The results indicate that the selected strains have an antilisterial activity, which is optimized at low temperatures. Moreover, when the strains were combined with EDTA it was observed a slight, but significant, inhibition of the gram-negative bacteria used in this study.**

Keywords: **antilisterial activity, biopreservation, bioprotective culture, lactic acid bacteria**

1 Introduction

Foodborne disease outbreaks are caused by the ingestion of contaminated food with pathogenic microorganisms. The World Health Organization considers that foodborne outbreaks are critical threats to the global health. The high rate of infections caused by human pathogens including *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* O157:H7 is a frequent reminder of the complex food web that links humans, animals and microbial population (Wolfe, Dunavan, & Diamond, 2007). Poor handling, incorrect storage and consumption of raw meat are one of the leading causes of foodborne diseases (Ozbey, Ertas, & Kok, 2006).

In Europe, invasive listeriosis has been reported to be an infection of great concern to public health due to its clinical severity (hospitalisation rate > 90%) and high fatality rate (20% to 30%), despite the correct treatment and its low incidence (Hernandez-Milian & Payeras-Cifre, 2014). Moreover, *Salmonella* sp. is reported as the cause of 22.5% of foodborne diseases associated with the consumption of meat and meat products (Hennekinne, Herbin, Firmesse, & Auvray, 2015). Finally, enterohemorrhagic *E. coli* (EHEC) is a typically food-born pathogen causing haemorrhagic colitis or haemolytic-uraemic syndrome. Typical EHEC strains produce Shiga-like toxins (named Shiga toxin producing *E. coli*, STEC) similar to those produced by *Shigella dysenteriae* making them the most virulent diarrhoeagenic *E. coli* known to date (Allocati, Masulli, Alexeyev, & Di Ilio, 2013).

Biopreservation has received the attention of food industry as a mean of naturally controlling the shelf-life and safety of food. Lactic acid bacteria (LAB) have significant potential for use in biopreservation and have been traditionally used as natural biopreservatives of food and feed (Castro, et al., 2017; Orihuel, et al., 2018; Tirloni, et al., 2014). *Carnobacteria* are ubiquitous LAB isolated from cold and temperate environments and can be found as natural microbiota of chilled meat, fish and dairy products (Laursen, et al., 2005). Among the species of *Carnobacterium*, two species, *Carnobacteria divergens* and *Carnobacteria maltaromaticum,* are frequently isolated from food and showed the ability to inhibit pathogenic and spoilage microorganisms in diverse food matrix, exerting their inhibitory effect as a result of the competition for nutrients and the production of bacteriocins (Leisner, Laursen, Prévost, Drider, & Dalgaard, 2007). Thus, their use as bioprotective cultures in food has been considered (Iskandar, et al., 2016; Leisner, Laursen, Prévost, Drider, & Dalgaard, 2007; Orihuel, et al., 2018).

The antimicrobial properties of *Carnobacterium* spp. have been studied *in vitro* (Hammi, et al., 2016; Tulini, et al., 2014), in cold-smoked salmon (Brillet-Viel, Pilet, Courcoux, Prévost, & Leroi, 2016; Brillet, Pilet, Prevost, Cardinal, & Leroi, 2005), ricotta

(Spanu, et al., 2018) and as a feed additive for rabbits to improve meat microbial quality and safety (Koné, et al., 2018).

In this context, this study aims to evaluate *in vitro* the bioprotective potential of *Carnobacterium maltaromaticum* isolated from vacuum-packaged beef against *L. monocytogenes*, *Salmonella* Typhimurium and *E. coli* O157:H7.

2 Material and methods

2.1 Bacterial isolates/strains

Three different isolates of C. maltaromaticum (CM_B824, CM_B827 and CM_B829), obtained from Australian vacuum packaged chilled beef (*longissimus thoracis et lumbrorum* muscle) with an extremely long shelf-life (140 days at −1 °C) were used in this study (Imazaki, et al., 2012). These isolates were selected among 11 after the sequencing of their genome, which revealed the existence of three main phylogenetic groups. Therefore, one isolate of each group was selected to be used in this research.

Three foodborne pathogenic bacteria (*L. monocytogenes* ATCC 19117, *Salmonella* Typhimurium ATCC 14028 and *E. coli* O157:H7 ATCC 35150) were used in this project.

2.2 Evaluation of the antimicrobial effect of C. maltaromaticum *in co-culture*

The antimicrobial effect of *C. maltaromaticum* in co-culture was determined using the following procedure: flasks with 30 mL BHI broth were inoculated with each isolate of *C. maltaromaticum* (6 $\log_{10}$ CFU/mL) and one of the pathogenic strains (3 $\log_{10}$ CFU/mL). Flasks with BHI were also inoculated with each pathogenic strain at 3 $\log_{10}$ CFU/mL alone as controls. The flasks were incubated at −1, 4 and 25 °C for 28 d, 14 d and 48 h, respectively, in a shaker (model Rotamax 120, Heidolph, Germany) at 150 rpm. Pathogenic bacteria counts were performed using specific chromogenic media: RAPID'*L.mono*, RAPID'*Salmonella* and RAPID'*E.coli* 2 (BioRad, Marnes, France). *C. maltaromaticum* concentration was estimated as the difference between counts on plate count agar (PCA) (BioRad, Marnes, France) and chromogenic media.

The influence of the addition of ethylenediaminetetraacetic acid (EDTA) on the antimicrobial effect of *C. maltaromaticum* on pathogens was investigated. Co-cultures were carried out in flasks containing BHI broth with EDTA 1 mM (VWR, Radnor, USA), incubated at 25 °C for 48 h, in a shaker (model Rotamax 120, Heidolph, Germany) at 150 rpm. Bacterial counts were performed following the same procedure described above.

To find the concentration of EDTA that would not interfere in the bacterial growth by itself, a previous experiment was conducted. Serial concentrations of EDTA (1, 5, 10, 20 and 40 mM) were added to the broth medium with each strain of pathogen. The concentration that showed no difference on growth compared to the negative control (broth without the addition of EDTA) was selected (1 mM).

2.4 Evaluation of the antimicrobial effect of the cell-free supernatant of C. maltaromaticum

To check if the antimicrobial effect of *C. maltaromaticum* was mediated by the production of antimicrobial molecules in the culture supernatant, tests were conducted as follows. Three tubes per *C. maltaromaticum* isolate were prepared with 10 mL of BHI broth and one colony of each isolate of *C. maltaromaticum*. The tubes were incubated at 25 °C for 48 h and centrifuged (Model Eppendorf Centrifuge 5804, Hamburg, Germany) at 15,557 g for 10 min. The supernatant of two tubes was treated with sodium hydroxide 1N (VWR, Radnor, USA) until pH 6.5. Finally, one supernatant treated with sodium hydroxide was filtered through 0.2 μm sterile Minisart syringe filters (Sartorius, Germany). The treated supernatants were inoculated in wells made in PCA plates previously spread with 100 μL each of the pathogenic bacteria cited above (6 $\log_{10}$ CFU/mL). Four treatments were applied on each plate: (i) sterile BHI broth (blank), (ii) centrifuged supernatant, (iii) centrifuged supernatant treated with sodium hydroxide (to neutralise organic acids eventually produced by *C. maltaromaticum*), and (iv) centrifuged supernatant treated with sodium hydroxide and filtered. The halo of inhibition was measured after 48 h of incubation at 37 °C, and all treatments were performed in triplicates.

2.4 Statistical analysis

Data was analysed by one-way ANOVA. The effect of inoculum (blank, CM_B824, CM_B827 and CM_B829) on the growth of *L. monocytogenes*, *S.* Thiphymurium and *E. coli* O157:H7 was calculated for co-cultures without EDTA at each incubation temperature (−1, 4 and 25 °C) and co-cultures with EDTA at 25 °C. When necessary, Tukey's test was used to assess differences between test groups. ANOVA was performed using VassarStats online.

3 Results

The antimicrobial effect of *C. maltaromaticum* against *L. monocytogenes*, *S.* Thiphymurium and *E. coli* O157:H7 was assessed in co-cultures at −1, 4 and 25 °C. At −1° C, *L. monocytogenes* alone reached a concentration of 6.6 $\log_{10}$ CFU/mL after 28 d.

When in co-culture with *C. maltaromaticum*, the *L. monocytogenes* load was < 1.0 (co-culture with isolate CM_824), 2.3 (co-culture with isolate CM_827) and 1.7 (co-culture with isolate CM_829) $\log_{10}$ CFU/ mL after incubation at the same temperature and time conditions (**Figure 1**). Moreover, *S.* Typhimurium and *E. coli* O157:H7 were not inhibited when co-cultured at −1 °C with any of the *C. maltaromaticum* isolates (data not shown in graphical form).

At 4° C, the three isolates of *C. maltaromaticum* (CM_B824, CM_B827 and CM_B829) inhibited the growth of *L. monocytogenes* ($P < 0.05$), showing a count reduction of a least 5.5 $\log_{10}$ CFU/mL when compared to the growth of *L. monocytogenes* alone (**Figure 2**). Nevertheless, none of the isolates did show any inhibition effect against *S.* Typhimurium and *E. coli* O157:H7 at 4 °C (data not shown in graphical form).

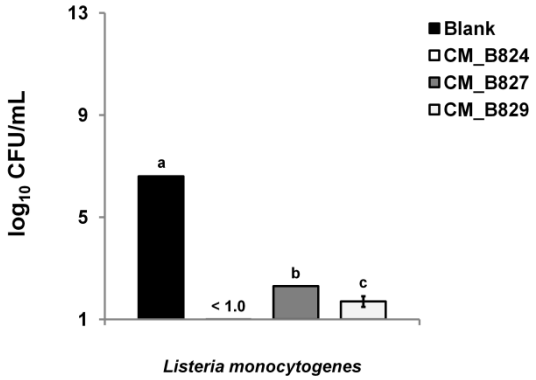

Figure 1 *Listeria monocytogenes* growth alone (blank) or in co-culture with *Carnobacterium maltaromaticum* (isolates CM_B824, CM_B827 and CM_B829) at −1 °C for 28 days.

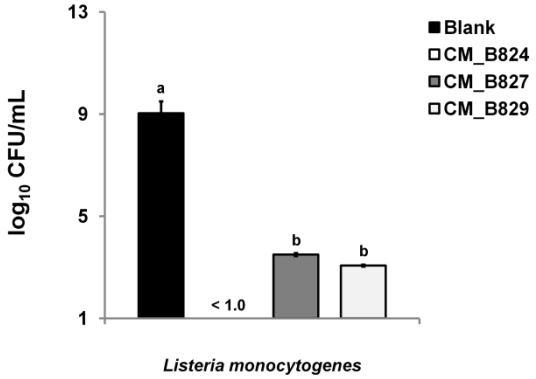

Figure 2 *Listeria monocytogenes* growth alone (blank) or in co-culture with *Carnobacterium maltaromaticum* (isolates CM_B824, CM_B827 and CM_B829) at 4 °C for 14 days.

Carnobacterium maltaromaticum isolates CM_B824 and CM_B827 showed an inhibition effect towards *L. monocytogenes* ($P < 0.05$) when in co- cultures at 25 °C (**Figure 3.a**). Regarding the two other pathogens, *C. maltaromaticum* isolates did not show any inhibitory effect when grown in co-culture with *S.* Typhimurium and *E. coli* O157:H7 at 25 °C (**Figures 3.b** and **3.c**). When EDTA was added in to the co-culture broths, isolates CM_B824 and CM_B827 inhibited the growth of *L. monocytogenes* and *S.* Typhimurium ($P < 0.05$) (**Figures 3.d** and **3.e**), and the three isolates (CM_B824, CM_B827 and CM_B829) inhibited the growth of *E. coli* O157:H7 ($P < 0.05$) (**Figure 3.f**). It should be noted that the inhibition effects observed at 25 °C were not strong.

Finally, the evaluation of the antimicrobial activity of cell-free supernatant of *C. maltaromaticum* (obtained after 48 h at 25 °C) by agar well diffusion assay did not highlight any inhibition effect of the supernatants against the tested pathogens.

4 Discussion

The food industry has a large interest in the potential use of LAB in biopreservation. Their inhibitory effect against pathogenic and spoilage bacteria (Ammor, Tauveron, Dufour, & Chevallier, 2006; Cizeikiene, Juodeikiene, Paskevicius, & Bartkiene, 2013) is an important indicator of their possible use as protective cultures, and applying then to the food matrix as antimicrobials would permit to act against foodborne pathogens, while replacing some synthetic conservatives (Engelhardt, Albano, Kiskó, Mohácsi-Farkas, & Teixeira, 2015; Huang, Ye, Yu, Wang, & Zhou, 2016).

In the present study, *C. maltaromaticum* showed an antilisterial activity, which was also observed by other authors. Alves, de Martinis, Destro, Vogel, and Gram (2005) reported an antilisterial activity of a *C. maltaromaticum* isolated from Brazilian smoked fish. Dos Reis, et al. (2011) also observed an antilisterial activity of *C. maltaromaticum* in fish models. Moreover, Hammi, et al. (2016) characterised a bacteriocin produced by *C. maltaromaticum* isolated from Camembert cheese, which showed a high activity against strains of *L. monocytogenes*.

Lactic acid bacteria (LAB) can inhibit pathogen microorganisms by synthesis of bacteriocins (Gómez-Sala, et al., 2016). However, Gram-negative bacteria are naturally resistant to the action of bacteriocins produced by Gram-positive bacteria, which are widely explored in foods (Prudêncio, dos Santos, & Vanetti, 2015). One of the most used strategies for the sensitisation of Gram-negative bacteria to the action of bacteriocins produced by Gram-positive bacteria is the combination of bacteriocins with EDTA. The chelator acts by promoting destabilisation of the outer membrane in part by releasing the lipopolysaccharide layer, allowing the bacteriocin to access the cytoplasmic membrane

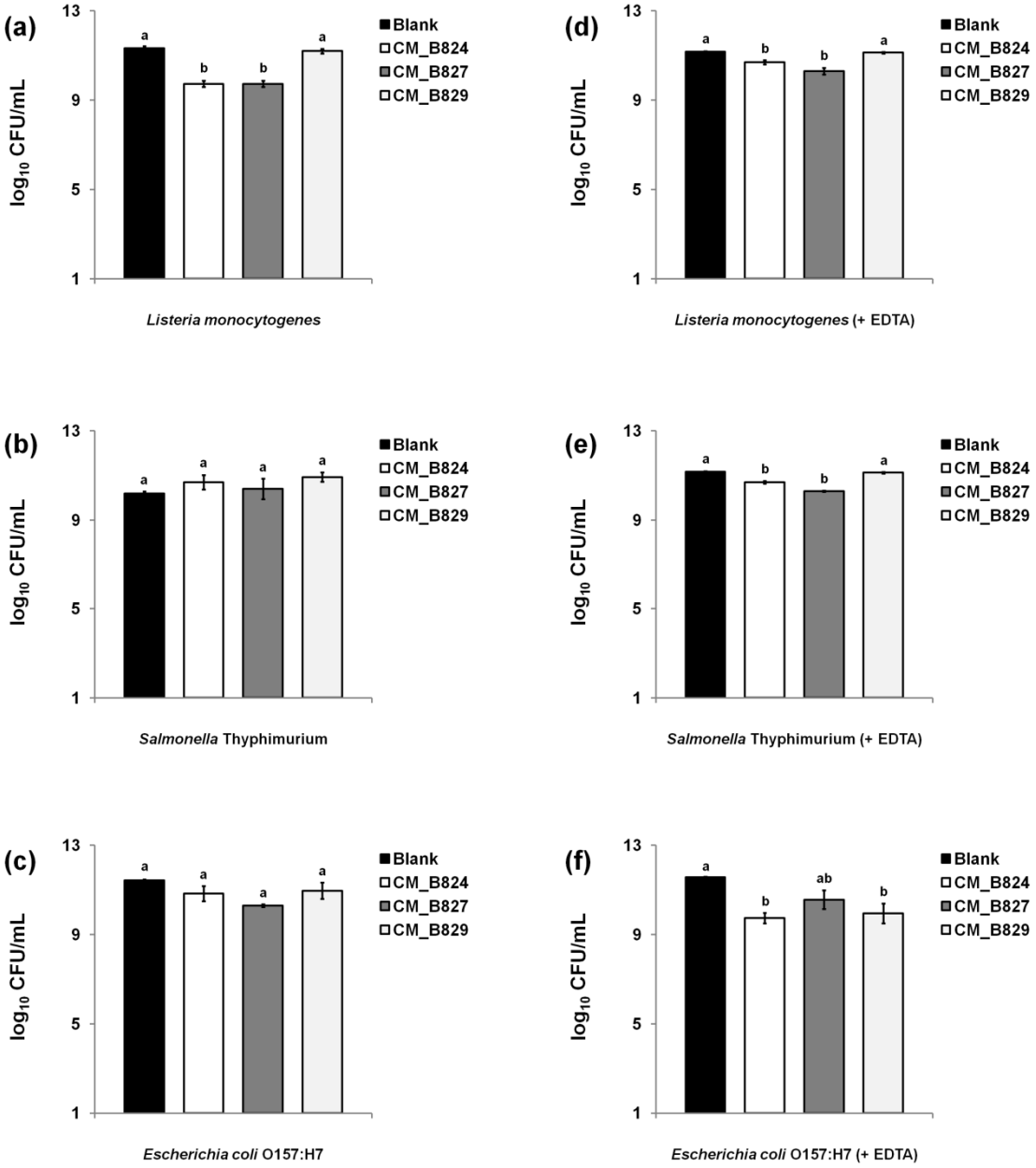

Figure 3 (a) *Listeria monocytogenes*, (b) *Salmonella* Thyphimurium and (c) *Escherichia coli* O157:H7 growth alone (blank) or in co-culture with *Carnobacterium maltaromaticum* (isolates CM_B824, CM_B827 and CM_B829) at 25 °C for 48 hours, and (d) *Listeria monocytogenes*, (e) *Salmonella* Thyphimurium and (f) *Escherichia coli* O157:H7 growth alone (blank) or in co-culture with *Carnobacterium maltaromaticum* (isolates CM_B824, CM_B827 and CM_B829) with addition of EDTA 1 mM at 25 °C for 48 hours.

(Prudêncio, Mantovani, Cecon, Prieto, & Vanetti, 2016). In the present study, an inhibition effect of *C. maltaromaticum* against *E. coli* O157:H7 and *S.* Thyphimurium was observed in co-cultures with the addition of EDTA.

As bacteriocins are compounds that are produced and released into the extracellular media, it would be possible to verify their production by studying the supernatant of target bacteria. In this study, the cell-free supernatant was not able to inhibit the growth of the

target pathogen bacteria. Thus, the isolates of *C. maltaromaticum* used in this research are not likely to produce bacteriocins under the studied conditions. Gursky, et al. (2006) demonstrated that bacteriocin activity was not observable in the supernatant of cultures of *C. maltaromaticum* grown in liquid media at 25 °C, but at temperatures less than 19 °C bacteriocin activity could be detected. Other variables, such as pH, medium composition and aeration could have influenced the production of bacteriocins by the isolates of *C. maltaromaticum* (Zhang, Kaur, Bowman, Ratkowsky, & Tamplin, 2017).

5 Conclusions

The three *C. maltaromaticum* isolates tested showed an antilisterial potential *in vitro*, which was more important at −1 and 4 than at 25 °C. Thus, the combination of two hurdles (refrigerated storage and addition of protective cultures) shows great potential to improve quality and food safety. To improve our work, it would be interesting to test the antimicrobial activity of the cell-free supernatant of *C. maltaromaticum* in other growth conditions (e.g., lower temperatures and anaerobiosis). The understanding of the effect of environmental factors on the inhibitory effect of *C. maltaromaticum* against other bacteria would contribute to the correct use of this LAB as a protective culture in meat and meat products.

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References

Allocati, N., Masulli, M., Alexeyev, M., & Di Ilio, C. (2013). *Escherichia coli* in Europe: An overview. *International Journal of Environmental Research and Public Health, 10*, 6235–6254.

Alves, V. F., De Martinis, E. C., Destro, M. T., Vogel, B. F., & Gram, L. (2005). Antilisterial activity of a *Carnobacterium piscicola* isolated from Brazilian smoked fish (surubim [*Pseudoplatystoma* sp.]) and its activity against a persistent strain of *Listeria monocytogenes* isolated from surubim. *Journal of Food Protection, 68*, 2068–2077.

Ammor, S., Tauveron, G., Dufour, E., & Chevallier, I. (2006). Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility: 1—Screening and characterization of the antibacterial compounds. *Food Control, 17*, 454–461.

Brillet-Viel, A., Pilet, M.-F., Courcoux, P., Prévost, H., & Leroi, F. (2016). Optimization of growth and bacteriocin activity of the food bioprotective *Carnobacterium divergens* V41 in an animal origin protein free medium. *Frontiers in Marine Science, 3*, 128.

Brillet, A., Pilet, M.-F., Prevost, H., Cardinal, M., & Leroi, F. (2005). Effect of inoculation of *Carnobacterium divergens* V41, a biopreservative strain against *Listeria monocytogenes* risk, on the microbiological, chemical and sensory quality of cold-smoked salmon. *International Journal of Food Microbiology, 104*, 309-324.

Castro, S. M., Kolomeytseva, M., Casquete, R., Silva, J., Queirós, R., Saraiva, J. A., & Teixeira, P. (2017). Biopreservation strategies in combination with mild high pressure treatments in traditional Portuguese ready-to-eat meat sausage. *Food Bioscience, 19*, 65–72.

Cizeikiene, D., Juodeikiene, G., Paskevicius, A., & Bartkiene, E. (2013). Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. *Food Control, 31*, 539–545.

dos Reis, F. B., de Souza, V. M., Thomaz, M. R. S., Fernandes, L. P., de Oliveira, W. P., & De Martinis, E. C. P. (2011). Use of *Carnobacterium maltaromaticum* cultures and hydroalcoholic extract of *Lippia sidoides* Cham. against *Listeria monocytogenes* in fish model systems. *International Journal of Food Microbiology, 146*, 228–234.

Engelhardt, T., Albano, H., Kiskó, G., Mohácsi-Farkas, C., & Teixeira, P. (2015). Antilisterial activity of bacteriocinogenic *Pediococcus acidilactici* HA6111-2 and *Lactobacillus plantarum* ESB 202 grown under pH and osmotic stress conditions. *Food Microbiology, 48*, 109–115.

Gómez-Sala, B., Herranz, C., Díaz-Freitas, B., Hernández, P. E., Sala, A., & Cintas, L. M. (2016). Strategies to increase the hygienic and economic value of fresh fish: Biopreservation using lactic acid bacteria of marine origin. *International Journal of Food Microbiology, 223*, 41–49.

Gursky, L. J., Martin, N. I., Derksen, D. J., van Belkum, M. J., Kaur, K., Vederas, J. C., Stiles, M. E., & McMullen, L. M. (2006). Production of piscicolin 126 by *Carnobacterium maltaromaticum* UAL26 is controlled by temperature and induction peptide concentration. *Archives of Microbiology, 186*, 317–325.

Hammi, I., Delalande, F., Belkhou, R., Marchioni, E., Cianferani, S., & Ennahar, S. (2016). Maltaricin CPN, a new class IIa bacteriocin produced by *Carnobacterium maltaromaticum* CPN isolated from mould-ripened cheese. *Journal of Applied Microbiology, 121*, 1268–1274.

Hennekinne, J.-A., Herbin, S., Firmesse, O., & Auvray, F. (2015). European food poisoning outbreaks involving meat and meat-based products. *Procedia Food Science, 5*, 93–96.

Hernandez-Milian, A., & Payeras-Cifre, A. (2014). What is new in listeriosis? *BioMed Research International, 2014*, 7 p.

Huang, Y., Ye, K., Yu, K., Wang, K., & Zhou, G. (2016). The potential influence of two *Enterococcus faecium* on the growth of *Listeria monocytogenes*. *Food Control, 67*, 18–24.

Imazaki, P. H., Tahiri, A., Rodrigues, A., Taminiau, B., Nezer, C., Daube, G., & Clinquart, A. (2012). Vacuum packaged beef with long shelf life: isolation and characterization of *Carnobacterium* strains. In *14èmes Journées Sciences du Muscle et Technologies des Viandes*. Caen.

Iskandar, C. F., Cailliez-Grimal, C., Rahman, A., Rondags, E., Remenant, B., Zagorec, M., Leisner, J. J., Borges, F., & Revol-Junelles, A.-M. (2016). Genes associated to lactose metabolism illustrate the high diversity of *Carnobacterium maltaromaticum*. *Food Microbiology, 58*, 79–86.

Koné, A. P., Zea, J. M. V., Gagné, D., Cinq-Mars, D., Guay, F., & Saucier, L. (2018). Application of *Carnobacterium maltaromaticum* as a feed additive for weaned rabbits to improve meat microbial quality and safety. *Meat Science, 135*, 174–188.

Laursen, B. G., Bay, L., Cleenwerck, I., Vancanneyt, M., Swings, J., Dalgaard, P., & Leisner, J. J. (2005). *Carnobacterium divergens* and *Carnobacterium maltaromaticum* as spoilers or protective cultures in meat and seafood: phenotypic and genotypic characterization. *Systematic and Applied Microbiology, 28*, 151–164.

Leisner, J. J., Laursen, B. G., Prévost, H., Drider, D., & Dalgaard, P. (2007). *Carnobacterium*: positive and negative effects in the environment and in foods. *FEMS Microbiology Reviews, 31*, 592–613.

Orihuel, A., Bonacina, J., Vildoza, M. J., Bru, E., Vignolo, G., Saavedra, L., & Fadda, S. (2018). Biocontrol of *Listeria monocytogenes* in a meat model using a combination of a bacteriocinogenic strain with curing additives. *Food Research International, 107*, 289–296.

Ozbey, G., Ertas, H. B., & Kok, F. (2006). Prevalence of *Listeria* species in camel sausages from retail markets in Aydin province in Turkey and RAPD analysis of *Listeria monocytogenesisolates*. *Irish Veterinary Journal, 59*, 342–344.

Prudêncio, C. V., dos Santos, M. T., & Vanetti, M. C. D. (2015). Strategies for the use of bacteriocins in Gram-negative bacteria: relevance in food microbiology. *Journal of Food Science and Technology, 52*, 5408–5417.

Prudêncio, C. V., Mantovani, H. C., Cecon, P. R., Prieto, M., & Vanetti, M. C. D. (2016). Temperature and pH influence the susceptibility of *Salmonella* Typhimurium to nisin combined with EDTA. *Food Control, 61*, 248–253.

Spanu, C., Piras, F., Mocci, A. M., Nieddu, G., De Santis, E. P. L., & Scarano, C. (2018). Use of *Carnobacterium* spp protective culture in MAP packed Ricotta fresca cheese to control *Pseudomonas* spp. *Food Microbiology, 74*, 50–56.

Tirloni, E., Cattaneo, P., Ripamonti, B., Agazzi, A., Bersani, C., & Stella, S. (2014). *In vitro* evaluation of *Lactobacillus animalis* SB310, *Lactobacillus paracasei* subsp. *paracasei* SB137 and their mixtures as potential bioprotective agents for raw meat. *Food Control, 41*, 63–68.

Tulini, F. L., Lohans, C. T., Bordon, K. C., Zheng, J., Arantes, E. C., Vederas, J. C., & De Martinis, E. C. (2014). Purification and characterization of antimicrobial peptides from fish isolate *Carnobacterium maltaromaticum* C2: Carnobacteriocin X and carnolysins A1 and A2. *International Journal of Food Microbiology, 173*, 81–88.

Wolfe, N. D., Dunavan, C. P., & Diamond, J. (2007). Origins of major human infectious diseases. *Nature, 447*, 279–283.

Zhang, P., Kaur, M., Bowman, J., Ratkowsky, D., & Tamplin, M. (2017). Effect of environmental factors on intra-specific inhibitory activity of *Carnobacterium maltaromaticum*. *Microorganisms, 5*, 59, 14 p.

Section III

General discussion

After the presentation of the experimental studies in **Section II**, a general discussion of the obtained results will be made in **Section III**. This section contains one chapter that makes an overview of the significant findings of this research and presents the practical implications and limitations of this study.

Chapter 6

Overview of significant findings, practical implications and limitations

6.1 Preservation of fresh meat with extremely long shelf life

The study of the preservation of fresh meat with extremely long shelf life (> 100 days) provided evidence that the use of sub-zero temperatures during vacuum storage is essential in meat shelf life extension since it retards meat deterioration, preventing Mb oxidation (discolouration) and the growth of certain spoilage bacteria, especially Enterobacteriaceae. It should be noted that the long shelf life displayed on these meats can only be achieved if and only if a storage temperature near the freezing point (−2°C) is respected throughout vacuum ageing. When a temperature of 4°C was applied during the last ⅓ of the shelf life to simulate a break in the cold chain, a decrease in the microbial and oxidative stability was observed. Jeremiah and Gibson (2001) had already highlighted that storage life could be more than doubled by storage at subzero temperatures. Therefore, it is crucial for operators in producer countries to ensure strict control of the cold chain during distribution if they want to consolidate export markets for chilled vacuum-packaged beef primals in geographically distant regions.

Furthermore, it was noticed a decline in the quality of these meats when they were repackaged in a high-O_2 atmosphere (retail display). Thus, it is not recommended that they be reconditioned, in the late stages of ageing, in consumer portions under

modified atmospheres rich in O_2. Such repackaging can, however, be envisaged for shorter ageing durations (generally less than or equal to ⅔ of the shelf life). Another possibility to prevent spoilage would be applying vacuum skin packaging for retail display. If a margin of safety is considered (break of the cold chain or possible repackaging in a high-O_2 atmosphere), then the shelf life attributed to these meats can be judged overestimated.

Taking into account Australian meat, not only its microbiological quality was excellent, it presented a high α-tocopherol content (6.1 µg/g) as well. Supplemental α-tocopherol in beef cattle diets can improve the shelf life of meat (Smith *et al.*, 1996) by minimising lipid oxidation and improving colour stability (Yang *et al.*, 2002; Suman *et al.*, 2014). Liu *et al.* (1995) considered 3.5 µg/g as the minimum α-tocopherol concentration that provides for near maximal suppression of lipid oxidation and MMb formation in fresh beef. Australian beef was largely above this threshold, while Brazilian meat, which also displayed a very long shelf life (120 days) presented an α-tocopherol content of only 3.2 µg/g. It should be noted that α-tocopherol content was not measured immediately after slaughter, and may have decreased during storage. In addition, the study of the composition of imported meats (e.g., fat content and fatty acid profile) and other endogenous antioxidant systems, including the MRA or antioxidant enzymes (e.g., SOD, CAT and GSH-Px) could have provided supplementary information to understand the oxidative stability of these meats.

Metagenetics analysis on imported meat revealed higher bacterial diversity in Brazilian, Irish and British meat when compared to Australian. In all cases, time contributed to the selection of fewer genera in the beef microbial environment. This result is in accordance with other studies on different food matrix (Ercolini, 2013; Delhalle *et al.*, 2016). Moreover, *C. maltaromaticum* was the dominant bacteria in Australian meat. This species has been used for biopreservation in meat products and seafood and is known for its inhibition properties against pathogenic and spoilage microorganisms (Leisner *et al.*, 2007). Hence, *C. maltaromaticum* may have played an essential role in the 140-day shelf life of Australian meat.

Finally, the lack of information related to the background of imported meats hinders the evaluation of the effect of production conditions (e.g., finishing system, fat score and carcase handling) on the quality of meat and differences observed between imported meat samples. Furthermore, designing an experimental scheme where samples were analysed at ⅔ and the end of shelf life generated results at different

ageing times, according to the origin of meat. These two constraints impeded an accurate comparison between meats of different origins.

6.2 Preservation of fresh meat from the Belgian Blue breed

The study of the preservation of fresh meat from the Belgian Blue breed showed a higher sensitivity of these meats to oxidation and microbial spoilage when compared to imported meats. However, it must be noted that the slaughter and storage conditions may not have been the same for meats from different origins.

The different combinations of studied parameters made it possible to identify those which are associated with better stability of meat:

a) ageing at −1°C > ageing at 4°C: temperature effect;
b) cull cow > young bull: effect of category (sex/age)
c) *longissimus thoracis et lumborum* > *rectus femoris*: effect of muscle
d) carcase ageing > vacuum ageing: effect of ageing technique.

When Belgian Blue meat was aged at −1°C, the onset of excessive bacterial growth was observed before oxidation phenomena. Specifically, the growth of the spoilage bacterium *B. thermosphacta* was the limiting parameter for vacuum ageing longer than 20 days at −1°C, permitting a subsequent 7-day shelf life under a high-O_2 atmosphere. Nielsen and Zeuthen (1986) showed that *B. thermosphacta* could grow at temperatures as low as −2°C. So, it is possible that subzero ageing can only show definite advantages when applied to meats with an excellent initial microbial quality. Also, extending vacuum ageing at 4°C for more than 20 days seems inappropriate for Belgian Blue beef, if it must be repackaged under a high-O_2 atmosphere, due to high microbial growth and Mb oxidation at this temperature. Finally, the duration of vacuum-packaged storage influenced microbial growth and lipid and pigment oxidation of beef during subsequent high-O_2 MAP storage: increasing ageing times promoted early oxidation and microbial growth during MAP storage.

With regard to the effect of category, meat from young bulls was more susceptible to Mb oxidation than meat from cull cows. Higher pigment oxidation was reported in muscles with higher Mb contents (Renerre *et al.*, 1996), and, in general, the Mb content is higher in muscles of bulls than those of cows at the same age (Lawrie and Ledward, 2006). However, myoglobin content increases as animals increase in age

(Miller, 2002). Therefore, it was not possible to conclude if the Mb content could explain the higher sensibility of young bulls to Mb oxidation when compared to cull cows. Concerning the fatty acid composition, meat from bulls presents higher proportions of PUFA than meat from cows (Venkata Reddy *et al.*, 2015). Since greater degrees of fatty acid unsaturation increases Mb oxidation (Chan *et al.*, 1997), differences in the fatty acid composition between young bulls and cull cows might also have explained the difference in pigment oxidation susceptibility. However, the fatty acid profile was not evaluated in these samples, and this hypothesis could be verified. Lastly, meat from young bulls presented an α-tocopherol content below the threshold proposed by Liu *et al.* (1995). Actually, the α-tocopherol content was 3.0 and 3.6 µg/g in meat from young bulls and cull cows, respectively. In this way, the lack of this antioxidant may have contributed to the low pigment stability of young bulls, confirming the importance of a correct assessment of dietary vitamin E supplementation to stabilise redness in meat.

In respect to the effect of muscle, from a microbiological point of view, *rectus femoris* was less stable than *longissimus thoracis et lumborum*. Two hypotheses have been put forward. Firstly, the anatomical location of the muscles could explain that they are more or less exposed to bacterial contaminations during slaughter and cutting. Secondly, differences in nutrient concentrations (e.g., glucose and amino acids) could lead to a selection of those micro-organisms that are adapted to the substrates present and promote their growth on meat.

Regarding the oxidative stability, *rectus femoris* was again more sensitive than *longissimus thoracis et lumborum*. Despite the higher sensitivity of *rectus femoris* to oxidation, it presented a higher α-tocopherol content than *longissimus thoracis et lumborum*. There are no clear explanations why skeletal muscles differ in α-tocopherol concentration; however, some hypotheses have been emitted. The first one is related to muscle fibre composition. Red fibres contain more but smaller mitochondria than white fibres. Smaller muscle fibres and reduced mitochondrial sizes should, in theory, provide greater surface areas with increased biological membrane volume, thereby providing more interactive sites and membrane surface for α-tocopherol incorporation (Porter and Palade, 1957). Current data on the fibre composition of different bovine muscles is variable (Ono *et al.*, 1996; Kirchofer *et al.*, 2002). For this reason, it is not easy to confirm if the red fibre content in *longissimus thoracis et lumborum* and *rectus femoris* contributed to their different α-tocopherol concentration. Then, differences in capillary density and capillary-to-fibre ratio could also explain the variation in α-tocopherol between muscles. In fact, high α-tocopherol concentration could be due to the residual

blood (with its associated α-tocopherol) entrapped in capillaries or to the great diffusion of α-tocopherol from more closely associated capillaries (Sheldon, 1984; Liu *et al.*, 1995). However, this hypothesis was not verified in the present study. Concerning other intrinsic factors that could retard oxidation in meat, MRA and CAT activity could be associated with the difference in oxidative stability observed between *longissimus thoracis et lumborum* and *rectus femoris*. The availability of nucleotides may explain differences in the MRA between muscles. Watts *et al.* (1966) demonstrated that increasing concentrations of nicotinamide adenine dinucleotide in meat increases MRA, and Faustman and Cassens (1991) showed that *longissimus thoracis et lumborum* present high concentrations of nicotinamide adenine dinucleotide. Lastly, CAT activity may be related to muscle metabolism. Christie and Stoward (1979) observed a higher CAT content in red fibres than in white fibres. Nevertheless, the nicotinamide adenine dinucleotide content and the fibre composition of muscles were not investigated in this research.

About the ageing techniques evaluated in this project, dry-ageing on the carcase could limit the oxidation of meat when it was repackaged in a high-O_2 atmosphere. Conversely, vacuum aged meat showed a high sensitivity to oxidation. The difference in the oxidative stability between dry- and wet-aged samples in the present research was explained by the experimental scheme. *Longissimus thoracis et lumborum* and *rectus femoris* are both entirely covered by superficial muscles from the back and thigh, respectively. In this study, vacuum aged samples were briefly in contact with air and light during retail cutting at the beginning of the experiment, while retail cutting of dry aged samples was performed seven days after. It is known that exposure to atmospheric O_2 and light can lead to beef discolouration (Andersen *et al.*, 1989; Lindahl, 2011), and may have contributed to the low colour stability of vacuum aged beef.

Furthermore, when these meats were repackaged in a modified atmosphere rich in O_2 and stored for 7 days at 4°C, the MRA values were zero. There was, therefore, no reducing activity in the meat, indicating the disappearance of mechanisms responsible for the reduction of MMb, and pointing out a disadvantage of meat display in high-O_2 atmospheres at 4°C. Finally, it should be highlighted that the biggest reason to dry age beef is to enhance its palatability attributes, yielding descriptive determinants such as brown-roasted, beefy/brothy, buttery, nutty, roasted nut and sweet (Kim *et al.*, 2018). There are currently two primary dry-ageing methods in the meat industry: conventional half carcases or quarters hanging and sub-primal dry-ageing, and the scientific literature on the former are limited. Therefore, beef ageing on the carcase should be considered as a subject for further studies.

In summary, extending the shelf life for vacuum-packaged Belgian Blue meat would be possible under the condition of strict respect of an ageing temperature close to the freezing point of meat and careful control of the hygiene at the slaughterhouse and cutting plant. In the present conditions, Belgian Blue meat should not exceed 20 days of vacuum-packaged ageing if they have to be repackaged in a high-O_2 atmosphere. Apart from the control of slaughterhouse hygiene conditions and the use of subzero chilling temperatures, an improvement of the antioxidant capacity of meat via production conditions (e.g., feed) is to be considered as an attractive way to increase the duration of meat preservation.

Lastly, differences in the experimental scheme used to evaluate the preservation of fresh meat from the Belgian Blue breed did not permit to compare all Belgian Blue meats between themselves and also not with imported meats. Furthermore, muscle fibre composition and metabolism were not studied. The assessment of these parameters could have given precious information to understand better the differences in oxidative stability found between cattle categories and muscles.

6.3 Biopreservation of fresh meat

In the study of the biopreservation of fresh meat, 11 isolates of *C. maltaromaticum* were obtained from Australian meat displaying a shelf life of 140 days. The assessment of physiological requirements revealed that *C. maltaromaticum* is adapted to low temperatures and O_2-depleted atmospheres. Therefore, its use as a protective culture in chilled meat and meat products should be associated with packaging systems such as vacuum packaging.

The genetic profiling revealed a low genomic variability between isolates from different samples and even batches. However, it is impossible to determine if this specific microorganism was added to these meats or if it was part of the naturally occurring microbiota that has been selected by the conservation conditions applied. The presence of genes coding for class IIa bacteriocins (*cbnBM1* for carnobacteriocin BM1 and *cbnB2* for carnobacteriocin B2) was highlighted in the major part of the isolates. Class II bacteriocins are highly active against *Listeria* strains (Zou *et al.*, 2018) and could also have potential applications against spoilage microorganisms (Ennahar *et al.*, 2000).

The inhibitory effect of *C. maltaromaticum* against spoilage and pathogenic microorganisms was evaluated *in situ* and *in vitro*. *Carnobacterium maltaromaticum* showed an inhibition effect against Enterobacteriaceae in commercial beef in an atmosphere without O_2. Furthermore, *C. maltaromaticum* showed an antagonist effect against *L. monocytogenes* in co-cultures, which was greater at −1 and 4 than at 25°C. When the co-cultures were supplemented with EDTA, which increases the permeability of the outer membrane of Gram-bacteria (Prudêncio *et al.*, 2016), *C. maltaromaticum* also inhibited the growth of *S.* Typhimurium and *E. coli* O157:H7.

The study of the cell-free supernatant of *C. maltaromaticum* did not evidence the production of bacteriocins. In a first glance, the incubation temperatures applied were probably too high to observe the activity of *C. maltaromaticum* bacteriocins (Gursky *et al.*, 2006). Besides temperature, other variables, including pH, medium composition and aeration, may have inhibited the production of bacteriocins by *C. maltaromaticum* (Zhang *et al.*, 2017).

Carnobacterium maltaromaticum may cause spoilage of meat and meat products (Laursen, et al., 2005) by producing molecules including 3-methyl-1-butanol, 1-octen-3-ol, butanoic acid and acetoin (Casaburi *et al.*, 2011). In the present study, sensory analyses were performed on beef patties inoculated with *C. maltaromaticum*. The results showed that the panellists could distinguish between inoculated and non-inoculated samples. However, one isolate (CM_B827) had a negligible negative impact on the sensory attributes of beef patties, and should, therefore, be used for further research on the potential use of *C. maltaromaticum* as a protective culture.

Finally, the use of biopreservation as an additional hurdle for fresh meat preservation should be taken as a promising alternative for controlling the growth of spoilage and pathogenic micro-organisms. “Natural” and “clean-label” are terms that are becoming increasingly popular with both consumers and food companies, and the use of micro-organisms generally recognised as safe meets the preference of consumers for “natural” additives rather than chemical ones. However, at this day, there is no specific legislation in the EU concerning biopreservation, and no other hurdle than cold and modified atmosphere may be applied to fresh meat (European Parliament and Council of the European Union, 2004). According to the Regulation (EU) No 2015/2283 (European Parliament and Council of the European Union, 2015), protective cultures should be considered as novel food, since they do not have a significant history of consumption within the EU before 1997. Before any novel food is approved for use on the European market, it must be rigorously assessed for safety. In this way, appropriate

regulation is necessary for the industrial development of protective cultures, whose effectiveness and safety must be demonstrated by scientific studies.

Conclusions and perspectives

This research permitted to study the microbial and physicochemical factors, such as spoilage bacteria growth and pigment and lipid oxidation, that determine the long-term preservation of chilled fresh beef at subzero temperatures.

Specific microbial environments and antioxidant capacity play an essential role in extended beef preservation. The selection of beneficial micro-organisms (e.g., LAB) and retardation of oxidation could be achieved with ageing at −1°C in low-O_2 packaging systems.

Further research on the antioxidant mechanisms and microbial ecosystems associated with meats with extremely long shelf life and, more specifically, the role of specific LAB, including *C. maltaromaticum*, will be a key to understand and control the extension of the shelf life of meat.

Future studies should take into account the background of meat samples. It was noted in this research that unknown production and distribution conditions might mislead the interpretation of results. Moreover, the present project showed that it is relevant to assess parameters such as fibre composition and muscle metabolism when studying the antioxidant capacity of meat. These parameters are intimately linked with pigment and lipid oxidation and should be evaluated together to understand oxidation phenomena in meat better. Finally, it is imperative to characterise the physiological

requirements of *C. maltaromaticum*, the conditions that allow this bacteria to exert its maximum biopreservative potential and the negative impact that it may have on sensory attributes in order to develop appropriate preservation strategies for meat and meat products.

References

– A –

ABE F. Exploration of the effects of high hydrostatic pressure on microbial growth, physiology and survival: perspectives from piezophysiology. *Bioscience, Biotechnology, and Biochemistry*, 2007, **71**, 2347-2357.

AHN D.U., MENDONÇA A.F., FENG X. The storage and preservation of meat: II — Nonthermal technologies. In: Toldrá F. (Ed.), Lawrie's meat science. Woodhead Publishing: Duxford (United Kingdom), 2017, 231-263.

AIDANI E., AGHAMOHAMMADI B., AKBARIAN M., MORSHEDI A., HADIDI M., GHASEMKHANI N., AKBARIAN A. Effect of chilling, freezing and thawing on meat quality: a review. *International Journal of Biosciences*, 2014, **5**, 159-169.

AIELLO L.C., WHEELER P. The expensive-tissue hypothesis: The brain and the digestive system in human and primate evolution. *Current Anthropology*, 1995, **36**, 199-221.

ALEXANDRATOS N., BRUINSMA J. World agriculture towards 2030/2050: the 2012 revision. Food and agriculture organization: Rome (Italy), 2012, 153 p.

ALLEN K.E., CORNFORTH D.P. Myoglobin oxidation in a model system as affected by nonheme iron and iron chelating agents. *Journal of Agricultural and Food Chemistry*, 2006, **54**, 10134-10140.

AMSA Meat color measurement guidelines: revised December 2012. American meat science association: Champaign, 2012, 135 p.

ANDERSEN H.J., BERTELSEN G., SKIBSTED L.H. Colour stability of minced beef. Ultraviolet barrier in packaging material reduces light-induced discoloration of frozen products during display. *Meat Science*, 1989, **25**, 155-159.

AWADA M., SOULAGE C.O., MEYNIER A., DEBARD C., PLAISANCIÉ P., BENOIT B., PICARD G., LOIZON E., CHAUVIN M.-A., ESTIENNE M., PERETTI N., GUICHARDANT M., LAGARDE M., GENOT C., MICHALSKI M.-C. Dietary oxidized n-3 PUFA induce oxidative stress and inflammation: Role of intestinal absorption of 4-HHE and reactivity in intestinal cells. *Journal of Lipid Research*, 2012, **53**, 2069-2080.

AYMERICH T., PICOUET P.A., MONFORT J.M. Decontamination technologies for meat products. *Meat Science*, 2008, **78**, 114-129.

– B –

BARCO L., BELLUCO S., ROCCATO A., RICCI A. A systematic review of studies on *Escherichia coli* and Enterobacteriaceae on beef carcasses at the slaughterhouse. *International Journal of Food Microbiology*, 2015, **207**, 30-39.

BEKHIT A.E., GEESINK G.H., MORTON J.D., BICKERSTAFFE R. Metmyoglobin reducing activity and colour stability of ovine *longissimus* muscle. *Meat Science*, 2001, **57**, 427-435.

BEKHIT A.E., FAUSTMAN C. Metmyoglobin reducing activity. *Meat Science*, 2005, **71**, 407-439.

BELCHER J.N. Industrial packaging developments for the global meat market. *Meat Science*, 2006, **74**, 143-148.

BELITZ H.-D., GROSCH W., SCHIEBERLE P. Food Chemistry. Springer: Berlin (Germany), 2009, 1116 p.

BERK Z. Food process engineering and technology. Academic Press: London (United Kingdom), 2018, 710 p.

BERNBOM N., LICHT T.R., BROGREN C.-H., JELLE B., JOHANSEN A.H., BADIOLA I., VOGENSEN F.K., NØRRUNG B. Effects of *Lactococcus lactis* on composition of intestinal microbiota: Role of nisin. *Applied and Environmental Microbiology*, 2006, **72**, 239-244.

BLAGOJEVIC B., ANTIC D., DUCIC M., BUNCIC S. Visual cleanliness scores of cattle at slaughter and microbial loads on the hides and the carcases. *Veterinary Record*, 2012, **170**, 563-563.

BOGDANOWICZ J., CIERACH M., ŻMIJEWSKI T. Effects of aging treatment and freezing/thawing methods on the quality attributes of beef from Limousin × Holstein-Friesian and Hereford × Holstein-Friesian crossbreeds. *Meat Science*, 2018, **137**, 71-76.

BORCH E., KANTMUERMANS M.L., BLIXT Y. Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology*, 1996, **33**, 103-120.

BREWER M.S., ZHU L.G., BIDNER B., MEISINGER D.J., MCKEITH F.K. Measuring pork color: Effects of bloom time, muscle, pH and relationship to instrumental parameters. *Meat Science*, 2001, **57**, 169-176.

BRODY A.L. Markets for MAP foods. In: Blakistone B.A. (Ed.), Principles and applications of modified atmosphere packaging of foods. Aspen Publishers: Gaithersburg (United States), 1999, 14-38.

BROWN W.D., MEBINE L.B. Autoxidation of oxymyoglobins. *The Journal of Biological Chemistry*, 1969, **244**, 6696-6701.

BROWN W.D., SNYDER H.E. Nonenzymatic reduction and oxidation of myoglobin and hemoglobin by nicotinamide adenine dinucleotides and flavins. *The Journal of Biological Chemistry*, 1969, **244**, 6702-6706.

BRYNJOLFSSON A. Future radiation sources and identification of irradiated foods. *Food Technology*, 1989, **43**, 84-89.

– C –

CAMPO M.M., NUTE G.R., HUGHES S.I., ENSER M., WOOD J.D., RICHARDSON R.I. Flavour perception of oxidation in beef. *Meat Science*, 2006, **72**, 303-311.

CANO-MUÑOZ G. Manual on meat cold store operation and management. Food and agriculture organization: Rome (Italy), 1991, 121 p.

CARLEZ A., VECIANA-NOGUES T., CHEFTEL J.-C. Changes in colour and myoglobin of minced beef meat due to high pressure processing. *LWT — Food Science and Technology*, 1995, **28**, 528-538.

CASABURI A., NASI A., FERROCINO I., DI MONACO R., MAURIELLO G., VILLANI F., ERCOLINI D. Spoilage-related activity of *Carnobacterium maltaromaticum* strains in air-stored and vacuum-packed meat. *Applied and Environmental Microbiology*, 2011, **77**, 7382-7393.

CASABURI A., DE FILIPPIS F., VILLANI F., ERCOLINI D. Activities of strains of *Brochothrix thermosphacta in vitro* and in meat. *Food Research International*, 2014, **62**, 366-374.

CASTELLANO P., BELFIORE C., FADDA S., VIGNOLO G. A review of bacteriocinogenic lactic acid bacteria used as bioprotective cultures in fresh meat produced in Argentina. *Meat Science*, 2008, **79**, 483-499.

CHAILLOU S., CHRISTIEANS S., RIVOLLIER M., LUCQUIN I., CHAMPOMIER-VERGÈS M.C., ZAGOREC M. Quantification and efficiency of *Lactobacillus sakei* strain mixtures used as protective cultures in ground beef. *Meat Science*, 2014, **97**, 332-338.

CHAN K.M., DECKER E.A. Endogenous skeletal muscle antioxidants. *Critical Reviews in Food Science and Nutrition*, 1994, **34**, 403-426.

CHAN W.K.M., FAUSTMAN C., DECKER E.A. Oxymyoglobin oxidation as affected by oxidation products of phosphatidylcholine liposomes. *Journal of Food Science*, 1997, **62**, 709-712.

CHAVES R.D., SILVA A.R., SANT'ANA A.S., CAMPANA F.B., MASSAGUER P.R. Gas-producing and spoilage potential of Enterobacteriaceae and lactic acid bacteria isolated from chilled vacuum-packaged beef. *International Journal of Food Science & Technology*, 2012, **47**, 1750-1756.

CHEFTEL J.C., CULIOLI J. Effects of high pressure on meat: A review. *Meat Science*, 1997, **46**, 211-236.

CHRISTIE K.N., STOWARD P.J. Catalase in skeletal muscle fibers. *Journal of Histochemistry & Cytochemistry*, 1979, **27**, 814-819.

CHURCH N. Developments in modified-atmosphere packaging and related technologies. *Trends in Food Science & Technology*, 1994, **5**, 345-352.

CLYDESDALE F.M., AHMED E.M. Colorimetry — Methodology and applications. *C R C Critical Reviews in Food Science and Nutrition*, 1978, **10**, 243-301.

COOPER G.M. The Cell: A molecular approach. Sinauer Associates: Sunderland (United States), 2000, 689 p.

CRAWFORD D.L., YU T.C., SINNHUBER R.O. Reaction mechanism, reaction of malonaldehyde with glycine. *Journal of Agricultural and Food Chemistry*, 1966, **14**, 182-184.

– D –

DAINTY R.H., MACKEY B.M. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. *Society for Applied Bacteriology Symposium Series*, 1992, **21**, 103S-114S.

DE CASTRO CARDOSO PEREIRA P.M., DOS REIS BALTAZAR VICENTE A.F. Meat nutritional composition and nutritive role in the human diet. *Meat Science*, 2013, **93**, 586-592.

DE HAAN C., STEINFELD H., BLACKBURN H. Livestock & the environment: Finding a balance. Food and agriculture organization: Rome (Italy), 2006, 115 p.

DE JONGHE V., COOREVITS A., VAN HOORDE K., MESSENS W., VAN LANDSCHOOT A., DE VOS P., HEYNDRICKX M. Influence of storage conditions on the growth of *Pseudomonas* species in refrigerated raw milk. *Applied and Environmental Microbiology*, 2011, **77**, 460-470.

DEAN R.T., FU S., STOCKER R., DAVIES M.J. Biochemistry and pathology of radical-mediated protein oxidation. *Biochemical Journal*, 1997, **324**, 1.

DECKER E.A., LIVISAY S.A., ZHOU S. Mechanisms of endogenous skeletal muscle antioxidants: Chemical and physical aspects. In: Decker E.A., Faustman C., Lopez-Bote C.J. (Eds.), Antioxidants in muscle foods. John Wiley & Sons Inc.: New York (United States), 39-47.

DECKER E.A., XU Z. Minimizing rancidity in muscle foods. *Food Technology*, 1998, **52**, 54-59.

DELHALLE L., KORSAK N., TAMINIAU B., NEZER C., BURTEAU S., DELCENSERIE V., POULLET J.B., DAUBE G. Exploring the bacterial diversity of Belgian steak tartare using metagenetics and quantitative real-time PCR analysis. *Journal of Food Protection*, 2016, **79**, 220-229.

DIAS DOS ANJOS GONÇALVES L., HILSDORF PICCOLI R., DE PAULA PERES A., VITAL SAÚDE A. Predictive modeling of *Pseudomonas fluorescens* growth under different temperature and pH values. *Brazilian Journal of Microbiology*, 2017, **48**, 352-358.

DOULGERAKI A.I., ERCOLINI D., VILLANI F., NYCHAS G.-J.E. Spoilage microbiota associated to the storage of raw meat in different conditions. *International Journal of Food Microbiology*, 2012, **157**, 130-141.

– E –

ECONOMOU T., POURNIS N., NTZIMANI A., SAVVAIDIS I.N. Nisin-EDTA treatments and modified atmosphere packaging to increase fresh chicken meat shelf-life. *Food Chemistry*, 2009, **114**, 1470-1476.

EGAN A.F. Lactic acid bacteria of meat and meat products. *Antonie Van Leeuwenhoek*, 1983, **49**, 327-336.

ELLIS D.I., GOODACRE R. Rapid and quantitative detection of the microbial spoilage of muscle foods: Current status and future trends. *Trends in Food Science & Technology*, 2001, **12**, 414-424.

ENNAHAR S., SASHIHARA T., SONOMOTO K., ISHIZAKI A. Class IIa bacteriocins: biosynthesis, structure and activity. *Fems Microbiology Reviews*, 2000, **24**, 85-106.

ENSER M., HALLETT K., HEWITT B., FURSEY G.A.J., WOOD J.D. Fatty acid content and composition of english beef, lamb and pork at retail. *Meat Science*, 1996, **42**, 443-456.

ERCOLINI D., RUSSO F., TORRIERI E., MASI P., VILLANI F. Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Applied and Environmental Microbiology*, 2006, **72**, 4663-4671.

ERCOLINI D., RUSSO F., BLAIOTTA G., PEPE O., MAURIELLO G., VILLANI F. Simultaneous detection of *Pseudomonas fragi*, *P. lundensis*, and *P. putida* from meat by use of a multiplex PCR assay targeting the *carA* gene. *Applied and Environmental Microbiology*, 2007, **73**, 2354-2359.

ERCOLINI D., RUSSO F., NASI A., FERRANTI P., VILLANI F. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential *in vitro* and in beef. *Applied and Environmental Microbiology*, 2009, **75**, 1990-2001.

ERCOLINI D. High-throughput sequencing and metagenomics: moving forward in the culture-independent analysis of food microbial ecology. *Applied and Environmental Microbiology*, 2013, **79**, 3148-3155.

ESTERBAUER H., GEBICKI J., PUHL H., JURGENS G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine*, 1992, **13**, 341-390.

ESTERBAUER H., MUSKIET F., HORROBIN D.F. Cytotoxicity and genotoxicity of lipid-oxidation products. *American Journal of Clinical Nutrition*, 1993, **57**, 779S-786S

ESTÉVEZ M. Protein carbonyls in meat systems: A review. *Meat Science*, 2011, **89**, 259-279.

ESTÉVEZ M., LUNA C. Dietary protein oxidation: A silent threat to human health? *Critical Reviews in Food Science and Nutrition*, 2017, **57**, 3781-3793.

EUROPEAN COMISSION The meat sector in the European Union. European Comission: Brussels (Belgium), 2004, 20 p.

EUROPEAN COMMISSION Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, 2005, **L 338**, 1-26.

EUROPEAN COMMISSION Commission Regulation (EC) No 101/2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcases. *Official Journal of the European Union*, 2013a, **L 34**, 1-3.

EUROPEAN COMMISSION EU beef farm report 2012 based on FADN data. European Union: Brussels (Belgium), 2013b, 110 p.

EUROPEAN PARLIAMENT AND COUNCIL OF THE EUROPEAN UNION Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. *Official Journal of the European Union*, 2004, **L 139**, 55-205.

EUROPEAN PARLIAMENT AND COUNCIL OF THE EUROPEAN UNION Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. *Official Journal of the European Union*, 2011, **L304**, 18-63.

EUROPEAN PARLIAMENT AND COUNCIL OF THE EUROPEAN UNION Regulation (EU) No 2015/2283 of the European Parliament and of the Council of 27 January 1997 on novel foods. *Official Journal of the European Union*, 2015, **L327**, 1-22.

EUROSTAT Agriculture, forestry and fishery statistics. European Union: Luxemburg (Luxemburg), 2013, 249 p.

EUROSTAT Agriculture, forestry and fishery statistics. European Union: Luxemburg (Luxemburg), 2017, 165 p.

– F –

FANG W., SUN J., LI Z.L., LE G., SHI Y. Effect of oxidated food protein on mice gut flora and redox state. *Chinese Journal of Microecology*, 2012, **24**, 193-196.

FAO Guidelines for slaughtering, meat cutting and further processing. Food and agriculture organization: Rome (Italy), 1991, 170 p.

FARBER J.M. Microbiological aspects of modified-atmosphere packaging technology — A review. *Journal of Food Protection*, 1991, **54**, 58-70.

FARKAS D.F., HOOVER D.G. High pressure processing. *Journal of Food Science*, 2000, **65**, 47-64.

FASFC Tableau avec les critères réglementaires et les limites d'action. [on line] Web address: http://www.afsca.be/publicationsthematiques/_documents/2018-01-16_Narval_microbio_VCT_JWS_ADK__CKS_5_jan_2018FR.xlsx, retrieved on 16/03/2018.

FAUSTMAN C., CASSENS R.G. The biochemichal basis for discoloration in fresh meat: A review. *Journal of Muscle Foods*, 1990, **1**, 217-243.

FAUSTMAN C., CASSENS R.G. The effect of cattle breed and muscle type on discoloration and various biochemical parameters in fresh beef. *Journal of Animal Science*, 1991, **69**, 184-193.

FAUSTMAN C., SUN Q., MANCINI R., SUMAN S.P. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. *Meat Science*, 2010a, **86**, 86-94.

FAUSTMAN C., YIN S., TATIYABORWORNTHAM N. Oxidation and protection of red meat. In: Decker E.A., Elias R.J., McClements D.J. (Eds.), Oxidation in foods and beverages and antioxidant applications. Woodhead Publishing: Duxford (United Kingdom), 2010b, 3-49.

FEURER C., CHRISTIEANS S., RIVOLLIER M., LEROY S., TALON R., CHAMPOMIER VERGÈS M.-C., ZAGOREC M., DESMONTS M.-H. Applications dans la filière des produits carnés. In: Zagorec M., Christiaens V. (Eds.), Flores protectrices pour la conservation des aliments. Éditions Quae: Versailles (France), 2013, 69-89.

FIEMS L.O., DE CAMPENEERE S., VAN CAELENBERGH W., DE BOEVER J.L., VANACKER J.M. Carcass and meat quality in double-muscled Belgian Blue bulls and cows. *Meat Science*, 2003, **63**, 345-352.

FRANCIS F.J., CLYDESDALE F.M. Food colorimetry: Theory and applications. The Avi Publishing Company, Inc.: Westport (United States), 1975, 477 p.

– G –

GARCÍA-LÓPEZ M.L., PRIETO M., OTERO A. The physiological attributes of Gram-negative bacteria associated with spoilage of meat and meat products. In: Davies A., Board R. (Eds.), The microbiology of meat and poultry. Blackie Academic & Progessional: London (United Kingdom), 1998, 1-34.

GEORGE P., STRATMANN C.J. The oxidation of myoglobin to metmyoglobin by oxygen. The relation between the first order rate constant and the partial pressure of oxygen. *Biochemical Journal*, 1952, **51**, 418-425.

GIDDING S.S., LICHTENSTEIN A.H., FAITH M.S., KARPYN A., MENNELLA J.A., POPKIN B., ROWE J., VAN HORN L., WHITSEL L. Implementing american heart association pediatric and adult nutrition guidelines. *Circulation*, 2009, **119**, 1161-1175.

GILL C.O., TAN K.H. Effect of carbon dioxide on growth of meat spoilage bacteria. *Applied and Environmental Microbiology*, 1980, **39**, 317-319.

GILL C.O. Extending the storage life of raw chilled meats. *Meat Science*, 1996, **43**, S99-S109.

GOTOH T., SHIKAMA K. Autoxidation of native oxymyoglobin from bovine heart muscle. *Archives of Biochemistry and Biophysics*, 1974, **163**, 476-481.

GRAHAM W.D., STEVENSON M.H., STEWART E.M. Effect of irradiation dose and irradiation temperature on the thiamin content of raw and cooked chicken breast meat. *Journal of the Science of Food and Agriculture*, 1998, **78**, 559-564.

GRAM L., RAVN L., RASCH M., BRUHN J.B., CHRISTENSEN A.B., GIVSKOV M. Food spoilage — Interactions between food spoilage bacteria. *International Journal of Food Microbiology*, 2002, **78**, 79-97.

GRANDIN T. The effect of stress on livestock and meat quality prior to and during slaughter. *International Journal for the Study of Animal Problems*, 1980, **1**, 313-337.

GREENE B.E., HSIN I.M., ZIPSER M.W. Retardation of oxidative color changes in raw ground beef. *Journal of Food Science*, 1971, **36**, 940-942.

GREENE B.E., CUMUZE T.H. Relationship between TBA numbers and inexperienced panelists' assessments of oxidized flavor in cooked beef. *Journal of Food Science*, 1982, **47**, 52-54.

GROBBEL J.P., DIKEMAN M.E., HUNT M.C., MILLIKEN G.A. Effects of packaging atmospheres on beef instrumental tenderness, fresh color stability, and internal cooked color. *Journal of Animal Science*, 2008, **86**, 1191-1199.

GRUNERT K.G. What's in a steak? A cross-cultural study on the quality perception of beef. *Food Quality and Preference*, 1997, **8**, 157-174.

GURER-ORHAN H., ERCAL N., MARE S., PENNATHUR S., ORHAN H., HEINECKE J.W. Misincorporation of free *m*-tyrosine into cellular proteins: A potential cytotoxic mechanism for oxidized amino acids. *Biochemical Journal*, 2006, **395**, 277-284.

GURSKY L.J., MARTIN N.I., DERKSEN D.J., VAN BELKUM M.J., KAUR K., VEDERAS J.C., STILES M.E., MCMULLEN L.M. Production of piscicolin 126 by *Carnobacterium maltaromaticum* UAL26 is controlled by temperature and induction peptide concentration. *Archives of Microbiology*, 2006, **186**, 317-325.

GUYON C., MEYNIER A., DE LAMBALLERIE M. Protein and lipid oxidation in meat: A review with emphasis on high-pressure treatments. *Trends in Food Science & Technology*, 2016, **50**, 131-143.

– H –

HANSEN E., JUNCHER D., HENCKEL P., KARLSSON A., BERTELSEN G., SKIBSTED L.H. Oxidative stability of chilled pork chops following long term freeze storage. *Meat Science*, 2004, **68**, 479-484.

HASTINGS J.W., STILES M.E., VON HOLY A. Bacteriocins of leuconostocs isolated from meat. *International Journal of Food Microbiology*, 1994, **24**, 75-81.

HINTLIAN C.B., HOTCHKISS J.H. Comparative growth of spoilage and pathogenic organisms on modified atmosphere-packaged cooked beef. *Journal of Food Protection*, 1987, **50**, 218-223.

HLADIK C.M., PASQUET P. The human adaptations to meat eating: a reappraisal. *Human Evolution*, 2002, **17**, 199-206.

HODGES J.H., CAHILL V.R., OCKERMAN H.W. Effect of vacuum packaging on weight loss, microbial growth and palatability of fresh beef wholesale cuts. *Journal of Food Science*, 1974, **39**, 143-146.

HOOD D.E., RIORDAN E.B. Discolouration in pre-packaged beef: Measurement by reflectance spectrophotometry and shopper discrimination. *International Journal of Food Science & Technology*, 1973, **8**, 333-343.

HOUTSMA P.C., DE WIT J.C., ROMBOUTS F.M. Minimum inhibitory concentration (MIC) of sodium lactate for pathogens and spoilage organisms occurring in meat products. *International Journal of Food Microbiology*, 1993, **20**, 247-257.

– I –

ILLIKOUD N., ROSSERO A., CHAUVET R., COURCOUX P., PILET M.-F., CHARRIER T., JAFFRÈS E., ZAGOREC M. Genotypic and phenotypic characterization of the food spoilage bacterium *Brochothrix thermosphacta*. *Food Microbiology*, 2018, in press.

IMAZAKI P.H., MARÉCHAL A., NEZER C., TAMINIAU B., DAUBE G., CLINQUART A. Influence of temperature on conservability of chilled vacuum packed beef from different origins. In: 57th International Congress of Meat Science and Technology (proceedings), Ghent (Belgium), 2011.

IMAZAKI P.H., TAHIRI A., RODRIGUES A., TAMINIAU B., NEZER C., DAUBE G., CLINQUART A. Vacuum packaged beef with long shelf life: Isolation and characterization of *Carnobacterium* strains. In: 14èmes Journées Sciences du Muscle et Technologies des Viandes (proceedings), Caen (France), 2012.

IMAZAKI P.H., DOUNY C., ELANSARY M., SCIPPO M.-L., CLINQUART A. Effect of muscle type, aging technique, and aging time on oxidative stability and antioxidant capacity of beef packed in high-oxygen atmosphere. *Journal of Food Processing and Preservation*, 2018, **42**, e13603.

– J –

JAMES S.J., JAMES C. Cold chain. In: Jensen W.K. (Ed.), Encyclopedia of meat sciences. Elsevier: Oxford (United Kingdom), 2004, 691-696.

JAY J.M., VILAI J.P., HUGHES M.E. Profile and activity of the bacterial biota of ground beef held from freshness to spoilage at 5–7 °C. *International Journal of Food Microbiology*, 2003, **81**, 105-111.

JEREMIAH L.E., GIBSON L.L. The influence of storage temperature and storage time on color stability, retail properties and case-life of retail-ready beef. *Food Research International*, 2001, **34**, 815-826.

JOHANSSON P., PAULIN L., SÄDE E., SALOVUORI N., ALATALO E.R., BJÖRKROTH K.J., AUVINEN P. Genome sequence of a food spoilage lactic acid bacterium, *Leuconostoc gasicomitatum* LMG 18811(T), in association with specific spoilage reactions. *Applied and Environmental Microbiology*, 2011, **77**, 4344-4351.

JONES R.J., HUSSEIN H.M., ZAGOREC M., BRIGHTWELL G., TAGG J.R. Isolation of lactic acid bacteria with inhibitory activity against pathogens and spoilage organisms associated with fresh meat. *Food Microbiology*, 2008, **25**, 228-234.

JONES R.J., WIKLUND E., ZAGOREC M., TAGG J.R. Evaluation of stored lamb bio-preserved using a three-strain cocktail of *Lactobacillus sakei*. *Meat Science*, 2010, **86**, 955-959.

JONGBERG S., LUND M.N., SKIBSTED L.H. Protein oxidation in meat and meat products. Challenges for antioxidative protection. In: Barbosa-Cánovas G.V., María Pastore G., Candoğan K., Medina Meza I.G., Caetano da Silva Lannes S., Buckle K., Yada R.Y., Rosenthal A. (Eds.), Global food security and wellness. Springer: New York (United States) 2017, 315-337.

JOO N.E., RITCHIE K., KAMARAJAN P., MIAO D., KAPILA Y.L. Nisin, an apoptogenic bacteriocin and food preservative, attenuates HNSCC tumorigenesis via CHAC1. *Cancer Medicine*, 2012, **1**, 295-305.

JUNG S., GHOUL M., DE LAMBALLERIE-ANTON M. Influence of high pressure on the color and microbial quality of beef meat. *LWT — Food Science and Technology*, 2003, **36**, 625-631.

– K –

KANNER J., SHEGALOVICH I., HAREL S., HAZAN B. Muscle lipid peroxidation dependent on oxygen and free metal ions. *Journal of Agricultural and Food Chemistry*, 1988, **36**, 409-412.

KAUR M., BOWMAN J.P., PORTEUS B., DANN A.L., TAMPLIN M. Effect of abattoir and cut on variations in microbial communities of vacuum-packaged beef. *Meat Science*, 2017, **131**, 34-39.

KERRY J.P., TYUFTIN A.A. Storage and preservation of raw meat and muscle-based food products: IV — Storage and packaging. In: Toldrá F. (Ed.), Lawrie's meat science. Woodhead Publishing: Duxford (United Kingdom), 2017, 297-327.

KESHAVARZIAN A., BANAN A., FARHADI A., KOMANDURI S., MUTLU E., ZHANG Y., FIELDS J.Z. (2003). Increases in free radicals and cytoskeletal protein oxidation and nitration in the colon of patients with inflammatory bowel disease. *Gut*, 2003, **52**, 720-728.

KIM Y.H., HUNT M.C., MANCINI R.A., SEYFERT M., LOUGHIN T.M., KROPF D.H., SMITH S. Mechanism for lactate-color stabilization in injection-enhanced beef. *Journal of Agricultural and Food Chemistry*, 2006, **54**, 7856-7862.

KIM Y.H., HUFF-LONERGAN E., SEBRANEK J.G., LONERGAN S.M. High-oxygen modified atmosphere packaging system induces lipid and myoglobin oxidation and protein polymerization. *Meat Science*, 2010, **85**, 759-767.

KIM Y.H.B., MEYERS B., KIM H.-W., LICEAGA A.M., LEMENAGER R.P. Effects of stepwise dry/wet-aging and freezing on meat quality of beef loins. *Meat Science*, 2017, **123**, 57-63.

KIM Y.H.B., MA D., SETYABRATA D., FAROUK M.M., LONERGAN S.M., HUFF-LONERGAN E., HUNT M.C. Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. *Meat Science*, 2018, **144**, 74-90.

KIRCHOFER K.S., CALKINS C.B., GWARTNEY B.L. Fiber-type composition of muscles of the beef chuck and round. *Journal of Animal Science*, 2002, **80**, 2872-2878.

KLAENHAMMER T.R. Genetics of bacteriocins produced by lactic acid bacteria. *Fems Microbiology Reviews*, 1993, **12**, 39-85.

KONÉ A.P., ZEA J.M.V., GAGNÉ D., CINQ-MARS D., GUAY F., SAUCIER L. Application of *Carnobacterium maltaromaticum* as a feed additive for weaned rabbits to improve meat microbial quality and safety. *Meat Science*, 2018, **135**, 174-188.

KOO O.-K., EGGLETON M., O'BRYAN C.A., CRANDALL P.G., RICKE S.C. Antimicrobial activity of lactic acid bacteria against *Listeria monocytogenes* on frankfurters formulated with and without lactate/diacetate. *Meat Science*, 2012, **92**, 533-537.

KOOS J., JANSENER K.E. Lactate — An opportunity to improve safety of processed meat and poultry. *Fleischwirtschaft*, 1995, **75**, 1296-1298.

KOUTSOUMANIS K., STAMATIOU A., SKANDAMIS P., NYCHAS G.J.E. Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat, and validation of the model under dynamic temperature conditions. *Applied and Environmental Microbiology*, 2006, **72**, 124-134.

KRAFT A.A. Psychrotrophic bacteria in foods: Disease and spoilage. CRC Press: Boca Raton, 1992, 277 p.

KRZYWICKI K. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Science*, 1979, **3**, 1-10.

– L –

LABADIE J. Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Science*, 1999, **52**, 299-305.

LAGERSTEDT Å., ENFÄLT L., JOHANSSON L., LUNDSTRÖM K. Effect of freezing on sensory quality, shear force and water loss in beef *M. longissimus dorsi*. *Meat Science*, 2008, **80**, 457-461.

LAMBERT A.D., SMITH J.P., DODDS K.L. Shelf life extension and microbiological safety of fresh meat — A review. *Food Microbiology*, 1991, **8**, 267-297.

LAURSEN B.G., BAY L., CLEENWERCK I., VANCANNEYT M., SWINGS J., DALGAARD P., LEISNER J.J. *Carnobacterium divergens* and *Carnobacterium maltaromaticum* as spoilers or protective cultures in meat and seafood: Phenotypic and genotypic characterization. *Systematic and Applied Microbiology*, 2005, **28**, 151-164.

LAWRIE R.A., LEDWARD D.A. Lawrie's meat science. Woodhead Publishing: Duxford (United Kingdom), 2006, 464 p.

LEDWARD D.A. Metmyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres. *Journal of Food Science*, 1970, **35**, 33-37.

LEISNER J.J., GREER G.G., DILTS B.D., STILES M.E. Effect of growth of selected lactic-acid bacteria on storage life of beef stored under vacuum and in air. *International Journal of Food Microbiology*, 1995, **26**, 231-243.

LEISNER J.J., LAURSEN B.G., PRÉVOST H., DRIDER D., DALGAARD P. *Carnobacterium*: positive and negative effects in the environment and in foods. *Fems Microbiology Reviews*, 2007, **31**, 592-613.

LEYGONIE C., BRITZ T.J., HOFFMAN L.C. Impact of freezing and thawing on the quality of meat: Review. *Meat Science*, 2012, **91**, 93-98.

LI D., ZHU Z., SUN D.-W. Effects of freezing on cell structure of fresh cellular food materials: A review. *Trends in Food Science & Technology*, 2018, **75**, 46-55.

LINDAHL G. Colour stability of steaks from large beef cuts aged under vacuum or high oxygen modified atmosphere. *Meat Science*, 2011, **87**, 428-435.

LINDH H., WILLIAMS H., OLSSON A., WIKSTRÖM F. Elucidating the indirect contributions of packaging to sustainable development: A terminology of packaging functions and features. *Packaging Technology and Science*, 2016, **29**, 225-246.

LIU Q., LANARI M.C., SCHAEFER D.M. A review of dietary vitamin E supplementation for improvement of beef quality. *Journal of Animal Science*, 1995, **73**, 3131-3140.

LUND M.N., LAMETSCH R., HVIID M.S., JENSEN O.N., SKIBSTED L.H. High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage. *Meat Science*, 2007, **77**, 295-303.

LUND M.N., HEINONEN M., BARON C.P., ESTÉVEZ M. Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research*, 2011, **55**, 83-95.

– M –

MA H.-J., LEDWARD D.A. High pressure/thermal treatment effects on the texture of beef muscle. *Meat Science*, 2004, **68**, 347-355.

MADDEN R.H., MURRAY K.A., GILMOUR A. Determination of the principal points of product contamination during beef carcass dressing processes in Northern Ireland. *Journal of Food Protection*, 2004, **67**, 1494-1496.

MANCINI R.A., HUNT M.C. Current research in meat color. *Meat Science*, 2005, **71**, 100-121.

MANCINI R.A., HUNT M.C., HACHMEISTER K.A., SEYFERT M.A., KROPF D.H., JOHNSON D.E., CUSICK S., MORROW C. The utility of lactate and rosemary in beef enhancement solutions: Effects on *longissimus* color changes during display. *Journal of Muscle Foods*, 2005, **16**, 27-36.

MANN N.J. A brief history of meat in the human diet and current health implications. *Meat Science*, 2018, **144**, 169-179.

MARTINAUD A., MERCIER Y., MARINOVA P., TASSY C., GATELLIER P., RENERRE M. Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *Journal of Agricultural and Food Chemistry*, 1997, **45**, 2481-2487.

MCMILLIN K.W., HUANG N.Y., HO C.P., SMITH B.S. Quality and shelf-life of meat in case-ready modified atmosphere packaging. In: Xiong Y.L., Ho C.-T., Shahidi F. (Eds.), Quality attributes of muscle foods. Kluwer Academic / Plenum Publishers: New York (United States), 1999, 73-93.

MCMILLIN K.W. Where is MAP going? A review and future potential of modified atmosphere packaging for meat. *Meat Science*, 2008, **80**, 43-65.

MCMILLIN K.W. Advancements in meat packaging. *Meat Science*, 2017, **132**, 153-162.

MCPHERRON S.P., ALEMSEGED Z., MAREAN C.W., WYNN J.G., REED R., GERAARDS D., BOBE R., BÉARAT H.A. Evidence for stone-tool-assisted consumption of animal tissues before 3.39 million years ago at Dikika, Ethiopia. *Nature*, 2010, **466**, 857-860.

MILLAR S.J., MOSS B.W., STEVENSON M.H. The effect of ionising radiation on the colour of beef, pork and lamb. *Meat Science*, 2000, **55**, 349-360.

MILLER R.K. Factors affecting the quality of raw meat. In: Kerry J., Kerry J., Ledward D. (Eds.), Meat Processing. Woodhead Publishing: 2002, 27-63.

MILLS J., DONNISON A., BRIGHTWELL G. Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: A review. *Meat Science*, 2014, **98**, 71-80.

MONSON F., SANUDO C., SIERRA I. Influence of breed and ageing time on the sensory meat quality and consumer acceptability in intensively reared beef. *Meat Science*, 2005, **71**, 471-479.

MONTEL M.C., MASSON F., TALON R. Bacterial role in flavour development. *Meat Science*, 1998, **49**, S111-S123.

MOR-MUR M., YUSTE J. High pressure processing applied to cooked sausage manufacture: physical properties and sensory analysis. *Meat Science*, 2003, **65**, 1187-1191.

MUELA E., SAÑUDO C., CAMPO M.M., MEDEL I., BELTRÁN J.A. Effect of freezing method and frozen storage duration on lamb sensory quality. *Meat Science*, 2012, **90**, 209-215.

– N –

NEWTON K.G., GILL C.O. The microbiology of DFD fresh meats: A review. *Meat Science*, 1981, **5**, 223-232.

NIELSEN H.-J.S., ZEUTHEN P. Growth of spoilage bacteria in broth and vacuum-packed bologna-type sausage at fluctuating temperatures and low temperature storage. *Journal of Food Protection*, 1986, **49**, 886-890.

NUBEL Table belge de composition des aliments. Nutrients Belgium: Brussels (Belgium), 2017, 112 p.

NYCHAS G.J., SKANDAMIS P.N., TASSOU C.C., KOUTSOUMANIS K.P. Meat spoilage during distribution. *Meat Science*, 2008, **78**, 77-89.

– O –

O'SULLIVAN M.G. The stability and shelf life of meat and poultry. In: Subramaniam P. (Ed.), The stability and shelf life of food. Woodhead Publishing: Duxford (United Kingdom), 2016, 521-543.

OECD Meat. In: OECD, FAO (Eds.), Agricultural outlook 2016-2025. Organisation for economic co-operation and development: Paris (France), 2016, 133 p.

OECD Meat. In: OECD, FAO (Eds.), Agricultural outlook 2017-2026. Organisation for economic co-operation and development: Paris (France), 2017, 137 p.

ONO Y., SOLOMON M.B., ELSASSER T.H., RUMSEY T.S., MOSELEY W.M. Effects of Synovex-S and recombinant bovine growth hormone (Somavubove) on growth responses of steers: II. Muscle morphology and proximate composition of muscles. *Journal of Animal Science*, 1996, **74**, 2929-2934.

– P –

PACHLOVÁ V., BUŇKOVÁ L., FLASAROVÁ R., SALEK R.-N., DLABAJOVÁ A., BUTOR I., BUŇKA F. Biogenic amine production by nonstarter strains of *Lactobacillus curvatus* and *Lactobacillus paracasei* in the model system of Dutch-type cheese. *LWT — Food Science and Technology*, 2018, **97**, 730-735.

PAPADOPOULOU O.S., DOULGERAKI A.I., BOTTA C., COCOLIN L., NYCHAS G.-J.E. Genotypic characterization of *Brochothrix thermosphacta* isolated during storage of minced pork under aerobic or modified atmosphere packaging conditions. *Meat Science*, 2012, **92**, 735-738.

PASTSART U., DE BOEVER M., CLAEYS E., DE SMET S. Effect of muscle and post-mortem rate of pH and temperature fall on antioxidant enzyme activities in beef. *Meat Science*, 2013, **93**, 681-686.

PEARSON A.M., LOVE J.D., SHORLAND F.B. “Warmed-over” flavor in meat, poultry, and fish. In: Chichester C.O., Mrak E.M., Stewart G.F. (Eds.), Advances in Food Research. Academic Press: Cambridge (United States), 1977, 1-74.

PEETERS A. Country pasture/forage resource profiles. Food and agriculture organization: Rome (Italy), 2010, 61 p.

PENNACCHIA C., ERCOLINI D., VILLANI F. Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. *Food Microbiology*, 2011, **28**, 84-93.

PEREIRA C.I., BARRETO CRESPO M.T., SAN ROMÃO M.V. Evidence for proteolytic activity and biogenic amines production in *Lactobacillus curvatus* and *L. homohiochii*. *International Journal of Food Microbiology*, 2001, **68**, 211-216.

PEREZ-CHABELA M.L., MATEO-OYAGUE J. Frozen meat: quality and shelf life. In: Hui Y.H., Cornillon P., Lagaretta I.G., Lim M.H., Murrell K.D., Nip W.K. (Eds.), Handbook of frozen foods. Marcel Dekker Inc.: New York (United States), 2004, 177-179.

PERRY N. Dry aging beef. *International Journal of Gastronomy and Food Science*, 2012, **1**, 78-80.

PICGIRARD L. Comparaison de muscles d'origine française et sud-américaine. *Viandes et Produits Carnés*, 2009, **27**, 171-177.

PIN C., GARCÍA DE FERNANDO G.D., ORDÓÑEZ J.A. Effect of modified atmosphere composition on the metabolism of glucose by *Brochothrix thermosphacta*. *Applied and Environmental Microbiology*, 2002, **68**, 4441-4447.

PORTER K.R., PALADE G.E. Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells. *Journal of Biophysical and Biochemical Cytology*, 1957, **3**, 269-300.

POTHAKOS V., DEVLIEGHERE F., VILLANI F., BJÖRKROTH J., ERCOLINI D. Lactic acid bacteria and their controversial role in fresh meat spoilage. *Meat Science*, 2015, **109**, 66-74.

PRUDÊNCIO C.V., MANTOVANI H.C., CECON P.R., PRIETO M., VANETTI M.C.D. Temperature and pH influence the susceptibility of *Salmonella* Typhimurium to nisin combined with EDTA. *Food Control*, 2016, **61**, 248-253.

PSOUNI E., JANKE A., GARWICZ M. Impact of carnivory on human development and evolution revealed by a new unifying model of weaning in mammals. *PLoS One*, 2012, **7**, e32452.

– Q –

QUIGLEY L., O'SULLIVAN O., STANTON C., BERESFORD T.P., ROSS R.P., FITZGERALD G.F., COTTER P.D. The complex microbiota of raw milk. *Fems Microbiology Reviews*, 2013, **37**, 664-698.

– R –

RAHARJO S., SOFOS J.N., SCHMIDT G.R. Improved speed, specificity, and limit of determination of an aqueous acid-extraction thiobarbituric acid-C_{18} method for measuring lipid-peroxidation in beef. *Journal of Agricultural and Food Chemistry*, 1992, **40**, 2182-2185.

RAY B., SANDINE W.E. Acetic, propionic, and lactic acids of starter culture bacteria as biopreservatives. In: Ray B., Daeschel M. (Eds.), Food preservatives of microbial origin. CRC Press: Boca Raton (United States), 1992, 103-136.

REMENANT B., JAFFRÈS E., DOUSSET X., PILET M.-F., ZAGOREC M. Bacterial spoilers of food: Behavior, fitness and functional properties. *Food Microbiology*, 2015, **45**, 45-53.

RENERRE M., DUMONT F., GATELLIER P. Antioxidant enzyme activities in beef in relation to oxidation of lipid and myoglobin. *Meat Science*, 1996, **43**, 111-121.

RÍOS COLOMBO N.S., CHALÓN M.C., NAVARRO S.A., BELLOMIO A. Pediocin-like bacteriocins: new perspectives on mechanism of action and immunity. *Current Genetics*, 2018, **64**, 345-351.

ROBERTSON, G.L. Food packaging: Principles and practices. CRC Press: Boca Raton (United States), 2012, 733 p.

ROSMINI M.R., PEREZ-ALVAREZ J.A., FERNANDEZ-LOPEZ J. Operational processes for frozen red meat. In: Hui Y.H., Cornillon P., Lagaretta I.G., Lim M.H., Murrell K.D., Nip W.K. (Eds.), Handbook of frozen foods. Marcel Dekker Inc.: New York (United States), 2004, 177-179.

– S –

SAKAI T., KUWAZURU S., YAMAUCHI K., UCHIDA K. A lipid peroxidation-derived aldehyde, 4-hydroxy-2-nonenal and ω6 fatty acids contents in meats. *Bioscience, Biotechnology, and Biochemistry*, 1995, **59**, 1379-1380.

SAKAI T., YAMAUCHI K., KUWAZURU S., GOTOH N. Relationships between 4-hydroxy-2-nonenal, 2-thiobarbituric acid reactive substances and n-6 polyunsaturated fatty acids in refrigerated and frozen pork. *Bioscience, Biotechnology, and Biochemistry*, 1998, **62**, 2028-2029.

SAKALA R.M., HAYASHIDANI H., KATO Y., KANEUCHI C., OGAWA M. Isolation and characterization of *Lactococcus piscium* strains from vacuum-packaged refrigerated beef. *Journal of Applied Microbiology*, 2002, **92**, 173-179.

SAMELIS J. Managing microbial spoilage in the meat industry. In: Blackburn C.d.W. (Ed.), Food spoilage microorganisms. Woodhead Publishing: Duxford (United Kingdom), 2006, 213-286.

SARZEAUD P., BÉCHEREL F., PERROT C. Impact of the CAP reform on the evolution of beef production and beef farming systems in Europe: Synthesis. In: Sarzeaud P., Dimitriadou A., Zjalic M. (Eds.), EU beef farming systems and CAP regulations. Wageningen Academic Publishers: Wageningen (Netherlands), 2008, 111-118.

SAVELL J.W., MUELLER S.L., BAIRD B.E. The chilling of carcasses. *Meat Science*, 2005, **70**, 449-459.

SERPEN A., GOKMEN V., FOGLIANO V. Total antioxidant capacities of raw and cooked meats. *Meat Science*, 2012, **90**, 60-65.

SHELDON B.W. Effect of dietary tocopherol on the oxidative stability of turkey meat. *Poultry Science*, 1984, **63**, 673-681.

SIES H., STAHL W., SEVANIAN A. Nutritional, dietary and postprandial oxidative stress. *Journal of Nutrition*, 2005, **135**, 96-972.

SIKES A., TORNBERG E., TUME R. A proposed mechanism of tenderising post-rigor beef using high pressure–heat treatment. *Meat Science*, 2010, **84**, 390-399.

SIU G.M., DRAPER H.H. A survey of the malonaldehyde content of retail meats and fish. *Journal of Food Science*, 1978, **43**, 1147-1149.

SMALL A.H., JENSON I.A.N., KIERMEIER A., SUMNER J. Vacuum-packed beef primals with extremely long shelf life have unusual microbiological counts. *Journal of Food Protection*, 2012, **75**, 1524-1527.

SMITH G.C., MORGAN J.B., SOFOS J.N., TATUM J.D. Supplemental vitamin E in beef cattle diets to improve shelf-life of beef. *Animal Feed Science and Technology*, 1996, **59**, 207-214.

SOGEPA Regards sur l'économie wallonne: Le secteur de la viande en Wallonie. Société wallonne de gestion et de participations: Liège (Belgium), 2016, 142 p.

SOHAIB M., ANJUM F.M., ARSHAD M.S., IMRAN M., IMRAN A., HUSSAIN S. Oxidative stability and lipid oxidation flavoring volatiles in antioxidants treated chicken meat patties during storage. *Lipids in Health and Disease*, 2017, **16**, 27.

STANBOROUGH T., FEGAN N., POWELL S.M., SINGH T., TAMPLIN M., CHANDRY P.S. Genomic and metabolic characterization of spoilage-associated *Pseudomonas* species. *International Journal of Food Microbiology*, 2018, **268**, 61-72.

STILES M.E., HASTINGS J.W. Bacteriocin production by lactic acid bacteria: Potential for use in meat preservation. *Trends in Food Science & Technology*, 1991, **2**, 247-251.

STRASBURG G.M., XIONG Y.L. Physiology and chemistry of edible muscle tissues. In: Damodaran S., Parkin K.L. (Eds.), Fennema's food chemistry. CRC Press: Boca Raton (United States), 2017, 955-1016.

SUMAN S.P., MANCINI R.A., JOSEPH P., RAMANATHAN R., KONDA M.K.R., DADY G., NAVEENA B.M., LÓPEZ-LÓPEZ I. Color-stabilizing effect of lactate on ground beef is packaging-dependent. *Meat Science*, 2010, **84**, 329-333.

SUMAN S.P., HUNT M.C., NAIR M.N., RENTFROW G. Improving beef color stability: practical strategies and underlying mechanisms. *Meat Science*, 2014, **98**, 490-504.

– T –

TANG J., FAUSTMAN C., HOAGLAND T.A. Krzywicki revisited: Equations for spectrophotometric determination of myoglobin redox forms in aqueous meat extracts. *Journal of Food Science*, 2004, **69**, C717-C720.

TANG J.L., FAUSTMAN C., HOAGLAND T.A., MANCINI R.A., SEYFERT M., HUNT M.C. Interactions between mitochondrial lipid oxidation and oxymyoglobin oxidation and the effects of vitamin E. *Journal of Agricultural and Food Chemistry*, 2005, **53**, 6073-6079.

TAPP W.N., 3RD, YANCEY J.W., APPLE J.K. How is the instrumental color of meat measured? *Meat Science*, 2011, **89**, 1-5.

TARLADGIS B.G., WATTS B.M., YOUNATHAN M.T., DUGAN L. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists Society*, 1960, **37**, 44-48.

TICHIVANGANA J.Z., MORRISSEY P.A. Metmyoglobin and inorganic metals as pro-oxidants in raw and cooked muscle systems. *Meat Science*, 1985, **15**, 107-116.

TORRICO D.D., HUTCHINGS S.C., HA M., BITTNER E.P., FUENTES S., WARNER R.D., DUNSHEA F.R. Novel techniques to understand consumer responses towards food products: A review with a focus on meat. *Meat Science*, 2018, **144**, 30-42.

TROUBRIDGE CRITCHELL J., RAYMOND J. A history of the frozen meat trade: An account of the development and present day methods of preparation, transport, and marketing of frozen and chilled meats. Constable & Company, Ltd.: London (United Kingdom), 1912, 442 p.

TRYFINOPOULOU P., TSAKALIDOU E., NYCHAS G.J.E. Characterization of *Pseudomonas* spp. associated with spoilage of gilt-head sea bream stored under various conditions. *Applied and Environmental Microbiology*, 2002, **68**, 65-72.

– V –

VAN ROOYEN L.A., ALLEN P., O'CONNOR D.I. The application of carbon monoxide in meat packaging needs to be re-evaluated within the EU: An overview. *Meat Science*, 2017, **132**, 179-188.

VENKATA REDDY B., SIVAKUMAR A.S., JEONG D.W., WOO Y.-B., PARK S.-J., LEE S.-Y., BYUN J.-Y., KIM C.-H., CHO S.-H., HWANG I. Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environments. *Animal Science Journal*, 2015, **86**, 1-16.

VERBEKE W., DE SMET S., VACKIER I., VAN OECKEL M.J., WARNANTS N., VAN KENHOVE P. Role of intrinsic search cues in the formation of consumer preferences and choice for pork chops. *Meat Science*, 2005, **69**, 343-354.

VERMEULEN A., RAGAERT P., RAJKOVIC A., SAMAPUNDO S., LOPEZ-GALVEZ F., DEVLIEGHERE F. New research on modified-atmosphere packaging and pathogen behaviour. In: Sofos J. (Ed.), Advances in microbial food safety. Woodhead Publishing: Duxford (United Kingdom), 2013, 340-354.

– W –

WALKER J. Discovering words in the kitchen. Shire Publications: Oxford (United Kingdom), 2010, 96 p.

WANG, W.-J., CROMPTON, R.H. The role of load-carrying in the evolution of modern body proportions. *Journal of Anatomy*, 2004, **204**, 417-430.

WATTS B.M., KENDRICK J., ZIPSER M.W., HUTCHINS B., SALEH B. Enzymatic reducing pathways in meat. *Journal of Food Science*, 1966, **31**, 855-862.

WEBB T. Useful report & infographics on palm oil, beef, leather and soy supply chains. [on line] Web address:
http://sustainablesmartbusiness.com/2016/01/useful-report-infographics-on-palm-oil-beef-leather-and-soy-supply-chains, retrieved on 05/03/2018.

WILLIAMS A.C., HILLS L.J. Meat and nicotinamide: A causal role in human evolution, History, and demographics. *International Journal of Tryptophan Research*, 2017, **10**, 1-23.

WOERNER D.R., SCANGA J.A., BELK K.E. Slaughet-line operation: Cattle. In: Devine C., Dikeman M. (Eds.), Encyclopedia of meat sciences. Academic Press: Cambridge (United States), 2014, 284-289.

WOOD J.D., NUTE G.R., RICHARDSON R.I., WHITTINGTON F.M., SOUTHWOOD O., PLASTOW G., MANSBRIDGE R., DA COSTA N., CHANG K.C. Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science*, 2004, **67**, 651-667.

WYNESS L., WEICHSELBAUM E., O'CONNOR A., WILLIAMS E.B., BENELAM B., RILEY H., STANNER S. Red meat in the diet: an update. *Nutrition Bulletin*, 2011, **36**, 34-77.

– X –

XIE F., SUN S., XU A., ZHENG S., XUE M., WU P., ZENG J.H., BAI L. Advanced oxidation protein products induce intestine epithelial cell death through a redox-dependent, c-jun N-terminal kinase and poly (ADP-ribose) polymerase-1-mediated pathway. *Cell Death & Disease*, 2014, **5**, e1006.

– Y –

YAM K.L., TAKHISTOV P.T., MILTZ J. Intelligent packaging: Concepts and applications. *Journal of Food Science*, 2005, **70**, R1-R10.

YANG A., LANARI M.C., BREWSTER M., TUME R.K. Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement. *Meat Science*, 2002, **60**, 41-50.

YOST C.K. Biopreservation. In: Smithers G. (Ed.), Encyclopedia of meat sciences. Academic Press: Oxford (United Kingdom), 2014, 76-82.

– Z –

ZACHAROF M.P., LOVITT R.W. Bacteriocins produced by lactic acid bacteria: A review article. *APCBEE Procedia*, 2012, **2**, 50-56.

ZAGOREC M., CHAMPOMIER-VERGÈS M.-C. Meat microbiology and spoilage. In: Toldrá F. (Ed.), Lawrie's Meat Science. Woodhead Publishing: Duxford (United Kingdom), 2017, 187-203.

ZHANG P., KAUR M., BOWMAN J., RATKOWSKY D., TAMPLIN M. Effect of environmental factors on intra-specific inhibitory activity of *Carnobacterium maltaromaticum*. *Microorganisms*, 2017, **5**, 59.

ZHAO Y., WELLS J.H., MCMILLIN K.W. Applications of dynamic modified atmosphere packaging systems for fresh red meats: Review. *Journal of Muscle Foods*, 1994, **5**, 299-328.

ZHOU G.H., XU X.L., LIU Y. Preservation technologies for fresh meat - A review. *Meat Science*, 2010, **86**, 119-128.

ZOU J., JIANG H., CHENG H., FANG J., HUANG G. Strategies for screening, purification and characterization of bacteriocins. *International Journal of Biological Macromolecules*, 2018, **117**, 781-789.

Curriculum vitae

Summary

Pedro H. Imazaki (°Rio de Janeiro, 15 February 1984) started his studies in veterinary medicine at the Fluminense Federal University in Niterói in 2001. In 2006, he graduated as Doctor of Veterinary Medicine. From 2007 to 2009, he had a two-year experience with endocrine disruptors under the supervision of Prof. Marie-Louise Scippo, and, in 2009 he received his Advanced Master diploma in Specialised Veterinary Medicine (module Veterinary Public Health: Food Science) at the University of Liège. In 2010, he started his doctoral research under the supervision of Prof. Antoine Clinquart, and his primary research topic became meat science. More specifically, his research interests were the influence of production and processing-related factors on the microbial and physicochemical quality of fresh meat; the study of long-term ageing of beef; the evaluation, prediction and optimisation of the shelf life of meat and meat products; and biopreservation of foodstuffs. In 2012, he became a graduate teaching assistant at the Department of Food Science of the University of Liège and, concomitantly with his research activities, he has conceptualised and performed teaching activities related to food quality and safety, food inspection and control of the food chain.

Scientific articles as first author

1. IMAZAKI P.H., ELANSARY M., SCIPPO M.-L., DAUBE G., CLINQUART A. Effect of sex and sub-zero storage temperature on the microbial and oxidative stability of beef packaged in high-oxygen atmosphere after different vacuum ageing times. *Meat Science* (*accepted*).

2. IMAZAKI P.H., DOUNY C., ELANSARY M., SCIPPO M.-L., CLINQUART A. Effect of muscle type, aging technique, and aging time on oxidative stability and antioxidant capacity of beef packed in high-oxygen atmosphere. *Journal of Food Processing and Preservation*, 2018, **42**, e13603.

3. IMAZAKI P.H., BROSE F., JAUNIAUX T., DAS K., MULLER M., SCIPPO M.-L. Estrogenic evaluation and organochlorine identification in blubber of North Sea harbour porpoise (*Phocoena phocoena*) stranded on the North Sea coast. *BioMed Research International*, 2015, **2015**, 438295.

4. IMAZAKI P.H., TAMINIAU B., FALL P.A., ELANSARY M., SCIPPO M.-L., DAUBE G., CLINQUART A. Oxidative stability and microbial ecology of beef with extremely long shelf-life. *Meat Science* (*revised version submitted*).

5. IMAZAKI P.H., JACQUES-HOUSSA C., KERGOURLAY G., DAUBE G., CLINQUART A. Phenotypic, genetic and functional characterisation of *Carnobacterium maltaromaticum* with potential as biopreservatives isolated from beef with extremely long shelf-life (*in preparation*).

Scientific articles as co-author

1. MÜLLER J.C., IMAZAKI P.H., BOARETO A.C., LOURENÇO E.L., GOLIN M., VECHI M.F., LOMBARDI N.F., MINATOVICZ B.C., SCIPPO M.-L., MARTINO-ANDRADE A.J., DALSENTER P.R. *In vivo* and *in vitro* estrogenic activity of the antidepressant fluoxetine. *Reproductive Toxicology*, 2012, **34**, 80-85.

2. MAIA DANIELSKI G., IMAZAKI P.H., DAUBE G., ERNLUND DE FREITAS MACEDO R., CLINQUART A. *In vitro* evaluation of the competing effect of *Carnobacterium maltaromaticum* isolated from vacuum-packed meat against food pathogens (*in preparation*).

Oral presentations in scientific meetings as presenting author

1. IMAZAKI P.H., MAIA DANIELSKI G., DAUBE G., ERNLUND DE FREITAS MACEDO R., CLINQUART A. The inhibitory effect of *Carnobacterium maltaromaticum* isolated from vacuum packed meat against *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium. BAMST Symposium, Melle, Belgium, 7/12/2017.

2. IMAZAKI P.H., MAIA DANIELSKI G., DAUBE G., ERNLUND DE FREITAS MACEDO R., CLINQUART A. *In vitro* evaluation of the competing effect of *Carnobacterium maltaromaticum* isolated from vacuum-packed beef with long shelf-life against three major food pathogens. 4th FARAH Day, Liège, Belgium, 13/10/2017.

3. MAIA DANIELSKI G., IMAZAKI P.H., DAUBE G., ERNLUND DE FREITAS MACEDO R., CLINQUART A. *In vitro* evaluation of the competing effect of *Carnobacterium maltaromaticum* isolated from vacuum packed meat against food pathogens. 63rd International Congress on Meat Science and Technology, Cork, Ireland, 15/8/2017.

4. IMAZAKI P.H., TEIXEIRA GONÇALVES A., KRANTZ M., THIMISTER J., CLINQUART A. Influence of aging time and technique (dry- vs. wet-aging) on tenderness, color and lipid stability of Belgian Blue beef. BAMST Symposium, Melle, Belgium, 8/12/2016.

5. IMAZAKI P.H., JACQUES-HOUSSA C., KERGOURLAY G., DAUBE G., CLINQUART A. Sensory quality of beef patties inoculated with strains of *Carnobacterium maltaromaticum* with potential as biopreservatives. BAMST Symposium, Melle, Belgium, 2/12/2015.

6. IMAZAKI P.H., JACQUES-HOUSSA C., KERGOURLAY G., DAUBE G., CLINQUART A. Evaluation of the sensory quality of beef patties inoculated with *Carnobacterium maltaromaticum* strains with biopreservative potential. 2nd FARAH Day, Liège, Belgium, 16/10/2015. [*best oral presentation award*]

7. IMAZAKI P.H., DOUNY C., SCIPPO M.-L., CLINQUART A. Impact of aging technique, muscle and previous vacuum storage time on oxidative stability of beef packaged under high-oxygen atmosphere. BAMST Symposium, Melle, Belgium, 11/12/2014.

8. IMAZAKI P.H., DOUNY C., SCIPPO M.-L., CLINQUART A. Stabilité oxydative des viandes blanc bleu belge conditionnées sous atmosphère riche en oxygène après maturation sous vide ou en carcasse. 15èmes Journées Sciences du Muscle et Technologies des Viandes, Clermont-Ferrand, France, 5/11/2014.

9. IMAZAKI P.H., TAHIRI A., TAMINIAU B., NEZER C., DAUBE G., CLINQUART A. Evaluation of the bacterial diversity and its evolution during storage of fresh beef from British and Belgian origins under different atmosphere and temperature conditions. BAMST Symposium, Kortrijk, Belgium, 10/12/2013.

Other oral presentations in scientific meetings

1. TAMINIAU B., NEZER C., DELHALLE L., ADOLPHE Y., IMAZAKI P.H., CLINQUART A., DAUBE G. L'analyse métagénomique ciblée au service de la microbiologie des aliments : Applications concrètes. 17th Conference on Food Microbiology, Brussel, Belgium, 21/9/2012.

2. TAMINIAU B., DELHALLE L., NEZER C., ADOLPHE Y., IMAZAKI P.H., CLINQUART A., DAUBE G. Metagenomic analysis as a tool to better characterize the bacterial content of food and food preparations. 23rd International ICFMH Symposium: FoodMicro, İstanbul, Turkey, 4/9/2012.

3. CLINQUART A., IMAZAKI, P.H., SÁNCHEZ MAINAR M., DELHALLE L., ADOLPHE Y., DURÉ R., DAUBE G. Extending the shelf life of fresh meat: What is technically and legally feasible? Euroepan Federation of Animal Science Annual Meeting, Stavanger, Norway, 29/8/2011.

4. DELHALLE L., ADOLPHE Y., CREVECOEUR S., IMAZAKI P.H., DAUBE G., CLINQUART A. A new tool to control meat products safety: A web based application of predictive microbiology models. 57th International Congress of Meat Science and Technology, Gent, Belgium, 11/8/2011.

Poster presentations in scientific meetings as presenting author

1. IMAZAKI P.H., TEIXEIRA GONÇALVES A., KRANTZ M., THIMISTER J., CLINQUART A. Wet-aging vs. dry-aging : Influence sur la tendreté et la stabilité oxydative des viandes Charolaises. 16èmes Journées Scientifiques du Muscle et des Technologies de la Viande, Paris, France, 21/11/2016. [*awarded with the medal of the* Académie de la viande]

2. IMAZAKI P.H., TEIXEIRA GONÇALVES A., KRANTZ M., THIMISTER J., CLINQUART A. Effect of aging time, aging technique (dry- vs. wet-aging) and packaging on tenderness, pigment and lipid stability of Belgian blue beef. 3rd FARAH Day, Liège, Belgium, 21/10/2016.

3. IMAZAKI P.H., JACQUES-HOUSSA C., KERGOURLAY G., DAUBE G., CLINQUART A. Sensory quality of beef patties inoculated with strains of *Carnobacterium maltaromaticum* with potential as biopreservatives. 61st International Congress of Meat Science and Technology, Clermont-Ferrand, France, 24/8/2015.

4. IMAZAKI P.H., TAHIRI A., NDEDI EKOLO F., DAUBE G., CLINQUART A. Morphological and functional characterization of *Carnobacterium maltaromaticum* isolated from vacuum-packed beef with long shelf life. 20ème Colloque du CBL, Lille, France, 17/6/2015.

5. IMAZAKI P.H., TAHIRI A., NDEDI EKOLO F., DAUBE G., CLINQUART A. Evaluation of morphological and functional characteristics of *Carnobacterium maltaromaticum* isolated from vacuum-packaged beef with long shelf life. 1st FARAH Day, Liège, Belgium, 17/10/2014.

6. IMAZAKI P.H., TAHIRI A., THIMISTER J., SCIPPO M.-L., CLINQUART A. Impact of aging technique and muscle on oxidative stability of beef packaged under high-oxygen atmosphere. 1st FARAH Day, Liège, Belgium, 17/10/2014.

7. IMAZAKI P.H., TAHIRI A., NDEDI EKOLO F., DAUBE G., CLINQUART A. *Carnobacterium maltaromaticum* isolated from vacuum-packed beef with long shelf life: Morphological and functional characterization. 19th Conference on Food Microbiology, Bruxelles, Belgium, 18/9/2014.

8. IMAZAKI P.H., TAHIRI A., NDEDI EKOLO F., DAUBE G., CLINQUART A. Morphological and functional characterization of *Carnobacterium maltaromaticum* isolated from vacuum-packed beef with long shelf life. 60th International Congress of Meat Science and Technology, Punta del Este, Uruguay, 18/8/2014.

9. IMAZAKI P.H., TAHIRI A., THIMISTER J., SCIPPO M.-L., CLINQUART A. Influence of aging technique and muscle on physicochemical stability and antioxidant capacity of high-oxygen atmosphere packed beef. 60th International Congress of Meat Science and Technology, Punta del Este, Uruguay, 18/8/2014.

10. IMAZAKI P.H., TAHIRI A., THIMISTER J., CLINQUART A. Influence of two breeds (Belgian Blue and Limousin) and previous storage time on pigment and lipid stability of high-oxygen atmosphere packaged beef. BAMST Symposium, Kortrijk, Belgium, 10/12/2013.

11. IMAZAKI P.H., TAHIRI A., TAMINIAU B., NEZER C., DAUBE G., CLINQUART A. Bacterial diversity and its evolution during storage of fresh beef from different origins under different atmosphere and temperature conditions. 3rd Scientific Meeting of the Faculty of Veterinary Medicine, Liège, Belgium, 11/10/2013.

12. IMAZAKI P.H., TAHIRI A., THIMISTER J., CLINQUART A. Influence of breed and previous storage time on color and lipid stability of beef packaged in high-oxygen atmosphere. 59th International Congress of Meat Science and Technology, İzmir, Turkey, 20/8/2013.

13. IMAZAKI P.H., TAHIRI A., BAPTISTA RODRIGUES A., TAMINIAU B., NEZER C., DAUBE G., CLINQUART A. Viandes bovines à longue durée de conservation conditionnées sous vide : Isolement et caractérisation de souches de *Carnobacterium*. 14èmes Journées Sciences du Muscle et Technologies des Viandes, Caen, France, 13/11/2012.

14. IMAZAKI P.H., SALMON O., TAHIRI A., CLINQUART A. Validation of a method for measuring the colour and determining the proportions of myoglobin redox forms on beef. 2nd Scientific Meeting of the Faculty of Veterinary Medicine, Liège, Belgium, 19/10/2012.

15. IMAZAKI P.H., TAHIRI A., DAUBE G., CLINQUART A. Effect of duration and temperature of previous vacuum-packed storage on the microbiological quality of Belgian Blue meat packed in high-oxygen atmosphere. 23rd International ICFMH Symposium: FoodMicro, İstanbul, Turkey, 5/9/2012.

16. IMAZAKI P.H., TAHIRI A., THIMISTER J., SCIPPO M.-L., CLINQUART A. Effect of duration and temperature of previous vacuum-packed storage on the oxidative stability of Belgian Blue meat packed in high-oxygen atmosphere. 58th International Congress of Meat Science and Technology, Montréal, Canada, 13/8/2012.

17. IMAZAKI P.H., TAMINIAU B., NEZER C., DAUBE G., CLINQUART A. Study of the microbial diversity in vacuum-packed chilled beef from different origins through a metagenomics approach. Biomedica, Liège, Belgium, 18/4/2012.

18. IMAZAKI P.H., NEZER C., TAMINIAU B., DAUBE G., CLINQUART A. Caractérisation des viandes bovines à très longue durée de conservation sous vide. 1st Scientific Meeting of the Faculty of Veterinary Medicine, Liège, Belgium, 9/12/2011. [*best poster award*]

19. IMAZAKI P.H., MARÉCHAL A., NEZER C., TAMINIAU B., DAUBE G., CLINQUART A. Évaluation de la diversité bactérienne et de son évolution pendant la conservation de la viande fraîche bovine de différentes origines emballée sous vide. Ecosystèmes Microbiens et Bioprotection des Aliments, Nantes, France, 18/11/2011.

20. IMAZAKI P.H., MARÉCHAL A., NEZER C., TAMINIAU B., DAUBE G., CLINQUART A. Influence of temperature on conservability of chilled vacuum packed beef from different origins. 57th International Congress of Meat Science and Technology, Gent, Belgium, 8/8/2011.

21. IMAZAKI P.H., BROSE F., REMACLE A.-S., VAN DER HEIDEN E., MULLER M., SCIPPO M.-L. Evaluation of the contamination of North Sea porpoises (*Phocoena phocoena*) by endocrine disrupting chemicals using CG-MS and *in vitro* assays. 5th Copenhagen Workshop on Endocrine Disrupters, København, Denmark, 20/5/2009.

22. IMAZAKI P.H., BROSE F., VAN DER HEIDEN E., MULLER M., SCIPPO M.-L. Evaluation of the endorcine disrupting chemicals contamination in the North Sea porpoise population using *in vitro* assays. Biomedica, Liège, Belgium, 1/4/2009.

23. IMAZAKI P.H., CIARLINI DE AZEVEDO I., BORGES MANO S. Avaliação bacteriológica, sensorial e composição centesimal de três formulações de carne de jacaré-de-papo-amarelo (*Caiman latirostris*) em conserva. XVI Seminário de Iniciação Científica, Niterói, Brazil, 7/11/2006.

24. IMAZAKI P.H., DE CASTRO BELTRÃO DA COSTA J., FERNANDES M. L., FIGUEIREDO DA SILVA M., BORGES MANO S. Avaliação nutricional da rotulagem nutricional obrigatória utilizada em salsichas normais e *light*. XV Seminário de Iniciação Científica, Niterói, Brazil, 8/11/2005.

25. IMAZAKI P.H., DE CASTRO BELTRÃO DA COSTA J., QUEIROZ DE FREITAS M., SILVA PARDI H., TEIXEIRA MÁRSICO E., FERNANDES M. L., FIGUEIREDO DA SILVA M., BORGES MANO S. Avaliação nutricional da rotulagem nutricional obrigatória utilizada em salsichas normais e *light*. XIV Seminário de Iniciação Científica, Niterói, Brazil, 9/11/2004.

Other poster presentations in scientific meetings

1. IMAZAKI P.H., TAHIRI A., TAMINIAU B., NEZER C., DAUBE G., CLINQUART A. La diversité bactérienne et son évolution pendant la conservation de viandes bovines fraîches de différentes origines conditionnées sous vide. 19èmes Journées 3R Rencontres Recherches Ruminants, Paris, France, 5/12/2012.

2. DELHALLE L., COLLIGNON B., DEHARD S., IMAZAKI P.H., DAUBE G., CLINQUART A. Chilling of heavy carcasses from double muscled cattle: Time-temperature evolution and predictive modelling of growth of *Listeria monocytogenes* and *Clostridium perfringens*. 15th Conference on Food Microbiology, Gent, Belgium, 16/9/2010.

3. DELHALLE L., COLLIGNON B., DEHARD S., IMAZAKI P.H., DAUBE G., CLINQUART A. Chilling of heavy carcasses from double muscled cattle: Time-temperature evolution and predictive modelling of growth of *Listeria monocytogenes* and *Clostridium perfringens*. 56th International Congress of Meat Science and Technology, Jeju-si, South Korea, 16/8/2010.

4. VAN DER HEIDEN E., REMACLE A.-S., IMAZAKI P.H., MULLER, M., RIBONNET L., PUSSEMIER L., LARONDELLE, Y., SCHNEIDER Y.-J., MAGHUIN-ROGISTER G., SCIPPO, M.-L. Reporter gene assays as screening tools to assess the endocrine disrupting potencies of 20 pesticides. Biomedica, Liège, Belgium, 1/4/2009.

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